"INVESTIGATION OF ALLELOPATHIC POTENTIAL IN *PASCALIA GLAUCA* ORTEGA."

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JULY, 2019

Certificate of the Guide

CERTIFIED that the work incorporated in the thesis "**Investigation of Allelopathic Potential in *Pascalia glauca* Ortega**." submitted by Mr. Ilahi Ismail Mujawar was carried out by the candidate under my supervision / guidance. Such material has been obtained from other sources has been duly acknowledged in the thesis.

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Declaration by the Candidate

I declare that the thesis entitled "**Investigation of Allelopathic Potential in *Pascalia glauca* Ortega**." submitted by me for the degree of Doctor of Philosophy is the record of work carried out by me during the period from 29/06/15 to 27/03/2019 under the guidance of Dr. M. B. Kanade and Co-guide Prin. Dr. C. V. Murumkar and has not formed the basis for the award of any degree, diploma, associate-ship, fellowship, titles in this or any other University or other institution of Higher learning.

I further declare that the material obtained from other sources has been duly acknowledged in the thesis.

Place: Mr. Ilahi Ismail Mujawar

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Dedicated
To
My Beloved Parents
and
Teachers

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Date :

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CHAPTER - I

INTRODUCTION

Agricultural practice has been a strong backbone of India. India is basically farming nation and developing country representing one of the largest and wide biodiversity in crop and crop patterns. Weeds and crops associate to each other. Agriculture is an unavoidable necessity for the existence of human life that is said to have its deep roots in the innovations since early civilization that started crop practice. Indian agriculture is likely to continue on this path for a long time though struggling for the improvement of agriculture practices to fulfill the necessities of agro dependent population by adopting innovative methods and techniques. Though, India contributed continuously and witnessed limitations. Among these, weeds pose an acute problem in agriculture alarming to urgent attention.

The invasion of exotic plant species is another major problem that threat to ecological diversity and number of invaders enters to snoop around that established themselves well and disrupts inherent, over competed against the natives. Weeds become tolerance in new habitats in agricultural ecosystem and disturbed crop field. Therefore, irretrievable loses in agro product. Besides these, shrinkage of cultivated land, unplanned industrialization in agricultural area contaminates the natural resources ultimately reduction in the crop yield. Further, natural calamities like irregular and erratic rainfall, storm, drought, and change in environmental scenarios, global warming was unexpectedly aggravated problem face Indian agriculture results into loss in productivity.

The weeds have been growing and co-associated with crops since the beginning of agriculture and their infestation is one of the reasons of yield loss in Indian crop system. Mukhopadhyaya (1992) stated that weeds in India cause an annual monitory loss of Rs. 19800 million. Weeds reduce crop yield by 5% in the most highly developed countries, 10% in the less developed countries and 25% in the least developed countries (Yau Lam *et al*., 2012). Number of experiments has been conducted by various researchers to understand the effect of different weed infestation on yield reduction of variety of crops. Several workers have investigated the effect of individual weed species on yield of crops. For example, the interference of wheat by Canada thistle (*Circium arvense)* has been found to reduce the wheat yield by more

than 40 % (Hagood, 1958). Dawson (1965) has found upto 94% loss in yield of sugarbeet in absence of control over the weed *Chenopodium album.* Islieb (1960) and Bandeen and Buchholtz (1967) found 32% and 90% loss in yield of potatoes and corm by quackgrass (*Agropyron repens*). *Setaria faberii* was found to decrease soybean yield by 21% reported by Knake and Slife (1962). About 21% grain yield reduction of sorghum by common milkweed (*Asclepias syriaca)* has been reported by Evetts and Burnside (1973). Purple nutsedge was found to cause 35-89% loss in yields of okra, green bean, cucumber, cabbage, tomato and garlic (William and Warren, 1975) and 23- 28% loss in rice yield (Okafor and De Datta, 1976). *Abutilon theophrasti* was found to cause heavy losses (14 to 100%) in yield of soybean (Eaton *et al.,* 1976) and cotton (Hagood *et al.,* 1980). Yellow nutsedge (C*yperus esculentus)* has been reported to cause 41% loss in corm (Stoller *et al.,* 1979) and 32% loss in seed cotton (Patterson *et al.,* 1980).

1.1. Native and exotic weed

Weeds are widespread biological constraint in crops that cause hidden damages of crops influencing the Indian economy and over lasting problem for agriculture results in shortage of food, fodder, fiber, fuel and agro based products for survive the ever-increasing population. Both the native and exotic weeds are involved in interrupting the crop system. Numbers of invaders entered, established well and over compete against the natives. They tolerated in new habitats in agricultural ecosystem and lowered the crop yields as well as impaired the quality of produce**.** This success is due to ability to out-compete locals has no new bearing on the nature of plant communities. Many invasive species are new and uncommon at their native places but very abundant and tolerant in their new habitats. Allelopathy has also been suggested that it is a mechanism for the impressive success of invasive plants in part because invaders often establish virtual monocultures where diverse communities once flourished. A large number of exotics have become naturalized in India and have affected the distribution of native flora to some extent. Their residues continuously mix into soil rhizosphere and disturbed the crop ecosystem. Some known terrestrial exotics plant species are *Eupatorium odoratum, Cytisus scoparius, Mikania micrantha, Lantena camera, Mimosa invisa, Ageratum conozoides, Pascalia glauca, Parthenium hysterophorus, Prosopis juliflora, Eucalyptus globulus,* etc. that inhibited

the various crop growth and its development (Patil, 2011; Chuiha *et al*., 1999; Soberero *et al*., 2004: Mujawar *et al*., 2016 and Batish *et al*., 2003b).

The invader or exotic weed species are new comers in specific region which compete with native species, influences the nearby plants and becomes a dominant succession and formation of new communities as a climax through the allelopathy. Exotic invaders and native weed species that impede in agricultural practice and degradation in environmental ecosystem (Inderjit, 2005). They provide alternate host for many microorganisms, become health hazardous to human and also increase total expenditure in inter-cultivation and harvesting process. The invasive weeds infesting the crop fields and other ecosystems have become a serious threat all over the India. They reduce the crop yield as well as loss the native phytodiversity, which is considered as the greatest damage to whole biosphere. The invasiveness of exotics, with their special characteristic features, is attributed to their rapid and ease growth that spread fast to form dense population over natives. They produce large number of seeds with effective seed dispersal ability, release of bioactive compounds, capability for stress tolerance, adapt in variety of ecological habitats; special potent of competence and defense mechanism to invade rapidly in the nature and crop fields as well.

Rapid regeneration and leaching of some novel biochemical called allelochemicals into the surroundings create a great pose in agricultural and horticultural ecosystem. They cause both positive and / or negative effect on the seed germination and seedling development in crops by utilization of allelopathic effects. Physiologically weeds and crop plants have similar demand and as a results the competition exists between weed and crop plants. Weeds compete for space, light, water, minerals and also influence the crop plants by secretion of toxic products into the environment. The significance of these toxic compounds may be realized from the fact that even if it is self tolerant, its presence, chemical nature and concentration in coincidence with other conditions may govern the type of its associates.

1.2 Allelopathy :

Allelopathy is the chemical interference mechanism to the plant because of the addition of secondary products, released into the soil rhizosphere. They are called as allelochemicals, acting on germination or as growth inhibitors. The inhibition may take place by one plant (or other organism) to another. They are influencing the

development and growth of nearby plants. These chemicals can originate either from whole or any part of donor plants and located in all types of plant tissues. Recent research suggests that allelopathic properties can deliver one species more invasive to native species and thus becomes potentially detrimental to both agricultural and naturalized settings.

The term “allelopathy” was first introduced by Austrian plant physiologist Molish in 1937 and refers to chemical interactions among plants. The word allelopathy has been derived from Greek word ‘allelon’ meaning mutual and ‘pathos’ meaning suffering to describe both the beneficial and detrimental chemical interactions of plants and micro organisms. Several definitions of allelopathy have been given to explain that the interference of one plant/weed to another plant/crop. According to Bonner (1950), it is the interspecific (antibiotic) as well as intraspecific (autotoxic) chemically acting together. Muller (1966) referred allelopathy to the injurious effects that one higher plant has on another through the production of chemical retardants that run away into the environment. Del Moral and Cates (1971) defined allelopathy as the inhibition of germination, growth and metabolism of one plant due to the release of organic chemicals by another. In recent years, plant ecologists have focused primarily on the harmful interactions and most workers now use the term in the sense implied by Rice (1974) that "any direct or indirect harmful effect by one plant on another through the production of chemical compounds that escape into the environment either through weathering, leaching, exudation or volatization".

According to Torres *et al*. (1996) allelopathy is any process involving secondary metabolites produced by plants, micro-organisms, viruses, fungi that influence the growth and development of agricultural and biochemical systems including positive or negative effects. These chemicals have no apparent metabolic, physiological or structural role for the producer but will have deleterious effects on other plants. In many cases they are believed to function as biochemical defenses. Biochemical compounds produced by donor species affect plant growth, development, yield and their quality. The influence by allelopathy inducing many changes in plant morphology, physiology as well as at cellular and organism level influences the gene expression and molecular level of the recipient plant. These useful or harmful effects have many inferences in crop ecosystem. The allelopathic potential of plants is mainly due to the allelochemicals present in them. In majority cases plants

contributing allelochemicals is said to be ‘donor plant’ and the plant being influenced by the released allelochemicals is recognized as the ‘target or receive plant’. Einhelling (1995) stated that allelopathic interactions involve addition of chemical substances or allelochemicals into environment which disorderd certain physiological processes of target plants. Hence, their isolation, characterization, bioassay and commercial use as well as impact on crop in sustainable agriculture has given the first priority by researchers in this field and experts have been concentrated to assess the target work particularly on influence of allelopathy since from long time.

The first hint of involvement and impact of allelopathy on agriculture crops was recorded by observations made by Theophrastus (ca 300B.C.) when he stated that chickpea did not reinvigorate the soil but rather, exhausted it completely with other legumes and destroyed weeds, particularly caltrop. Plinious (I A.D.) reported that chickpea, barley, fenugreek and *Vicia* destroy or burn up farmland (Rice, 1984). Credit for the first ever report pertaining to weed allelopathy is given by de Candolle (1832) who described the damaging effects of root exudates of Canada thistle *(Circium arvense)* on the growth of neighboring oat plants. Some workers recorded with positive results leading to enhanced weed suppression and reduced herbicide inputs while others with mixed results (Barnes and Putnam, 1983, 1987; Burgos *et al*., 1999; Einhellig and Rasmussen, 1989; Masiunas *et al*., 1995; Moyer *et al*., 2000; Petersen *et al*., 2001 and Sene *et al.*, 2001). Today, the number of publications in this field has increased exponentially as physiologists, soil scientists, weed scientists and natural product chemists continue to study this challenging area (Macias, 2002) and helped to protect plant biodiversity and enhance weed management strategies in a variety of ecosystem including sustainable agricultural ecosystem. Most of the weeds have adverse effect on germination and growth of test crops or crop cultivars. Several workers have reported that lower concentrations of weed extracts or leachates may be stimulatory or may not have any effect but higher concentrations of almost all weeds are usually detrimental to crop growth.

1.3 Allelopathy and weed :

Allelopathic effect of weeds in crop field is considered as one of the major threat in agriculture that reduces crop yield and increases the cost of productivity. The agro ecosystems all over the world are threatened by the native and invasive weeds. These weeds held an unexplainable place in agriculture, because they compete with

crops for enjoying benefits of resources and have coevolved, thereby causing about 34% loss in agricultural crop yield (Jabran *et al*., 2015 and Mujawar *et al*., 2016). These weeds successfully occupy and spread over the whole agricultural field, displacing the crops, suppressing their growth and reduce metabolic process in them. The potential yield reductions by the weeds in some important crops were recorded as wheat 23%, soybean 37%, rice 37%, maize 40%, cotton 36%, and potatoes 30% (Oerke, 2006). They act as a shield for the crop plants for available nutrients, space, light and moisture (Gulzar *et al*., 2015a). Hence, in the presence of weed, physiological activities and growth of crops are mostly negatively affected (Rajcanand Swanton, 2001). In addition to this, they deteriorate crop quality, block water paths in crop ecosystem and cause health problems in humans. Not only crop ecosystem but other natural or manmade ecosystems such as follow lands, grass lands, forest areas, garden, parks, pathways and pavements also greatly affected and disturbed (Singh *et al*., 2003c). Weeds cause unappealing loss in crop productivity, local biosociology and health hazards to living.

Weeds have always played an important role throughout the domestication of crop plants, which necessitated practicing weed control measures. There are number of methods of weed control that have been practiced like hand pulling, cutting, physically smothering weeds and burning the remnants of weeds but this has created number of other problems like weed residues affecting the next crop growth, anxious and omitting in farm workers in case of poisonous weeds (Mujawar *et al*., 2016b). However, from the beginning of agriculture, hand weeding, mechanical weeding and herbicide applications have been most relied upon weed control methods (Chauvel *et al*., 2012 and Jabran *et al*., 2015). These weed control methods have served to keep lower weed infestations and improve the crop productivity. Despite the significant contribution of these weed control methods in improving crop productivity, certain challenges are also associated with them making it urgent to develop diversity in the current weed control methods. Additionally, the costs of weed eradication are also huge. Pimentel *et al*. (2001) has estimated out that the loss in crop yield due to weeds in the U.S. is about 12% and it costs nearly US$35 billion to control. In addition to the direct losses, approximately $4 billion is spent each year on herbicides used to control pest weeds (Inderjit, 2008). In the development of sustainable agriculture to some extent one can solve these problems through allelopathy.

1.4 Causes of action in Allelopathy :

Allelopathy refers to the negative possessions of higher plants of one species considered as the donor plant, on the seed germination, its growth and development of plants of another recipient species. It can be separated from other mechanisms of plant interference because such detrimental effect is exerted only through release of chemical inhibitors called allelochemicals by the donor species to the plant environment. Therefore, it is different from competition which involves the removal or reduction of some growth factors from the environment that are required by some other plant sharing the same habitat. Thus, allelopathy and competition together constitute the concept of interference. Allelochemicals are non-nutritional chemicals especially secondary metabolites produced by one organism and affecting the growth, behavior and population biology of other organisms or species (Reese, 1979). Inderjit, (2001) pointed out that the released chemicals in soil may act as allelochemical in one situation but not in another. Allelochemicals are considered as natural plant secondary products of the main metabolic pathway that has been recognized as phenolic acids, coumarins, terpenoids, flavonoids, alkaloids, glycosides and glucosinolate (Barnes and Putnam 1986 and Blum, 1995). Plant phenolics originate from shikimic acid pathway and terpenoids from mevalonic pathway (Inderjit *et al*., 1995 and Mallik 2002). They were escape into environment under specific condition (Chou, 1999) and for the self defense against herbivores, predators, pests and microbes (Swain, 1977). They were influencing positively or negatively or with mixed results on the growth and development of vegetation have been reported by dozens of researchers from India and other countries. Different weeds and their parts of extracts reported to produce different effects on various crops. Such results have a great potentiality of allelopathy and it has been importance to develop sustainable agro ecosystem also.

The allelochemicals released from one species may inhibit the growth of other species at certain concentration or may stimulate the growth of the same or different species at lower concentration (Narwal, 1994). Oudhia *et al*. (1988) argued that stimulatory allelochemicals can be used to develop eco- friendly, cheap and effective green growth promoter. The stimulatory effect of such a weed on crops can be utilized to increase the rate of germination, seedling growth and dry matter production in crops as it also gives new idea and hope for natural herbicides (Cardellina, 1988). Allelochemicals in the selective action can suppress plant growth and regulate species diversity in their habitat (Lambert *et al*., 1991 and Rizvi *et al*., 1999). The majority of

allelopathic plants tend to store the protective chemicals or allelochemicals in bound form, mainly in the tissues of leaves which latter defoliation on ground decompose it and release allelochemicals in the rhizosphere soil environment (Putnum and Duke, 1978 and Fisher, 1979). Some phenolic compounds present in rhizosphere soil are reported to promote growth and some others to suppress growth and development of plants depends upon their concentration therefore allelopathic activity due to phenolic inhibitors mostly occurs in nutrient poor soils (Stowe and Osborn, 1980).

Allelochemicals interfere with physiological processes of target plant inhibiting respiration (Rice, 1984) and energy transfer responsible for ATP synthesis (Moreland and Novizky, 1985), causes loss of plasma membrane integrity resulting in cellular leakage (Abbas *et al*., 1992). Allelochemicals suppressed mineral uptake by plants, reduced enzyme activities, photosynthesis and respiration, collectively results in retardation of the plant growth. They showed both inhibitory and stimulatory effects (Waller *et al*., 1995 and Mitzutani, 1999) and various factors like concentration, flux rate, age, metabolic state and environmental conditions determine their toxicity (Kohli *et al*., 1993, Weidenhamer, 1996, Gallet and Pellissier, 1997 and Nilson *et al*., 1998). The target plant shows varied responses to allelochemicals which released from the donor plant and mostly known to be concentration dependent either stimulation at low concentration and repellence when its concentration increased (Lovett *et al*., 1989). Mostly affected plants shows inhibition of seed germination with retard root and shoot length of seedlings, decoloration, lack of root hairs, reduced dry weight accumulation and ultimately loss in net productivity.

Several findings after 1950s have shown that allelopathic interactions between crops and weeds are also partly responsible for loss in yield of crops besides competition. These findings are based on a large number of bioassays involving treatment on different crops on germination by weed extracts and leachates, root exudates and also by chemicals isolated from weed species (allelochemicals) using pot culture, glass house and field experiments. The water dissolved allelochemicals from various plant parts of different weeds that influenced various plants or crops has been done and well documented by Rice (1979); Gill and Sanddhu (1996); Pawar and Chavan (1999); Chou (1999); Kalita *et al*. (1999).

Bromus tectorum was reported to cause 40 percent yield reduction in wheat (Rydrych and Muzik, 1968). The weeds *Amaranthus blitoides*, *A. gracilis*, *A. retroflexus*, *Ammi majus*, *Amigallis foemina*, *Plantago lanceolata* and *Rumex crispus*

were studied for allelopathic impact against the wheat and bajara that showed repressive impact (Qasem 1994b; 1995a, b, c). Chuiha *et al*. (1999) found that aqueous extracts, leachates and volatile chemicals of *Ageratium conyzoides* L. had negative effect on germination of wheat (*Triticum aestivum* L.). Batish *et al*. (2003b) recoded that extracts of *Parthenium hysterophorus* L. on *Brassica compestris* L. and *B. rapa* L. reduced seed germination and dry weight. Emetrio *et al*. (2004) studied effect of *Lepidium draba* L. shoot extract on the *Glycine max* (L.) Merr., *Hordium vulgare* L., *Nicotiana tobacum* L., *Triticum aestivum* L. and *Zea mays* L. and recorded reduction in radical elongation. Allelopathic impact of aqueous extract of leaf and ground fruit of genus *Solanum lycocarpum* L. reduced germination and early growth in *Sesamum indicum* L. (Aires *et al*., 2005). Water extract of stem, leaf and root of *Tagetes pretense,* T. *repens, Melilotus officinalis* and *Vicia villosa* subdued the seed germination and seedling growth of *Lolium multiflorum* (Caixia *et al*., 2005). Xiao Xia *et al*. (2007) explained weed-weed interaction in the treatment of whole plant, leaves and roots extract of *Vicia villosa* inhibited seed germination and seedling growth of another weeds *viz. Veronica persica, Poa annua* and *Echinochloa crus-galli*. Influence of allelochemicals released by *Excoecaria agallocha* L. and showed inhibitory effects on seed germination and seedling growth of pulses including *Vigna radiate, V. mungo, Arachis hypogaea* and millets like *Pennisetum typhodes, Eleusine coracana* have been reported by Kavitha *et al*. (2012). Hossain (2012) remarked that when debris of siam weed (*Chromolaena odorata*) was used in pot experiment, it reduced the seedling emergence of groundnut and other crops. Garbage combines with *Parthenium* weed, induce microbes and scale back the allelopathic impact that stirred up the seed germination in crop *Arachis hypogaea* (Rajiv *et al*., 2013). The allelopathic effects of aqueous and ethanolic extracts of ten common native and exotic weeds (*Alternanthera sessilis, Amaranthus tricolor, Cardiospermim helicacabum, Corchorus olitorius, Cyperus rotundus, Euphorbia heterophylla, E. hirta, Phyllanthus amerus, Portulaca oleracea and Vicoa indica*) greatly affect the seed health, regular growth and yield of *Triticum aestivum* (Dhole *et al*., 2013) and same weeds extracts treated against cotton seeds also showed same results reported by Dhole *et al*. (2014).

Madany and Ahmed (2015) knew the cinnamic acids, carboxylic acids and flavonoids from *Euphorbia helioscopia* that tested against wheat and pea impaired seed germination and cretaceous growth of seedlings due to impact of phenolics present in it. The study by Shinde (2016) revealed that the aqueous extract of

Parthenium hysterophorus had allelopathic potentiality and showed detrimental effect on seed germination and seedling growth of groundnut (*Arachis hypogaea*) however the length of plumule and radical increased in wheat (*Triticum aestivum*). Rezaul *et al*. (2017) pointed out that the aqueous extract of siam weed (*Chromolaena odorata*) had inhibitory allelopathic effects on the germination and seedling growth of rice, groundnut, mustard and chickpea. They further stated that higher concentration exhibited greater inhibitory effect than the lower concentration in aqueous extract. Saira Siyar *et al*. (2017) studied allelopathic effect of *Chenopodium album* and *Phalaris minor* and found that the seed germination and growth of wheat was affected. Recently, Mujawar *et al*. (2016b, 2017 and 2018) observed that the seed germination and seedling growth retarded as well as photosynthetic pigments was reduced at higher concentration of stem, leaf and flower aqueous extract of *Pascalia glauca* Ortega in wheat and groundnut. Furthermore they found that the leaf extract have more potentiality of negative effect than the stem and flower extracts of weed *P. glauca* on wheat (*Triticum aestivum*) and groundnut (*Arachis hypogaea*) seedling.

1.5 *Pascalia glauca* Ortega :

Pascalia glauca Ortega (Asteraceae) is a poisonous perennial weed found in crop field from Maharashtra, India since, 1992 as well as grasslands in Argentina. It's native to South America and widespread in "Pampas" of Argentina where its referred to as 'Agricultural plaque' that broken crops and of farm animals like cows, pigs and horses causing acute deadly hepatotoxicosis due to presence of atractyloside. Today, it has spread in Chile, Brazil, South America, Argentina, Uruguay, New Zealand and India (Carretero, 1988; Soberero *et al*., 2004; Randoll, 2007 and Mujawar, 2013). In India, it was introduced since 1985 from Tamil Nadu (Bhattacharya *et al*., 1985) and from Islampur of Sangli district of Maharashtra (Mujawar, 2013) reported this weed.

Pascalia glauca Ortega belongs to subtribe Ecliptinae of tribe Heliantheae under family Asteraceae. Earlier their nomenclature was *Wedelia glauca* (Ort.) Hoffmann ex Hicken (Jacquin, 1760). Recently *W. glauca* is nomenclature as *Pascalia glauca* Ortega (Manuel Crespo and Peno-Martin, 2014).

1.6 Work on *Pascalia* allelopathy :

There is no any report available from India and other countries on allelopathic effect of *Pascalia glauca*. This weed has allelopathic potential first time reported by

Soberero *et al*. (2004) and only once work where they strongly argued that few phytotoxins soluble in water are discharged into surrounding that showed marked inhibition in seed germination and radical growth of *Lycopersicon esculentum, Cucumis sativus* and *Raphanus sativus*. Therefore, present work undertaken to assessed the allelopathic influence of various parts of *P. glauca*. The allelopathic potentiality of various parts of *P. glauca* have been first time screened primarily from India and investigated that it has been inhibitory effect of the aqueous and methanol extract on the seed germination, seedling growth and photosynthetic pigments of wheat and groundnut (Mujawar *et al*., 2016b, 2017 and 2018). Chemically, the plant determined presence of tetra cyclic diterpene and it is unhealthy to cows when feed (Cassabuono and Pomilio, 2000). Schteingart and Pomilio (1948) observed that its deadly action is due to acylated and sulfated tetra cyclic diterpene monoglycoside causing metabolic failure in mice. Many researchers have worked only on its chemistry and toxicity (Marzocca, 1979; Petetin and Molinari. 1982; Ragonese and Millano, 1984; Collazo and Riet-Correa, 1996 and Dias Timm and Riet-Correa, 1997). Besides these works, there is no any type of allelopathic work. Unfortunately, it had been completely neglected from India and also from abroad. Therefore, present work proposed first time for allelopathic potentiality of various parts of *P. glauca* on the wheat and groundnut crop to evaluate seed germination bioassay, biochemical, physiological and enzymological effects from studied area of Islampur, Sangli district of Maharashtra, India.

1.7 Test crops :

Wheat (*Triticum aestivum* L., Family – Poaceae)

Wheat is one in all the most important cereal crop in worldwide and stands next to jawar, maize and rice that turning into the most staple food which offer sixty percentage take advantage of protein demand for Indian population. In 2007 world production of wheat were 607 million tons, making it the third most-produced cereal after maize (784 million tons) and rice (651 million tons) (FAO, 2007). It is a typical rabbi season food crop in the study area of Islampur, Maharashtra and becomes main dietary food. It's cultivated on a lot of area than the other economic crops because of their most significant foodstuff supply for human. The employment of grains were not solely as a food supply; however additionally a supply in varied food and agro

industries, bakeshops, bakery products and created variety of different preparation helpful to human.

Groundnut (*Arachis hypogaea* L., Family - Leguminosae. Sub-family - Fabaceae, Papilionaceae)

Groundnut is that the vital and major annual seed oil crop of our country cultivated in "kharif" and "rabbi" seasons. It's cultivated principally for oilseeds to extract the edible oil and food. Seeds of groundnut are far most cost-effective supply of proteins, lipids and fatty acids and wealthy supply of minerals and vitamins. Groundnut is an important oilseed crop of India, occupying about 7.0 million hectare area, scattered over 260 districts of 12 states (Jat *et al*., 2011). India has a diverse climate; as such groundnut is grown throughout the year in kharif, rabbi, summer and spring seasons in one or other part of the country. Area wise, about 85% groundnut is grown during the kharif season under rainfed situations where the vagaries of monsoon and seasonal biotic and abiotic stresses attenuating to low productivity (Devi Dayal, 2004). Giri *et al*. (1998) reported an average yield loss of 89% due to weed infestation in irrigated summer groundnut. Groundnut plays crucial role within the agricultural and industrial economy of the country. Seeds used for edible oil, as a food when preserved and in vegetable preparations, seeds and oil utilized in Ayurvedic medicines. Whole dry and fresh plants were used as fodder for cattle's feed and are employed as manure for improvement of soil health and forage for domestic animals.

1.8 Objectives of present work :

The present study has been undertaken due to newly introduced weed *Pascalia glauca* Ortega, in India especially Maharashtra as it is well naturalized in some other states in India and farmers are now facing problems because of their economic loss. In India only taxonomical reports have been published (Mujawar, 2013) and there is no any allelopathic detrimental effect on crops has to be confirmed in cultivars of various crops in India. Looking into consideration of allelopathic potential of *P. glauca* the present study was designed to evaluate the allelopathic effects of *P. glauca* on wheat (*Triticum aestivum* L.) and groundnut (*Arcachis hypogaea* L.) crops first time from studied area.

The prime objective of present study was to screen the field survey critically and observe the damage of the crop productivity due to the growth of *Pascalia glauca* Ortega in the wheat and groundnut fields. The disturbance in weed flora in the field and native flora nearby area has been surveyed and were documented. The data was collected for the allelopathic study using various parameters. The critical and keen observations were made to analyze the allelopathic potential of this newly recorded exotic and invasive weed *P. glauca* on the test crops wheat and groundnut on the basis of parameters like seed germination response and seedlings behavior. The allelopathic effect of *P. glauca* has been investigated with well planned protocol to understand the interference of *P. glauca* on the wheat and groundnut crop plant like,

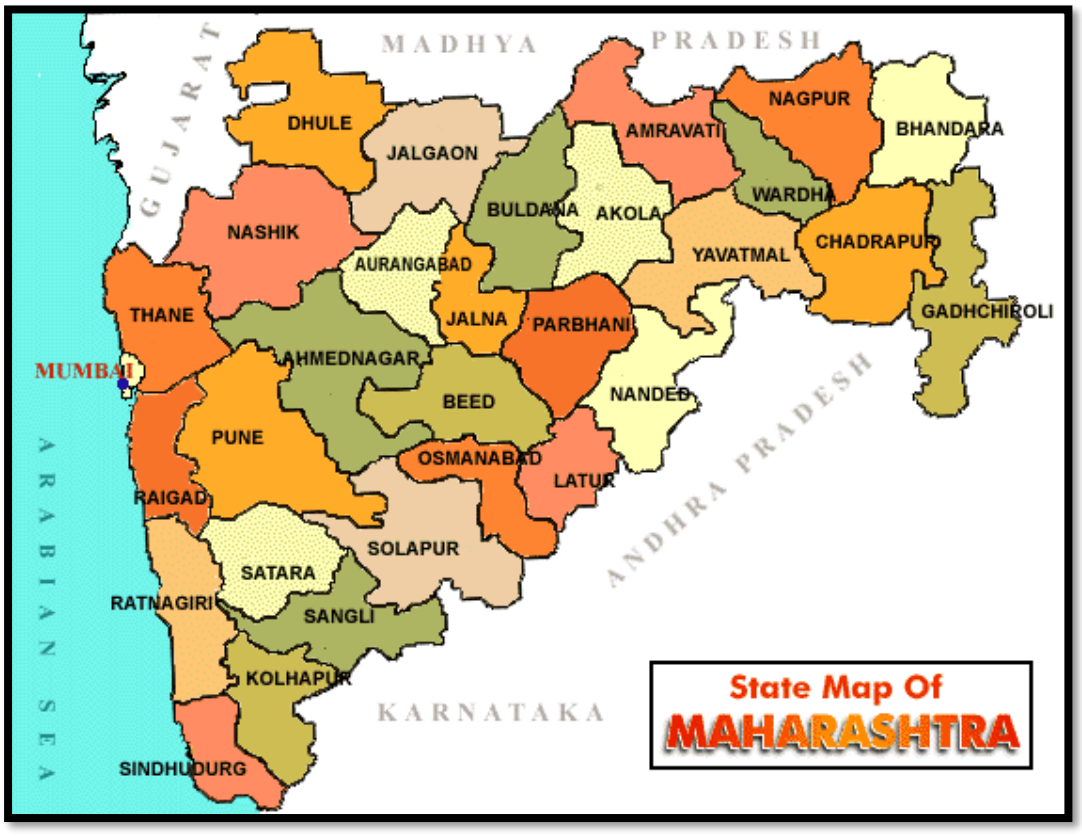
- To make keen observations and collect field data from infested area.
- Study has been undertaken for detail description and taxonomy for their correct identification.
- To study the effect of *Pascalia glauca* Ortega on rhizosphere soil from infected field and non-infected to analyze mineral nutrients and organic and inorganic matters.
- To analyze the allelopathic potential using aqueous and methanol extracts on the test crop seedlings.
- To study the effect of the aqueous and methanol extract of *P. glauca* weed on physiological, biochemical and enzymological response of test crops.

The present work of this thesis is divided into five chapters *viz*, Introduction, Review of Literature, Material and Methods, Results and Discussion and Bibliography. The extensive literature survey carried out by referring recent journals, review articles, books, monographs were listed at the end of the thesis under Bibliography. Almost all efforts have been taken to keep update of this work and the findings are justifying the hypothesis of the present work.

Plate - 1 : a) Heavy infestation of *Pascalia glauca* Ortega in crop field

b) Single plant habit of *P. glauca*

c) Flower close up of *P. glauca*



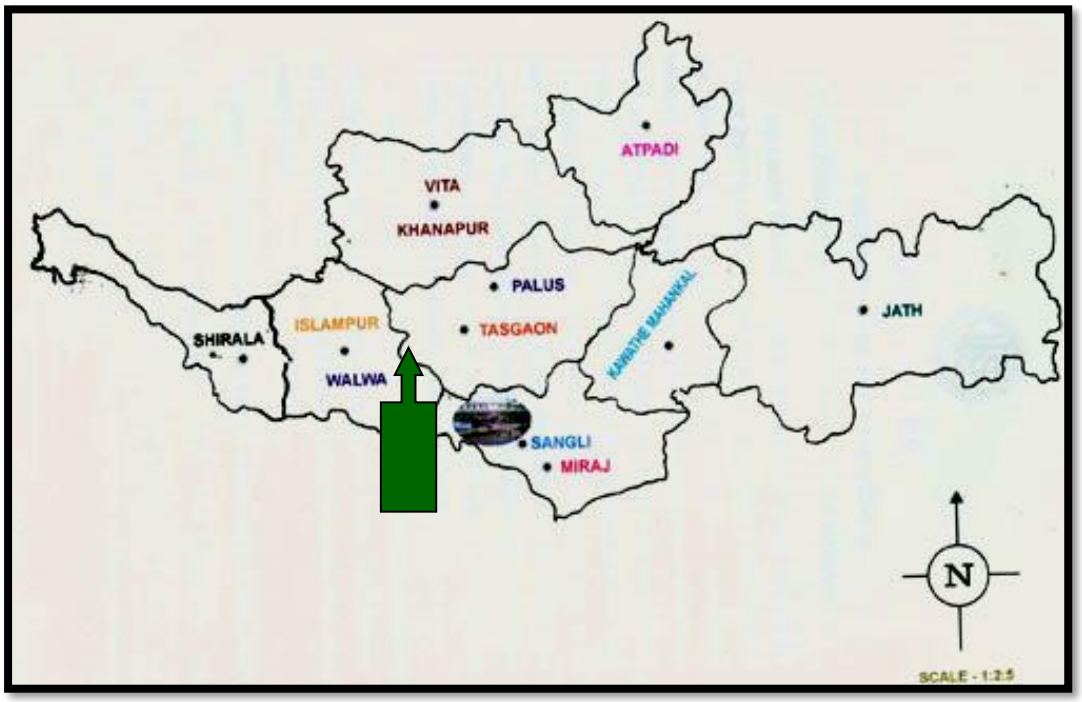


Plate - 2: **Map of Sangli District and Walwa taluka: *Pascalia glauca* Ortega reported and distribution from Walwa taluka, Maharashtra.**

CHAPTER - II

REVIEW OF LITERATURE

2.1 *Pascalia glauca* Ortega :

Pascalia glauca Ortega is one in all the unhealthful invaders introduced in some Indian states as well as geographic region, since from 1990. In India *P. glauca* Ortega was first recorded in Tamil Nadu state (Bhattacharya *et al*., 1995) after study has been undertaken for detail description and illustration for their correct identification from Islampur of Sangli district of Maharashtra (Mujawar, 2013). Additionally, he had been contributed for his elaborated field study and toxicity for grazing animals by that they die when consumed. Recently, Garad, *et al*. (2015) reported from the Solapur district of Maharashtra region as toxic species and Gloria *et al*. (2016) newly recorded for flora of Western geographic region of Andalusian as a dangerous invasive weed. Fast growth and easy spread through its stolons, it becomes threats in put down cultivation in crop field. Because of its toxic nature, farmers and farm workers suffers from headache, nervousness, anxiety and omitting throughout field works (Mujawar *et al*., 2013 and 2016). It can infest in each variety of crop to form varied issues including down the farmers' socio-economy. It stands warning signals and challenge to agriculture specialists, researchers and veterinarians. The demand has been immediately increased to understand their influence on the various crops growth and development.

2.2 Allelopathic potential of *P. glauca* Ortega:

Allelopathic potentiality of *Pascalia glauca* Ortega has been almost neglected by Indian as well as other workers even it was considered as 'Agricultural Plaque' in Argentina but well experimented under veterinary science due to death of domestic animals. Sobrero *et al*. (2004) studied the allelopathic effect of *P. glauca* Ortega (*Wedelia glauca*) and pointed out that, it has allelopathic potential and affected on seed germination and growth of tomato, cucumber and radish. They stated that some of the phytotoxins are soluble and released when aqueous extract prepared. The leaves have maximum allelopathic potential activity than other parts which showed marked inhibition effect in seed germination and radical elongation of *Lycopersicon esculentum*, *Cucumis sativus* and *Raphanus sativus*.

During present work, review of *Pascalia glauca* Ortega had been summarized on its threats in the various crop fields by Mujawar *et al*. (2016a). Mujawar, *et al*. (2016b) studied the influence of different concentrations of stem, leaf and flower aqueous extract on wheat seeds and they stated that the leaves extract have more potential intence to reduce the seed germination and seedling growth. Similar results had been observed in methanol extract of stem, leaves and flower of *P. glauca* where suppressed the seed germination and seedling growth of wheat (Mujawar *et al*., 2017a). Mujawar *et al*. (2017b) argued that the seed germination of groundnut decreased when treated with aqueous extract of stem, leaves and flower of *P. glauca* at higher concentration. Photosynthetic pigments were greater reduction in the stem, leaf and flower aqueous extract of *P. glauca* after the treatments on wheat and groundnut seedling (Mujawar *et al*., 2018).

2.3 Chemistry of *P. glauca* and intoxication in domestic animals:

Pascalia glauca Ortega is majorly investigated underneath their chemical determination and most of the workers concentrated on their toxicity to animal husbandry from Argentina. Oberti *et al*. (1980) determined the diterpenes and sterols like, 15-alpha-cinnamoylox-ent-kaur-16-en-19-oic acid from *P. glauca* (*Wedelia glauca*). Eichholazer (1981) had known the atractyloside from *Pascalia* plant. Schteingart and Pomilio (1984) declared its deadly action is due to acylated and sulfated tetracyclic diterpene monoglycoside cause metabolic process failure in mice. These substances found to be unhealthy secondary metabolites to chemical structure. Asteraceae (Compositae) members are wealthy in metabolic products and majority of them stored in special secretary tissues of stem, root and leaves. They are in the forms of terpenes, diterpenes, triterpenes, sesquterpenes, fatty acids, amino acids, alkaloids, flavonoids and coumarins (Damages, B. 1988). *Pascalia* has chemically characterized by presence of tetracyclic diterpines and it becomes unhealthy to cows eat (Cassabuono and Pomilio, 2000). Vieira (2001) recognized the chemical ent-kaur-16-en-19-oic acid from *Pascalia* plant. Giannitti *et al*. (2012) reported that death of livestock because of *P. glauca* intoxication in domestic animals at the time of feeding in feedlot operations if contaminated with hay. The toxicity is due to presence of hepatotoxic terpenoid caused acute deadly in grazing animals in Argentina and major cause of significant economic losses in the agriculture sector (Giannitti *et al*., 2013) and powerful inhibitor of cellular respiration and ATP synthesis (Costa *et al*., 2013)

they further pointed out that cattle are most frequently poisoned and this poisoning is hyperacute or acute. Various workers including Marzocca (1979); Petetin and Molinari (1982); Ragonese and Millano (1984); Collazo and Riet-Correa (1996); Tapia *et al*. (1996); Dias Timm and Riet-Correa (1997) and Cristina Yagueddu *et al*. (1998) paid attention on its toxicity and chemistry and they advocated after their experimentation, consumption of *P. glauca* (*Wedelia glauca*) were ascertained acute intoxication in sheep and such domestic animals results into death at the vegetative stage consumption.

2.4 Allelopathy:

Involvement of chemical substances which released from plants and it was influenced on the near plants; have potential in allelopathy (Tuky, 1969). According to Whittaker (1970) the metabolic substances made during metabolic activity and discharged by the plants have probably concerned in allelopathy. Release of biochemical from plants involves in allelopathy phenomenon through direct or indirect, helpful or harmful effects of one plant upon others referred to as allelochemicals that escape into the surroundings (Molisch, 1937; Rizvi and Rizvi, 1992). Allelopathy has received increasing attention explaining vegetation patterns in plant communities and is considered important regarding crop weed interactions which result in dominance of one species over another (Mandal *et al.*, 1998). Usually they showed harmful effect and signifies injurious impact on neighbors under natural conditions. Different groups of plants such as algae, lichens, crops, annual and perennial weeds show allelopathic interactions (Rice, 1984; Horsley, 1991; Inderjit and Dakshini, 1994). Today, term ‘allelopathy’ is most often used to refer for chemical mediated negative interference between plants (Willis, 2004).

Weeds are major threats in the crop ecosystem of various crops including pulses, oil yielding and cereals. These weeds destructed the metabolic activity in crop by escaping the secondary chemicals, called allelochemicals. These allelochemicals released in the surrounding environment may affect in different ways. They affect internal as well as external morphology, growth, biological and physiological processes such as uptake of nutrients, photosynthesis and respiration, organic and inorganic constituents, enzymology of crop.

2.5 Allelochemicals, origin and allelopathy:

Majority of weeds in crop ecosystem and such other plants released secondary chemicals during metabolic activity either from whole plant or plant parts including root, stem, leaves, flowers, fruits and even rhizome and seeds called allelochemicals (Singh *et al*., 2003 and Ahmad *et al.*, 2011) and involved in allelopathy mechanism. Allelopathy is a unique mechanism and may offers eco-friendly solution in the sustainable agriculture ecosystem. It helps in the growth development and regulation, weed management and disease control at the same time to enhance the productivity of crop, safe food supply for increasing global population (Arora *et al*., 2015). According to Rice (1984), allelopathy is the influence of one plant over the other plant by releasing the secondary metabolites or byproducts during different physiological process in its surrounding environment. These allelochemicals discharged in rhizosphere soil through leaching or decomposition, vitalization or root exudation thereby them affecting the growth of near plant. Mostly they were associated with defense mechanism of plants but these novel compounds may be involved in various metabolic activity in species and ecological functions. It is been considered as natural phenomenon in which different plants or organisms release the secondary metabolites and affect the function of other plants or organism in their positive or negative vicinity (Cheema *et al*., 2012 and Farooq *et al*., 2011). These compounds, termed as allelochemicals in the form of flavonoides, alkaloids, phenolics, momilactone, jasmonates, glucosinolates, hydroxamic acid, brassinosteriods, amino acids, terpenoids and carbohydrates (Adaramola *et al*., 2016). These chemicals are nonnutritional and can be synthesized in any part of the plants (Bonanomi *et al*., 2006 and Rice, 1984). Mostly they have inhibitory action on neighboring plant species. This inhibitory action is attributed to interruption in various physiological process and blockage their activity within the plant (Iqbal *et al*., 2007). But some studies have been reported the promotory effect when used in low concentrations that improve the plant growth (Narwal, 2000).

Number of researchers identified various allelochemical compounds from different part of the weed species and recorded their effect on the neighboring plants. Hira *et al*. (2000) detected various dithiolane oxides as well as zeylanoxide-A, epizeylanoxide-A and zeylanoxide-B from the *Sphenoclea zeyanica* Gaertn. *Vulpia myuros* (L.) C. Gmelin have phenolic resin acids as well as carboxylic acid, ferulic acid, hydroxy acid, carboxylic acid, catechol and hydrocinnamic acid (An *et al*.,

2001). Mandal (2001) isolated caffeic acid from *Leonurus sibricus* L. *Echinochloa cruss-galli* L., *Fagopyum esculentum* Moench. contains carbpxamode allelochemicals as well as fagomine, 4-piperidone, 2-piperidine wood alcohol (Iqbal *et al*., 2002). Kato-Naguchi (2003b) identified carotenoids cluster includes cis-trans-xanthoxin and trans-xanthoxin from the genus *Pueraria thunbergiana*. Ambica *et al*. (2003) found phenolics like *p*-hydroxybenozoic acid, vanillic acid, caffeic, protocatechuic, transcinnamic genitistic syringic, ferulic, O-coumaric, *p*-coumaric and hydroxy acids in exotic plant *Lantana camara* L. Weidenhamer and Romeo (2004) separated gallic acids, qauercetin hydroquinone and rhamnetin phenolics within the plant *Polygonella myriophylla* (Small) Horton. *Tephrosia candida* L. mulching detected the *p*-hydrozybenzoic acid, salicylic acid, syringic acid and syringaldehyde allelochemicals by Huang *et al*. (2007).

Sisodia and Siddgqui (2008) known gallic acid, *p*-coumaric, cinnamic acid, anisic acid, *p*-hydroxybenzoic and ferulic acid from *Croton bonplandianum* L. Jamil *et al*. (2009) found that the various forms of essential oils in *Eucalyptus*. Root hairs of *Sorghum bicolor* L. residues exudates the allelochemicals like gallic acid, protocateuic acid, syringic acid, vanillic acid, *p*-hydroxybenzoic acid, *p*-coumaric acid, carboxylic acid ferulic acid, *m*-coumaric acid, caffeic acids and sorgoleone (Chung *et al*., 2011). Dube *et al*. (2012) found that the presence of cynamide+ and scopoletin within the hairy vetch and oat. Islam *et al*. (2014) isolated vitegnuside-I from the leaves of *Leucas aspera* L. *Elymus repens* growth intensity reduces due to cyclic hydroxamic associate acids+ produces if apply to the *Secale cereal* L. (Ringselle *et al*., 2015). *Lablab purpureus* that discharged the allelochemicals identified as gallic acid, chlorogenic acid and rutin and they affect the growth of *Paraknoxia parviflora* and *Portulaca quadrifida* (Mwangi *et al*., 2015). Monoterpene like alpha-pinene, beta-phellandrene and alpha-cedrol allelochemical detected from *Thuja orientalis* (Ismail *et al*., 2015). Arora *et al*. (2015) detected the volatile oils *viz*. nepetalactone, transpulegol, 1, 8-cineole from *Tagetes minuta* L.

2.6 Allelopathy, seed germination and seedling growth:

Seed germination, seedling development and their growth is one of the prime important parameter in the crop life cycle. Their bioassay studies have basic need in the allelopathy research to investigate the allelopathic effect of weed on crop. Several weeds and their related species have been tested and reported to possess allelopathic

effects on germination, seedling growth and entire plants growth in various crops from different parts of world by different workers including *Ageratum conyzoides* (Oudhia, 2000; Oudhia, 2001; Oudhia and Tripathi, 2001; Gogoi *et al.*, 2002; Saxena *et al.*, 2003; Dongre *et al.*, 2004 and Dongre and Yadav, 2005). *Amaranthus indica* (Bratoeff *et al.*, 1996), *Amaranthus palmeri* (Dailey *et al.*, 1997), *Amaranthus viridis* (Kwon *et al.*, 1997), *Amaranthus retroflexus, A. gracilis* and *A. blitoides* (Qasem, 1995); *Amaranthus spinosus* (Ambika and Suma, 1999); *Anagallis arvensis* (Dongre *et al.*, 2004 and Dongre and Yadav, 2005), *Blumea lacera* (Oudhia, 1999 and 2000); *Commelina benghalensis* (Prasad and Srivastava, 1991), *Cyperus rotundus* (Pandey and Padhiar, 2000; Quayyum *et al.*, 2000; Meena, 2001; Ameena and George, 2002; Agarwal *et al.*, 2002 and Catunda *et al.*, 2002); *Chenopodium album* (Mallik *et al.*, 1994; Alam *et al.*, 1997 and Jafari and Kholdebarin, 2002), *Chenopodium murale* (Qasem, 1995); *Cynodon dactylon* (Kalita *et al.*, 1998; Oudhia, 1999; Alam *et al.*, 2001 and Gonzalez *et al.*, 2002), *Convolvulus arvensis* (Alam *et al.*, 1998), *Desmodium oxyphyllum* (Mizuno *et al.*, 1992), *Desmodium styracifolium* (Yang *et al.*, 1993), *Echinochloa crus-galli* (Velu and Raj Gopal, 1996b), *Eclipta alba* (Zhang and Chenyayan, 1996; Zhang and Chen, 1996; Yahara *et al.*, 1997; Dongre *et al.*, 2004 and Dongre and Yadav, 2005), *Eleusine indica* (Gogoi *et al.*,2002), *Ipomoea carnea* (Jadhav *et al.*, 1997), *Imperata cylindrical* (Hussain *et al.*, 1992), *Lathyrus sativus* (Lambein *et al.*, 1993 and Sawhney *et al.*, 1996), *Melilotous indica* (Macias *et al.*,1996 and Sexena *et al.*, 2003), *Melilotus messanensis* (Saxena and Nigam, 1997 and Macias *et al.*, 1997), *M. alba* (Al-Hazimi and Al-Andis, 2000), *Parthenium hysterophorus* (Oudhia, 2000; Gupta, 2000; Pahwa *et al.*, 2000; Kalia *et al.*, 2000; Batish *et al.*, 2002; Sasikumar *et al.*, 2002; Singh *et al.*, 2002; Saxena *et al.*, 2003; Narwal *et al.*, 2003; Dongre *et al.*, 2004 and Dongre and Yadav, 2005), *Phyllanthus niruri* (Hassarajanis and Mulchandani, 1990; Dongre *et al.*, 2004 and Dongre and Yadav, 2005), *Pluchea lanceolata* (Chawla *et al.*, 1990, 1991; Srivastava *et al.*, 1990; Inderjit and Dakshini, 1994a, 1994c,1996; Inderjit, 1998, 2002; Dongre *et al.*, 2004 and Dongre and Yadav 2005), *Polygonum lapathifolium* (Ahmed *et al.*, 1990), *Polygonum plebeium* (Dongre *et al.*, 2004 and Dongreand Yadav, 2005), *Polygonum sachalinense* (Inoue *et al.*, 1992), *Polygonum hydropiper* (Agarwal *et al.*, 2002), *Solanum myriacanthum* (Chattopadhyay, 1995) and *Solanum nigrum* (Agarwal *et al.*, 2002).

Allelopathic effect of *Croton bonplandianum* reduced growth of wheat reported by Sarkar and Chakraborty (2010). Aqueous extract of *Jatropha curcas* stimulate the seed germination shoot growth of *Sesamum indicum* (Rejila and Vijaykumar, 2011). Influence of allelochemicals released by *Exocoecaria agalloch* L. on seed germination and seedling growth of pulses and millets (Kavitha *et al*., 2012). Ahlam Al-Watban and Hediat Salama (2012) studied allelopathic inhibitory activity of *Artemisia monosperma* on seed germination and seedling growth of *Phaseolus vulgare* L. Various inhibitors are present in leaf leachate and leaf extract of *Eupatorium odoratum* have inhibitory impact on seed germination of mung bean (Maiti *et al*., 2013). Silva *et al*. (2014) stated that the volatiles released from fresh and dry leaves of *Heterothalamus psiadiodies* Less. and *Baccharis patens* Baker. have strongly allelopathic potentiality and were inhibited the seed germination and root-shoot length of lettuce and onion. Santos *et al*. (2015) argued that aqueous and solvent extract of *Tagetes patula* and *T. erecta* contain allelochemical compounds that decreased the seed germination and seedling growth of *Lactuca sativa* at higher concentration. Mandal *et al*. (2016) examined the allelopathic effect of *Andrographis paniculata* Nees. leaf extract on seed germination and seedling growth of four wheat genotype(Sonalika, VL-914, Euguanda-6 and Mond's Aids). They found the aqueous leaf extracts reduced seed germination and seedling growth, germination relative index and vigor index. Aqueous extract of *Salvia plebia* R. Brown. strongly affected the germination, plumule growth, radical growth, chlorophyll content and fresh and dry weights of *Zea mays* var. 30-25 Hybrid, *Triticum a**e**stivum* var. Pirsabak-04 and *Sorghum bicolor* L. (Husna *et al*., 2016). Experimental study had been conducted by Adhikari (2017) and advocated that the seedlings growth of *Phaseolus radiates* L. has been declined in the treatment of various concentrations of whole plant, leaves and fruit leachate of *Xanthium indicum*. Tuber germination and length of shoot of *Cyprus rotundus* was significantly decreased after the treatment of Sesamum plant leachate that helpful in the biological control of nutsedge growth (Imtiyaz *et al*., 2017). Hamed *et al*. (2018) found that aqueousleaf extract of *Datura stramonium* L. reduced seed germination of *Trigonella foenum-graecum* and *Lepidium sativum*.

2.7 Allelopathy and wheat:

2.7.1 Seed germination and seedling growth of wheat:

The allelopathic effect of various weeds and their various parts has been studied by various researchers from different parts of world and they concluded that majorly weed had detrimental effect on the growth and development of wheat (*Triticum aestivum* L.). In early days of research on allelopathy various experts were experiented effect of different weeds on wheat crop. Aqueous extract of *Chenopodiun album* significantly decreased seed germination, root growth and coleoptile elongation in wheat. Anaya and Gomez-Pompa (1971) demonstrated that extracts of leaves and fruits of *Schinus molle* L. inhibit germination and seedling growth of cucumber and wheat. Guantes and Mercado (1975) have reported that horse purslane (*Trianthema portulacastrum* L.) residue significantly reduced the germination and seedling growth of wheat, chickpea and mustard. Infestation of weeds reduces the quality of agricultural products and poses a recurrent and ubiquitous threat to agricultural productivity. It is assumed that weeds reduce more than 10% of crop yield (Robert, 1982). Chaghtai *et al*. (1986) reported the negative allelopathic potentiality of *Fumaria indica* on the germination and growth of *Triticum aestivum*. Sharma and Nathawat (1987) studied the allelopathic effect of *Argemone mexicana* on *Triticum* cultiver, *Brassica compestris* var. sarson, *Raphanus sativus* and *Pennisetum typhoides* and found significant inhibition of germination and seedling growth of these test crops. Ranjangam (1989) demonstrated work on effect of aqueous extracts of a pteridophytic plant *Marsilea minuta* L. which inhibit seed germination, growth and reduce plant height, dry weight and chlorophyll contents of two rice cultivars *viz.* ADT-31 and IR-50.

Gupta *et al*. (1992) reported that harmful effects of horse purslane (*Trianthema portulacastrum* L.) on germination and seedling growth of radish, wheat, pigeon pea and sorghum; further they stated that the dry aqueous leachate of horse purslane was found not to be inhibitory for wheat up to 5% concentration but further increase in concentration from 5% to 10% reduces seed germination in wheat and chick pea. Agarwal and Anand (1992a) found the shoot and inflorescence extract of *Parthenium hysterophorus* highly toxic to germination and plant growth of green gram and wheat causing reduction in their caloric coefficient and overall growth. Agrawal and Anand (1992b) further found reduction in root and shoot dry weight, and also decrease in net and gross primary productivity of wheat and green gram in a pot

culture experiment involving treatment of *P. hysterophorus* extract. Joshi *et al.* (1996) studied inhibition of germination and growth ofcrops *viz., Triticum aestivum* L., *Raphanus sativus* L., *Brassica campestris* L. and *Eleusine corocana* (L.) Gaertn by using extract of *Fraxinus micrantha* L. a deciduous tree. Blaise *et al.* (1997) assessed influence of *Eucalyptus* leaves, litter and root amended soils on seedling growth of wheat, maize and cowpea and they found that reduction in height of wheat and maize. Leaf extracts of *Acacia auriculiformis* and *Acacia nilotica* were shown to be highly toxic to wheat growth (Kamal *et al.,* 1997). Increasing the concentration of extract of all plant parts of *Chrozophora rottleri* has been showed that the increase in inhibitory effects on germination and seedlings growth of tested crops including mustard, rice and wheat (Mandal *et al.,* 1998). Yadav and Chauhan (1998) found that soaking of wheat seeds in root leachates and root and leaf extracts of *Pluchea lanceolata* results significant reduction in germination percentage and seedling growth. Oudhia (2000) demonstrated experiment and found that 10% (w/v) extracts of root, stem, leaf and mixture of stem and leaf of *Parthenium hysteroporus* cause significant decrease in germination and seedling growth of wheat. Aqueous extracts of *Helianthus annus* have also been reported to inhibit the germination and seedling growth of wheat where the roots getting greater impact in comparison to shoots (Ghafar *et al.,* 2000). Sharma *et al.* (2000) investigated effect of extracts of poplar (*Populus deltoides*) on germination and seedling growth of wheat and observed thatfresh leaf extracts inhibited wheat germination significantly but causedstimulatory effects on shoot and root growth, dry weight of wheat.

Alam *et al.* (2001) found to be significant inhibition in germination percentage, and root-shoot length of wheat by leaf extracts (2%) of bermuda grass. Gonzalez *et al.* (2002) stated that the inhibition of seed germination, dry matter accumulation and rate of respiration in seedling of oat, wheat, sorghum and bean treated with stem and leaf extracts of *Cynodon dactylon.* Agrawal *et al.* (2002) noticed about 46% reduction in plumule length of wheat seedlings treated with extracts of *Cyprus rotundus.* Saxena *et al.* (2003) occurred that some phenolic compounds isolated from *Ageratum conyzoides* and they found the inhibition of germination, seedling length, seedling dry weight and uptake of labeled phosphorus and zinc in seedling of three varieties of wheat with increasing concentration (5 and 10%) of *A. conyzoides* extracts. Application of *Sphaeranthus indicus* extracts (higher concentration) decreased germination percentage, root and shoot length of *Oryza*

sativa, *Triticum aestivum* and *Vigna radiata* L. (Lodha, 2004). Wang *et al*. (2005) conducted experiments to evaluate the allelopathic effects of volatile oil from *Ambrosia trifida* L. on maize, wheat and barnyard grass and they found that except barnyard grass, it inhibited germination and growth of maize and wheat.

Kaysak *et al*. (2006) argued that aqueous leachates of *Morus alba* and *Toona ciliata* reduced seed germination and down length of radical of *Cicer arietinum*, *Zea mays*, *Triticum aestivum*, *Phaseolus vulgaris* and *Glycine max*. Maharjan *et al*. (2007) found that the extract of *Parthenium hysterophorus* L. had strong inhibitory effect to root and shoot elongation of *Oryza sativa* L., *Zea mays* L., *Triticum aestivum* L., *Aphanus sativus* L., *Brassica campestris* L., *Brassica oleracea* L., *Artemisia dubia* Wall ex. Besser and *Ageratina adenophora* (Spreng.) King and H. E. Robins. Rhizosphere soil and root residues of *Chenopodium murale* L. exhibit inhibitory effect on seedling length and seedling dry weight of *Triticum aestivum* L. Only a partial amelioration in growth inhibition occurred upon charcoal supplementation or nitrogen fertilization in these amended soils (Batish *et al*., 2007a). High residue levels of *Hordeum spontaneum* amended in soil and exudates from tillers reduced mature plant height, fresh, dry weights and yield of *Triticum aestivum* L. (Hamidi *et al*., 2008). Khan *et al*. (2008) demonstrated experiment on allelopathic effect of *Eucalyptus camaldulensis* on seed germination of wheat and reported that decrease in the germination and seedling growth. Sisodia and Siddiqui, (2009) stated that *Triticum aestivum* L., *Brassica rapa* L., *Brassica oleracea* var. *Botrytis* L., *Spinacea oleracea* L., *Melilotus alba* Medik., *Vicia sativa* L. and *Medicago hispida* Gaertn. root length, shoot length and dry weight of seedlings decreased significantly when plants were grown in rhizosphere soil of *Croton bonplandianum* Baill. Root extracts (up to 5%) of *Chenopodium album* L. stimulated growth of roots and shoots in wheat whereas leaf extracts inhibited the growth (Dave and Jain, 2009).

Triticum aestivum L., *Cicer arietinum* L. and *Lens culinaris* Medic. seedling emergence, seedling vigor index and total dry weight were significantly reduced in rhizosphere soil and aqueous extract of various organs of *Euphorbia helioscopia* L. (Tanveer *et al*., 2010). Siddiqui *et al*. (2010) determined negative effect of *Phylaris minor* with other weeds on growth of wheat cultivars. Prasad *et al*. (2011) demonstrated allelopathic effects of walnut leaf extracts on seed germination and seedling growth of wheat and they found that seed germination and growth was inhibited. El-Fattah A. *et al*. (2011) showed that the extract of weed *Alternaria*

princeps and *Launai sonchadis* on wheat seed and found suppression of seed germination and seedlings growth. Aqueous extracts *Phalaris minor* L., *Chenopodium murale* L., *Sonchus oleraceus* L., *Cyanodon dactylon* L. and *Convolvulus arvensis* L. extracts caused inhibitory effects on seed germination, seedling length of *Triticum aestivum* L. (Ankita and Chabbi, 2012). Katoch *et al*. (2012) found that the incorporation of weed residue of *Eupatorium adenophorum* Spreng., *Ageratum conyzoides* L. and *Lantana camera* L. in soil had inhibitory effect on seed germination percentage, shoot length and physiology of *Triticum aestivum* cv. HPW-42, *Oryza sativa* cv. Hasanshrasativa I Basmati and *Zea mays* cv. Girija, *Oryza sativa* L. Concentrated leaf extracts of *Chenopodium album* L. had detrimental effects on plant height, number of tillers and spike length of *Triticum aestivum* L. (Majeed *et al*., 2012). Treatment of dry leachates in higher concentrations (1/10, w/v) from all plant parts (leaves, stem, root, ovary wall and seed) of *Jatropha curcas* bring about significant reduction in germination percentage, length of root and shoot, fresh and dry weight of roots and shoot in seedlings of wheat (Tomar, 2012).

Aqueous and organic extract and residue of *Xanthium italicum* Moretti incorporation reduced seedling growth and affected photosynthetic pigment content of *Triticum aestivum* L. (Shao *et al*., 2013a). Further, they stated that main alkaloid harmaline present in *Peganum harmala* L. inhibit root length and shoot length especially dicots (lettuce and amaranth) and monocot plant (wheat and ryegrass) and shown to be inhibiting root elongation at a very low concentration where as harmine exhibited much weaker non-selective inhibitory effect. Variable phytotoxicity effect was observed in leaf extract of *Coronopus didymus* L. which caused the greatest inhibition in growth development of *Triticum aestivum* L. (Khaliq *et al*., 2013b). Amoghein *et al.* (2013) found to be plant debris and mulch management with *Avena fatua* L. and *Secale cereale* L. had a significant inhibitory effect on *Triticum aestivum* L. germination parameters. Gella *et al.* (2013) reported the decrease in seed germination, radical, plumule length and biomass of wheat seedlings after the treatment of aqueous extract of *Amaranthus hybridus, Parthenium hysterophorus, Datura stramonium* and *Argemone maxicana* leaf, stem and root parts and they found that the seed germination, seedlings growth and biomass production of wheat was reduced. *Triticum aestivum* sp. *vulgare* L., *Secale cereale* L., *Lupinus luteus* L. and *Brassica napus* sp. *oleifera* L. affected germination, length, weight and number of roots and leaves decreased by aqueous extract of Cannabis *sativa* L. (Pudelko *et al*.,

2014). Root exudates from rhizosphere soil of *Euphorbia himalayensis* (Klotzsch) Boiss. exhibit negative allelopathic effect on *Triticum aestivum* L., *Lactuca sativa* L., *Poa annua* L., *Festuca rubra* L. and *Trifolium pretense* L. (Liu *et al*., 2014). Root, shoot elongation and biomass accumulation had been drastically reduced in wheat after the application of the methanol extract of various weeds from Nanded district (Dhole *et al.,* 2014). Golzardi *et al.* (2014) found that *Cynanchum acutum* L. on increasing the concentration rate of aqueous extract, decreasing germination percentage, radical and shoot length of *Triticum aestivum* L.

Aqueous extract *Salvia plebia* R. Brown. strongly affected the germination, plumule growth, radical growth, chlorophyll content and fresh and dry weights of *Zea mays* var. 30-25 Hybrid, *Triticum a**e**stivum* var. Pirsabak-04 and *Sorghum bicolor* L. (Husna *et al*., 2016). Weed extract of *Impomoea carnea, Malchara capitata* and *Alternanthera sessilis* had the most adverse allelopathic effect on seed germination, decrease in radical and plumule length of wheat (Joshi and Joshi, 2016). Mandal *et al*. (2016) examined the allelopathic effect of *Andrographis paniculata* Nees. leaf extract on seed germination and seedling growth of four wheat genotype (Sonalika, VL-914, Euguanda-6 and Mond's Aids). They found the aqueous leaf extracts reduced seed germination and seedling growth, germination relative index and vigor index. The root and shoot length, fresh weight and dry weight decreased with increase in the concentrations of the leaf extracts but the allelopathic effects of leaf extract of *Andographis paniculata* were more in Mond's Aidsvariety than the other varieties. Mujawar *et al*. (2017) demonstrated the effect of methanol extract of *Pascalia glauca* Ortega on wheat and the results indicated that seed germination, root length, shoot length, seedling growth, fresh and dry weight of wheat seedling was substantially reduced. Radical and plumule length of *Triticum aestivum* L. showed declining trend with increasing doses of aqueous extract of the weeds *Chenopodium album, Avena fauta* and *Phalaris minor* at the same time dry weight was drastically decreased gradually with increasing concentration of extract of these weeds (Saira *et al*., 2017). Abdul *et al*. (2017) advocated that aqueous extract of sugarcane root, stem peel and leaf showed healthy effect on wheat growth but leaf extract have inhibitory effect on seed germination and they suggested that wheat could be cultivated in sequential rotation in field conditions.

2.7.2 Allelopathic Physiological and biochemical influence on wheat:

Allelochemicals are mostly present within the allelopathic plants. They have more potential to have an effect on the varied organic chemistry and enzymological method in plant life cycle at different levels. They acts at cellular membrane, nutrient uptake, closing and gap of stomata aperture, chemical action, respiration, water relation, plant product, protein activity level. Majority of the allelochemicals were discharged by the plants that free into the rhizosphere that initial contact with roots of plants so have an effect on the emergence of receiver plant roots therefore ultimately have an effect on the mineral uptake of various nutrients from the soil.

Pande *et al.* (1998) investigated allelochemicals from petroleum extract of *Prunus amygdalus* Batsch. *viz. P*-sitosterol, naringenin while ethyl acetate extract contained aromadendrin, *P*-sitosterol-5-D-glucoside, persicogenin-3'-glucoside. Bioassay studies revealed inhibition of germination, seedling growth in finger millet and wheat and they found that theethyl ether extract have more potential in the reduction of germination and growth than the light petroleum and water extract. At the same time they found persicogenin-3-glucoside, Naringenin and Aromadendrin were the most potent inhibitors. Raj and Tripathi (1984) stated that the adverse impact of allelochemicals on photosynthesis, chlorophyll content, accumulation of carbohydrates and proteins may be due to higher concentration treatments while Tripathi *et al*. (2000) explained the various stimulatory allelochemicals present in weed helping for improving the yield attributes at lower concentration treatments. Prasad *et al.* (1999) investigated aqueous and organic solvent extracts of *Rhamnus virgatus* Roxb. were toxic to wheat, lentil, finger millet, and black gram. Soil amended with residues of *R. virgatus* influenced germination, growth, protein, amino acids, carbohydrates, pigments, nitrogen, and phosphorus and potassium contents of test plants. The allelochemicals detected from it, containing chrysophanol, 7-O-methyl kaempferol, 7-O-methylkaempferol- 3O-B-rhamnioside, entepiazelechin - kaemferol may be affect and reduced germination, growth and yield of test crop plants in an order of wheat >finger millet >lentil >blackgram. Bhatt and Chauhan (2000) dealt with the investigation of *Quercus glauca* Thunb. and *Q. leucotrichophora* A. Camus, (Fagaceae) and effects of aqueous extracts of dried and fresh leaves, leaf litter and bark on crops *viz.* wheat, mustard and lentil. *Quercus* species reduced germination, shoot and root length, chlorophyll a, chlorophyll b and carotenoids of these three-test crops. They further stated that inhibitory effects are attributed to high

tannin contents, triterpenoids, 3-sitosterol and mixture of leucoantho-cyanidins and quercetin. Bark extract of *Q. leucotrichophora* A. Camus. promoted plumule length and growth of lentil. *Q. glauca* Thunb. slightly promoted germination in wheat.

Venkateshwarlu *et al.* (2001) bioassayed methanol extracts of leaves of *Mangifera indica* L. on wheat, radish and okra. They identified the allelopathin present in the leaves as mangiferin (1,3,6,7- tetra hydroxy 2-c-b-glucopyranosylxanthone). Except radish, germination in wheat and okra was affected by mangiferin (200ppm) level. It also affected chlorophyll contents in radish. Aliotta *et al.* (2002) isolated 17 phytotoxic polyphenols from olive *(Olea europaea* L.) by oil mill wastewater and were used for bioassay study of radish and wheat. They found individual toxicity of each polyphenols was much lower but synergic action of all mixed polyphenols was more. Nanofiltration and reverse osmosis fractions of oil mill wastewater sludge on dilution showed inhibition of germination in both radish and wheat. They detected polyphenolic compounds from reverse osmosis fraction of oil mill wastewater sludge these allelopathin was inhibitory to both radish and wheat.

High content of proline in salt stressed wheat plants did not negatively affect the enzymes activities, but it protects the enzymes set and the cell walls from the saline factors (Hanafy *et al*., 2002). Mandal *et al*. (2003) also observed that sugar content of wheat seedlings decreases with increase in leachate concentration of *Dalbergia sissoo, Acacia lenticularis, Bombax cieba* and *Populus deltoids*; further they stated the protein content decreased and free amino acids increased in leaf extracts concentrations. Decrease in protein might be due to disturbance in protein metabolism. Bernet *et al*. (2003) reported reduction in yield of sorghum and wheat due to allelopathic effects of sunflower. Irshad and Cheema (2004) reported that increase in grain yield, spike length, grains per spike in wheat at lower concentration treatment of sorghum extract. Higher proline content in wheat after water stress has been reported by Vendruscolo *et al*. (2007). Sarkar and Chakraborty (2010) reported that allelopathic impact of *Croton bonplandianum* cause decrease in chlorophyll and starches in wheat and mustard. 40-60% of amino acids absorbed carbon in *Triticum aestivum* L. (Hill *et al*., 2011). Abu-Romman (2011) noticed that *Achillea bieberstenii* affected negatively on germination, radical and shoot length as well as reduction in the amount of chlorophyll- a, b, total chlorophyll, carotenoids and protein content of *Capsicum annuum* L.

The biomass and chlorophyll content were reduced by the treatment of aqueous extract of *Cassia tora* L. in the *Brassica compestris* L. was investigated by Sarkar *et al*., (2012). Wafana Shikry and Rola (2012) declared that chloride causes reduction in nitrate content in salt stressed wheat. Chlorophyll and carotenoid contents were also greatly reduced in wheat seedlings treated with dry leachates (1/10, w/v) of leaves and ovary wall of *Jatropha curcas* and *Jatropha gossypifolia* further, reduction in growth, biomass and pigment content of wheat seedlings in response to leachates from various parts of both *Jatropha* species is an indicator of its phytotoxicity to wheat seedlings. (Tomar, 2012). Asif Tanveer *et al*. (2012) concluded that phenolic compounds in aqueous extract of *Euphorbia dracunculaoides* have allelopathic effects on chickpea and wheat growth. Wheat seedlings when treated at higher concentrations of leachates (1/10, w/v) of *Jatropha curcas* brings down nitrate reductase (NR), aspartate and alanine aminotransferases activity (Tomar Nishasingh, 2012). Khliq *et al*. (2013) reported that the aqueous leaf extract of *Coronopus didymus* L. accelerated the more reduction in dry biomass and chlorophyll content of wheat seedlings. Chlorophyll content, protein and carbohydrates were reduced in wheat after the treatment of *Eclipta alba* L. (Gulzar and Siddiqui, 2014).

Madany and Ahmed (2015) identified the cinnamic acids, carboxylic acid acids and flavonoids from aqueous extract of *Euphorbia helioscopia* that tested against wheat and pea impaired seed germination and cretaceous growth of seedlings due to impact of phenolics compounds in aqueous extract. Hozayn *et al*. (2015) declared that the *Casurina equisetifolia* L. leaf litter will increase the extent of macro and micro nutrients within the crop field of wheat. The allelopathic potential of *Cuscuta reflexa* causes the stimulatory and repressive impact on seed germination, biomass, chlorophyll and carotenoides contents of wheat (Salgude *et al*., 2015). Joshi and Joshi (2016) reported that the total protein and total chlorophyll pigments of wheat significantly decrease after the treatment of aqueous leaf extract of *Alternanthera sessilis* and *Ipomoea carnea*. Mandal *et al*. (2016) demonstrated the allelopathic effect of *Andrographis paniculata* Nees. leaf extract and was examined on seed germination and seedling growth of four wheat genotype (Sonalika, VL-914, Euguanda-6 and Mond's Aids). They found the aqueous leaf extracts reduced seed germination and seedling growth, root and shoot length, root and shoot biomass in the high concentration of the leaf extracts. They further observed declining effect of extract in reducing sugar, non-reducing sugar, total sugar and soluble protein and

stimulatory effect on total free amino acids contents of wheat seedlings. Mujawar *et al*. (2018) demonstrated the experiment to study of allelopathic effect of aqueous extract of *Pascalia glauca* Ortega on wheat seedlings and concluded that aqueous leaf extract of *Pascalia glauca* Ortega showed more allelopathic effects than the stem and flower extract on photosynthetic pigments in the reduction of chlorophyll- a, b, total chlorophyll and carotenoides of *Triticum aestivum* L. and *Arachis hypogaea* L.

