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ARTICLE

Allelopathic Effect of *Quisqualis indica* L. Flower Extract on the Germination of *Cicer arietinum* L.

Priyanka L. P., Raveendran P. B.*

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Abstract

Allelopathy can be defined as any direct or indirect harmful or beneficial effect of one plant on another through the production of chemicals that it releases into the environment. Allelochemicals are present in several parts of plants that are known to interfere seed germination and growth of neighbouring or successional plants by releasing allelochemicals in their environment. These compounds can be regarded as 'natural herbicides'. With this background a study was designed to study the allelopathic influence of flower extract (aqueous) of *Quisqualis indica* L. on morphological and biochemical parameters of seed germination and seedling growth in *Cicer arietinum*. The treated seeds show only 45% percentage germination upto 6th day when compared to 100% germination obtained in control group. The percentage of growth inhibition of radicle and plumule is almost upto 100% in the initial days in treated cases, and the condition is not significantly improving through the later days. Interestingly the concentration of protein was significantly higher in treated seeds (73.28mg/g Fresh Weight) when compared to control seeds (28.50mg/g Fresh Weight). The treated seeds contains higher amount of proline (1.15mg/g Fresh Weight) when compared to the control seeds (0.98mg/g Fresh Weight). Interestingly the activity of peroxidise at 6th day is is still higher in treated seeds (0.27mg/min/mL) when compared to the control seeds (0.15mg/min/mL). The activity of catalase is significantly higher in treated seeds (0.06mg/min/mL) as compared to control seeds (0.03mg/min/mL). The PPO activity was more evident towards the initial stages (0.08mg/min/mL) of germination but steadily depleting to negligible activity towards 6th day (0.01mg/min/mL) in treated seeds. From the study it is clear that aqueous extract from the flowers of Quisqualis indica is causing inhibition of normal seed germination and its growth. Various biochemical parameters analyzed also confirms the findings.

Keywords: Allelopathy, *Cicer arietinum*, *Quisqualis indic*a, Seed Germination, Growth Inhibition, Antioxidative Enzyme

Introduction

The phenomenon of allelopathy, where a plant species chemically interferes with the germination, growth or development of other plant species has been known for over 2000 years. Allelopathy can be defined as any direct or indirect harmful or beneficial effect of one plant on another through the production of chemicals that it releases into the environment.

In 1996, the International Allelopathy Society defined allelopathy as follows: "Any process involving secondary metabolites produced by plants, micro-organisms, viruses, and fungi that influence the growth and development of agricultural and biological systems (excluding animals), including positive and negative effects". Chemicals released from plants and imposing allelopathic influences are termed as allelochemicals or allelochemics. Haig (2008) classified allelochemicals into several categories such as glucosinolates, phenolic compounds, terpenoids, alkaloids, hydroxamic acids, and other organic compounds (flavonoids, quinines, polyacetylenes). Allelochemicals can be present in several parts of plants including roots, rhizomes, leaves, stems, pollen, seeds and flowers. Allelochemicals are released into the environment by root exudation, leaching from aboveground parts, and volatilisation and/or by decomposition of plant material (Rice 1984). Allelochemicals are present in several parts of plants that are known to interfere seed germination and growth of neighbouring or successional plants by releasing allelochemicals in their environment (Xuan *et al.*, 2004; Kruse *et al.*, 2000). The strength of the allelopathic effect can vary with the target species and with the type of plant tissue extract. The search and development of new herbicides through the identification of active compounds from allelopathic plants is an interesting research and development area. These compounds can be regarded as 'natural herbicides'. With this background a study was designed to study the allelopathic influence of flower extract (aqueous) of *Quisqualis indica*.L. on morphological and biochemical parameters of seed germination and seedling growth in *Cicer arietinum*.

Post Graduate Department of Botany and Research Centre, Mahatma Gandhi College, Thiruvananthapuram, Kerala, India.
*Corresponding Author email: praveenrcc@gmail.com

Materials and Methods

Plant material

The flowers of *Quisqualis indica*.L. (family: Combretaceae) were collected from the garden of M.G College, Department of Botany. After that flowers dried in an oven at 25°C for 48 hours. The flowers were ground mechanically into a coarse powder and kept into an air-tight container for use in the study.