2.8 Allelopathy and groundnut:

2.8.1 Seed germination and seedling growth of groundnut:

Eyini *et al*. (1989) studied the allelopathic effect of water extracts of the fallen leaves of *Bambusa arundinacea* on the growth of *Arachis hypogaea* and they found that the water extracts exhibited the growth and the decrease in leaf area, plant height, total chlorophyll and protein content is proportional to the increase in concentration of the leaf extract**.** Tiwari *et al*. (1985) showed inhibitory effect of most conspicuous root washing extracts of weeds on germination and root and hypocotyl length of soybean, groundnut and greengram. *Eucalyptus globules* had phytotoxic effect on the seed germination and radical growth of groundnut and corn (Jayakumar *et al*., 1990). They aggressively postulated that the root growth was more sensitive to the increasing concentration of the aqueous extract of *E. globules* in comparison to seed germination of groundnut. Prasad and Srivastava (1991) found that the extracts of *Echinochloa crus-galli* (barnyard grass) are inhibitory to seed germination and seedling growth of groundnut. Aqueous plant extract of *Aegeratum conyziodes* and *Lantana camera* caused significant reduction in groundnut seed germination and root and shoot length; and delayed germination by 4-7 days (Prasad and Srivastava, 1991). Aqueous extract of *Cyperus rotundus* and *Digera muricata* reduced seed germination by 10-30% in groundnut (Vijay Kumar, 1991). Agarwal and Kohli (1992) screening of crops *Arachis hypogaea*, *Ricinus communis* and *Gossypium hirsutum* seeds for seed germination against leachates of *Parthenium hysterophorus* and showed reduction in seed germination with drastic decrease in seed vigor and seedling growth. Suseelamma, (1992) demonstrated the allelopathic potentiality of *Digeria muricata* on the seed germination and early seedling growth of horse gram, jowar and groundnut crop plants, the common semi arid crops in Andhra Pradesh and found that the inhibition of seed germination and their growth after the extract treatment. Singh and Hajarika (1996) experiments on groundnut found that *Galinsoga parviflora* and

Bidenspilosa were inhibitory to seed germination and radical and plumule growth of groundnut plant. Oudhia (1999c) stated that the medicinal weed plant *Calotropis gigantea* stem extract of 216 hours was found to be most harmful extract in groundnut fields of Chhattisgarh (India) in which the seed germination and growth were affected. Aqueous plant extract of *Aegeratum conyziodes* and *Lantana camera* caused significant reduction in groundnut seed germination, root and shoot length; and delayed germination by 2-3 days (Ghosh *et al*., 2000). Ghosh *et al*. (2000) demonstrated allelopathic effect of aqueous extracts of seven weeds *viz*. *Chromolaena odorata, Degeria muricata, Chenopodium album, Ageratumconyzoides, Cirsium arvensis, Abutilon indicum* and *Cyperus rotudus* and reported that these weeds cause significant reductions in seed germination, shoot and root length of groundnut. Karikari *et al*. (2000) investigated the allelopathic effect of five weed species from Botswana, *viz*. *Cynodon dactylon, Chromolaena odorata, Argemone maxicana, Biden spolosa* and *Cyperus rotundus* on *Arachis hypogaea* and *Sorghum bicolor* var. *Segaolane*. They found that seed germination, radical and plumule length, seedling survival and dry weight were inhibited under laboratory condition while residues of these weeds were reduced plant height, leaf area and shoot dry weight under greenhouse and field conditions, thus crop growth and yield was lost in groundnut and sorghum.

Jennings and Nelson (2002) stated that leaf extract of *Cyprus tuberosus* inhibited seedling, root and shoot growth as well as disorder observed in the morphology of seedlings. Patil *et al*. (2002) studied the effect of leaf litter leachates of *Casuarina equisetifolia* L. on wheat var. DWR-162 and groundnut var. JL-24 and observed that the wheat was sensitive but groundnut was tolerant to treated leachates on seedling growth. Singh and Singh (2003) stated that the allelopathic effects of leaf leachate of *Eucalyptus* on seed of green gram, black gram and groundnut and they observed that inhibit the seed germination and seedling growth. Channappagoudar *et al*. (2005) argued that the allelopathic effect of aqueous extracts of weed *Commelina benghalensis* and *Cyprus rotundus* had greater inhibitory effect on germination, seedling length and seedling vigor index of the groundnut seeds. They further stated that the 10% concentration of extract caused more harmful effects than 5%. Ismail and TetVun (2007) stated that the allelopathic effect of leaf extract of *Chromolaena odorata* was most inhibitory response to seed germination than the root extract as well as suppressed fresh weight of crops *viz*. rice, maize, chickpea and groundnut. Kazinczi

et al. (2007) demonstrated the experiment to investigate the allelopathic effects of *Choromolaena odorata* weed on seed germination and seedling growth of groundnut. They found that aqueous leaf and flower extract complete inhibited germination at the 10% concentration while root extract had no effect on seed germination. Further, they noted that the aqueous extract of stem, leaf and flower had stimulatory effect on shoot length in all concentrations.

Lawan *et al*. (2011) demonstrated the allelopathic influence of *Eucalyptus citriodora, E. camaldulensis, E. globules* aqueous leaf extract tested against the *Arachis hypogaea* seed showed great inhibitory effect on seed germination and root elongation in *E. camaldulensis* than the *E. citriodora* and *E. globules*. They further stated that all three plants are allelopathic in nature, but *E. camaldulensis* exhibited the highest allelopathic potentials. Belel and Rahimatula (2012) stated that allelochemicals released by *Cyprus tuberosus* water extract of both leaf and seeds have an inhibiting effect on growth of both root and shoot of groundnut also the toxic chemical has more effect on groundnut seedlings. Besides the root inhibition, morphological disorders like root twisting and distorting were also observed. Hossain (2012) remarked that when the plant debris of siam weed (*Chromolaena odorata)* at the rate of 1 gm debris per 100 gm of soil was used in pot experiment, it reduced the seedling emergence of rice, mustard, groundnut and chickpea by 16.44, 54.93, 52.25 and 26.73%, respectively. Parthsarthi *et al*. (2012) concluded that the groundnut seed germination, radical and plumule length decreased in the higher concentration of leaf extract of *Parthenium hysterophorus* and they also found that the radical length trend showed a rapid reduction than the plumule length because the radical had more area of root surface exposed to the allelochemicals. Phytotoxic effect have been introduced due to allelochemicals present in the leaf extract of *Excoecaria agallocha* and cause the synergistic activity in the retardation of seed germination and growth of groundnut (Kavitha *et al*., 2012).

Saritha and Sreeramulu (2013) stated that *Celosia argentea* leaf extract showed more inhibitory effect on growth of seedlings of groundnut. Rajiv *et al*. (2013) demonstrated the experiment to assess the allelopathic effects of composts mixture of *Parthenium hysterophorus* with soil (1:3) for seed germination and seedling growth of *Arachis hypogea* and they found that in early 45 days old compost was very sensitive to seed germination, radical and plumule elongation but after 60 days germination percentage, radical and plumule lengths of groundnut increased and

allelopathic inhibitory effects significantly reduced. At the same time they observed that *Parthenium* alone compost has moderate inhibition effect on germination percentage compared to other treatment. Kaveriammal *et al*. (2013) stated that at the 20 and 25% aqueous extract of *Lawsonia inermis* showed maximum inhibition of seed germination of green gram, black gram and groundnut seeds. Additionally they reported that the root and shoot length of green gram, black gram and groundnut seedlings were reduced by the treatment of 25%. Usha *et al*. (2013) tested water extract of six dominant weeds *viz. Apilia africana, Emiliasonchifolia, Crotalaria retusa, Chromolaena odorata, Panicum maxicana* and *Cyperus esculentus* against test crops maize, melon, okra, cow pea, soybean and groundnut and they found that the seed germination was greatly inhibited but *C. esculentus* have highest inhibitory effect than the other tested weeds. Ghetiya (2014) demonstrated the allelopathic effect of *Trachyspermum ammi* and *Mentha arvensis* on *Arachis hypogaea* (cv.G-6 and cv. G-20) seed and found that the *Trachyspermum ammi* had inhibitory effect on the biomass of *A. hypogaea* G-20 and *Mentha arvensis* L. had positive allelopathic potential for both the varieties of *A. hypogaea*. He also focused on the field studies of groundnut verities and observed that the fresh weight, biomass and net production of both varieties of *A. hypogaea* were inhibited in the presence of *T. ammi*, whereas, both verities were stimulated by *M. arvensis* L.

More than 50% reduction of seed germination of *Arachis hypogaea* was recorded in the pots treated with ground leaves of *Eucalyptus camaldulensis* as well as the growth of root, shoot and dry weight of groundnut drastically reduced in the higher treatment (Ghanuni *et al*., 2015). Shinde (2016) stated that aqueous extract of *Parthenium hysterophorus* showed allelopathic effect against *Arachis hypogaea* and *Vigna radiata* and the overall results showed the legumes are more susceptible to allelopathic effect of *P. hysterophorus* as compared to cereals. Shinde (2016) experimented the aqueous extract of *Parthenium hysterophorus* L. root and aerial part were tested on groundnut seed for their allelopathic effect on seed germination, growth seedling and seed borne infection at various concentrations in laboratory condition and observed that, seed germination of groundnut was highly affected. He further stated that the growth of radical and plumule of groundnut were decreased with increasing concentration of extract as compared to that of control. Karim *et al*. (2017) concluded that in the treatment of stem, leaves and root at different concentrations (2.5, 3.5 and 4.0%) of the extracts of siam weed (*Chromolaena*

odorata has been significantly affected seed germination (23.3%), patterns of root (37.7%) and shoot growth (26.33) and dry matter accumulation (17.5%) of groundnut and the average percent inhibition was recorded by 24.7%. Mujawar *et al*. (2017) conducted the experiment to assessed the allelopathic effect of *Pascalia glauca* Ortega stem, leaf and flower aqueous extract on groundnut seed (*Arachis hypogaea*) and they found that leaves of *Pascalia* have potent germination inhibitor than the other parts therefore, seed germination, root and shoot length and biomass of groundnut seedlings were maximum declined in the higher concentration of leaf extract treatment than the stem and flower extract but they have least allelopathic potential.

2.8.2 Allelopathic Physiological and biochemical influence on groundnut:

Physiological, enzymological and biochemical study of any plant has been core importance within the allelopathy to understanding their cardinal impact of weed extracts of various concentrations treatment against the crops for the agricultural development. Number of researchers were studied such aspects and specify the influence of different weed extracts on varied crops, most of them are repressive or suppressed the metabolic activity of crop and declined in productivity. Huge contributory work had been done by various scientist on such parameters like chlorophylls, carotenoids, proteins, carbohydrates, phenols, proline, amino acids contents, mineral constituents and inhibitor enzymes like enzyme, catalase, peroxidase, lipid perioxidase, polyphenol enzyme, nitrite enzyme, nitrate enzyme in many allelopathic plants were investigated and records bucketed since from several years.

Allelopathic plants have potentiality due to synthesizing allelochemicals have an effect on the varied metabolic activity of recessive plants at different levels like cellular, membrane porosity, nutrient uptake, closing and opening of stomata aperture, chemical action, respiration, water relation, plant product and protein activity level. Majority of the allelochemicals were discharged by the allelopathic plants that free into the rhizosphere and initial contact with roots of receiver plant so ultimately have an effect on the mineral uptake of the plant species as a result of roots are eventually related to uptake of nutrients from the soil. Eyini *et al*. (1989) studied the allelopathic effect of aqueous extracts of the fallen leaves of *Bambusa arundinacea* on the growth of *Arachis hypogaea* and they found that the water extracts decrease in total

chlorophyll and protein content is proportional to the increase in concentration of the leaf extract. Several of phenolic compounds, such as vanillic, p-coumeric, p-hydroxybenzoic and protochatechuic acid, tested alone and in combinations, were able to inhibit the enzymatic activity of all or most of glycolytic enzymes (Muscolo *et al*., 2001). The results obtained from study by Tolulope *et al.* (2016) showed that the aqueous and methanolic extracts of *Tithonia diversifolia* at different concentrations had effects on protein concentration, proline content, amount of chlorophyll and the activities of some antioxidant enzymes like superoxide dismutase and catalase in *V. unguiculata*. Sharma and Sengupta (1987) found that the activity of protease enzyme inhibited after the seven days treatment of cycloheximide in the groundnut seedlings. Patil *et al*. (2002) studied the effect of leaf litter leachates of *Casuarina equisetifolia* L on wheat var. DWR-162 and groundnut var. JL-24 and they observed the wheat was sensitive but groundnut doesn't show any negative effect and was tolerant to treated leachates on seedling growth. Mujawar *et al*. (2018) stated that the allelopathic effect stem, leaves and flower extract of *Pascalia glauca* Ortega decrease the chlorophyll a, b, total chlophyll and carotenoides content in grounddnut and it would possibly be attributed with the aid of a number of allelochemicals existing in *Pascalia glauca* Ortega.

2.9 Rhizosphere soil, surroundings and allelopathy:

Allelopathy and surroundings were interconnected to every allelopathic system has an effect on the organisms as well as plants, animals and microorganisms in natural and manmade surroundings. Over such scenario, particularly plants are exposed to varied stresses like environmental, natural, allelopathic, drought, space, light, temperature, wet and nutrient. Various environmental factors like temperature, light, soil type, precipitation, convenience of nutrients and water; modify allelopathic effects had been reportable (Khan *et al*., 1999, Catherine *et al*., 2008 and Ahmed *et al*., 2008). Throughout stress conditions plants discharged biological compounds, referred to as allelochemicals that tolerated in plants and additionally changed by low and/or high temperatures (Glass, 1976 and Einhelling, 1987), nutrient stresses (Stowe and Osborn, 1980; Hall *et al*., 1983 and Singer and Ord, 1991), drought (Einhelling, 1987).

Einhelling and Eckrich (1984) declared that environmental conditions modify the expansion interference of allelochemicals. Hall *et al*. (1983) prompt that nutrient

are standing of soil might have an impression on allelopathic effects. Nutrient deficiencies might be need additive or synergistic effects on allelopathy that interference with nutrient uptake and consequent reduction in nutrient accumulation is vital mechanisms of the action of phenolic resin compounds (Einhelling 1987 and 1995). Increase in inhibitory effects with decline in nutrients has additionally been detected by Blez and Hurle (2004). Allelochemicals and their interactions with rhizosphere soil, nutrients in soil are prime importance within the allelopathy. Phenolic resin compounds are major constituents in soil and also found in plant. They are cosmopolitan within the sort of secondary metabolites (Rice, 1984; Enhelling, 1995) including phenolic resin acids, cumarins, flavonoids, quinines and tannins that are synthesized within the shikimic acid pathway and their accumulation increased by varied environmental stresses (Kuiter, 1990). The number and composition of allelochemicals within the sort of phenolics are variable in plant and soil that chargeable for growth behavior on completely different degree and depends upon environmental factors, organic phenomenon and abiotic factors; largely they are water soluble substances (Einhelling, 1996; Rigosa *et al*., 1999). When they released into soil, it has added and change in phenolics content and become part of the soil organic matter (Whitehead *et al*., 1981, 1982; Inderjit, 1996; Martens, 2002b; Kobayashi, 2004). Phenolics found in varied bonds like free, reversible and irreversible sure. Free phenolics considerably play a phytotoxic role in soil surroundings (Huang *et al*., 1999) within the sort of ferulic acids, *p*-coumaric acids, vanillic acids, protocatechuic acids (Whitehead *et al*., 1982; Chou and Lee 1991; Li *et al*., 1992), *p*-hydroxybenzoic acids (Kuitors and Dennemen, 1987), caffic acid (Lodhi, 1976, 1978) and salicylic acids (Shindo *et al*., 1978; Jalal and browse, 1983). These are major supply of allelochemical in rhizosphere soil that generated directly or indirectly from precursor compounds discharged into the basis o root zone of plant and at the same time change the structure through the organic chemistry process throughout the action of microbes of upper organisms (Tang *et al*., 1989).

Allelopathic interaction depends upon the degree of allelochemicals present within the soil rhizosphere, soil texture, soil pH, accessible element and clay organic matter influencing its uptake that change the physiology and organic chemistry characteristics of soil (Blum, 1995). Polyvalent small nutrient components make possible the transformation of phenolic resin compounds (Pal *et al*., 1994). Water soluble phenols have inhibitory potentiality in higher plants as they come back to

rhizosphere through natural process and decomposition (Einhilling and Ramsaus, 1979). Soil sort and soil wet have an effect on the supply and persistence of allelochemicals (Patrick 1964; Wang *et al*., 1971; McCalla and Norstadt, 1974). Quantities of allelochemicals in soil rhizosphere show extreme variations (Wang *et al*., 1967; Turner and Rice, 1975; Chou and patron saint, 1976 and Lodhi, 1978). Sandy soil surroundings of *Prosopis juliiflora* was found to be a lot of unhealthy than clay soil in its section to seedlings of *Indigofera linnaei*, due to retention of wet in clay impede to diluting impact or there are higher growth possibilities of microorganisms in clay that decompose the allelochemicals a lot of quicker than sandy soil (Goel *et al*., 1989). Temperature and soil wet together with the age of tissue and organic process stage are vital factors within the production of allelochemicals (Burgos *et al*., 1999). Chlorogenic acid once added to soil inhibits the uptake of phosphorus in *Amaranthus retroflexus* L. However, addition of nutrients NPK to soil nullifies the impact of chrorogenic acid (Hall *et al*., 1983). The intensity of affects depends on the species sensitivity, duration, quantity and concentration of phenolic resin acids synthesized around root zone of plant (Blum and Rebbeck, 1989 and Lehman and Blum, 1999).

CHAPTER - III

MATERIAL AND METHODS

3.1. Survey and procurement of *Pascalia glauca* Ortega :

a) Study area :

Urun-Islampur area is about 2km irrigated crop field in Walwa taluka of Sangli district of Maharashtra, India where present weed *Pascalia glauca* Ortega firstly reported by Mujawar Ilahi (2013) and Mujawar *et al.* (2016a). Site lies between the latitude of 17º 7' 4" North and longitude 74º 16' 7" East. The climate is dry throughout the year. June to September is the south-west Manson receives 692.4mm (27.26") annual rain fall. The average temperature is in between 22.7ºC (72.9º F) to 37.5ºC (99.5ºF). Black alluvial and black cotton origin soil containing granular black to poor gravely soil. One half part of taluka mainly irrigated on Krishna and Warna river uplifting schemes for the watering the crops. Weed contaminated firstly in the cucumis and tomato plot simultaneously it spread and grow in all types of crops including sugarcane, wheat, groundnut, soybean, jawar and all vegetable fields.

b) Weeds in wheat and groundnut crop fields from study area :

Number of weed species was inhabited from both wheat and groundnut crop fields in the studied area. They are enlisted as *Physalis minima, Argemone maxicana, Alternanthera tenella, A. sesilis, Amaranthus viridis, A. spinosus, Digera muricata. Portulaca oleracea, Triumthemum portulacastrum, Trichodesma indicum, Tridax procumbens, Convolvulus arvensis, Stemodia viscosa, Leucas longifolia, Euphorbia hirta, E. geniculata, E. hyperecifolia, Cleome viscosa, Anagallis arvensis, Corchorus olitorius, Oxalis corniculata, Chrozophora rottlerio, Phyllanthus amarus, Cynotis cristata, C, axillaris Commelina benghalensis, C. nudiflora, Oldenlandia corybosa, Spermacoce hispida, Cynodon dactylon, Arundinella ciliata, Cyperus rotundus, Chloris vargata,* and *Degitaria ciliaris* (Mujawar Ilahi, 2013). This natural weed floras unremarkably happen in crop field is replaced by a unique *Pascalia glauca* Ortega invasion and battle with natives. Even though common and dominated native weed *Cynodon dactylon, Cyperus rotundus, Alternanthera tenella* from crop field fully eradicated by their growth was most vital observation. The growth and development of different species naturally adult within the studied field area are pause

and eaten up all by *Pascalia glauca* to spread monoculture greenery credited tolerance of *Pascalia glauca* with competence may be under allelopathic proficiency (Mujawar Ilahi, 2013 and Mujawar *et al*., 2016a).

c) Morphology and Taxonomy of *Pascalia glauca* Ortega :

Plant is annual or perennial stoloniferous herb; about 18-30 cm. in height. Stem is erect, glabrous and sparsely hirsute with longitudinal striations. Leaves simple, opposite, sessile, oblong-lanceolate, glabrous to scabrous with minutely hairs; hairs gland based apprising, base narrow with 2-3 or often 2 dentate teeth and acuminate apex, entire margin towards apex, slightly dentate at lower side. Flowers are in pedunculate solitary, terminal or axillary heads; heads hemispherical to companulate. Involucral bracts 2-3 seriate; inner is membranous and outer is leafy. Receptacle was flat or convex and scales prominent. Ray florets are carpellate with one row, fertile or sometimes sterile, ligulate and corolla yellow. Disc florets are bisexual, tubular and 5-lobed. Anthers were auriculate or truncate base, ovate or slightly acute apex and exserted. Style branched, filiform and hairy outside. Achenes obovoid, compressed, angled and glabrous flattened in ray florets. Disc is usually hairy, 4-angled, compressed and thick. Papus is short or lacerate scales with 2 minute awns and awns absent in ray florets.

Pascalia glauca Ortega is monotypic species included under the family Asteraceae. It is poisonous weed to domestic animals, health hazardous to human that becomes great barrier in crop field (Mujawar *et al*., 2016) and grasslands in Argentina (Soberero *et al*., 2004), totally reflected on agro economy. Tribe Helianthaeae of Asteraceae comprising over 1500 species of which it is monotypic genera, native to Central and South America widely spread in Chile, Brazil, South America Argentina, Uruguay, New Zealand and India (Carretero, 1988; Randoll, 2007 and Mujawar Ilahi, 2013). It was commonly known as ‘Sunchillo, destroyer, yuuo toad’. It is perennial weed, well established and naturalized in grasslands of ‘Pumpas’ of Argentina since 1932. It is considered to be one of the worst broadleaf weed and was declared as a pest of agriculture, called as ‘Agricultural Plaque’ in Argentina affecting maize, cotton, potato, sunflower, alfalfa, fruit forest, gardens, roadside ditches, waste lands and pastures (Sobrero *et al*., 2004; Marzocca, 1979 and Petetin and Mollnari, 1982) that toxic to sheep, pigs, cattle, horses, buffalos and goats (Ragnoese and Correa, 1997; Callazo and RietCorrea, 1996; Dias Timm and Riet-Correa, 1997; Mujawar,

2013 and Mujawar *et al*., 2016). It is dangerous for grazing and domestic animals which cause lethal hepatotoxicosis, death of animals when and even accidently consume (Mujawar, 2013 and Gianitti *et al*., 2013).

Earlier subtribe Ecliptinae of tribe Heliantheae belongs to under family Asteraceae, present weed species included and nomenclature as *Wedelia glauca* (Ort.) Hoffmann ex Hicken (Jacquin, 1760). Recently Manuel Crespo and Peno-Martin (2014) this name *Wedelia glauca* is replaced and the current nomenclature as *Pascalia glauca* Ortega has received its present status.

d) Procurement of various plant parts of *Pascalia glauca* Ortega :

Fresh plant material of *Pascalia glauca* Ortega were collected from study area i.e. from fields of Islampur, Sangli district of Maharashtra in paper bags. These materials washed thoroughly and cleaned. Healthy plant material was selected from collection lots and subjected for further analysis.

e) Procurement of test crop seeds :

i) *Triticum aestivum* L. (Wheat)

ii) *Arachis hypogaea* L. (Groundnut)

The certified seeds of test crop wheat (*Triticum aestivum* L.) variety c.v. trimbak and groundnut (*Arachis hypogaea* L.) local variety were procured from the registered seed shop from the Islampur, of Walwa taluka, district Sangli, Maharashtra.

3.2. Rhizosphere soil analysis :

a) Collection of soil samples :

Soil samples infested with *Pascalia glauca* Ortetga and non-infested, about 50m. away from *P. glauca* infested field from agricultural crop were collected at the depth of 5-15cm and 10cm radius that brought to laboratory and spread on a paper. Three soil samples were collected; one form infested field of *P. glauca* at fully vegetative growth stage, second from flowering stage and third non infested field soil serves as control. Clean to remove stones, gravels and other coarse residues, larger pieces of soil were broken by hand. The air dried soil samples were crushed in mortar and pestle and sieved through 2mm sieve. These samples were used further for analysis of various physical and chemical characters.

b) Physical Characters :

i) pH :

Soil extracts were prepared by mixing dried soil and distilled water in the 1:2 (w/v.) as, 20g soil was mixed into 40ml distilled water. The slurry of each soil samples was stirred thoroughly for 1h on electric shaker and kept undisturbed for 30min. The solutions of respective soils were taken in beakers and pH was determined using Digital pH meter (Eutech Instuments, Singapore).

ii) Electrical Conductivity (E.C.) :

From the above soil solution electrical conductivity was measured with the help of Conductivity Meter (Eutech Instuments, Singapore).

iii) Organic Carbon :

Organic carbon was estimated by a rapid titration method of Walkley and Black (1934). In this experimental procedure, potassium dichromate ($K_2Cr_20_7$) and conc. H_2SO_4 reagents were added to 1g of the soil sample. The solution is swirled and allowed to cool. Prior to adding water to halt the reaction, orthophosphate H_3PO_4 is added to the digestive mixture to eliminate interferences from the ferric (Fe^{+++}) iron that may be present in the sample.

1g of the prepared soil sample was taken in a 500ml conical flask. To this, 10ml of 0.1667 M $K_2Cr_20_7$ solution and 20ml of concentrated H_2SO_4 containing Ag_2S0_4 was added, mixed thoroughly and finally allowed the reaction to complete for 30min. The reaction mixture was diluted with 200ml with distilled water and 10ml of H_3PO_4 and then to this 10ml of NaF solution and 2ml of diphenylamine indicator was added. The final solution was titrated with standard 0.5M $FeS0_4$ solution till a brilliant green color was obtained. Simultaneously, a blank without sample was also run.

iv) Organic Matter :

It is function of carbon and thus was calculated percentage of OM = 5 OC X 1.724

c) Chemical Characters :

Available Total Nitrogen (N) :

Alkaline permanganate method of Bremner (1960) was followed for estimation of the available nitrogen content. Oven dried, powdered 0.5g of soil sample was taken in Kjeldahl flask with a pinch of microsalt (200g K_2SO_4+ 5g $CUSO_4$, dehydrated) and to it 5ml H_2SO_4 (1:1) was added. Few glass beads were added to avoid bumping and the material was digested on low flame. After complete digestion a faint yellow solution was obtained which was cooled to room temperature, transferred to volumetric flask and diluted to 100ml with distilled water.

In very clean Nessler's tube, 1ml of soil extract and different concentrations of standard ammonium sulphate solution (0.236g Ammonium sulphate dissolved in water and few drops of H_2SO_4 were added and the volume was made 1000ml. This solution contains 0.05mg of nitrogen per ml) were taken. In control tube 1ml distilled water was taken. To this, 1 drop 8% KH_2SO_4 was added and volume was made 35ml with distilled water. To this, 15ml Nessler's reagent is added (Reagent A: 7g KI+ 10g HgI_2 in 40ml distilled water, Reagent B: 10g NaOH in 50ml water, A and B are to be mixed in proportion of 4:5 at the time of estimation). After 15min, the absorbance of the chromophore developed was read at 520nm using ELICO spectrophotometer. Nitrogen value was calculated from the standard curve. Values are expressed as mg/g dry matter.

d) Available Macronutrients in Soil :

i) Phosphorous (P^{5+})

The method of Chapman and Pratt (1961) was followed for estimation of phosphorus in soil (P^{5+}). Here, phosphorus reacts with 'Molybdate Vanadate reagent' to give yellow colour complex. By estimating calorimetrically the intensity of the colour developed and by comparing it with the colour intensity of known standards, phosphorus content was estimated.

To 1ml of acid digest in a test tube, 2ml of 2N HNO_3 was added with 1ml Molybdate Vanadate reagent (1.25g of ammonium molybdate in 500ml 1N HNO_3 + 25g of ammonium vanadate in 500ml distilled water mixed in equal volumes) and volume was made to 10ml with distilled water. The ingredients were mixed well and allowed to react for 20min. After 20 min, color intensity developed was measured at

420nm spectrophotometrically using a reaction blank containing no phosphorus. With the help of standard curve, amount of phosphorus in the soil was calculated. Values are expressed as mg/g dry matter.

ii) Potassium (K^+)**:**

The soil sample was acid digested following the method of Toth *et al*. (1948). The acid digest served as sample. Potassium was estimated according to standard flame photometric process employing ELICO flame photometer. Absorbance of flame colour intensity was measured on flame photometer with specific colour filter for potassium. For standardization, various concentrations of K^+ were prepared ranging from 1 - 10ppm by diluting stock solution of KCl (100ppm) from the galvanometer readings. Potassium was estimated using calibration curve of known concentration (K^+). Values are expressed as mg/g dry matter.

iii) Calcium (Ca^+) :

Calcium was also estimated from the sample solution flame photometrically using the same procedure of Toth *et al*. (1948) to get acid digest. The acid digest served as sample. For Standardization, various concentrations of Ca^+ were prepared ranging from 50-200ppm by diluting stock solution of $CaCO_3$ (100ppm). From galvanometer readings, calcium was estimated using calibration curve of known concentration (Ca^+). Values are expressed as mg/g dry matter.

iv) Magnesium (Mg^+) :

Magnesium was estimated following the method of Drosdoff and Nearpass (1948). To 5ml of acid digest in a 50ml volumetric flask, following reagents were added in a sequential order and mixed thoroughly: 1ml hydroxylamine (5% w/v), 5ml starch compensating solution (equal volume of freshly prepared 2% starch solution and compensating solution: 3.7g calcium chloride, 0.6g of trisodium phosphate all dissolved in distilled water containing 10ml concentrated HCL and then volume made to 1lit.), 1ml Thiazole yellow (0.1% aqueous) and 5ml 2.5N NaOH volume was made to 50ml with distilled water and after 30min colour intensity was measured at 525nm spectrophotometrically. Reagent blank was prepared in the same manner as above except sample was replaced by distilled water. A standard curve for Mg^+ was also prepared with the help of different concentrations of Mg^+ from stock solution of Mg^+

(100ppm) and with this Mg^{+} content in the sample was calculated. Values are expressed as mg/g dry matter.

v) Clorides (Cl) :

Estimation of Cl from the soil was done by using potassium chromate indicator as per the method given by Black (1973). To the 20ml of soil solution, added 1ml of K_2CrO_7 as indicator (Dissolve 1g of K_2CrO_7 in 100ml of H_2O) yellow colour developed. This soil solution was titrated against $AgNO_3$ till red precipitates appeared. Values are expressed in percentage.

e) Available Micronutrients in Soil :

Iron, Manganese, Zinc and Copper :

DTPA (diethylene triamine penta acetic acid) extraction method was followed (Lindsay and Norvell, 1978) by using AAS for above mentioned micronutrients analysis.

About 5g of soil sample was taken in extraction flask. To this, 40ml of DTPA was added using calibrated dispenser and then sample was shook on electric shaker table at 200rpm for 2hr. After this, samples were filtered using Whatman filter paper No.2 and finally filtrate was used for analysis on Atomic Absorption Spectrophotometer (Perkin-Elmer - 3030). Results are expressed as mg/gm dry matter.

f) Estimation of total phenolic content from soil :

For this estimation, 1:5 w/v soil solution (prepared as above) were used. The amount of total phenolics was determined from 1ml of these extract using Swain and Hills (1959) method.

3.3. Preparation of plant material for laboratory bioassay study :

Fresh and well collected plant material of *Pascalia glauca* Ortega previously was brought to further process in laboratory. Debris and unwanted material remove from bagged material and dry under shade. From the dry material separated the different parts of weed plant parts *viz.* stem, leaves and flowers were separated and stored separately. Dried parts of plants were crushed to powder using laboratory

grinder, fine powder filtered through the 2 mm sieve and stored into brown colored plastic bottles until it was subjected for the preparation of different concentration of aqueous and methanol extracts.

3.4. Preparation of extracts of *Pascalia glauca* Ortega and its concentrations :

a) Preparation of the aqueous extracts :

Aqueous extracts were prepared by soaking 10 g stem, leaf and flower powder of *P. glauca* Ortega separately in 100ml distilled water for 24 hours. Then it was filtered using Whatman filter paper No. 1. The filtrate considered as stock solutions (100% concentration) and stored in brown plastic bottles. Stock solution was further diluted using distilled water into different concentrations *viz.* 5%, 10%, 15% and 20%. The control treatments were made by using distilled water (0%).

b) Preparation of the methanol extracts :

Methanol extracts were prepared by method Veeraragavan *et al.,* (2016). Here 10 g of stem, leaves and flower powder of Pascalia glauca Ortega was separately poured into 100ml of 80% methanol in volumetric flask for 24 hours. The extract was filtered through Whatman filter paper No. 1. This extract poured in 250 ml of conical flask placed on rotary shaker for overnight. The flask was placed in water bath and allowed to evaporate the methanol trace and final volume was made into 100 ml using distilled water. This extract treated as stock solution (100%), stored in brown plastic bottles kept cold storage before used for experiment. Further dilutions of 5%, 10%, 15% and 20% were prepared using distilled water as per standard protocol.

3.5. Seed germination, root and shoot length and biomass of wheat and groundnut :

Uniform and healthy, surface sterilized seeds of wheat and groundnut were used for seed germination studies under normal laboratory conditions. Fifteen seeds of wheat and ten seeds of groundnut were placed in petriplates containing 15 ml each of various concentrations of a aqueous or methanol extracts (5%, 10%, 15% and 20%) of stem, leaves and flower of Pascalia glauca Ortega with control having distilled water. Further, the extract treatments were provides to seeds as per their requirement.

The seed germination performance, root length, shoots length, were measured and recorded at 120 hours of seed germination of both test crops.

Biomass of seedlings was done after 120 hours of germination through randomly selected five seedlings for fresh weight. These seedlings were kept in oven at 80^0C for 48 hours and dry weight was recorded. Same seedlings were used for various biochemical estimations.

3.6. Inorganic Constituents :

Analysis of various inorganic constituents like nitrogen, phosphorus, potassium, calcium, magnesium and microelements like iron, manganese, copper, sodium and zinc from seedlings of wheat and groundnut were estimated after 192 hours of seed germination using slandered methods. For this, Oven dried plant material served as sample for analyzing various inorganic constituents. Methods for analysis of inorganic elements followed the method of soil mineral elements. Microelements *viz*. iron, manganese, zinc, copper was estimated on Atomic Absorption Spectrophotometer (Perkin-EImer-3030). The values are expressed as mg/g dry tissue.

3.7. Organic constituents :

a) Carbohydrates :

i) Total sugars :

The sugars were estimated by adapting the method of Nelson (1944). The soluble sugars were extracted from 0.5g of oven dried powder of seedlings with 80% alcohol. The extract was filtered through Buchner's funnel using Whatman's filter paper No.1. The filtrate thus obtained was condensed on water bath at about 5ml. To this 2g lead acetate and potassium oxalate (1:1) were added for decolonization, 40ml distilled water was added and aliquot was filtered. The volume of filtrate was measured and it served as an extract for determination of reducing sugars. A 20ml aliquot of this extract was hydrolyzed with 2ml concentrated HCl by autoclaving at 15lbs atmospheric pressure for half an hour. The volume of the filtrate was measured and this filtrate was used for the estimation of total soluble sugars.

The extract of reducing sugars was employed to estimate soluble sugars. The Phenol-sulphuric acid method described by Dey (1990) with slight modification was followed for estimation of the soluble sugars. In a test tube 0.2ml plant extract was taken to which 1ml phenol was carefully added and mixed thoroughly. After which 5ml of concentrated H_2SO_4 was added very carefully to the above test tube. The

mixture was vertically agitated thoroughly by using a broad end of glass rod. Then mixture was kept for cool to room temperature and the absorbance was taken at 485nm. The standard glucose (0.1mg ml^{-1}) was used for estimation of soluble sugars. The values are expressed in mg g^{-1} dry tissue.

ii) Starch :

Nelson (1944) method was adapted for the estimation of starch. The oven dried powders of plant material about 500mg were extracted with 50ml of 80% ethyl alcohol for the estimation of starch. Whatman filter paper No.1 was used to filter the extract through Buchner's funnel. The filter paper along with the insoluble residue was transferred to a 100ml conical flask containing a mixture of 50ml distilled water and 5ml concentrated HCl and the contents were hydrolyzed at 15lbs pressure for half an hour in autoclave for the estimation of starch. These conical flasks were brought to room temperature and anhydrous sodium carbonate was used to neutralize the contents and filtered through Buchner's funnel. The filtrate thus obtained was measured, which contains reducing sugars (glucose) formed as a result of hydrolysis of starch. The starch content in the residue is equivalent to the amount of glucose in the filtrate. A set of separate test tubes containing 0.1ml filtrates was employed for estimation of starch. In another set of test tubes different concentrations of standard glucose (0.1mg ml^{-1}) were taken. Final volume of 1ml in each test tube was made using distilled water. Instead of filtrate/standard glucose 1ml distilled water was taken as a blank. To above test tubes 1ml Somogyi's alkaline copper tartarate reagent (made as, 4g $CuSO_4$, $5H_2O$, 24g anhydrous Na_2CO_3, 16g Na-K-tartarate and 180g anhydrous Na_2SO_4 dissolved in 1liter distilled water) was added and then the tubes were kept in boiling water bath for 10 minutes. After cooling to room temperature, 1 ml Nelson's Arsenomolybdate reagent (It was from, 25g ammonium molybdate dissolved in 450ml distilled water, 3g sodium arsenate dissolved in 25ml distilled water, 21ml concentrated HCl. These ingredients were mixed well and incubated for 48hrs at 37^oC) was added using burette, reaction mixture was diluted to 10ml using distilled water. The readings were recorded at 560nm on Shimadzu, UV-190 double beam spectrophotometer. The calibration curve of standard glucose (0.1mg ml^{-1}) was used to calculate the amount of reducing sugars/starch and the values were expressed as mg g^{-1} dry tissue.

3.8. Photosynthetic pigments :

a) Chlorophylls :

Total Chlorophylls were estimated according to the method of Arnon (1949). Chlorophylls were extracted in chilled 80% acetone from 0.5g seedling of wheat and groundnut treated with different concentration of stem, leaves and flower aqueous and methanol extract. The extract was filtered through Buchner funnel using Whatman filter paper No.l. Residue was washed repeatedly with 80% acetone. Collected the washings in the same filtrate, the volume of the filtrate were made 100ml with 80% acetone. The absorbance was read at 645nm and 663nm wavelength spectrophotometrically using Elico spectrophotometer.

b) Carotenoids :

Carotenoids were estimated by reading the absorbance of above acetone extract at 480nm (Kirk and Allen, 1965). Total carotenoids were estimated using Liaaen-Jensen and Jensen (1971) formula. Values of carotenoids are expressed in mg g^{-1} fresh tissue.

3.9 Total free amino acids :

Total free amino acids have been estimated by method of Moore and Stein (1948). Plant seedling's sample 0.5g of was weighed and homogenized with 5ml of 80% ethanol. For this, homogenized was centrifuged at 15000 rpm for 15min. The residue was re- extracted with 5ml of 80% ethanol and centrifuged. Supernatants were collected and used for quantitative estimation of total free amino acids. 1ml of ninhydrin reagent (prepared by warming 1.25gm of ninhydrin in 30ml glacial acetic acid and 20ml 6M phosphoric acid with agitation, cooled and stored at 4^0C) was added to 0.1ml of extract in test tubes. Volume was made up to 2ml with distilled water. Tubes were heated in a boiling water bath for 20min. To each, 5ml of diluent solvent (Equal volumes of water and n-propanol), was added and the contents were mixed well. After 15 min, the absorbance of the developed purple colour was read at 570nm spectrophotometrically. A standard curve was prepared by using L-leucine (0.1mg/ml). Using the standard curve, the amount of free amino acids present in the samples was calculated. Free amino acid content was expressed in terms of in as mg g^{-1} fresh tissue.

3.10 Total proteins :

The total soluble proteins were estimated according to the method of Lowry *et al*. (1951). For this experiment, 0.5g plant material was homogenized in 10ml 0.1M phosphate buffer (pH 7.0) and then filtered through Whatman filter paper No.l. Collected filtrate was kept in ice-bath and used as source for protein estimation. From the source, 0.5ml crude supernatant was taken in test tube and diluted to 1ml with distilled water. To this, 5ml of reagent C (Alkaline copper tartarate which is mixture of reagent A 48ml (2% Na_2C0_3 in 0.1N NaOH) + B 1ml (1% NaK Tartrate in H_2O) and C lml (0.5% $CuS0_4$, $5H_2O$ in H_2O) was added. Solution was mixed well and allowed to stand for 15min, at room temperature. After 15min, 0.5ml Folin Ciocalteu's reagent (prepared by dissolving 10g sodium tungstate and 2.5g sodium molybdate in 70ml water + 5 ml 85% phosphoric acid + 10 ml concentrated hydrochloric acid. refluxed for 10hr with addition of 15g of lithium sulfate, 5ml water and 1 drop bromine. Refluxed for 15min cooled to room temperature and brought to 100ml with water) was added rapidly with immediate mixing. This was allowed to stand for next 30min and the developed colour intensity was measured at 660nm spectrophotometrically. Reagent A (made as, 2% Na_2C0_3 in 0.1N NaOH) without source served as blank. Protein concentration was calculated by comparing with standard curve of different concentrations of BSA (Bovine Serum Albumin - 0.1mg/ml). Amount of proteins is expressed as mg g^{-1} fresh wt.

3.11 a) Free Proline :

Free proline content was determined according to the method of Bates *et al.* (1973). For this, 0.5gm of fresh seedlings plant material was homogenized in 10ml sulphoslaicylic acid (3.0%) and then filtered through Whatman filter paper No.l. 2ml of this filtrate was reacted with 2 ml glacial acetic acid and 2ml acid Ninhydrin reagent (It was made from 1.25gm of Ninhydrin in a mixture of 30ml of glacial acetic acid and 20ml of 6M orthophosphoric acid) in a test tube for one hour at 100^0C in boiling water bath. Similar procedure was followed using standard proline solution (1ug/ml proline). After boiling, the reaction was terminated by transferring the test tubes immediately to ice bath. The colour developed in reaction mixture was extracted using 4ml toluene. The reaction mixture was mixed vigorously with test tube stirrer for 15-20sec. and allowed to settle. Reaction mixture was then brought to room temperature and absorbance of toluene chromophore was measured at 520nm

spectrophotometrically using toluene blank as blank. Free proline content was calculated from calibration curve and the values of free proline are expressed as mg g^{-1} fresh tissue.

b) Total Polyphenols :

Total polyphenols were determined according to the method of Folin and Denis (1915). Fresh seedlings material (0.5g) was homogenized in 30ml 80% acetone and filtered through Buchner Funnel. The residue was washed several times with 80% acetone and the final volume of the filtrate was made 100ml with 80% acetone. 2ml extract along with series of standards (standard tannic acid 0.1mg/ml) was taken in Nessler's tube, to this 10ml 20% Na_2C0_3 and 2ml Folin Denis Reagent (750ml of distilled water, 100g sodium tungstate, 20g phosphomolybdic acid and 50ml ortho phosphoric acid, mixture was refluxed for 2hrs, cooled and diluted to 1litre) were added. The final volume was made 50ml with distilled water. After 20min. absorbance of developed color was read at 660nm spectrophotometrically using reagent blank. Total polyphenols were calculated using standard curve of tannic acid and are expressed as mg g^{-1} fresh tissue.

3.12 Enzyme Estimation :

a) Catalase (E.C. 1.11.1.6) :

The activity of enzyme catalase was done by modified method of Herbert (1955). 2g of seedlings from each treatment of concentrations from aqueous and methanol extract were washed with distilled water and homogenized in pre-chilled mortar and pestle with 15ml cold 0.1M acetate buffer (pH 5.0). The extract was filtered through four layered of muslin cloth. The filtrate was centrifuged twice at 6000rpm for 5min and the supernatant was used for enzyme assay. All the operations were carried out at 0-4^0C. The assay mixture was prepared by mixing 1ml of 0.045M H_2O_2 in phosphate buffer (pH 6.8) and 1ml aliquot of enzyme. Assay mixture was incubated at room temperature for 1min and then the reaction was terminated by the addition of 5ml of 5N H_2SO_4. To this 1ml 10% aqueous KI solution and a drop of 2% ammonium molybdate were added. The amount of H_2O_2 utilized by liberation of iodine was determined by titrate the reaction mixture with 0.01M sodium thiosulphate using starch indicator. The difference between 0min and 1min reaction was used as

enzyme activity. Activity of enzyme is expressed as mg H_2O_2 broken down min^{-1} mg^{-1} protein.

b) Peroxidase (E.C. 1.11.1.7) :

Maehly and Chance (1954) method was used for study of enzyme peroxidase. The enzyme was extracted from seedlings subjected to different concentration of treatments by using cold distilled water as an extraction medium. The remaining procedure was same to the ones described for catalase. The assay consists of 2ml 0.1M phosphate buffer (pH 7.0), 1ml 20mM guiacol and 1ml enzyme source. The reaction was started by adding 0.04ml, 10mM H_2O_2 and oxidation of guiacol was studied by measuring the change in optical density at 470nm on double beam spectrophotometer (Shimadzu-190, Japan).

c) Superoxide dismutase (E.C. 1.15.1.1) :

Superoxide dismutase activity was determined following the method described by Giannopolities and Ries (1977) with slight modifications. Enzyme was extracted by homogenizing 0.5g fresh seedlings treated with different extract concentration and control in 10ml, 150mM cold potassium phosphate buffer (pH 7.8) containing 1% PVP (to protect the enzyme from the action of polyphenols). Then it was filtered through the 4-layered muslin cloth and the filtrate obtained was centrifuged at 10,000rpm for 20min at 0-4^{0}C. The supernatant was used as an enzyme source. An enzyme assay mixture contained 2ml potassium phosphate buffer pH 7-8, 0.2ml methionine (13mM), 0.1ml Nitroblue tetrazolium (75uM), 0.5ml EDTA (0.1mM), 0.1ml enzyme and 0.1ml riboflavin (2M) was added lastly. The total volume of the assay mixture was measured at 560nm on UV-VIS double beam spectrophotometer (Shimadzu-190, Japan). Then the assay mixture was exposed to full sunlight for 30min again the changed absorbance was read at 560nm. The enzyme activity is expressed as ΔO.D. $h^{-1}mg^{-1}$ of protein.

d) Lipid Peroxidation :

The Thiobarbitutric Acid (TBA) assay for lipid peroxidasation was carried out according to the method of Heath and Packer (1968). 500mg of seedlings tissue from different concentration treatments and control were homogenized in 10ml 0.5% Thiobarbituric acid in 20% Trichloro Acetic acid (TCA). The mixture was incubated

at 90^0C in a shaking water bath for 30minutes and reaction was stopped by placing the reaction tubes in ice bath. After centrifugation at 10,000rpm for 30min. the amount of MDH-TBA complex in the supernatant was determined by recording its absorbance at 535nm and corrected for the non-specific absorbance by subtracting the absorbance value obtained at 600nm. The final values are expressed as µmole MDA g^{-1} fresh tissue.

e) Polyphenol Oxidase (E.C. 1.10.3.2) :

The activity of an oxidative enzyme polyphenol oxidase was studied spectrophotometrically by using the extraction and assay procedure suggested by Mahadevan and Sridhar (1982) with slight modification to suit our laboratory condition. 0.5g seedlings material was cleanly washed and cut into small pieces and extracted in 15ml cold 0.1M phosphate buffer (pH 6.1) in pre-chilled mortar with pestle. The homogenate was filtered through 4 layered muslin cloth and centrifuged at 10,000rpm at 4^0C using refrigerated centrifuge. The supernatant was used for assaying the enzyme activity.

In order to score the activity of polyphenol oxidase the oxidation of catechol was measured from the reaction mixture containing 2ml phosphate buffer (pH 6.1), 0.5ml enzyme extract and 1ml 0.01M catechol at 495nm. The change in the absorbance upto 180sec. was measured. The control reaction was maintained with heated enzyme.

f) Nitrate reductase (E.C. 1.6.6.1) :

This enzyme was determined by following the method of Jaworsky (1971). The sample of groundnut seedlings were cut into small pieces and incubated in 10ml incubation medium containing 1ml KNO_3, 2ml 5% n-propanol, 5ml 0.2M phosphate buffer (pH 7.5) and 2ml 0.5% Triton-x-100, for 1h in dark. After incubation, 1ml of the reaction medium was taken out for determination of nitrate and was mixed with 1ml each of 1% sulfanilamide in 1M HCl and 0.02% NEEDA. The absorbance was read at 540nm on double beam Shimadzu spectrophotometer using reagent blank. The standard curve was prepared with 0.03mM KNO_2 (0.0026 mg NO_2 ml^{-1}) against a mixture of 1ml incubation medium, 1ml sulfanilamide and 1ml NEEDA as a blank.

After determination of NR activity, the plant material from the incubation medium was removed carefully and washed thoroughly first with distilled water and

then with 0.2M phosphate buffer (pH 7.5). It was then homogenised in 10ml 0.2M phosphate buffer (pH 7.0). The extract was filtered through Whatman filter paper No.1. The filtrate was used for the estimation of enzyme nitrate (NO^-_3) and nitrite (NO^-_2) reductase and proteins were estimated by method of Lowry *et al*. (1951) in the fresh tissue.

g) Nitrite reductase (E.C. 1.6.6.4) :

The nitrite reductase activity was determined by the method of Jaworsky (1971). Followed the same procedure as already described for nitrate reductase except that KNO_3 was replaced by 0.3mM KNO_2 in the incubation medium and the incubation was done in light.

The amount of KNO_2 remained in the incubation medium after the enzymatic activity of 1h was determined by recording the optical density of the reaction mixture containing 1ml incubation medium, 1ml sulfanilamide and 1ml NEEDA. The difference between the two readings, one at 0min and the other after the enzymatic reaction, gave the amount of KNO_2 utilized by the enzyme. The standard curve of KNO_2 was prepared as described for nitrate reductase. For the preparation of blank, 2ml n-propanol, 2ml triton-x-100 and 6ml phosphate buffer (pH 7.5) were mixed well and from this 1ml was mixed with 1ml sulfanilamide and 1ml NEEDA. Nitrate, nitrite and proteins in the fresh tissue were determined.

3.13 Phytochemical analysis :

a) Phytochemical screening tests of *Pascalia glauca* Ortega from root/stolon, stem, leaves and flower extracts :

The healthy plant parts including root/stolon, stem, leaves and flower were collected from *Pascalia glauca* weed plant were shade dried at room temperature for two weeks then individually homogenized to a fine powder and stored in an airtight brown bottles until the further process. Aqueous and methanol extract prepared from various plant parts powder as per described earlier in this chapter. The preliminary phytochemical analysis was carried out on the aqueous and methanol extracts using standard procedures (Krishnavignesh and Mahalakshmipriya, 2013 and Patil and Khan, 2017) to identify the phytochemical constituents.

i) Test for alkaloids :

The 0.5 ml extract were treated with few drops of 1ml 2N HCl to this few drops of Mayer's reagent / Dragandorf reagent and Hager's reagent were added. Orange precipitate, Orange color, White or Yellow precipitate shows the presence of alkaloids.

ii) Test for flavonoids :

Test tubes containing 0.5ml of test extracts, 5-10 drops of dilute HCl and small piece of zinc or magnesium were added and the solution was boiled for few min. In the presence of flavanoids, reddish pink or dirty brown color is produced.

iii) Test for steroids:

The extract was mixed with 2ml of chloroform and concentrated sulphuric acid was added sidewise. A red colour produced in the lower chloroform layer indicated the presence of steroids.

iv) Test for terpenoids : Salkwoski reaction :

The 0.5ml of the test extracts was mixed in 0.2ml of chloroform and conc. H_2SO_4 (0.3ml) was carefully added to form a layer. A reddish brown coloration in the inter phase formed indicate the presence of terpenoids.

v) Test for Tannins :

To 1-2ml of test extracts, few drops of 5% aqueous Fecl3 solution were added. A bluish black color, which disappears on addition of a few ml of dilute H_2SO_4 followed by the formation of yellowish brown precipitate indicate the presence of tannins.

vi) Test for Saponins :

The extract was mixed with 5 ml of distilled water in a test tube and it was shaken vigorously. The formation of stable foam was taken as an indication for the presence of saponins.

b) Extraction, isolation and identification of allelochemicals in *Pascalia glauca* extracts :

i) Liquid chromatography mass spectra (LCMS) analysis :

Liquid Chromatography-Mass Spectrometry (LCMS) is an analytical chemistry technique that combines the physical separation capabilities of liquid chromatography (or HPLC) with the mass analysis capabilities of mass spectrometry. Detection of different allelochemicals was done from methanol whole plant extract of *Pascalia glauca* by using the method of Harborne (1984), Liquid chromatography mass spectrometry (LCMS) analysis was carried out using equipment API QSTAR pulsar Germany. Samples dissolved in methanol and continuous run fully flow. The data dependent acquisition, during which compound precursor ions were detected by scanning in the range from 50 to 2000 m/z with column flow rate of 5µl/min. Two solvent were used including solvent A and B. Solvent A consisted of 90% (water in 0.1% Trifluro acetic acid) and solvent B consisted of 10% (acetonitrile in 0.1% Trifluro acetic acid) and 30µl sample were injected onto the column.

ii) Gas chromatography-mass spectrometry (GC-MS) analysis :

Gas chromatography-mass spectrometry (GC-MS) analysis of the methanol extract of the *Pascalia glauca* was carried out for the phytochemical investigation of methanolic extract was performed (Hites, 2016) on a GC-MS equipment (Shimadzu). Experimental conditions of GC-MS system were follows by using Sh-rxi-5sil capillary standard non-polar column with dimension 30Mts, ID: 0.25 mm and film thickness was 0. 25µm. Helium (He) was used as the carrier gas at a constant flow of 1 ml/minute and an injection volume of 1.0 µl. In the gas chromatography part, the temperature programming was set with initial oven temperature at 50°C and held for 2 minutes and the temperature of the oven was raised to 250°C for 10 minutes and raised at 5°C/minute. Samples dissolved in methanol were run fully at a range of 50-650 m/z and the results were compared by using NIST 14 library search programme. The components were identified by comparison of their mass spectra with those of mass spectral library and retention time either with those of authentic compounds or with literature values.

3.14 Statistical analysis :

The data collected from the various experiment were analyzed for test of significance and compared with treatment means following online one way Anova Tukeys HSD Post-hoc Test Inference significance at $p < 0.05$ and $P < 0.01$ level of probability. Online one way anova was done from following web sites,

http://astatsa.com/OneWay_Anova_with_TukeyHSD/

http://www.socscistatistics.com/tests/anova/default2.aspx

CHAPTER - IV

RESULTS AND DISCUSSION

A) Physico-chemical analysis of rhizosphere soil from invaded and non-invaded region of *Pascalia glauca* Ortega.

1) PH, electrical conductivity, organic carbon, organic matters and phenolic analysis of rhizosphere and non-rhizosphere soil of *P. glauca* Ortega :

The soil was collected from *Pascalia glauca* Ortega invaded area as well as from non-invaded area from studied region Islampur, district Sangli. It was analyzed some physico-chemical characteristics for this three criteria were selected i.e. a) Soil under vegetative growth of *P. glauca* stage b) Soil fully flowered stage of *P. glauca* and c) Soil free from *P. glauca* growth stage, it was considered as control soil.

The control as well as invaded *P. glauca* area, the soils were alkaline and a little significant difference was observed among these soils. The pH was recorded least from the flowering stage (7.20) than vegetative stage (7.25) as compared to the control (7.70). The electrical conductivity was found maximum in *P. glauca* invaded site at vegetative stage (0.38mmhos/cm) followed in sequence by flowering stage (0.30mmhos/cm) and control soil (0.24mmhos/cm). In *P. glauca* invaded soil at vegetative stage, the amount of organic carbon (1.65%) was maximum followed by flowering stage (1.28%) and little in control (1.02%). Organic matters also highly increased in the vegetative stage (2.84%) than flowering stage (2.34%) in comparison to the control soil (1.75%). The soils were also analyzed for total phenols content. In *P. glauca* invaded soil, the maximum amount (3.83ppm) of total phenols was found i. e. 3.83 ppm at vegetative stage followed by flowering stage (3.26ppm) and control soil (1.83ppm) (Table – 1).

2. Macro and micro nutrients of soil :

The macro and micro-nutrients amount was assessed from the rhizosphere soil invaded by the *Pascalia glauca* at the time of fully vegetative growth, flowering stage and control serve as free field area from *P. glauca* dipicted in Table-2.

The maximum amount of nitrogen (N) (272.39Kg/ha), Phosphorus (P) (84.50Kg/ha), Potassium (K) (290.20Kg/ha), Sodium (Na) (167.50Kg/ha), Calcium

(Ca) 0.82 %, Magnesium (Mg) 0.23%, and Chloride (Cl) 18.14g/100g were determined at vegetative stage in *P. glauca* invaded soil. Nitrogen (N), Phosphorus (P), Potassium (K), Sodium (Na) theywere in the large amount at vegetative stage of *Pascalia* while Calcium (0.98%), Magnesium (0.32%), and Chloride (23.33%) were recorded high at flowering stage. Control soil recordedless amount than the others that indicates increase the macronutrient in infested soil than control soil. The infested soil with vegetative stage of *Pascalia* showed rich in macronutrients than flowering stage and control soil stage.

The micro-nutrients was determined including iron (Fe) (5.82ppm), manganese (Mn) (2.98ppm), zinc (Zn) (0.92ppm), and copper (Cu) (0.88ppm), the maximum amount of respective element or nutrient was found in soil at vegetative stage, followed by flowering stage iron (4.70ppm), manganese (2.29ppm), zinc (0.82ppm), copper (0.72ppm) and control soil determined micronutrients in the amount of iron (1.18ppm), manganese (1.27ppm), zinc (0.52ppm), copper (0.47ppm). Micronutrients are also rich in the vegetative stage of *Pascalia* invaded soil than the others. Macronutrient including nitrogen, phosphorus, calcium and sodium and micro nutrients like iron, zinc, manganese and copper were large in amount within the vegetative growth of *Pascalia* invaded soil.

Discussion:

The data revealed that the macronutrients and micronutrients as well as pH, electric conductivity, organic carbon, organic matter and total phenols has raised in the vegetative stage than flowering stage of weed *Pascalia glauca* Ortega in infested soil of wheat and groundnut crop in comparison to the uninfected soil control sample.

The rhizosphere soil invaded with *Pascalia glauca* and its allelopathic effect indicates its inhibitory impact on growth of crop. In field observations, the growth of both wheat and groundnut test crops when cultivated in *P. glauca* invaded soil showed reducing growth potentiality that affected crops metabolism and their growth has been suppressed. The pH, electric conductivity, organic carbon, organic matter and phenols have been more in the vegetative stage followed by flowering stage over the control. It indicates that more inhibitory compounds released at the time of vegetative growth of *Pascalia*. The increment of values of soil characters depicted in the Table - 1 in the series of vegetative stage > flowering stage > control.

Table- 1: Physioco-chemical analysis of rhizosphere soil of *Pascalia glauca* Ortega.

Soil characters	Control soil(free from *Pascalia* field)	*Pascalia* invaded field soil at vegetative stage	*Pascalia* invaded field soil at flowering stage
pH	7.70	7.25	7.20
E.C.	0.24mmhos/cm	0.38mmhos/cm	0.30mmhos/cm
O.C.	1.02%	1.65%	1.28%
O.M.	1.75%	2.84%	2.34%
Totalphenols	1.83ppm	3.83ppm	3.26ppm

Table- 2: Macro and micronutrient analysis of rhizosphere soil of *Pascalia glauca* Ortega.

Nutrients	Control soil(free from *Pascalia* field)	*Pascalia* invaded field soil at vegetative stage of *Pascalia*	*Pascalia* invaded field soil at flowering stage of *Pascalia*
Nitrogen-N	98.25Kg/ha	167.50Kg/ha	116.33Kg/ha
Phosphorus- P	62.26Kg/ha	84.50Kg/ha	75.50Kg/ha
Potassium- K	216.78Kg/ha	290.20Kg/ha	275.33Kg/ha
Sodium- Na	117.50Kg/ha	167.50Kg/ha	155.50Kg/ha
Calcium- Ca	0.45%	0.82 %	0.98%
Magnesium- Mg	0.09%	0.23%	0.32%
Cloride- Cl	6.12g/100g soil	18.14g/100g soil	23.33g/100g soil
Iron- Fe	1.18ppm	5.82ppm	4.70ppm
Magnese- Mn	1.27ppm	2.98ppm	2.29ppm
Zinc- Zn	0.52ppm	0.92ppm	0.82ppm
Cooper- Cu	0.47ppm	0.88ppm	0.72ppm

The N, P, K, and Na were detected maximum in the vegetative stage while Ca, Mg and Cl were found to be higher from the flowering stage. The micronutrients Fe, Mn, Zn and Cu were increased when *Pascalia* was fully grown at the vegetative stage. The control soil has been determined minimum in comparisons with vegetative stage and flowering stage soil. That means the invaded soil have increased nutrients over the control thus at the same time the growth of *Pascalia* some inhibitant biochemical also released in full swings that mixed into soil rhizosphere and influenced on the growth of crop plant grown in field.

The pH of soil was not much more change but control (7.70) soil has more pH than the vegetative (7.25) and flowering (7.20) stage of *Pascalia* grown in the field. It was reduced after the growth of weed in both the stages. The electric conductivity has been increased 1.58fold in vegetative and 1.25fold in flowering stage of weed. Same things have been observed in organic carbon that boosted by 1.61fold in vegetative and 1.25fold in flowering stage in comparable to the control. Organic matter increased with 1.62fold in vegetative and 1.33fold in flowering while total phenols determined maximum encourages in vegetative (2.09fold) than the flowering stage (1.78fold) over the control, indicates more influence the vegetative part of weed i.e. stem and leaves than the flowering parts that delayed the growth and development of crop during flush greenery of *P. glauca* in the standing crop field that receives and enjoyed benefits more natural resources in competence with crops.

The macronutrients in the invaded soil must be heightened than the control soil. N, P, K nutrients were more increased in the vegetative stage than the flowering stage of *Pascalia* their average increments was determined 1.46fold more in vegetative growth stage than the flowering stage (1.22fold). That means both the stages affect the crop plants but more hindered the growth during its vegetative growth and if it eradicated through using some eco friendly, it can be minimize their alleopathic influence. Ca, Mg, Cl nutrients were detected maximum at the flowering stage of weed that determined 3.17fold more than the vegetative stage (2.44fold) compared with control. Cl was increased higher (3.81fold) followed by Mg (3.55fold) and Ca with 2.17fold over the control at flowering stage while in vegetative stage Cl (2.96fold), 2.55fold more Mg and 1.82fold more Ca over the control. Micronutrients were not far more in the average increments both are very nearer in vegetative (2.72fold more) and flowering stage (2.22fold) over the control. In comparisons Fe,

Mn, Zn and Cu, the Fe (4.93fold more) in vegetative stage was detected maximum than the other stages.

From the analysis of rhizosphere soil clearly indicates that increased phenolics in invaded soil than control may be adversely affects the early growth of both wheat and groundnut test plants. Various recent studies (Batish *et al.,* 2006a, 2007a; Sisodia and Siddiqui, 2009; Raoof and Siddiqui, 2012a; Gulzar *et al*., 2011; Gulzar *et al.,* 2014cand Gulzar and Siddiqui, 2015) have evaluated the phytotoxic activity of rhizosphere soil in which the growth of the test plants reduced that supports our finding. The presence of phenolics might be negatively affecting the growth of other plants (Sarkar and Chakraborty, 2010). Same condition has been seen in crop field soil inhabited by *Pascalia* therefore, wheat and groundnut seedlings may be affected on their growth and developments were confirming from above researchers. The phytotoxic compound 'Juglone' was detected from *Juglans nigra* in the rhizosphere soil was the classical example indicating that the inhibitors released from the plant accumulate in the soil (Rietveld, 1983). Number of other experts has been observed to be responsible for bringing about inhibitory effects on other surrounding plants (Bais *et al*., 2002 and Corey and Jorge, 2008). Same phenomenon has been reported by El-Khatib *et al*. (2003) where strong inhibitory effect of rhizosphere soil invaded with *Medicago sativa* plant that affect the other plants. The amount of all the nutrients including macro and microelements was higher from *P. glauca* invaded field soil compared to control soil and hence they are ruled out as none of the estimated nutrients was to be deficient in rhizosphere soil and for subdue the growth effect on test plants. The analyzed rhizosphere soil indicated that there is definite role of allelopathy in reducing the crop growth, same statement postulated by Batish *et al*. (2007a).

The phenolics were detected maximum amount in rhizosphere soil from *Pascalia* invaded field in comparison to control soil in our study. Such findings have vital role in the retardation of test crop growth and other plants in the field; have causing appreciable injury in the growing plants (Rice, 1984 and 1995; Qasem and Foy, 2001; Weston and Duke, 2003 and Batish *et al*., 2007b). Present results suggested that soil invade with *P. glauca*, contains some growth inhibitory substances that reduce the growth of test plant seedlings. These compounds are released from plant by various mechanisms like leachation, exudation through roots and decompositions of residues. Root exudates are considered as one of the major means

of communication between rhizosphere and various microorganisms residing there which is complex mixture of compounds that are responsible for underground interactions (Bais *et al*., 2004). Physical and chemical properties of soil also alter and inhibit growth of competing plants (Nardi *et al*., 2000; Norsworthy and Meehan, 2005 and Kong *et al*., 2006).

In ecological terms, the rooting zone and rhizosphere is a very competitive environment where the roots of neighboring species and microorganisms compete for space, water, nutrients and gases. Roots also perform several more specialized roles in the rhizosphere, which rely on the synthesis and exudation of metabolites in addition to providing mechanical support, water and nutrients. Root secretions comprise the majority of low molecular mass constituents such as amino acids, organic acids, sugars, phenolics and other secondary metabolites (Bertin *et al*., 2003). Root produced allelochemicals are generally associated with the reduction in neighboring plant growth and resistance to or suppression of plant pathogens, soil microbes and other herbivores. The presence of phenolics in the rhizosphere soil of *Pascalia* invaded fields indicates that these might have been released from the weed plant. Same results stated by Batish *et al*. (2007a). Our results were closely nearer and present work in the right line to above workers.

The percentage of organic carbon and organic matter found to be maximum in soil supporting plants from *Pascalia* at vegetative stage followed by soil from flowering stage and control. The reason for this could be that foliage at vegetative stage was quite expanded and thus, its death and decay adds more organic matter and organic carbon and also the number of plants was greenery thicate at this stage and when the plant reaches the flowering stage, its foliage gets comparatively smaller and it has been largely over taken by its inflorescence and flowers.

It becomes clear from the study that soil supporting *Pascalia glauca* invaded plants either at vegetative and flowering stage is not deficient in any of nutrients rather the status of both macro and micronutrients are better in comparison to control favoring the better growth of *Pascalia glauca* thus well compete with crops receives more and more nutrients and enjoy their benefits. The phytotoxic effect induced by rhizosphere soil of *P. glauca* indicates that the allelopathic effect could be due to the presence of phenolics. Therefore, our work has been in the right line of above workers.

Inhibitors or allelochemicals are released or accumulated in the soil by natural agencies of water leachation, decomposition, root exudation and volatilization Allelochemicals may be released through any one of these or through all of these modes. Besides, retention, transport of allelopathic chemicals in soil and physicochemical and biological components of the soil can influence the fate of allelopathic chemicals and thus of allelopathy, in soil (Inderjit, 2001). Our study support all such viewspostulated by various allelopathic experts that some of the chemicals were escapedmostly called as allelochemicals from the *Pascalia* incorporating with soil and their influence has been inconvenient on test crop wheat and groundnut growth and its development in field.

B) Effect of aqueous and methanol extracts of *Pascalia glauca* Ortega on seed germination and growth parameters of wheat and groundnut.

1. Seed germination:

The study has been demonstrated to evaluate the allelopathic effect of aqueous and methanol extract of stem, leaves and flower of *Pascalia glauca* Ortega on two local test crops *viz*. wheat and groundnut commonly cultivated in the studied area *viz*. wheat and groundnut for germination bioassay. Observations recorded after five days of the bioassay which experimented in wet petriplated of respected extract are presented here.

1.1 Wheat (*Triticum aestivum* L.)

Seed germination :

The data represented in Table- 3 and Plate- 3 and 4 showed reductions in the seed germination from lower concentration (5%) to higher concentration (20%) in all tested extracts. The concentration of 20% from all extracts of both aqueous and methanol has been significantly reduced seed germination. Aqueous extract of all tested 5% concentration have been recorded lowest and similar inhibition of seed germination (93.33%). Methanol extract showed significant seed germination inhibition in all tested concentrations except 5 and 10% flower extract. The lowest seed germination (66.66%) has been observed in 20% aqueous leaves extract and 44.44% in methanol leaves extract that means 1.50 and 2.25 fold inhibited the seed germination over the control respectively. Thus, in present investigation the inhibitory

effect of all aqueous and methanol extracts was found to be concentration dependant and methanol extract had more detrimental effect on seed germination.

The results expressed that the seed germination in wheat under treatment of aqueous extract of *Pascalia glauca* of stem, leaves and flower as this has gradually declined with increasing the concentration of extract from 5% to 20% comparable to control. There was 93.33% seed germination recorded at 5% aqueous extract from stem, leaves and flower over the control. Stem and leaves extracts found similar inhibition seed germination from 10% (91.10% and91.10%) to 20% (73.33% and 66.66%) but in flower extracts showed slow inhibition of seed germination from 10% (88.88%), 15% (86.66%) to 20% (84.44%) concentration respectively.

Methanol extract of stem, leaves and flower of *Pascalia glauca* gradually retarded seed germination in wheat from lower concentration 5% (62.22%, 64.44%, 77.33% respectively) to 20% higher concentration (51.10%, 44.44%, 59.99%) respectively over the control. The methanol extract recorded lowest seed germination (44.44%) in 20% concentration from leaves extract. The inhibition of seed germination increased from 5% to 20% concentration over the control.

The lowest seed germination has been observed at highest concentration (20%) of aqueous leaves extract (66.66%) i.e. seed germination inhibited 1.50fold while 1.36fold in aqueous stem followed by 1.18fold from aqueous flower extract over control. The seed germination was inhibited in the series of sequence leaves > stem > flower.

Methanol leaves extract has been detected as major source of seed inhibitor that inhibited 2.25fold seed germination from 20% concentration followed by 1.95fold in stem extract and 1.66fold seed germination inhibited in flower extract over control.

Leaves extract has been investigated so far major source of allelopathic component which may be released in the soil environment that inhibited more seed germination than the stem and flower extracts.

Groundnut (*Arachis hypogaea* L.)