Morphological analysis of seeds treated with flower extract

The powdered plant material (20g) was extracted with 300mL distilled water. The extract was filtered and used for treatment. The test samples for allelopathic bioassay were prepared freshly from the dry extract. Different concentrations were prepared by dissolving in distilled water immediately prior to use. Healthy uniform seeds of gram (*Cicer arietinum* L., family: Fabaceae) were collected. The seeds were soaked in distilled water for 12h. Then the seeds were surface sterilized with 70% ethanol for 2min, then rinsed with distilled water for several times for complete removal of the sterilant. This procedure was performed under aseptic conditions. The surface sterilized seeds were placed evenly in sterilized glass plates. Each Plate contained 20 seeds. Then equal volume (5mL) of the test samples were introduced into each Plate. Similar volume of distilled water was used as control. Then all the Petri dishes were incubated in dark at room temperature (24-26°C). Allelopathic behavior was evaluated by recording the number of germinated seeds and radicle length using a millimeter ruler, during every day of treatment. The indicating parameters viz., germination percentage and percentage inhibition of radicle growth were calculated by the following formulae:

Germination Percentage = Number of germinated seeds/ Total number of seeds ×100

Percentage Inhibition of Radicle Growth= (X–Y)/X ×100.

Where, X= Control mean radicle length and Y= Treated mean radicle length.

The extract concentration for 50% radicle length inhibition (IC50) was determined by plotting percentage inhibition of radicle growth with respect to control against treatment concentration.

Biochemical analysis of seeds treated with flower extract

Estimation of Protein by Folin's Lowry method

Pipette out 0.1, 0.5, 1mL of seed extract in to a series of clean dry test tube. Make up the volume to 1mL in all the test tube. A tube with 1mL distilled water serve as the blank. Add 5mL of reagent C to each tube including the blank. Mix well and allow to stand for 10 minutes. Then add 0.5mL of Folin's reagent, mix well and incubate at room temperature for 30 minutes. Measure the absorbancy at 680nm.

Estimation of Glucose by Anthrone Method

Pipette out 0.1, 0.5, 1mL sample to separate test tubes. Make the volume to 1mL with distilled water. Add 4mL of Anthrone reagent and mix rapidly. Place the tubes in a boiling water bath for 10 minutes. Cool and read the absorbancy at 630nm against a reagent blank.

Estimation of Proline

Proline was estimated from the oven dried plant material, following the method described by Bates *et al.* (1973). 0.2g plant material was homogenized in 10mL, 3% sulfosalicylic acid and the extract was filtered through Whatman No. 1 filter paper. For assay, 2mL of the filtrate was mixed with 2mL of glacial acetic acid and 2mL of acid Ninhydrin reagent (mixture of 1.25g Ninhydrin, 30mL glacial acetic acid and 20mL 6M orthophosphoric acid, heated for few minutes so that Ninhydrin was completely dissolved and kept in freeze at 0°C). The contents were boiled for 1h on boiling water bath and then cooled rapidly in ice bath. 4mL toluene was added to each test tube and vigorously shaken for few seconds. The absorbance of the toluene chromophore was recorded at 520nm against toluene as a blank.

Estimation of Photosynthetic Pigments

i) Chlorophylls:

Chlorophylls were estimated following the method of Arnon (1949). Randomly sampled fresh material was brought to laboratory, washed with distilled water and blotted to dry. Chlorophylls were extracted in 80% chilled acetone. From 0.5g of fresh plant material homogenized in cold mortar with pestle in dark. A pinch of $MgCO_3$ was added to neutralize the acids released during extraction. The extract was filtered through Whatman No.1 filter paper using Buchner's funnel under suction. Final volume of the filtrate was made to 100mL with 80% acetone. The filtrate was transferred into a conical flask wrapped with black paper to prevent photo-oxidation of the pigments. Absorbance was read at 663nm and 645nm on a double beam spectrophotometer using 80% acetone as a blank.

Chlorophylls were estimated using the following formulae

Chlorophyll 'a' = 12.7 x A663 œ 2.69 x A645 -------X

Chlorophyll 'b' = 22.9 x A645 œ 4.68 x A663 --------Y

Total chlorophylls (a+b) = (8.02 xA663) + (20.20 x A645) --------Z

ii) Carotenoids

Carotenoids were extracted from the weighed amount of material as per the procedure described for chlorophylls earlier. Carotenoids were estimated following the method described by Kirk and Allen (1965). The absorbance was recorded at 480nm on a double beam spectrophotometer.

Isolation and assay of Peroxidase

Homogenize 1g seed in chilled 0.1M Phosphate buffer pH

6.8. Filter the homogenate through a cheese cloth and centrifuge at 10,000rpm for 10 minutes. Collect the supernatant and make up to 10mL. Reaction mixture contained 2mL of 0.1M Phosphate buffer, 0.5mL Guaiacol, 0.5mL of supernatant. 3.5mL of 0.1M Phosphate buffer and 0.5mL of supernatant function as the control. Peroxidase activity can be measured by recording the increase in absorbance at 436nm. Take the absorbance immediately after the addition of enzyme extract and at the peak.

Isolation and assay of Catalase

Homogenize 1g seed in chilled 0.1M Phosphate buffer pH 6.8. Filter the homogenate through a cheese cloth and centrifuge at 10,000rpm for 10minutes. Collect the supernatant and make up to 10mL. The reaction mixture contained 3mL 0.05M H_2O_2 in 100mL ice cold buffer (pH 7) and 0.5mL enzyme extract. Measure the increase in absorbance at 470nm immediately and at the peak of activity.

Isolation and assay of Polyphenol oxidase

Homogenise 1g fresh plant tissue in 10mL chilled 0.1M Citrate phosphate buffer pH 7. Filter the homogenate through cheese cloth and centrifuge at 10,000rpm for 20 minutes. Collect the supernatant and made up to 10mL. The reaction mixture contained 2.5mL 0.1M Citrate phosphate buffer, 1mL 0.5% catechol and 0.5mL enzyme extract. Control solution contained 3mL 0.1M Citrate phosphate buffer and 1mL 0.5% Catechol. Polyphenol oxidase activity can be measured by recording the absorbance at 420nm. Take the reading immediately at initial stage and at peak of activity of enzyme.

Results and Discussion

Morphological analysis: Seed germination Studies

The seeds of Cicer arietinum were pre-soaked in distilled water for 1h and treated with aqueous flower extract. Data were recorded upto 6th day for each day. Various parameters like germination percentage, mean radicle length, mean plumule length, percentage inhibition of radicle growth and percentage inhibition of plumule growth were observed and analyzed. Treated and Control seeds at various stages of germination is shown in Figure 1.

The treated seeds show only 45% percentage germination upto 6th day when compared to 100% germination obtained in control group (Table 1; Fig. 2). The 1:4 dilution is shown to be significantly affecting the seed germination of Cicer arietinum. The mean radicle length observed for all the 6 days were shown in the table (Table 1). it is very much clear that the treated seeds are showing delayed and distorted growth pattern (Fig. 1). Similar is the case of plumule growth as shown in the table (Table 1). The percentage of growth inhibition of radicle and plumule is almost upto 100% in the initial days in treated cases, and the condition is not significantly improving through the later days (Table 1, Fig. 3) It is clear from the results that the aqueous flower extracts of Quisqualis had inhibitory effect on seedling growth at the present concentrations. Swaminathan *et al.* (1990) showed that the Parthenium hysterophoorus leachates obtained from the leaves, stem and flowers significantly inhibited the plumule growth of cowpea. While, in sorghum only radical growth was affected. The inhibition was attributed to the unsaturated lactones found in plant parts of weed species. The mean weight of the germinating seeds was also recorded at two day intervals upto 6 days and is shown in figure 4. The treated seeds are underweight when compared to control seeds at 6th day of experiment.