Seed germination :

The gradual reduction of seed germination has been observed in groundnut with increasing the concentration of aqueous extracts of *Pascalia glauca* Ortega stem, leaves and flower as depicted in Table-3 and Plate- 5 and 6. The highest inhibition of

seed germination has been found in the 20% concentration of aqueous leaves extract (40.00%) and methanol flower extract (33.33%) in present investigation. Further, significant inhibition is recorded in the 15% and 20% concentration of all tested extract in aqueous as well as in methanol extract while all lower concentration in aqueous extract of stem have insignificant results. Methanol extract of all concentrations showed more significant results in the groundnut seed germination. 15% Aqueous stem and leaves extract have similar inhibition i.e. 50.00% in seed germination at the same time 15% methanol leaves and flower extract also found identical results with 40.00% inhibition over control. The higher concentration (20%) of aqueous stem extract seed germination inhibition has been defeated by 2.30fold, aqueous leaves extract by 2.5fold and flower extract by 2fold in comparison to the control. There is 3 fold increased seed germination inhibition at higher concentration (20%) of methanol flower extract than the leaves (2.72fold) and 2.30fold in the stem.

Aqueous extract of stem in 20% concentration has shown 43.33% seed germination followed by 15% (50.00%), 10% (60.66%) and 5% with 66.60%. Aqueous leaves extract recorded highest inhibition (40.00%) of seed germination in 20% concentration while at 15% concentration have 50.00%, 10% recorded53.33% and 5% have 60.00% seed germination. Aqueous extract of flower responds slow seed inhibition from 5% (70.00%) followed by 10% (66.60%), then increased in 15% (53.33%) and 20% with 50.00%.

Methanol extract of stem decreased seed germination (66.66%) in 5%, (63.66%) in 10%, (53.33%) in 15% and 20% concentration have 43.33%. Methanol extract of leaves showed gradual suppressing the seed germination from 5% (56.66%), 10% (53.33%), 15% (40.00%) and in 20% concentration have been recorded maximum seed germination inhibition (36.66%). Methanol flower extract treatment observed drastic change in curtailment values of seed germination i.e. 33.33% in 20% concentration was higher within all tested concentration. Remaining methanol extract concentrations observed 66.33% in 5%, 56.66% in 10% and 40.00% in 15% concentration.

From above observations it is noted that the effect of allelopathic potentiality is more injurious in groundnut than wheat. Also leaves extract has shown more influence on germination than other weed part extracts. These findings clearly indicate the harmful allelopathic effect of this weed in laboratory conditions and the

same will be true even in field conditions. Methanol extracts more deleterious than the aqueous extracts.

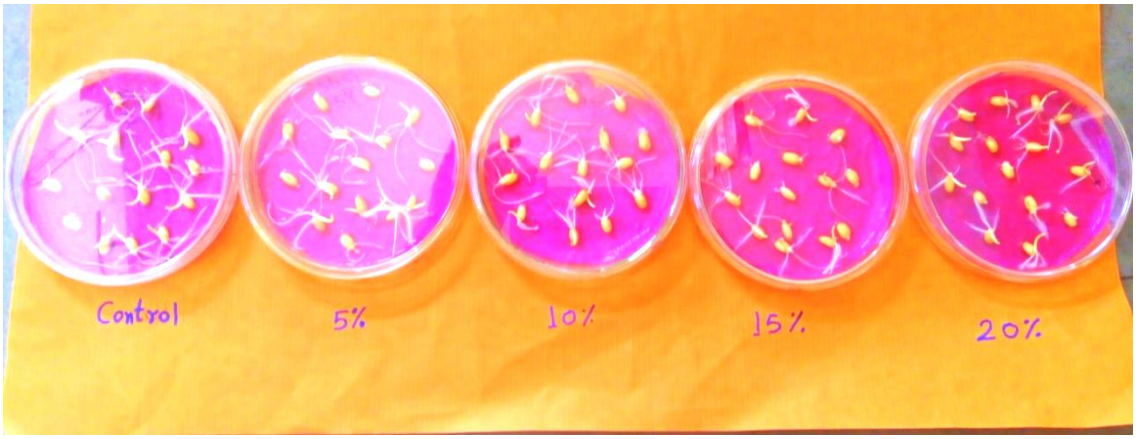
Table-3 : Effect of aqueous and methanol extracts of stem, leaves and flowers of *Pascalia glauca* Ortega on wheat and groundnut seed germination percentage at 120 hours.

Plant part extract	Concen-trations	5%		10%		15%		20%	
	Control	Aqueous	Methanol	Aqueous	Methanol	Aqueous	Methanol	Aqueous	Methanol
Wheat seedling									
Stem	100.00 ±0.00	**93.33** ±0.00	62.22** ±3.84	**91.10** ±3.85	60.66** ±0.00	77.77* ±7.69	53.33** ±5.77	73.33** ±6.67	51.10** ±3.85
Leaves		**93.33** ±0.00	64.44** ±5.77	**91.10** ±3.85	55.55** ±3.85	84.44 ±3.84	46.66** ±0.00	**66.66**** ±6.66	**44.44**** ±3.84
Flower		**93.33** ±0.00	73.33** ±0.00	88.88 ±3.85	71.10** ±13.87	86.66* ±0.00	40.00** ±10.00	84.44 ±3.84	59.99** ±6.66
Groundnut seedling									
Stem	100.00 ±0.00	**66.66**** ±5.77	**66.66**** ±5.77	60.00** ±10.00	63.33** ±5.77	**50.00**** ±10.00	53.33** ±5.77	**43.33**** ±5.77	**43.33**** ±5.77
Leaves		60.00** ±10.00	56.66** ±5.77	**53.33**** ±5.77	**53.33**** ±5.77	**50.00**** ±10.00	**40.00**** ±10.00	**40.00**** ±10.00	**36.66**** ±5.77
Flower		70.00** ±10.00	63.33** ±5.773	66.66** ±5.77	56.66** ±5.77	53.33** ±5.77	**40.00**** ±10.00	50.00** ±10.00	**33.33**** ±5.77

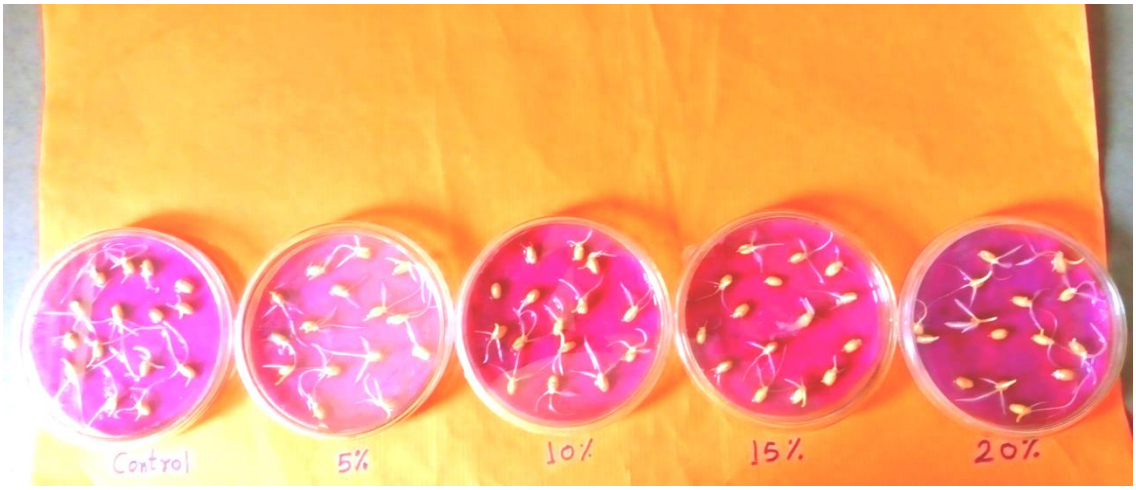
One way Anova: Tukeys HSD Post-hoc Test Inference. **significant at P < 0.01. *Significant at P < 0.05. Otherwise insignificant result. ±Standard deviation.

Plate- 3 : Effect of aqueous extract of stem, leaves and flowers of *Pascalia glauca* Ortega. on wheat seed germination growth after 72 hrs.

Aqueous stem extract



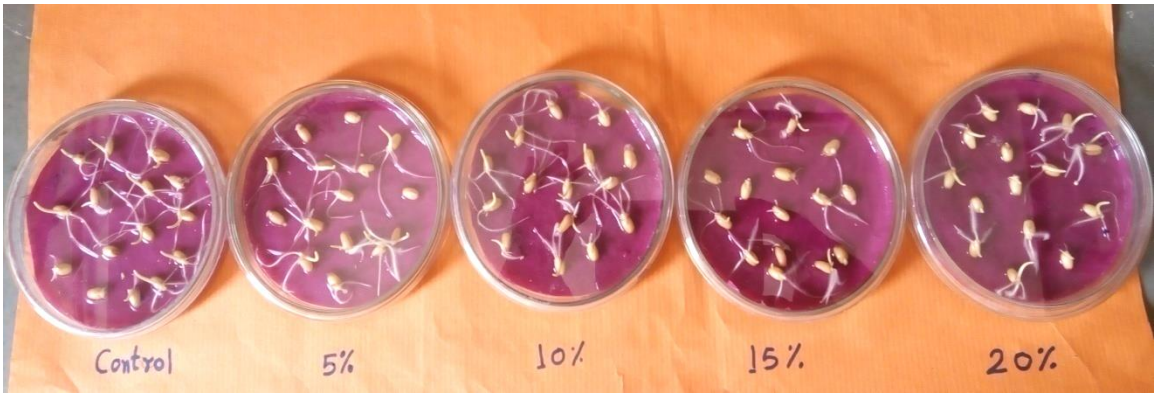
Aqueous leaves extract



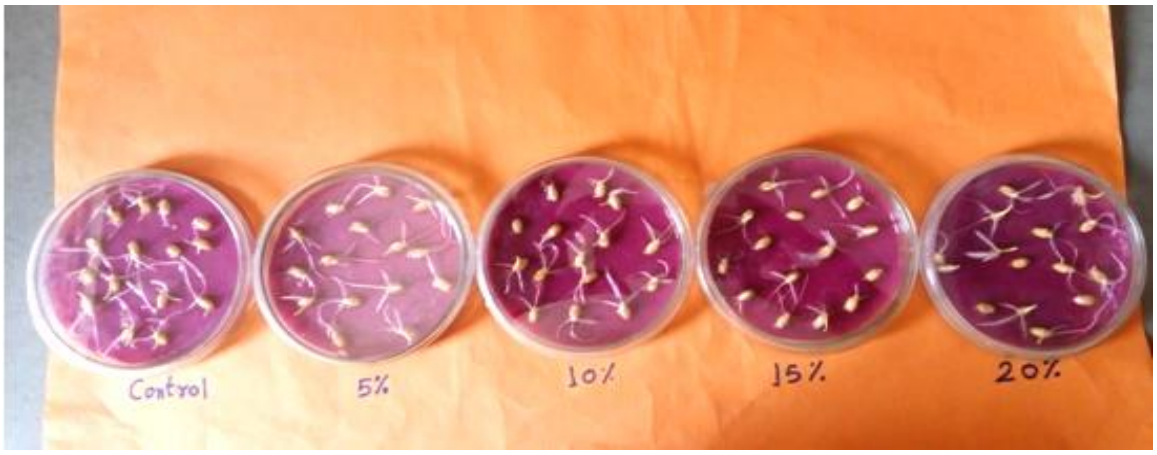
Aqueous flower extract

Plate- 4 : Effect of methanol extract of stem, leaves and flowers of *Pascalia glauca* Ortega. on wheat seed germination growth after 72 hrs.

Methanol Stem extract



Methanol leaves extract



Methanol flower extract

Plate- 5 : Effect of aqueous extract of stem, leaves and flowers of *Pascalia glauca* Ortega. on groundnut seedling after 72 hrs.

Aqueous stem extract

Aqueous leaves extract

Aqueous flower extract

Control **5%** **10%** **15%** **20%**

Plate-6 : Effect of methanol extract of stem, leaves and flowers of *Pascalia glauca* Ortega on groundnut seedling after 72hrs.

Methanol stem extract

Methanol leaves extract

Methanol flower extract

Control **5%** **10%** **15%** **20%**

2. Seedling growth :

2.1 Wheat : Root length

The gradual reduction has been observed in root length after 120 hours in all tested concentrations depicted in Table-4 and Plate- 7 and 8. The aqueous leaves extract has remarkable reduced root length than in stem and flower extracts. The highest root length inhibition (0.9cm) was recorded in 20% aqueous leaves extract and lowest in 5% aqueous stem extract (8.4cm). The degree of reduction was fast in the aqueous leaves extract from 15% (3.9cm) to 20% (0.9cm) followed by 10% (4.6cm) and 5% (6.5cm).

Aqueous stem extract in 5% (8.4cm); 10% (5.7cm); 15% (4.5cm) and 20% exhibited 1.1cm root length. In higher 20% concentration of aqueous stem (1.1cm) and leaves (0.9) showed nearly similar suppression of the wheat root length seedling. The aqueous flower extract recorded gradual reduction in root length from 5% (7.4cm); 10% (6.6cm); 15% (4.7cm) to 20% (2.5cm).

The methanol extract of leaves recorded maximum reduction (2.1cm) of root length than in stem extract (3.3cm) and flower extract (3.4cm) at higher concentration 20%. There is similar reduction observed in stem and flower methanol extract.

Methanol extract of stem has recorded gradual decline in root length at 5% (8.1cm); 10% (7.4cm) and 15% have 5.1cm. In methanol leaves extract root length was suppressed from 5% (6.2cm); 10% (5.5cm); 15% (4.4cm) to 20% (2.1cm). Methanol extract of flower showed nearly similar reduction in 5% (7.6cm) and10% (7.1cm) while 15% concentration has 5.4cm and 3.4cm in 20%.

The remarkable damage has been found in the root length of wheat seedling in the aqueous leaves extract than methanol extract. The greatest reduction in the length of root i.e. 11.42fold was observed at higher concentration (20%) of aqueous leaves extract while stem extract damaged 9.14fold and flower extract comparatively less i. e. 4.28fold in comparison to the control.

Methanol extract of stem has shown deduction of 2.81fold root length, leaves extract have 4.35fold and flower extract with 2.74fold at higher concentration (20%) as comparable to the control. Similar to that of aqueous extract, methanol leaves extract also have maximum reduction in the root length.

Comparable to methanol extract, aqueous extract of stem, leaves and flower has more and strong damaging effect on root length of wheat seedling. Furthermore,

all the concentrations of aqueous and methanol extracts of stem, leaves and flower had showed the remarkably significant results of root length reduction at $P < 0.01$ in wheat seedling.

2.2 Wheat : Shoot length

Similar trend of reduction has observed in shoot length reduction as that of root length at higher concentrations. Aqueous leaves extract strongly subdued shoot length by 0.9cm at 20% concentration followed by 3.1cm in 15% concentration; like this, aqueous stem extract 1.4cm in 20% and 4.9cm in 15% concentration while aqueous flower extract showed 1.9cm in 20% and 4.1cm in 15%. Remaining concentrations of stem, leaves and flower aqueous extracts from 5% and 10% recorded gradual decrease in seedling shoot length.

Sudden reduction in shoot length of wheat seedling has observed in methanol leaves extract from 15% (4.2cm) to 20% (2.6cm) concentration. The methanol extract of stem lowered shoot length from 5% (6.3cm); 10% (5.2cm); 15% (4.3cm) and in 20% have more reduction by 2.56cm. Methanol leaves extract in 5% (5.8cm); 10% (5.7cm) and in 15% recorded as 4.2cm and 20% with 2.6cm. Flower methanol extract showed gradual decline in the length of shoot from 5% (6.0cm); 10% (5.9cm); 15% (4.8cm) and in 20% with 2.2cm.

There has been significant reduction in the shoot length of wheat seedling at 20% higher concentration of aqueous stem extract nearly 6.93fold, leaves extract 10.52fold while in flower extract with 4.96fold in comparison to that of control. The highest reduction in the shoot length has been put on the record i.e. 10.52 fold in the aqueous leaves extract than the stem and flower extract. The sequence of shoot length reduction in the series of leaves > stem > flower.

Methanol stem extract showed shoot length reduction about 3.37fold, leaves extract with 3.18fold while flower extract have 3.75fold at higher concentration (20%).

All the concentrations of aqueous and methanol extract of stem, leaves and flower have remarkable significant results at $P < 0.01$.

2.3 Groundnut : Root length

The effect on root and shoot length of the groundnut seedling has declined when treated with various concentrations of stem, leaves and flower aqueous and methanol extract depicted in Table- 4 and Plate- 9 and 10. The lowest length (2.2cm) was recorded at higher concentration (20%) of aqueous leaves extract while maximum length (8.1cm) from the 5% concentration of aqueous stem extract. Present Table showed noticeable reduction from the 10% to 20% concentration while insignificant at 5% concentration as well as slow and negligible reduction was seen from 5% and 10% concentration. At the same time, effect of aqueous stem extract and flower extract showed similar results at 20%. The greatest reduction of root length (0.5cm) was determined in higher concentration (20%) of methanol leaves extract.

Aqueous stem extract showed gradual reduction in root length of groundnut in 5% concentration (8.1cm.) followed by 10% (7.4cm), 15% (5.3cm) and 20% found 3.6cm. Aqueous leaves extract have greater effect in reduction of root length and determined 2.2cm at 20% concentration then trailed at 15% (4.4cm), 10% (5.5cm) and 5% (6.2cm). Aqueous extract of flower slowly reduced root length from 5% (7.4cm), 7.1cm in 10%, 5.7cm in 15% and 3.6cm at 20% concentration.

Methanol extract of stem observed gradual repression in the length of root from 5% (5.0cm), 4.4cm in 10%, 3.4cm in 15% to higher concentration 20% with 1.8cm. Methanol leaves extract showed gradually reduced from 5% (5.6cm), 5.4cm in 10%, 3.8cm in 15% and maximum in 20% (0.5cm) concentration. Methanol extract of flower decreased 6.4cm in 5%; 4.9cm in 10%; 3.7cm in 15% and 2.2cm in 20% concentration.

Aqueous extract of leaves determined 4.16fold reduction in the root length of groundnut seedling than stem (2.62fold) and flower (2.63fold) which was more or less identical at 20% concentration. It indicates that leaves extract was affect almost doubled and created more negative effect on the root length than the stem and leaves extracts.

Methanol extract of leaves showed 19fold decline in the reduction of seedling root length than the control and in comparison to this, methanol stem extract had 5.27fold and flower extract 4.31fold reduction.

2.4 Groundnut : Shoot length

The lowest shoot length 1.2cm was recorded in aqueous leaves extract followed by 2.4cm in flower extract and 2.8cm in stem extract. 5% and 10% of aqueous stem extract and flower extract showed negligible reduction of shoot length comparable to control. The lowest 0.4cm shoot length was found in 20% methanol leaves extract which was almost 15.75fold more reduction in the shoot length as compared to control. There was a similar (5.7cm) from 5% aqueous and methanol stem extract as well as 10% aqueous stem and flower extract (5.4cm) have identical shoot length. There were similar negative effect and identical reduction in the shoot length of groundnut seedlings from 10% (3.3cm), 15% (2.8cm) and 20% (1.0cm) concentration of methanol stem and flower extract.

Compared with control (6.3cm), aqueous stem extract showed reduction in shoot length at the 20% concentration (2.8cm) followed by 15% (4.4cm), 10% (5.4cm) and 5% (5.9cm). Aqueous leaves extract greatly affected groundnut seedling shoot length at 20% concentration (1.2cm) it has been lowest length observed in all tested concentration followed by in 15% (3.7cm.), 10% (4.3cm.) and 5% (5.1cm.). Aqueous flower extract in 5% concentration recorded 5.7cm, 10% have 5.4cm; 15% with 4.7cm and in 20% was recorded as 2.4cm shoot length.

Methanol extract of flower showed 5.7cm in 5%; 10% (3.3cm); 2.8cm in 15%and 1.0cm in 20% concentration. Methanol extracts of leaves showed maximum decreased (0.4cm) shoot length at higher concentration (20%) followed by 2.4cm (15%), 3.4cm in 10% and 4.7cm in 5% concentration. Methanol stem extract found to be nearly similar reduction in the shoot length as compared to the methanol flower extract. Maximum decline (1.0cm) shoot length was recorded at 20% higher concentration of methanol extract of stem and flower while it gradually decreased in methanol stem extract from 5% (4.8cm), 10% (3.3cm) and 15% with 2.8cm.

There was about 5.25fold reduction of shoot length in the aqueous leaves extract followed by 2.62fold from the aqueous flower extract and 2.25fold in the aqueous stem extract in comparison to the control.

Methanol leaves extract determined 15.75fold more reduction of shoot length in comparison to the control. Methanol stem and flower extract have similar declined shoot length nearly 6.30 fold to that of control.

3. Biomass of seedlings :

3.1 Wheat : Fresh and dry weight of seedling

The biomass of wheat seedling showed more reduction in the aqueous leaves extract at higher concentration treatment after 120 hours is explicated in Table- 5. Fresh weight of wheat seedlings showed maximum reduction from aqueousleaves extract (0.640mg) than stem (0.725mg) and flower (0.959mg) at higher concentration (20%). Similar results were found in the reduction of dry weight under the treatment of aqueous leaf extract (0.120mg) than stem (0.135mg) and flower (0.174mg). Aqueous extract of flower showed increase dry weight at 5% (0.325mg) and 10% (0.375mg) in comparison to control (0.249mg). There was significant decrease in the biomass of wheat seedling in all tested concentrations. Similarly, methanol extract also found to have similar results and leaves extract have more potentiality in the biomass reduction under treatment.

Progressive declined in fresh weight of wheat seedling with increasing the concentration treatments from 5% to 20%. Aqueous stem extract declined fresh weight from 5% concentration (1.296mg), 10% with 1.079mg and in 15% have 0.828mg fresh weight. Aqueous leaves extract decreased fresh weight in 5% (1.377mg); 10% (0.923mg); 15% (0.692mg) while aqueous flower extract recorded fresh weight in 5% (1.469mg) followed by 10% (1.088mg); and 15% with 0.970mg.

Similar trend of reduction occurs in dry weight while treating with aqueous extract of stem from 5% (0.211mg), 10% (0.204mg), and 15% (0.169mg) to 20% (0.135mg) in wheat seedling. Aqueous leaves extract determined declining trends in dry weight from 5% (0.212mg), 10% (0.210mg), 15% (0.151mg) to 20% (0.102mg) while in aqueous flower extract increased dry weight at 5% (0.325mg) and 10% (0.375mg) in comparable to control (0.249mg) but further at 15% concentration it decreased with 0.206mg and 20% with 0.174mg.

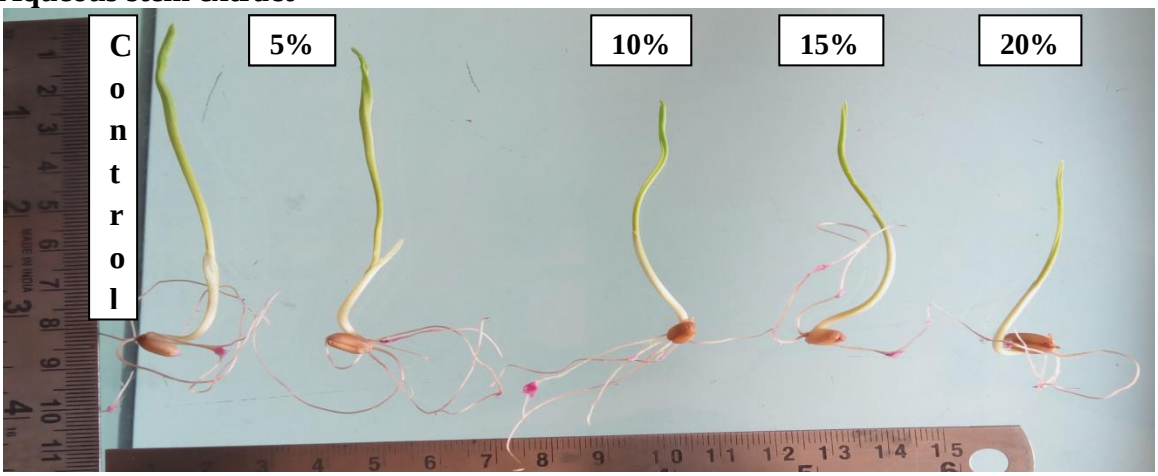
Table - 4 : Effect of aqueous and methanol extracts of stem, leaves and flowers of *Pascalia glauca* Ortega. on wheat and groundnut seedling growth at 120 hrs. (cm)

Plant part extract	Seedlings growth (cm)								
	Conce-ntratio-ns	5%		10%		15%		20%	
	Control	Aqueous	Methanol	Aqueous	Methanol	Aqueous	Methanol	Aqueous	Methanol
Wheat seedling Root length (cm)									
Stem	10.8 ±0.06	8.4** ±0.15	8.1** ±0.07	5.7** ±0.11	7.4** ±0.10	4.5** ±0.04	5.2** ±0.11	1.1** ±0.03	**3.3**** ±0.25
Leaves		6.5** ±0.06	6.2** ±0.07	4.6** ±0.04	5.5** ±0.11	3.9** ±0.10	4.4** ±0.14	**0.9**** ±0.03	**2.1**** ±0.08
Flower		7.4** ±0.06	7.6** ±0.22	6.6** ±0.03	7.1** ±0.35	4.7** ±0.06	5.4** ±0.33	2.5** ±0.09	**3.4**** ±0.13
Shoot length (cm)									
Stem	9.6 ±0.27	7.3** ±0.46	6.3** ±0.40	6.3** ±0.16	5.2** ±0.17	4.9** ±0.20	4.3** ±0.14	1.4** ±0.23	2.5** ±0.22
Leaves		5.6** ±0.20	5.8** ±0.86	4.3** ±0.12	5.7** ±0.57	3.1** ±0.21	4.2** ±0.12	**0.9**** ±0.11	**2.6**** ±0.12
Flower		6.6** ±0.18	6.0** ±0.29	5.3** ±0.22	5.9** ±0.63	4.1** ±0.32	4.8** ±0.09	1.9** ±0.21	2.2** ±0.17
Groundnut seedling Root length (cm)									
Stem	9.5 ±0.56	8.1* ±0.55	5.0** ±0.54	7.4** ±0.94	4.4** ±0.31	5.3** ±0.39	3.4** ±0.30	3.6** ±0.37	1.8** ±0.31
Leaves		6.2** ±0.32	5.6 ±0.19	5.5** ±0.23	5.4* ±0.15	4.4** ±1.12	3.8** ±0.67	**2.2**** ±0.82	**0.5**** ±0.74
Flower		7.4** ±0.36	6.4 ±0.19	7.1** ±0.31	4.9** ±0.87	5.7** ±0.27	3.7** ±0.78	3.6** ±0.40	2.2** ±0.52
Shoot length (cm)									
Stem	6.3 ±0.88	5.9 ±0.45	4.8 ±0.33	**5.4** ±0.71	**3.3**** ±0.25	4.4** ±0.22	**2.8**** ±0.46	2.8** ±0.43	**1.0**** ±0.63
Leaves		5.1 ±0.66	4.7 ±0.11	4.3** ±0.37	3.4** ±0.19	3.7** ±0.33	2.4** ±0.25	**1.2**** ±0.82	**0.4**** ±0.60
Flower		**5.7** ±0.35	**5.7** ±0.45	**5.4** ±0.71	**3.3**** ±0.25	4.7** ±0.25	**2.8**** ±0.46	2.4** ±0.43	**1.0**** ±0.63

One way Anova: Tukeys HSD Post-hoc Test Inference. **Significant at P < 0.01. *Significant at P < 0.05. Otherwise insignificant result. ±Standard deviation.

Plate- 7 : **Effect of aqueous extracts of stem, leaves and flowers of *Pascalia glauca* Ortega on wheat seedlings growth after 120 hrs.**

Aqueous stem extract



Aqueous leaves extract

Aqueous flowers extract

Plate- 8 : **Effect of methanol extracts of stem, leaves and flowers of *Pascalia glauca* Ortega. on wheat seedlings growth after 120 hrs.**

Methanol stem extract

Methanol leaves extract

Methanol flowers extract

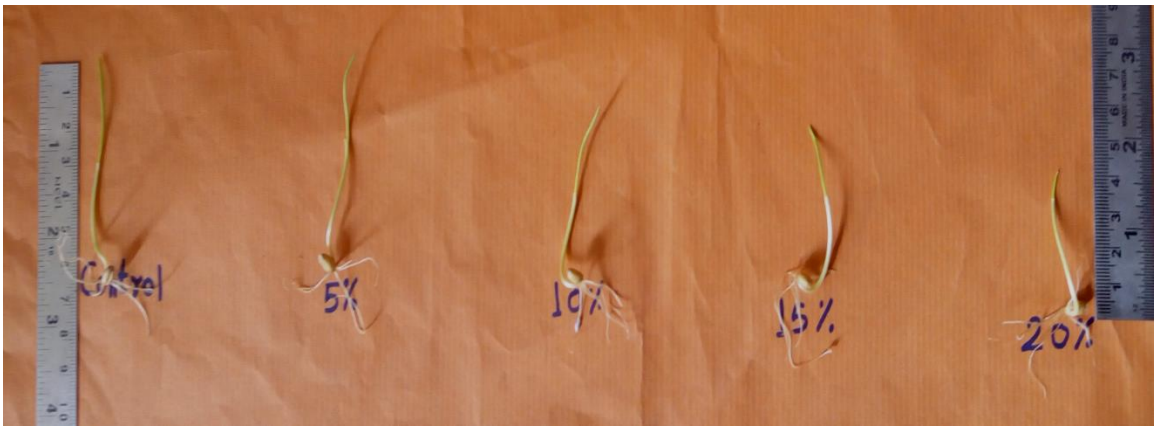


Plate- 9 : **Effect of aqueous extracts of stem, leaves and flowers of *Pascalia glauca* Ortega. on groundnut seedling after 120 hours.**

Aqueous stem extract

Aqueous leaves extract

Aqueous flowers extract

Plate- 10 : **Effect of methanol extracts of stem, leaves and flowers of *Pascalia glauca* Ortega. on groundnut seedling after 120 hours.**

Methanol stem extract

Methanol leaves extract

Methanol flowers extract

Maximum reduction in fresh weight in methanol extract of leaves (0.651mg) has been recorded than the stem extracts (0.735mg) and flower extract (0.713mg) comparable to the control in higher concentration (20%). The 15% concentration of stem (0.868mg) and flower (0.808mg) has been recorded nearly the same. The methanol extract of stem showed decline in the fresh weight from 5% (1.366mg) to 10% (0.931) while leaves extract recorded 1.120mg in 5%, 0.888mg in 10% and 15% have 0.770mg. Methanol extract of flower maximum decreased the fresh weight (0.713mg) from 20% concentration treatment followed by 15% (0.808mg) while in 10% (1.023mg) and 5% have 1.311mg which was very slow decreasing rate.

The decreased dry weights were recorded in stem methanol extract (0.107mg) followed in flower with 0.108mg and leaves extract treatment accumulated 0.111mg at 20% higher concentration as compare to control. Methanol extract of stem reduced dry weight with increase in treatment from 5% with 0.205mg, 10% (0.187mg) and 15% with 0.179mg while leaves extract recorded 0.203mg in 5%, 0.174mg in 10% and 0.152mg in 15%. Methanol extract of flower had minimal effect on dry weight at 5% (0.210mg) as compared to control and then depletion at 10% (0.170mg) and in 15% (0.143mg).

Fresh weight of wheat seedlings in aqueous extract of leaves recorded more than doubled i.e. 2.66fold decrease followed by stem extract 2.34 fold and flower extract count down at 1.77fold in comparison to the control at 20% concentration. The severity in reduction in the fresh weight of wheat seedling as leaves > stem > flower that has been observed which notifies that leaves are the important source of allelolpathic potentiality.

The methanol leaves extract determined more than doubled reduction in fresh weight by 2.61fold, flower extract with 2.38fold and stem extract have 2.31fold in the higher concentration (20%) within all tested extract.

Dry weight of wheat seedlings in aqueous extract of leaves has been greater decrease (2.44fold) in comparison to control where aqueous stem extract decreased 1.84fold and aqueous flower extract 1.43fold.

Methanol extract of stem decreased 2.32fold, leaves extract 2.26fold and flower extract have 2.30fold declined the dry weight of wheat seedlings in comparison to control. Methanol stem and flower extract was similar decrease in dry weight.

3.2 Groundnut: Fresh and dry weight of seedlings

The aqueous and methanol extract at different concentration (5, 10, 15 and 20%) from various parts of *Pascalia glauca* stem, leaves and flower extracts treated against groundnut seeds showed the expected negative impact on fresh weight and dry weight is presented in Table-5. The higher reduction in fresh weight was determined in 20% concentration of aqueous (4.313mg) and methanol (3.470mg) extract of leaves. Aqueous stem extract from 5% and 10% was found nearly similar reduction of fresh weight as well as from 10% aqueous leaves and flower extract. Further in 15% methanol extract of leaves and flower also found nearly similar reduction in fresh weight. There was slow reduction has been observed in lower concentration of methanol extract from 5% to 10% concentration. All the tested concentrations were seen statistically significant results in the reduction of fresh weight. Leaves extract was more pronounced effect than the others.

Aqueous stem extract decreased fresh weight of groundnut seedlings *viz*. 7.340mg in 5%, 7.423mg in 10%, 5.690mg in 15% while 4.500mg in 20% concentration over to control. The maximum reduction (4.313mg) has been observed when treated with 20% concentration of aqueous leaves extract followed by 5.466mg in 15%, 6.196mg in 10% and 6.786mg in 5% concentration over the control (8.406mg.). Same trend of fresh weight depletion has found in the aqueous flower extract at 5% (7.040mg), 10% (6.156mg), 15% (5.343mg) and 20% concentration (4.763mg).

Methanol extract of stem, leaves and flower also showed gradual decrease from lower 5% to higher 20% concentration. Methanol stem extract decreased concentration fresh weight in 20% (4.046mg) concentration followed by 15% (5.480mg), 10% (6.820mg) and in 5% it was little trailed by 7.363mg over the control (7.783mg). There is noticeable reduction in higher concentration 20% of methanol leaves extract of 3.470mg followed by 15% (4.640mg), 10% (6.136mg) and5% with 6.770mg. Methanol flower extract gradually suppressed the fresh weight from 5% (6.710mg), 10% (5.156mg), and 15% (4.643mg) to 20% (3.863mg).

Maximum decrease in dry weight of groundnut seedlings has been observed (0.933mg) in aqueous leaves extract at higher concentration (20%), followed by 1.013mg in stem extract and 1.030mg in flower aqueous extract treatment. 5% and 10% stem and flower extract have nearly similar response in reduction of dry weight

with 1.666mg and 1.606mg and 1.680mg and 1.570mg respectively. Leaves aqueous extract declined dry weight from 5% (1.560mg) and 10% (1.513mg) dry weight.

Methanol extract of stem in 5% concentration dry weight of groundnut weighed 1.410mg, 1.2033mg in 10%, 1.246mg in 15% and 20% concentration have 1.080mg. Significant reduction (0.880mg) observed in methanol leaf extract in 20% concentration followed by 1.133mg in 15%, 1.150mg in 10% while 5% concentration have 1.480mg dry weight of groundnut seedlings as compare to control. Flower extract recorded gradual reduction in dry weight with increase in concentration of treatment, 5% (1.553mg), 10% (1.776mg), 15% with 1.083mg and 1.006mg at 20% concentration.

Aqueous extract of stem has showed the decrease in fresh weight of groundnut seedling by 1.88fold over the control, 1.94fold in leaves extract and 1.76fold in flower extract at the higher concentration of extract (20%).

Similarly, methanol extract of leaves greatly suppressed fresh weight in the 20% concentration that determined 2.24fold over the control followed by flower (2.01 fold) and stem (1.92fold).

Methanol extract of leaves and flower has been recorded maximum reduction in the fresh weight in comparable to the aqueous extract of leaves and flower while stem methanol extract had not much difference in the reduction.

Dry weight of groundnut seedlings has been reduced at higher concentration 20% by 2.43fold in aqueous leaves extract followed by aqueous extract of stem (2.24fold) and flower extract with 2.20fold.

Methanol extract of leaves highly decreased dry weight and determined about 2.17fold while methanol stem extract have 1.77fold and flower extract with 1.90fold comparable to the control. Statistically, all extracts showed significant results.

Table- 5 : Effect of aqueous and methanol extracts of stem, leaves and flowers of *Pascalia glauca* Ortega on fresh and dry weight of wheat and groundnut seedlings at 120 hrs. (mg)

Plant part extract	Biomass (mg)								
	Concentrations	5%		10%		15%		20%	
	Control	Aqueous	Methanol	Aqueous	Methanol	Aqueous	Methanol	Aqueous	Methanol
Wheat seedlings Fresh weight (mg)									
Stem	1.702 ±0.201	1.296* ±0.160	1.366** ±0.017	1.079** ±0.064	0.931** ±0.020	0.828** ±0.045	0.868** ±0.020	0.725** ±0015	0.735** ±0.047
Leaves		1.377* ±0.165	1.122** ±0.024	0.923** ±0.018	0.888** ±0.028	0.692** ±0.036	0.770** ±0.022	0.640** ±0.025	0.651** ±0.031
Flower		1.469 ±0.066	1.311** ±0.073	1.088** ±0.020	1.023** ±0.032	0.970** ±0.026	0.808** ±0.049	0.959** ±0.030	0.713** ±0.040
Dry weight (mg)									
Stem	0.249 ±0.011	0.211** ±0.002	0.205** ±0.005	0.204** ±0.003	0.187** ±0.015	0.169** ±0.007	0.179** ±0.003	0.135** ±0.010	0.107** ±0.017
Leaves		0.212** ±0.006	0.203** ±0.013	0.210** ±0.005	0.174** ±0.009	0.151** ±0.012	0.152** ±0.007	0.102** ±0.009	0.111** ±0.014
Flower		0.325** ±0.015	0.210* ±0.018	0.375** ±0.009	0.170** ±0.008	0.206* ±0.019	0.143** ±0.007	0.174** ±0.007	0.108** ±0.013
Groundnut seedlings Fresh weight (mg)									
Stem	8.406 ±0.179	7.340** ±0.325	7.363 ±0.227	7.423** ±0.325	6.820** ±0.070	5.690** ±0.227	5.480** ±0.329	4.500** ±0.271	4.046** ±0.205
Leaves		6.786** ±0.283	6.770** ±0.045	6.196** ±0.141	6.136** ±0.037	5.466** ±0.478	4.640** ±0.253	4.313** ±0.410	3.470** ±0.554
Flower		7.040* ±0.130	6.710** ±0.385	6.156** ±0.922	5.156** ±0.020	5.343** ±0.330	4.643** ±0.238	4.763** ±0.400	3.863** ±0.061
Dry weight (mg)									
Stem	2.273 ±0.107	1.666** ±0.215	1.410 ±0.250	1.606** ±0.090	0.870** ±0.476	1.113** ±0.100	1.246* ±0.070	1.013** ±0.015	1.080* ±0.060
Leaves		1.560** ±0.395	1.480** ±0.153	1.513** ±0.115	1.150** ±0.045	0.996** ±0.115	1.133** ±0.125	0.933** ±0.060	0.880** ±0.088
Flower		1.680 ±0.286	1.553** ±0.106	1.570* ±0.415	1.176** ±0.061	1.223** ±0.120	1.083** ±0.064	1.030** ±0.052	1.006** ±0.005

*Values are mean of three determinations.
One way Anova: Tukeys HSD Post-hoc Test Inference. **Significant at $P < 0.01$.

*Significant at $P < 0.05$. Otherwise insignificant result. ±Standard deviation.

Discussion:

Potentiality of various parts of *Pascalia glauca* Ortega after bioassay analysis observed varied response in aqueous and methanol extract. Mostly it was concentration dependent and positively detrimental from higher concentration of stem, leaves and flower extracts in both media i. e. aqueous and methanol on seed germination, seedling growth and fresh and dry weight of wheat and groundnut.

Seed germination is an important event in the life cycle of the plant that determines survival potential. The capacity of seed germination lowers on the release of substances present in surrounding and that also influence the seedlings growth. Concentration of allelochemicals, developmental stage of plant and environmental conditions affect the sensitivity of target species (Inderjit and Weiner, 2001 and Inderjit, 2001). The inhibition of seed germination and reduction in seedlings growth has affected towards the compounds present or escaped from the plant parts and those also disturb the metabolic activity in the test seeds which further results ultimately in the loss of crop productivity. The degree of decrease mostly depends on the amount of allelochemicals discharged from the weed plant parts that gets mixed up in crop environment; affect them and hamper the seedlings growth and development. Weed plants produce an array of chemicals with different bioactivities and a single compound alone is rarely responsible for the complicated biological process like seed germination (Inderjit, 2006).

Aqueous leaves extracts of *Helianthus annus* have reported to inhibit the germination and seedling growth of wheat in treated seedlings (Ghafar *et al.,* 2000). Tefera (2002) and Maharjan *et al.* (2007) also noticed that leaves are the most potent parts exhibiting allelopathic interactions. Allelochemicals probably interfere with enzymes involved in mobilization of nutrients necessary for germination, thereby affecting cell division consequently reducing the elongation of seedlings (Ashrafi *et al.,* 2008). Aqueous extracts of *Jatropha curcas* inhibited the seedling growth of *Capsicum annum* and degree of inhibition increases with increasing concentrations of extract (Rejila and Vijaykumar, 2011).

The explication and scrutiny of results showed strong apprehension of seed germination and seedling growth dynamics of wheat under influence of different concentration treatments of *Pascalia glauca* Ortega parts. Suppression in germination and delayed in growth due to its allelopathic compounds released, mostly they are water soluble and that are encroaching with basic skeleton and functions of tested

species (Duke and Dayan, 2006) and acting additively or synergistically (Einhellig, 1996). The degree of inhibition decreases with increasing concentration in extracts that exhibiting a strong reciprocal correlation with dose response relationship.

Ghodake *et al*. (2012) pointed out that *Euphorbia genuculata* and *E. maicrophylla* inhibited seed germination of wheat. Root, shoot elongation and biomass accumulation were significantly retarded in wheat by applying the aqueous extract of *E. dracunculaoides* Lam. (Tanveer *et al*., 2012). All studied weeds including *Amaranthus hybridus, Parthenium hsterophorus, Datura stramonium* and *Argemone maxicana* showed the reduction of seed germination; reduction in radical and plumule length as well as biomass of wheat (Gella *et al*., 2013). Nasira *et al*. (2013) experimented that when *Asphodelus tenuifolius, Euphorbia hirta, Fumaria indica* were used in the form of powder with different amounts mixed with uniform amount of soil and in this when seeds were grown to assess the allelopathic effect on wheat, they found that *Asphodelus tenuifolius* considerably increased the percentage and rate of germination of wheat, while *Euphorbia hirta* and *Fumaria indica* were reduced seed germination, fresh and dry weight of wheat.

Golzardi *et al*. (2014) found that *Cynanchum acutum* L. on increasing the concentration of aqueous extract, decreasing germination percentage, hamper the radicle and shoot length of *Triticum aestivum* L. Wasim Ahmad *et al*. (2014) stated that, the aqueous extract of *Avena fatula* showed significant allelopathic effect on seed germination and growth parameters of wheat. *Moringa* leaf, bark and root extract reduced seed germination and plumule length of wheat (Noorshaila Sharmin, 2014).

Mustapha and Rahimatu (2015) observed that leaf and seed extract of the nutgrass (*Cyperus tuberosus*) at the higher the concentration had the stronger inhibitory effect on germination of cowpea (*Vigna unguiculata* (L.) Walp.) and also found that the leaves extract had more allelopathic effect than seed extract. Salgude *et al*. (2015) has been studied the allelopathic effect of *Cuscuta reflexa* Roxb. on seed germination, growth, root-shoot length and biomass of wheat that caused both the inhibitory as well as stimulatory effect. Aqueous extract *Salvia plebia* R. Brown strongly affected the germination, plumule and radical growth, chlorophyll content and fresh and dry weight of *Zea mays* var. 30-25 Hybrid, *Triticum a**e**stivum* var. Pirsabak-04 and *Sorghum bicolor* L. (Husna *et al*., 2016). Ravlic, *et al*. (2016) demonstrated the experiment to assesse allelopathic effect of creeping thistle (*Cirsium arvense*), field poppy (*Papaver rhoeas*), scentless mayweed (*Tripleurospermum*

inodorum), redroot pigweed (*Amaranthus retroflexus*), black nightshade (*Solanum nigrum*) and Johnson grass (*Sorghum halepense*) extracts on test crop wheat; and found significant negative effect of weed extracts on seed germination, seedling growth and reduced biomass. Joshi and Joshi (2016) found that the allelopathic activities of *Alternanthera sessilis* had more positive impact on seed germination with decrease in the length of plumule of wheat. Radicle and plumule length of *Triticum aestivum* L. showed declining trend with increasing doses of aqueous extract of the weeds *Chenopodium album, Avena fauta* and *Phalaris minor* at the same time dry weight was drastically decreased gradually with increasing concentration of extract of above weeds (Saira *et al*., 2017). Lehoczky *et al*. (2017) stated that water extract of *Silybum marianum* (L.) Gaertn. decreased fresh weight, dry weight and length of the radicle and shoot of wheat (*Triticum aestivum* L.) at higher concentrations.

Prasad and Srivastava (1991) found that the extracts of *Echinochloa crus-galli* (barnyard grass) are inhibitory to seed germination and seedling growth of groundnut. Ghosh *et al*. (2000) investigate the aqueous plant extract of *Aegeratum conyziodes* and *Lantana camera* caused significant reduction in groundnut germination and root and shoots length; and delayed germination by 2-3 days. Jennings and Nelson, (2002) stated that leaf extract of *Cyprus tuberosus* inhibited seed germination, seedling growth and root and shoot growth.

Lawan *et al*. (2011) demonstrated the allelopathic influence of *Eucalyptus citriodora, E. camaldulensis, E. globules* aqueous leaf extract when tested against the *Arachis hypogaea* seed showed inhibitory effect on seed germination and root elongation. Femina *et al*. (2012) reported that the aqueous leaves extract of *Tridax procumbens* L. had inhibitory effect on germination, root, shoot elongation and fresh and dry weight of leguminous crops. Rose and Anita (2012) reported that *Euphorbia hirta* L. extract had negative effect on germination and growth in groundnut. Pytotoxicity of allelochemicals present in the leaf extract of *Excoecaria agallocha* L. may cause synergistic activity on retardation of growth of groundnut (Kavitha *et al*., 2012). Parthasarthi *et al*. (2012) concluded that, groundnut seeds not germinated in higher concentration of leaf extract of *Parthenium hysterophorus* L. Hossain (2012) examined in his experiment conducted on the plant debris of siam weed (*Chromolaena odorata)* at the rate of 1gm debris per 100 gm of soil was used in pot experiment, it reduced the seedling emergence of rice, mustard, groundnut and chickpea by 16.44, 54.93, 52.25 and 26.73%, respectively.

Sarita and Sreeramulu (2013) stated that *Celosia argentea* L. leaf extract with increasing concentration have more inhibitory potentiality on seed germination and seedling growth of groundnut. Kaveriammal *et al*. (2013) stated that at the 20 and 25% aqueous extract of *Lawsonia inermis* showed maximum inhibition of seed germination of green gram, black gram and groundnut seeds. Usha *et al*. (2013) tested water extract of six dominant weeds, *viz*. *Apilia Africana, Emilia sonchifolia, Crotalaria retusa, Chromolaena odorata, Panicum maxicana* and *Cyperus esculentus* against test crops namely maize, melon, okra, cow pea, soybean and groundnut and they found that the seed germination was greatly inhibited but *C. esculentus* had highest inhibitory effect than the other tested weeds. Prasad *et al*. (2014) advocated that leaf leachates of *Parthenium*, *Hyptis* and *Tridax* treated on the black gram (*Vigna mungo*), all the leachates at 5.0% concentration, significantly reduced seed germination, root and stem length and dry matter. Four weed species, *Cyperus esculentus* (Della), *Axonopus compressus* (Itsit), *Convulvulus arvensis* (Lehli) and *Parthenium hysterophorus* were tested against rice (*Oryza sativa* L.) and showed significant detrimental effect on germination rate, plumule length, radicle length, fresh weight and dry weight of rice seedlings (Muhammad *et al*., 2014).

Ghetiya (2014) demonstrated the allelopathic effect of *Trachyspermum ammi* and *Mentha arvensis*on *Arachis hypogaea* (cv.G-6 and cv. G-20) seed and found that they had more inhibitory effect on the seed germination and growth as well as biomass of *A.hypogaea* cv.G-20 than cv. G-6. Ghanuni *et al*. (2015) advocated that *Arachis hypogaea* seed germination was significantly reduced to more than 50% in the pots treated with ground leaves of *Eucalyptus camaldulensis* as well as root, shoot length and dry weight of peanut drastically declined in the higher treatment of *Eucalyptus* ground leaves. The findings outcome from experiment conducted by Mujawar *et al*. (2016) showed weed parts of *Pascalia glauca* interpreted the reduction in the seed germination and seedling growth of *Arachis hypogaea* L. with progressively increase in concentration of leaves, stem and flower aqueous extract of it. Aqueous extract of *Parthenium hysterophorus* L. root and aerial parts were tested on groundnut seeds for their allelopathic effect on seed germination and seedling growth at various concentrations in laboratory condition and observed that, seed germination of groundnut (*Arachis hypogaea* L.) was highly affected and their growth of radicle and plumule was decreased with increasing concentration of extract as compared to that of control (Shinde, 2016). The treatment of stem, leaves and root at

different concentrations of the extracts of Siam weed (*Chromolaena odorata*) significantly affected the seed germination, length of root and shoot, growth as well as dry matter accumulation of groundnut (*Arachis hypogea*) and the average percent inhibition was recorded 24.7% (Karim *et al*., 2017). Neha *et al*. (2017) stated that effects of dichloromethane and double distilled water soluble fractions of *Echinochloa colona* L. and *Cyperus iria* L. root and aerial part extracts reduced seed germination and suppressed early seedling growth of rice and soybean. Novak *et al*. (2018) found that extract of *Abutilon theophrasti* Med., *Ambrosia elatior* L., *Datura stramonium* L., *Xanthium strumarium* L., *Ailanthus altissima* (Mill.) Swingle., *Amorpha fruticosa* L., *Reynoutria japonica* Hout. and *Solidago gigantea* Aiton. have great inhibitory potentiality to inhibited the seed germination, radicle and plumule length of oat (*Avena sativa* L.), oilseed rape (*Brassica napus* subsp. *oleifera*) and sunflower (*Helianthus annuus* L.). Further they stated that the allelopathic effect of perennial species are maximum than the annual species. Hamed *et al*. (2018) revealed that *Datura stominum* aqueous leaf extract inhibited seed germination and retarded the activities of the f enzymes of *Trigonell foenum-graecum* and *Lipedium sativum*. Rita *et al*. (2018) experimented three weed extract on maize seed and showed an inhibitory effect of plant extracts of *Amaranthus retroflexus* that inhibited the germination, negative effect on the shoot length and weight, but positive effect on the root length and weight of maize while *Datura stramonium* extracts showed declining the shoot and root length of germinating maize but *Panicum miliaceum* extract did not show any significant difference stimulatory or inhibitory effects. Our results in present investigation are in the line of well documented above researchers findings.

The higher concentration levels of aqueous extract of *Pascalia glauca* Ortega (*Wedelia glauca*) inhibit the seed germination and early stages of growth of tomato, cucumis and radish (Sobrero *et al*., 2004). Our present work supports their findings that *P. glauca* has a significant allelopathic detrimental potential that affects the seed germination and seedlings growth in both wheat and groundnut. Also, leaf extract has more allelopathic detrimental potentiality than stem and flower extract. Aqueous extract have more potent source in the inhibition of seed germination than that of methanol extract. In seedling growth and biomass accumulation, the detrimental effect of aqueous and methanol extract was not stronger indicating the slow response.

Pascalia weed is newly introduced to India and in particular Maharashtra becomes naturalized. It is one of the major weed now in studies region. The findings

of leaves, stem and flower extract in aqueous and methanol media indicates its potentiality of solubility. Obviously aqueous extracts are more lethal and can produce more overlasting effect on crops. This has clearly reflected in our experiments. The aqueous extracts of leaves and stem in particular are showing strong inhibiting effect on seed germination. Further, it is indicating slow growth rate of seedlings resulting slowed emergence of radicle and plumule. This is found in delayed seedling growth.

The allelopathic effect of compounds present in leaves, stem and in flowers to some extend are offering the metabolism at seed germination stage and further during emergence of radicle and plumule, as this has resulted in reduction in biomass both fresh weight and dry weight. As there is tendency of high moisture percentage at higher concentrations, this may be due to protective response from test crop by inducing water holding capacity in particular.

Here one should not forget that the composition of aqueous and methanol extract may differ become of solubility of bioactive chemicals accordingly there will be influencing the germination process differently. The response effect will be the real level of further studies.

2. Inorganic constituents :

I. Effect of aqueous and methanol extracts of stem, leaves and flowers of *Pascalia glauca* Ortega. on macronutrients of wheat seedlings:

2.1. Wheat seedlings :

2.1.1 Nitrogen (N) **:**

It has predominant role in different metabolic activities in plants, being the constituents of protein, nucleic acids, nucleotides, phytohormones etc. It has also regulates photosynthesis, carbohydrates metabolism, and biosynthesis of pigments, water use efficiency and secondary metabolites. The influence of allelopathy on mineral nutrition was studied by Buchholtz (1971).

The wheat seedlings when treated with different concentrations of aqueous stem, leaves and flower extract of *Pascalia glauca* Ortega, affects on nitrogen content depicted in Table 6. The nitrogen was elevated from the lower treatment of 5% aqueous stem (0.300), leaves (0.316) and flower (0.376) extract while methanol stem (0.293), leaves (0.310) and flower (0.333) extract. Then it was suddenly inhibited in 10% but again slower in 15% while it becomes rapid reduction in 20% concentrations.

The maximum decreased nitrogen determined in aqueous (0.008) and methanol (0.006) leaves extract. Identical inhibition has been found from 15% aqueous stem and flower extract (0.073).

Aqueous stem extract from 5% concentration increased (0.300) then suddenly decreased from 10% (0.170), 15% (0.116) and maximum from 20% (0.110). Same trend has been determined in aqueous leaves extract from 5% (0.316) promoted while it was start to decreased from 10% (0.176), it suddenly maximum reduced from 15% (0.073) and at higher concentration 20% (0.008) while in the flower extract it responds promoting from 5% (0.376), further it was greatly repressed from 10% (0.186) 15% (0.073) and in 20% (0.056) over control (0.243).

Methanol extract also response promotory activity at lower 5% concentration of stem extract (0.293), leaves extract (0.370) and flower extract determined 0.333. The nitrogen has been reduced gradually in methanol stem extract from 10% (0.146), 15% (0.106) and 20% (0.080). Leaves extract showed decreasing response of the nitrogen content from 10% (0.216) and 15% (0.086) while it was abruptly decreased from 20% (0.006). Methanol flower extract has found inhibition trend from 10% (0.126), 15% (0.093) and in 20% (0.043).

Maximum 30.37fold reductions of nitrogen content was determined from aqueous leaves extract followed by flower extract (4.33fold) and stem extract (2.20fold) in higher concentration (20%) in wheat seedlings.

Methanol leaves extract determined higher decreasing (38.83fold) nitrogen of wheat seedlings while flower extract inhibited by 5.41fold and stem extract by 2.91fold at 20% concentration comparing with control.

2.1.2 Phosphorus (P) :

Phosphorus is a major inorganic component of many metabolically important molecules such as sugar phosphates, nucleotides, nucleic acids, phospholipids and coenzymes (Marschner, 2002). It plays an important role in plant metabolism by supplying energy required for metabolic processes (Lal, 2002). Phosphorus is essential for almost all aspects of plant metabolism including seed germination, photosynthesis, protein formation and flower and fruit formation. Phosphorus plays central role in growth and development of crops.

Phosphorus has been increased from the lower concentration 5% and 10% while it was decreased suddenly from 15% and 20% concentrations of aqueous stem,

leaves and flower extracts. It was increased only from 5% concentration of methanol stem, leaves and flower extract. Similar increased from 5% and 10% aqueous flower extract while decreased from 20% stem and flower extract. Phosphorus was maximum decreased from methanol (0.042) and aqueous (0.063) leaves extract in higher 20% concentration.

Aqueous extract of stem, leaves and flowers of *P. glauca* has determined stimulatory results of phosphorus at lower concentration in 5% 0.266, 0.275 and 0.292; 10% as 0.242, 0.257, and 0.292 respectively over the control (0.223) while at higher 15% phosphorus content was decreased in aqueous stem extract by 0.137, leaves extract with 0.122 and 0.136 in flower extract. 20% concentration showed great reduction in the aqueous leaves extract (0.063) followed by identical decreased from stem extract and flower extract (0.112). Stem and flower extract represented similar inhibition at 20% concentration (Table- 6).

Methanol extract was found stimulatory results only in lower 5% concentration of stem extract (0.274); leaves extract (0.255) and flower extract (0.273). From the 10% to 20% concentration it becomes gradually decreased phosphorus content in all extracts. 10% aqueous stem extract concentration determined 0.152, leaves extract with 0.125 and 0.156 in flower extract. It has been more reduction occurred in 15% leaves extract (0.070), than the stem extract (0.118) and flower (0.119) which was nearly similar. At higher concentration (20%) methanol leaves extract found maximum reduction (0.042) followed by stem (0.094) and flower extract (0.106).

The maximum increment of reduction (3.52 fold) has been occurred in the 20% concentration of aqueous leaves extract while aqueous stem and flower extract was recorded similar reduction (1.97 fold) in the phosphorus of wheat seedlings.

Methanol extract of stem decreased phosphorus content by 2.45 fold, 5.40 fold in leaves extract and 2.17 fold in flower methanol extract over control at 20% concentration.

2.1.3 Potassium (K) :

Potassium is one of the most important mineral, after phosphorus and nitrogen that has been necessary for normal plant growth and metabolism. It is necessary for formation of sugars, starches and protein synthesis and it helps in pod growth (Gore *et al*., 2011), cell division in roots and other plant parts as well as in cell extension

(Lindhauer,1989). It is also required to improve stem rigidity, cold hardiness, enhances flavor and color of fruit and vegetable crops as well as increases oil content. Potassium provides necessary osmotic potential for water uptake by plant cells. It is involved in photo-reduction and photo-phosphorylation, nitrogen turnover, opening and closing of stomata (Pfluger and Mengel, 1972).

5% methanol leaves and stem extract responds the minor promoting activity of potassium as well as both aqueous and methanol stem and flower extract from 5% and 10% concentrations determined increasing the potassium. Maximum reduction has been found in aqueous (0.39) and methanol (0.27) leaves extract at 20% concentration. Potassium was highest increased from the lower treatment (5%) of aqueous (3.32) and methanol (3.06) flower extract. 10% methanol stem and flower extract does not determined significant increment of potassium.

Aqueous extract of stem at lower concentration (5%) has been increased 2.81 and little down in 10% 2.75 but it was promoted over the control (2.58). Same trend followed in the leaves extract from 5% (2.91) and 10% (2.12) and flower extract from 5% (3.32) and 10% (2.95). From the higher concentration of stem extract is abruptly declined in 20% (0.56) followed by 15% (1.23) whereas aqueous leaves extract crushed potassium more from 20% (0.39) and 15% (0.77) over the other extracts. Aqueous flower extract determined gradual reduction from 15% (1.71) and 20% (1.63).

Methanol extract of stem, leaves and flower has been increased potassium content when treated at lower concentration (5%) that determined as 2.74, 2.64 and 3.06 respectively over control and within this flower extract found highest increased (3.06) potassium content of wheat seedlings. 10% concentration of stem and flower extract endorsed by 2.45 and 2.44 while leaves extract reduced (1.34) potassium. From the 15% concentration it becomes decreased in stem extract (1.17), leaves extract (0.68) and flower extract with 1.15. At higher concentration 20% it determined higher inhibition in leaves extract (0.27) followed by stem (0.60) and flower (0.96) extract.

Potassium content was highly decreased (6.56fold) in the aqueous leaves extract over control than the stem extract (4.61fold) and flower extract (1.57fold) at 20% concentration.

Methanol leaves extract determined maximum 8.72fold inhibition of potassium in wheat seedlings than the stem 3.97fold and 2.50fold in flower extract in comparison to control at 20% concentration.

2.1.4 Calcium (Ca) :

Calcium is an essential and important macronutrient content in plants. It is taken up by plants through root system and transported to shoot via the xylem. It regulates many physiological processes (Tuteja and Mahajan, 2007). Calcium has been responsible for cellular stabilization that maintains cell integrity and membrane permeability. It helps in pollen germination and activates number of enzymes for cell division and involved in protein synthesis and carbohydrate transfer. Calcium has been helpful to increase the net absorption of potassium (Ortiz *et al.*, 1994) Calcium is one of the prime elements for functioning as central communication point in overall 'signalling web' and plays key role in signal transduction pathways (Tuteja and Sopory, 2008).

Calcium was increased in 5% aqueous and methanol stem, leaves and flower extract and insignificantly increased from 10% aqueous flower extract while significantly increased from 10% methanol stem extract. Maximum calcium was inhibited in aqueous (0.072) and methanol (0.041) leaves extract.

Calcium content in wheat seedlings increased from the 5% aqueous and methanol stem, leaves and flower as well as from 10% aqueous and methanol extract of stem and flower. 10% aqueous leaves extract treatment, decreased by 0.425. In the 15% concentration it starts to suppress in stem extract (0.272); maximum decreased (0.110) in leaves extract while it was found nearly doubled reduction in flower extract (0.375). In the higher 20% concentration of leaves extract found highly reduction of calcium content (0.072) followed by stem (0.113) and flower (0.218) extract.

Methanol extract has been elevated from 5% concentration in stem extract (0.708); leaves extract (0.694) and flower extract (0.645). Same trends have been observed in the 10% concentration over control.10% methanol stem extract (0.656) leaves extract (0.626) and flower extract (0.621) determined minor increased. 15% concentration showed reduction of calcium in stem extract (0.375); more in leaves (0.102) and flower extract (0.262) found more than triple fold over previous concentration. 20% concentration determined rapidly increased in inhibition of

calcium content in leaves extract (0.041) followed by stem extract (0.112) and flower extract (0.164).

The maximum reduction (8.53fold) of calcium content was found in aqueous extract of leaves followed by 5.94fold in stem extract and 2.83fold in flower extract at 20% concentration in wheat seedlings.

There is huge amount of calcium was declined (15.36 fold) in the methanol extract of leaves than the stem extract (5.60 fold) and flower extract (3.82 fold) at the 20% concentration over to control in the wheat seedlings.

2.1.5 Magnesium (Mg) **:**

Magnesium plays major role in plant mineral nutrition. It is important component of chlorophyll molecule which is a basic component of light reactions of photosynthesis. Magnesium is strongly electropositive and a mobile, divalent element in plant. It serves as a cofactor in most of the enzymes that activate phosphorylation process. Several enzymes involved in carbohydrate metabolism require magnesium as an activator. Magnesium is keeping its vital role in protein synthesis. Mg uptake is influenced by some factors like Mn^{2+}, K^{+}, NH_3 and low pH. Thus, high concentration of Mg^{2+} and K^{+} are essential in the chloroplast and cytoplasm to maintain high pH (6.5 to 7.5) (O' Neal and Joy, 1974).

Magnesium was determined promoting trend when treated against wheat seedlings at lower 5% and 10% concentrations of aqueous and methanol stem, leaves and flower extract of *Pascalia glauca* Ortega. There is more elevation of magnesium in 5% but it was insignificantly increased in10% concentrations from both extract. But after the increase in concentrations of extract from 15% to 20% it was quickly decreased. Maximum inhibition has found from 20% extract of methanol (0.030) and aqueous (0.040) leaves over control. 15% and 20% aqueous leaves and flower extract decreased magnesium suddenly fast, parallel and nearly similar in comparison to 10% concentrations. Insignificant identical increase in magnesium from 10% methanol stem, leaves and flower extract.

The aqueous extract of stem; leaves and flower at lower 5% and 10% concentrations, magnesium has been endorsed 0.366; 0.317 and 0.334 from 5% and 0.284; 0.294 and 0.290 from 10% extract respectively. It was soon reduced in aqueous stem extract from 15% (0.122) and maximum from 20% (0.092) while in leaves extract rapidly reduced from 15% (0.074) and maximum from 20% (0.040)

concentration than the other extract. 15% aqueous flower extracts found 0.073reduction while highest inhibition determined from 20% (0.037) concentration.

Methanol extract heightened magnesium levels of wheat seedlings from 5% stem extract (0.346); leaves extract (0.340) and flower extract (0.371). Same trend has been observed in 10% concentration of stem extract (0.290); leaves (0.290) and flower extract (0.293) that means stem, leaves and flower extract found more or less identical increased in the elevation of magnesium. At the higher concentration it was reduced from 15% (0.172) and 20% (0.082) methanol stem extract while methanol leaves extract was suddenly decreased from 10% concentration (0.074) and maximum reduction at 20% (0.040). Methanol flower extract from 15% concentration reduced by 0.073 and 20% by 0.037 over control.

Aqueous flower extract highly decreased magnesium about 7.10fold after treatment in the 20 % concentration than leaves 3.08fold and 7.62fold stem extract over the control in the wheat seedlings.

9.43fold increments in the reduction of magnesium content from the methanol leaves extract followed by stem (3.52 fold) and flower (3.03 fold) extract.

II. Effect of aqueous and methanol extracts of stem, leaves and flowers of *Pascalia glauca* Ortega. on macronutrients of groundnut seedlings:

2.2 Groundnut seedlings:

2.2.1 Nitrogen (N) :

Nitrogen content of groundnut seedlings have determined decreasing trends from lower (5%) to higher concentrations (20%) when treated with aqueous and methanol stem, leaves and flower extract of *Pascalia glauca*. Maximum nitrogen content was declined at 20% aqueous (1.05) and methanol (0.93) extract of leaves (Table- 7).

Aqueous stem extract from lower concentration of 5% and 10% it has been determined declining activity in nitrogen content by 2.12 and 2.02; leaves extract 1.92 and 1.75 and flower extract with 2.12 and 2.06 respectively over the control (2.19). The higher concentration of extract at 15% and 20% it was decreased by 1.78 and 1.26 in stem extract; leaves extract recorded 1.60 and 1.05 while the aqueous extract of flower determined 1.81 and 1.57 respectively in comparisons to lower concentration and control.

Methanol extract of stem, leaves and flower recorded decreasing results from 5% in groundnut seedlings as 2.17; 2.87 and 2.05 respectively over the control. The reduction of nitrogen has been observed gradually in stem extract from 10% (1.96), 15% (1.69) to 20% (1.38). Leaves extract declined successively in 10% (1.55) and 15% (1.40) but it has been suddenly decreased in higher concentration (20%) was determined 0.93. Flower extract at 10% concentration has been decreased by 1.87; 15% with 1.70 and at 20% have 1.50.

Aqueous extract of stem was reduced by 1.73fold nitrogen content of groundnut seedling, 2.08fold in leaves extract and 1.39fold in flower extract in comparison to control at 20% concentration.

Table- 6 : Effect of aqueous and methanol extract of stem, leaves and flowers of *Pascalia glauca* on macronutrient of wheat seedlings (mg g^{-1} dry weight).

Plant part extract	Wheat seedlings								
	Nitrogen (N)								
	Concentrations	5%		10%		15%		20%	
	Control	Aqueous	Methanol	Aqueous	Methanol	Aqueous	Methanol	Aqueous	Methanol
Stem	0.243 ±0.045	**0.300** ±0.036	**0.293** ±0.035	0.170 ±0.020	0.146* ±0.025	0.116** ±0.025	0.106** ±0.030	0.110** ±0.035	0.080** ±0.010
Leaves		**0.316** ±0.035	**0.370**** ±0.030	0.176 ±0.025	0.216 ±0.040	**0.073**** ±0.025	0.086** ±0.015	**0.008**** ±0.020	**0.006**** ±0.025
Flower		**0.376**** ±0.040	**0.333**** ±0.030	0.186 ±0.030	0.126** ±0.025	**0.073**** ±0.025	0.093** ±0.025	**0.056**** ±0.020	0.043** ±0.011
Phosphorus (P)									
Stem	0.223 ±0.003	**0.266**** ±0.004	**0.274**** ±0.003	**0.242**** ±0.003	0.152** ±0.003	0.137** ±0.003	0.118** ±0.001	**0.112**** ±0.003	0.094** ±0.002
Leaves		**0.275**** ±0.004	**0.255**** ±0.005	**0.257**** ±0.001	0.125** ±0.003	0.122** ±0.003	0.070** ±0.004	**0.063**** ±0.003	**0.042**** ±0.003
Flower		**0.292**** ±0.005	**0.273**** ±0.004	**0.292**** ±0.003	0.156** ±0.006	0.136** ±0.003	0.119** ±0.003	**0.112**** ±0.003	0.106** ±0.002
Potassium (K)									
Stem	2.58 ±0.035	**2.81**** ±0.061	**2.74**** ±0.040	**2.75**** ±0.051	**2.45** ±0.026	1.23** ±0.030	1.17** ±0.030	0.56** ±0.040	0.60** ±0.030
Leaves		**2.91**** ±0.036	**2.64**** ±0.035	2.12** ±0.020	1.34** ±0.025	0.77** ±0.030	0.68** ±0.083	**0.39**** ±0.030	**0.27**** ±0.025
Flower		**3.32**** ±0.060	**3.06**** **±0.075**	**2.95**** ±0.030	**2.44** ±0.040	1.71** ±0.055	1.15** ±0.030	1.63** ±0.047	0.96** ±0.015
Calcium (Ca)									
Stem	0.617 ±0.005	**0.678**** ±0.004	**0.708**** ±0.004	**0.657**** ±0.005	**0.656**** ±0.004	0.272** ±0.005	0.375** ±0.003	0.113** ±0.003	0.112** ±0.002
Leaves		**0.643**** ±0.004	**0.694**** ±0.004	0.425** ±0.005	0.626 ±0.004	0.110** ±0.003	0.102** ±0.004	**0.072**** ±0.006	**0.041**** ±0.003
Flower		**0.675**** ±0.003	**0.645*** ±0.004	**0.624** ±0.003	0.621 ±0.006	0.375** ±0.004	0.262** ±0.005	0.218** ±0.004	0.164** ±0.005
Magnesium (Mg)									
Stem	0.284 ±0.004	**0.366**** ±0.004	**0.346**** ±0.004	**0.284** ±0.002	**0.290** ±0.003	0.122** ±0.004	0.172** ±0.004	0.092** ±0.004	0.082** ±0.004
Leaves		**0.317**** ±0.005	**0.340**** ±0.004	**0.294** ±0.002	**0.290** ±0.002	**0.074**** ±0.004	0.083** ±0.003	**0.040**** ±0.002	**0.030**** ±0.005
Flower		**0.334**** ±0.006	**0.371**** ±0.005	**0.290** ±0.002	**0.293** ±0.003	**0.073**** ±0.005	0.126** ±0.004	**0.037**** ±0.005	0.095** ±0.003

One way Anova: Tukeys HSD Post-hoc Test Inference. **Significant at $P < 0.01$.

*Significant at $P < 0.05$.Otherwise insignificant result. ±Standard deviation.

The highest supersession of nitrogen content (2.35fold) in the groundnut seedlings was occurred in the methanol extract of leaves trailed by 1.58fold in stem extract and 1.46fold in the flower extract at the 20% concentration over the control.

2.2.2 Phosphorus (P) :

Phosphorus content conquered more in aqueous extract than methanol extract in groundnut seedlings. Aqueous stem extract suppressed the phosphorus contents in higher concentration than lower concentration. 5% and 10% concentration of stem, leaves and flower extract increased the activity of nitrogen by 192.66 and 159.33; 173.66 and 139.33; 189.66 and 156.33 respectively over the control (111.00). Higher concentration of aqueous stem extract in 15% (51.00); leaves (34.00) and in flower extract it was 54.66 while 20% concentration decreased maximum in leaves (18.00), stem extract (33.00) and in flower extract (28.66).

Methanol extract of stem, leaves and flower extract has been recorded stimulatory effect at 5% concentration by 183.33, 180.33 and 180.66 that means nearly similar effect but after the concentration has been increased, there was declined from 10% to 20%. 10% stem extract determined (72.66), leaves extract (78.00) and flower extract (73.66). 15% stem extract (54.33), leaves extract (74.66) and flower extract (63.00) while at 20% concentration it has been suppressed in stem extract (41.66), leaves (64.66) and flower (45.66) in comparison to control.

Aqueous leaves extract has been reduced maximum (6.16 fold) phosphorus content than the stem extract (3.36 fold) and flower extract (3.87 fold) over to control in the groundnut seedlings at 20% concentration.

Methanol stem extract reduced phosphorus content by 2.66fold, followed by flower extract 2.43fold while 1.71fold in leaves extract from the 20% concentration in groundnut seedlings.

2.2.3 Potassium (K) :

5% concentration of aqueous stem and leaves found increasing potassium content of groundnut seedlings while 5% flower and 10% stem extract unchangeable to that of control i.e. similar while 10% leaves extract negligible increased (286.00). It decreased maximum from the aqueous (62.00) and methanol (82.00) leaves extract at 20%. Only 5% methanol extract of stem, leaves and flower have increasing potassium trends while others decreasing trends.

Aqueous extract of stem increased the potassium when treated at lower concentration 5% by 342.66 and in 10% with 280.66. Same increasing results has been continued in the 5% leaves aqueous extracts that determined 317.33 and 286.00. The 15% higher concentration of extract observed reduction of potassium in stem extract (129.33) and 20% (81.66). The leaves extract from 15% (140.66) and 20% (62.00) found gradually decreasing while flower extract occurred unchangeable from 5% (280.33) and identical to control then it becomes decrease from 10% (252.00), 15% (146.00) and 20% (111.66).

Methanol extract treatment from 5% concentration showed increasing trends in stem (375.00), leaves (366.66) and flower (332.66) comparable to the control. 10% methanol extract responds repressed results in stem (251.66), leaves (295.00) and flower (236.66) extract. The decreasing results determined from 15% concentration in stem (130.66), leaves (114.00) and flower extract (113.33). The 20% higher concentration reduced the potassium in all extract including stem (117.33), leaves (82.00) and flower extract (105.00).

Aqueous stem extract reduced potassium content by 3.43fold, 4.52fold in leaves extract and 2.51fold in flower extract at 20% concentration.

Methanol leaves extract decreased potassium more about 2.19fold than the stem extract (1.53fold) and flower extract (1.71fold) over control from 20% concentration in groundnut seedlings.

2.2.4 Calcium (Ca) :

Aqueous and methanol extract of stem, leaves and flower at 5% as well as 10% concentration aqueous stem and flower has been determined increasing the calcium content. Maximum calcium decreased in aqueous (71.66) and methanol (75.33) leaves extract. 15% aqueous stem and 10% leaves extracts found to be identical reduction in calcium i. e. 282.00.

5% aqueous stem extract have been increasing calcium activity by 362.33, leaves with 372.00 and flower extract with 354.00. 10% stem extract with 350.66 and 342.00 in flower extract. 15% concentrations of aqueous stem extract (282.00); leaves extract (95.00) and flower extract (144.66) while 20% concentration calculated 114.00 in stem extract, 71.66 in leaves extract and 105.00 in flower extract has been determined decreasing calcium content of groundnut seedlings.

Methanol extract of stem and flower extract have similar promoting result from 5% concentration that determined 372.66 while leaves extract have 346.66 over control. Methanol extract of stem responded differently in various concentration of extract, 10% (264.00), 15% (136.00) and 20% (116.00). Continuities the reduction of calcium in leaves extract from 10% (174.33), 15% (137.66) and maximum from 20% (75.33) while flower extract reduced from 10% (217.33), 15% (179.66) and 20% (144.33).

From the 20% higher concentration of aqueous leaves extract 4.63fold decrease calcium followed by 3.16fold in flower extract and stem extract decreased by 2.91fold. Maximum 4.41fold inhibition of calcium has been determined in methanol leaves extract trailed by 2.86fold in stem extract and 2.30fold in flower extract from 20% concentration in groundnut seedlings.

2.2.5 Magnesium (Mg) :

Magnesium content of groundnut seedlings has been highly decreased in aqueous (93.33) and methanol (71.00) leaves extract from 20% concentration. Elevation of magnesium was found in 5% aqueous stem and leaves extract as well as 5% and 10% flower extract did not significant in decreasing magnesium. Surprisingly, 5% and 10% methanol stem, leaves and flower extract has been determined increasing the magnesium content where as 15% and 20% concentrations were found decreasing magnesium of groundnut seedlings in all treated extracts. Furthermore, the degree of inhibition has been rapidly increased from 15% to 20% in leaves extract while it was nearly identical in stem and flower extract.

5% aqueous extract of stem (634.66) and leaves (680.66) have increasing magnesium while flower extract was similar to control (545.33) from 5% lower concentration. Magnesium has been continuously decreasing from the 10% in aqueous stem extract (496.33); leaves (513.33) and flower (502.66). In 15% concentration it was suddenly decreased in stem extract (326.00); leaves (196.66) and flower (260.33) in comparison to the previous concentration. At the higher 20% concentration of aqueous stem extract it was determined 234.66 and it was greatly reduced in leaves (93.33) while in flower extract with 133.00.

Methanol extract of stem (561.00), leaves (592.33) and flower (653.33) at 5% lower concentration, magnesium has been activated more and increasing in all treated extract. Same promoting trend has been continued in the 10% concentration of

methanol stem extract (534.00), leaves (575.66) and flower extract have 544.00. 5% concentration of methanol flower extract has been maximum endorsing (653.33) magnesium than the leaves and stem in comparison to the control. At the higher concentration of 15% and 20% it has been decreased well in 15% stem extract (260.00); leaves (171.33) and flower (207.33) while in 20% concentration of stem extract it was reduced by 181.00; maximum inhibition in leaves (71.00) while slow in flower (175.33) extract.

Aqueous extract of leaves maximum heightened the reduction (5.78 fold) in the magnesium content trailed by flower extract (4.06 fold) and stem extract (2.30 fold) in the groundnut seedlings at higher concentration level (20%).

Maximum reduction (7.61fold) of magnesium has been determined from 20% concentration methanol leaves extract while 2.98fold in stem extract and 3.08fold in flower extract in groundnut seedlings.

III. Effect of aqueous and methanol extracts of stem, leaves and flowers of *Pascalia glauca* Ortega. on micronutrients of wheat seedlings :

2.3.1 Wheat seedlings :

3.1.1 Zinc (Zn) :

Zinc has been structural, functional as well as regulatory cofactor of number of enzymes including Cu-Zn superoxide dismutase, carbonic unhydrase, proteinase, peptidases, glutamic acid dehydrogenase, isomerases, aldolases, transphosphorylases. It may also play an important role in nitrogen metabolism (Reddy and Rao, 1979). Zinc is essential to carbohydrate metabolism, protein synthesis and inter nodal elongation. Zinc acts as an activator of several enzymes alcoholic dehydrogenase, pyridine nucleotide, dehydrogenase and carbonic unhydrase.

Table- 7 : Effect of aqueous and methanol extract of stem, leaves and flowers of *Pascalia glauca* Ortega. on macronutrient of groundnut seedlings (mg g^{-1} dry weight).

Plant part extract	Groundnut seedlings								
	Nitrogen (N)								
	Concentrations	5%		10%		15%		20%	
	Control	Aqueous	Methanol	Aqueous	Methanol	Aqueous	Methanol	Aqueous	Methanol
Stem	2.19 ±0.070	2.12 ±0.026	2.17 ±0.036	2.02 ±0.030	1.96* ±0.070	1.78** ±0.080	1.69** ±0.050	1.26** ±0.050	1.38** ±0.060
Leaves		1.92** ±0.045	1.87** ±0.047	1.75** ±0.030	1.55** ±0.051	1.60** 0.060	1.40** ±0.020	1.05** ±0.045	0.93** ±0.101
Flower		2.12 ±0.025	2.05 ±0.015	2.06** ±0.030	1.87** ±0.081	1.81** ±0.041	1.70** ±±049	1.57** ±0.055	1.50** ±0.060
Phosphorus (P)									
Stem	111.00 ±4.582	**192.66**** ±5.033	**183.33**** ±3.055	**159.33**** ±5.033	72.66** ±5.033	51.00** ±4.582	54.33** ±4.041	33.00** ±4.582	**41.66**** ±3.511
Leaves		**173.66**** ±3.511	**180.33**** ±2.516	**139.33**** ±3.055	78.00** ±6.557	34.00** ±4.000	74.66** ±3.055	**18.00**** ±4.000	64.66** ±4.163
Flower		**189.66**** ±4.509	**180.66**** ±3.055	**156.33**** ±6.027	73.66** ±3.214	54.66** ±4.163	63.00** ±5.567	28.66** ±3.055	45.66** ±4.041
Potassium (K)									
Stem	280.33 ±5.507	**342.66**** ±5.033	**375.00**** ±4.000	**280.66** ±4.163	251.66** ±5.507	129.33** ±7.023	130.66** ±4.509	81.66** ±4.725	117.33** ±5.033
Leaves		**317.33**** ±7.023	**366.66*** ±6.110	**286.00** ±5.000	295.00 ±52.048	140.66** ±5.033	114.00** ±4.000	**62.00**** ±6.000	**82.00**** ±4.000
Flower		**280.33** ±5.507	**332.66**** ±5.033	252.00** ±8.185	236.66** ±4.163	146.00** ±4.000	113.33** ±4.163	111.66** ±4.041	105.00** ±7.000
Calcium (Ca)									
Stem	332.33 ±6.027	**362.33**** ±2.516	**372.66**** ±8.326	**350.66*** ±6.429	264.00** ±5.000	282.00** ±6.000	136.00** ±4.000	114.00** ±4.000	116.00** ±6.000
Leaves		**372.00**** ±7.211	**346.66*** ±6.110	282.00** ±4.000	174.33** ±7.371	95.00** ±7.000	137.66** ±5.507	**71.66**** ±4.509	**75.33**** ±7.023
Flower		**354.00**** ±5.567	**372.66**** ±8.326	**342.00** ±4.000	217.33** ±7.023	144.66** ±7.023	179.66** ±9.712	105.00** ±6.557	144.33** ±7.505
Magnesium (Mg)									
Stem	540.33 ±4.509	**634.66**** ±6.110	**561.00**** ±5.567	496.33** ±8.504	**534.00**** ±4.000	326.00** ±6.000	260.00** ±5.000	234.66** ±9.018	181.00** ±3.605
Leaves		**680.66**** ±7.023	**592.33**** ±5.507	513.33** ±4.163	**575.66**** ±7.767	196.66** ±11.718	171.33** ±6.110	**93.33**** ±5.033	**71.00**** ±4.582
Flower		540.33 ±7.234	**653.33**** ±7.023	502.66 ±7.023	**544.00**** ±3.605	260.33** ±4.509	207.33** ±10.263	133.00** ±66.775	175.33** ±7.023

One way Anova: Tukeys HSD Post-hoc Test Inference. **Significant at $P < 0.01$.

*Significant at $P < 0.05$. Otherwise insignificant result. ±Standard deviation.

Aqueous stem, leaves and flower extract of *Pascalia glauca* when treated with wheat seedlings at lower concentration of 5% stem (0.242) and 10% (0.228) extract has been expressed endorsing activity. Then it was greatly reduced in 15% (0.095) and 20% (0.031). Aqueous leaves extract found increasing only from 5% (0.214) and from 10% it was gradually decreased (0.134), in 15% (0.052) to 20% (0.020). Aqueous flower extract decreased from 5% (0.169), 10% (0.170), 15% (0.126) and 20% (0.114) concentration.

Methanol extract at 5% concentration zinc has been risen the activity from stem extract (0.236) and leaves (0.212) while in flower extract it was 0.162. 10% concentration it was decreased in methanol stem (0.115) and leaves (0.110) extract while negligible increased from flower (168) extract. 15% concentration showed gradually decreased in stem extract (0.112) and flower (0.125) but it was highly decreased in leaves (0.051). Zinc content was maximum decreased from 20% higher concentration of leaves (0.024) followed by stem (0.031) and flower extract (0.113) over the control (Table - 8).

Aqueous extract of leaves have greatly suppressed (8.65fold) the zinc content followed by 4.67fold in stem extract and 1.52fold in flower extract from the 20% concentrations over to control in wheat seedlings.

Methanol extract of leaves was found maximum reduction (7.207fold) of zinc content of the wheat seedlings while stem extract determined 5.58fold and 1.53fold flower extract from the higher concentration (20%).

3.1.2 Iron (Fe) :

Iron is important micronutrient of many enzymes associated with energy transfer, nitrogen reduction and fixation as well as lignin formation. It has been the greatest biological importance because of its alliance with proteins and play vital role in the biosynthesis of chlorophyll. It is essential for the young growing parts of plants. There are two groups of iron containing proteins, heme proteins and iron sulphur proteins (Sandmann and Bogger, 1983). Iron is also a constituent of leghaemoglobin which is participated in the mechanism of nitrogen fixation in the leguminous plants. The iron acts as an activator for the enzymes involved in number of metabolic reactions including catalase, peroxidase and cytochrome oxidase (Campbell, 1988).

From the 5% lower concentration extracts, iron content was promoted in wheat seedlings that determined in aqueous stem (0.317), leaves (0.325) and flower

(0.328) over the control. After the aqueous extract concentration dose increased iron content reduce in 10% stem and flower extract (0.212) which was similar while leaves extract by 0.196. Same trend has been found from the 15% concentration in stem extract (0.082), leaves (0.065) and flower extract (0.085). 20% higher concentration it was maximum decreased in the leaves extract (0.033) followed by stem (0.050) and flower (0.055).

Methanol extract of stem, leaves and flower has been determined increasing iron content in the 5% (0.214, 0.190 and 212) and 10% concentration (0.207, 0.180 and 0.193) respectively. After the concentration increased by 15% it was suppressed in the methanol stem (0.082), leaves (0.063) and flower extract (0.082). 20% concentration largely declined iron content of wheat seedlings from stem extract (0.068), leaves extract (0.047) and flower extract (0.058).

There is 6.66fold increment of reduction in the iron content from aqueous extract of leaves while 4.33fold in stem extract and 3.97fold in flower extract at 20% concentration after the treatment to the wheat seedlings.

Iron content of wheat seedlings maximum decreased about 4.68fold frommethanol leaves extract followed by stem extract (3.23fold), and 3.79fold in flower extract at 20% concentration.

3.1.3 Copper (Cu) :

Copper is an essential for normal plant growth and their development, although it is potentially toxic. It participates in various physiological processes and an essential cofactor for many metalloproteins. It acts as a structural element and participated in regulatory proteins and photosynthesis electron transport, mitochondrial respiration, oxidative stress responses, cell wall metabolism and hormone signaling (Marschner, 1995 and Raven *et al*., 1999). It plays significant role in signaling of transcription and protein trafficking machinery, oxidative phosphorylation and iron mobilization.

Aqueous extract of stem at 5% concentration (0.003) did not change while leaves (0.004) and flower (0.005) extract determined promoting over control. 10% concentration also unchangeable trend in stem extract (0.003) over control but it was negligible increased in leaves and flower (0.004). At higher concentration 15% stem and flower extract it was similarly decreased (0.001) but maximum in leaves (0.0009)

extract. In 20% concentration it was higher and similar reduction (0.0005) found in stem and leaves extract while flower extract have 0.001.

5% methanol extract of stem, leaves and flower (0.004) was determined minor and identical endorsing copper over control. 10% concentration in stem and leaves extract (0.003) unchanged while flower extract (0.004) minor increased. Similar reduction found at 15% concentration from stem, leaves and flower extract (0.001) while 20% concentration increased in reduction of copper content from stem extract (0.0007); maximum (0.0004) in leaves extract and flower extract with 0.001 in wheat seedlings.

There is identical 6.80fold reduction of copper was occurred in the aqueous extract of stem and leaves while 3.00fold in the flower extract in wheat seedlings at the higher concentration (20%).

Maximum impediment of copper content has been recorded (7.50fold) in the methanol extract of leaves followed by 4.28fold in stem extract and 3.00fold in the flower extract from the 20% concentration of treated wheat seedlings.

3.1.4 Manganese (Mn) :

Manganese is another most essential micronutrient in plants that acts as an enzyme activator and plays vital role in oxidation reduction process, respiration and nitrogen metabolism. It is absorbed as $Mn2^{+}$ and translocated predominantly as the free divalent cation in the xylem from the roots to the shoot. It plays a direct role in photosynthesis as it is involved in photoxidation of water during photolysis of water and involved in biosynthesis of fatty acids. It is also related to stability of thylakoid structure. Mn is also a constituent of enzyme superoxide dismutase (Sevilla *et al*., 1980).

Aqueous extract from 5% of stem and flower unchanged i.e. 0.003 over control. It was increased in the leaves extract from 5% (0.005) and 10% (0.004). 10% concentration of aqueous stem extract has reduced by 0.002 and flower extract not changed that remains steady over control. 15% concentration determined similar reduction of manganese in stem, leaves and flower (0.001) extract while maximum reduction was found in 20% concentration of leaves (0.0003) followed by stem (0.0008) and flower (0.001) depicted in (Table - 8) .

Increasing activity of manganese has found in all extract of 5% concentration of methanol stem (0.004), leaves (0.005) and flower (0.004) extract in groundnut

seedlings in comparison to control. Unchanged results from the 10% stem extract (0.003) but increased in leaves (0.004) while flower extract decreased by 0.002 manganese content. Methanol stem, leaves and flower extract has been greatly declined with similar (0.001) results were found from15% concentration. 20% concentration of stem extract (0.0008) and leaves (0.0005) has been suddenly decreased while slowly decreased in flower extract (0.001) over the control.

Maximum manganese content was reduced (10.00fold) in the aqueous leaves extract then in stem (3.75fold) and flower extract (3.00fold) from the 20% concentration.

Methanol leaves extract also determined maximum suppression (6.00fold) of manganese content followed by 3.75fold in stem extract and 3.00fold in flower extract when wheat seedlings treated with 20% concentration.

IV. Effect of aqueous and methanol extracts of stem, leaves and flowers of *Pascalia glauca* Ortega. on micronutrients of groundnut seedlings :

3.4.2 Groundnut seedlings :

4.2.1 Zinc (Zn) :

Aqueous stem extract found increasing zinc content of groundnut seedlings at 5% (2.48) and 10% (2.25) concentration. Leaves extract has 2.12 and 1.66 while flower extract with 1.96 and 1.82 respectively over the control. The higher concentration of extract at 15% and 20% it has been more reduced by 0.65 and 0.54 in stem extract; 0.39 and 0.20 in leaves extract while 1.48 and 1.06 in flower extract respectively in comparisons to control (Table - 9).

Methanol extract of stem, leaves and flower also found to be increasing zinc content only in 5% lower concentration by 2.73; 2.81 and 3.23 respectively over the control. Gradual suppression of zinc content was found in stem extract from 10% (1.71), 15% (0.44) to 20% (0.21). Leaves extract declined successively from 10% (1.28) and 15% (0.90) but it was suddenly reduced (0.36) from higher concentration (20%). Flower extract was reduced zinc content from10% (1.41); 15% (1.50) and 20% have 1.31.

Table- 8 : Effect of aqueous and methanol extract of stem, leaves and flowers of *Pascalia glauca* Ortega on micronutrient of wheat seedlings (mg g^{-1} dry weight).

Plant part extract	Wheat seedling								
	Zinc (Zn)								
	Concent -rations	5%		10%		15%		20%	
	Control	Aqueous	Methanol	Aqueous	Methanol	Aqueous	Methanol	Aqueous	Methanol
Stem	0.173 ±0.003	**0.242**** ±0.007	**0.236**** ±0.003	**0.228**** ±0.002	0.115** ±0.003	0.095** ±0.007	0.112** ±0.002	0.037** ±0.004	0.031** ±0.003
Leaves		**0.214**** ±0.002	**0.212**** ±0.002	0.134** ±0.003	0.110** ±0.003	0.052** ±0.003	0.051** ±0.004	**0.020**** ±0.003	**0.024**** ±0.002
Flower		0.169 ±0.002	0.162 ±0.003	0.170 ±0.003	**0.168** ±0.017	0.126** ±0.002	0.125** ±0.003	0.114** ±0.003	0.113** ±0.003
Iron (Fe)									
Stem	0.220 ±0.004	**0.317**** ±0.006	**0.214**** ±0.003	**0.212** ±0.002	**0.207**** ±0.005	0.082** ±0.005	**0.082**** ±0.006	0.050** ±0.004	0.068** ±0.003
Leaves		**0.325**** ±0.003	**0.190**** ±0.004	0.196** ±0.005	**0.180**** ±0.002	0.065** ±0.003	0.063** ±0.003	**0.033**** ±0.005	**0.047**** ±0.004
Flower		**0.328**** ±0.002	**0.212**** ±0.003	**0.212** ±0.003	**0.193**** ±0.007	0.085** ±0.004	**0.082**** ±0.013	0.055** ±0.003	0.058** ±0.004
Copper (Cu)									
Stem	0.003 ±0.0003	**0.003** ±0.0003	**0.004*** ±0.0001	**0.003** ±0.0002	**0.003** ±0.0002	**0.001**** ±0.0002	**0.001**** ±0.0003	**0.0005**** ±0.0002	0.0007** ±0.0003
Leaves		**0.004**** ±0.0004	**0.004** ±0.0003	**0.004**** ±0.0002	**0.003** ±0.0003	0.0009** ±0.0002	**0.001**** ±0.0003	**0.0005**** ±0.0002	**0.0004**** ±0.0002
Flower		**0.005**** ±0.0005	**0.004*** ±0.0002	**0.004**** ±0.0004	**0.004** ±0.0003	**0.001**** ±0.0003	**0.001**** ±0.0002	**0.001**** ±0.0003	**0.001**** ±0.0002
Manganese (Mn)									
Stem	0.003 ±0.0003	**0.003** ±0.0001	**0.004** ±0.0002	0.002** ±0.0003	0.003 ±0.0003	**0.001**** ±0.0001	**0.001**** ±0.0002	0.0008** ±0.0002	0.0008** ±0.0002
Leaves		**0.005**** ±0.0004	**0.005**** ±0.0003	**0.004**** ±0.0003	**0.004**** ±0.0003	**0.001**** ±0.0002	**0.001**** ±0.0002	**0.0003**** ±0.0001	**0.0005**** ±0.0002
Flower		**0.003** ±0.0003	**0.004** ±0.0003	0.003 ±0.0003	0.002* ±0.0003	**0.001**** ±0.0002	**0.001**** ±0.0002	0.001** ±0.0002	0.001** ±0.0002

One way Anova: Tukeys HSD Post-hoc Test Inference. **Significant at $P < 0.01$.

*Significant at $P < 0.05$. Otherwise insignificant result. ± Standard deviation.

Aqueous extract of leaves at 20% higher concentration showed highest reduction by 7.50fold while 2.77fold in stem extract and 1.40fold in flower extract in groundnut seedlings.

Maximum reduction of zinc was recorded in methanol stem extract (7.14fold) followed by leaves extract (.16fold) and 1.14fold in flower extract at 20% concentration.

4.2.2 Iron (Fe) :

Promoting trends of iron content was found in aqueous stem extract at 5% (92.66) and 10% (73.66) while it was reduced from 15% (30.00) and 20% (10.00) concentration. Same trend was followed in the leaves extract from the 5% (93.33) and 10% (71.00) concentration; but it was greatly reduced from 15% (38.66) and 20% (9.33). Flower extract increased iron content from 5% (85.00) and 10% (71.33) while highly reduced from 15% (32.00) and 20% (21.33) concentration over the control in groundnut seedling.

Methanol extract was also showed same trend of increasing iron content from stem extract at 5% (82.00) but reduced at 10% (64.66), 15% (22.00) and 20% (14.00) in comparison to control. Methanol extract of leaves found increasing trends of iron content from 5% (96.00) and 10% (70.66) while it was suddenly decreased from 15% (10.00) and 20% concentration (6.66). Flower extracts from 5% (76.66) determined increased but it was reduced iron content of groundnut from 10% (57.33), 15% (22.33) and 20% (15.00).

Maximum reduction about 7.35fold of iron content in groundnut has been found in the aqueous leaves extract treatment followed by 6.86fold in stem and 3.21fold in flower extract at 20% concentration.

Iron content of groundnut was maximum reduced in the methanol leaves extract (10.30fold) while 4.90fold in stem and 4.57fold in flower extract at 20% concentration Methanol leaves extract dominantly participated in the decreasing iron content over the aqueous extract.

4.2.3 Copper (Cu) :

Aqueous stem extract was found stimulatory activity of copper content of groundnut seedlings from 5% concentration (3.68) and 10% (2.17) over the control while it was reduced from 15% (0.80) and in 20% (0.49). 5% leaves extract extended

increasing copper content by 3.60 while same trend from 10% (2.39) but it was reduced from 15% (0.83) and 20% (0.40). Flower extract showed increasing trend in the treatment of 5% (3.51) and 10% (2.42) but it was decreased in 15% (1.16) and 20% (0.50) concentration.

5% methanol extract of stem increased copper content of groundnut by 3.14; leaves (2.94) and flower (3.61). 10% concentration of stem extract was increased by 2.98; leaves (2.76) and flower (2.44). There was progressively decreased copper content from 15% stem extract (0.76); leaves (0.61) and in flower extract (1.20). Same trend has been observed from 20% concentration of stem extract (0.62); leaves (0.37) and flower extract (0.62).

Nearly similar reduction of copper content of groundnut seedlings has occurred in the aqueous stem extract (3.79fold) and flower extract (3.72fold) while leaves extract highly reduced about 4.65fold at 20% concentration.

Methanol extract of leaves was determined maximum increments of reduction (5.02fold) in the copper content of groundnut seedlings while stem and flower extract decreased similar (3.00fold) reduction after the treatment of 20% concentration.

4.2.4 Manganese (Mn) :

There is increasing trends in the Manganese content of groundnut seedlings from 5% aqueous extract of stem (7.60), leaves extract (7.16) and flower extract (6.17) over the control. Similar trend was found in the 10% stem extract (6.16), leaves extract (6.03) and flower extract (5.86). When the treatment of extract was boosted at higher concentration level from 15% concentration, manganese content was reduced by 4.37in stem extract, 3.24 in leaves extract and 3.21 in flower extract. 20% higher concentration showed maximum decreased manganese content in leaves extract (0.87) followed by stem (2.29) and flower extract (2.16) in the groundnut seedlings (Table-9).

Methanol extract at the lower concentration of 5% methanol stem extract increased manganese content by 7.19 while leaves extract by 7.71 and flower extract with 7.16 in the groundnut seedlings. Similar increased activity has found in the 10% concentration of stem extract (6.18) and flower (5.91) but leaves extract showed decreased (4.12) over the control. When treatment of extract has been extended from 15% concentration it was found to be great reduction in the methanol stem extract (2.11), leaves extract (0.92) and flower extract (2.06). Similar decreasing trend has

followed from 20% higher concentration of stem extract (1.56), leaves extract (0.32) and flower extract (1.18).

When the groundnut seedlings treated with higher concentration (20%) of aqueous leaves extract and found to be maximum reduction (6.71fold) then 2.70fold in flower extract and 2.55fold in stem extract in comparable to the control.

Methanol extract of leaves was reduced manganese content of groundnut seedlings by 18.25fold followed by 4.94fold in flower extract and 3.74fold in stem extract at the higher concentration (20%).

Discussion:

The mineral constituents play major role in the growth, development and dominance of the weeds in the crop ecosystems. All living organisms require a continuous supply of large number of substances from outside to complete their life cycle. This supply is called as nutrition. The essential nutrients required by higher plants are exclusively of inorganic nature. A plant for normal optimal growth requires sixteen different elements. Green plants have comparatively simple nutrient requirements and are classified as macronutrients (N, P, K, Ca, Mg, and Na) and micronutrients (Fe, Mn, Cu, Zn, Mo, B and Cl). Macronutrients are found and needed in plants in relatively higher amounts than micronutrients. Both of them are essential for almost all metabolic processes. Hence this aspects was attempted by many allelopathic workers, within that selected five macronutrients (N, P, K, Ca and Mg) and four micronutrients (Zn, Fe, Cu and Mn) to assessed well from wheat and groundnut seedlings in the affection of various weed parts extract including stem, leaves and flower of *Pascalia glauca* Ortega to investigated their amount of contents in the seedlings after the various treatments under different concentrations. The review of literature cleared that such work does not take more attention in the allelopathy in general and additionally no work has been reported from India and abroad with reference to the *P. glauca* in particular therefore, focus the light on influence of stem, leaves and flower aqueous and methanol extract on wheat and groundnut seedlings.

Table- 9 : Effect of aqueous and methanol extract of stem, leaves and flowers of *Pascalia glauca* Ortega on micronutrient of groundnut seedlings (mg g^{-1} dry weight).

Plant part extract	Groundnut seedlings								
	Zinc (Zn)								
	Concentrations	5%		10%		15%		20%	
	Control	Aqueous	Methanol	Aqueous	Methanol	Aqueous	Methanol	Aqueous	Methanol
Stem	1.50 ±0.045	**2.48**** ±0.065	**2.73**** ±0.035	**2.25**** ±0.061	1.71** ±0.041	0.65** ±030	0.44** ±0.030	0.54** ±0.0400	**0.21**** ±0.041
Leaves		**2.12**** ±0.050	**2.81**** ±0.055	**1.66**** ±0.040	1.28** ±0.040	0.39** ±0.030	0.90** ±0.061	**0.20**** ±0.030	0.36** ±0.040
Flower		**1.96**** ±0.040	**3.23**** ±0.050	**1.82**** ±0.040	1.41** ±0.5988	1.48 ±0.040	1.50* ±0.040	1.06** ±0.075	1.31** ±0.070
	Iron (Fe)								
Stem	68.66 ±3.511	**92.66**** ±3.055	**82.00**** ±6.000	**73.66** ±4.163	**64.66** ±4.509	30.00** ±4.000	22.00** ±6.000	10.00** ±3.000	14.00** ±4.000
Leaves		**93.33**** ±5.033	**96.00**** ±3.605	**71.00** ±5.567	**70.66**** ±3.055	38.66** ±4.041	10.00** ±2.000	**9.33**** ±2.516	**6.66**** ±2.516
Flower		**85.00*** ±5.000	**76.66**** ±2.081	**71.33** ±7.023	**57.33** ±5.033	32.00** ±6.000	22.33** ±4.509	21.33** ±5.033	15.00** ±3.000
	Copper (Cu)								
Stem	1.86 ±0.032	**3.68**** ±0.035	**3.14**** ±0.041	**2.17**** ±+-0.061	**2.98**** ±0.065	0.80** ±0.072	0.76** ±0.040	0.49** ±0.055	0.62** ±0.060
Leaves		**3.60**** ±0.055	**2.94**** ±0.030	**2.39**** ±0.055	**2.76**** ±0.045	0.83** ±0.070	0.61** ±0.070	**0.40**** ±0.040	**0.37**** ±0.075
Flower		**3.51**** ±0.050	**3.61**** ±0.096	**2.42**** ±0.045	**2.44**** ±0.040	1.16** ±0.0503	1.20** ±0.040	0.50** ±0.050	0.62** ±0.040
	Manganese (Mn)								
Stem	5.84 ±0.083	**7.60**** ±0.065	**7.19**** ±0.040	**6.16**** ±0.050	**6.18**** ±0.070	4.37** ±0.050	2.11** ±0.041	2.29** ±0.050	1.56** ±0.061
Leaves		**7.16**** ±0.060	**7.71**** ±0.136	**6.03** ±0.102	4.12** ±0.035	3.24** ±0.111	0.92** ±0.060	**0.87**** ±0.070	**0.32**** ±0.050
Flower		**6.17**** ±0.050	**7.16**** ±0.060	**5.86** ±0.131	**5.91**** ±0.056	3.21** ±0.070	2.06** ±0.110	2.16** ±0.030	1.18** ±0.050

One way Anova: Tukeys HSD Post-hoc Test Inference. **Significant at $P < 0.01$.

*Significant at $P < 0.05$.Otherwise insignificant result. ±Standard deviation.

Major effects of allelochemicals on nutrient uptake have also been reported by Crawley (1997) and Lambers *et al.* (1998). Many workers paid attention on such work including, Saswade and Dhumal (2004) reported that higher contents of minerals like N, P, K, Mg, Ca, Cu, Mn, Fe and Zn in the weed leaves of *Celosia, Euphorbia* and *Solanum* at lower concentrations. Similarly Dim *et al.* (2004) and Feng *et al*. (2006) noted significant contents of essential minerals in leaves of *Ageratum*. The work of Ghayal and Dhumal (2006) indicated higher mineral content in leaves of dominant invasive weeds like *Synedrella* and *Cassia* growing luxuriantly in the campus of University of Pune. Bhalerao (2003), Pawar (2004), Jadhav (2006) and Vaiddya (2009) reported that leaf extract of *Tectaria, Pteridium, Parthenium, Eichhornia, Pistia, Azolla* and *Ageratum* they were rich in different types of macro and micro nutrients. Chambers and Holm (1965) observed reduction in nutrient contents of plants under the influence of allelochemicals. Our work is in the line of above workers and found that majority of nutrients were increased in lower concentrations and when increased extract concentrations they were inhibited under the influence of stem, leaves and flower extract treatment on wheat and groundnut seedlings in the conformity of results.

Nitrogen has predominant role in plants, being the constituents of protein, nucleic acids, nucleotides, phytohormones etc. It has also regulates photosynthesis, carbohydrates metabolism and biosynthesis of pigments, water use efficiency and secondary metabolites. The influence of allelopathy on mineral nutrition was studied by Buchholtz (1971) found that the average values of nitrogen content are 1.5% on dry weight basis in terrestrial plants. The allelopathy research workers like Bararpour and Oliver (1999), Jadhav (2006), Vaidya (2009) recorded increase in nitrogen content of wheat, chickpea, mungbean, fenugreek and sorghum due to lower concentration of leaf extract of *Echhornia, Pistia, Azolla* and *Ageratum*. While reduction in nitrogen content was recorded by Odhia (2001), Bajaj (2005), Bhajwa (2004), Jadhav (2006) Facknath (2006), Vaidya (2009) in rice, sunflower, maize, wheat, mungbean, sorghum and chickpea due to higher concentration of extracts of *Agemone, Echhornia, Pistia, Azola, Wedelia* and *Ageratum*. Present work advocated that the lower concentration of *Pascalia glauca* extracts has been stimulatory effect and at higher concentrations of aqueous and methanol extract has been inhibitory effect in the right line of above researchers.

Role of phosphorus in many metabolic processes leading to healthy, vigorous growth and higher yields in crop plants is well established. Soil pH has a major role in making the phosphorous available to the plants. Phosphate plays important roles in energy metabolism, carbohydrate and protein metabolism, cell membranes structure, stability and functioning. It is also an important constituent of nucleic acids, phosphoproteins, lipoproteins and nucleoproteins. It has very important role in activities of different enzymes. Plants require 1% potassium for their optimal growth (Epstein, 1972). It plays a significant role in plant growth and developmental processes such as photosynthesis (Peoples and Koch, 1979), translocation of proteins and carbohydrates (Marschner, 1997), stability of ribosome, protein synthesis, nitrogen turnover, activation of enzymes, stomatal movement, nyctinastic and seismonastic movements, and cell extension (Suelter, 1970 and Shankar *et al*., 2013). Leaf extract and leaf leachate treatment of *Chromolena odorata,* there is decline in phosphorus contents were observed in both *Crotolaria verrucosa* and *C. retusa* (Dethe and Gaikwad, 2017). Present investigation determined decreasing the phosphous content in the seedlings of wheat and groundnut at higher concentration, indicates that our results were conformity of above researchers.

Potassium is also the most important major, essential nutrient required by the plants. Potassium has different roles in various metabolic processes like enzymatic activities, defense mechanism, ionic balance, stomatal functioning etc. The deficiency or excess accumulation of K, both have hazardous effects on plants, Hence level of K must be investigated under allelopathic influence, which will help to understand the metabolic status and functioning of the treated plants. The increase in K content in different crops treated with the extract or residues of different allelopathic weeds (Mahmood, 2004; Batish *et al*., 2005; Ambika and Smitha, 2005; Ruan *et al*., 2005; Facknath, 2006; Supe *et al*., 1997 and Kaith and Awasthi, 1998). Although, the work has done by Vaidya, (2009) in the test crops mungbean and sorghum K content was reduced in higher concentration of weed species. As reported by Mitra *et al*. (1998) and Dutta and Duha (1999) they stated that the potassium helps to enhance the different physiological and biochemical processes along with Mg, which in turn influences growth and yield. The higher content of K in the treated crop seedlings might be directly or indirectly responsible for its stimulated uptake at lower concentration of *Pascalia glauca* extract therefore, results of present work are in the right path of above researchers.

Calcium has many roles in plants and is required in differing amount depending on the process in which it is involved. It plays important role in cell division, growth and involved in different metabolic process in plants (Kauss, 1987). The positive or negative influence on Ca content due to low or high concentration treatments of allelopathic plants has important role for providing the explanation to increase or decrease in growth and different physiological parameters. Calcium has long been known to be essential for structural and functional integrity of plant membrane (Epstein, 1972). According to Clark (1984) the activities of many enzymes have been either stimulated or inhibited by calcium. The major role carried out by calcium in plants is to bind with proteins, nucleic acids and lipids to affect cell adhesion, membrane chromatin organization and enzyme conformation (Clarkson and Hanson, 1980). Positive effect of allelopathic treatments of extracts or leachates in different crops like mungbean, chickpea and sorghum was reported by Bhalerao (2003), Jadhav (2006), Vaidya (2009). The negative influence on calcium content due to higher concentration treatments was noted in cucumber and *Linum* by Ruan *et al*. (2005) and Riess and Herth (1978). The higher contents of calcium might have positively influenced on chlorophyll contents, sugars, starch, phednols, proteins, nitrogen along with improved growth and yield in all the test crops (Reiss and Herth, 1978 and Sinha and Dube, 2000). Our results were on the same track with negative effect when treated at heigher concentration of extract which found conformity of above workers.

Magnesium is a small, mobile and strongly electropositive divalent cation in the plants, found both in bound as well as free form (Gilbert, 1957). Well known role of Mg is its contribution to the center of the chlorophyll molecule. It is a part of ring structure of chlorophyll molecule, the photosynthetic pigment in chloroplast. It is a cofactor of several enzymatic reactions involved in organic acid synthesis. The Mg content in plant tissue is always high, but it varies from plant to plant and organ to organ. Mg has wide roles in plant metabolism, it acts as cofactor in biosynthesis of sucrose, activates carboxylating enzymes, stimulate Co_2 fixation rate (Mengel and Kerkby, 1978). The various allelopathic treatments may cause heighten or deaden in Mg content of treated plants, which depend on degree of concentration applied to the test plants. Many workers reported accumulation of Mg is due to lower concentration treatments of leaf extract including Ram and Bose (2000), Bhalerao (2003), Mahmood (2004), Vaidya (2009) and Dethe and Gaikwad (2017). Present work

clearly indicates the lower concentration of extract of *Pascalia glauca* Ortega increase in magnesium content while in higher concentration decrease in magnesium content therefore, present investigation ruled that increase or decrease in inorganic content depends on the degree of extract concentration that confirm our work on the right line of above workers.

Zinc is essential for carbohydrate metabolism and regulation of consumption of sugars, nitrogen metabolism, protein synthesis, auxin synthesis, particularly IAA synthesis, as well as for sexual fertilization and development of reproductive parts. Many enzymes require zinc for their activity. Zinc acts either as a metal component or as a functional, structural or regulatory cofactor of a large number of enzymes. It is required for chlorophyll biosynthesis. It participates in synthesis of indole acetic acid from its precursor; tryphtophan (Skoog, 1940 and Tsui, 1948). Zn plays a role in membrane stability by regulating the level of oxidizing O_2 species (Pinton *et al.*, 1994). Zn contents are also positively or negatively affect when under allelopathic treatments of extracts. Number of workers has been worked and find out such results including Ram and Bose (2000), Tripathy *et al*. (1999), Jadhav (2006), Bhalerao (2003), Ruan *et al*. (2005), Batish *et al*. (2005) and Vaidya (2009). They reported that increasing the content of Zn in different crops due to treatments of lower concentration of leaf extracts of weeds. Present investigation also determined zinc content was increased from the lower concentration of aqueous and methanol extract.

Iron is an immobile element in living cells. It is absorbed by plant roots as Fe2+ or as Fe chelate. Fe chelates are soluble and therefore available to roots. It is involved in oxidation, reduction reactions, ferredoxin formation and chlorophyll synthesis (Spillar and Teny, 1980). Fe is stored in stroma of chloroplast as phytoferritin, which can store about 5000 atoms of Fe3+ (Marschner, 1986). Increased iron content along with other essential nutrients might have significantly improved the chlorophyll contents, total carbohydrates, protein and phenols along with increase in height and number of leaves per plant such type of findings reported by Dube *et al*. (2003) in vegetable crop radish. There is significant decrease in iron content was recorded in the mugbean, chickpea and sorghum at higher concentration treatments of *Celosia* and *Euphorbia* (Saswade, 2007). Present investigation in the corroborated above workers in their findings and work has been confirmed that both the stimulatory and inhibitory activity has been observed in the seedlings of wheat and

groundnut when treated with different concentration of aqueous and methanol stem, leaves and flower extract of *Pascalia glauca* Ortega.

Copper as a cupric ion is an essential trace element for algae and higher plants. Copper provides metabolic control over auxin synthesis (Skoog, 1940). It is involved in protein and carbohydrate metabolism. It plays an important role in reproductive growth as well as another trace element whose requirement is known in photosynthesis. It plays an important role in nitrogen metabolism (Hallsworth *et al.,* 1960). Deficiency of copper induces the activity of ferric reductase enzyme which involved in Fe uptake (Kochian, 2000). According to Mizumo *et al*. (1982), the copper deficient leaves exhibit low soluble carbohydrates than normal leaves during vegetative stage. According to Shrivastava and Gupta (1996) it is important in photosynthesis and accumulation of various metabolites, flower setting and grain filling in crops. Bhalerao (2003), Jadhav (2006), Vaidya (2009) explained that incarease in copper content of strawbedrry, sorgthum, chickpea, wheat munbean because of lower concentration of teatments of extract of *Tectaria, Pteridium, Eichhornia, Pistia, Azolla, Ageratum* and sesamum. Same results has been determined from present investigation when test crop seedlings treated at lower concentration 5% and 10% stem, leaves and flower aqueous and methanol extract of *Pascalia glauca* thus, it can be confirmered that the extract of weed species inhanced the copper content. Furthermore, at the heigher concentration of same extract decreased the copper content in the wheat and groundnut seedlings, same results has been postulated by Batish *et al*. (2005) reported decrease in copper due to soil amendment of *Parthenium.*

Manganese is involved in shikimic acid pathway and enhances the resistance of plants to various diseases. Marschner (1986) advocated, it is directly involved as a component of the biotin enzyme in the biosynthesis of fatty acids. Superoxide dismutase incorporating manganese in mitochondria plays an important role in scavenging of free radical (Jimenez *et al.,* 1998). The allelopathic treatments may cause increase or decrease in Mg content of treated plants, which depend on degree of concentration of extract. The researchers including Baziramkenga *et al*. (1994), Khurana *et al*. (1999) Ram and Bose (2000), Bhalerao (2003), Mahmood (2004) Jadhav (2006) and Vaidya (2009) reported that the increase in Mg in soybean, fababean, sorghum, chickpea mungbean, wheat strawberry and cucumber due to lower concentration of leaf extract treatment of *Ipomoea, Cuminum, Allium,*

Hyoscanmus, tectaria, Pteridium, Eichhornia, Pistia, Azolla, Ageratina and sesamum. Our investigation also showed at lower concentration of stem, leaves and flower extract of *P. glauca* has been increased the Mn content in the wheat and groundnut seedlings. Allelopathic treatment of *Chromolena odorata,* there is decline in manganese content were observed in both *Crotolaria verrucosa* and *C. retusa* (Dethe and Gaikawad, 2017). Present results in the line of this work where the nutrients were decreased at higher concentration.

3. Effect of aqueous and methanol extract of stem, leaves and flowers of *Pascalia glauca* Ortega. on photosynthetic pigments :

3.1 Wheat seedlings :

The results on photosynthetic pigments expressed in the Table- 10 and Fig.-1 to 4 showed that the amount of chlorophyll a, chlorophyll b, total chlorophylls and carotenoids pigments in test crops wheat and groundnut from the study area was decreasing.

Wheat seedlings pigments respond concentration dependent decreasing and increasing. The chlorophyll a was declined highest from 20% aqueous leaves extract (0.040) and stem extract (0.042) nearly similar and from methanol leaves extract (0.037) which was higher inhibition within both extract. Lower concentration (5%) of aqueous extract determined nearly similar reduction in leaves extract (0.212) and flower extract (0.215) as well as methanol stem extract (0.208) and flower extract (0.212). Statistically, all tested concentrations represented significant results in the reduction of chlorophyll a pigment. Aqueous extract have more potentiality than the methanol extract but methanol leaves extract determined important source in chlorophyll a reduction. Same that of chlorophyll a, chlorophyll b has been found to be decreasing trend in the pigments. Highest rate of decreasing chlorophyll b was found at the higher concentration from 20% (0.070) in the aqueous extract of leaves.

The total chlorophylls pigments declined (0.109) in the aqueous stem extract and leaves extract (0.110) was nearly identical than that of the methanol leaves extract (0.131).

The carotenoids pigments have been highly reduced at higher concentration (20%) of aqueous leaves extract (0.066) and methanol leaves extract (0.052).

a) Chlorophyll- a :

Aqueous extract of stem slowly decreased the chlorophyll a in 5% concentration (0.221) then it was suddenly reduced in the 10% (0.162), 15% (0.092) to 20% (0.042). Great reduction was determined in the aqueous leaves extract from 10% (0.0154), 15% (0.079) to 20% (0.040) over the control. Aqueous flower extract slowly reduced from 5% (0.215) to 10% (0.195) then it was rapidly decreased from 15% (0.105) to 20% (0.072).

Methanol extract of stem gradually declined from 5% (0.208), 10% (0.146), 15% (0.121) to 20% (0.106). Methanol leaves extract declined higher in higher concentration 20% (0.037) followed by 15% (0.075), 10% (0.136) and slow from 5% (0.199). Methanol extract of flower has reduced highly at the higher concentration 20% (0.112) and 15% (0.171) and it was slow at 5% (0.212) and 10% (0.205) concentration.

Chlorophyll a pigment from aqueous extract of stem reduced 6.31fold, leaves extract 6.74fold and flower extract with 3.70fold over control in 20% higher concentration.

Methanol extract of leaves was determined by 6.34fold reduction then in stem (2.26fold) and flower extract (2.14fold).

Both extracts of leaves maximum reduced the chlorophyll a pigments than stem and flowers extract. Aqueous extract of stem, leaves and flower more affect than the methanol extract.

b) Chlorophyll- b :

Aqueous extract of stem decreased gradually from 5% (0.504) to 10% (0.411) then it was abruptly increased in the suppression of chlorophyll b pigments at 15% (0.262) and 20% (0.067). The maximum reduction was observed from lower concentration 5% (0.468) then it was gradually declined in the 10% (0.314) to 15% (0.234) and 20% (0.070) in the aqueous leaves extract. Aqueous flower extract reduced chlorophyll b step by step from 5% (0.512), 10% (0.441), 15% (0.333) and in the 20% concentration of extract it was maximum declined (0.160).

Chlorophyll-b slowly decreased in methanol stem extract from 5% (0.534) to 10% (0.516) and reduction rate was increased in 15% (0.310) and 20% (0.125) concentration. Methanol leaves extract greatly affected the chlorophyll- b pigments from lower concentrations 5% (0.312), 10% (0.303) again it speed up in 15% (0.191)

and 20% (0.093) concentration. In methanol flower extract it was slowly decreased from 5% (0.505), 10% (0.466) then maximum declined at 15% (0.272) and 20% (0.134).

There is 9.44fold reduction was observed in the chlorophyll-b pigment at higher concentration (20%) of aqueous stem extract followed by leaves extract (9.08fold) and flower extract with 3.96fold in the wheat seedling in sequence of stem > leaves > flower.

Methanol extract of leaves detected maximum reduction (5.81fold) in the chlorophyll-b pigment at 20% concentration followed by methanol stem extracts (4.33 fold) and flower extract (4.03fold).

c) Total chlorophylls :

Total chlorophylls of wheat seedlings has gradually reduced in aqueous stem extract from 5% (0.725) and 10% (0.573) while highest reduction found from 15% (0.354) and 20% (0.109). Aqueous leaves extract was observed the maximum reduction in 20% (0.110) concentration followed by 15% (0.313), 10% (0.437) and minimum decreased in 5% (0.681). Same reduction trend was determined in the aqueous flower extract from 5% (0.727), 10% (0.636), 15% (0.438) to 20% (0.232).

Slow reduction was found in methanol stem (0.742) and flower (0.717) extract from 5% concentration then gradually decreased in 10% (0.662) and (0.672) then it was decreased rapidly from 15% (0.431) and (0.442) and 20% higher concentration (0.231) and (0.245) respectively. Nearly similar reduction was determined in methanol stem and flower extract. Higher amount of total chlorophylls decrease in methanol leaves extract from 20% (0.130) followed by 15% (0.266), 10% (0.439) and 5% (0.511) over to control.

Similar increase in the reduction of total chlorophyll has been determined from aqueous stem (8.18fold) and leaves (8.20fold) extract while flower extract decreased 3.89fold from 20% concentration.

Methanol extracts of stem (3.38fold) and flower extract (3.19fold) increased nearly similar reduction in the total chlorophylls of wheat seedlings. Leaves extract showed maximum declined (5.97fold) at higher 20% concentration.

d) Carotenoids :

Carotenoid was maximum declined (0.066) in aqueous leaves extract from 20% concentration than the stem extract (0.082) and flower extract (0.105). From other concentrations of aqueous stem extract it has found gradual reduction in 5% (0.192), 10% (0.122) and 15% (0.104) while it was slowly reduced from the 5% concentration (0.211) of aqueous flower extract followed by 10% (0.185) and 15% (0.151) concentration. Aqueous leaves extract suddenly decreased the carotenoids from the 5% (0.171), 10% (0.161) and 15% concentration (0.103) in comparison to control.

Methanol extract of stem and flower, carotenoids decreased nearly similar in lower concentration 5% (0.235 and 0.243), 10% (0.192 and 0.196) and in 15% it was reduced by 0.121 and 0.133 and at 20% concentration by 0.095 and 0.090 respectively. Maximum reduction was found in higher concentration of methanol leaves extract at 20% (0.052) followed by 15% (0.101), 10% (0.112) and 5% (0.120).

There was 3.98fold reduction of carotenoids determined in the aqueous leaves extract followed by stem extract (3.20fold) and flower extract (2.50fold) at higher concentration 20% over control. While methanol extract of leaves maximum reduced (4.71fold) carotenoids then in flower extracts (2.72fold) and stem extract (2.57fold) at higher concentration (20%).

3.2 Groundnut seedlings :

The groundnut chlorophyll pigments response variously at difference concentrations depicted in Table- 11 and Fig.- 5 to 8. The lower concentration (5%) of aqueous stem extract showed promoting the chlorophyll-a by 0.878.The chlorophyll-b pigments was also promoted in the 5% aqueous stem extract (1.068) and flower extract(1.114) and in 10% aqueous(1.221) and 5% methanol (1.122) flower extract.

Total chlorophyll pigments has promoting trend in the lower concentration (5%) of aqueous extract of stem (1.946) and flower (1.933) while aqueous flower extract in10% concentration promoted by 1.948. 5% methanol flower extract was also promoted by 1.908 in comparison of control. Methanol extract of stem in 5% concentration the carotenoids has been occurred negligible promoting by 0.230 and in methanol flower extract it was determined by 0.246comparable to control (0.227).

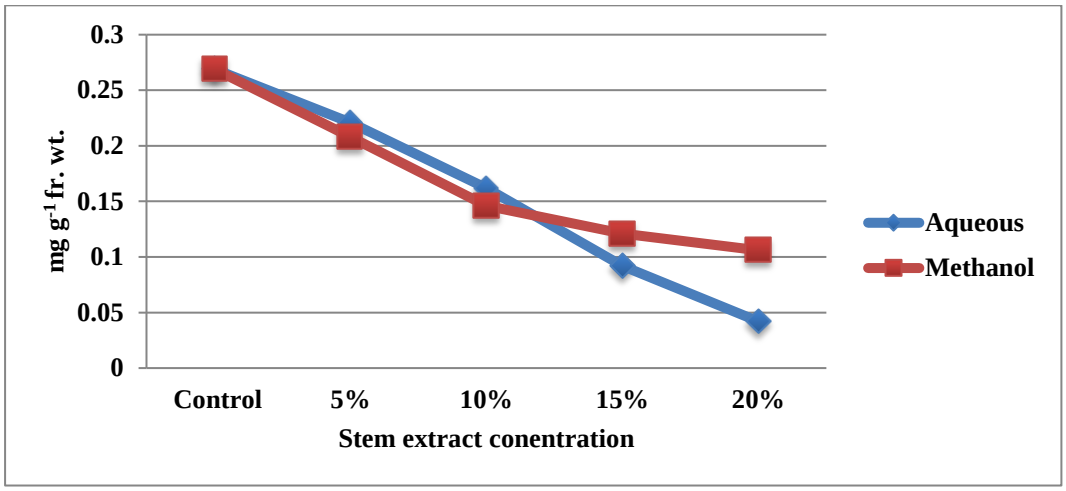
Table - 10 : Effect of aqueous and methanol extracts of stem, leaves and flowers of *Pascalia glauca* Ortega. on photosynthetic pigments of wheat seedlings at 192 hrs. (mg g^{-1} fresh weight).

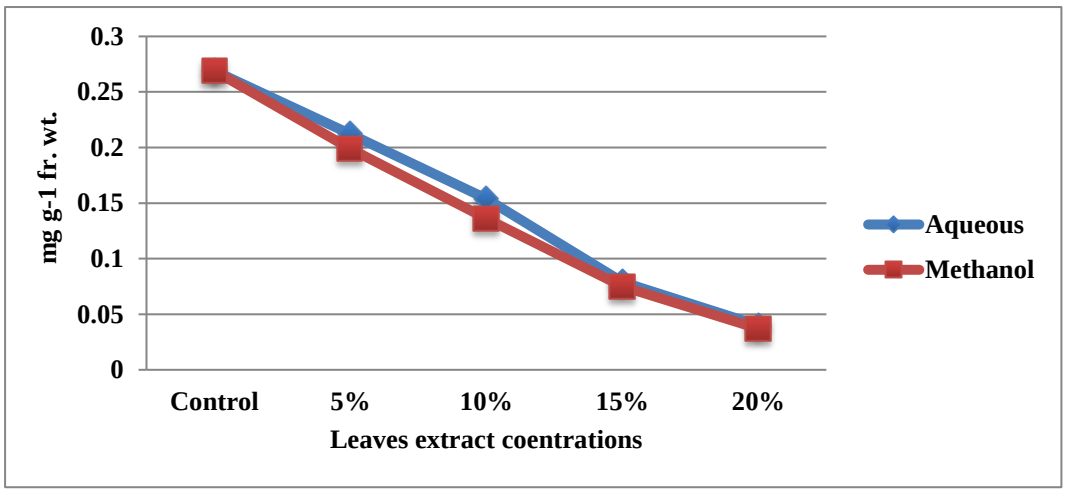
Plant part extract	Chlorophyll - a								
	Concentrations	5%		10%		15%		20%	
	Control	Aqueous	Methanol	Aqueous	Methanol	Aqueous	Methanol	Aqueous	Methanol
Stem	0.269 ±0.006	0.221** ±0.011	0.208** ±0.002	0.162** ±0.002	0.146** ±0.002	0.092** ±0.002	0.121** ±0.004	**0.042**** ±0.005	0.106** ±0.004
Leaves		0.212** ±0.002	0.199** ±0.005	0.154** ±0.004	0.136** ±0.004	0.079** ±0.007	0.075** ±0.003	**0.040**** ±0.002	**0.037**** ±0.001
Flower		0.215** ±0.004	0.212** ±0.002	0.195** ±0.006	0.205** ±0.003	0.105** ±0.003	0.171** ±0.003	0.072** ±0.005	0.112** ±0.006
Chlorophyll-b									
Stem	0.635 ±0.006	0.504** ±0.005	0.534 ±0.002	0.411** ±0.004	0.516 ±0.025	0.262** ±0.005	0.310** ±0.004	**0.067**** ±0.005	0.125** ±0.003
Leaves		0.468** ±0.008	0.312** ±0.003	0.314** ±0.003	0.303** ±0.002	0.234** ±0.004	0.191** ±0.003	**0.070**** ±0.002	**0.093**** ±0.004
Flower		0.512** ±0.003	0.505 ±0.0132	0.441** ±0.003	0.466** ±0.004	0.333** ±0.002	0.272** ±0.004	0.160** ±0.004	0.134** ±0.003
Total Chlorophyll									
Stem	0.904 ±0.0005	0.725** ±0.0004	0.742** ±0.0004	0.573** ±0.0005	0.662** ±0.0005	0.354** ±0.0005	0.431** ±0.0003	**0.109**** ±0.0004	0.231** ±0.0004
Leaves		0.680** ±0.0005	0.511** ±0.0004	0.468** ±0.0007	0.439** ±0.0003	0.313** ±0.0004	0.266** ±0.0005	**0.110**** ±0.0005	**0.130**** ±0.0004
Flower		0.727** ±0.0005	0.717** ±0.0007	0.636** ±0.0004	0.671** ±0.0011	0.438** ±0.0002	0.443** ±0.0007	0.232** ±0.0004	0.246** ±0.0015
Carotenoids									
Stem	0.262 ±0.003	0.192** ±0.002	0.235* ±0.003	0.122** ±0.003	0.192** ±0.004	0.104** ±0.003	0.121** ±0.003	0.082** ±0.002	0.095** ±0.003
Leaves		0.171** ±0.004	0.120** ±0.004	0.161** ±0.003	0.112** ±0.004	0.103** ±0.002	0.101** ±0.002	**0.066**** ±0.003	**0.052**** ±0.002
Flower		0.211** ±0.004	0.243 ±0.003	0.185** ±0.003	0.196** ±0.002	0.151** ±0.005	0.133** ±0.001	0.105** ±0.003	0.090** ±0.002

One way Anova: Tukeys HSD Post-hoc Test Inference. **Significant at $P < 0.01$.

*Significant at $P < 0.05$. Otherwise insignificant result. ±Standard deviation.

Fig. - 1 : **Effect of aqueous and methanol extracts of stem, leaves and flowers of *Pascalia glauca* Ortega. on chlorophyll - a of wheat seedlings at 192hrs.**





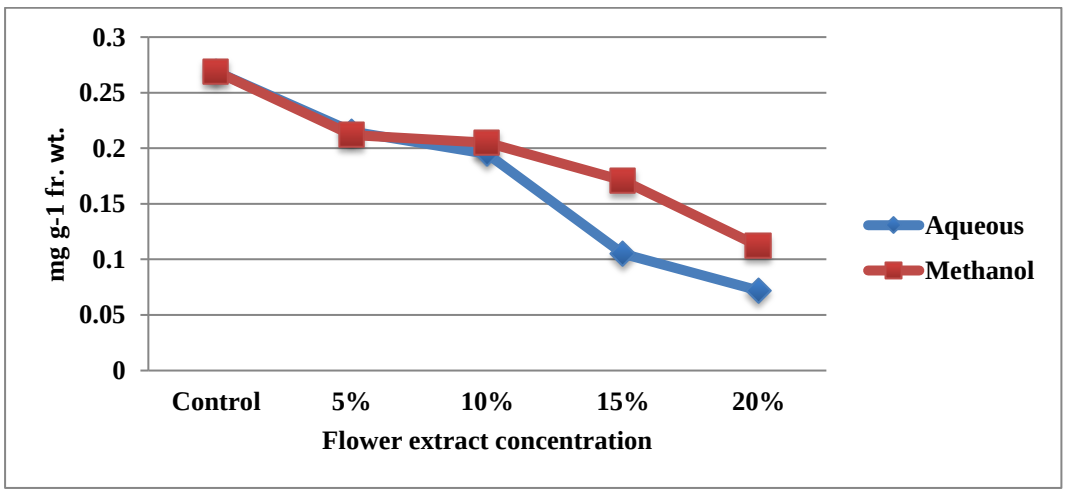
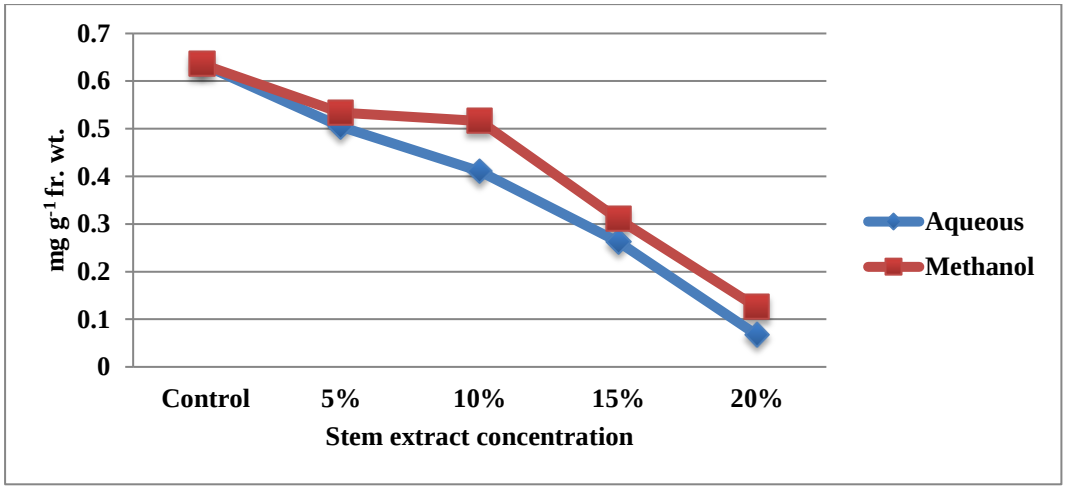
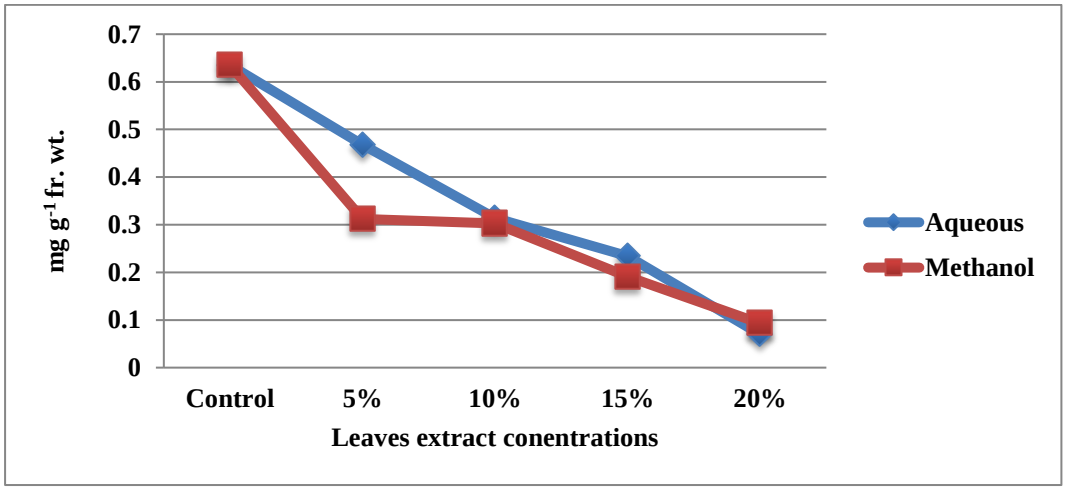


Fig. - 2 : **Effect of aqueous and methanol extracts of stem, leaves and flowers of *Pascalia glauca* Ortega. on chlorophyll - b of wheat seedlings at 192hrs.**





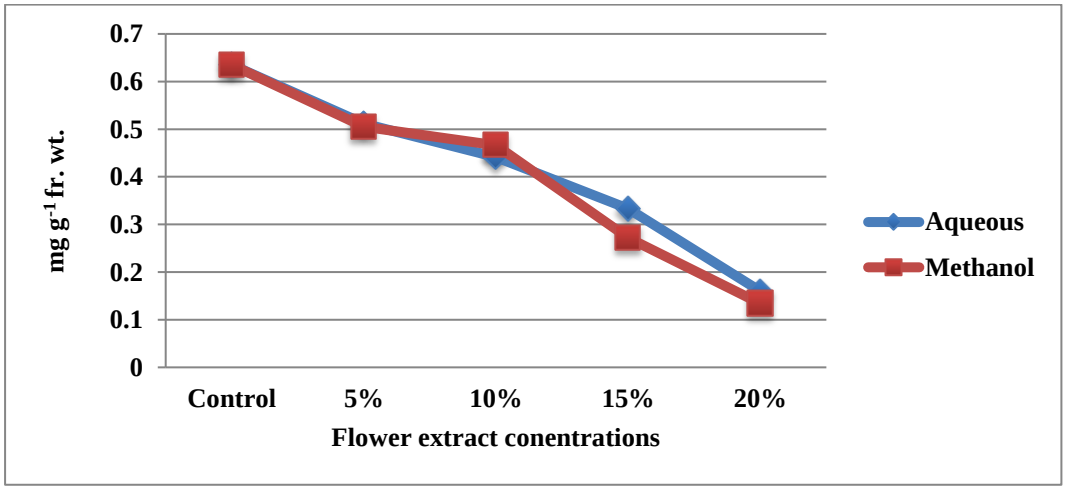
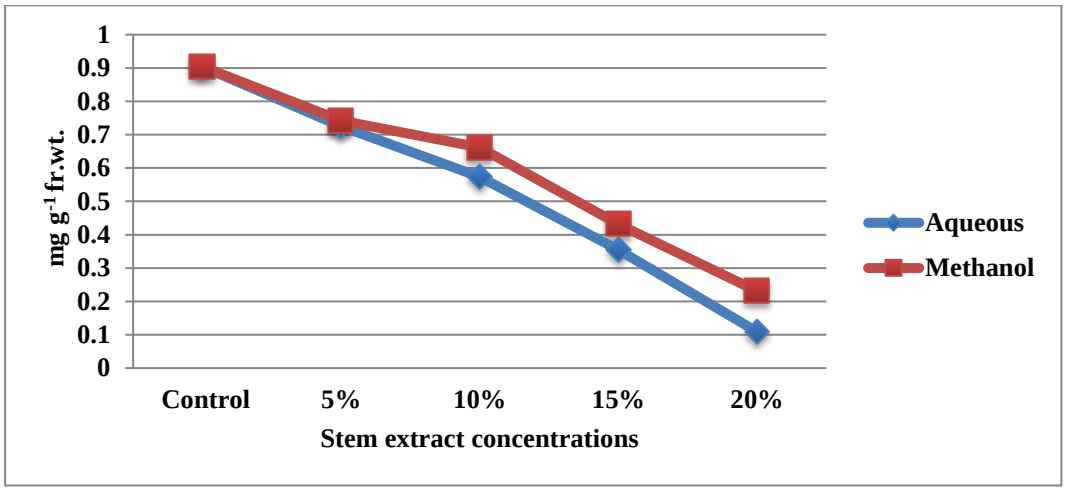
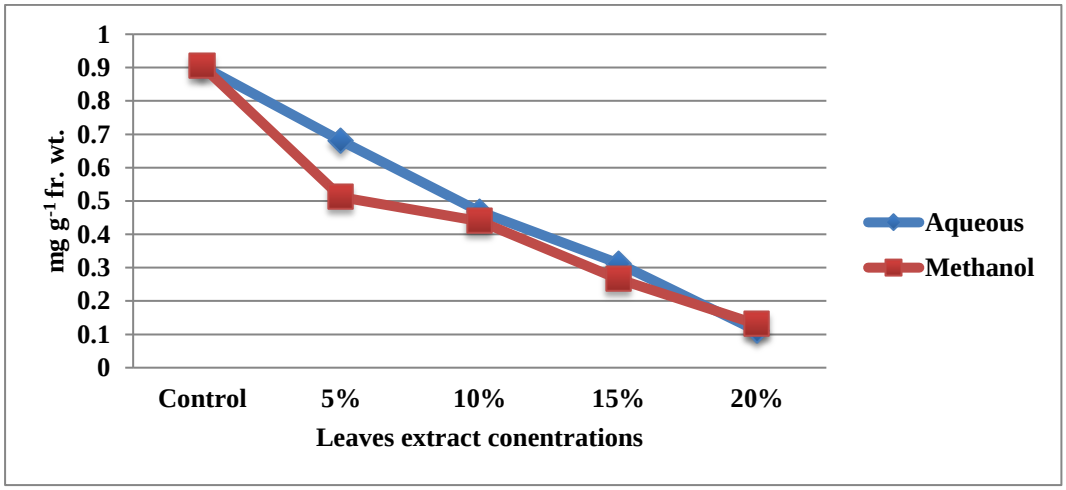


Fig. - 3 : **Effect of aqueous and methanol extracts of stem, leaves and flowers of *Pascalia glauca* Ortega. on total chlorophylls of wheat seedlings at 192hr.**





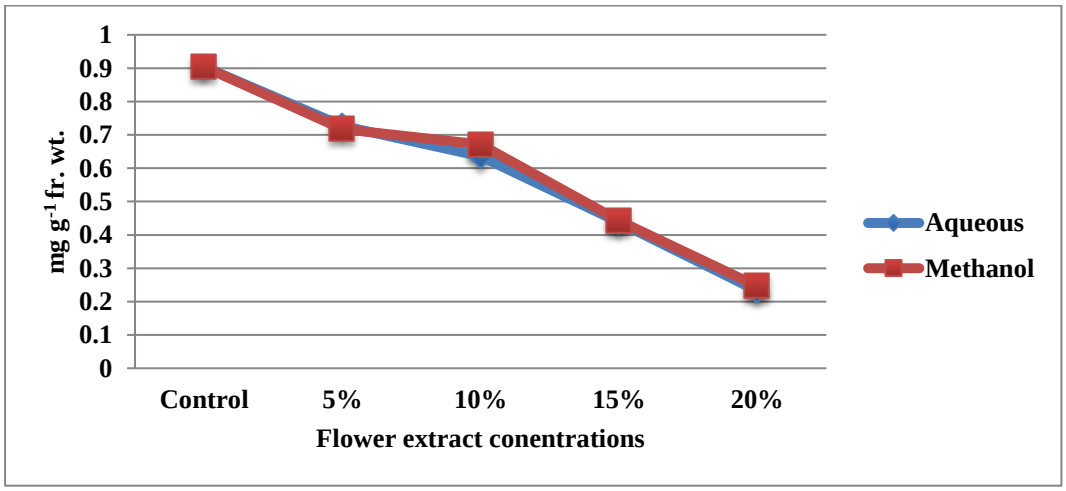
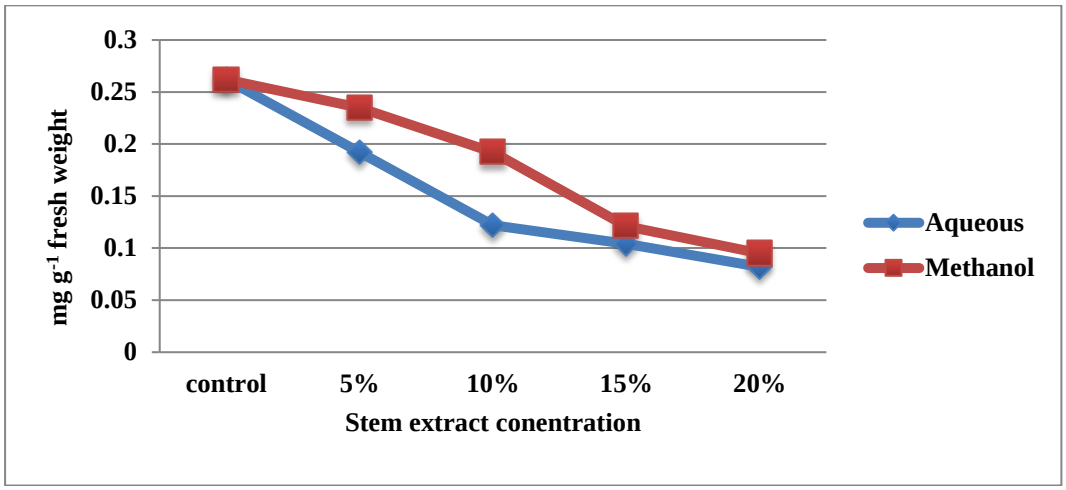
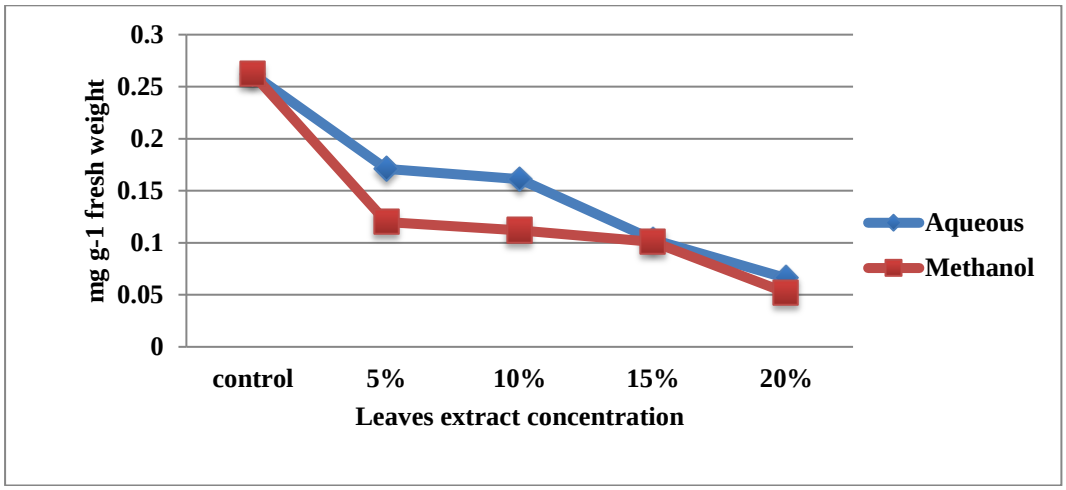
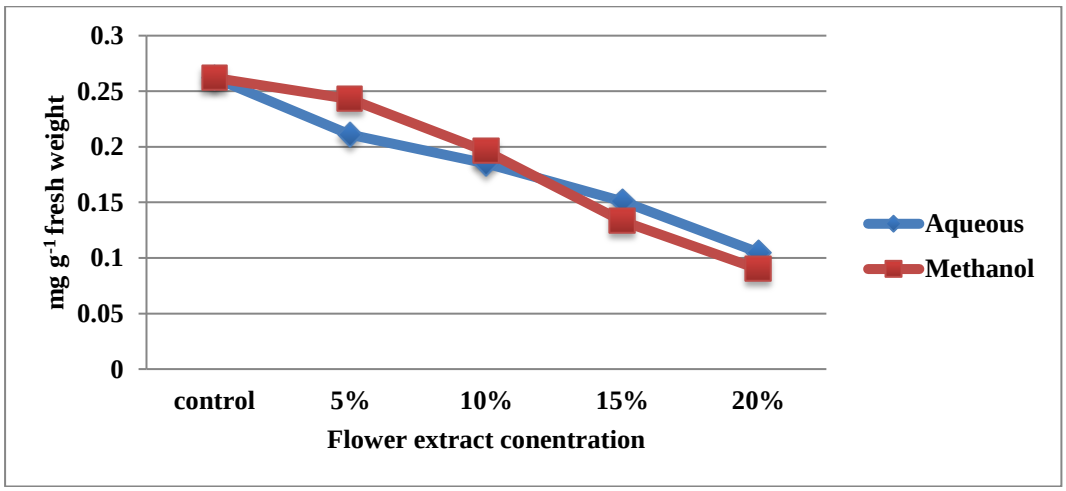


Fig. - 4 : **Effect of aqueous and methanol extracts of stem, leaves and flowers of *Pascalia glauca* Ortega. on carotenoids of wheat seedlings at 192hr.**







a) Chlorophyll-a :

Chlorophyll-a pigment increased from 5% aqueous stem extract (0.878) immediately it was start to decrease from the 10% concentration (0.679) and 15% (0.414) and finally maximum reduction determined from 20% (0.303) as compare to control (0.843). Aqueous leaves extract suppressed slowly from 5% (0.783) and 10% (0.662) then it was speeds up in 15% (0.474) and 20% (0.279) concentration. Same trend of reduction has been found in the aqueous flower extract in 5% (0.819), 10% (720), 15% (0.532) and 20% (0.362).