BIOCHEMICAL ANALYSIS

Photosynthetic Pigments content

Chlorophyll and carotenoids content in germinating seeds is shown in Figure 5. It is clear from the values that in the treated seeds the chlorophyll content is considerably lower (3.18mg/g Fresh Weight) than that of the control seeds (8.52mg/g Fresh Weight). Similar is the case with all the observations on successive days (Fig. 5). It also reflects that Chlorophyll b is predominant over Chlorophyll a which is

Figure 1. *Quisqualis indica* **floral extract Treated and Control** *Cicer arietinum* **seeds at various stages of germination**

Table 1. Morphological Features of Germinating *Cicer arietinum* Seeds

	Mean Radicle Length (cm ± SD)		Mean Plumule Length (cm ± SD)		% Growth Inhibition of	
Days	Treated	Control	Treated	Control	Radicle	Plumule
1	0	1.74 ± 0.19	0	0	100.00	
2	0.6 ± 0.26	2.9 ± 0.67	0	0.68 ± 0.13	79.31	100.00
3	0.9 ± 0.24	4.9 ± 0.89	0.36 ± 0.35	3.54 ± 0.75	81.63	89.83
4	1.14 ± 0.18	5.38 ± 0.89	0.86 ± 0.09	5.18 ± 0.64	78.81	83.40
5	1.32 ± 0.22	5.46 ± 82	1.18 ± 0.13	9.02 ± 1.04	75.82	86.92
6	1.5 ± 0.16	5.96 ± 0.72	1.54 ± 0.15	12.1 ± 1.75	74.83	87.27

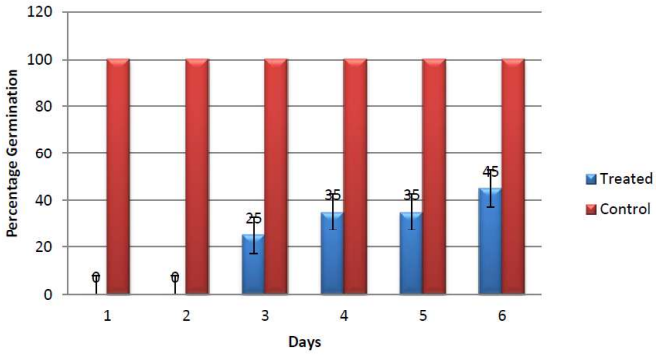


Figure 2. Cumulative Percentage of Seed Germination

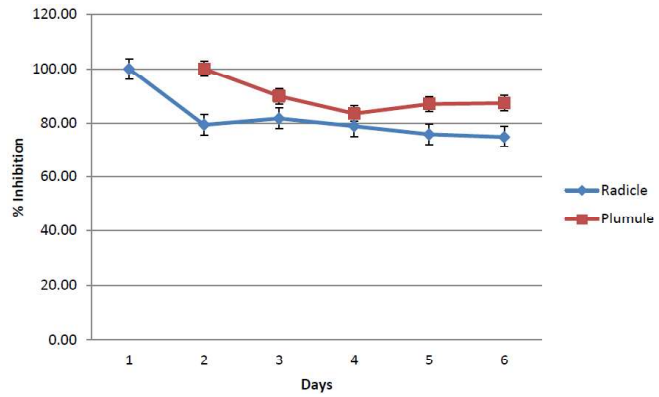


Figure 3. Percentage inhibition of Radicle & Plumule Growth

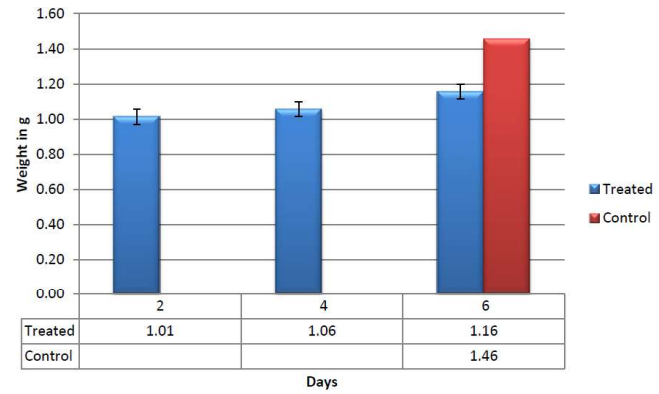


Figure 4. Fresh Weight of Treated and Control Seeds

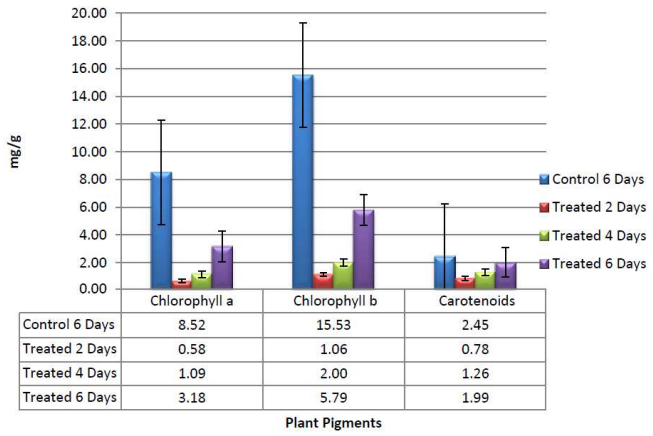
quite uncommon and leads to Chl a/b ratio less than 1. It is usually more than 2 in case of higher plants. The level and state of chlorophyll pigments in the leaf tissue is thus one of the important factors which determine the overall photosynthetic efficiency of the plants. This level is influenced by both endogenous factors such as developmental stage as well as environmental factors. The treated seeds show (1.99mg/g Fresh Weight) a little difference in the carotenoids concentration when compared to control (2.45mg/g Fresh Weight) on 6th day (Fig. 5).

Protein content

It was observed that the protein content of the germinating seeds was shown to increase steadily from start of experiment to 6th day. Interestingly the concentration of protein was significantly higher in treated seeds (73.28mg/g Fresh Weight) when compared to control seeds (28.50mg/g Fresh Weight) on 6th day of experiment (Table 2). The protein content is highly affected by treatment of weed extracts and stimulated more than two and three times than control by *Alternanthera sessilis*, [L.] R.Br., and *Cyanodon dactylon*, [L.] Pers. Respectively. Both the weed extracts caused significantly stimulation in protein content of jowar seedlings than control. Maximum stimulation caused by 75% Conc (Abhinav A. *et al.*, 2014).

Proline content

Content of proline during germination in treated vs. Control seeds are shown in Table 2. It reflects the same order of concentration levels as that of amino acids and amides. The treated seeds contains higher amount of proline (1.15mg/g Fresh Weight) when compared to the control seeds (0.98mg/g Fresh Weight) on 6th day of experiment. The initial level was lower and is steadily increasing attaining an optimum level, in treated cases from start of experiment to 6th day (Table 2). Proline is one of the important amino acids in plants. It is observed that free proline accumulates in plants in response to various types of environmental stresses, such as drought, salinity, high temperature, nutrient deficiency and exposure to heavy metals and high acidity (Oncel *et al.*, 2000; Ruiz *et al.*, 2002). In view of Matysik



	Chlorophyll a	Chlorophyll b	Carotenoids
Control 6 Days	8.52	15.53	2.45
Treated 2 Days	0.58	1.06	0.78
Treated 4 Days	1.09	2.00	1.26
Treated 6 Days	3.18	5.79	1.99

Figure 5. Concentration of Different Plant Pigments in Treated & Control Seed Germination

et al. (2002) proline is a proteinogenic amino acid which functions as an osmolyte, free radical scavenger, electron sink and stabilizer of macromolecules and component of cell wall. Proline is considered to play a major role in adjustment to osmotic stresses (Voetberg and Sharp, 1991).