Chlorophyll-a pigment has found gradual inhibitory results in groundnut seedling from 5% methanol extract of stem (0.774), 10% (575), 15 % (360) to 20% (0.322) concentration. Methanol extract of leaves decreased maximum chlorophyll-a from 20% (0.272) concentration followed by 15% (0.368), 10% (0.630) and 5% (0.728). Methanol extract of flower have reduced slow but surely from 5% (0.786), 10% (0.692), 15% (0.632) to 20% (0.360) concentration.

There is 3.01fold highest reduction of chlorophyll-a has been determined from the higher concentration (20%) of aqueous leaves extract followed by stem extract (2.78fold) and in flower extract (2.33fold).

Methanol extract of stem has been crushed 2.54fold chlorophyll-a pigment while 3.01fold in leaves extract and 2.27fold in flower extract in the higher concentration (20%).

Comparing methanol extract, the aqueous extract not much more difference and nearly similar reduction of chlorophyll-a pigment. In both the extracts in all concentrations except aqueous stem extract in 5% concentration, it occurred promoting while rest of all concentrations showed reduction in the chlorophyll-a pigment. The maximum reduction has found in the aqueous and methanol extract of leaves at higher 20% concentration.

b) Chlorophyll-b :

There is negligible increased chlorophyll-b from aqueous extract of stem in 5% concentration (1.068) over to control (1.066) in the groundnut seedlings further, it was reduced from 10% (0.718), 15% (0.656) and maximum declined fro at 20% (0.554) . At the same way it was promoted in 5% aqueous flower extract (1.114) but noticeable increasing chlorophyll-b from the 10% concentration (1.221) in comparison to the control. Then it decreased in 15% concentration by 0.588 and in

20% with 0.436. Aqueous leaves extract little declined in 5% (1.028) then rate of decreasing increased from 10% (0.728), 15% (0.522) while maximum reduction was found in 20% (0.410) concentration.

Methanol extract of stem has slowly but constantly decreased chlorophyll b from 5% (1.017), 10% (0.736), 15% (0.639) to 20% (0.404) concentration. Methanol extract of leaves has negligible inhibited in 5% concentration (1.025). The rate of declining chlorophyll-b became fasten from 10% (0.628), 15% (0.445) and 20% (0.370) concentration. Flower methanol extract observed promoted activity of chlorophyll-b in the 5% (1.122) concentration but it was decrease in 10% (1.039), 15% (1.016) and 20% (0.723) concentration of flower extract.

The reduction of chlorophyll-b has determined about 1.92fold in aqueous extract of stem, 2.60fold in leaves extract and 2.44fold in flower extract from higher concentration (20%). Methanol leaves extract increased maximum reduction of chlorophyll-b (2.78fold) followed by stem extract (2.53fold) and flower extract (1.41fold)at higher concentration 20%.

Comparatively, methanol leaves extract in higher concentration more inhibitory effect than aqueous extract of leaves. Additionally, lower concentration of 5% aqueous leaves and flower extract increased the chlorophyll-b but the activity has been noticeable increasing in the10% flower extract. Methanol leaves and flower extract from lower concentration (5%) also determined promotory results. These results were vital from present investigation.

c) Total chlorophylls :

Aqueous extract of stem at lower 5% concentration (5%) total chlorophyll pigments has shown increasing activity (1.946) in comparison to the control (1.909), but reduction was slowly but surely found from 10% (1.397), 15% (1.070) and 20% (0.857). Aqueous leaves extract showed gradual reduction in 5% (1.792) and 10% (1.390), 15% (0.996) and 20% (0.689). Aqueous extract of flower surprisingly increasing results in lower concentrations of 5% (1.933) and 10% (1.948) then it was gradually reduced in 15% (1.120) and 20% (0.798).

Methanol extract of flower determined not noticeable increasing (1.908) in total chlorophyll at 5% lower concentration while rest of concentrations found decreasing in 10% (1.731), 15% (1.648) and 20% (1.083). Methanol extract of stem observed gradual reduction in 5% (1.791), 10% (1.311), 15% (0.999), 20% (0.727).

Leaves extract influenced maximum and noticeable reduction in 20% (0.642) followed by 15% (813), 10% (1.257) and 5% (1.753).

Aqueous extract of stem determined about 2.22fold decreased total chlorophylls in groundnut seedlings but more in leaves extract (2.76fold) and flower (2.39fold) extract at 20% concentrations.

Methanol extract of leaves observed highest reduction (2.87fold) in total chlorophyll pigments followed by stem extract (2.53fold) and flower extract (1.70 fold) in comparison to control at 20% concentrations.

The most important and very impressive results found from 5% concentration of stem and flower as well as 10% flower extract of aqueous media and 5% methanol extract has determined increasing activity in total chlorophyll of groundnut seedling.

d) Carotenoids :

Aqueous extract of stem immediately affected from 5% concentration (0.192) so carotenoids decreased from 0.227 control treatments and it can continuous in 10% (0.127) and in 15% (0.111), 20% (0.071) concentration. Same trends has been occurred in the aqueous extract of leaves from 5% (0.156), 10% (0.138), 15% (0.103) and in 20% (0.058) concentration. Aqueous flower extract has determined gradual reduction in 5% (0.230), 10% (0.175), 15% (140) and 20% (0.114) concentration.

Methanol extract of stem and flower showed slow promoting activity of carotenoids in 5% concentration (0.230) and (0.246) respectively. Rest of concentrations in stem extract occurred gradual reduction from 10% (0.178) to 15% (0.111) then it was maximum inhibited in 20% (0.076). Methanol extract of flower decreased from 10% (0.187), 15% (0.122) and 20% (0.092). Methanol extract of leaves has been greatly affect on the activity of carotenoids and it reduced from 5% (0.122) followed by 10% (0.110), 15% (0.093) and 20% (0.073) concentration.

Carotenoids pigment suppressed about 3.89fold in aqueous stem extract, maximum 4.76fold in leaves extract and 2.42fold in flower extract in comparison to control at 20% concentration in groundnut seedlings.

Carotenoids was decreased more about 3.09fold from methanol leaves extract followed by 2.95 fold in methanol stem extract and 2.45 fold in flower extract at 20% in groundnut seedlings.

Discussion:

Photosynthetic pigments in wheat seedling variedly acknowledged when treated with different concentration of stem, leaves and flower extract. They are mostly maximum inhibited in the higher concentration of extracts in general and leaves extract in particular. Lower concentrations did not vividly respond either decreasing or increasing trend of pigments in wheat seedlings but it was some exceptions in groundnut seedling where it was increasing. Stem and flower extract activate nearly similar on wheat and groundnut seedlings. Flower extract did not have more effect as compare to the stem extract from lower concentration. The chlorophyll was declined higher from aqueous leaves extract in both test crops. The degree of inhibition of pigments were mostly in the series of leaves > stem> flower extract in general from aqueous and methanol extract. Gradual declining trend has determined in lower concentration 5% and 10% while the activity has increased from the higher concentrations from 15% and 20% in both extract.

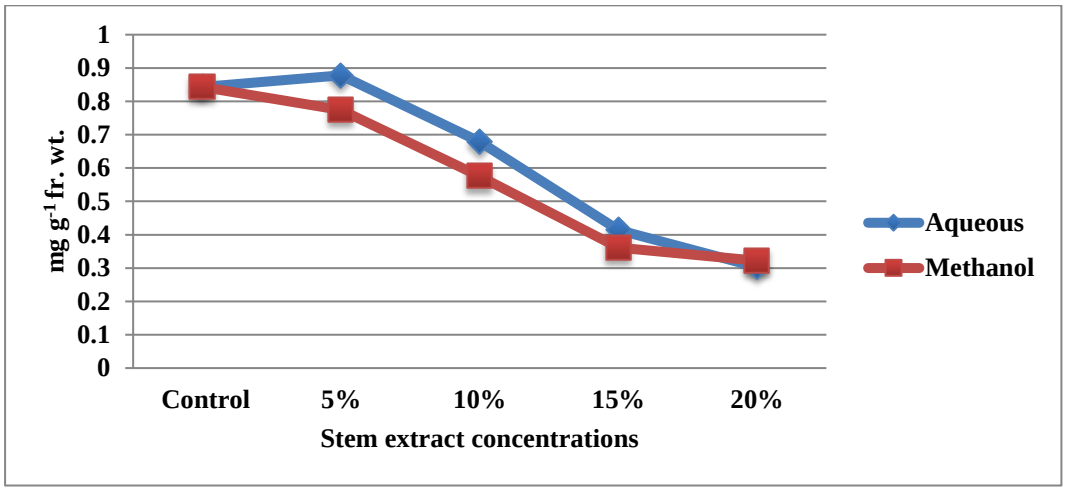
It is very clear from the results in present investigation that *Pascalia glauca* Ortega extract of stem, leaves and flower exert mostly progressively negative effect from lower to higher concentrations on the various pigments of wheat and groundnut. Effect of different treatments of aqueous and methanol extract on photosynthetic pigment content in the series of order aqueous extract > methanol extract on wheat seedling while more fluctuating results was found on groundnut seedling.

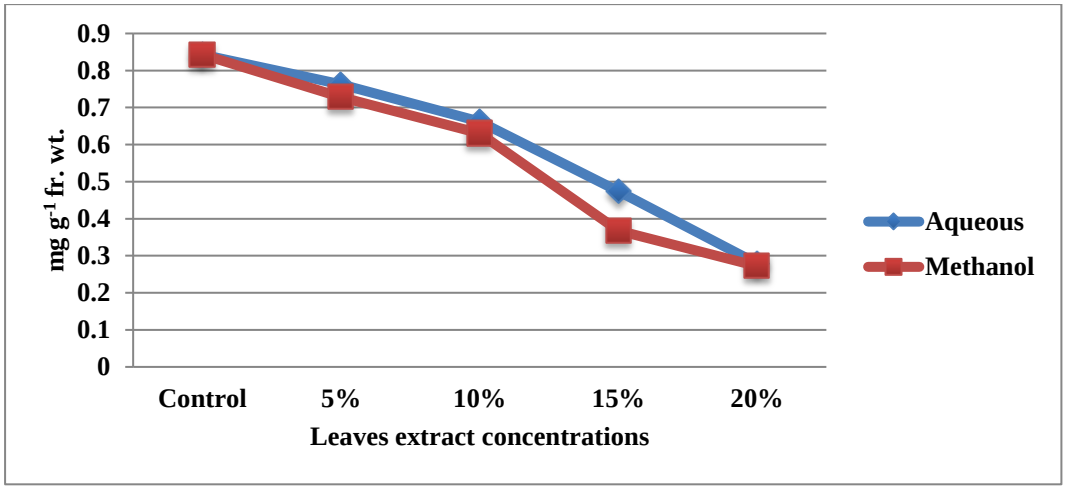
Table - 11 : Effect of aqueous and methanol extracts of stem, leaves and flowers of *Pascalia glauca* Ortega. on photosynthetic pigments of groundnut seedlings at 192 hrs. (mg g^{-1}fresh weight).

Plant part extract	Chlorophyll – a								
	Concentr -ations	5%		10%		15%		20%	
	Control	Aqueous	Methanol	Aqueous	Methanol	Aqueous	Methanol	Aqueous	Methanol
Stem	0.843 ±0.019	**0.878** ±0.015	0.774 ±0.0180	0.679** ±0.013	0.575** ±0.012	0.414** ±0.010	0.3607** ±0.029	0.303** ±0.013	0.322** ±0.015
Leaves		0.763** ±0.022	0.728** ±0.020	0.662** ±0.015	0.630** ±0.022	0.474** ±0.007	0.368** ±0.028	**0.279**** ±0.014	**0.272**** ±0.013
Flower		0.819 ±0.009	0.786 ±0.010	0.720** ±0.019	0.692** ±0.016	0.532** ±0.022	0.632** ±0.026	0.362** ±0.032	0.360** ±0.012
	Chlorophyll-b								
Stem	1.066 ±0.017	**1.068** ±0.031	1.01 ±0.01	0.718** ±0.009	0.736** ±0.021	0.656** ±0.030	0.639** ±0.019	0.554** ±0.034	0.404** ±0.016
Leaves		1.028 ±0.020	**1.025** ±0.024	0.728** ±0.014	0.628** ±0.016	0.522** ±0.014	0.445** ±0.021	**0.410**** ±0.022	**0.370**** ±0.011
Flower		**1.114** ±0.018	**1.122**** ±0.011	**1.221**** ±0.012	**1.039** ±0.025	0.588** ±0.012	1.016 ±0.018	0.436** ±0.027	0.723** ±0.014
	Total Chlorophyll								
Stem	1.909 ±0.000	**1.946**** ±0.0005	1.791** ±0.0009	1.397** ±0.0003	1.311** ±0.0004	1.070** ±0.0004	0.999** ±0.0002	0.857** ±0.0004	0.727** ±0.0005
Leaves		1.792** ±0.0006	1.753** ±0.0005	1.390** ±0.0007	1.257** ±0.002	0.996** ±0.0002	0.813** ±0.0005	**0.689**** ±0.0002	**0.642**** ±0.0002
Flower		**1.933**** ±0.0003	**1.908**** ±0.0004	**1.948**** ±0.0005	1.731** ±0.0003	1.120** ±0.000	1.648** ±0.0007	0.798** ±0.0002	1.083** ±0.0004
	Carotenoids								
stem	0.227 ±0.016	0.192** ±0.024	**0.230*** ±0.008	0.127** ±0.018	0.178* ±0.013	0.111** ±0.006	0.111** ±0.010	0.071** ±0.010	0.076** ±0.020
Leaves		0.156** ±0.013	0.122** ±0.011	0.138** ±0.021	0.110** ±0.006	0.103** ±0.008	0.093** ±0.010	**0.058**** ±0.012	**0.073**** ±0.021
Flower		0.230* ±0.017	**0.246** ±0.008	0.175** ±0.009	0.187** ±0.011	0.140** ±0.012	0.122** ±0.012	0.114** ±0.011	0.092** ±0.004

One way Anova: Tukeys HSD Post-hoc Test Inference. **significant at P < 0.01. *Significant at P < 0.05. Otherwise insignificant result. ±Standard deviation.

Fig. - 5 : **Effect of aqueous and methanol extracts of stem, leaves and flowers of *Pascalia glauca* Ortega. on chlorophyll-a of groundnut seedlings at 192 hrs.**





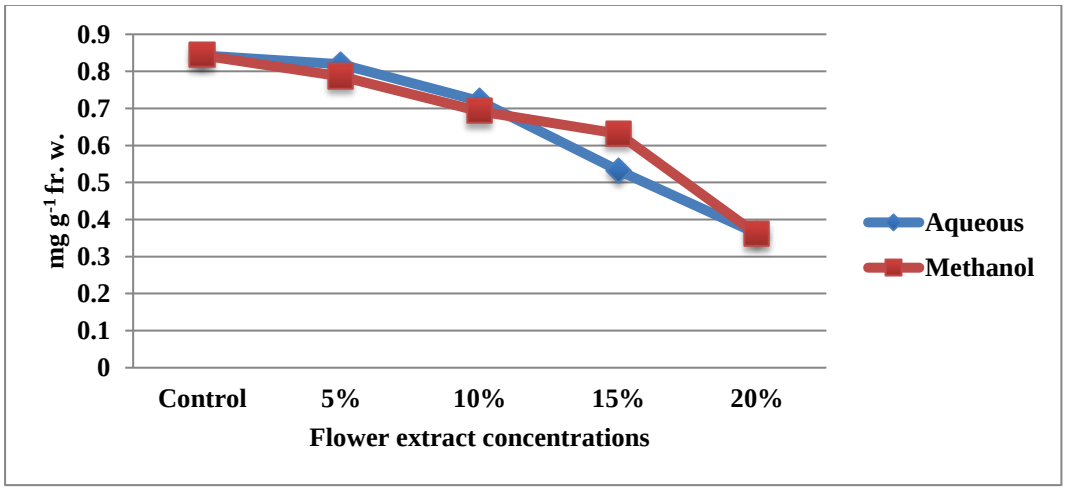
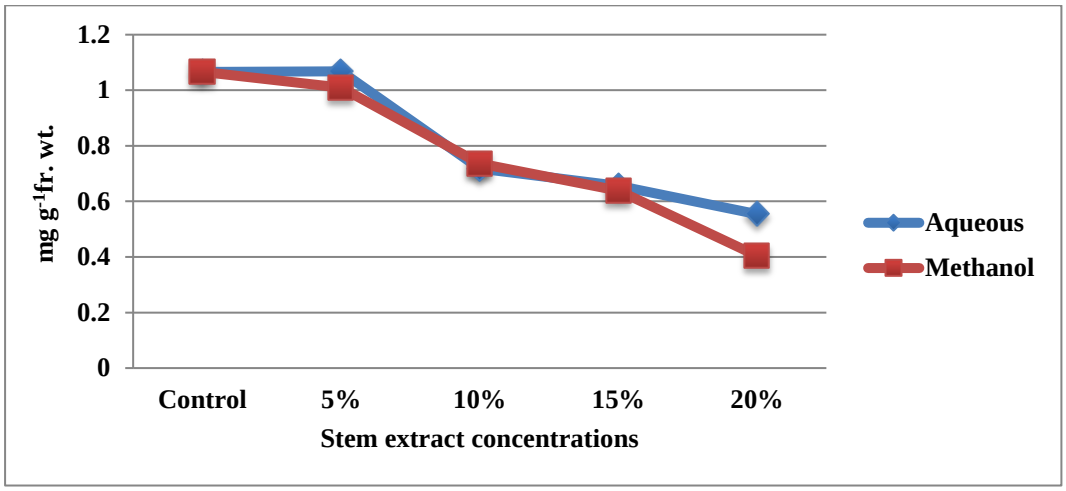
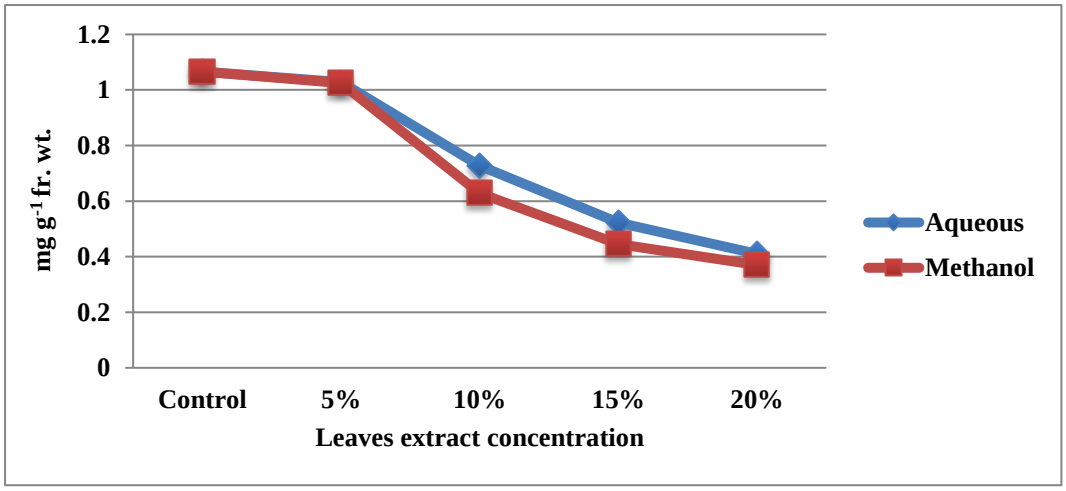


Fig. - 6 : **Effect of aqueous and methanol extracts of stem, leaves and flowers of *Pascalia glauca* Ortega. on chlorophyll- b of groundnut seedlings at 192 hrs.**





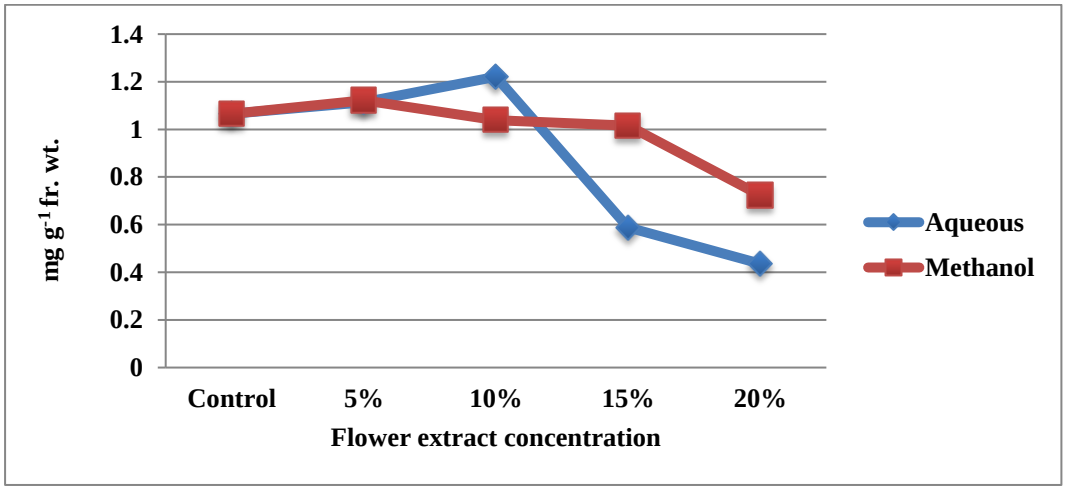
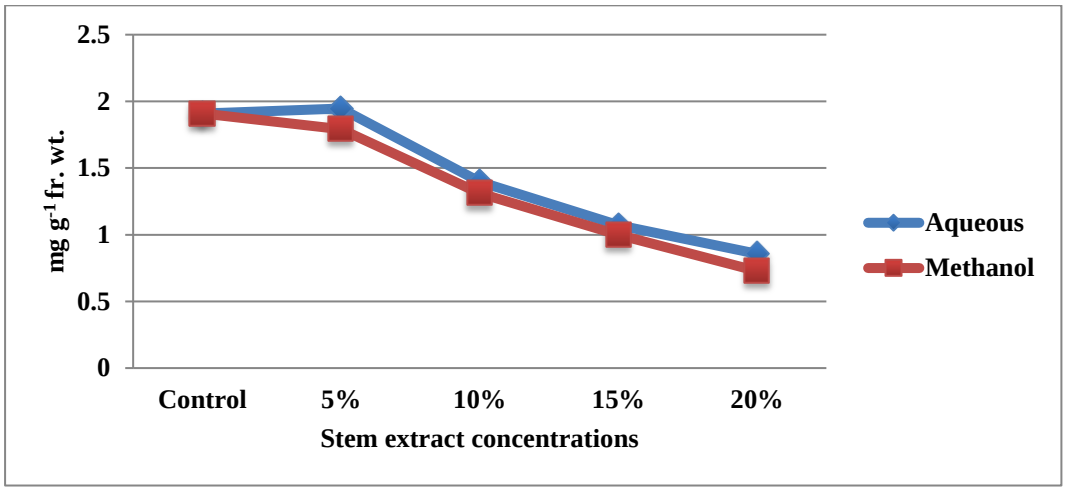
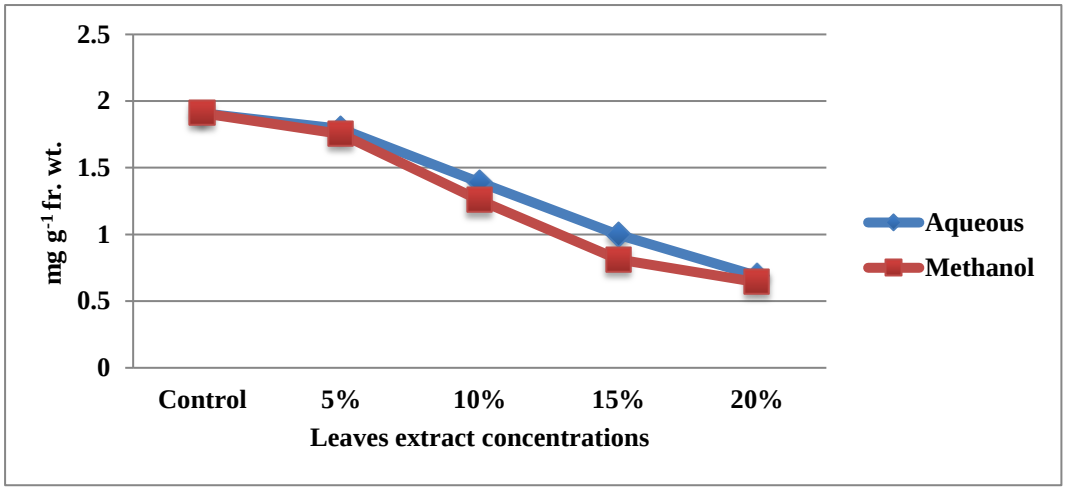


Fig. - 7 : **Effect of aqueous and methanol extracts of stem, leaves and flowers of *Pascalia glauca* Ortega. on total chlorophylls of groundnut seedlings at 192 hrs.**





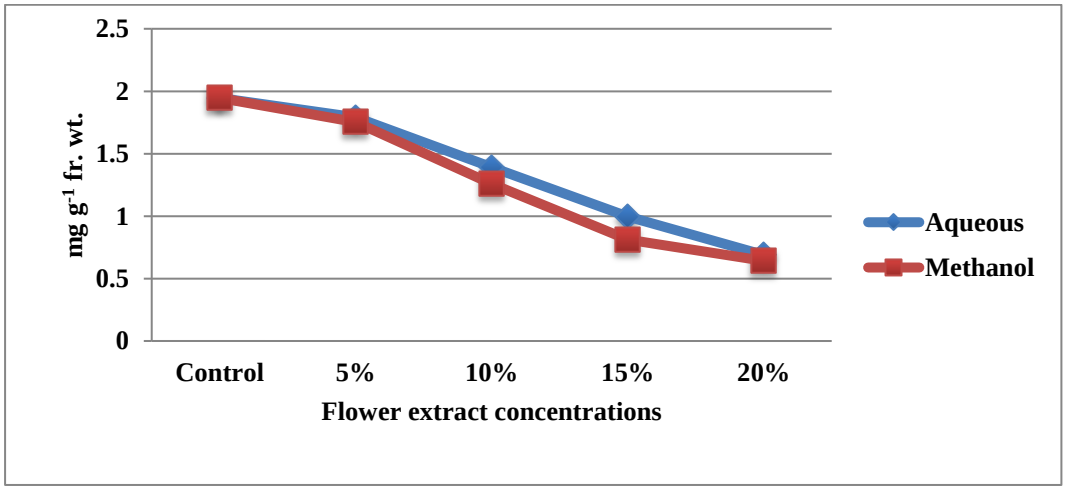
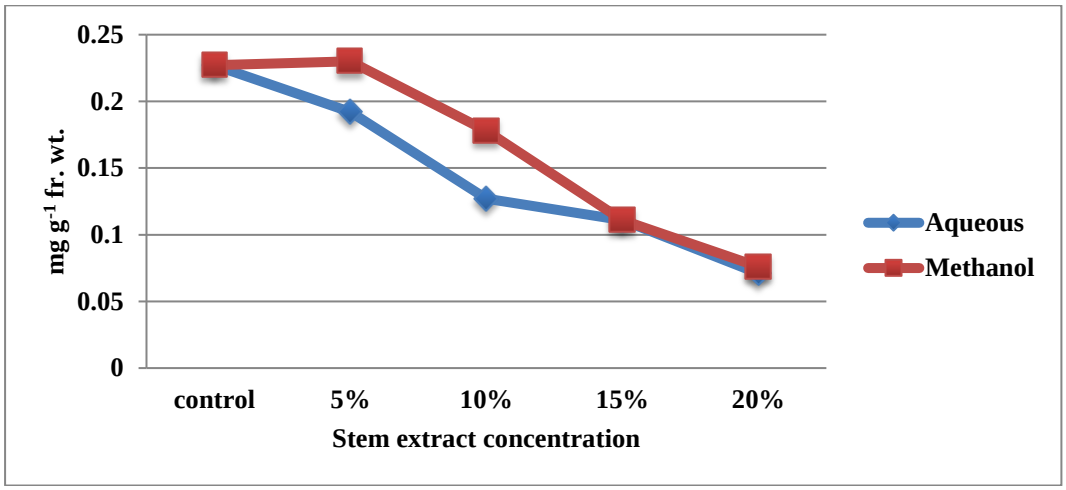
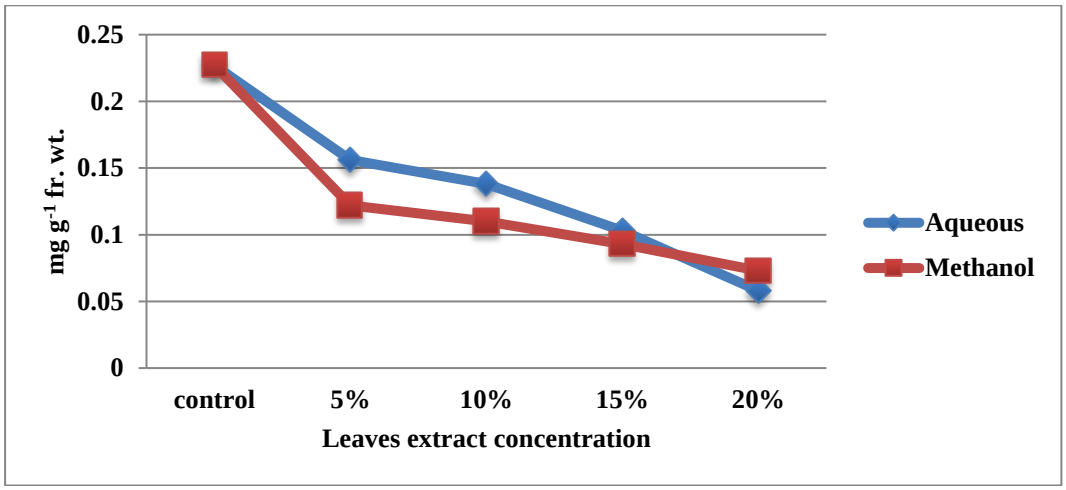
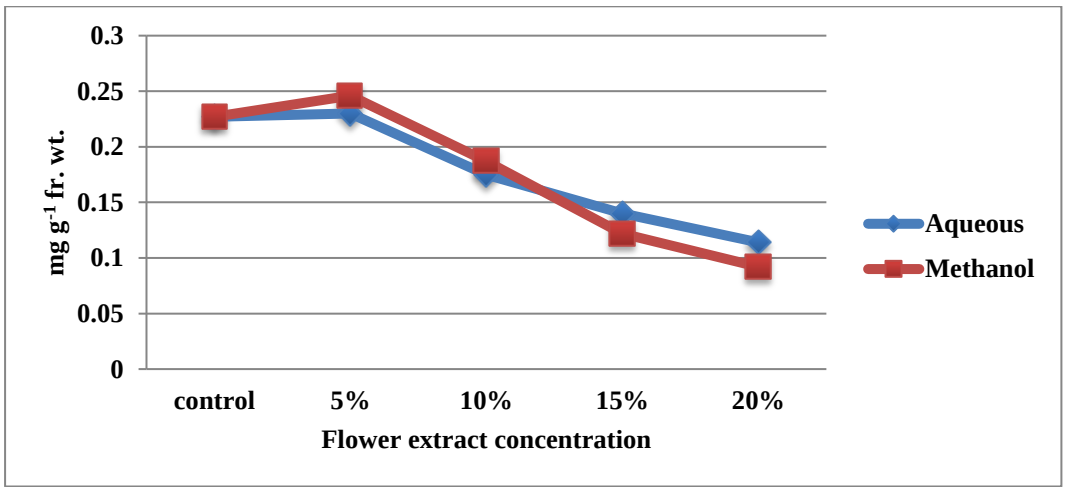


Fig. - 8 : **Effect of aqueous and methanol extracts of stem, leaves and flowers of *Pascalia glauca* Ortega. on carotenoids of groundnut seedlings at 192 hrs.**







Chlorophyll-a, b, total chlorophyll raised from 5 and 10% aqueous extract, 5% methanol extract of flower as well as carotenoids from 5% methanol extract of flower found increasing trend, was important investigation in present work. Methanol has more influence than the aqueous extract on groundnut seedling. Leaves extract have determined more potentiality on the decreasing the pigments might be more detrimental bioactive compound synthesis than the stem and flower from both aqueous and methanol extract.

Chlorophylls are important green pigments in plants performing the mechanism of photosynthesis and highly efficient in absorbing solar energy. Photosynthetic pigments constitute the photosystems. Chlorophylls act as an electron gun and participated in the conversion of solar energy into chemical energy. Chlorophylls are basically the magnesium chelates of closed tetrapyrrole rings derived from protoporphyrin through a number of steps which together with iron porphyrin represents the end products of porphyrin metabolism in plants. Chlorophylls are belongs to a class of lipids. Higher plants are characterized by the presence of Chlorophyll a and Chlorophyll b, which are part and parcel of the photosynthetic apparatus. Among these two pigments chlorophyll a plays a key role in light reactions as it appears as the key pigment in the leaves while Chlorophyll b plays an accessory role. As the chlorophylls plays a key role in light reactions, its content as well as the state of these pigments have direct influence on the photosynthetic efficiency of the plant. Induced chlorophyll a fluorescence *in vivo* reflects underlying changes in pigment composition and the electron transport through PS II. Chlorophyll fluorescence yield is used as a measure of photosynthetic efficiency. Being essential component of photosynthesis, chlorophyll contents are positively co-related with photosynthetic rate. Assessment of pigment content has also become an effective means of monitoring plant growth and estimating photosynthetic productivity (Chen *et al*., 2007).

The concentration of chlorophyll affects the photosynthetic rate as they absorb the light energy without which the reactions cannot proceed. There is gradual increase in Chlorophyll a level appears with increase in the crop age (Gupta and Goyal, 2010). Chlorophyll deficiency results in chlorosis of leaves. Carotenoids are associated with chlorophyll-binding proteins in chloroplasts however, chromoplasts developed carotenoids lipoprotein structure to sequester carotenoids (Vishnevetsky *et al*., 1999). Carotenoids are accessory pigments and chemically tetraterpenoids that

naturally occurring in the chloroplasts and chromoplasts of plants. They absorb light energy, useful in photosynthesis and they protect chlorophylls from photodamage. In higher plants carotenoids function as antenna pigments, involved in harvesting and transmitting radiant energy with some losses to chlorophyll a molecules. Carotenoids act as carriers of oxygen through epoxidation.

Physiological status of the plant depends on the chlorophyll a and b total chlorophyll and carotenoids concentration that correlated to the photosynthetic potential of a plant (Young and Britton, 1990). Chlorophylls are important molecules which act as core component of pigment complexes surrounded the photosynthetic membrane and play a vital role in photosynthesis metabolism (Siddiqui and Zaman, 2005). Several researchers have postulated that chlorophyll content and ion uptake reduced significantly in nearby plants due to allelochemicals released by donner plant species (Al-saadawi *et al.*, 1986). Plant growth inhibition is achieved through disruption of photosynthesis as well as reduced chlorophyll development (Einhellig and Souza, 1992). Cumarins compounds have been found to inhibit the seed germination, growth and likely interference in photosynthetic products like chlorophyll, respiration, nutrient uptake and metabolism (Abenavoli *et al*., 2001, Razia *et al*., 2010 and Abenavoli *et al*., 2004). Kang *et al*. (2010) reported that chlorophyll content and photosynthetic rate increased at the early stage of groundnut, whereas decreased continuously at the later growth stages. Our results support their results and findings where the aqueous stem and flower extract and methanol extract of flower at lower concentration (5%) increased chlorophyll a, b and total chlorophyll in the early growth stages of groundnut.

Salicylic acid a well known allelochemical promoted the chlorophyll pigments. Photosynthetic pigments such as chlorophyll a, chlorophyll b, carotenoids and total pigments elevated in salicylic acids and Baruim treated maize plants subjected to salinity stress. Singh and Usha (2003) recorded increase in total chlorophyll content in wheat seedlings in response to salicylic acids treatment. Allelochemicals leaching from plants with phenolic property may partially block the biosynthetic pathway of chlorophyll or stimulate the degrading pathway of chlorophyll and reduce photosynthesis process (Siddiqui and Zaman, 2005). The allelochemicals released to the environment by poisonous plant species, have significant effects on neighboring plants by reducing the rate of photosynthesis and respiration processes (Gniazdowska and Bogatek, 2007). Salicylic acids increased

chlorophyll contents in strawberry plants (Karlidag *et al*., 2009). Hayat *et al*. (2009) emphasized that salicylic acids treatments to heat stressed mustard plant increased the levels of chlorophyll. Salicylic Acids administration enhanced about 38.66% chlorophyll concentration in the leaves of corn (Farahbakhsh and Saiid, 2010). Singh *et al*. (2010) found that salicylic acids increases chlorophyll content in isolated cucumber seedlings. Many researchers have reported that chlorophyll content and other physiological parameters were reduced significantly by allelochemicals present in donor species (Alsaadawi *et al*., 1986). Decreased photosynthetic products like chlorophyll pigments were indication of presence of allelopathic compounds like phenolic acids released by plants in the surrounding (Patterson, 1981 and Yue *et al*., 2003). Peng *et al*. (2004) stated that allelochemicals affect the photosynthetic activity in plant by destroying chlorophyll molecules. Allelochemicals responsible for breaking of the chlorophyll molecules by activation of pyrrolica ring and phytol chain through which inhibit the synthesis of chlorophyll, automatically reduction in chlorophyll content in the plant (Blum *et al*., 1993).

Yang *et al*. (2006) reported that the reduction of chlorophyll pigments in rice seedlings was caused by leachates of *Ageratina adenophora*. The allelopathic effect of *Sinapis arvensis* root and shoot organs aqueous extracts at 0.5% and 1.5% concentrations against *Brassica napus* revealed that shoot extract inhibited chlorophyll-a and total chlorophyll at 1.5% concentration and Chlorophyll bwas reduced at 1.5% concentration of root extract (Haddadchi and Khorasani, 2006). Total chlorophyll contents and the amount of proteins and carbohydrates of *Cicer arientinum* and *Pisum sativum* were reduced when crops were grown in soil amended with 5, 10, 20 and 40 g residue kg-1 soil of *C. murale* (Batish *et al*., 2007a). Otusanya (2008) demonstrated that leachates of *Tithonia diverifolia*inhibited the photosynthetic pigments in *Lycopersicon esculentum* and *Capsicum annum*.

Oyerinde *et al.* (2009) who revealed the decrease in chlorophyll-a, chlorophyll-b and total chlorophyll accumulation in young plants of maize after being treated with fresh shoot aqueous extract of *Tithonia diversifolia* which possess allelopathic characteristics. Saeid Abu-Romman (2011) noticed that the *Achillea biebersteinii* affected negatively on germination percentage, radical and shoot length as well as chlorophyll a, b, total chlorophyll, carotenoids and protein content of *Capsicum annum* L. The chlorophylls were hampered by the treatment of aqueous extract of *Cassia tora* L. in *Brassica campestris* L. was investigated by the Sarkar *et*

al. (2012). The stimulatory effect was observed at lower concentrations while higher concentrations were observed to cause inhibitory effect on photosynthetic pigments viz. chlorophyll *a*, *b* and total chlorophyll (Kavitha and Arumugam, 2012). Ibrahim *et al*. (2013) stated that allelopathic effect of leaves of GM and non GM extract significantly suppressed the amount of chlorophyll content. The growth development and chlorophyll contents of wheat seedlings had been reduced by *Coronopus didymus* L. (Khaliq *et al*., 2013). Chlorophyll content was reduced in *Chenopodium album* L., *Chenopodium murale* L., *Cassia tora* L. and *Cassia sophera* L. after the treatment of aqueous leaf leachate of *Tinospora cordifolia* (Abdul Raoof and Siddiqui, 2013). Omezzine *et al*. (2014) declared that *Trigonella foenum-graceum* extract cut chlorophyll contents in lettuce due to allelopathic potential of mixoploid. Our results are in these lines of agreement with above findings where photosynthetic pigments were suppressed in the higher concentration of *Pascalia glauca* aqueous as well as methanol extract on the wheat and groundnut seedlings.

Gulzar and Siddiqui (2014) reported chlorophyll contents were reduced in weeds namely, *Amaranthus spinosus* L., *Cassia tora* L. and *Cassia sophera* L. after the treatment of *Eclipta alba* extract. Leela *et al*. (2014) demonstrated the aqueous leaf extract of *Casurina equisetifolia* L. against rice which showed significant reduction in the chlorophyll content. Similar results regarding the effects of allelochemicals released by *Azdirechata indica* leaf extract on *Vigna radiate* studied by Shruti *et al*. (2014) and they further stated that the aqueous leaf extract of *Azadirachta indica* showed both inhibitory and stimulatory effects on photosynthetic pigments, carbohydrate, protein and phenol content in *Vigna radiata* (green gram) seedlings and they observed that aqueous leaf extract of *Azadirachata indica* had been stimulatory effect at lower concentration while 5% and 10%, 15%, and 20% concentration showed inhibitory effect on chlorophyll a, b, total chlorophyll and carotenoids that reduced in the seedlings of *Vigna radiata* may be due to presence of some allelochemicals in leaf extract. Present results has been emphasizing on the allelopathic potential of *Pascalia glauca* Ortega that reduce the chlorophyll a, b, total chlorophyll and carotenoids content and leaves are considered to be most important source because of all pigments were suppressed in the higher concentration of aqueous as well as methanol extract. It might be attributed by various allelochemicals present in *P. glauca* Ortega.

Allelopathic effect of *Croton bonplandianum* has been cause reduction in chlorophyll content of *Triticum aestivam* and *Brassica campestris.* was reported by Sarkar and Chakraborty (2010). Mahmoud (2015) stated that aqueous leaves extract (10 and 20%) concentration of *Amaranthus cruentus, Sinapis arvensis, Sisymbrium irio and Sonchus oleraceus* weeds from Egypt strongly affected on *Triticum aestivum* and *Vicia faba* seedlings and reduced chlorophyll a, b and total chlorophyll. Salgude *et al*. (2015) demonstrated the experiment for effect of aqueous extracts of *Cuscuta reflexa* on the wheat seedlings and they observed that in all the concentrations of *C. reflexa* were decreased the chlorophyll a, b, total chlorophyll and carotenoid where the maximum chlorophyll and carotenoids reduced in the higher concentration (100%) and minimum suppression has been noted in the lower concentration (25%). The aqueous effect of *Hyptis sauveolens, Ricinus communis, Alternanthera sessilis, Ipomoea carnea, Malacara capitata* and *Cymbopogon citratus* on wheat seedlings had been studied by Joshi and Joshi (2016) and who observed that significant reduction total chlorophyll content of wheat seedlings in all the concentrations of above tested weeds but *Ipomoea carnea* and *Cymbopogon citratus* suppressed more total chlorophyll content in wheat. Same results presented by Patil and Khade (2017) who stated that the higher concentration of aqueous extract of *Celosia argentea* inhibited the photosynthetic pigments in *Vigna aconitifolia* and *Trigonella foenum graecum*. Chlorophyll contents of *Parthenium hysterophorus* seeds decreased when treated with aqueous leaf extract of *Datura metel*, additionally he stated that the chlorophyll was completely inhibited in higher (50 and 75%) concentration of *D. metel* (Ramachandran, 2017).

Suppression of chlorophyll pigments in groundnut after the treatment of extracts of bamboo (Eyini *et al*., 1989). Kavitha *et al.* (2012) examined that the leaf extract of *Excoecaria agallocha* continuously reduced the total chlorophyll pigments with increasing the concentration of extract from 2% to 25% in the *Arachis hypogaea*. Allelochemicals released by weeds disturb numerous essential physiological processes in crop plants such as protein synthesis, chlorophyll synthesis and maintenance, photosynthetic rate and respiration their by impact upon plant growth and development (Zohaib *et al.,* 2016*).*

Present innovation is kept on the right track to express additively or synergistically allelopathic effect of *Pascalia glauca* Ortega on wheat and groundnut seedlings as compare to above advocatory statements. Grater reduction of

photosynthetic pigments has been found in higher concentration of both extract. Aqueous and methanol leaf extract determined more allelopathic effects than the stem and flower on photosynthetic pigments. Statistically, our results were significant at $P < 0.01$ in the reduction of chlorophyll-a, b, total chlorophyll and carotenoids from present investigation in all concentration of aqueous and methanol extracts in wheat seedling.

Fluctuating results were found in the groundnut seedlings where the lower concentration (5%) of aqueous stem extract insignificantly increased chlorophyll-a, 5% flower extract insignificantly decreased chlorophyll-a while 5% methanol stem and flower extract showed insignificant decreased chlorophyll-a. Chlorophyll-b in 5% aqueous stem, and flower extract insignificantly increased results while 5% aqueous leaves extract insignificantly decreased. Chlorophyll-b insignificantly increased in 5% methanol leaves and 10% flower extract while 5% methanol stem and15% flower extract insignificantly decreased in groundnut seedling. Total chlorophyll in 5% aqueous stem and flower extract showed significantly increased while 5% methanol flower extract significantly increased total chlorophyll pigment. Carotenoids pigments occurred insignificantly increased in the 5% methanol stem and flower extractof the groundnut seedlings.

4. Effect of aqueous and methanol extracts of stem, leaves and flowers of *Pascalia glauca* Ortega. on sugars of wheat and groundnut seedlings :

4.1 Wheat seedlings :

The major end products of photosynthetic carbon metabolism are sucrose and starch while glucose is a substrate for respiration. Sucrose is the prime end product of photosynthetic carbon assimilation and involved in assimilation partitioning. Sugars are considered as product of hydrolytic processes participated in energy production, sugar sensing and signaling systems. Soluble sugars are major constituents of osmotic adjustment maintaining turgor pressure and stabilizing cellular membranes (Morgan, 1994 and El-Tayeb and Ahmed, 2010). Sucrose decomposes to glucose and fructose and starch breaks-down to glucose increases osmotic pressure of cells (Benbella, 1999). Sugar responsible genes give various defensive responses and cellular expansion (Koch, 1996).

The carbohydrate synthesized in every green plant by the process of photosynthesis to fulfill energy requirement. Carbohydrate contents including

reducing sugars, total sugars and starch has play significant role in the metabolic activity in the plant. Synthesis and accumulation of carbohydrates has been formidable and essential requirement for all metabolic process almost in all green plants. In plants carbohydrates represent one of the great consequence groups of organic compounds that form a connection between photosynthesis and respiration useful in the plant yield that depends upon its carbohydrate status.

Total carbohydrates and their constituents including reducing sugars, total sugars and starch have shown reduction from lower concentration to higher concentration both in aqueous and methanol extract of stem, leaves and flower of *Pascalia glauca* depicted in Table-12. Lower concentrations showed minor effect while the rate of carbohydrates reduction was much more drastic in the higher concentrations of both extract.

Maximum inhibition of total sugar (4.05) and starch (3.47) has found in the aqueous stem extract at 20% concentration while reducing sugar (3.10) from leaves extract. Higher inhibition of reducing sugars (2.15), total sugars (2.06) and starch (2.12) determined in higher concentration (20%) of methanol leaves extract was nearly the same. Maximum decline of total carbohydrates determined from aqueous stem extract (10.86) and methanol leaves extract (6.31) over control while minimum in the aqueous (19.41) and methanol (18.45) flower extract against control.

4.1.1 Reducing sugars:

The declining trend of reducing sugars have found as per extract concentrations treatment increasing from 5% (5.43), 10%(4.20), 15% (3.33) to 20% (3.31). Leaves aqueous extract suppressed greater amount of reduction from 20% (3.10) followed by 15% (4.10), 10% (4.38) and minor reduction in 5% (5.23) as compare to control. The flower extract has been inhibited slowly from 5% (5.80) and 10% (5.39) then it increased in the reduction from 15% (4.47) to 20% (3.98) concentration.

Reducing sugars decreased at 5% methanol extract of stem (5.11), 10% (4.54), 15% (4.14) and maximum reduced in 20% (2.99) concentration. The leaves extract highly affected wheat seedlings and maximum reduction was observed from 20% (2.15) followed by 15% (3.52), 10% (4.54), and 5% (5.09) while methanol extractof flower was suppressed almost equal in 5% and 10% (5.14) then it became slowly in 15% (4.57) and more decreased in 20% (3.40) concentration.

Reducing sugars of wheat seedlings highly decreased from higher concentration (20%) of leaves extract (1.87fold) followed by stem (1.75fold) and flower extract (1.46fold) over control.

Methanol extract of flower decreased by 1.71fold reducing sugars of wheat seedlings and in stem (1.99fold) while maximum reduction was seen about 2.70fold in leaves extract from 20% higher concentration treatment over the control.

4.1.2 Total sugars:

Aqueous extract of leaves reflected more reduction in the total sugars at 20% (4.08) concentration followed by 15% (5.16), 10% (5.60) and at 5% concentration (6.18). In stem extract treatment total sugars declined from 5% (6.52), 10% (5.20), 15% (4.52) to 20% have higher reduction (4.05). The flower aqueous extract has showed minor reduction in 5% (6.71) trailed gradually from 10% (6.22) to 15% (6.13) and maximum in 20% (5.38) concentration in comparison to the control.

Methanol extract of leaves had maximum total sugars at the 20% (2.06) concentration than the control (6.87); doubled in the 15% (3.72) while lowered from 5% (5.12) to 10% (4.16). Stem extract reduced from 5% (6.17) to 10% (5.61) then in 15% (3.72) and more in 20% (2.48) concentration. In the methanol extract of flower it reduced progressively from 5% (6.25), 10% (6.16), 15% (6.04) while in 20% it has decreased suddenly (3.17).

The total sugars have reduced 1.69fold in aqueous stem extract, 1.68fold in leaves extract that means it was similar reduction while 1.27fold in aqueous flower extract at 20% concentration over the control i.e. stem > leaves > flower.

Methanol extract of leaves has been declined 3.33fold which was maximum reduction in the total sugars in comparison to stem (2.77fold) and flower (2.16fold).

4.1.3 Starch:

Starch is another important component of carbohydrates decreased to (3.47) nearly double in the wheat seedlings at higher concentration (20%) of aqueous stem extract over to the control (7.16) while in remaining concentration from 5% (6.67), 10 (5.38) to 15% (4.44). Aqueous leaves extract decreased slowly from 5% (6.86) to 10% (6.28) and then it fasten in 15% (5.71) and 20% (4.44) concentration. Theaqueous flower extract was decreased starch from 5% (6.26), 10% (6.78), 15% (6.69) and 20% (6.23).

Methanol extract of stem gradually decreased the starch content of wheat from 5% (6.12), 10% (5.06), 15% (4.16) and in 20% it reduced more (2.67) in compression to the control (7.07). Leaves extract declined gradually in 5% (6.22) and 10% (5.45) then suddenly it decreased in 15% (3.22) and 20% concentration starch has maximum decreased (2.12) i.e. more than triple fold in comparable to the control. In methanol flower extract it was successively decreased from 5% (7.02), 10% (6.28) and 15% (6.16) and 20% (5.08) concentration over control.

Aqueous extract of stem had reduced starch 2.06fold, 1.61fold in leaves extract and 1.14fold in flower extract at 20% concentration in wheat seedlings over to the control.

There is 3.37fold reduction of total sugars of wheat seedlings was noticed in the methanol extract of stem and leaves extract which is highest in all extracts and 1.40fold was recorded in the flower extract as compared to the control.

4.1.4 Total carbohydrates:

Total carbohydrate content has been reduced more in the aqueous stem extract (10.86) over the control (19.85) at the 20% concentration and also there was major decrease in the methanol extract of leaves (6.31) in comparison to the control (18.67).

Aqueous stem extract greatly affected the total carbohydrate content in wheat seedling that declined with increasing the concentration of extract from 5% (18.61), 10% (14.70), 15% (12.23) to 20% (10.86). Aqueous leaves extract resulted in decreased total carbohydrate maximum in 20% (11.60) concentration followed by 15% (14.92), 10% (16.19) and in 5% with 18.24. Flower methanol extract treatments reduced very slowly from 5% (19.41) to 10% (18.42), 15% (17.30) and 20% (15.59).

Methanol extract treatment has greatly affected than the aqueous extract which recorded in stem, leaves and flower extract. In methanol stem extract more reduced total carbohydrates at 20% (8.27) concentration trailed by 15% (12.08), 10% (15.92), 5% (17.45).In leaves extract declined triple fold from 20% concentration (6.31) comparable to control and same trend was recorded from 5% (16.44), 10% (14.17) to 15% (9.85). In methanol flower extract total carbohydrates decreased with increase in the concentration of extract and it has gradually decreased from 5% (18.45), 10% (17.55) to 15% (16.76) and in 20% (11.64).

Aqueous extract of stem in higher concentration (20%) total carbohydrates of wheat seedlings has reduced by 1.82fold, 1.70fold in leaves and 1.27fold in flower extract over the control.

In methanol extract of stem, total carbohydrates likely to be decreased by 2.39 fold, 3.13fold in leaves and 1.70 fold in flower extract in comparison to control at 20% concentration.

4.2 Groundnut seedlings :

Reducing sugars, total sugars, starch and total carbohydrates was reduced from lower concentration to higher concentration both in aqueous and methanol extract of stem, leaves and flowers of *Pascalia glauca* Ortega. in groundnut seedlings. Lower concentrations had minor inhibition while the rate of reduction was significant at the higher concentrations (20%) in both extracts (Table – 13).

4.2.1 Reducing sugars :

Aqueous extract of stem showed slow decline in the reducing sugars of groundnut seedlings in 5% (2.16) and 10% (2.03) concentration while in the 15% it was more decreased (1.70) and in 20% (0.97). Leaves extract greatly affected it and progressively reduced from 5% (2.15), 10% (1.06), 15% (1.52) while at higher 20% concentration it was maximum reduction (0.93) in comparison to the control (2.19). 5% aqueous flower extract had negligible (2.05) affected on groundnut but from 10% concentration (1.90) gradual decrease continued in 15% (1.59) and 20% (1.04) concentration over control.

Methanol extract of stem reported suppression in reducing sugars from 5% (1.89), 10% (1.77), 15% (1.51) and in 20% (1.01) concentration. Same decline trend has been found in the 5% flower extract (2.07); 10% (1.85), 15% (1.71) and 20% (1.32) concentration over to control. Significant reduction has been found in methanol leaves extract in higher concentration 20% (0.82) followed by 15% (1.61), 10% (1.66) and low in 5% (1.81) concentration.

Aqueous extract of stem indicated 1.25fold reduction in reducing sugars of the groundnut seedlings over the control, while 2.35fold in aqueous leaves extract and 2.10fold in flower extract at 20% concentration.

Table-12 : Effect of aqueous and methanol extracts of stem, leaves and flowers of *Pascalia glauca* Ortega. on sugars of wheat seedlings at 192hr (mg g^{-1}dry weight).

Plant part extract	Reducing sugars (mg g^{-1} dry weight)								
	Concent-rations	5%		10%		15%		20%	
	Control	Aqueous	Methanol	Aqueous	Methanol	Aqueous	Methanol	Aqueous	Methanol
Stem	5.82 ±0.080	5.43 ±0.115	5.11** ±0.041	4.20** ±0.060	4.54** ±0.091	3.33** ±0.315	4.14** ±0.070	3.33** ±0.291	2.99** ±0.088
Leaves		5.23** ±0.130	5.09* ±0.030	4.38** ±0.209	4.54** ±0.130	4.10** ±0.190	3.52** ±0.060	**3.10**** ±0.072	**2.15**** ±0.130
Flower		**5.80** ±0.077	**5.14**** ±0.020	5.39 ±0.284	**5.14**** ±0.040	4.47** ±0.177	4.57** ±0.050	3.98** ±0.122	3.40** ±0.087
Total sugars (mg g^{-1} dry weight)									
Stem	6.87 ±0.040	6.52** ±0.060	6.17 ±0.065	5.20** ±0.058	5.61** ±0.065	4.52** ±0.052	3.72** ±0.050	**4.05**** ±0.143	2.48** ±0.040
Leaves		6.18** ±0.060	5.12** ±0.052	5.60** ±0.080	4.16** ±0.052	5.16** ±0.060	3.14** ±0.075	4.08** ±0.045	**2.06**** ±0.030
Flower		**6.71** ±0.030	**6.25** ±0.0009	6.22** ±0.070	6.16 ±0.001	6.13** ±0.041	6.04* ±0.004	5.38** ±0.189	3.17** ±0.002
Starch (mg g^{-1} dry weight)									
Stem	7.16 ±0.040	6.67** ±0.050	6.12** ±0.050	5.38** ±0.060	5.06** ±0.052	4.44** ±0.080	4.16** ±0.060	**3.47**** ±0.240	**2.12**** ±0.040
Leaves		**6.86**** ±0.072	6.22** ±0.083	6.28** ±0.060	5.45** ±0.111	5.71** ±0.070	3.22** ±0.100	4.44** ±0.130	**2.12**** ±0.040
Flower		6.26 ±0.080	**7.02** ±0.025	6.78* ±0.136	6.28** ±0.070	6.69** ±0.177	6.16** ±0.060	6.23** ±0.080	5.08** ±0.070
Total carbohydrates (mg g^{-1} dry weight)									
Stem	19.85 ±0.053	18.61** ±0.073	17.45 ±0.042	14.70** ±0.158	15.92** ±1.183	12.23** ±0.070	12.08** ±0.043	**10.86**** ±0.045	8.27** ±0.238
Leaves		18.24** ±0.048	16.44** ±0.034	16.19** ±0.071	14.17** ±0.031	14.92** ±0.048	9.85** ±0.037	11.60** ±0.085	**6.31**** ±0.068
Flower		**19.41**** ±0.176	**18.45**** ±0.034	18.42** ±0.029	17.55** ±0.037	17.30** ±0.035	16.76** ±0.033	15.59** ±0.048	11.64** ±0.035

One way Anova: Tukeys HSD Post-hoc Test Inference. ** significant at P < 0.01.
* Significant at P < 0.05. Otherwise insignificant result.±Standard deviation.

Maximum 2.32fold decrease in reducing sugars found at 20% concentration extract of leaves extract while by 1.89fold suppression in methanol extract of stem and 1.45 fold in the flower extract in the series of sequence of leaves > stem > flower.

4.2.2 Total sugars :

Aqueous stem extract has slow decrement of total sugars in 5% (4.41) and 10% (4.34), in the 15% (3.72) and 20% (3.11) concentration. Aqueous leaves extract greatly affected groundnut seedlings and more low values of total sugars in higher concentration 20% (2.92) were recorded in comparison with control followed by 15% (3.31) whereas there was less reduction in the 5% (4.38) and 10% (4.33). Aqueous flower extract not affected and remains unchangeable from 5% (4.47) as that of control then it was progressively subdued in the 10% (4.27), 15% (3.62) to 20% (3.09).

Methanol extract of stem and flower showed same trend of reduction as that of aqueous extract but in the leaves extract it was highly suppressed at the 20% (2.87). Stem extract treatment showed slowly decreased from 5% concentration with 4.30, 10% (4.21), 15% (4.02) and 20% (2.88) concentration. Methanol extract of leaves showed reduction and recorded at 5% (4.20), 10% (4.08), 15% (3.01) and 20% (2.87) while flower extract treatment had very slow decrement of total sugars in 5% (4.19) and 10% (4.10) then it suddenly fall off in the 15% (3.92) and 20% (3.12) concentration comparable to the control.

There is 1.53fold high reduction of total sugars of groundnut in aqueous leaves extract followed by stem extract (1.43fold) and 1.44fold in flower extract in 20% concentration over control.

Methanol leaves and stem extracts continued the similar suppression of total sugars by 1.55fold and 1.43fold in the methanol flower extract over control from higher concentration (20%).

4.2.3 Starch :

Starch content in the groundnut seedling progressively decreased in the stem aqueous extract at lower 5% (8.84) and 10% (8.20) concentrations then higher reduction from 15% (7.48) and 20% concentration with 6.25. It was maximum declined in the higher concentration of 20% concentration of leaves extract (6.08)

followed by in 15% (7.18), 10% (8.45) and 5% (8.54). Flower aqueous extract decreased slowly from 5% (7.93), 10% (7.79), 15% 7.20) and it has highest decrease at 20% (6.67) concentration.

Methanol extract of stem had less content of starch in the groundnut seedlings in 5% (8.14) and in 10% (8.02), in 15% (7.88) and highest decrease at 20% (6.51) as compared to control. Leaves methanol extract repressed bit by bit form 5% (7.95), 10% (7.85), 15% (7.02) and it has been abruptly decreased in 20% concentration as 5.97. The flower methanol extract had starch at 5% (8.11), in 10% (7.09) then it slightly decreased from 15% (6.91) and 20% (6.06) concentration in groundnut seedlings.

The reduction of starch of groundnut in the aqueous extract of stem has recorded almost 1.45fold decline in leaves extract, 1.41fold in stem extract and 1.32fold in flower extract over the control at 20% higher concentration.

In the methanol extract of leaves it decreased 1.48fold, nearly similar in flower extract 1.46fold and from stem extract 1.35fold at 20% concentration over control.

4.2.4 Total carbohydrates :

Aqueous stem extract treatment showed lowered carbohydrates from 5% (15.42) and 10% (14.5) then significant decrease in 15% (12.90) and 20% (10.34). Leaves extract had boosted more reduction of total carbohydrates at 20% (9.93) followed by 15% (11.56), 10% (14.93) while in 5% concentration it was very slow (15.08) over control. Similar trend has been observed in the treatment of aqueous extract of flower from the 5% (14.45) to 10% (13.97) then high reduction in 15% (12.42) to 20% (10.80).

Methanol extract of stem showed slow decrease from 5% (14.34) and 10(14.01) while it still lowered in 15% (14.41) with highest in the 20% (10.41). Leaves extract treatment reduced carbohydrates at higher 20% concentration (9.67) followed by 15% (11.66), 10% (13.60) and in 5% it was 13.96 as comparable to the control. Methanol extract of flower decreased from the lower concentration 5% (14.38) to 10% (13.05) further in 15% (12.55) to the higher 20% (10.50) concentration.

The high difference in the total carbohydrates has been recorded about 1.59fold in methanol extract of leves while 1.56fold in aqueous extract leaves was found from higher 20% concentration. Similar trend from an aqueous stem extract

showed elevation 1.50fold and 1.43fold in flower extract. Same way, total carbohydrates decreased in the methanol extract of stem at 1.47fold and 1.46 fold reduction in flower extract in the 20% concentration over control.

Discussion:

The effect of aqueous and methanol extracts of stem, leaves and flowers of *Pascalia glauca* Ortega on wheat seedlings showed different impressions when treated at different concentrations of extract depicted in Table-12 and Fig.- 37 to 40. Majority of concentrations have progressively suppressed the content of reducing sugars, total sugars, starch and total carbohydrates with increasing the concentration of stem, leaves and flower extracts. Lower concentration treatments at 5% and 10% showed slow and steady decline while higher concentration at 15% and 20% have greater increase in reduction of different constituents of carbohydrates.

Wheat seedlings variedly responded after the treatment of aqueous and methanol extract. Reducing sugars significantly decreased in aqueous (3.10) and methanol (2.15) leaves extract and lowest in aqueous flower (5.82) and methanol leaves and stem (5.14) extract. Total sugar highly influenced in aqueous stem and decreased by (4.05) and methanol (2.06) leaves extract while lower in aqueous (6.71) and methanol (6.25) flower extract. Maximum starch content was reduced in the aqueous stem (3.47) and methanol leaves extract (2.12) where as lowest was found in aqueous leaves (6.86) and Methanol (7.02) flower extract. Higher reduction of total carbohydrates found in aqueous stem (10.86) and methanol (6.31) leaves extract and lower in aqueous (19.41) and methanol (18.45) flower extract.

Groundnut seedlings response in divergent concentrations of aqueous and methanol extracts of stem, leaves and flowers of *Pascalia glauca* Ortega was concentration dependent. The result give an insight of data expressed in the Table -13 and Fig.- 41 to 44 showing constant reduction in the reducing sugars, total sugars, starch and total carbohydrates in the different concentrations of stem, leaves and flower extracts. The higher concentrations of extract in all treatments has been greatly subdued the total carbohydrates and their components in both the extracts, further flower extract little declined in comparable to stem while both extract of leaves enhance the inhibition.

Lower concentrations at 5% and 10% has expressed miner while higher concentrations showed higher decrease. Leaves are the major source of

allelochemicals in present findings than stem and flower. Reproductive part like flower are non significant in lower concentrations to release the allelochemicals and mostly vegetative parts play cardinal role in releasing the allelochemicals that influence on the total carbohydrate balance making ultimately detrimental effect on growth and development of host crops.

With increasing the concentrations of methanol leaves extract result in decrease the carbohydrates at seedling establishment and its growth is an important parameter as it bears direct relationship with photosynthesis in future resulting fall in productivity of crop. Thus, the aim of our study is to find the reasons of affecting the growth of crop wheat and groundnut and ultimate loss in productivity.

Released allelochemicals from donor weed plants enter into the environment of recipient crop plant that affects the germination, establishment of growth. Carbohydrates and its components play a vital role in plant metabolism. Carbon skeleton of a plant body is the main structural and functional component, which is involved in almost all anabolic and catabolic processes. Fluctuations in carbohydrate metabolism will give clear indications of status of plant development and its effect on yield.