Soluble Carbohydrate content

The concentration of soluble carbohydrate in germinating seeds is shown in Table 2. It is clear from the results that the level of soluble carbohydrate in treated seeds (102.77mg/g Fresh Weight) is comparable to those of control seeds (106.94mg/g Fresh Weight) on the 6th day of experiment. It was observed that the concentration is decreasing from a higher point on 2nd day to a lower point on 4th day and is then increasing afterwards (Table 2). This may be due to the rapid mobilization of reserve carbohydrates during initial days and its depletion on later days due to utilization for metabolic processes. Carbohydrates, a final product of photosynthesis occupy a very important place in the primary metabolism of all green plants. These are the major products of photosynthetic carbon assimilation and the major substrates for respiration. Thus, the level of carbohydrates in the plant tissue gives an indirect idea of the metabolic status of the plant tissue as well as the energy content of the plant tissue. Carbohydrates provide carbon skeletons for wide range of carbon compounds present in the plant tissue. These compounds include various secondary metabolites, some of which have a definite medicinal value. Starch is the storage carbohydrate

ANTIOXIDATIVE ENZYMES

Peroxidase activity

The peroxidase activity in seed germination is shown in the Table 3. The results show that the activity of peroxidase is in its highest during initial stages of germination (2nd day) (1.60mg/min/mg) and steadily decreasing towards the 6th day (0.27mg/min/mg) of experiment in treated seeds. Inter-

Table 2. Biochemical Analysis of Germinating *Cicer arietinum* Seeds

Treated Days	Protein (mg/g)	Proline (mg/g)	Soluble Carbohydrate (mg/g)
2	39.11	0.63	112.80
4	70.36	1.22	80.44
6	73.28	1.15	102.77
Control 6 Days	28.50	0.98	106.94

Table 3. Anti-oxidative Enzymes' Study of Germinating *Cicer arietinum* Seeds

Treated Days	Peroxidase		Catalase		Polyphenol Oxidase	
	Enzyme activity (mg/min/mL)	Specific Activity (mg/min/mg)	Enzyme activity (mg/min/mL)	Specific Activity (mg/min/mg)	Enzyme activity (mg/min/mL)	Specific Activity (mg/min/mg)
2	6.27	1.60	1.43	0.04	3.3	0.08
4	7.91	1.12	4.4	0.06	0.5	0.01
6	2.00	0.27	4.2	0.06	0.65	0.01
Control 6 Days	0.44	0.15	0.8	0.03	7.93	0.28

estingly the activity of peroxidise at 6th day is is still higher in treated seeds (0.27mg/min/mg) when compared to the control seeds (0.15mg/min/mg) (Table 3). A group of enzymes that catalyse the oxidation reactions in the biochemical pathways of plant tissues are peroxidases. Peroxidases contain a heme cofactor in their active site, or redox-active cysteine or selenocysteine residues. These are found in different parts of cell in different forms. Peroxidase typically catalyzes the following reaction ROOR' + electron donor (2 e^-) + $2H^+ \rightarrow$ ROH + R'OH. Peroxidase plays an important role in growth and development of plants through its control over auxin catabolism (Ray 1962; Hinman and Lang, 1965), H_2O_2 formation (Gross *et al.*, 1977) and lignin (Halliwell, 1978) and ethylene biosynthesis (Liberman, 1979). Peroxidase is an indicator of respiration rate (Horovitz *et al.*, 1968). It may be involved in catabolism of chlorophylls in senescent leaves (Matile, 1980). Peroxidases are also known to play a part in increasing plant's defense against pathogens (Karthikeyan *et al.*, 2005).