The increase or decrease in carbohydrates has become the importance in the allelopathy research; hence several allelopathy workers like Tripathi *et al*., (2000), Padhy *et al*., (2000), Bhalerao (2003), and Singh and Singh (2003) they published and discussed their role. Gabar *et al*. (2004) had reported increase in total carbohydrate and starch contents of *Vigna*, *Capsicum*, fingermillet, sorghum mungbean, pea, rice horse gram, *Mimosa*, maize etc. due to the lower concentrations treatments of aqueous leaf extract/ Leachates of *Tectona*, *Pteridium*, *Parhenium, Andrographis, Eupatorium* and *Ageratum*. However, reduction in carbohydrates in various crops was also noted at lower concentration treatment of extracts of various weed species (Ghafar *et al*., 2000; Mandal *et. al*., 2003; El-Kahtib and Hegazy, 2004; Dhumal and Ghayal, 2004 and Garge *et al*., 2005). Inhibitory effect on total carbohydrates and starch has been studied at higher concentration of extract treatments of weed parts and some allelopathic forest plants have affected the crops and vegetables reported by several workers including Singh *et al*. (2002), Singh *et al*. (2003), Thapar and Singh (2006), Vaidya and Dhumal (2007) in tomato, cucumber, cotton, wheat rice, mungbean, pea and sorghum. Soil incorporated *Chenopodiu mmurale* L with root and shoot amended soil decreased the carbohydrates, dry matter, nutrient uptake and soluble proteins in

Table- 13 : **Effect of aqueous and methanol extracts of stem, leaves and flowers of *Pascalia glauca* Ortega on sugars of groundnut seedlings. (mg g^{-1} dry weight).**

Plant part extract	Reducing sugars(mg g^{-1} dry weight)								
	Concent-rations	5%		10%		15%		20%	
	Control	Aqueous	Methanol	Aqueous	Methanol	Aqueous	Methanol	Aqueous	Methanol
Stem	2.19 ±0.083	2.16 ±0.030	1.89 ±0.070	2.03 ±0.060	1.77 ±0.045	1.70** ±0.080	1.51** ±0.045	0.97** ±0.061	1.01** ±0.060
Leaves		2.15 ±0.030	1.81 ±0.041	1.06** ±0.040	1.66** ±0.061	1.52** ±0.061	1.61** ±0.065	**0.93**** ±0.055	**0.82**** ±0.055
Flower		2.05 ±0.061	2.07 ±0.075	1.90** ±0.030	1.85 ±0.036	1.59** ±0.045	1.71* ±0.065	1.04** ±0.091	1.32** ±0.060
Total sugars (mg g^{-1} dry weight)									
Stem	4.47 ±0.050	4.41 ±0.030	4.30** ±0.040	4.34 ±0.050	4.21** ±0.061	3.72** ±0.060	4.02** ±0.050	3.11** ±0.045	2.88** ±0.050
Leaves		4.38 ±0.061	4.20** ±0.040	4.33 ±0.070	4.08** ±0.061	3.31** ±0.065	3.01** ±0.075	**2.92**** ±0.050	**2.87**** ±0.040
Flower		4.47 ±0.030	4.19** ±0.045	4.27** ±0.050	4.10** ±0.045	3.62** ±0.045	3.92** ±0.072	3.09** ±0.030	3.12** ±0.040
Starch (mg g^{-1} dry weight)									
Stem	8.85 ±0.061	8.84 ±0.061	8.14** ±0.040	8.20** ±0.045	8.02** ±0.080	7.48** ±0.065	7.88** ±0.040	6.25** ±0.075	6.51** ±0.065
Leaves		8.54** ±0.080	7.95** ±0.065	8.45** ±0.041	7.85** ±0.113	7.18** ±0.065	7.02** ±0.070	**6.08**** ±0.105	**5.97**** ±0.096
Flower		7.93** ±0.036	8.11** ±0.070	7.79** ±0.050	7.09** ±0.050	7.20** ±0.045	6.91** ±0.055	6.67** ±0.041	6.06** ±0.104
Total carbohydrates (mg g^{-1} dry weight)									
Stem	15.51 ±0.0005	15.42 ±0.0006	14.34** ±0.002	14.58 ±0.009	14.01** ±0.001	12.90** ±0.002	14.41** ±0.001	10.34** ±0.001	10.41** ±0.001
Leaves		15.08 ±0.001	13.96** ±0.003	14.93 ±0.002	13.60** ±0.001	11.56** ±0.0009	11.66** ±0.003	**9.93**** ±0.0005	**9.67**** ±0.001
Flower		14.45 ±0.0009	14.38** ±0.003	13.97** ±0.0009	13.05** ±0.002	12.42** ±0.002	12.55** ±0.003	10.80** ±0.001	10.50** ±0.002

One way Anova: Tukeys HSD Post-hoc Test Inference. ** Significant at $P < 0.01$.
* Significant at $P < 0.05$. Otherwise insignificant result. ± Standard deviation.

the *Cucumis sativus* L., *Melilotus indicus* (L.) All. *Trifolium alexandrine* L. and *Triticum aestivum* L. (El-Katib *et al*., 2003). Carbohydrates metabolism under stress causes interference with biosynthetic processes in wheat because of sensitivity of allelochemicals present in treated extract (Singh and Rao, 2003).

The reduction of carbohydrate content of wheat and groundnut must proportional change in metabolism at the time of germination in aqueous and methanol extract of leaves possibly because of in the deterioration indicating the allelopathic effect of *Pascalia glauca* Ortega extract. In the line of present results explained by Maiti *et al*. (2010) stated that protein and carbohydrates play important in shifting the metabolic activity of mung bean when treated with *Lantana camera*. Reduction of carbohydrates has been reported in the ragi seedling influenced when treated with *Eucalyptus globules* leaf litter leachate (Pattnaik, 1998 and Padhy *et al*., 2000). Abdul Roof and Siddiqui (2013) stated that the increase in carbohydrates and decline in chlorophyll content along with protein in aqueous leachate of *Tinospora cordifolia* when treated with extract from some selected weeds due to allelochemicals present in it. Sarkar and Chakraborty (2010) reported that allelopathic effect of *Croton bonplandianum* caused reduction in chlorophyll and carbohydrates in wheat and mustard. Same results postulated by Abu-Romman *et al*. (2010) in wheat when treated with *Euphorbia hierosolymitana*. Different and important view put forth by Kowther *et al*. (2010) they experimented allelopathic potentiality of aqueous extract of leaves and tuber of *Cyperus rotundus* on weed *Chorchorus* and *Echinochloa* under greenhouse condition and they found that the growth of weeds was inhibited in pot while on other hand at the same time elevated the carbohydrates, protein and oil percentage of soybean, ultimately here the growth and yield increased became of allelopathic potentiality. Thus, highly allelopathic potentiality of *P. glauca* might be helpful in future for ecofriendly development of sustainable agriculture system.

Degradation of storage carbohydrates of common bean *Phaseolus vulgaris* L. seedlings was significantly retarded with increasing the concentration of *Artemisia monosperma* aerial parts extract (Ahlam and Hediat, 2012). They further observed the reduction of total soluble sugars in the aqueous extract. Higher the concentrations of leaf extract / leachates of *Eupatorium*; more was the accumulation of amino acids and carbohydrates in mung bean (Maiti *et al*., 2013). Tejinderpal *et al*. (2014) stated that biochemical parameters like carbohydrates of wheat were greatly affected by leaves extracts of *Populus deltoids* at low concentration. They further added that if

concentration was increased from 0.01 to 10% the total sugars reduced. Leaf extracts and leaf leachates of *Hyptis suaveolens* decreased the insoluble carbohydrates in the cotyledons of mung bean seeds because of stronger allelopathic action at higher concentration as there might be positive correlation between seed germination and metabolism of treated seeds (Maiti *et al*., 2015). The phytotoxic compounds present in the various types of leachates of the *Xanthium indicum* which promotes the suppression of carbohydrates and protein content in seedlings of *Phaselus radiatus* L (Adhikary, 2017). It suggested that the variety of environmental stresses lead to excessive production of reactive oxygen species causing progressive oxidative damage and ultimately cell death due to damage of cell membranes (Duke and Dayan, 2006).

In present findings the total carbohydrates and their components were found declined with increasing concentration of aqueous and methanol extracts of *Pascalia glauca*. Thus, the aqueous and methanol extract of *P. glauca* have shown strong deleterious effect on growth of wheat and groundnut seedlings and such activity implied that the *P. glauca* has allelopathic potentiality and might be posses some inhibitor biochemical components that weakened metabolism the growth and development of these test plants resulting into loss in this valuable organic constituents.

Carbohydrates are the major organic constituents. During seed germination, there is mobilization of stored carbohydrates like starch from cotyledons towards growing axis of primordial of radicle and plumule. From our experiments, it is noted that due to active biomolecules present in *Pascalia glauca* the germination mechanism is hampered and this is reflected in lowered biomass. This is in accordance with the findings of reduction in various components of carbohydrates especially reducing and total sugars indicating disturbed mobilization of their sugars. Also reduced starch content may be disturbed inhibitory effect of starchy break-down. The effect of disturbed carbohydrate metabolism is a common cause effect reported in both extracts i.e. aqueous and methanol the allelopathic compound is obviously strong inhibition of carbohydrate metabolism. This has further confirmed through both test crops namely wheat and groundnut. It is but natural that hampered carbohydrate metabolism is a major cause of reduced biomass in both there test crops and will lead to stunted growth and development at vegetative level. This assumption will be confirmed become of our further findings.

5. Effect of aqueous and methanol extracts of stem, leaves and flowers of *Pascalia glauca* Ortega. on total nitrogen content of wheat and groundnut seedlings :

Nitrogen is an essential component of amino acids, proteins, growth hormones, chlorophylls and vitamins that played vital role in development of plant and their life. It is a part of other metabolites including purines, pyrimidines, porphyyrines and many other coenzymes. Porphyyrines are essential in photosynthesis and respiration while coenzymes for enzyme activities. It is also a basic component of metabolic regulation and building block of proteins which influences the enzymatic complement during response to various environmental conditions (Huffakar and Peterson, 1974). Nitrogen metabolism has essential and play important major factor in the development of vegetative growth of plant especially in the growth of stem and leaves.

5.1 Wheat seedlings :

The treatments of various concentrations of stem, leaves and flower extracts of *Pascalia glauca* on nitrogen content of wheat seedlings depicted in Table - 14. Aqueous extract of stem has seen slow and regularly reduction of nitrogen from 5% (0.190), 10% (0.163), 15% (0.126) to 20% (0.110). Flower extract showed slower reduction activity only in lower concentrations at 5% (0.166) and 10% (0.133) while it was rapidly reduced at 15% (0.070) and 20% (0.050). Aqueous leaves extract seen minor reduction at 5% (0.212) but nitrogen reduction increased from 10% (0.017), 15% (0.013) and 20% (0.005) over the control. Aqueous flower extract found slow reduction from 5% (0.166) and 10% (0.133) but it was noticeable increased in inhibition of total nitrogen content of wheat seedlings in 15% (0.070) and 20% (0.050).

Methanol extract also showed same trends in the reduction of nitrogen content that of aqueous extract of wheat seedlings but leaves extract have more potent to decrease greater amount of total nitrogen content. Nitrogen was declined slow from 5% methanol stem extract (0.190), leaves extract (0.156)and flower extract (0.200) while in the 10% concentration it was slower in stem (0.153) and flower extract (0.123) but leaves extract (0.018) has found maximum reduction. 15% and 20% concentration recognized more decreasing in nitrogen content of wheat seedlings in stem extract (0.103 and 0.083) while remarkably declined in methanol leaves extract

(0.016 and 0.005) and methanol flower extracts decreased nitrogen by 0.066 and 0.045 respectively.

Highly reduction of nitrogen content was seen in aqueous (31.42fold) and methanol (44fold) leaves extract which was noticeable. Stem extract in aqueous media recorded about 2fold and methanol solvent with 2.65fold reduction. 4.40fold reduction of total nitrogen was found in aqueous and 4.88fold in methanol flower extract in wheat seedlings at 20% concentration. Nitrogen content was more sensitive to methanol extract.

5.2 Groundnut seedlings :

Under the different treatments of aqueous stem, leaves and flower extract concentrations nitrogen content of groundnut has shown steadily decline from lower (5%) to higher (20%) concentration. The aqueous stem extract was found slow and continuous reduction of total nitrogen from 5% (2.11), 10% (2.02), 15% (1.83) to 20% (1.41). The aqueous leaves extract showed great effect on the groundnut seedlings and gradually reduced nitrogen content from 5% (1.92), 10% (1.75), 15% (1.61) to 20% (1.26). Flower aqueous extract decreased nitrogen from 5% (2.12), 10% (2.04) and 15% (1.73) to higher concentration at 20% with 1.64 (Table – 14).

There is steady reduction of total nitrogen content was seen from 5% methanol stem extract (2.10) and flower extract (2.05) but it increased in methanol leaves extract (1.81) over control. The concentration increased at 10%, it decreased (2.03) in methanol stem extract, flower extract (1.94) and methanol leaves extract (1.54). Same trend has been observed in 15% concentration in methanol stem extract (1.73), flower extract (1.62) and leaves extract (1.40). The higher concentration 20% flower extract reduced by 1.43 followed by stem extract with 1.38and leaves extract have maximum reduction of nitrogen content (1.15).

Aqueous extract of stem has found great declining the total nitrogen content at the 20% concentration of leaves (1.73fold) followed by stem (1.54fold) and flower extract with 1.32fold reduction in groundnut seedlings.

Methanol extract of stem showed 1.57fold, 1.89fold in leaves and 1.52fold reduction of nitrogen content of groundnut seedlings in flower extract over the control at 20% concentration.

Discussion:

The aqueous extract from all concentrations of stem, leaves and flower of *Pascalia glauca* total nitrogen content was decreased in the wheat seedlings due to allelochemicals have soluble nature can be attributed to the field conditions. Large amount of dry material of *P. glauca* shed to the ground and in next growing season, after showers it would have leached large quantities of inhibitors from the dry aerial parts of the *Pascalia* and there is possibility of formation of different concentrations of inhibitors. From present study it is quite evident that the allelopathic potentiality of weed parts virtually depends on the concentrations of inhibitors in the different plant parts and the water availability in the soil.

Same trend has been observed in the groundnut seedlings when treated different concentrations of stem, leaves and flower methanol extract as that of wheat seedlings. The maximum reduction of nitrogen has been takes place in the 20% concentration of leaves extract in comparison to that of aqueous leaves extract.

Nitrogen uptake and its partitioning in metabolism is a major factor in stem and leaf growth in the development of vegetative growth. It is essential to improve the crop growth and their productivity (Gallacher and Sprent, 1978). Nitrogen with carbon and oxygen develops an integral part of the complex repeating structure which is important in the molecular architecture. The requirement of nitrogen in groundnut is much higher than cereals at the same time; it is essential in early stages growth that helps in developing root nodules and fixes atmospheric nitrogen with the help of *Rhizobium*. Organic nitrogen can constitute a significant nitrogen source for wheat crop and that there is an interaction between the uptake of inorganic and organic nitrogen (Gioseffi *et al*., 2012).

According to Singh and Usha (2003) salycylic acid allelochemical maintained nitrogen content in wheat leaves; our results showed decreased the amount of nitrogen at higher concentrations of extract indicates that the presence of more allelochemical compounds in treated extracts their by reduced the total nitrogen in wheat and groundnut seedlings. Similarly Singh and Singh (2007) were found that salycyclic acid causes induction in nitrate assimilation through the induction of total nitrogen. Sarangthem and Singh (2003) found that foliar spray of salycyclic acid enhanced the nitrogen levels in *Phaseolus valgaris*. Karlidag *et al*. (2009) demonstrated that salycyclic acid enhanced the uptake of nitrogen in salt stressed strawberry plants.

Table- 14 : Effect of aqueous and methanol extracts of stem, leaves and flowers of *Pascalia glauca* Ortega on total nitrogen of wheat and groundnut seedling.

Plant part extract	Concent-rations	Total nitrogen (mg g^{-1} dry weight)							
		Wheat seedlings							
		5%		10%		15%		20%	
	Control	Aqueous	Methanol	Aqueous	Methanol	Aqueous	Methanol	Aqueous	Methanol
Stem	0.220 ±0.020	**0.190** ±0.030	**0.190** ±0.010	0.163 ±0.020	0.163 ±0.020	0.126** ±0.025	0.103** ±0.015	0.110** ±0.030	0.083** ±0.011
Leaves		0.212 ±0.003	0.156 ±0.032	0.163 ±0.020	0.018** ±0.002	0.013** ±0.002	0.016** ±0.003	0**.007**** ±0.001	**0.005**** ±0.001
Flower		0.166 ±0.025	0.200 ±0.020	0.133** ±0.015	0.123** ±0.020	0.070** ±0.020	0.066** ±0.003	0.050** ±0.020	0.045** ±0.003
Groundnut seedlings									
Stem	2.18 ±0.040	**2.11** ±0.026	**2.10** ±0.017	2.02 ±0.030	2.03* ±0.025	1.83** ±0.030	1.73** ±0.045	1.41** ±0.182	1.38** ±0.060
Leaves		1.92** ±0.045	1.81** ±0.047	1.75** ±0.030	1.54** ±0.025	1.61** ±0.036	1.40** ±0.020	**1.26**** ±0.050	**1.15**** ±0.035
Flower		**2.12** ±0.025	2.05* ±0.015	2.04** ±0.030	1.94** ±0.020	1.73** ±0.051	1.62** ±0.040	1.64** ±0.010	1.43** ±0.035

One way Anova: Tukeys HSD Post-hoc Test Inference. **Significant at $P < 0.01$.
* Significant at $P < 0.05$. Otherwise insignificant result. ± Standard deviation.

Similar findings have been shown by El-Tayeb (2005) in barley, Gunes *et al*. (2005, 2007) in maize and Yildirim *et al*. (2008) for cucumber plant. Hayat *et al*. (2009) stated that well known allelochemical salicyclic acids increased leaf nitrogen content in Indian mustard seedlings.

Hussain *et al*. (2010) reported that exogenously applied salicyclic acids increases leaf and root nitrogen content in salt stressed pearl-millet. Study of Khan *et al*. (2010) on mungbean and they revealed that application of salicyclic acids increased nitrogen content under salinity condition. Thornton and Robinson (2005) stated that the importance of organic nitrogen compounds as nitrogen source for plants is the possible interaction between organic and inorganic nitrogen forms during absorption by plant roots. In agricultural cropping systems depends on recycling and decomposition of organic nitrogen sources from domestication of animals and remnants of crops (e.g. animal manure based, low-input or organic agriculture), amino acids may represent a large nitrogen input (El-Naggar *et al*., 2009) and an important plant-available nitrogen pool (Jones *et al*., 2002). Rasmussen and Kuzyakov (2009) reported that the simultaneous uptake of inorganic carbon derived from mineralization of organic compounds interfered with root uptake of dual-labeled organic nitrogen. It will be of similar importance to assess the interactions between organic and inorganic forms during absorption by plant roots in order to establish the significance of organic nitrogen compounds for plant nitrogen nutrition. Similar statement postulated by Rasmussen *et al*. (2010). Number of workers postulated that directly inhibitory effect of extract was related to the allelochemical concentrations and weeds are more competitive in leaching the plant nutrients from the soil (Adrian *et al*., 2000 and Lixf *et al*., 2010).

The analysis of nitrogen status during experiment under different concentrations of extracts in wheat and groundnut seedlings showed there was continuous declining the nitrogen content with increase in concentration of extracts. Our results showed negative effect of extracts of various parts *Pascalia glauca* indicates some allele chemicals dissolved both in water and methanol solvent results into declining the nitrogen content of groundnut. Further, significantly decrease in nitrogen content at higher concentration levels of treatment leads to affect the seedlings and morphological signals visualized like yellowing and weaken the seedlings. So starvation of nitrogen availability to seedlings and showed its deficiency results into might be decrease in root and nodulation formation. Furthermore, there is

no work on allelopathic plant extract treatment and their effect of total nitrogen. Present investigation indicates that there decrease in total nitrogen content with increasing the concentrations of aqueous and methanol extract of stem, leaves and flower of *P. glauca* because allelochemicals present in weed parts are release and mixed into rhizosphere soil. They were affected availability of nitrogen content to the seedlings ultimately resulting the growth and development of wheat and groundnut seedlings.

6. Effect of aqueous and methanol extracts of stem, leaves and flowers of *Pascalia glauca* Ortega. on protein content of wheat and groundnut seedlings :

6.1 Wheat seedlings :

The results depicted in Table - 15 showed protein contents of wheat and groundnut seedling in reduction sequence from lower concentration (5%) to higher (20%) concentration of stem, leaves and flower extract of *Pascalia glauca.* Aqueous leaves extract greatly decreased protein content from wheat seedlings in comparable to stem and flower.

Aqueous stem extract has declined protein from 5% (1.08), 10% (0.95), 15% (0.78) to 20% (0.67) over the control. Leaves extract decreased maximum protein at higher 20% concentration (0.04) followed by 15% (0.07), 10% (0.10) and 5% have 0.12. Gradual reduction of protein determined in flower extract from 5% (1.01), 10% (0.78), 15% (0.42) to 20% (0.20) over control.

Methanol extract of stem and flower has showed more or less same trend of reduction of protein content of wheat seedlings as that of aqueous extract. Methanol stem extract shown gradual reduction of protein in 5% (1.11), 0.87 in 10%, 0.62 in 15% and 0.52 in 20% concentrations. Methanol flower extract has found decreasing protein trend from 5% (1.11), 10% (0.78), 15% (0.37) to 20% (0.24). But leaves extract has been greatly decreased protein content from 5% (1.03), 10% (0.12), 15% (0.09) to 20% (0.03) over the control.

Aqueous extract of stem was seen about 1.76fold protein content reduction of wheat seedlings, 29.50fold in leaves extract and 5.90fold in flower extract in the wheat seedlings at 20% concentration of extract.

Methanol extract of stem has determined 2.26fold protein content of wheat seedlings reduction, 39.33fold in leaves extract and 4.91fold in flower extract at the 20% concentration in wheat seedlings.

6.2 Groundnut seedlings :

In the aqueous stem extract total amount to protein was decreased progressively in groundnut seedlings from 5% (12.18), 10% (11.64), 15% (10.47) to 20% (9.01) over the control (12.41). Leaves extract has slowly reduced protein content from 5% (12.10) and 10% (11.24) while more from 15% (9.59 t) to 20% (8.09). Flower extract showed gradual reduction in protein content from 5% (12.20), 10% (11.73), 15% (10.18) to 20% have 9.19 over control (Table - 15).

Progressively reduction of protein content was found in methanol extract of stem from 5% (11.99) and 10% (11.60), 15% (9.68) and 20% with 8.16 in groundnut seedlings. Methanol leaves extract found maximum reduction in protein content than the aqueous extract and it was found from 5% (10.34), slowly decreased in 10% (8.92) and 15% (8.14); but it was highly reduced at 20% (6.10). Flower extract showed gradual decreased protein content from 5% (11.60) and 10% (11.12) while the degree of reduction has becomes increased from 15% (9.41) to 20% (8.31) over control.

Aqueous extract of stem reduced protein content by 1.37fold, 1.53fold in leaves extract and 1.35fold in flower extract over control in the groundnut seedlings.

Methanol extract of leaves determined maximum reduction (2.03fold) of protein content followed by 1.52fold in stem extract and 1.49fold in flower extract over the control in groundnut seedlings and comparatively it was more than the aqueous extract.

Discussion:

Gradual protein reduction has seen in aqueous stem extract while sudden decreased protein content of wheat seedlings in aqueous leaves and flower extract from lower (5%) to higher (20%) concentration. Methanol extract showed slower reduction of protein content at lower concentration of stem and flower extract while in leaves extract it was rapidly in wheat seedlings. Aqueous and methanol extract of leaves highly affected on the protein content and decreased from lower to higher

concentration in wheat seedlings. There is gradual but surely decrease in protein content of groundnut seedlings from lower to higher concentration.

The growth and development of the plant depends on the synthesis of various metabolites, including protein which later play important role in the different metabolic activities to form chemical basis of life. Plants synthesize proteins in response to abiotic and biotic stress. Increase in protein content plays an important role in plant defense (War *et al*., 2011).

Aqueous and methanol extract of stem, leaves and flower of *Pascalia glauca* found decreased protein content in wheat and groundnut seedlings from lower concentration to higher concentrations in groundnut seedlings. In this line of our investigation, some reports recorded significant decreased in protein content in different crops and vegetable plants including tomato, cucumis, mungbean, chickpea, sorghum when treated higher concentration of leaf extract of alloleopathic plants including *Chenopodium*, *Parthenium*, *Ageratum* and aquatic weeds by El-Khtib *et al*. (2004); Bhakat *et al*. (2005); Singh and Thaper (2003); Jadhav (2006) and Vaidya and Dhumal (2007). Increase or decrease in protein might be due to stimulation of protein synthesis or reduction of protein due to different types of allelochemicals present in the extracts of allelopathic plants (Schuab *et al*., 2001 and Sukul, 2001). Similar explanation can be given by them for the increase or decrease in the protein content of mungbean, chickpea and sorghum treated with lower as well as higher concentration of leaf extracts of *Celosia* and *Euphorbia*.

Decrease in total protein content may be due to increase in phenol content because many phenolic acids such as Ferulic acid, chlorogenic acid, vanillic acid, and *p*-coumaric acid are known to reduce the incorporation of certain amino acid into proteins and thus reduce the rate of protein synthesis (Husain *et al*., 2010). Decline the protein contents when treated with leaf and whole plant extracts of *Xanthium indicum* in seedlings of *Phaseolus radiates* may be due to the interference of phytotoxic and other phenolic compounds present in the plant parts (Adhikary, 2017), additionally he also stated that decreasing trend of protein level in leaves was comparatively more in treated seedlings than other parts and control.

Our results are in line of above work where protein was decreased more in leaves extract than stem and flower of *Pascalia glauca* aqueous and methanol extract. We also found at higher concentration of extract more affected than lower concentrations of weed parts extract in protein reduction.

Table- 15 : Effect of aqueous and methanol extracts of stem, leaves and flowers of *Pascalia glauca* on protein content of wheat and groundnut seedling.

Plant part extract	Concent-rations	Proteins (mg g^{-1} fresh weight)							
		Wheat seedlings							
		5%		10%		15%		20%	
	Control	Aqueous	Methanol	Aqueous	Methanol	Aqueous	Methanol	Aqueous	Methanol
Stem	1.18 ±0.020	**1.08*** ±0.020	**1.11** ±0.030	0.95** ±0.050	0.87** ±0.009	0.78** ±0.003	0.62** ±0.010	0.67** ±0.006	0.52** ±0.010
Leaves		0.12** ±0.004	1.03** ±0.041	0.10** ±0.004	0.12** ±0.005	0.07** ±0.003	0.09** ±0.003	**0.04**** ±0.002	**0.03**** ±0.005
Flower		1.01** ±0.004	**1.11** ±0.036	0.78** ±0.004	0.78** ±0.006	0.42** ±0.006	0.37** ±0.017	0.20** ±0.004	0.24** ±0.011
Groundnut seedlings									
Stem	12.41 ±0.085	12.18 ±0.035	**11.99** ±0.062	11.64** ±0.090	11.60** ±0.070	10.47** ±0.202	9.68** ±0.165	9.01** ±0.115	8.16** ±0.050
Leaves		12.10 ±0.030	10.34** ±0.092	11.24** ±0.180	8.92** ±0.050	9.59** ±0.309	8.14** ±0.036	**8.09**** ±0.196	**6.10**** ±0.180
Flower		**12.20*** ±0.091	11.60** ±0.070	11.73** ±0.085	11.12** ±0.035	10.18** ±0.035	9.41** ±0.050	9.19** ±0.075	8.31** ±0.070

One way Anova: Tukeys HSD Post-hoc Test Inference. **Significant at $P < 0.01$.
* Significant at $P < 0.05$. Otherwise insignificant result. ± Standard deviation.

Comerson and Julin (1980) have reported the repression of protein synthesis in lettuce leaves affected by allelopathic compounds. Similarly, Pattnaik (1998) and Padhy *et al*. (2000) have reported the adverse effect of allelochemicals of *Eucalyptus globules* on protein content in leaves of several crop plants. Phenolic acids are major allelochemicals in extract interfere with protein synthesis (Baziramakenga *et al*., 1997). Moreover, in salt- and water-stressed plant parts, the protein content also decreases owing to decreased rate of protein synthesis and increased rate of proteolysis (Dubey and Rani, 1990).

The multiple effects either increasing or decreasing protein, enzymes and amino acids resulting from allelochemicals include effect on cell division, production of plant hormones, membrane permeability, germination of pollen grains, mineral uptake, movement of stomata, pigment synthesis, photosynthesis, respiration, protein synthesis, nitrogen fixation, and specific enzyme activities (El-Khatib *et al*., 2004; Pisula and Meiners, 2010; Kim and Lee, 2011; Djurdjevic *et al*., 2012; and Mansour, 2013). Imam *et al*. (2006); Abu-Romman *et al*. (2012); Ibrahim *et al*. (2013); Gulzar and Siddiqui (2014b) all they reported the amount of protein was decreased when treated with plant extract. Reduction in the rate of protein synthesis occurred by incorporation of certain amino acids into proteins (Baziramakenga *et al*., 1997).

Our findings in the line of results stated by Tejindarpal *et al*. (2014) that after the treatments of extract of *Populus deltoides* on wheat (*Triticum aestivum* L.) seedlings, maximum reduction of protein content were recorded. Similar observations have on record by Pawar and Chavan (2004). Significantly decreased protein content at higher concentration of *Pascalia glauca* stem, leaves and flower suggest that it may be happens due to presence of more amounts of allelochemicals present in *P. glauca* which can reduce protein synthesis in wheat and groundnut seedlings. Similar effect was reported in *Brassica napus* and *Triticum aestivum* (Ullha *et al*., 2013), *Vicia faba* and maize (Saleh and Madany, 2013), *T. durum* (Abu-Roman *et al*., 2010), *Achillea biebesteinii* (Abu-Roman, 2011) and *Cucurbita pepo* (Hamed and Gawad, 2015).

7. Effect of aqueous and methanol extracts of stem, leaves and flowers of *Pascalia glauca* Ortega. on total amino acids of wheat and groundnut seedlings :

7.1 Wheat seedlings :

Amino acids are building blocks of proteins and plays important role in growth and development of plants. The 22 amino acids are standard, naturally incorporated into polypeptides are called as proteinogenic or natural amino acids (Creighton and Thomas, 1993). Amino acids plays double role in plants, it acts as precursor for proteins and functions as transport and storage compounds for nitrogen. Amino acids are primary products of nitrogen assimilation. In addition to proteinogenic amino acids, several other amino acids present in plants that are called as non proteinogenic or non standard amino acids. Plant can synthesize other amino acids with the help of transaminsaes (Jones *et al*., 2000). Amino acid, glycine stimulates chlorophyll formation and affects the plant growth rate. Valine affect root and seed formation, methionine increases the fruit maturity and accelerate the capacity of the plant in the disease resistance, maintain hormonal balance and increase the chlorophyll formation. Aspartate and arginine improve plant response to biotic and abiotic stresses and lysine increases the crop growth and yield.

The maximum amount of amino acids increased in aqueous flower extract (7.93) followed by aqueous (7.83) and methanol (7.80) extract of leaves at 20% higher concentration. It was noticed that minimum increased amino acids from aqueous (3.59) leaves and methanol flower (3.15) extract in wheat seedlings. All the concentration of stem, leaves and flower extract showed steadily increased the amino acids of wheat seedlings (Table- 16).

Wheat seedlings were responded gradual increment of amino acids from 5% (3.91); 10% (5.71); 15% (6.10) to 20% (6.18) aqueous stem extract. The maximum amino acids increased from 20% aqueous leaves extract (7.83) over control followed by 15% (5.93), 10% (4.69) and 5% (3.59). Same trends have found in aqueous flower extract from 5% (3.72); 10% (6.10); 15% (6.97) to 20% (7.93).

The amino acids also elevated in methanol stem extract from 5% (3.23) and 10% (6.07), 15% (7.51) and 20% (7.63) concentrations. Similarly, in methanol leaves extract it increased from 5% (4.19), 10% (6.24), 15% (7.41) to 20% (7.80) concentration. Methanol flower extract showed higher elevation of amino acids at

20% (6.87) concentration followed by 15% (6.18), 10 % (4.40), and 5% (3.15) concentration.

Aqueous extracts of stem increased amino acids content of wheat seedlings about 3.02fold, leaves extract with 3.85fold and flower extracts have 3.88foldat the higher concentration 20%. Leaves and flower extract nearly similar increments.

20% concentration of methanol extract of stem elevated amino acid by 3.74fold, maximum (3.82fold) in methanol extract of leaves while 3.36fold in flower extract in wheat seedlings.

7.2 Groundnut seedlings :

Amino acids in groundnut seedlings have decreased from lower concentrations 5% and 10% aqueous and methanol extracts of stem, leaves and flower but at higher concentration 15% and 20% it was increased both in aqueous and methanol extract of stem and flower. Aqueous and methanol extract of leaves has gradually and continuously decreased amino acids from 5% to 20% concentration over control. The highest increment of amino acid was found in 20% methanol extract while lowest reduction seen from aqueous leaves extract (2.13) and methanol leaves extract (2.14) which is nearly same at 20% (Table- 16).

Amino acid content of groundnut seedlings was decreased in lower concentration from 5% (4.71) and 10% (4.60) aqueous stem extract but increased from 15% (4.92) and 20% (4.94). Aqueous leaves extract showed gradually decreased in amino acid content of groundnut seedlings from 5% (3.10), 10% (2.84), 15% (2.80) to 20% (2.13). Aqueous flower extract found decrease in amino acids from 5% (4.79) and 10% (4.75) but increased from 15% (5.02) and 20% (5.06).

Methanol extracts response as that of aqueous extract after the treatments from lower (5%) to higher concentration (10%).Amino acid content of groundnut seedlings was inhibited in lower concentration from 5% (4.23) and 10% (4.13) aqueous stem extract but increased from 15% (4.96) and 20% (5.00). Aqueous leaves extract decreased amino acid content of groundnut seedlings slowly but surely from 5% (3.72), 10% (3.23), 15% (2.80) to 20% (2.14). Aqueous flower extract found decrease in amino acids from 5% (4.16) and 10% (4.71) but increased from 15% (5.93) and 20% (5.35).

Statistically, aqueous extract of stem in 15% was determined insignificant results in increasing amino acids while 5% stem extract showed significant decreasing

results at $P < 0.05$ and 10% concentration significantly inhibited amino acids at $P < 0.01$.Aqueous flower extract from 5%, 10% and 15% insignificant results at $P < 0.01$ but 15% and 20% stem and flower extract was seen significantly increasing amino acids results at $P < 0.01$.

Discussion:

Our results showed increasing amino acids trend in wheat seedlings from aqueous and methanol extract of leaves when treated with different concentrations of stem, leaves and flower extracts of *Pascalia glauca*. Minimum increased in 5% aqueous leaves (3.59) and 5% methanol flower (3.15) extract. Lower concentration treatments showed slow increasing but at higher concentrations 20% of aqueous flower (7.93) and methanol leaves (7.80) extract have maximum increased amino acids in wheat seedlings.There was promoting the amino acids of groundnut seedlings in the higher concentration of 15% methanol stem (5.06) and flower extract (5.03) and 20% methanol stem (5.25) and flower extract (5.35). Rest of concentrations reduced total amino acids in groundnut seedlings.

Amino acids are considered as building blocks of proteins and are primary products of nitrogen assimilation. Nitrogen is first assimilated into glutamate which formed from alpha-Ketoglutarate and ammonia in plant mitochondria while plants can also synthesize other amino acids with the help of transaminsaes (Jones *et al*., 2000). Amino acids regulates plant growth and important as modulator of growth and cell differentiation. They also affect metabolism and morphogenesis (Basu *et al*., 1989). Secondary metabolites such as phenols and quinones are pose toxicity to plants by formation of radicals. Increased amount of free amino acids has been observed upon protein degradation due to the allelochemicals (Singh and Thapar, 2003). Our results same in this line to increase the amino acids and decreased the protein in wheat and groundnut seedlings. The higher amount of proline in wheat increase the amino acids was reported by Vendruseolo *et al*. (2007) and Poustini *et al*. (2007). Rao and Rao (1979) stated that increase in proline in salt stressed *Arachis hypogaea* might be due to attributed in increment of total amino acids. So amino acids showed link with increase in proline content. The absorbed amino acids carbon will always be lost in the form of CO_2 produced during deamination and breaks-down of the carbon skeleton in the TCA cycle (Nasholm and Persson, 2001) while in wheat this amount is

Table- 16 : Effect of aqueous and methanol extracts of stem, leaves and flowers of *Pascalia glauca* on total amino acids of wheat and groundnut seedling. (mg g^{-1} fresh weight).

Plant part extract	Concent-rations	Amino acids (mg g^{-1} fresh weight)							
		Wheat seedlings							
		5%		10%		15%		20%	
	Control	Aqueous	Methanol	Aqueous	Methanol	Aqueous	Methanol	Aqueous	Methanol
Stem	2.04 ±0.0306	3.91** ±0.030	3.23** ±0.565	5.17** ±0.050	6.07** ±0.025	6.10** ±0.030	**7.51**** ±0.081	6.18** ±0.060	7.63** ±0.060
Leaves		3.59** ±0.043	4.19** ±0.090	4.69** ±0.112	6.24** ±0.060	5.93** ±0.085	7.41** ±0.047	7.83** ±0.130	7.80** ±0.151
Flower		3.72** ±0.040	**3.15**** ±0.035	6.10** ±0.056	4.40** ±0.040	6.97** ±0.125	6.18** ±0.075	**7.93**** ±0.040	6.87** ±0.045
Groundnut seedlings									
Stem	4.86 ±0.040	4.71* ±0.055	4.23 ±0.041	4.60** ±0.073	4.13** ±0.030	4.92 ±0.040	**5.06**** ±0.040	4.94 ±0.045	5.25** ±0.105
Leaves		3.10** ±0.062	3.72** ±0.040	**2.84**** ±0.058	3.23** ±0.050	2.80** ±0.075	2.80** ±0.020	2.13** ±0.036	2.14** ±0.025
Flower		4.79 ±0.035	4.16** . ±0300	4.75 ±0.030	4.71** ±0.041	5.02 ±0.087	5.03** ±0.041	**5.06**** ±0.080	**5.35**** ±0.075

One way Anova: Tukeys HSD Post-hoc Test Inference. **Significant at $P < 0.01$.
* Significant at $P < 0.05$. Otherwise insignificant result. ± Standard deviation.

60-80% of the absorbed amino acid carbon (Hill *et al*., 2011) or in process related to photorespiration (Bauwe *et al*., 2010). Photorespiration may have played an important role in the assimilation of the nitrogen derived from the absorbed amino acids (Gioseffi *et al*., 2012).

Amino acids were increased, when treated with *Eupatorium odorata* leaf extracts against mung beans (Maiti *et al*., 2013). Further, they stated that the effect of *E. odorata* leaves extract containing putative allelochemicals rendered harmful effect on plant growth even under stressful condition. Maximum concentrations of extracts were more injurious; additionally the allelochemicals in plants stress drastically inhibited metabolism in mung bean that showed increase in amino acid in seed kernels of mung bean (Maiti *et al*., 2010 and 2013). Increase in amino acids may be considered as adaptive mechanism to increase the stress to tolerance (Alam and Salama, 2012). Madany and Saleh (2015) stated that *E. helioscopia* extract significantly increased the amino acids of wheat and pea seedlings in higher concentrations. Similar results postulated by AL Wakeel *et al*. (2013) and Saleh and Madany (2015).

Our results agreement with above researchers where amino acids increased in the treatments of stem, leaves and flower extract of *Pascalia glauca* in wheat seedlings and groundnut seedlings at higher concentrations. It indicates that *P. glauca* became tolerance in both crops which was considered as adaptive mechanism of weed plant because of it newly enters in study area and need to environmentally adapt in crop ecosystem. Continuous increased amino acids in wheat seedlings has been observed from both and all concentrations of aqueous and methanol extract of *P. glauca* results into the breaks-down of protein therefore protein content was declined in the wheat and groundnut seedlings. It arrowed that more putative allelochemicals present in treated extract and influenced on the growth and development of test crops. Thus, they can act as potential weapons for interference and might be affected growth of crop and becomes tolerance in newly adapted habitat. So present work is in the right line and similar observations is in advocating with earlier reports (Singh and Thapar, 2003; Bhakat *et al*., 2006 and Maiti *et al*., 2008 and 2010).

Further, maximum enhancement of amino acids was found at 20% aqueous (3.83fold) and methanol (4.05fold) leaves extract in wheat seedlings and 1.24fold in aqueous and 1.08fold in methanol flower extract in groundnut seedlings over control. It indicates *Pascalia* under biotic and abiotic stress and induced tolerance ultimately

provides protection against natives. Kirmizi and Guleryuz (2006) advocated that the free amino acids content increases during the seed germination due to combined activities of proterolytic enzymes. Madany and Saleh (2015) also pointed that increase in protease activity, decrease in protein results into increasing the amino acids and proline content that protect the plants and help in dominancy over natives and crops. Same observations and results noted in present investigation that more decrease in the protein contents and increase in the amino acids in wheat and groundnut seedlings thus our work is in right line support above researchers.

8. Effect of aqueous and methanol extracts of stem, leaves and flowers of *Pascalia glauca* Ortega. on free proline of wheat and groundnut seedlings :

Proline is a proteinogenic amino acid, and is essential for primary metabolism. Proline accumulates in many plant species in response to environmental stress. Proline metabolism has a complex effect on development and stress responses, and that proline accumulation is important for the tolerance of certain adverse environmental conditions (Hong *et al*., 2000). Proline can act as a signaling molecule to modulate mitochondrial functions, influence cell proliferation or cell death and trigger specific gene expression, which can be essential for plant recovery from stress. Proline was considered as an inert compatible osmolyte that protects subcellular structures and macromolecules under osmotic stress (Kavi Kishor *et al*., 2005). Proline has able to protect protein integrity and enhance the activities of different enzymes. Proline is usually considered to be a metabolite with protective functions, several reports show that, under certain conditions, exogenous proline can be deleterious to plants and can inhibit growth, cell division and cell death in plants (Maggio *et al*., 2002).

8.1 Wheat seedlings :

The lower concentration (5%) of aqueous and methanol stem, leaves and flower found to be decreasing trends in the free proline content of wheat seedlings while the concentrations of extract increased from 10% to 20% it was increased as seen in the Table - 17. Aqueous leaves extract was the potent source for the increasing free proline content of wheat seedlings followed by methanol stem, leaves and flower extract.

The aqueous extract of stem, leaves and flower in lower concentration (5%) the proline was decreased and determined from stem (6.41) and leaves (6.58) while in flower extract it reduced by 5.70 in comparison to the control (6.65). Proline was further increased from 10% concentration of stem (7.26), leaves (8.43) and in flower extract has 7.17. Similar increasing trends was observed from 15% aqueous stem extract (9.88), leaves (10.33) and flower (8.15) while in 20% it was determined higher increment in proline content from aqueous leaves (12.73) then stem (11.17) and flower (8.76) extract.

Methanol extract of stem, leaves and flower decreased the proline at 5% lower concentration and recorded 6.12, 6.18 and 5.04 respectively. Free prolein has found increased from 10% stem extract (7.73), 15% (10.08) and 20% (10.72). Methanol leaves extract showed increasing trend from 10% (7.73) 15% (9.84) and in 20% recorded 10.17. In 10% methanol flower extract proline has decreased by 6.16, then increased in 15% (8.12) and in 20% (9.88) in wheat seedlings.

Aqueous extract of stem endorse the free proline content by 1.67fold, 1.91fold in leaves extract and 1.31fold in flower extract over the control in wheat seedlings at 20% concentration.

Methanol extract of stem showed increased free proline content of wheat seedlings by 1.61fold, 1.52fold in leaves extract and 1.48fold in flower extract over the control at 20% concentration.

8.2 Groundnut seedling :

Maximum free proline has increasing from the groundnut seedlings in the higher concentration (20%) of aqueous leaves extract (16.14) and minimum (8.87) in aqueous flower extract. In methanol extract leaves has found more potent in the increasing the proline (18.46) than the stem and flower. More interesting observation was decreased free proline from the 5% aqueous (8.18) flower extract and from 10% methanol (8.64) flower extract. Gradual increment was found in methanol stem and flower extract while methanol leaves extract showed relatively fasten increment of free proline than the others in groundnut seedlings (Table - 17).

5% aqueous extract of stem elevated free proline by 9.09, 10% (9.89), 15% (10.39) while 20% found higher increment i.e. 12.12 over the control (8.74) in groundnut seedlings. The aqueous leaves extract maximum increased the free proline from 5% concentration extract 9.51, 10% (10.28), 15% (14.34) while at 20%

concentration relatively maximum (16.14) than others. Aqueous flower extract minutely endorse free proline in 5% concentration (8.87), 10% (8.96), 15% (9.20) and in 20% (9.96).

Methanol flower extract decreased free proline at 5% (8.18) and 10% (8.64) in comparable to control (8.74). It was again increased in 15% (9.98) and 20% with 10.54. Methanol extract of leaves had maximum increased proline from 5% (9.96), 10% (10.88), and 15% (14.63) to 20% was noticed 18.46 and the degree of increment was higher than the stem and flower extract. Methanol stem extract increased proline from 5% (9.00), 10% (9.74), 15% (10.57) while at the higher concentration 20% it was found 11.73 over the control in the groundnut seedlings.

In higher concentration (20%) free proline content of groundnut seedlings increased in the treatment of aqueous stem extract by 1.38fold, 1.84fold in leaves and 1.13fold in flower over the control.

Methanol extract of leaves has seen maximum increase by 2.11fold free proline of groundnut seedlings, 1.34fold in stem and 1.20fold in flower extract over control.

Discussion:

Chenier *et al*. (2013) argued that proline accumulation is accompanied by the oxidation of NADPH hence increased the ratio of NADP+/NADPH that in turn promotes the oxidative pentose phosphate pathway providing precursors for phenolic biosynthesis via shikimic acid pathway. Phenolic phytochemicals can alter the activity and function of metabolic activity after passing through the plant cell (Le *et al*., 2010). Increased synthesis of secondary metabolites under stressful conditions believed to protect the cellular structures from oxidative damage (Buchanan *et al*., 2000).

Our results from present investigation are in agreement with these above findings and observations were showed from studied area where *Pascalia glauca* becomes dominated over other weeds and also wheat and groundnut crop. This incresed phenolic contents might be helping the plant to survive *P. glauca* in adverse conditions and adapted in the new habitat in studied region.

Increased free proline accumulation was indication of stress of proline which is common stress marker (Al Wakeel *et al*., 2013 and Saleh and Madany, 2015) that induces tolerance and it can be provides the protection against biotic and abiotic

Table- 17 : Effect of aqueous and methanol extracts of stem, leaves and flowers of *Pascalia glauca* Ortega on free proline of wheat and groundnut seedlings. (mg g^{-1} fresh weight).

Plant part extract	Concentrations	Free proline (mg g^{-1} fresh weight)							
		Wheat seedlings							
		5%		10%		15%		20%	
	Control	Aqueous	Methanol	Aqueous	Methanol	Aqueous	Methanol	Aqueous	Methanol
Stem	6.65 ±0.060	6.41* ±0.141	6.12 ±0.037	7.26** ±0.061	7.73** ±0.077	9.88** ±0.040	10.08** ±0.060	11.17** ±0.062	**10.72**** ±0.066
Leaves		6.58 ±0.045	6.18 ±0.036	8.43** ±0.125	7.71** ±0.058	10.33** ±0.104	9.84** ±0.040	**12.73**** ±0.072	10.17** ±0.061
Flower		**5.70**** ±0.060	**5.04**** ±0.050	7.17** ±0.070	6.16 ±0.060	8.15** ±0.070	8.12** ±0.112	8.76** ±0.102	9.88** ±0.080
Groundnut seedlings									
Plant part extract	Concentrations	5%		10%		15%		20%	
	Control	Aqueous	Methanol	Aqueous	Methanol	Aqueous	Methanol	Aqueous	Methanol
Stem	8.74 ±0.050	9.09** ±0.045	9.00 ±0.105	9.89** ±0.045	9.74** ±0.060	10.39** ±0.061	10.57** ±0.075	12.12** ±0.055	11.73** ±0.508
Leaves		9.51** ±0.061	9.96** ±0.087	10.28** ±0.116	10.88** ±0.075	14.34** ±0.080	14.63** ±0.117	**16.14**** ±0.090	**18.46**** ±0.085
Flower		**8.87** ±0.060	8.18** ±0.060	8.96** ±0.015	**8.64** ±0.100	9.20** ±0.072	9.98** ±0.102	9.96** ±0.020	10.54** ±0.140

One way Anova: Tukeys HSD Post-hoc Test Inference. **Significant at P < 0.01.

*Significant at P < 0.05. Otherwise insignificant result. ±Standard deviation.

stresses. Same results have been postulated by Al Wakeel *et al*. (2013) in study of *Acacia nilotica* aqueous extract against pea and Saleh and Madany (2015) in the study of salinity stress treated against wheat seedlings. Saleh *et al*. (2014) stated that proline is known to accumulate under phenolic exposure. Batish *et al*. (2007) showed that proline content of chickpea increased 5-times when the soil amended with the *Chenopodium murale* residues that are rich in inhibitory phenolic acids which induce stress conditions affecting the plant growth. Proline increased when treated with aqueous extract of *Euphorbia helioscopia* against wheat and pea (Madany and Saleh, 2015). The negative effects on crop growth and other parameters might be due to its higher allelopathic nature causing alterations in various physiological and biochemical activities in treated plants. Results revealed by Tejinderpal Khaket *et al*. (2014) and they postulated that germination and other biochemical and physiological parameters of wheat were severely affected by senescence poplar leaves (*Populus deltoids)* even at very low concentration; further they explained proline content of wheat leaves increased from 1.2 to 20.1% with an increase in poplar leaves concentration from 0.01 to 10%, respectively. Our results had shown similar findings where leaves of *Pascalia glauca* extract increased the maximum free proline content at higher concentration (20%) of extract. Proline content is positively correlated up to a significant level with stress severity which may be either due to inhibition of protein oxidation or due to breaked-down of protein from its precursors (Mohammed and Sen, 1987). Free proline is also involved in intracellular osmotic adjustment (Subbarao, 2001 and D'Souza and Devaraj, 2010).

Our results in present investigation revealed that the stem, leaves and flower aqueous and methanol extracts of *Pascalia glauca* Ortega. treated against wheat and groundnut seedlings increased the free proline with increasing the concentration of extracts. It promotes the stress condition in crop plants and ultimately affects the test crop growth. Leaves are considered to be major source of proline and affect the growth and development of crops that confirm in compliance with above workers.

9. Effect of aqueous and methanol extracts of stem, leaves and flowers of *Pascalia glauca* Ortega. on total polyphenols of wheat and groundnut seedlings :

The ecological and physiological activities of phenolic compounds in the plants are diverse and highly variable and the high amount of phenols and flavonoids in extracts may explain their high antioxidative activities (Roitto *et al*., 2005). Plant

phenolics include several groups of compounds such as simple phenols, phenolic acids, flavonoids, isoflavonoids, tannis and lignins. A phenol plays a major role in stimulation of protein and ammonia assimilation. Phenols and polyphenolic compounds such as flavonoids, act as antioxidants and cytotoxic effects of oxygen radicals in plant cells are removed (Lavid *et al*., 2001). Phenols are secondary metabolites that play vital role in defense mechanism in plnats. The changes in such secondary metabolites either increased or decreased may cause sensitivity or resistance towards the treatments of extracts of various allelopathic plants. Phenolic acids are potential allelochemicals because of their high water solubility and plant growth inhibitory properties (Inderjit, 1996).

9.1 Wheat seedlings :

Total polyphenols in the wheat seedlings found little decrease at lower concentration (5%) in aqueous and methanol extract of stem, leaves and flower of *Pascalia glauca*. It was increased from 10% concentrations and higher increment of polyphenols seen at 20% aqueous leaves (1.87) and methanol (0.94) stem extract over control (0.58). The elevation of polyphenol has observed from 10% to 20% concentrations of aqueous and methanol extract. From results aqueous extract determined more potent over the methanol extract in increasing polyphenols in wheat seedlings.

The values depicted in the Table - 18 showed, the total polyphenols has decreased in wheat seedlings, at lower concentration (5%) from aqueous stem extract (0.51), leaves (0.47) and flower extract (0.37) over control. More interesting results was after the increasing extract concentrations, it was increased in all treated extracts from 10% aqueous stem extract (0.81), leaves extract have 0.93 and flower extract with 0.68. 15% concentration of aqueous stem extract has increased by 1.01 while leaves extract by 1.06 and flower extract with 0.81. Maximum polyphenols increased at 20% concentration of aqueous leaves (1.87) followed by stem extract (1.05) and aqueous flower extract (0.92) in wheat seedlings.

The values of total polyphenols decreased like that of aqueous extract in the lower concentration (5%) of methanol stem, leaves and flower extract as 0.49, 0.38 and 0.47 respectively. Treatments of extract increased in concentration from 10% to 20% have gradually increased. Methanol extract of stem at 10% (0.63), 15% (0.88) to 20% (0.94) concentration has seen increasing trend comparable to the control (0.58).

Methanol extract of leaves also highly increased the polyphenol contents of wheat from 10% (0.57), 15% (0.87) and 20% with 0.86. Methanol flower extract also found promoting values from 10% concentration (0.58), 15% (0.68) and 20% (0.85) in wheat seedlings.

Maximum polyphenol content of wheat seedlings increased in aqueous leaves extract about 3.22fold followed by stem (1.05fold) and flower (1.52fold) at higher concentration (20%).

Polyphenol content of wheat seedlings in methanol extract of stem promoted higher about 1.62fold, 1.48fold in leaves extract and 1.46fold in flower extract at 20% concentration.

9.2 Groundnut seedlings :

Different concentrations of aqueous extracts of stem, leaves and flower of *Pascalia glauca* when treated against the test plant groundnut seedlings, they reflecte different response of the total polyphenols depicted in Table - 18. The increased concentration of extract increased polyphenols contents. It was found that gradually increase of polyphenols in all concentrations from the lower concenstration (5%) to higher concentraion (20%) in groundnut seedlings. Heighest and fasten increament of polyphenols determined in the aqueous leaves extract (12.17) and methanol leaves extract (14.45) over to control. The increased trend in polyphenols was important findings in present investigation. Only 5% aquous flower extract (4.42) found significantly decreased.

Aqueous stem extract showed increasing polyphenols of groundnut seedlings at 5% (5.88), 10% (6.64), 15% (7.97) and 20% incresed by 8.25 in comparison to rest of concentration. Aqueous leaves extract has more influenced on groundnut seedligss and maximum increased total polyphenols from 20% (12.17) concentration trailed by 9.71 in 15% extract, 10% have 8.10 and in lower concentratrion (5%) level it increased by 6.31over the control. Aqueous flower extract at 5% decreased by 4.42which was contrasting as compare to other concentrations over control. Then polyphenols increased gradually in 10% (5.64), 15% (7.16) and 20% have 8.92.

Methanol extract of stem, leaves and flower was increased the polyphenol content of groundnut as 6.92, 8.17 and 6.10 respectively in the 5% concentration. 10% concentration of stem extract showed 7.33; 8.74 in leaves extract and 6.57 in flower extract. 15% concentration of stem extract has increased by 7.99, 10.11 in leaves

extract and 7.17 in methanol flower extract. At at the higher concentration 20% stem extarct, total polyphenols determined 9.52, highest increased in leaves extract i.e. 14.45 while flower extract with 8.92 in the groundnut seedlings.

Highest increased total polyphenols in aqueous leaves extract 1.84fold followed by 1.38fold in the aqueous stem extract and 1.13fold in flower extract at 20% concentration of extract in groundnut seedlings.

Methanol extract of stem endorse total polyphenols of groundnut by 1.34fold, 2.11fold in leaves extract and 1.20fold in the flower extract at the 20% concentration.

Discussion :

Phenolics are the compounds with multi array of allelochemical activities and most important in allelopathy. They are water soluble and leach from leaves, stem and roots into the soil solution (Katase, 1993 and Zhu and Mallik, 1994). Many researchers have found that the inhibitory substances involved in allelopathy are terpenoids and phenolic substances (Alexa *et al*., 2004; Chaves and Escudero, 2006; Khanh *et al*., 2007). The quantitative analysis of aqueous extract and of *Artemisia* containing the phenolic compounds and flavonoids might be implicated allelochemicals agents (Ahlam Al-Watban and Salama, 2012). Many allelopathic experts and researchers like Batish *et al*. (2005 and 2004), Singh *et al*. (2003), Vaidya and Dhumal (2007), Vaidya (2009), Gabar *et al*. (2004), Djanaguiraman *et al*. (2004a and b) and Patil and Govindwar (2006) noticed that increaseing phenolic contents in black gram, corn, sorghum, radish, mustard, maize and rice due to higher concentrations of leaf extracts and residues of *Parthenium* and *Ageratum,Tectona, Pteridium* and *Adrographis*.

Increased synthesis of secondary metabolites under stressful conditions believed to protect the cellular structures from oxidative damage (Buchanan *et al*., 2000). As reported by Banerjee and Kalloo (1989) and Ambika and Smitha (2005) the significant increase in phenolic contents at higher concentration treatments may be due to induction of stress, which might be playing an important role in providing biotic and abiotic stress resistances in plants for their survive.

Table- 18 : Effect of aqueous and methanol extracts of stem, leaves and flowers of *Pascalia glauca* Ortega on total polyphenols of wheat and groundnut seedlings.

Plant part extract	Concentrations	Total polyphenoles (mg g^{-1} fresh weight)							
		Wheat seedlings							
		5%		10%		15%		20%	
	Control	Aqueous	Methanol	Aqueous	Methanol	Aqueous	Methanol	Aqueous	Methanol
Stem	0.58 ±0.060	0.51 ±0.045	0.49 ±0.030	0.81** ±0.035	0.63 ±0.045	1.01** ±0.090	0.88** ±0.035	1.05** ±0.062	**0.94**** ±0.041
Leaves		**0.47** ±0.04	**0.38** ±0.032	0.93** ±0.030	0.57 ±0.092	1.60** ±0.060	0.87** ±0.090	**1.87**** ±0.041	0.86** ±0.131
Flower		0.37** ±0.025	0.47 ±0.035	0.68* ±0.030	0.58 ±0.035	0.81** ±0.030	0.68* ±0.040	0.92** ±0.030	0.85** ±0.070
Groundnut seedlings									
Plant part extract	Concentrations	5%		10%		15%		20%	
	Control	Aqueous	Methanol	Aqueous	Methanol	Aqueous	Methanol	Aqueous	Methanol
Stem	5.20 ±0.050	5.88** ±0.032	6.92** ±0.040	6.64** ±0.030	7.33** ±0.055	7.97** ±0.070	7.99** ±0.045	8.25** ±0.065	9.52** ±0.112
Leaves		6.31** ±0.056	8.17** ±0.070	8.10** ±0.061	8.74** ±0.025	9.71** ±0.041	10.11** ±0.030	**12.17**** ±0.050	**14.45**** ±0.086
Flower		**4.42**** ±0.040	**6.10**** ±0.040	5.64** ±0.040	6.57 ±0.050	7.16** ±0.050	7.17** ±0.070	7.55** ±0.060	8.92** ±0.040

One way Anova: Tukeys HSD Post-hoc Test Inference. **Significant at $P < 0.01$.

*Significant at $P < 0.05$. Otherwise insignificant result. ±Standard deviation.

Increased proline accumulation was indication of stress of proline which is common stress marker (Saleh and Madany, 2015) that induces tolerance and it can be provides the protection against biotic and abiotic stresses. Same results have been postulated by Al Wakeel *et al.* (2013) in study of *Acacia nilotica* aqueous extract against pea and Saleh and Madany, (2015) in the study of salinity stress treated against wheat seedlings. Saleh *et al.* (2014) stated that proline is known to accumulate under phenolic exposure.

Our results from present investigation are in agreement with these above findings and observations were showed from studied area where *Pascalia glauca* becomes dominated over other weeds and also wheat and groundnut crop. This incresed phenolic contents might be helping the weeplant to survive in adverse conditions and adapted in the new habitat.

Batish *et al*. (2007) argued that the *Chenopodium murale* residues that are rich in inhibitory phenolic acids which induce stress conditions affecting the plant growth. Phenolic compounds and proline increased when treated with aqueous extract of *Euphorbia helioscopia* against wheat and pea (Madany and Saleh, 2015). The negative effects on crop growth and other parameters might be due to its higher allelopathic nature of weed plants present in crop field causing alterations in various physiological and biochemical activities in treated plants. Adverse effects on phenolic compounds in plants are well reported by Pavleuchenko (2002) and Singh *et al*. (2002). Enhanced phenol content was found to be responsible for reducing seedling growth in various abiotic stresses due to allelopathic conditions.

Phenolic compounds interfere with phosphorylation pathway or decreased synthesis of carbohydrates, proteins, nucleic acids, and secondary metabolites and also interfere with cell division, mineral uptake, and some other biosynthetic processes (Das *et al*., 2012 and Pawar and Chavan, 2004). Results revealed by Tejinderpal Khaket *et al*. (2014) and they postulated that germination and other biochemical and physiological parameters of wheat were severely affected by senescence poplar leaves (*Populus deltoids)* even at very low concentration due to phenolics compounds. Our results were in the right line findings of above researchers where leaves of *Pascalia glauca* extract increased the maximum secondary metabolites like proline and polyphenols of wheat and groundnut seedlings at higher concentration (20%) of extract.

Present investigation revealed that the stem, leaves and flower aqueous and methanol extracts treated against wheat and groundnut seedlings increased the total polyphenols and proline with increasing the concentration of extracts. Leaves are major source of polyphenols and works like allelochemicals and affect the growth and development of crops that confirm in compliance with above workers.

10. Effect of aqueous and methanol extract of stem, leaves and flowers of *Pascalia glauca* Ortega. on different enzymes :

10.1 Wheat seedlings :

10.1.1 Catalase (EC 1.11.1.6) :

Catalase enzyme is a powerful and potentially harmful oxidizing agent having heme containing redox which involved in the decomposition of hydrogen peroxide (Scandalios, 1997). The plants exposed to different environmental stresses that causes promotion of hydrogen peroxide generation (Bartosoz, 1997). Hydrogen peroxide (H_2O_2) is a harmful byproduct, to avoid metabolic damage it must be immediately converted into less toxic and less reactive gaseous oxygen and water molecules. Catalase uses hydrogen peroxide to oxidize toxins such as phenols, formic acid, formaldehyde and alcohols (Karra-Chaabouni, 2003 and Ozturk *et al*., 2007). Catalase plays vital role in plant defense, aging and senescence (Mura *et al*., 2007 and Conarth *et al*., 1995) and their actively involved in photorespiration and symbiotic nitrogen fixation, Catalase and superoxide dismutase are combinely involved in dismutation of superoxide radicals (Achuba, 2003). Biotic and abiotic stresses like high temperatures, UV radiations, ozone exposure, gravity, wounding, pathogen attack, can induce rapid changes in H_2O_2 levels, leading to a variety of responses in plants (Alvarez *et al*., 1998; Neill *et al*., 2002b and Blackman and Hardham, 2008).

The lower 5% aqueous stem extract recorded increasing catalase in wheat seedlings with 1.13, leaves extract have 1.27 but flower extract decreased by 1.05 in comparison to the control (1.22). Similar trend of reduction was followed at 10% concentration of aqueous stem extract (1.09), leaves extract (1.05) and flower extract (1.17). Reduction of catalase activity has speed up in the 15% and 20% higher concentration of stem, leaves and flower extracts. Catalase recorded 0.94 in 15% stem, 0.84 in leaves and flower extract have 0.85. 20% aqueous leaves extract showed

more reduction (0.30) in catalase activity of wheat seedlings followed by stem extract 0.72, and 0.51 in flower extract (Table - 19 and Fig.- 9 to 13).

5% methanol extract of stem reduced catalase (1.15) but it can monute increased in methanol leaves (1.23) extract, while again it decreased in flower (1.05) extract over control (1.22). Gradually catalase has decreased from 10% aqueous stem (1.12), leaves (1.18) extract and flower extract with 1.03. The increment of reduction of catalase was further continued at 15% (0.92) and 20% (0.57) concentration of flower. Methanol leaves extract from 15% (0.71) and 20% (0.61) concentration determined decreasing trend. Same trends has found in 15% (0.81) and 20% (0.65) methanol stem extract in wheat.

Aqueous stem extract reduced catalase by 1.70fold while 4.06fold in leaves extract and 2.39fold in flower extract in comparison to control in the wheat seedlings. The highest reduction of catalase of wheat seedlings was seen in leaves extract followed by flower and stem extract at 20% concentration.

Methanol flower extract (2.14fold) was detected highest reduction followed by leaves extract (2fold) and stem extract (1.87fold) from 20% concentration.

10.1.2 Peroxidase (EC 1.11.1.7) :

Peroxidases are heme containing glycoproteins, are universally found in plant. Fernandes *et al*. (2006) explained that peroxidase protects the plant cells against the destructive effects of hydrogen perioxide by its decomposition. Further, Murugan and Sumitha (2006) stated that the role of peroxidase catalysis was the generation of hydrogen perioxide which involved in various post enzymatic reactions. It also catalyzes the oxidative cross-linking of phenolic groups in the cell wall (Fry, 1986 and Schopter, 1996). Peroxidases control the endogenous levels of auxin and involved in the process of plant growth and defense (Gasper *et al*., 1982 and Greppin *et al*., 1986). Wielinder (1985) explained that peroxidase in higher plants is glycoprotein and calcium proteins and its heme synthesis occurs in the mitochondria (Chibbar and Van Huystee, 1986). Peroxidase becomes inactive in the absence of heme molecule (Chibbar *et al*., 1984).

Aqueous and methanol extract of stem, leaves and flower of *Pascalia glauca* when treated with different concentrations showed various changes in the perioxidase enzyme against the wheat seedlings. Perioxidase has decreased from the 5% lower concentration to the 20% higher concentration whereas the leaves extract has been

shown maximum reduction in perioxidase of wheat seedlings. Maximum reduction seen in aqueous (0.004) and methanol (0.005) leaves extract at higher 20% concentration. 5% aqueous stem and flower showed similar reduction while10% methanol stem and leaves also similar reduction.

Aqueous stem extract responses gradually decreased from 5% (0.040), 10% (0.022), and 15% (0.011) to the 20% higher concentration with 0.009 over the control. Same trend was observed in the aqueous flower extract and recorded as 0.040, 0.037, 0.032and 0.011 with respective to the 5% to 20% concentration. In comparison with stem and flower, aqueous leaves extract determined maximum reduction from the higher concentration 20% with 0.004 followed by 15% (0.020), 10% (0.030) and in 5% have 0.039.

Methanol extract of stem determined more reduction in the 20% (0.011), followed by 15% (0.025), 10% (0.031) while minor in the 5% with 0.039. Methanol extract of leaves rapidly decreased from the 15% (0.015) to 20% (0.005) while the slowly reduced from the 5% (0.036) to 10% (0.030). Methanol flower extract reduced gradually from the 5% (0.043), 10% (0.037), 15% (0.021) to 20% (0.018) against the control.

Wheat seedlings showed maximum perioxidase enzyme decreased about 11fold from the aqueous leaves extract at 20% higher concentration followed by stem extract by 4.88fold and flower extract by 4fold while methanol extract of leaves decreased perixodase enzyme of wheat seedlings by 8.80fold, stem 4fold and 2.44fold in flower extract in comparison to control.

10.1.3 Superoxide Dismutase (SOD) (EC 1.15.1.1) :

Superoxide dismutase (SOD) catalyzes the dismutation of superoxide anion radicals to yield hydrogen peroxide and molecular oxygen. The key role of superoxide dismutase is to prevent the oxidation of biological molecules by scavenging O_2-radicals generated in various physiological processes (Fridovich, 1995). Plant cells develop superoxide dismutase as a defense system against ROS (reactive oxygen species) which are produced in both unstressed and stressed cells. Superoxide dismutase plays an important role in metal homeostasis (Abdel-Ghany *et al*., 2005). Cu has been acts as the primary regulator of cytosolic and stromal superoxide dismutase activity (Cohu and Pilon, 2007 and Yamasaki *et al*., 2007). Plants protect themselves from toxic effects of reactive oxygen species created by numerous stresses

with the help of antioxidant enzymes including SOD induced in plants (Omidi, 2010 and He *et al*., 2011).

The wheat seedlings were variously responded against the treatment of aqueous and methanol extract of stem, leaves and flower of *Pascalia glauca*. Superoxide dismutase (SOD) was reduced from 15% and 20% concentration in both extract while 5% aqueous stem and flower extract determined increasing activity but decreasing in aqueous leaves extract. Maximum reduction determined in aqueous (0.380) and methanol (0.243) leaves extract from 20% higher concentration.

The most important observation from aqueous stem extract, superoxide dismutase activity was nearly similar increased in 5% stem (0.910) and flower (0.900) extract, it was slowly decreased at 10% (0.843) while degree of reduction was increased from 15% (0.606) and 20% (0.466) over the control. Aqueous leaves extract greatly affect on the wheat seedlings and maximum superoxide dismutase activity decreased at 20% higher concentration (0.380) followed by 0.593 in 15%. Slower and minute reduction has found in 5% (0.826) and in 10% (0.810). The flower extract response same trend in lower 5% (0.900) as that of stem extract where superoxide dismutase insignificantly increased, further it was decreased at 10% (0.813), 15% (0.723) and 20% (0.503).

Methanol extract of stem decreased the superoxide dismutase enzyme activity of wheat seedlings from 5% (0.726), 10% (0.646), 15% (0.520) to 20% with 0.323. Same trend of reduction has seen in methanol flower extract at 5 % (0.713), 10% (0.640), 15% (0.563) and 20% with 0.356. Methanol extract of leaves reduced maximum superoxide dismutase from 5% (0.706), 10% (0.606), 15% (0.276) and 20% (0.243).

Maximum superoxide dismutase of wheat seedlings has reduced by 2.23fold in aqueous leaves extract of *Pascalia* followed by 1.82fold in aqueous stem extract and 1.68fold in flower extract over the control at 20% concentration.

There was more than triple fold (3.49) superoxide dismutase decreased from the methanol extract of leaves followed by 2.63fold in stem extract and 2.38fold in flower extract at (20%) concentration in wheat seedlings.

10.1.4 Lipid perioxidase :

Lipid peroxidation refers to the oxidative degradation of lipids. Lipid peroxidation is a complex process in which polyunsaturated fatty acids in biological

membrane systems undergo changes by chain reactions and form lipid hydroperoxides which decompose double bonds of unsaturated fatty acids and disrupt membrane lipids (Isamah *et al*., 2000). Malondialdehyde (MDA) is a final product of lipid peroxidation and the levels of lipid peroxidation are usually measured as the amount of MDA (Heath and Packer, 1968). MDA act as an indicator of the rate of oxidative processes in plant cell. According to Marnett (1999) malondialdehyde is an end product of lipid peroxidation may be mutagenic or carcinogenic. Higher lipid peroxidation related with increased phosphorylase activity which results into accumulation of free glucose (Achuba, 2006). Reactive oxygen species at physiological concentration are not considered as harmful but their toxicity arises by metal ion dependent conversion into hydroxyl radicals and such toxicities are able to destruction of tissues (Foyer *et al*., 1994 and Bowler *et al*., 1992).

Lipid perioxidation of wheat seedlings slowly decreased at lower (5%) concentration of aqueous and methanol stem, leaves and flower extract. 5% aqueous flower and methanol leaves extract as well as 10% aqueous stem extract was found significantly decrease results. The higher reduction has shown similar from aqueous and methanol (0.430) leaves extract at 20% concentration while lowest deceased in aqueous leaves (0.930) and methanol stem extract (0.863) at 5% concentration. It was significantly and gradually decreased from 10%, 15% and 20% concentration from both aqueous and methanol extracts except 10% aqueous stem extract.

The results showed gradual reduction in the lipid perioxidase activity in the perioxidation from the 5% concentration to 20% of aqueous extracts. Aqueous stem extract reduced by 0.903 in the 5%, 0.846 in 10%, 0.573 in 15% and 0.533 in the 20% concentration. Leaves extract slowly reduced from 5% (0.930) to 10% (0.806) then degree of reduction has been increased at the 15% (0.713) and 20% concentration (0.430). Flower extract determined 0.826reduction in 5% then it becomes increased reduction in 10% (0.710), 0.556 in 15% and 0.463 in 20%concentration over the control.

Methanol extract of stem determined gradually reduction from 5% (0.863) and 10% (0.730) then immediately the rate of decreasing lipid perioxidase activity has been increased in 15% (0.570) and 20% (0.436) concentration. Methanol extract of leaves was determined maximum declining from 20% (0.430) followed by 15% (0.523), 10% (0.693) and minimum from 5% (0.810) concentration. Flower extract

more reduced at 20% (0.513) concentration while it was reduced gradually from 5% (0.840), 10% (0.646) to 15% (0.606) over the control.

Aqueous extract of stem has increased 1.78fold reduction of lipid perioxidase activity of wheat seedlings while 2.21fold in leaves extract and 2.05fold in aqueous flower extract at higher 20% concentration while methanol extract of leaves at higher 20% concentration found similar inhibition activity of lipid perioxidase from leaves 2.21fold and 2.18fold in stem while 1.85fold in flower extract.

10.1.5 Polyphenol oxidase (EC 1.10.3.2) :

Polyphenol oxidase is enzyme catalyses containing copper metallo and catalyses the oxidation of phenolics to quinones (Kosuge, 1969 and Robb, 1984 They are predominant enzymes found in plants, fungi, bacteria and animals. They are usually found in membrane bound chloroplast particularly in plants. Kosuge (1969) stated that there is a direct correlation between their activity and accumulation of phenols. Polyphenol oxidase catalyzes the oxidation of phenolic compounds. Vaughn *et al*. (1998) argued that it is mediated in the Mehler reaction i.e. photoreduction of molecular oxygen in plants. Immunological analysis with antibodies to broad bean polyphenol oxidase confirmed structural similarities found in higher plants (Flurkey, 1986). The product of polyphenol oxidase activities, quinone reacts with cellular factors and forms brown spots-melanin that deteriorates fruits and vegetables results in losses in nutrient quality of fruits and economic losses (Sanchez *et al.*, 1997 and George, 2010).

The aqueous and methanol extract of stem, leaves and flowers of *Pascalia glauca* Ortega.when treated with wheat seedlings has found reduction of polyphenol oxidase activity with increasing concentration of extract from lower to higher concentration. Higher reduction was found in aqueous (0.005) and methanol (0.004) leaves extracts. Aqueous stem and flower extract found slow and nearly identical reduction. Lower concentration (5%) of methanol extract showed nearly unchangeable reduction. 10% methanol stem and flower reduced (0.012) while stem and leaves extract showed similar (0.004) inhibition from 20% concentration.

Aqueous extract of stem affect on polyphenol oxidase activity of wheat seedlings when treated at 5% lower concentration that slowly and nearly similar reduction has occurred from stem (0.009), leaves (0.011) and flower extract (0.010) over to control. 10% concentration of stem and leaves extract found right to similar

inhibition (0.008) and flower extract seen very minute reduction by 0.007. Aqueous extract of stem have 0.005 in 15% concentrations while in leaves extract determined 0.009 and flower extract recorded 0.008. 20% concentration of stem and leaves reduced similar (0.005) polyphenol oxidase of wheat seedlings and flower extract with 0.006.

Lower concentration (5%) of methanol extract of stem and leaves have similar (0.011) reduction and flower extract near to it (0.012) as compare to the control that means it was minor effect. 10% concentration determined identical reduction from the stem and flower extract (0.012) while methanol leaves extract recorded 0.009. 15% concentration slowly decreased from stem extract (0.009); leaves extract (0.006) to flower extract (0.011). The higher 20% concentration have maximum decreased and determined nearly similar from stem (0.005) and leaves extract (0.004) while flower extract determined 0.009.

Aqueous extract of stem and leaves decreased similar 2.20fold polyphenol enzyme activity and flower extract found 1.83fold over control while methanol extract of leaves determined maximum reduction (3.25fold) in the polyphenol enzymes activity followed by 3.02fold in stem extract and 1.44fold in flower extract over the control in the series of reduction sequence leaves > stem > flower at 20% higher concentration in the wheat seedlings.

10.2 Groundnut seedlings:

10.2.1 Catalase (EC 1.11.1.6) :

Aqueous and methanol extract of stem leaves and flowers of *Pascalia glauca* reduced the activity of different enzymes activity of the groundnut seedlings depicted in Table- 20 and Fig. 14 to 18.Aqueous extract of leaves determined higher reduction (0.74) in catalase enzyme activity from 20% concentration and aqueous stem (2.19) and flower (2.18) extract found minor increment in reduction when treated with 5% concentration. Rapidly increased reduction of catalase was seen at higher concentration. 20% stem and flower extract shown identical reduction (1.22). Maximum catalase activity reduction was found in methanol leaves extract (0.21) and aqueous leaves extract (0.74) further, methanol leaves and flower extract have similar reduction at 5% (2.14) concentration.

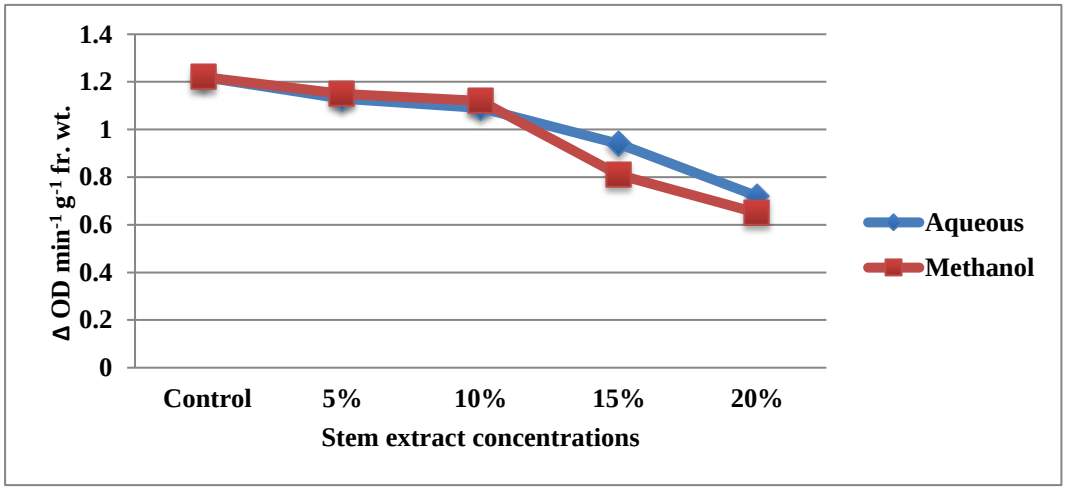
Table- 19 : Effect of aqueous and methanol extract of stem, leaves and flowers of *Pascalia glauca* Ortega. on different enzymes of wheat seedlings (Δ OD $min^{-1}g^{-1}$fresh weight).

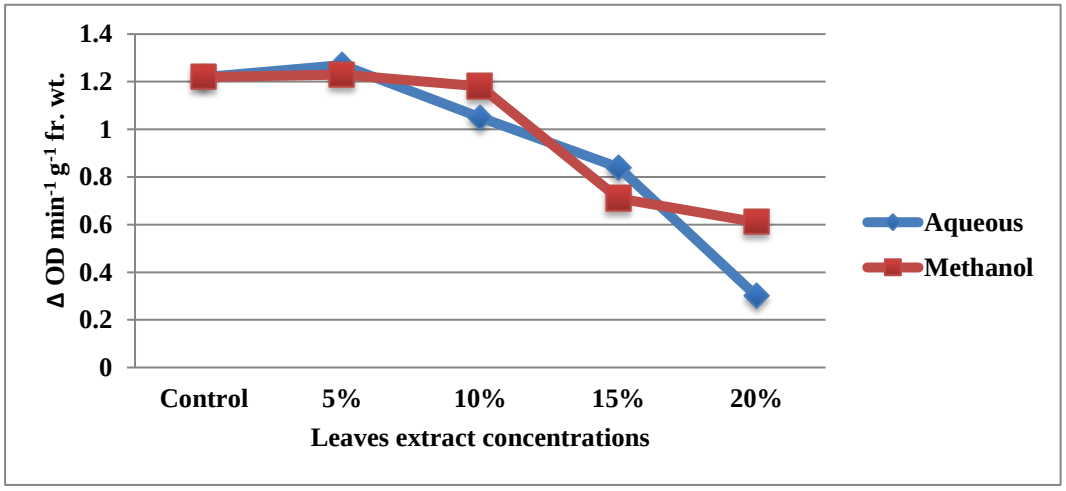
Plant part extract	Wheat seedlings								
	Catalase								
	Concentrations	5%		10%		15%		20%	
	Control	Aqueous	Methanol	Aqueous	Methanol	Aqueous	Methanol	Aqueous	Methanol
Stem	1.22 ±0.050	**1.13** ±0.035	**1.15** ±0.030	1.09** ±0.020	**1.12** ±0.036	0.94** ±0.030	0.81** ±0.041	0.72** ±0.036	0.65** ±0.035
Leaves		**1.27** ±0.045	**1.23*** ±0.055	1.05* ±0.035	**1.18** ±0.060	0.84** ±0.041	0.71** ±0.060	**0.30**** ±0.080	0.61** ±0.035
Flower		**1.05*** ±0.030	**1.05** ±0.030	1.17 ±0.030	1.03 ±0.030	0.85** ±0.045	0.92** ±0.050	0.51** ±0.085	**0.57**** ±0.040
Perioxidase									
Stem	0.044 ±0.002	**0.040** ±0.001	0.039* ±0.002	0.022** ±0.004	**0.031**** ±0.003	0.011** ±0.002	0.025** ±0.002	0.009** ±0.003	0.011** ±0.003
Leaves		0.039 ±0.002	0.036* ±0.004	0.030** ±0.003	**0.030**** ±0.003	0.020** ±0.003	0.015** ±0.003	**0.004**** ±0.002	**0.005**** ±0.001
Flower		**0.040** ±0.002	**0.043** ±0.003	0.037 ±0.005	0.037 ±0.002	0.032** ±0.0006	0.021** ±0.003	0.011** ±0.003	0.018** ±0.003
Superoxide dismutase									
Stem	0.850 ±0.045	**0.910** ±0.026	**0.726** ±0.040	0.843 ±0.040	0.646* ±0.025	0.606** ±0.055	0.520** ±0.055	0.466** ±0.045	0.323** ±0.051
Leaves		**0.826 ±0.040**	0.706 ±0.025	0.810 ±0.040	0.606** ±0.020	0.593** ±0.050	0.276** ±0.060	**0.380**** ±0.060	**0.243**** ±0.032
Flower		**0.900** ±0.026	0.713 ±0.025	0.813 ±0.035	0.640* ±0.036	0.723* ±0.045	0.563** ±0.040	0.503** ±0.050	0.356** ±0.035
Lipid perioxidation									
Stem	0.953 ±0.030	0.903 ±0.015	**0.863** ±0.030	0.846* ±0.045	0.730** ±0.030	0.573** ±0.035	0.570** ±0.020	0.533** ±0.047	0.436** ±0.035
Leaves		**0.930** ±0.026	0.810* ±0.030	0.806** ±0.055	0.693** ±0.070	0.713** ±0.030	0.523** ±0.045	**0.430**** ±0.036	**0.430**** ±0.030
Flower		0.826* ±0.040	0.840 ±0.045	0.710** ±0.045	0.646** ±0.045	0.556** ±0.041	0.606** ±0.030	0.463** ±0.045	0.513** ±0.030
Polyphenol oxidase									
Stem	0.011 ±0.005	0.009 ±0.002	**0.011** ±0.004	**0.008** ±0.003	**0.012** ±0.002	0.005 ±0.002	0.009 ±0.005	**0.005** ±0.003	**0.005** ±0.003
Leaves		**0.011** ±0.006	**0.011** ±0.003	**0.008** ±0.003	0.009 ±0.004	0.009 ±0.002	0.006 ±0.002	**0.005** ±0.003	**0.004*** ±0.001
Flower		**0.010** ±0.003	**0.012** ±0.003	**0.007** ±0.002	**0.012** ±0.004	0.008 ±0.003	0.011 ±0.003	0.006 ±0.0030	0.009 ±0.004

One way Anova: Tukeys HSD Post-hoc Test Inference. **Significant at $P < 0.01$.

*Significant at $P < 0.05$. Otherwise insignificant result. ±Standard deviation.

Fig. - 9 : **Effect of aqueous and methanol extract of stem, leaves and flowers of *Pascaliaglauca* Ortega. on catalase enzymes of wheat seedlings.**





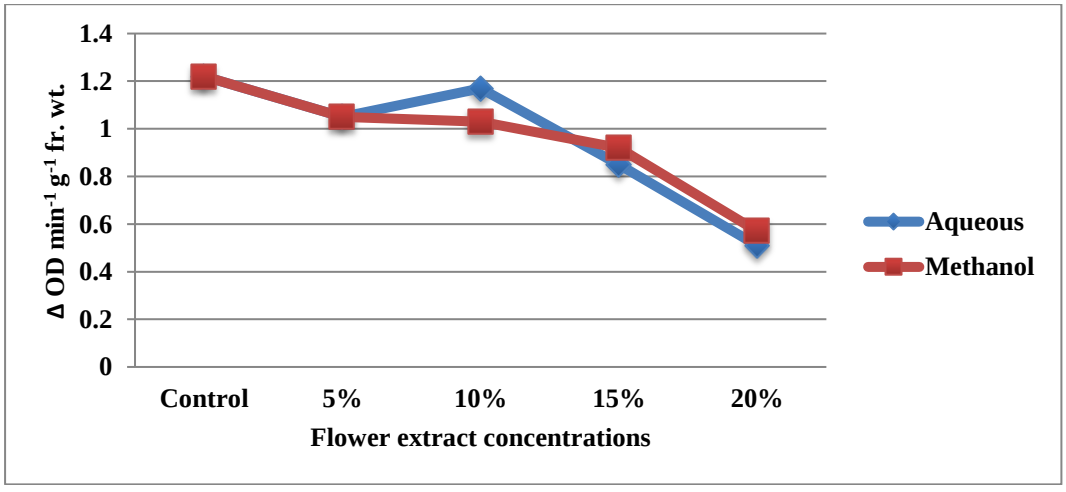


Fig. - 10 : **Effect of aqueous and methanol extract of stem, leaves and flowers of *Pascalia glauca* Ortega. on perioxidase enzymes of wheat seedlings.**

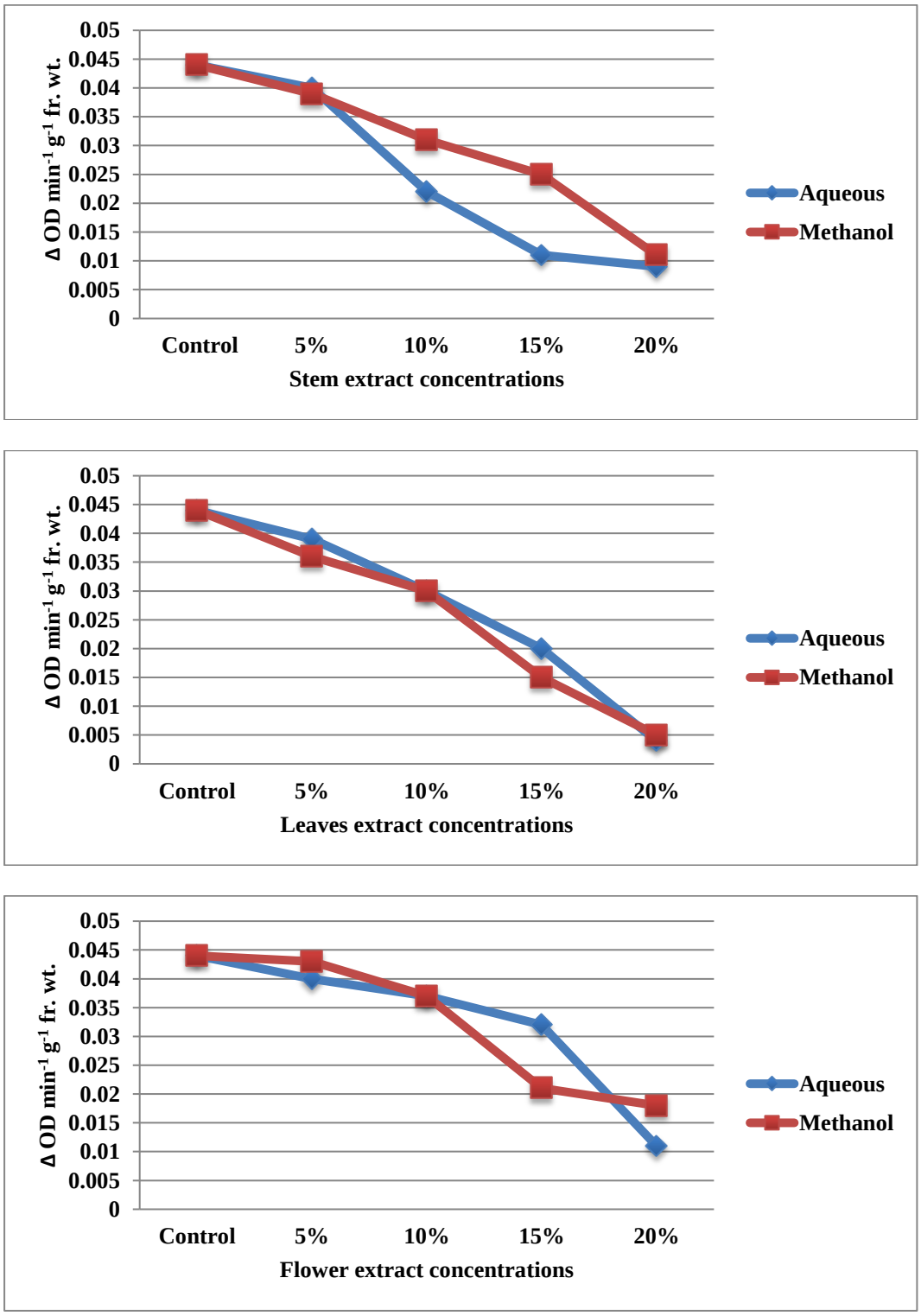


Fig. - 11 : **Effect of aqueous and methanol extract of stem, leaves and flowers of *Pascalia glauca* Ortega. on superoxidase dismutase enzymes of wheat seedlings.**

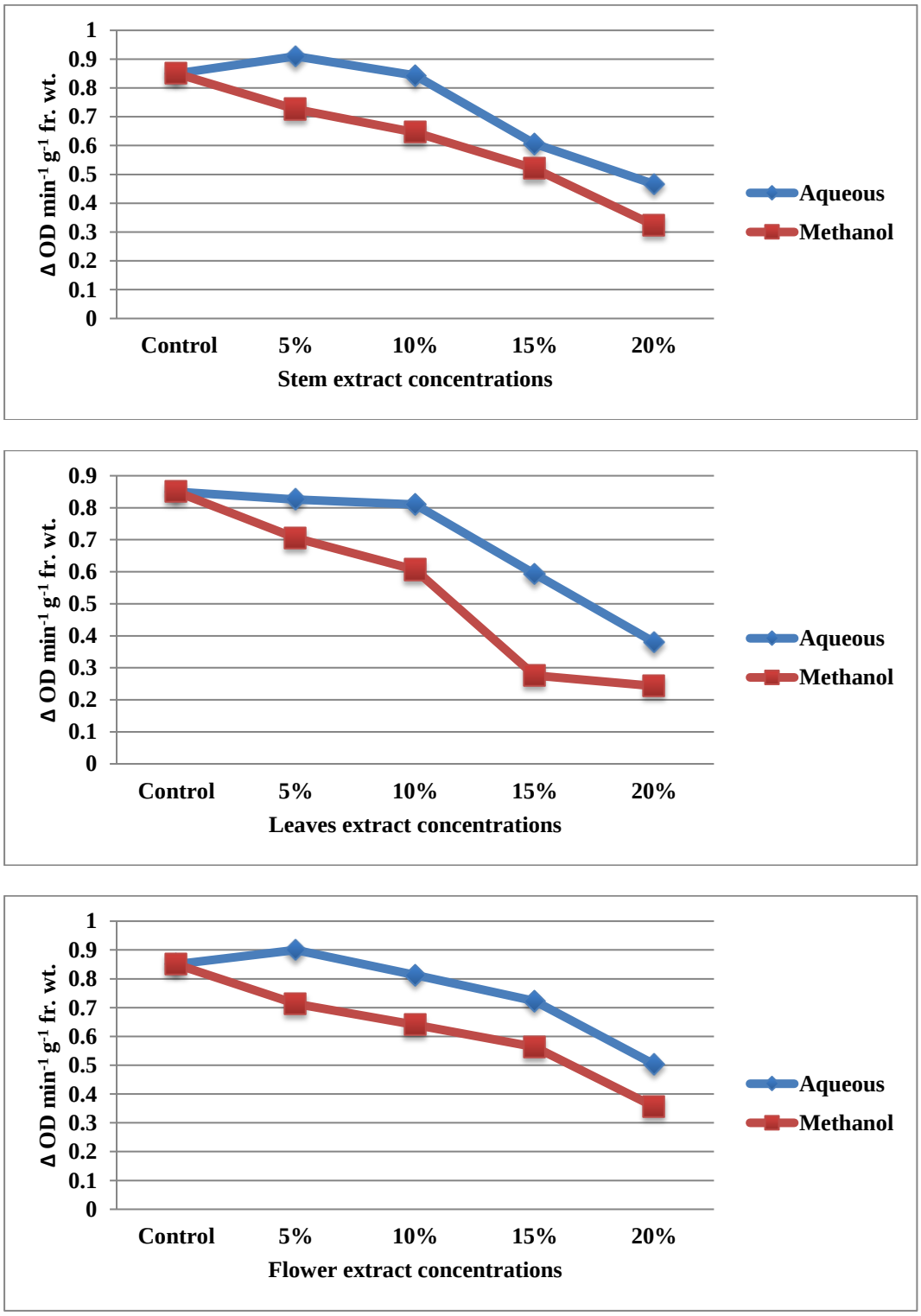


Fig. - 12 : **Effect of aqueous and methanol extract of stem, leaves and flowers of *Pascalia glauca*Ortega.on lipid perioxidase enzymes of wheat seedlings.**

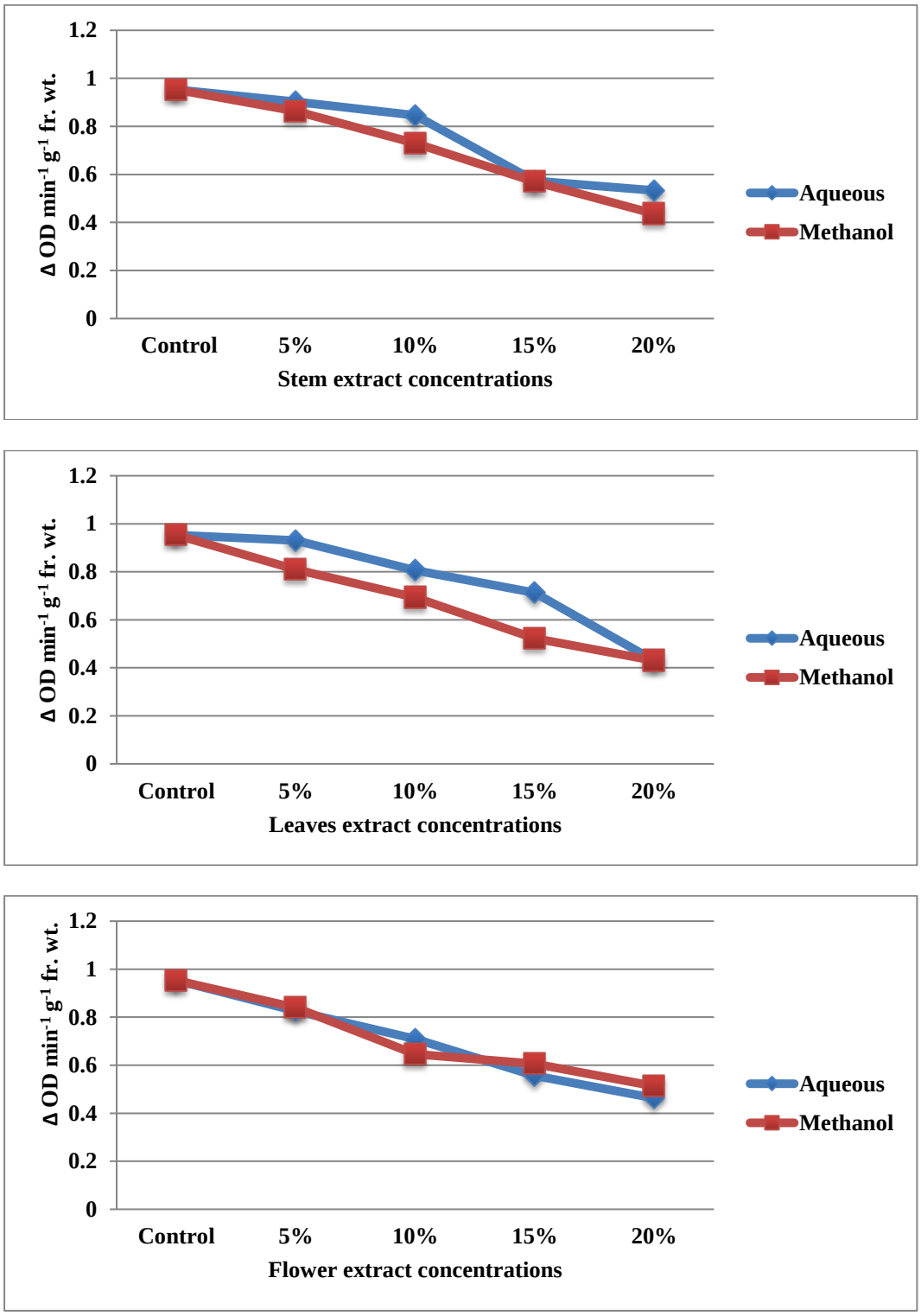
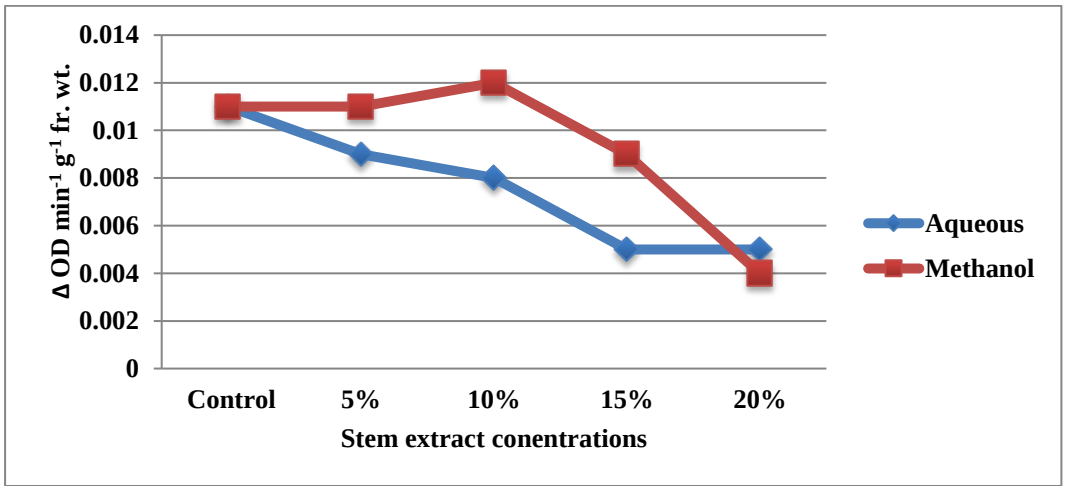
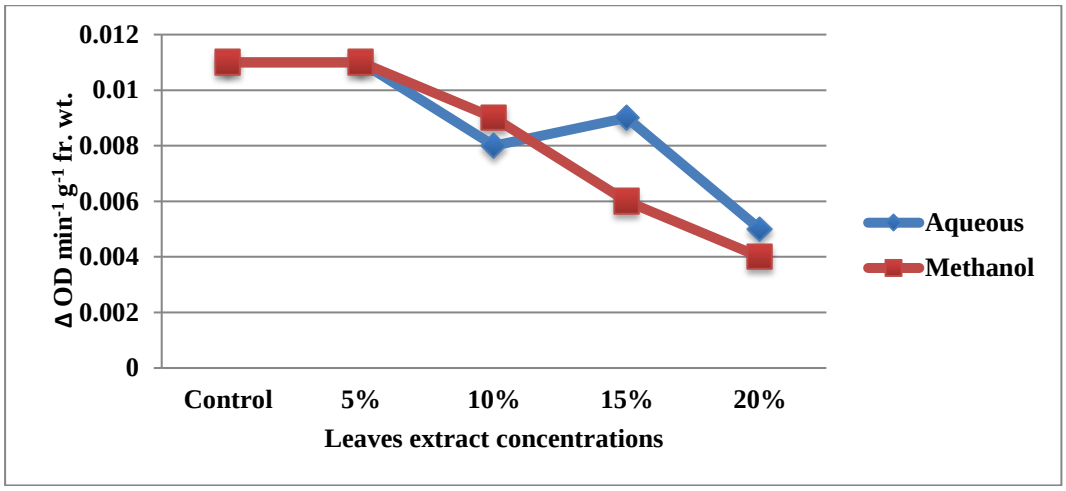
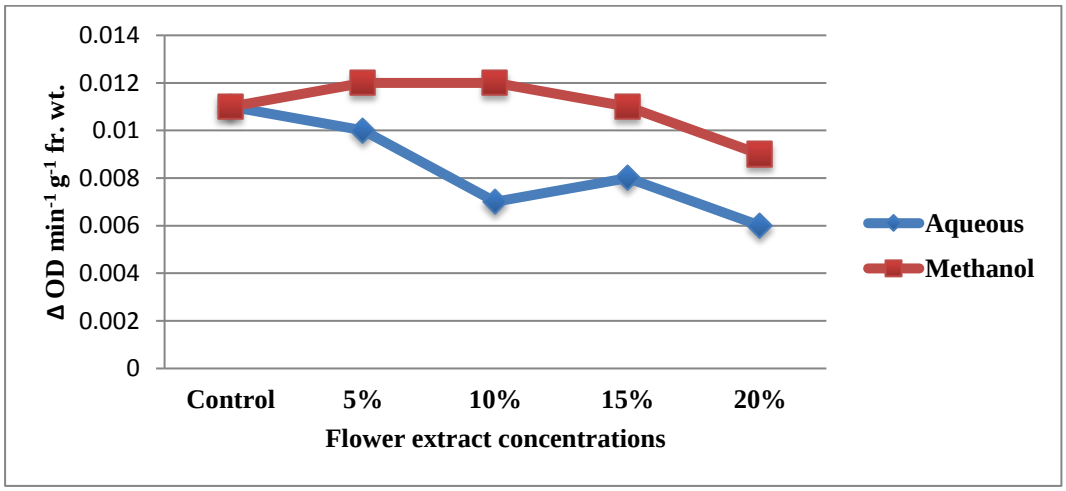


Fig. - 13 : **Effect of aqueous and methanol extract of stem, leaves and flowers of *Pascalia glauca* Ortega. on polyphenol oxidase enzymes of wheat seedlings.**







The reduction of catalase enzyme activity of groundnut seedlings have showed at lower (5%) concentration of aqueous stem extract (2.19) then negligible in 10% (2.18) latter it becomes rapid in 15% (1.76) to 20% higher concentration (1.22). The aqueous leaves extract found highest reduction from the 20% concentration (0.74) means triple fold over control, followed by 15% (1.07), 10% (1.87) and in 5% (2.13). Aqueous flower extract minutely affected at lower concentration 5% (2.18) and 10% (2.07) then it was more reduced in 15% (1.86) and 20% (1.22) concentration over the control.

Methanol stem extract has observed slower reduction trend at the lower concentration 5% (2.08) and 10% (1.90), leaves extract (2.14 and 1.64) and flower extract (2.14 and 2.02)respectively in comparison to the control. Methanol leaves and flower extracts was determined similar reduction. As concentration of extract increased, the degree of reduction also increased at 15% stem (1.77), leaves (0.94) and flower (1.86) extract while at 20% concentration of stem extract catalase activity of groundnut seedlings has found (1.10), leaves has maximum reduction (0.21) and flower extract with 1.18 over control.

Aqueous extract of stem decreased 2.07fold catalase enzyme, 10.85fold in leaves extract and 1.93fold in flower extract in groundnut seedlings while methanol extract of stem determined 1.99fold reduction catalase activity, highest 10.52fold in leaves extract and 1.86in flower extract at 20% concentration.

10.2.2 Perioxidase (EC 1.11.1.7) :

Similar reduction of perioxidase enzyme activity of groundnut determined in aqueous extract of stem and flower (0.153) from 20% concentration and 10% methanol stem (0.330) and flower extract (0.333) was seen nearly similar were the vital investigation in present work. The higher reduction was found in aqueous (0.096) and methanol (0.076) leaves extract at 20% concentration. Slower and gradual inhibition was determined in the lower concentrations of aqueous stem, leaves and flower extract from 5% and 10% concentration while in methanol extract it was rapidly reduced in comparison to aqueous extract. Leaves extract was found sudden decrease of perioxidase activity from lower to higher concentration over to control.

The activity of perioxidase enzyme progressively decreased from lower to higher concentrations in aqueous extract of stem. It was little slower down in the aqueous stem extract from 5% (0.486) to 10% (0.463) then it was rapidly more

reduction observed from 15% (0.323) to 20% (0.153). Similar and unchanged decreased trend has recorded in the 5% and 10% aqueous extract of flower (0.426), but it was sudden reduction found at 15% (0.286) and 20% higher concentration (0.153). Aqueous leaves extract greatly reduced its activity in the 5% (0.386), 10% (0.306) whereas it has more reduced in 15% (0.196) and 20% (0.096) concentration.

Methanol extract of stem and flower has found more or less nearly same reduction in all concentration that represented 0.546 and 530 from 5%, 0.330 and 0.333 in 10%, 0.216 and 0.240from 15% while 20% concentration has recorded 0.140 and 0.166 respectively. Methanol leaves extract seen maximum inhibition of perioxidase activity from 5% (0.446), 10% (0.230), 15% (0.123) to 20% have higher decreased (0.076) in comparable to the stem and flower extract.

Aqueous extract of stem and flower was decreased 3.65fold while aqueous leaves extract suppressed 5.79fold perioxidase enzyme activity of groundnut seedlings while methanol extract of leaves has seen maximum reduction (7.36fold) followed by stem extract by 4fold and 3.37fold in the flower extract over control from 20% concentration.

Methanol leaves extract found more potent in the reduction of perioxidase activity of groundnut seedlings over all other extracts of *Pascalia glauca* Ortega.

10.2.3 Superoxide dismutase (EC 1.15.1.1) :

Maximum decrease enzyme activity of superoxide dismutase of groundnut seedlings from aqueous (0.153) and methanol (0.050) leaves extract at 20% concentration. Similar reduction was found in aqueous stem and flower (0.593) extract at 10% concentration. Gradual and slower reduction has seen in aqueous stem, leaves and flower as well as in methanol extract but aqueous and methanol leaves extract shown much rapidly reduction of superoxide dismutase enzyme activity from lower concentration (5%) to higher concentration (20%). Comparing aqueous extract, methanol extract have more potentiality to affect the enzyme activity and reduced maximum superoxide dismutase activity of groundnut seedlings from all treated concentrations.

Groundnut seedlings were affected and reduced the superoxide dismutase enzyme activity when treated with 5% aqueous stem extract (0.636); leaves extract (0.583) and in flower extract (0.563). 10% concentration recorded 0.593 in stem extract, 0.526in leaves extract and 0.593in flower extract that means stem and flower

extract showed similar reduction while 15% concentration decreased by 0.356 the superoxide dismutase in leaves, 0.523 in stem and 0.513 in flower extract. From the higher concentration level 20% leaves extract inhibited maximum (0.153) superoxide dismutase activity of groundnut followed by 0.306 in flower extract and 0.310 in stem extract.

Methanol extract of stem determined progressively decrease in superoxide dismutase enzyme of groundnut seedlings from 5% (0.576), 10% (0.426), 15% (0.320) and 20% with 0.190. Methanol extract of leaves was found maximum reduction at 20% (0.050) followed by 15% (0.080), 10% (0.200) and 5% concentration with 0.313. Flower extract gradually reduced enzyme at 5% (0.640), 10% (0.553) while it was sudden decreased at 15% (0.396) and 20% (0.286) concentration.

Aqueous extract of stem expressed minor reduction (2.185fold) while maximum enzyme decreased (4.41fold) in leaves extract then 2.20fold from flower extract in 20% concentration of groundnut seedlings.

At 20% concentration of methanol extract of leaves was maximum decreased superoxide dismutase enzyme about 13.52fold then 3.55fold in stem extract and 2.36fold in the methanol flower extract of *Pascalia glauca* in the groundnut seedlings.

10.2.4 Lipid perioxidase :

The degree of reduction increased maximum in lipid perioxidase of groundnut seedlings has determined in the leaves extracts than stem and flower in both aqueous and methanol extract. Further, methanol extract of leaves have more able to release maximum biochemical that affect negatively on metabolic activity of enzyme activity thus decrease in the lipid perioxidation of groundnut seedlings from higher concentration (20%). The maximum reduction was found at 20% aqueous (0.022) and methanol (0.006) leaves extract. Same reduction (0.065) has seen at 20% methanol stem and flower extract.

Aqueous extract of stem, leaves and flower of *Pascalia glauca* when treated with groundnut seedlings at 5% concentration, it reduced lipid perioxidase activity by 0.082; leaves extract with 0.074and flower extract minor increased by 0.086. 10% concentration of stem extract decreased by 0.070; leaves with 0.071and flower extract found negligible increasing perioxidase (0.084) over control.5% (0.086) and 10% (0.084) aqueous flower extract showed negligible promoting activity over to control.

15% concentration response decreasing trend in the treatments of aqueous extract of stem (0.049); leaves (0.050) and flower extract (0.064). 20% aqueous stem extract decreased by 0.035 leaves extract (0.022) and flower extract by 0.042 over the control.

Methanol extract of stem was reduced lipid perioxidase activity of groundnut by 0.082; leaves (0.062) and flower (0.076) at 5% concentration. 10% concentration of stem decreased by 0.065; leaves (0.052) and flower with 0.065. 15% concentrations decreased 0.053 in stem; 0.023 in leaves and 0.052 in flower extract. At higher concentration of 20% it maximum decreased in activity from leaves extract (0.006) followed by stem (0.029) and in flower (0.034) extract.

Aqueous stem extract reduced 2.51fold; leaves extract 3.77fold and flower extract determined 1.97 fold at 20% concentration of groundnut seedlings.

Methanol extract of stem reduced lipid peroxidase enzyme activity of groundnut seedlings by 2.88fold; leaves extract was reduced maximum (13.33fold) lipid peroxidase of groundnut seedlings in comparison to other extract while flower extract reduced 2.46fold over the control at higher 20% concentration.

10.2.5 Polyphenol oxidase (EC 1.10.3.2) :

The different concentrations of aqueous and methanol extracts of stem, leaves and flowers of *Pascalia glauca* Ortega. has responds decreasing trend of the polyphenol oxidase activity of groundnut seedlings. The results expressed with increasing extract concentration decrease in enzyme activity of groundnut seedlings. Maximum reduction was found in aqueous (0.012) and methanol (0.010) leaves extract at 20% concentration while lowest at 5% aqueous stem and flower extract determined same reduction (0.066). Methanol leaves extract showed higher (6.94fold) than aqueous leaves (5.85fold) extract. Slower reduction recorded at lower concentration (5%) in aqueous and methanol extract while the degree of decreasing enzyme increased from 10% to 20%.

With comparison of control (0.072) aqueous extract of leaves determined maximum reduction (0.012) in the polyphenol oxidase of groundnut seedlings at higher concentration (20%) than the stem (0.033) and flower (0.029) extract. Reduction has been trailed by 0.028 in 15% concentrations, 10% (0.043) and 5% have 0.062from aqueous leaves extract. Aqueous stem extract treatment showed minutely reduction at 5% (0.066), 10% (0.052), then it increased at 15% (0.040) and 20%

(0.033) Aqueous flower extract was seen gradually declined trends from 5% (0.066), 10% (0.070), 15% (0.052) to 20% (0.029) concentrations over the control in groundnut seedlings.

Methanol extract of stem decreased gradually in polyphenol oxidase activity of groundnut seedlings from 5% (0.074), 10% (0.065), 15% (0.048) and 20% (0.016) concentration. Methanol extract of leaves at higher 20% concentration showed maximum reduction (0.010) in polyphenol oxidase activity followed by 15% (0.032), 10% (0.049) and 5% with 0.062 over the control (0.074). Flower extract also reduced from 5% (0.065), 10% (0.042), 15% (0.036) to 20% (0.019) concentration.

Aqueous extract of stem decreased 2.18fold polyphenol oxidase enzyme activity of groundnut seedlings, 6.00fold in leaves extract and 2.48fold in flower extract in over control at 20% concentration.

Methanol extract of leaves was determined maximum reduction (7.20fold) in the polyphenol enzymes of groundnut seedlings followed by 4.55fold in stem extract and 3.78fold in flower extract from 20% concentration in the groundnut seedlings.

10.2.6 Nitrate reductase (NR; EC 1.6.6.4) :

In the higher plants, nitrate is main source of nitrogen for growth and their development that obtained from soil in the form of inorganic nitrogen (Beevers and Hageman, 1980). Migge and Becker (1996), they stated that nitrate assimilation requires nitrate uptake, reduction of nitrate, conversion of nitrate to ammonia and incorporation of ammonia to organic compounds for development of plant growth. Several factors regulate the nitrate assimilation in plants including availability of nitrate, growth regulators, light and other environmental parameters (Campbell, 1996; Padgett and Leonard, 1996 and Ruiz *et al.,* 1998). Nitrate induces genes essential for the uptake and reduction of nitrate, ammonia assimilation, the oxidative pentose pathway, glycolysis and organic acid metabolism (Wang *et al.,* 2004 and Gutierrez *et al.,* 2007).

Mostly the organic nitrogen in biosphere about 99% is derived from the assimilation of nitrate. Nitrate reductase (NR) is a key enzyme of assimilatory nitrogen metabolism. Chavan (1987) was reported that in groundnut it plays an important role in nitrogen metabolism. Nitrate is the important source of nitrogen for plants growing under various field conditions while nitrate reductase has been regulated cellular concentration of nitrate. Enzyme nitrate reductase plays paramount

role in biosynthesis of amino acids and regulation of protein synthesis (Harris, 2000). Sinha and Nicholas (1981) argued that NR activity is generally associated with protein synthesis and plant growth, which are affected by abiotic stresses. Reduction of nitrate by nitrate reductase enzyme has been considered as a rate limiting step of nitrogen assimilation in plants and its position in leguminous crops like groundnut due to symbiotic nitrogen fixation is complicated (Beevers and Hageman, 1980). Sengupta and Sharma (1986) investigated the interaction between plant growth regulators and nitrogen metabolizing enzymes in groundnut.

The effect of stem, leaves and flower aqueous and methanol extract of *Pascalia glauca* on nitrate reductase enzyme when treated with different concentrations against the groundnut seedlings that responds variably as depicted in Table- 20 and Fig.- 19 and 20. Aqueous stem and flower extract decreased nitrate reductase activity of groundnut seedlings steadily but in leaves extract it was maximum decreased at higher concentration. Maximum reduction was seen in 20% aqueous (0.47) and methanol (0.63) leaves extract and minimum in the treatment of 5% methanol stem extract (1.82). Lower concentration 5% aqueous and methanol stem and flower extract found nearly similar reduction. Nitrate reductase enzyme slower but progressively decrease from 5% to 20% concentrations of all extracts.

Aqueous stem extract was decreased nitrate reductase enzyme activity of groundnut seedlings from 5% (1.74), 10% (1.73), 15% (1.52) to 20% (1.27). Similar trends has seen in aqueous flower extract and decrease nitrate enzymes gradually from 5% (1.73), 10% (1.47), 15% (1.30) and in 20% (1.18) while in leaves extract in 5% (1.75) and 10% (1.64) decreasing rate of enzyme activity was increased from 15% (1.36) and 20% (0.47) concentration over the control (1.85).

Methanol extract of stem has reduced activity of nitrate reductase of groundnut from 5% (1.82), 10% (1.62), and 15% (1.35) to 20% (1.12). The methanol extract of leaves decreased nitrate reductase from 5% (1.71), 10% (1.32), 15% (1.27) while maximum reduction seen in 20% (0.63). Methanol flower extract has found reduction of nitrate reductase activity of groundnut seedlings from 5% (1.81); 10% (1.64); 15% (1.50) and 20% with (0.81).

Higher concentration (20%) of aqueous stem extract increased 1.46fold reduction. The maximum inhibition was found in aqueous leaves extract (3.92fold) while flower extract determined 1.57fold over control in groundnut seedlings.

Methanol extract of stem decreased 1.12fold nitrate reductase of groundnut seedlings, 2.93fold in leaves and 2.28fold in flower extract at 20% concentration.

10.2.7 Nitrite Reductase (NiR; EC 1.6.6.4) :

Nitrite reductase is second important enzyme in the pathway of nitrate reduction. A multi heme nitrite reductase reduces nitrite to variety of products. It catalyzes the formation of nitric oxide or nitrous oxide (Kroneck *et al.,* 1992). Payne *et al*. (1985) stated that the nitrite binds to reduced heme during nitrite reduction process. Nitrite reductase is located in plastids of leaf and roottissues (Miflin, 1974). The activity of enzyme has been reported to localize outside the chloroplast (Grant and Canvin, 1970). Cytochrome C nitrite reductase (CcNiR) is a multiheme enzyme plays an important part in the biogeochemical nitrogen cycle. Nitrite reduction in plants is coupled to noncyclic photosynthetic electron transport at the level of ferredoxin (Hucklesby *et al*., 1981).

Nitrite reductase was gradually decreased in the 5% and 10% concentrations while it became rapidly reduction in 15% and 20% in methanol extract of stem, leaves and flower while it was slower decrease at 5% aqueous extract and it becomes fast from 10%, 15% to 20% concentration of stem, leaves and flower extract. The maximum reduction was found in aqueous (0.008) and methanol (0.010) leaves extract.

Progressively decreased nitrite reductase activity of groundnut seedlings from 5% aqueous stem extract (0.045); leaves extract (0.039) and flower extract with 0.039, similar reductions in leaves and flower. Gradual reduction of nitrite reductase has found from 10% concentration of aqueous stem extract (0.032); leaves extract (0.028) and flower extract with 0.024. It was continued at 15% concentration of aqueous stem extract (0.024), leaves extract (0.013) and flower extract determined 0.015. At higher concentration 20% it has been more decreased from stem extract (0.012); leaves (0.008) and in flower extract (0.012). Aqueous stem and flower extract showed similar reduction in the nitrite reductase enzymes activity of groundnut seedlings.

Methanol extract of stem and leaves showed minor reduction of nitrite reductase activity of groundnut seedlings (0.049 and 0.050) respectively while flower extract have 0.047 at 5% lower concentration. 10% concentration found 0.043 reduction in stem extract; leaves (0.040) and flower extract 0.030. 15% concentration of stem extract decreased 0.031; 0.024 in leaves extract while flower extract was

determined maximum (0.020) over the control. At 20% higher concentration it was reduced in stem extract (0.021); maximum reduction in leaves extract (0.010) and flower extract (0.017) over control.

In higher concentration (20%) of aqueous leaves extract determined maximum reduction of nitrite reductase (6.37fold) followed by similar reduction in aqueous stem and flower extract (4.25fold) in groundnut seedlings.

Methanol extract of stem decreased 2.42fold nitrite reductase enzyme activity of groundnut seedlings, 5.10fold in leaves extract and 3fold in flower extract at 20% concentration.

Discussion :

Allelopathy is the complex phenomenon that mechanized in plant and crop ecosystem and it concerns the effects on neighboring life through breakdown products of their metabolites. Biochemical compounds were released from the donor and/weed plants by the various biological and physiological process that hoard allelochemicals have potentiality to cause either beneficial or mostly harmful effect on the nearby plants or crop. They ruled the multidimensional and wide spectrum role in the physiology; biology and enzymology of receptor plant species might be alter or change their activity. They detain the plant growth through increasing the seed dormancy that unease and/broach the essential metabolism due to which it can alter the mineral uptake, engender the different stress and influence on the photosynthesis and respiration, hurdling in the protein and specific enzyme activity, impaired the hormonal balance in the crop plants.

Enzymes are macromolecular biological catalysts that stimulate chemical reactions. They have been considered as protein molecules in cells which work as catalysts that speed up chemical reactions. Enzymological study of plant has been prime importance in the allelopathy to understanding the effect of weed extracts of different concentration on the crops for the agricultural development. Number of researchers have been studied such aspects that explain the influence of weed extracts on various crops, most of them are inhibitory or suppressed the metabolic activity of crop and finally declined in their productivity. Dozens of contributors work on antioxidant enzymes like catalase, peroxidase, superoxide dismutase, lipid perioxidase, polyphenol oxidase, nitrite reductase, and nitrate reductase in several allelopathic plants were investigated and record put in bucket to publish and examined

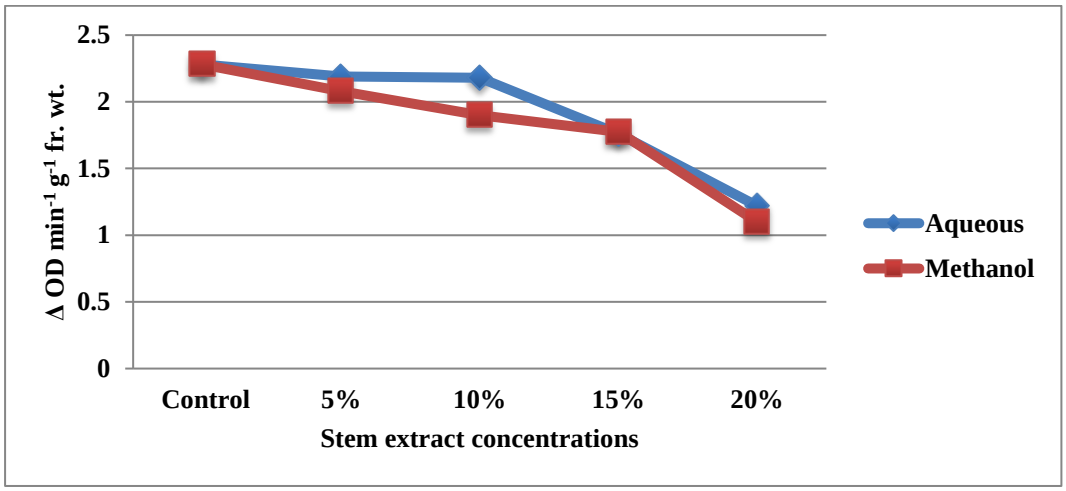
Table- 20 : Effect of aqueous and methanol extracts of stem, leaves and flowers of *Pascalia glauca* Ortega on different enzymes of groundnut seedlings (ΔOD min^{-1} g^{-1} fr. wt.).

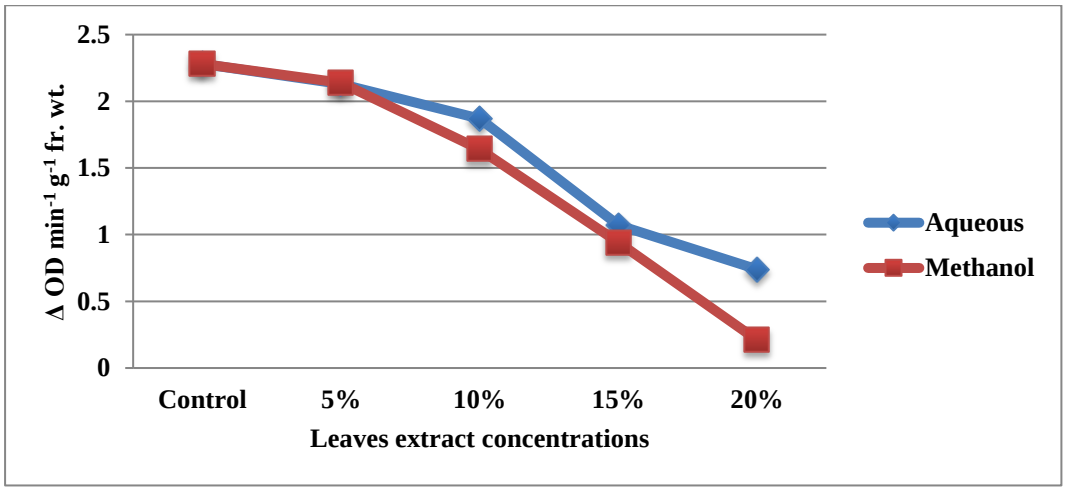
Plant part extract	Groundnut seedlings								
	Catalase								
	Concentrations	5%		10%		15%		20%	
	Control	Aqueous	Methanol	Aqueous	Methanol	Aqueous	Methanol	Aqueous	Methanol
Stem	2.28 ±0.035	**2.19** ±0.025	2.08* ±0.030	2.18 ±0.025	1.90** ±0.025	1.76** ±0.040	1.77** ±0.045	**1.22**** ±0.050	1.10** ±0.060
Leaves		2.13* ±0.035	**2.14** ±0.040	1.87** ±0.040	1.64** ±0.045	1.07** ±0.050	0.94** ±0.030	**0.74**** ±0.051	**0.21**** ±0.045
Flower		**2.18** ±0.060	**2.14** ±0.040	2.07** ±0.040	2.02* ±0.065	1.86** ±0.060	1.86** ±0.050	**1.22**** ±0.055	1.18** ±0.090
Perioxidase									
Stem	0.560 ±0.040	**0.486** ±0.040	**0.546** ±0.025	0.463 ±0.035	**0.330**** ±0.045	0.323** ±0.035	0.216** ±0.020	**0.153**** ±0.030	0.140** ±0.020
Leaves		0.386** ±0.050	0.446** ±0.030	0.306** ±0.030	0.230** ±0.055	0.196** ±0.025	0.123** ±0.025	**0.096**** ±0.025	**0.076**** ±0.015
Flower		0.426** ±0.025	0.530* ±0.026	0.426** ±0.037	**0.333**** ±0.040	0.286** ±0.030	0.240** ±0.030	**0.153**** ±0.025	0.166** ±0.025
Superoxide dismutase									
Stem	0.676 ±0.025	**0.636** ±0.025	0.576 ±0.025	**0.593*** ±0.030	0.426** ±0.030	0.523** ±0.025	0.320** ±0.020	0.310** ±0.020	0.190** ±0.026
Leaves		0.583* ±0.035	0.313** ±0.040	0.526** ±0.025	0.200** ±0.020	0.356** ±0.025	0.080** ±0.020	**0.153**** ±0.030	**0.050**** ±0.030
Flower		0.563* ±0.040	**0.640** ±0.020	**0.593** ±0.051	0.553* ±0.030	0.513** ±0.030	0.396** ±0.025	0.306** ±0.025	0.286** ±0.030
Lipid perioxidase									
Stem	0.083 ±0.002	0.082 ±0.002	**0.082** ±0.002	0.070** ±0.002	**0.065**** ±0.003	0.049** ±0.003	**0.053**** ±0.003	0.035** ±0.003	0.029** ±0.003
Leaves		0.074 ±0.002	0.062** ±0.002	0.071 ±0.015	0.052** ±0.002	0.050** ±0.003	0.023** ±0.003	**0.022**** ±0.004	**0.006**** ±0.002
Flower		**0.086** ±0.002	0.076* ±0.003	**0.084** ±0.003	**0.065**** ±0.003	0.064** ±0.002	**0.052**** ±0.002	0.042** ±0.002	0.034** ±0.003
Polyphenol oxidase									
Stem	0.072 ±0.002	**0.066** ±0.002	**0.074** ±0.002	0.052** ±0.002	0.065* ±0.002	0.040** ±0.003	0.048** ±0.003	0.033** ±0.002	0.016** ±0.004
Leaves		0.062* ±0.002	0.062* ±0.004	0.043** ±0.003	0.049** ±0.005	0.028** ±0.004	0.032** ±0.004	**0.012**** ±0.002	**0.010**** ±0.003
Flower		**0.066** ±0.002	0.065 ±0.006	0.070 ±0.003	0.042** ±0.004	0.052** ±0.002	0.036** ±0.004	0.029** ±0.003	0.019** ±0.003
Nitratereductase (µg g^{-1}dry wt.)									
Stem	1.85 ±0.035	1.74* ±0.036	**1.82** ±0.136	1.73* ±0.015	1.62* ±0.025	1.52** ±0.055	1.35** ±0.036	1.27** ±0.055	1.12** ±0.026
Leaves		**1.75** ±0.035	**1.82** ±0.136	1.64 ±0.040	1.62* ±0.025	1.36** ±0.243	1.27 ±0.574	**0.47**** ±0.030	**0.63**** ±0.050
Flower		1.73* ±0.040	1.81 ±0.036	1.47** ±0.030	1.64** ±0.040	1.30** ±0.040	1.50** ±0.030	1.18** ±0.052	0.81** ±0.030
Nitrite reductase (nmol N_2consumed $min^{-1}mg^{-1}$)									
Stem	0.051 ±0.003	**0.045** ±0.004	0.049 ±0.003	0.032** ±0.004	0.043 ±0.003	0.024** ±0.002	0.031** ±0.003	**0.012**** ±0.002	0.021** ±0.003
Leaves		**0.039**** ±0.003	**0.050** ±0.002	0.028** ±0.005	0.040** ±0.004	0.013** ±0.003	0.024** ±0.002	**0.008**** ±0.002	**0.010**** ±0.003
Flower		**0.039**** ±0.005	0.047 ±0.002	0.024** ±0.002	0.030** ±0.004	0.015** ±0.003	0.020** ±0.004	**0.012**** ±0.003	0.017** ±0.002

One way Anova: Tukeys HSD Post-hoc Test Inference. **Significant at P < 0.01.

*Significant at P < 0.05. Otherwise insignificant result.±Standard deviation.

Fig. - 14 : **Effect of aqueous and methanol extracts of stem, leaves and flowers of *Pascalia glauca* Ortega on catalase enzymes of groundnut seedlings.**





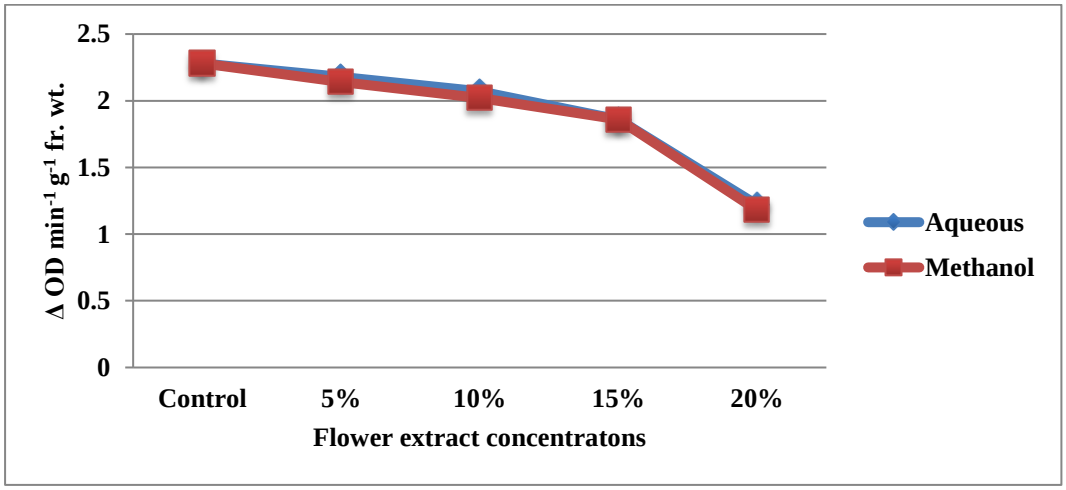
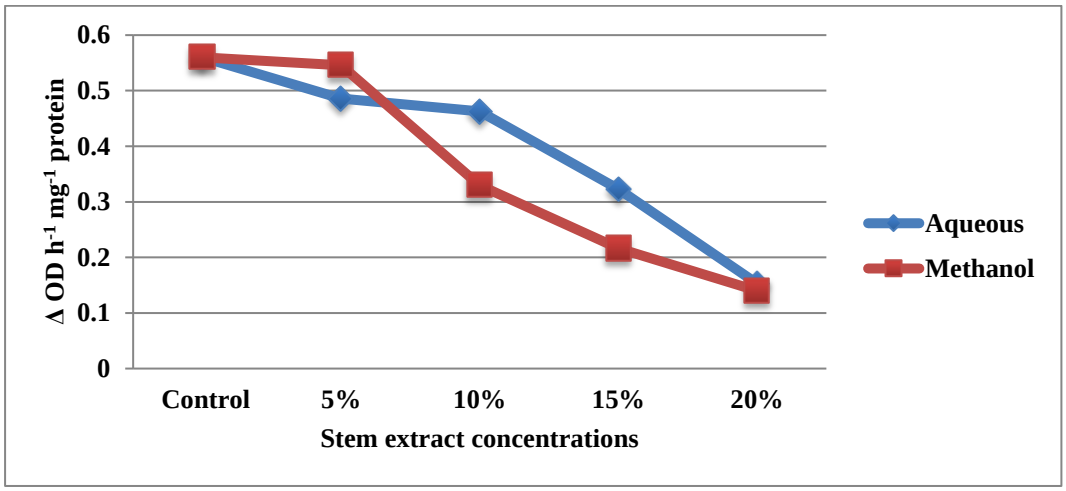
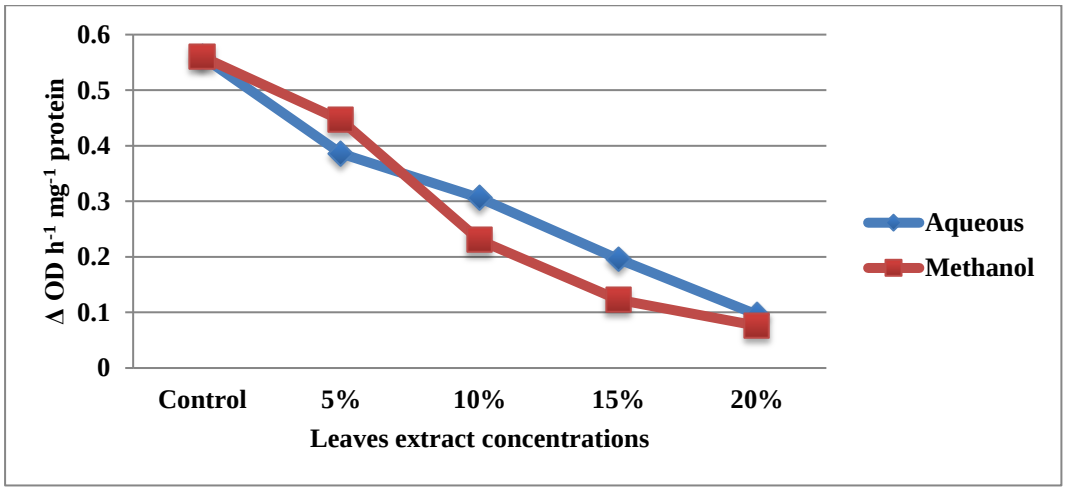


Fig. - 15 : **Effect of aqueous and methanol extracts of stem, leaves and flowers of *Pascalia glauca* Ortega on perioxidase enzymes of groundnut seedlings.**





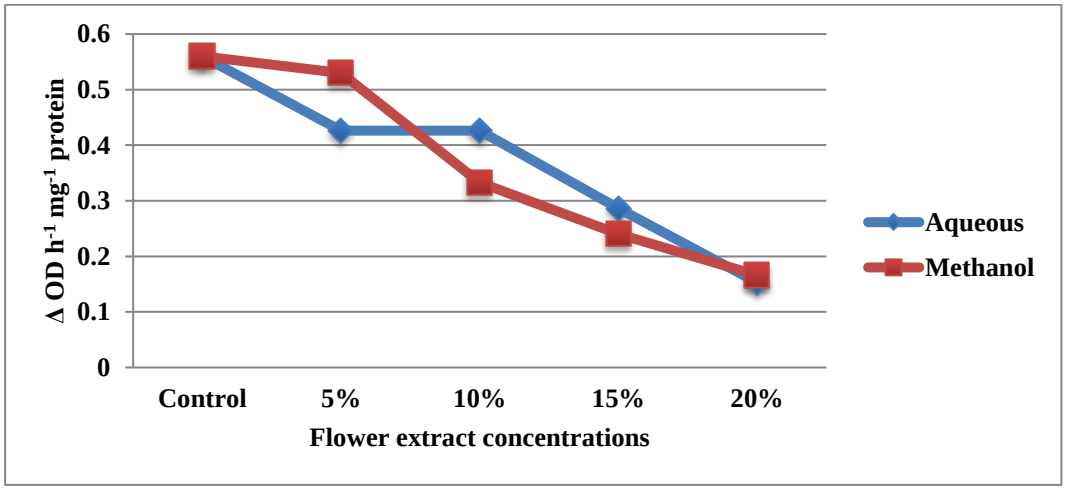
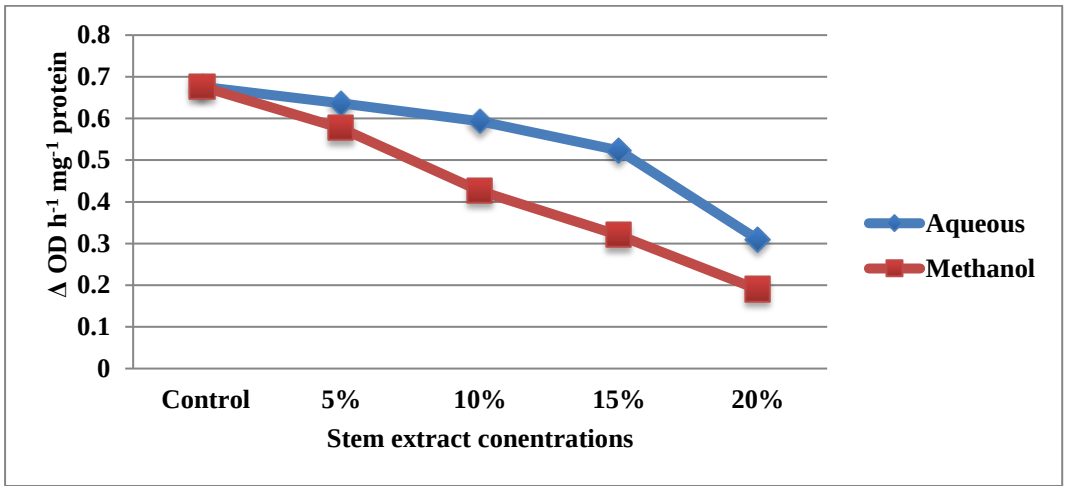
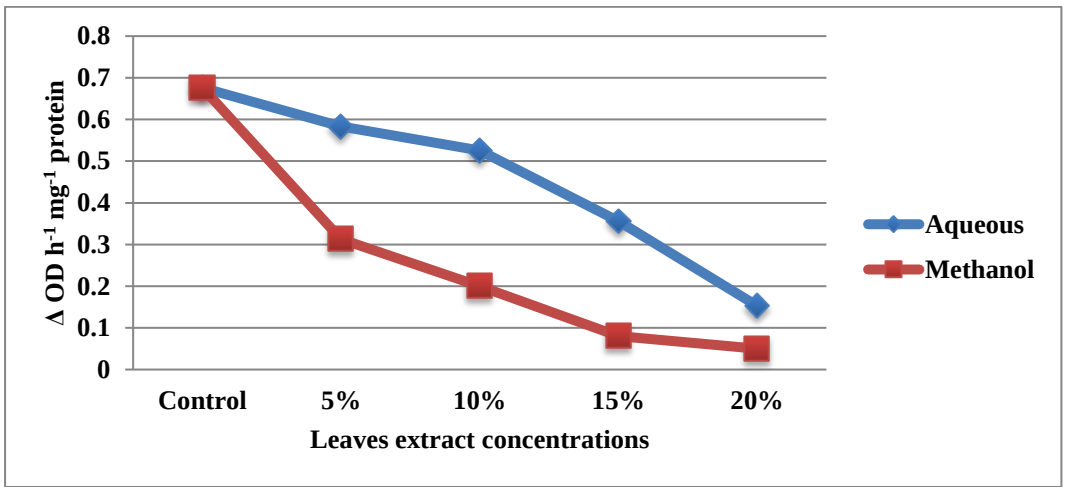


Fig. - 16 : Effect of aqueous and methanol extracts of stem, leaves and flowers of *Pascalia glauca* Ortega. on superoxidase dismutase enzymes of groundnut seedlings.