Catalase Activity

Activity of Catalase enzyme is shown in the Table 3. The results of catalase activity in seed germination reveal that the activity of this enzyme is significantly higher in treated seeds (0.06mg/min/mg) as compared to control seeds (0.03mg/min/mg). It is also observed that the level of catalase activity is steadily increasing from start of experimennt attaining an optimum point and their after maintaining a steady level towards the 6th day in treated seeds (Table 3). Hydrogen peroxide acts as a precursor of more cytotoxic or highly reactive oxygen derivatives such as peroxynitrite or OH. So, it is very important to scavenge excess H_2O_2 (Pang *et al.*, 2005). The reaction of catalase in the decomposition of hydrogen peroxide is: $2H_2O_2 \rightarrow 2\ H_2O + O_2$. Catalase can also oxidize different toxins, such as formaldehyde, formic acid, phenols, and alcohols. In doing so, it uses hydrogen peroxide according to the following reaction: $H_2O_2 + H_2R \rightarrow 2H_2O + R$. Catalase protects the cell from toxic effects of hydrogen peroxide, by catalyzing its decomposition into molecular oxygen and water without the production of free radical. It is an effective antioxidative enzyme in preventing oxidative damage (Willekens *et al.*, 1995; Mittler, 2002). Hydrogen peroxide concentrations generally increase in response to various abiotic and biotic stresses and takes part in the reactive oxygen regulatory network (Mittler *et al.*, 2004). Therefore, it is important that H_2O_2 is scavenged rapidly and systematically by the antioxidative defence system. According to Guo *et al.* (2006) catalase and ascorbate peroxidase are primary H_2O_2 scavenging enzymes. The electron transfer chain of the chloroplast is the best-documented source of H_2O_2 (Asada, 1994). Catalase catalyzes decomposition of H_2O_2 to water and oxygen, which does not need reducing substrate for its activity (Mittler, 2002). Catalase has been found predominantly in leaf peroxisomes (in higher plants), where it functions chiefly to remove H_2O_2 formed during photorespiration in C3 species (Dat *et al.*, 2000).

Polyphenol Oxidase Activity (ppo)

The present results (Table 3) show that Polyphenol Oxidase activity is significantly higher in control seeds (0.28mg/min/mg) when compared to the treated seeds (0.01mg/min/mg) on the 6th day of experiment. The PPO activity was more evident towards the initial stages (0.08mg/min/mg) of germination but steadily depleting to negligible activity towards 6th day (0.01mg/min/mg) in treated seeds (Table 3). PPOs are almost ubiquitous among all groups of organisms. Polyphenol oxidases (PPO) are copper-binding metalloenzymes of secondary plant metabolism that catalyze the oxidation of polyphenols and their derivatives to quinones, with simultaneous oxygen reduction. Thus these enzymes bring about the oxidation of hydroxyphenols to their quinone derivatives, which then spontaneously polymerize. (Steffens *et al.*, 1994). Depending upon their reaction mechanism and substrate they are grouped into three different classes (Mayer, 1987) as Catechol oxidases (EC.1.10.3.2) that oxidize o-diphenols to o-quinones. Laccases (EC. 1.10.3.1) that oxidise p-diphenols to p-diquinones and tyrosinases (EC. 1.14.18.1) that are catachol oxidases. Catachol oxidases also have a function of hydroxylation of monophenols to o-diphenols. After formation of o-quinone, the pathway can proceed spontaneously, since this compound is not stable and undergoes an intramolecular cycling yielding L-dopachrome, a wellœknown red orange intermediate of melanin synthesis. Then L-dopachrome decarboxylation yields dihydroxyindole, and further rearrangements and polymerization of these units leads to melanin (Solano *et al.*, 1997) or quinones can form covalent bonds with nucleophilic residues on proteins or free amino acids (Steffens *et al.*, 1994). Due to this quinone tanning, dark coloured reaction products are formed, which are insoluble and can serve as wound protection layers (Waterman and Mole, 1994).

Conclusion

From the study it is clear that aqueous extract from the flowers of *Quisqualis indica* is causing inhibition of normal seed germination and its growth. Various biochemical parameters analyzed also confirms the findings. Action of allelochemicals in target plant is diverse, and affects a large number of biochemical effects of allelochemicals action are detected at molecular, structural, biochemical, physiological and ecological levels of plant organization. The search and development of new herbicides through the identification of active compounds from allelopathic plants is an interesting research and development area.

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