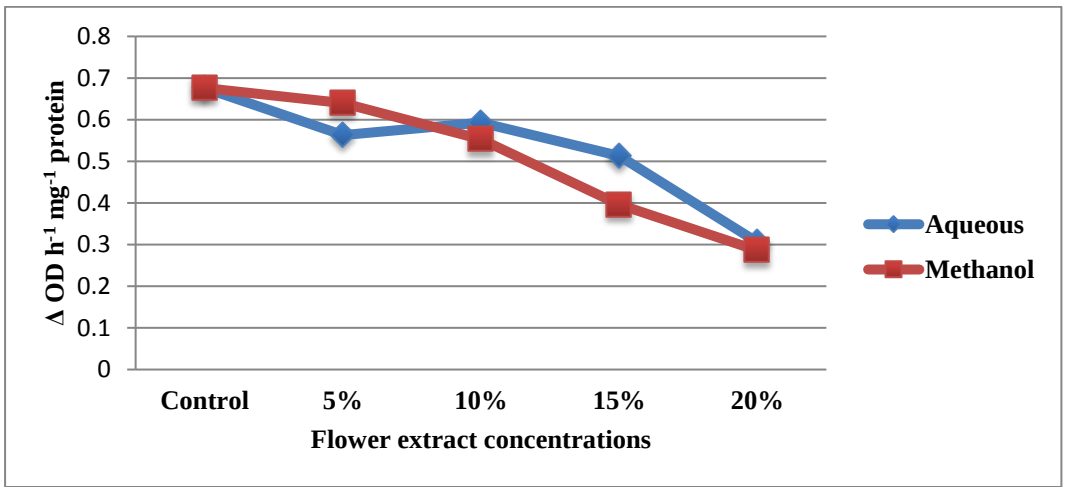
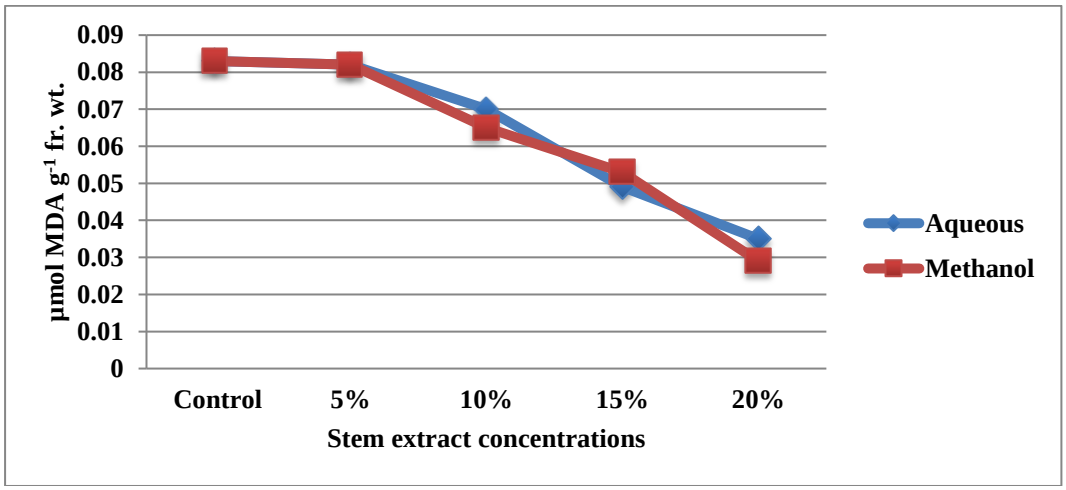
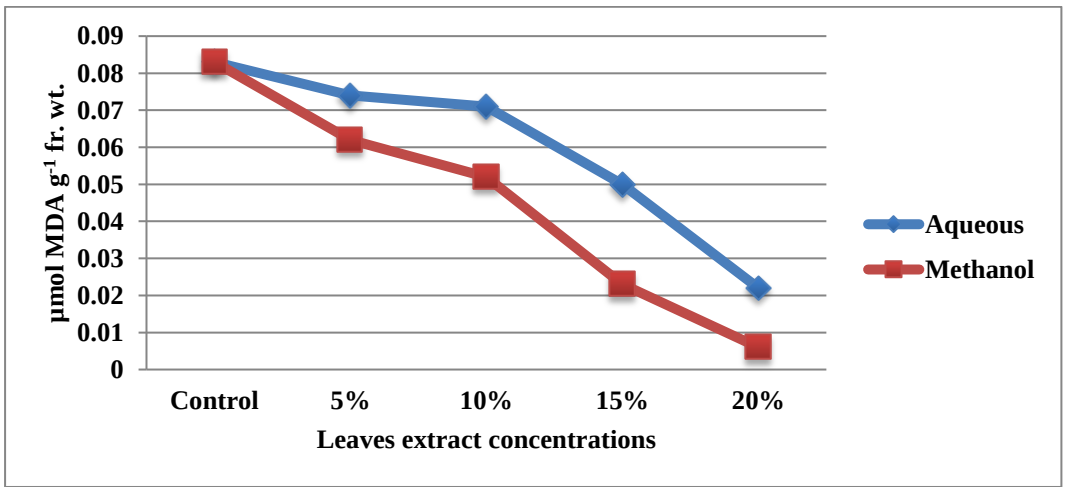


Fig. - 17 : **Effect of aqueous and methanol extracts of stem, leaves and flowers of *Pascalia glauca* Ortega. on lipid perioxidase enzymes of groundnut seedlings.**





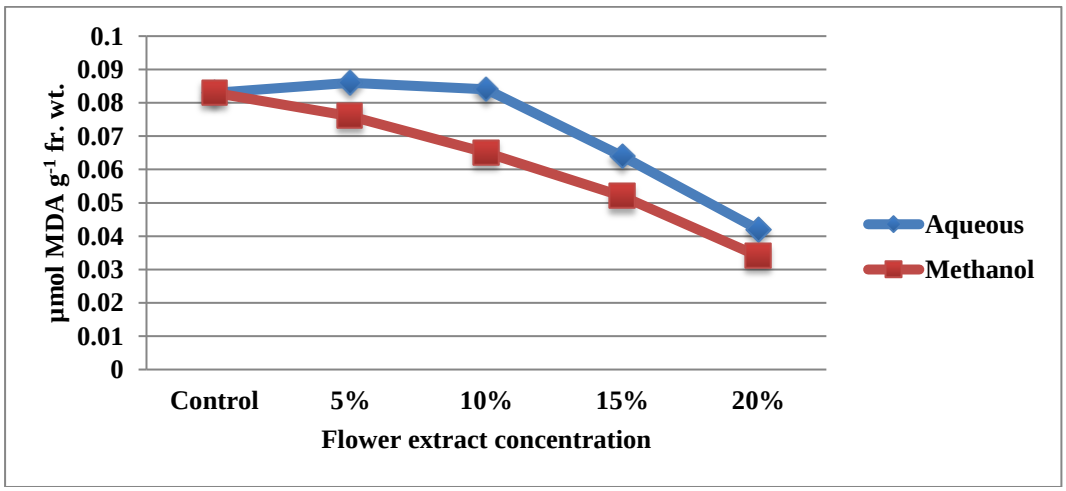
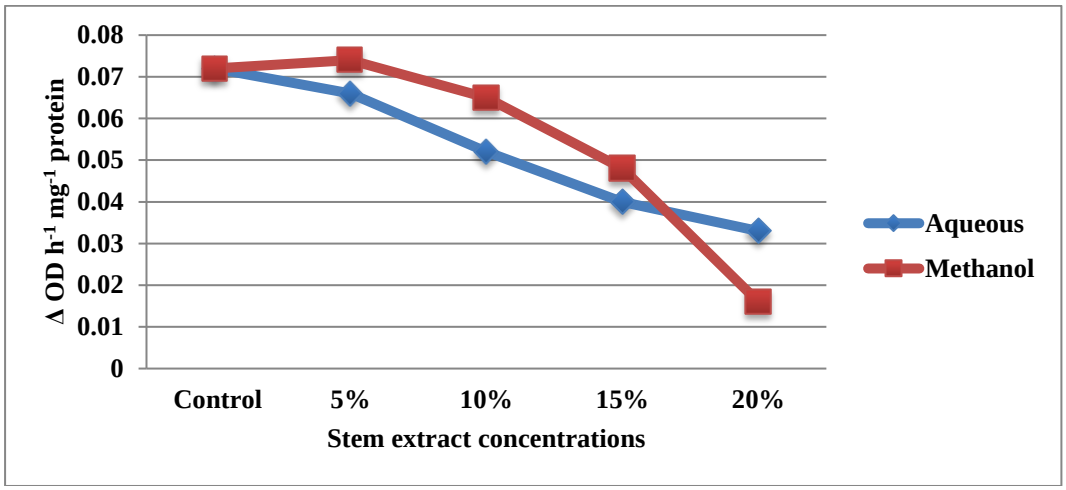
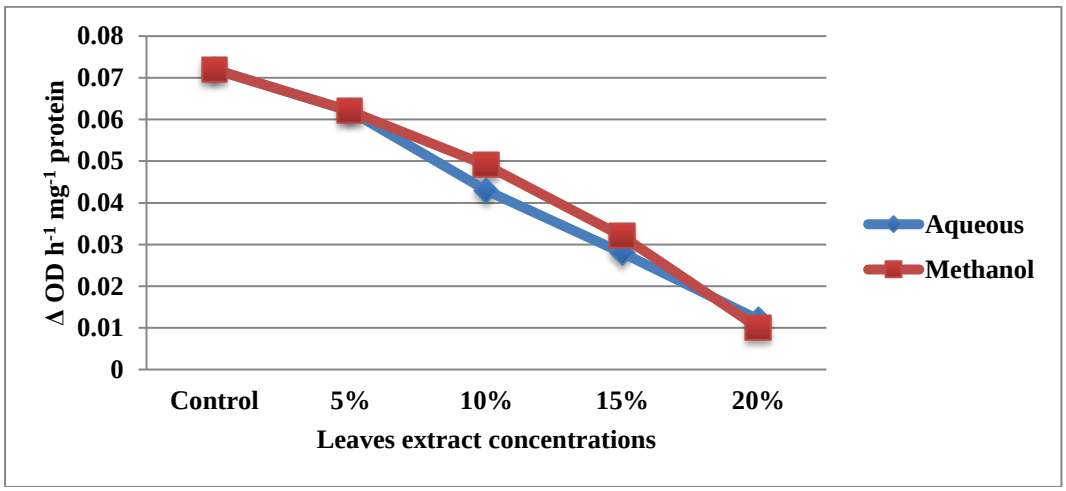


Fig. - 18 : **Effect of aqueous and methanol extracts of stem, leaves and flowers of *Pascalia glauca* Ortega. on polyphenol oxidase enzymes of groundnut seedlings.**





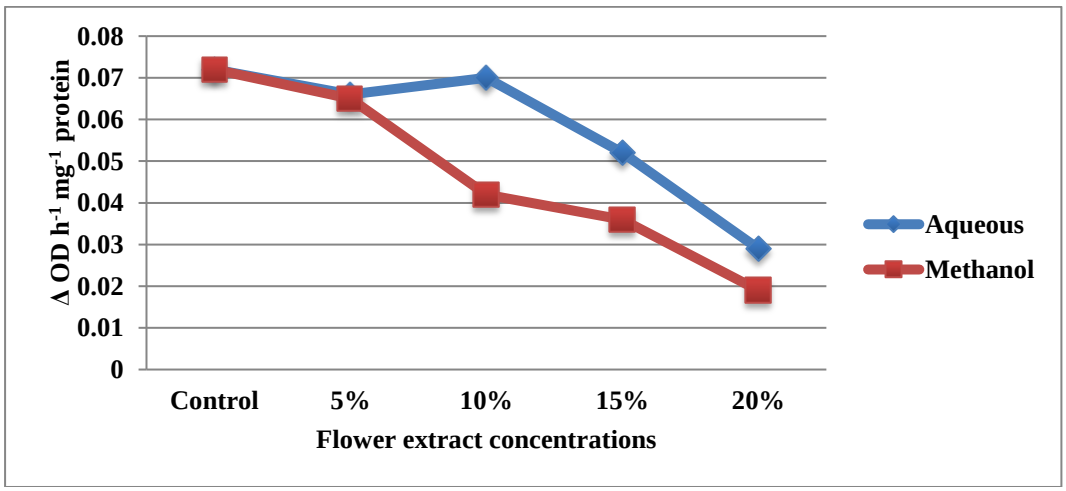
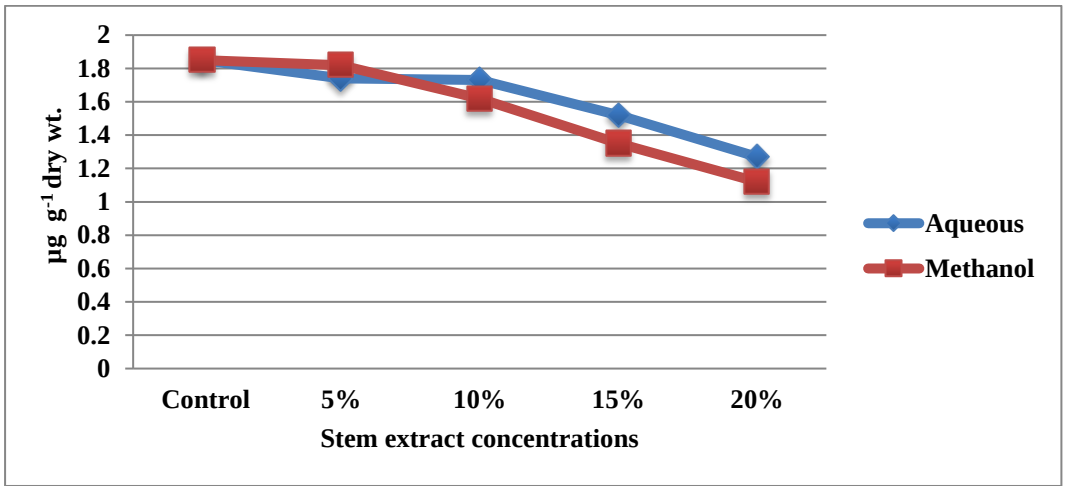
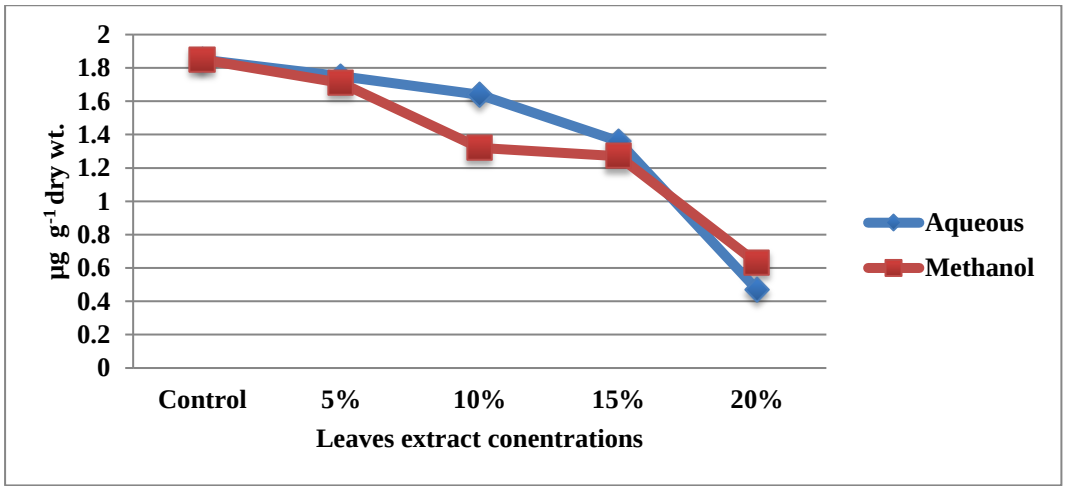


Fig. - 19 : **Effect of aqueous and methanol extracts of stem, leaves and flowers of *Pascalia glauca* Ortega. on nitrate reductase enzymes of groundnut seedlings.**





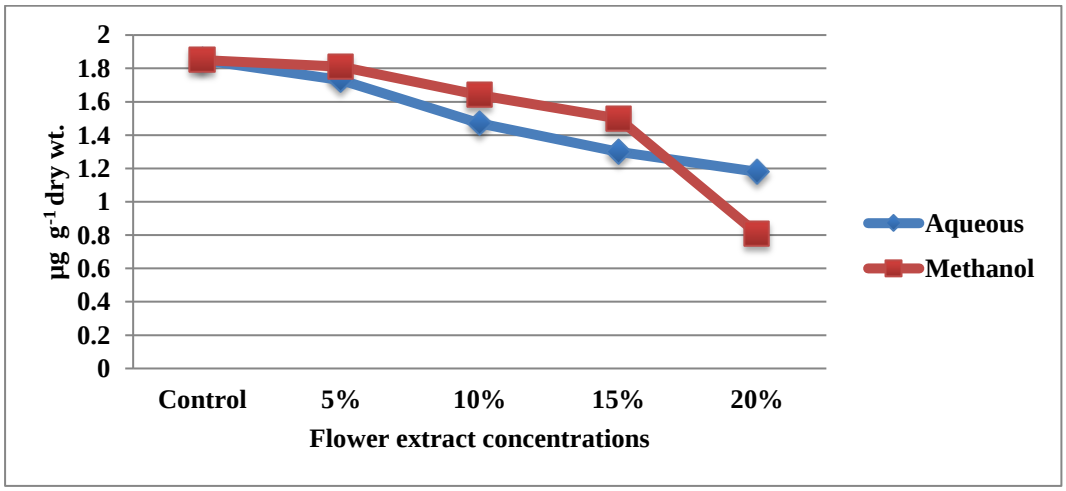
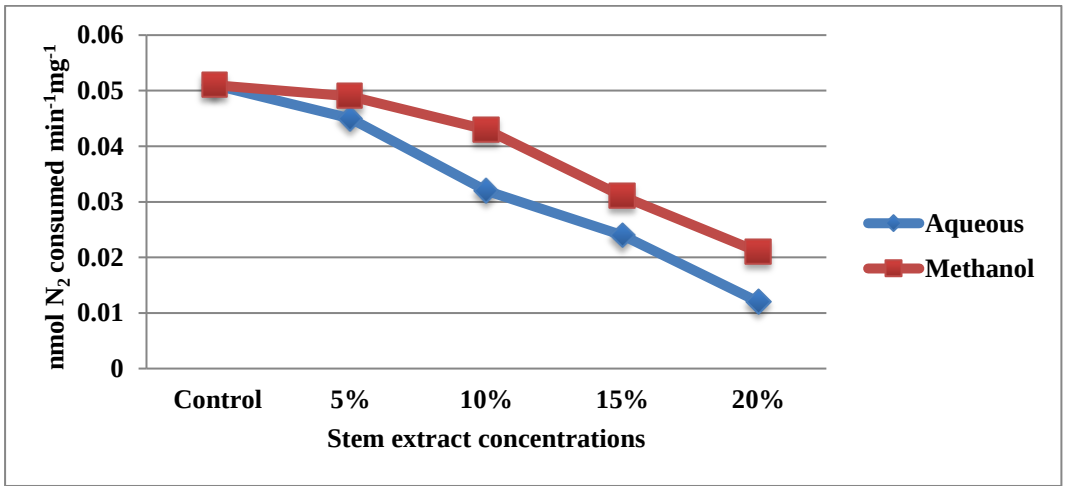
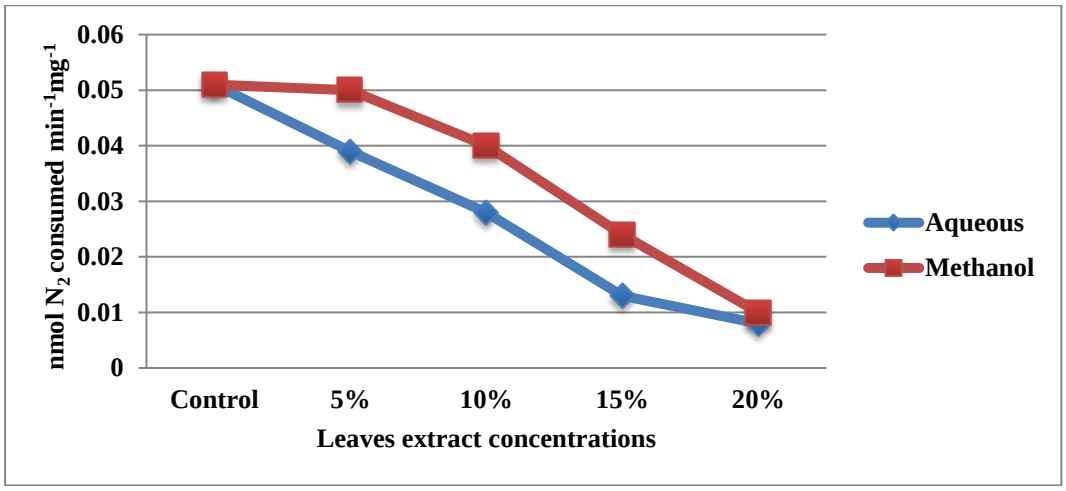
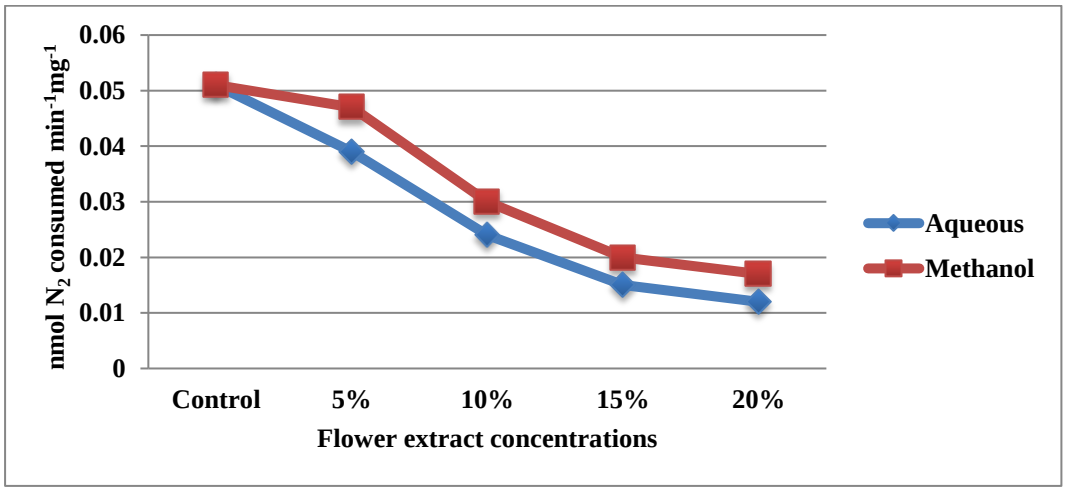


Fig. - 20 : **Effect of aqueous and methanol extracts of stem, leaves and flowers of *Pascalia glauca* Ortega. on nitrite reductase enzymes of groundnut seedlings.**







judgments in reputed scientific journals in different years well known Rosa *et al*. (2009), Maiti *et al*. (2010), Anjum *et al*. (2010), Manimegalai and Manikandan (2010), Fini *et al*. (2011), Naeem *et al*. (2012), Tanveer *et al*. (2012), Mohamed *et al*. (2012), Maiti *et al*. (2013), Rajiv *et al*. (2013), Kim *et al*. (2013), Maristela Imatomi *et al*. (2013), Omezzine *et al*. (2014), Shruti *et al*. (2014), Oliveiva *et al*. (2014), Mahmoud and Ahmed (2015), Saleh and Madany (2015), Babar *et al*. (2015), Amb and Ahluwalia (2016), Saira Siyar *et al*. (2017), Adhikary (2017) and Imtiyaz Hussain *et al*. (2017).

The intensity of inhibition was directly proportional to the concentration of the extract used and was concentration dependent. As the concentration of extract increased the catalase, peroxidase and polyphenol oxidase content has been found to be gradual decreased in black gram and green gram (Manimegalai and Manikandam, 2010). The intensity of the inhibition increased as the concentration of leaf extract increased. Tefera (2002) and Maharjan *et al.* (2007) noticed that leaves are the most potent parts exhibiting allelopathic interactions. Our results were in the line of above workers. There are numerous reports that dehydrogenase, catalase, amylase and peroxidase enzymes seem to play a vital role during germination and growth (Bhattacharjee *et al.,* 2003, Bhakat *et al.,* 2006, Isfan and Shariati, 2007 and Amoo *et al*., 2008). Maiti *et al*. (2010) explained that drastic reduction of proteins and catalase when treated with *Lantana camera* on test plants. Phototoxic substances may act in many biological processes such as retarding the photosynthesis, respiration and enzymatic activities, resulting in the retardation of plant growth. They may also interfere with the action of plant growth regulators (Chou, 1980). Xu Tao *et al.* (1999) have found significant inhibition in chlorophyll content, soluble protein content and peroxidase activity of all the test plants, namely radish, mungbean and ryegrass treated with volatile oil of *Ageratum conyzoides* in Hoagland solution culture.

Padhy *et al*. (2000) reported that seed germination and seedling growth hindered due to impairment of various metabolic activity that regulated by different enzymes in the vicinity of allelopathic plants. Reduction in nitrate reductase activity in response to weed extracts has also been reported by Gogoi *et al.* (2002).The results obtained from the study by Krishna *et al*. (2003) in which they revealed that the leaf extract of *Tectona grandis* exhibited inhibitory effects in enzymes catalase, perioxidase and polyphenol oxidase in higher concentration extract treatment against test plants. Further, they explained the level of catalase, perioxidase and polyphenol

oxidase showed a gradual decreased when treated with 10, 25, 50, and 75 % and in 100% leaf extract of treated seedlings showed an inhibitory effect. Our results showed cleared conformity with these results that determined the gradual increased in reduction of above enzyme when treated with *Pascalia glauca* extract on wheat and groundnut seedlings. The allelopathic effect of extracts from teak leaves has been tested on solanaceae species such as tomato (*Lycopersicum esculentum),* eggplant (*Solanum melongena*) and pepper (*Capsicum annum*) (Krishna *et al.,* 2003). The extracts significantly inhibited metabolic activities, germination and growth of plant species. *Tectona grandis* has also shown high allelopathic activity on wheat (*Triticum aestivum*) (Krishna *et al.,* 2003). Lipid peroxidase increased membrane damage in increasing allelochemical treatment (Apel and Hirt, 2004). Maiti *et al*. (2010) explained that *Lantana camera* leaf extract declined the protein level and significantly slow down the activity of enzyme dehydrogenase and catalase of mung bean. The interaction of allelochemicals with enzymatic activities might have regulated the energy metabolism and thus consequently resulted in impairment of germination behavior and metabolism of the test seed samples (Sodaeizadeh *et al*., 2009, Anjum *et al*., 2010 and Maiti *et al*., 2013).Our results corroborated with above workers.

The concentration of extract intensity of *Tectona grandis* increased results were gradual decreasing enzyme like catalse, peroxidase and polyphenol oxidase of black gram and green gram (Manimegalai and Manikandam, 2010). Kasi Viswanath Kotapatil *et al*. (2014) stated that drought stress reduces the availability of CO_2 in leaves and inhibits the carbon fixation and generation of reactive oxygen species which leads to oxidative stress. Catalase is an enzyme responsible for the degradation of hydrogen peroxide present in plant leaves (Kasi Viswanath Kotapatil *et al*., 2014). In our investigation, catalase activity was decreased in test samples when compared to controls from higher concentrations of *Pascalia glauca* extracts. Allelochemicals, present in sesame leachate inhibited sprouting of tubers of the nutsedge along with decrease in growth, photosynthetic pigment and activity of antioxidant enzymes reflects that sesamum leachate may help control weed that contributed as bioherbicide to reduce the use of weedicides and pesticides for the sustainable agriculture (Imtiyaz Hussain *et al*., 2016).Thus, our results were same in line of above said statement and might be helpful in control of weed because of our field observations showed dominant weed *Cynodon dactylon* has completely eradicated by the growth of *P. glauca* from studied area. Kalita *et al.* (1998) reported the inhibition of nitrate

reductase activity of rice plants grown in soils amalgamated with different amounts of weed residue of *Cynodon dactylon.* Jafari and Kholdebarin (2002) found that the presence of leaf extracts of *Chenopodium album* significantly delayed the complete oxidation of nitrite into nitrate. Reduction in nitrate reductase activity in response to weed extracts has also been reported by Gogoi *et al.* (2002). Environmental stresses can result into oxidative stress through increased production of reactive oxygen species (ROS) that can induce changes in cellular metabolism through oxidative damage to membranes, proteins and nucleic acid and may result in lipid peroxidation and protein denaturation (Imlay, 2003; Garg and Manchanda, 2009 and Gill and Tuteja, 2010). Superoxide dismutase is the first enzyme in the detoxifying process that catalyses the dismutation of O_2- to H_2O_2 and O_2 and is considered to be an essential component of antioxidant defense system in plants (Fridovich, 1986). Our findings has been determined decreased superoxide dismutase, lipid perioxidase and polyphenol oxidase from 20% higher concentration when treating with stem, leaves and flower extracts of *Pascalia glauca* has greatly influenced on test crops growth point out indicated that the effect of extracts on the metabolic activities and enzyme degradation, protein denaturation might be results into the oxidative damage and reduce the growth and development of wheat and groundnut seedlings.

Peroxidases are a group of oxido-reductases which are present in most of the plant tissues. Ascorbate peroxidase is involved in scavenging of H_2O_2 and forms an important part of the antioxidant system (Dabrowska *et al.,* 2007). Peroxidase is the first enzyme to alter its activity under stress (Srivalli *et al.,* 2003). According to Koca *et al*. (2007) salinity leads to decrease in superoxide dismutase activity in salt sensitive plants of *Sesame indicum* L. than salt tolerant ones (Akbar *et al*., 2009).The reduction of seed germination occurs due to imbalance in metabolism and metabolite transport regulated by various enzyme activities from seeds (Malele *et al*., 2003 and Maiti *et al*., 2008). Destruction of lipid components of membrane by lipid peroxidation causes membrane impairment and leakage (Halliwell, 1994). Qian *et al*. (2009) reported that allelochemicals stress cause oxidative damage and trigger the synthesis of reactive oxygen species (ROS) to disrupt the subcellular structure. The increase of melondialdehyde (MDA) formation is a direct consequence of increase ROS formation and thus unsaturated fatty acid peroxidation.

The biochemical changes in studied enzyme that suppressed their activity and the growth parameters were significantly reduced in seedlings of mung beans when

pretreated with leaf extracts of *Lantena camera* from each concentration (Maiti *et al*., 2010). The concentration of extract increased, the catalase, peroxidase and polyphenol oxidase content of black gram and green gram showed a gradual decrease further, the intensity of the inhibition increased as the concentration of leaf extract increased (Manimegalai and Manikandam, 2010). Leaf extracts of *Gmelina arborea* inhibition the activity of some hydrolytic enzymes amylase, catalase and acid phosphatase in legumes seeds (Ramakrishnan *et al*., 2014). These above results were in the line of our work, where the impairments of seed germination and disturbances in metabolism due to either alter or change and impede the enzyme activities when treated with higher concentration of extract of *Pascalia glauca* containing some inhibiting substances or allelochemicals escaped by plant and mix into soil that affect on wheat and groundnut seedlings.

There are many reports that antioxidative enzymes play important role during the seed germination (Terry *et al*., 2008, Maiti *et al*., 2009, Ghayal *et al*., 2011, Bhakat and Maiti, 2012 and Mahmood *et al*., 2013) and are greatly influenced by some putative allelochemicals present in the leaf extracts. Membrane damage under the influence of secondary metabolites possess high amount of melondialdehyde (MDA) content which linked to lipid peroxidation in cucumber and sorghum roots (Zeng *et al*., 2001) Superoxide dismutase protects the cell damage by detoxifying reactive oxygen species. Decreased activity of reactive oxygen species in plants when treated at higher concentration of leachate indicated the failure of superoxide dismutase defense. The inhibiting the activity of superoxide dismutase has been at higher concentration of treatment (Imtiyaz *et al*., 2017). The result of the work conducted by Gulzar and Siddiqui (2017) indicated that catalase, peroxidase and superoxidase dismutase activity decreased in brassica receiving leaf, fruit and flower extracts of *Calatropus procera* additionally, maximum significant decrease in activity of catalase, peroxidaseand superoxidase dismutase was found with (20 and 40%) aqueous extract of leaf and their results can be inferred that extracts posses some allelochemicals which might have significantly and non-significantly increased and decreased the antioxidant enzyme activity in brassica. Our results corroborated with above workers, where aqueous and methanol extract of stem, leaves and flower extract of *Pascalia glauca* suppressed the activity of above enzymes at 15% and 20% concentration in wheat and groundnut seedlings. On the other hand, there is absolutely opposite results has been put on desk by Hamid and Ahmad (2017) in *Cucurbita pepo*

seedlings expressed significant enhancement of many physiological parameters including the activity of antioxidant enzymes like perioxidase, superoxide dismutase, catalase, lipid peroxidation and proline contents. Same results stated by Tolulope *et al*. (2016) in *Vigna unguiculata* seedlings when treated with *Tithonia diversifolia* extract. Our findings also determined similar results in increasing the some enzyme content after the treatment of lower concentration of *Pascalia glauca* extract, from 5% lower concentrations methanol flower extract slightly increased in superoxide dismutase, 5% aqueous flower extract elevated lipid peroxidase while polyphenol oxidase increased in 5% stem extract in the groundnut seedlings. Catalase has been increased from 5% aqueous leaves and 5% as well as 10% stem and leaves extract in the wheat seedlings.

On the basis of our experimental results on germination and growth behavior, biochemical changes in general and enzymes in particular when the seeds of wheat and groundnut pretreated with different concentrations of aqueous and methanol extracts of stem, leaves and flower of *Pascalia glauca*, determined few of lower concentrations of extract have promotory results but majorly with increasing extract concentrations declining in the enzyme activity of catalase, perioxidase, superoxide dismutase, lipid perioxidase, polyphenol oxidase, nitrate and nitrite reductase. It might be due to the some substances interfere the metabolism of crop wheat and groundnut due to presence of inhibitor biochemical in the surrounding.

It is evident from our observations that catalase, peroxidase, superoxide dismutase, lipid perioxidation and polyphenol oxidase enzyme in germinating wheat and groundnut seeds were quite sensitive to leaf extract of *Pascalia glauca*. Although, a marked decreased of the enzyme activity has been caused by all parts of *Pascalia* including stem, leaves and flower extract in the wheat and groundnut seedlings at 20% higher concentration. Additionally, both the aqueous and methanol extract have potentiality to alter the oxidative enzyme activity but aqueous extract seen more inhibitory effect than methanol extract. In the overall experiment decrease in all studied enzymes activity by leaf extract treatment may pose limitation on scavenging of ROS as well as cell wall biogenesis in the germinating wheat and groundnut seedlings and this in turn can lead to affect seedling growth and its growth adversely.

C) Phytochemical investigation :

11.1 Phytochemical screening of *Pascalia glauca* Ortega from root, stem, leaves and flower extracts :

The phytochemical analysis was done from the aqueous and methanol extracts of *Pascalia glauca* using standard procedures to identify the positive or negative test of the phytochemical constituents from the root/stolon, stem, leaves and flower (Patil and Khan, 2017). Our preliminary phytochemical screening test showed presence of alkaloids, flavonoids, steroids, triterpenoids, tannins and saponins in various plant parts depicted in Table-21.

Aqueous root/stolon extract has positive test for alkaloids, flavonoids, steroids while methanol extract showed negative test in steroids but positive test for alkaloids, flavonoids, triterpenoids, tannins and saponins. The presence of alkaloids, flavonoids, triterpenoids and tannins was detected from aqueous stem extract and methanol extract of stem showed presence of alkaloids, flavonoids, steroids, tannins and saponin but triterpenoids absent. Steroids and saponins showed negative test from aqueous leaves extract but alkaloids, flavonoids, triterpenoids and tannins have positive test and methanol extract detected alkaloids, flavonoids, steroids, triterpenoids and saponins. Only flavonoids and triterpinoids test was positive in aqueous flower extract but methanol extract showed presence of alkaloids, flavonoids and triterpinoids.

Aqueous and methanol stem and leaves extracts has more potent source for the secondary metabolites. Root/stolon methanol extract showed positive test in all tested phytochemicals except triterpenoids. Flower extract showed positively results only for the flavonoides and triterpenoides.

11.2 Extraction, isolation and identification of allelochemicals in *Pascalia glauca* Ortega extracts :

The alleolepathic action was responded by a number of identified allelochemicals. Such chemicals were identified from plant extract of *Pascalia glauca* by using Liquid Chromatography Mass Spectra (LCMS) and Gas Chromatography Mass Spectra (GC-MS) study as shown in the Table- 22, 23 and 24 and Fig.-21.

a) Liquid chromatography mass spectra (LCMS) analysis :

Liquid Chromatography-Mass Spectrometry (LCMS) is an analytical chemistry technique that combines the physical separation capabilities of liquid chromatography (or HPLC) with the mass analysis capabilities of mass spectrometry. It is widely used method of sample ionization prior to analysis and is frequently coupled with mass spectrometry. Detection of different allelochemicals was identified from methanol extract of whole plant *Pascalia glauca* by using advanced techniques of LCMS. The monotypic genera *Pascalia glauca* Ortega belongs to family Asteraceae mainly constituted by *ent*-kaur-16-ene-19-oic acid, wedelolactone, atractyloside, sesquiterpenes, steroids, tannic acids, flavonoides and its derivatives, present in various parts of plant.

In *Pascalia glauca* Ortega majorly identified compounds including monoterpines, diterpines, triterpines, triterpine saponins, sesquiterpines, kaurenes diterpinoides, polyacetylenes and flavonoides. It contains lactones, luteolin and kaurenoic acid and wedelolactone group of compounds. These identified compounds as per laborataory data are tabulated in Table - 22 including, a-Terpinyl acetate, 3-Cyclohexane 1-ol, 3-Cyclohexane 1-ol,4Methyl-1 (R), a-Phellendrene, D-Limonene, N-Butyl-4-methylpyridinium, Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl, Kaur-16-en-19-ol, Methyl Ent-16-Kauren-19-Oate, Kaur-16-en-18-oic acid, methyl ester, S-(6-chloro-1-hexyl) S'-methyl dithiocarbonate, 9-Octadecenamide and Caryophyllene. These compounds and their identified derivatives have important role in the plant development. It might be influenced on the seed germination and development of seedlings as well as on the certain biochemical activity of wheat and groundnut. From literature review, these compounds also have pharmaceutical potentiality may be helpful for findings potent drugs in future.

b) Gas chromatography-mass spectrometry (GC-MS) analysis :

The results form GC-MS analysis a number of allelochemicals were identified from flavenoides and essential oils in the *Pascalia glauca* weed which are vital role in seed germination and seedlings development and certain biochemical activity. From the GC-MS study number of active identified biochemical compounds depicted in the Table-23. Biologically active compounds called allelochemicals in the form of different derivatives of alkaloids, flavonoids, steroids, tannins, triterpenoids and saponins which maybe enhanced in the presence of the aqueous and methanol extract

and stronger extraction capacity of both solvent media that may yield a greater number of active constituents responsible for the allelopathic activity. Based on the retention time, it has been compared with the available library and literatures to find the compounds. The various compounds identified through the library search were tabled (Table- 24). From the tabulated data it summarized that the presence of vital compounds like a-Terpinyl acetate, D-Limonene, alpha-Phellandrene, Kaur-16-en-19-ol, Terpinen-4-ol, Tumerone, Cyclohexene, aR-Turmerone, Caryophyllene and they will play a important role in finding potent allelopathic activity which were responsible for sensitivity and selectivity in the seedlings development and different biological activity in wheat and groundnut growth. These results obtained from present investigation and it is evident that this plant will also play an important role in finding potent drugs in the future.

Discussion :

From the analysis of results some biochemical compounds were identified through GS-MS and advanced liquid chromatographic mass spectra (LCMS) study. This is important part of our experiment clearly indicates the existence of allelopathic potential of experimental weed plant *Pascalia glauca* and its allelopathic action was triggered by a number of identified allelochemicals which were established by above study. Present investigation, an effort was taken to analyze potential of the aqueous and methanol extracts of *Pascalia glauca* and revealed its phytochemical richness. Preliminary phytochemical analysis has performed to check the presence of secondary metabolites and results were depicted in Table- 21.

Aqueous and methanol root, stem and leaves showed presence of five compounds *viz*. alkaloids, flavonoids, triterpenoids, tannins and saponins. The presence of different phytocompounds showed their potential in extraction. These phytochemical compounds under the different classes were well known for allelopathic action against various stages of seedlings development and biochemical activity in wheat and groundnut. Out of six phytochemical compounds screening in present work showed four groups were more active from aqueous extracts while five in methanol extract.

Table- 21 : Phytochemical screening of aqueous and methanol extracts of stem, leaves and flowers of *Pascalia glauca* Ortega.

Name of phytochemicals	Extracts							
	Stem		leaves		Root		flower	
	A. E.	M. E.	A. E.	M. E.	A. E.	M. E	A. E	M.E.
Alkaloids	+	+	+	+	+	+	-	+
Flavonoids	+	+	+	+	+	+	+	+
Steroids	-	+	-	+	+	-	-	-
Triterpenoids	+	-	+	+	-	+	+	+
Tannins	+	+	+	-	-	+	-	-
Saponins	-	+	-	+	-	+	-	-

" +" Presence of the compound; "– " Absence of the compound.
"A.E." Aqueous extract; "M.E." Methanol extract.

Table- 22 : LCMS identified allelocompounds from whole plant methanol extract of *Pascalia glauca* Ortega.

Sr. No.	Name of compound	Nature of compound	Retenti-on time in minutes	Molecular formula	Molecular weight	Area %
1	a-Terpinyl acetate	Acetate group	5.46	$C_{12}H_{20}O_2$	196	2.40
2	3-Cyclohexane 1-ol	Amino group	7.70	$C_{10}H_{18}O$	154	12.60
3	3-Cyclohexane 1-ol,4Methyl-1 (R)	Amino group	7.78	$C_{10}H_{18}O$	154	12.60
4	a-Phellendrene	Monoterpene Volatile oil	8.369	$C_{10}H_{16}$	136	2.48
5	D-Limonene	Aromatic	9.078	$C_{10}H_{16}$	136	1.96
6	N-Butyl-4-methylpyridinium	Alkaloid	17.93	$C_{10}H_{16}N$	150	5.25
7	Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl-	Volatile compound	22.397	C_9H_{12}	120	1.75
8	Kaur-16-en-19-ol	Diterpene alkaloid	29.60	$C_{20}H_{32}O$	288	0.94
9	Methyl Ent-16-Kauren-19-Oate	Diterpene alkaloid	29.60	$C_{21}H_{32}O_2$	316	0.94
10	Kaur-16-en-18-oic acid, methyl ester	Diterpene alkaloid	29.60	$C_{21}H_{32}O_2$	316	0.94
11	S-(6-chloro-1-hexyl) S'-methyl dithiocarbonate	Sulpher compound	30.85	$C_8H_{15}ClOS_2$	226	3.42
12	9-Octadecenamide	Amide group	35.60	$C_{18}H_{35}NO$	281	6.88
13	beta.-Bisabolene	Sesquiterpenes	23.105	$C_{15}H_{24}$	204	0.42
14	Caryophyllene	Sesquiterpenes	20.823	$C_{15}H_{24}$	204	0.51
15	n-Tridecan-1-ol	Alcohol	25.171	$C_{13}H_{28}O$	200	5.09
16	Methyl 5,12-octadecadienoate	Ester	36.059	$C_{19}H_{34}O_2$	294	9.56
17	9-Octadecenoic acid, methyl ester, (E)	Ester	36.182	$C_{19}H_{34}O_2$	294	15.56
18	Oleic anhydride	Fatty acids	42.156	$C_{36}H_{66}O_3$	546	14.87
19	Ethanol, 2-(9,12-octadecadienyloxy)-, (Z,Z)	Double-bond Steriod group	43.006	$C_{20}H_{38}O_2$	310	15.55
20	Glycidyl oleate	Fatty acids	43.114	$C_{21}H_{38}O_3$	338	39.38

Table-23 : GC-MS identified compounds from extracts of *Pascalia glauca* Ortega.

Sr.no.	Name of the compound	Root extract	Stem extract	Flower extract	Leaves extract
1	a-Terpinyl acetate	-	+	+	-
2	9-Octadecenamide	-	+	-	-
3	a.-Phellandrene	+	+	+	-
4	D-Limonene	+		+	-
5	Caryophyllene	+	+	+	-
6	Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl	+	+	+	-
7	(1S,5S)-2-Methyl-5-((R)-6-methylhept-5-en-2-yl)bicyclo[3.1.0]hex-2-ene	+	+	+	-
8	Cyclohexene, 3-(1,5-dimethyl-4-hexenyl)-6-methylene-, [S-(R*,S*)]	+	+	+	-
9	aR-Turmerone	+	+	+	-
10	Tumerone	+	+	+	-
11	Curlone	+	+	+	-
12	3-Cyclohexen-1-ol 4-metylethyl)- (R)	+	-	-	-
13	Benzene, 1,2,3-trimethyl	-	+	-	-
14	beta.-Bisabolene	-	+	-	-
15	n-Tridecan-1-ol	-	-	-	+
16	Methyl 5,12-octadecadienoate	-	-	-	+
17	9-Octadecenoic acid, methyl ester, (E)	-	-	-	+
18	Oleic anhydride	-	-	-	+
19	Ethanol, 2-(9,12-octadecadienyloxy)-, (Z,Z)	-	-	-	+
20	Glycidyl oleate	-	-	-	+

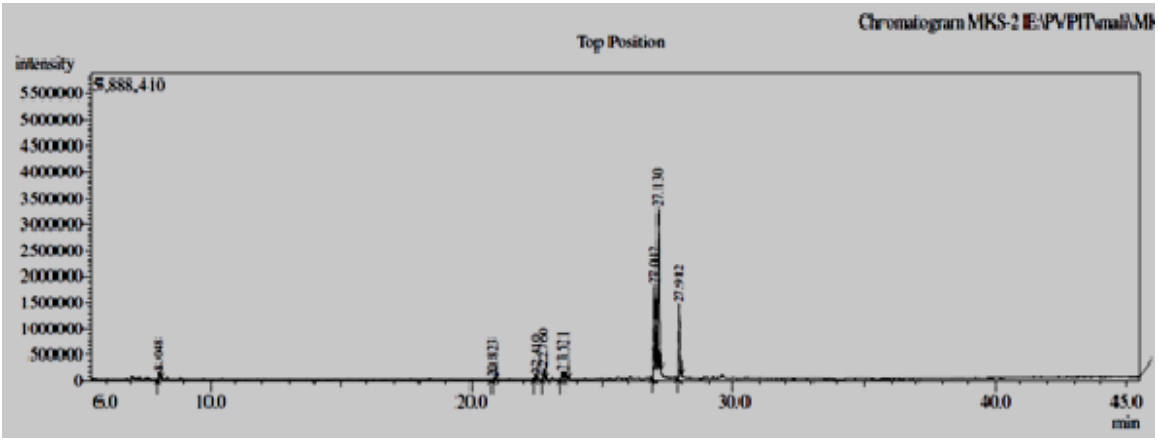
"+" Presence of the compound; "- " Absence of the compound.

Table- 24 : GC-MS identified important compounds from extract of *Pascalia glauca* Ortega.

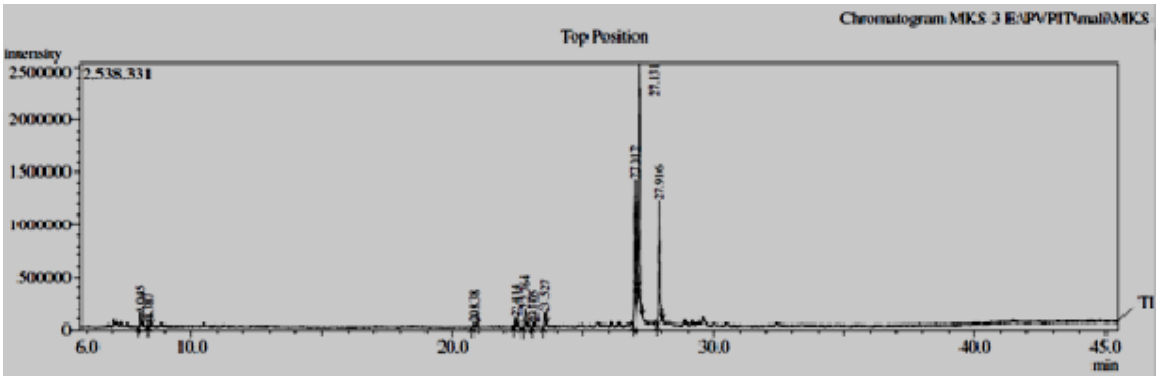
Sr.No.	Name of compound	Retention Time in Minutes	Area	Area%
1	alpha-Phellandrene	8.369	165426	0.42
2	Benzene, 1,2,3-trimethyl	8.045	398490	2.34
3	D-Limonene	9.078	67670	0.93
4	Caryophyllene	20.818	154891	0.39
5	Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl	22.397	677811	1.71
6	(1S,5S)-2-Methyl-5-((R)-6-methylhept-5-en-2-yl)bicyclo[3.1.0]hex-2-ene	22.750	1106902	2.79
7	Cyclohexene, 3-(1,5-dimethyl-4-hexenyl)-6-methylene-, [S-(R*,S*)]	23.512	801995	2.02
8	aR-Turmerone	27.011	11739647	29.60
9	Tumerone	27.131	14094267	35.53
10	Curlone	27.909	10162575	25.62
11	a-Terpinyl acetate	5.46	1105910	2.37
12	9-Octadecenamide	35.66	165895	6.68
13	3-Cyclohexen-1-ol 4-metylethyl)-(R)	7.73	951894	13.68
14	beta.-Bisabolene	23.105	70957	0.42
15	n-Tridecan-1-ol	25.171	34105	5.09
16	Methyl 5,12-octadecadienoate	36.059	64098	9.56
17	9-Octadecenoic acid, methyl ester, (E)	36.182	104333	15.56
18	Oleic anhydride	42.156	99659	14.8
19	Ethanol, 2-(9,12-octadecadienyloxy), (Z,Z)	43.006	104230	15.55
20	Glycidyl oleate	43.114	263975	39.38

Fig.-21. GC-MS and LCMS peaks of root, stem, leaves, flower and whole plant extract of *Pascalia glauca* Ortega.

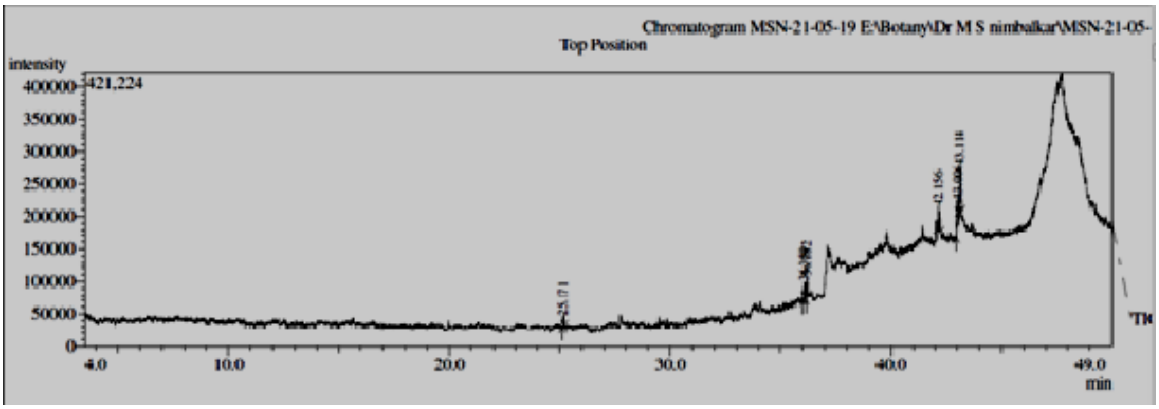
Root extract peak.



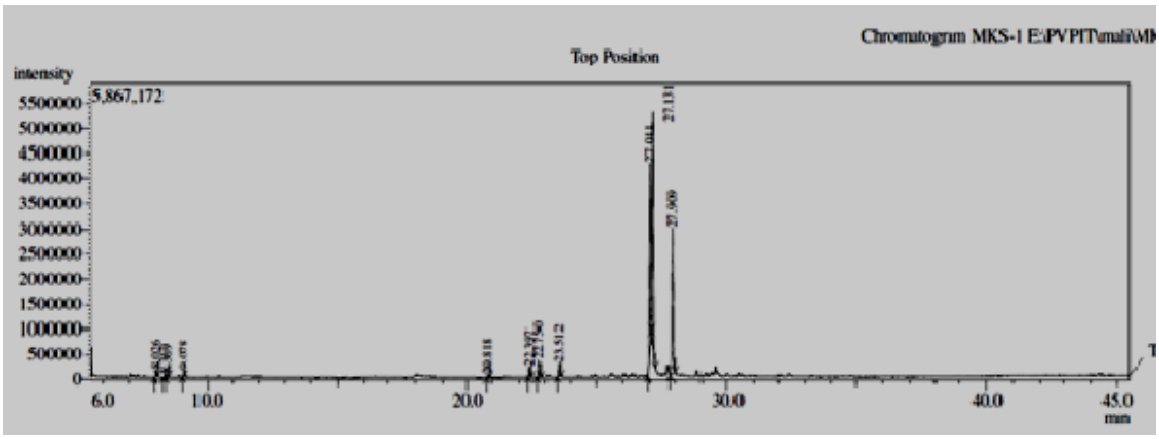
Stem extract peak.



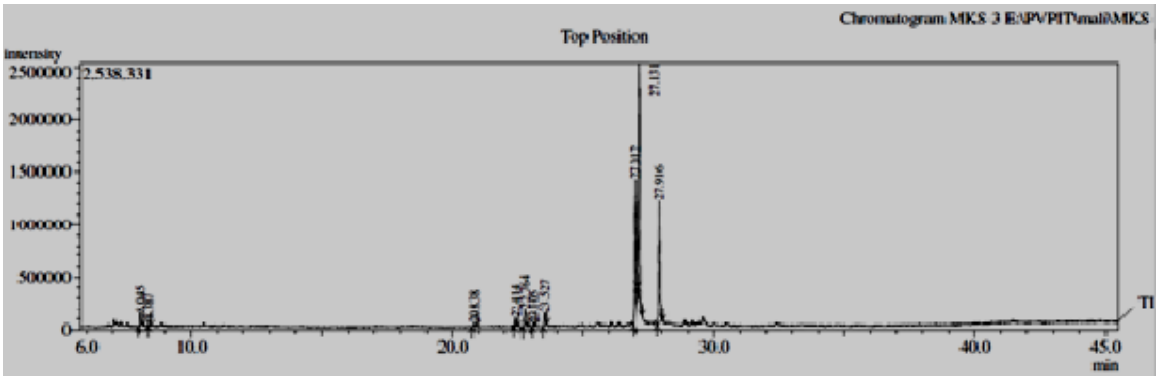
Leaves extract peak.



Flower extract peak.



Whole plant extract.



One thing is cleared that the aqueous and methanol flower extracts showed few of phytochemical compound groups positively present including alkaloids, flavonoides and triterpenoides and other groups showed negative approaches. Therefore, lower concentration of flower extracts not more richness of allelochemicals than the root, stem and leaves extracts but at higher concentration their activity against wheat and groundnut was noticeable in present study. Methanol extract of root, stem and leaves had more active phytochemical compounds than the aqueous extracts and was found to retain higher amount of phytoconstituents which possessed most potent and actively responds their selectivity in allelopathic influence on the studied parameters from both wheat and groundnut seedlings which showed on the seedlings growth development and in biochemical parameters.

Pascalia glauca had many bioactive compounds from various groups of chemicals including monoterpines, diterpines, triterpines, triterpine saponins, sesquterpines, kaurenes diterpinoides, polyacetylenes and flavonoides. From present study various bioactive compounds were identified in the extracts of *Pascalia glauca* through LCMS study and GS-MS spectra study. There were 20 different compounds identified in various parts including root/stolon, stem, leaves and flower of *Pascalia glauca* are depicted in Table-23. The most vital bioactive compounds has identified including a-Terpinyl acetate, 9-Octadecenamide, Mesitylene, a.-Phellandrene, D-Limonene, Caryophyllene, Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl, (1S,5S)-2-Methyl-5-((R)-6-methylhept-5-en-2-yl)bicyclo[3.1.0]hex-2-ene, Cyclohexene, 3-(1,5-dimethyl-4-hexenyl)-6-methylene-[S-(R*,S*)], aR-Turmerone, Tumerone, Curlone, 3-Cyclohexen-1-ol 4-metylethyl)-(R), Benzene, 1,2,3-trimethyl-beta.-Bisabolene. The other compounds like Wedelolide A, Wedelolide B, Wedeliatrilolactone B.Wedelia-seco- kaurenolide, (3 α)-3-(cinnamoyloxy)-17- hydroxy-*ent*-kaur-15-en-19 oic acid (Wedelidin A). The steroidal compounds like stigmasterol, (7α)-7-hydroxystigmasterol, (3β)-3-hydroxy stigmasta-5, 22-dien-7-one, *p*-cymene, caffeic acid, caffeine alkloides, cyclic terpenes, a-phellandrene and a-pinene. Some of these compounds were also identified by various researchers from allelopathic weed species of *Wedielia* (Narbortao, 2005; Krishnavignesh and Mahalakshmipriya, 2017; Rehana and Nagarajan, 2013 and Balekar *et al*., 2014).

An exhaustive survey of literature on the *Pascalia* and *Wedelia* and other genus under family Asteraceae deals with presence of many bioactive chemicals in the form of alkaloids, flavonoids, phenols, saponins, triterpenols and other such

secondary metabolites. Alkaloids are often toxic to human beings and many have physiological activities, hence they are widely used in medicines. Other species allied to *Pascalia glauca* like *Wedelia trilobata* revealed that the presence of tannin, saponins, flavonoids, phenol, terpenoids constitute major groups of phytoconstituents of this plant (Balekar *et al*., 2014). Nasreenhusain and Anilkumar (2015) stated that *Wedelia trilobata*, *Achyranthes aspera* and *Chrysanthemum* showed the presence of different phytochemical compounds and absence of some of them as well. Balekar *et al*. (2014) studied *Wedelia trilobat*a leaves, stem and flower essential oil and was characterized by a high percentage of hydrocarbon sesquiterpenes, hydrocarbon monoterpenes and low levels of oxygenated sesquiterpenes. *Pascalia glauca* (*Wedelia glauca*) toxicity is attributed to the presence of a hepatotoxic terpenoid called atractyloside, a powerful inhibitor of cellular respiration and ATP synthesis (Lemaster and Sowers, 1979). The atractyloside inhibits the ADP/ATP carrier through the membrane of the organelles, preventing the oxidative phosphorylation by blocking the translocation of adenine dinucleotide (Obatomi and Bach, 1998). The consequence is an initial alteration of the intrahepatic circulation with hepatocyte necrosis and periacinal haemorrhage (centrilobular). Such findings are common to acute hepatotoxic compounds in the death of domestic animals (Giannitti *et al*., 2013). Further, it was cleared that the toxic substance is atractyloside and related kauran type of acids. These toxins were responsible for animal death due to wedeloside with unusual features in its structures. The distribution of hydroxyl group in kauran system is quite unusual and the toxicity is results into death if accidentally *Pascalia glauca* mix with other forage (Giannitti *et al*., 2013 and Mujawar, 2013). Our observations from field survey strongly support this view because of many domestic animals death after the grazing in studied field. However, in ayurvedic and ethnomedicine, plants and their derivatives play an important role as therapeutic use. Plants derived compounds exhibited significant activity against cancer and it proved to be effective in combating bacterial, fungal, viral infections, inflammatory, arthritis, diabetic, wound healing and much more.

The presence of vital compounds like a-Terpinyl acetate, dl-Limonene, a-Phellandrene, Kaur-16-en-19-ol, Terpinen-4-ol, which were already been reported for their antitumour, anti HIV activity, antiaflatoxigenic activity, angiogenesis, antiseptic activity, antiulcer activity (Priyanka *et al.*, 2010). Our investigation also identified above compounds from present weed therefore the potentiality of *Pascalia glauca* has

pharmaceutical importance other than its toxic activity. Vighnaganesh and Mahalaxmipriya (2017) examined phytochemical screening and antimicrobial activity of stem extracts of *Pascalia glauca* (*Wedelia glauca*).

It is an interesting source of potential bioactive molecules, as steroidal compounds, flavonoids, diterpenoids derivatives, phytosteroids, with antioxidant, anti-inflammatory, antimicrobial, hepatoprotective activity, analgesic and antihistamine, anti-implantation, antiasthmatic activities, and anticancer activity. Thus, GC-MS and LCMS analysis of phytoconstituents in *P. glauca* gives a clear picture of the pharmaceutical value. Although, such type of analysis is the first step towards understanding the nature of active principles in this weed and this type of study will be helpful for further detailed study. Further investigations into the pharmacological importance and their diversity and detailed phytochemistry may add new knowledge to the information in the traditional medical systems. In addition to this, at the same situation, it is a time to find better efficient antibiotics with zero side effects. *P. glauca* enriched several phytocompounds with diverse activities which possess the efficacy to inhibit the growth of different pathogens causing various infections. On further analysis this weed plant will be hope a good healthcare and alternative formulation of synthetic drugs.

CHAPTER - V

SUMMARY AND CONCLUSION

Summary :

a) **Rhizosphere soil analysis from invaded and non invaded region of *Pascalia glauca* Ortega** : The root zone soil was analyzed at vegetative and flowering stage of *P. glauca*. In soil the inorganic elements of like N, P, K, and Na were found to be at maximum level at the vegetative stage while Ca, Mg and Cl were found to be at higher side at flowering stage. The micronutrients Fe, Mn, Zn and Cu were increased when *P. glauca* was fully grown at the vegetative stage. The control soil showed minimum amount of elements in comparisons with vegetative stage and flowering stage soil. It is interesting to note that, *P. glauca* invaded soil has high level of nutrients over the control soil.

b) **Effect of aqueous and methanol extracts of *Pascalia glauca* Ortega on various parameters of test crop plants *viz*. wheat and groundnut :**

   1) **Seed germination and seedlings growth :** Overall impact of aqueous and methanol extracts of *P. glauca* Ortega on seed germination and growth of wheat and groundnut was negative. The higher concentrations of the extracts decreased the seed germination capacity. Maximum reduction in seed germination of both the test plants was recorded in 20% aqueous and methanol extracts of leaves, stem and flowers. The remarkable inhibition was found in the root and shoot length of wheat seedlings in the aqueous leaves extract than methanol extracts. Similarly, aqueous and methanol extracts decreased the biomass of test crop seedlings. Root and shoot length as well as fresh and dry weight has more reduced remarkably in methanol leaves extracts than aqueous leaves extracts**.**

   2) **Inorganic constituents :** The macroelements such as nitrogen, phosphorus, potassium, calcium and magnesium were increased only at 5 and 10% aqueous and methanol extracts of stem, leaves and flower treatment in wheat and groundnut seedlings. However, the higher concentrations hampered the content of macroelements in both crops. It was also recorded that the methanol extracts reduced the macroelements much significantly compared to aqueous ones. The microelements such as zinc, iron, copper and manganese were

increased only at 5 and 10% aqueous and methanol extracts in wheat and groundnut seedlings. However, only leaves extract caused more reduction in microelements than the stem and flower in both wheat and groundnut seedlings.

3) **Photosynthetic pigments :** Aqueous and methanol leaf extracts strongly affected photosynthetic pigments in both the test plants compared to stem and flower extracts. Maximum reduction of chlorophyll a, b, total chlorophylls and carotenoides of wheat and groundnut seedlings was seen in the aqueous and methanol extracts of leaves extracts at 20% concentration. Carotenoides have gradually decreased in groundnut from concentrations of aqueous extracts compared to methanol extracts.
4) **Total sugars :** Carbohydrates and their constituents like reducing sugars, total sugars and starch of wheat seedlings have shown decreasing trend from lower concentration (5%) to higher concentration (20%) of both extracts. Lower concentrations had minor reduction effect on carbohydrate content while more drastic reduction was at higher concentrations of both aqueous and methanol extracts. The maximum inhibition of these sugars of groundnut seedlings was found in methanol leaves extracts than the aqueous one.
5) **Total nitrogen :** Highly reduced nitrogen content of wheat seedlings was observed in 20% of methanol and aqueous leaves extract. Compared to aqueous extract, methanol extract of stem, leaves and flower showed more influence in the reduction of total nitrogen content of groundnut seedlings.
6) **Protein content :** In both the test crop plants all aqueous and methanol extracts showed gradual decrease in total protein content. Maximum reduction of protein has been recorded in wheat and groundnut seedlings treated with methanol leaves extractthan aqueous leaves extracts.
7) **Total** a**mino acids :** Amino acids content of wheat seedlings showed increase at higher concentration (20%) of both the aqueous and methanol extract of stem, leaves and flower compared to control. It is interesting to note that, the level of amino acids was more enhanced in aqueous flower extract. In groundnut seedlings highest reduction was found in aqueous and methanol leaves extract.
8) **Free Proline :** The lower concentration (5%) extracts of aqueous and methanol of stem, leaves and flower hadminimal impact on the proline content

in wheat seedlings with the exception of flower extract. High proline content was recorded from 10% to 20% and the highest value was recorded at highest concentrations of both the extracts. Similar trend was noticed in proline of groundnut seedlings. Thus both wheat and groundnut seedlings showed similar tendency of proline accumulation in both aqueous and methanol extracts of *P. glauca.*

9) **Total polyphenols :** Total polyphenol content of wheat seedlings was showing slight decrease at lower concentration (5%) but continuously increased from 10% to 20% concentrations of aqueous and methanol extract of stem, leaves and flower of *P. glauca*. The highest accumulation has seen at the 20% aqueous leaves extract followed by methanol stem extract over control.In groundnut seedlings 5% aqueous flower extract showed minor decrease in total polyphonols. Aqueous and methanol leaves extract showed highest accumulation of polyphenol content in groundnut seedlings than the stem and flower extract.

10) **Enzyme catalase** : The activity of enzyme catalase of wheat and groundnut seedlings showed decrease with increasing the concentrations of stem, leaves and flower extract of *P. glauca.* The aqueous leaves and methanol flower extract recorded highest decrease im catalase activity in wheat seedlings. Groundnut seedlings have also reported maximum decrease at 20% methanol and aqueous extract. A slightly increase in catalase activity was found in 5% methanol extract.

11) **Enzyme peroxidase** : The peroxidase enzyme showed decreasing activity in the wheat seedlings after the treatment of aqueous and methanol extracts of stem, leaves and flower of *P. glauca* from lower (5%) to higher (20%) concentrations. Maximum decrease in peroxidase enzyme activity was noticed from aqueous and methanol leaves extract at 20% concentration in wheat seedlings. The higher reduction of peroxidase activity of groundnut was found in methanol and aqueous leaves extract at 20% concentration.

12) **Enzyme superoxide dismutase** : Enhanced superoxide dismutase activity of wheat seedlings in 5% aqueous stem and flower extract is most noticeable. Further, it was reduced in the treatments of from 10%, 15% and 20% higher concentration in both aqueous and methanol extract. Maximum decrease in superoxide dismutase activity was noticed in groundnut seedlings at methanol

leaves extract than aqueous one at 20% concentration. Not noticeable reduction of superoxide dismutase activity of groundnut seedlings has been recorded in both aqueous and methanol extracts.

13) **Enzyme lipid peroxidase :** Activity of enzyme lipid peroxidase activity of wheat seedlings gradually decreased from lower 5% to higher 20% concentration of aqueous and methanol stem, leaves and flower extract. Maximum and similar reduction of enzyme lipid peroxidase activity has found from methanol and aqueous extract of leaves at 20% concentration. Aqueous extract of stem, leaves and flower of *P. glauca* when subjected to groundnut seedlings expressed gradual reduction in the activity of lipid peroxidase from lower 5% to higher 20% concentrations.

14) **Enzyme polyphenol oxidase :** Only 20% methanol leaves extract has showed noticeable reduction in activity of this enzyme compared to control. The various concentration treatments of stem, leaves and flower extract of *P.glauca* showed a decreasing trend on the activity of polyphenol oxidase in groundnut seedlings. Aqueous and methanol extract of leaves has strongly inhibited activity of polyphenol oxidase enzyme in groundnut seedlings. Enhancement of polyphenol oxidase activity at 5% methanol stem extract was not much noticeable ashigher decrease was recorded in 20% concentration of methanol and aqueous leaves extract.

15) **Enzyme nitrate reductase activity of groundnut seedlings :** The activity of nitrate reductase enzyme of groundnut seedlings showed decreasing trend from 5% lower concentration to 20% higher concentration of stem, leaves and flower extracts of *P. glauca*. Aqueous stem and flower extract decreased nitrate reductase steadily but in leaves extract, it has seen gradual reduction and maximum was recorded at higher concentration of leaves extract. Aqueous leaves extract at 20% showed more reduction of nitrate reductase enzyme activity than the methanol leaves extract in groundnut seedlings.

16) **Enzyme nitrite reductase activity of groundnut seedlings** : Nitrite reductase gradually decreased in 5% and 10% concentrations while highest reduction was seen in groundnut seedlings at 15% and 20% in methanol extract of stem, leaves and flower. Aqueous extract of leaves had more impact than the methanol extract for inhibition of nitrite reductase enzyme.

c) **Phytochemical screening and isolation, identification of allelochemicals of *Pascalia glauca* Ortega from root, stem, leaves and flower extracts :** Preliminary phytochemical screening tests showed presence of alkaloids, flavonoids, steroids, triterpenoids, tannins and saponins in various plant parts of *P. glauca*. Aqueous and methanol extracts of stem and leaves was more potential source of the secondary metabolites over the others. A number of bioactivated compounds were identified and tabulated from different plant parts using methanol extract through LCMS advanced technique. The vital bioactive compounds were identified and tabulated from GS-MS techniques. Some of the important bioactive compounds identified are alkaloids, flavonoids, steroids, triterpenoids, tannins and saponins. These biochemical might be play a important role in finding potent allelopathic activity which were responsible for sensitivity and selectivity in the seed germination and seedlings development as well as different biological activity in wheat and groundnut growth.

Conclusion :

In the present investigation study of allelopathic effect of newly introduced weed plant species *Pascalia glauca* Ortega from Islampur, Sangli district of Maharashtra on wheat and groundnut test plants was undertaken. It is very clear from the results of present work that, the effect of stem, leaves and flower aqueous and methanol extracts of *P. glauca* Ortega has caused strong allelopathic effect on wheat and groundnut seed germination, seedlings growth and its overall physiology.

The soil analysis showed significant increase in phenolic compounds while decrease the organic, inorganic matters in weed infected soil, thus starving the crop from it and affect on the their growth and development. Phenolic content in the rhizosphere soil was high compared to control soil and might be responsible for reducing the growth of wheat and groundnut seedlings.

The inhibitory effect exerted by different concentrations of extracts in all studied parameters was found to be concentration dependent. Both the crops wheat and groundnut responded differently to the aqueous and methanol extract of stem, leaves and flower of *P. glauca* Ortega. Mostly leaves extract have more strong negative potential in the reduction of studied parameters than the stem and flower extracts.

All concentrations of stem, leaves and flower aqueous and methanol extract of *Pascalia glauca* Ortega reduced seed germination and reducing growth of both the test plants. Decreasing photosynthetic pigments in seedlings of wheat and groundnut indicates that, some allelopathic mediated compounds, because of presence of phenolics, may interfere with synthesis of porphyrin precursors of chlorophyll pigment biosynthesis resulting in overall decrease.

Decrease in sugars and proteins related shifting and altering the metabolism in germinating seeds due to presence of phytotoxins in extract. Leaching of carbohydrates from germinating seeds might be possible because of the damage of plasma membrane and imbalance the metabolic activity. The oxidating enzymes *viz*. catalase, peroxidase, superoxide dismutase, lipid peroxidase, polyphenol oxidase of wheat and groundnut indicate hampered metabolism and their decrease at high concentrations may be inhibitory effect of allelochemicals over oxidation process. Important nitrogen metabolism enzymes like nitrate and nitrite reductase of groundnut and their decreased activities indicate disturbed nitrogen metabolism in germinating seeds of groundnut.

The aqueous and methanol extract showing increasing the levels of amino acids, phenolics and proline content are indicative of some allelochemicals present and affecting the growth and seedlings development of both test crops. As these cannot providing protective mechanism to control damage at cellular level.

Phytochemical screening of *P. glauca* Ortega root, stem, leaves and flower tests showed presence of alkaloids, flavonoids, steroids, triterpenoids, tannins and saponins. Aqueous and methanol root, stem and leaves extracts seems to have more potent source of the secondary metabolites than the flower extract.

Various groups of secondary metabolites identified from weed plant *P. glauca* are terpenoids, flavonoids and polyacetylenes as well as steroids, eudesmanolide lactones, luteolin and kaurenoic acid, sesquiterpenoids, triterpenoid and diterpenoid and Benzene derivatives.

Essential oils, alkaloids and terpenoids are valuable plant products. Most constituents of essential oil belong to the large group of terpenes. The essential oil obtained from this plant was analyzed by GC-MS showed presence of a- pinene, a-phellandrene, aR-Turmerone, Tumerone, Curlone, D-limonene and caryophyllene.

The presence of a-Terpinyl acetate, D-Limonene, a-Phellandrene, Kaur-16-en-19-ol, Terpinen-4-ol, have already been reported for their antitumour, anti HIV,

antiaflatoxigenic, angiogenesis, antiseptic and antiulcer activity. With results obtained from present study it is clear that this plant may play an important role in finding the potent drugs in the future.

All these aspects suggest *Pascalia glauca* Ortega has allelopathic potentiality because of presence of number of allelochemicals in root, stem, leaves and flower which have strong inhibitory capability over host plant metabolism and further may provide source of phytomedicines.

Our study identified the influence of *Pascalia glauca* Ortega on seed germination, development and metabolism of wheat and groundnut seedlings. This impact is majorly negative as expected in present study. The numbers of allelochemicals identified from *P. glauca* weed parts must be intervening the host metabolism to affect its growth and development pattern and surely will influence the yield at the end. But also it will provide possibility to use allelopathic potentiality against different weeds and their control of other plants from field as a new source through phytomedicines. This present study opens new area for further investigation of allelochemicals present and their mode of action against theropic uses.

Pascalia glauca Ortega, a new introduced weed, thus has provided a new source of potential against the cancer treatment. A new trend of standardization of local medicines for strong theropic use may be the new path of further research and this weed is a promising candidate for the same. Also the inhibitory effect of this weed through leachate chemicals clearly indicates a danger zone for active crop present in field and the eradication of this weed through bioactive remedies may be a important measure in coming years. A further research in the mode of action of these allelochemicals on host plant metabolism is an urgent need and such studies may form further line of research.

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PUBLICATIONS

1. **Mujawar Ilahi**, M. B. Kanade and C. V. Murumkar (2016a): A review on *Pascalia glauca* Ortega as Poisonous Weed Barrier in Crop of Sangli District, Maharashtra. *Proce., International conference on "Plant research and resource management"* Organized by T. C. College, Baramati (MS) India.11-13 Feb., : pp 181.

2. **Mujawar Ilahi**, Mahadev Kanade and Chandrashekhar Murumkar**.** (2016b): Investigation of allelopathic effect of *Pascalia glauca* Ortega on seed germination and seedling growth of wheat. *Indian Journal of Fundamental and Applied Life Sciences* ISSN: 2231– 6345 (Online) Vol. 6 (3) July-September : pp. 50-55.

3. **Mujawar Ilahi,** Mahadev Kanade and Chandrashekhar Murumkar. (2017a): Effect of methanol extract of *Pascalia glauca* Ortega on wheat seed germination studies *International Journal of Food, Agriculture and Veterinary Sciences* ISSN: 2277-209X (Online) Vol.7 (3) September-December, pp.69-74.

4. **Mujawar Ilahi,** Mahadev Kanade and Chandrashekhar Murumkar, (2017b): Allelopathic potential of *Pascalia glauca* Ortega aqueous extract against seed germination and seedling growth of groundnut. *Bioscience Discovery*, 8 (1): 35-39.

5. **Mujawar Ilahi,** Mahadev Kanade and Chandrashekhar Murumkar, (2018): Allelopathic Effect of *Pascalia glauca* Ortega. Aqueous extract on Photosynthetic Pigments of *Triticum aestivum* L. and *Arachis hypogea* L. Seedlings. International *Journal of Creative Research Thoughts* Vol. 6 (2): 643-653.

LIST OF SEMINAR / CONFERENCES ATTENDANDED

1. **Mujawar Ilahi**, M.B. Kanade and C.V. Murumkar (2016): A review on *Pascalia glauca* Ortega as Poisonous Weed Barrier in Crop of Sangli District, Maharashtra. Attendance and presentation of paper. *International conference on "Plant research and resource management"* Org. T. C. College, Baramati (MS) India.11-13 Feb., 2016.

2. **Mujawar Ilahi**, M. B. Kanade and C. V. Murumkar (2017): Effect of methanol extract of *Pascalia glauca* Ortega on wheat seed germination studies. **Paper presentation** and **chair person for oral session**. National conference on, *"Emerging trends classical in life sciences (ETC-LS 2017)".* Organized by Department of Botany and Zoology, Y. C. Warana Mahavidyalaya, Warnanagar, Dist: Kolhapur. (M.S.). India. 28-1-2017.