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Studies on the allelopathic effect of aquatic invasive plants on *Cicer arietinum* L.

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ABSTRACT

In the present study, an attempt has been made to study the effect of two invasive aquatic plants Eichhornia crassipes (Mart.) Solms and Pistia stratiotes L. common weeds seen in the Cochin backwaters, on seed germination, seedling growth and the total phenolic content of Cicer arietinum L. The objective of this study is to determine the rate of germination, radicle emergence, plumule elongation, biomass, chlorophyll and the phenolic content of the cultivar seeds exposed to five concentrations of the leaf extracts of E.crassipes and P.stratiotes viz., 0.01ppm,0.1ppm, 1ppm,4 ppmand 10 ppm. In general the seeds of C. arietinum showed a slight decrease in the rate of germination, radicle elongation, plumule growth, biomass, phenol and chlorophyll content in 0.01 ppm over the control.There was gradual decrease in rate of germination, radicle emergence, plumule elongation, biomass and the chlorophyll content with increasing concentrations. The maximum inhibition was observed in highest concentration of both the extracts(10ppm). The total chlorophyll and chlorophyll a content decreased as the concentration of the leaf extract increased, extracts of E.crassipes showed a 50% decrease while it was 20% in P. stratiotes. The chlorophyll b recorded a marginal increase in the P. stratiotes treated seeds of C. arietinum. This research contributes to the phytotoxicity assessment database, and besides to lay the foundation for the exploitation of the secondary metabolites in these weeds for industry and medicine.

KEY WORDS: *Allelopathy, chlorophyll, Phenols, seed germination, Seedling*

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I. INTRODUCTION

Allelopathy can be defined as chemical interactions between and among both plants and microorganisms through the release of biologically active chemical compounds into the environment (Andrzej1977). Allelochemistry, the production and release of toxic chemicals produced by one species that affect a receiving susceptible species, has been the subject of diverse degrees of scientific enquiry (Tiffany et al. 2004). Recent advances in plant biology have permitted the revamp of allelochemistry as a biologically and ecologically sound explanation for plant invasion and plant–plant communication in the rhizosphere. (lalitha et al. 2012).

Weeds are a constant constraint to rice production and cause yield losses in all rice production systems and in all seasons Although excessive weed growth may provide protective cover in water for small fish growth it may also interfere with fish harvesting (Brij and Goel 1993). Dense growth of aquatic weeds may provide ideal habitat for the development of mosquitoes causing malaria, encephality filarasis and serve as vectors for disease causing organisms and can greatly reduce the aesthetic value of water bodies from a recreational point of view (Kumar and Singh 1987). Aquatic weeds can assimilate large quantities of nutrients from the water reducing their availability for planktonic algae. They may also cause reduction in oxygen levels and present gaseous exchange with water resulting in adverse fish production. (Wium et al. 1982).Allelopathy can influence the competition between different photoautotrophs for resources and change the succession of species, for example, in phytoplankton communities. Application of allelochemicals from *P. stratiotes* to inhibit *M. aeruginosa* has a high degree of ecological for treating water subjected to algae blooms (Xiang Wu 2013).

Our increasing knowledge of allelopathy helps greatly in our understanding of many ecological phenomena, such as succession and patterning of agroecosystems. Research has been particularly active in relation to the roles of allelopathy in agriculture. The basic approach used in allelopathic research for agricultural crops has been to screen both crop plants and natural vegetation for their capacity to suppress weeds (Jefferson and Pennacchio 2003). To demonstrate allelopathy, plant origin, production, and identification of allelochemicals must be established as well as persistence in the environment over time in concentrations sufficient to affect plant species (Hesammi and Farshidi 2012). Allelopathy may be a key mechanism by which some native trees could reduce the abundance and impact of exotic species. It is a recognized tool for weed management in agriculture and agroforestry (Cummings et al. 2012).

Cicer arietinum L. also known as chickpea is an important pulse crop with a diverse array of potential nutritional and health benefits. It is one of the most important food legume crops and has the useful ability to fix atmospheric nitrogen through its root nodules. Chickpea consumed all over the world, especially in the Afro-Asian countries and is a good source of carbohydrates and protein, and protein quality is considered to be better than other pulses (Jukanti et al. 2012). Allelopathy can also induce negative effects to the processes of germination, growth and development of plants or disrupt some physiological and metabolic processes. *Pistia stratiotes* and *Eichhornia crassipes* are the two most common aquatic weeds that flourish in Vembanad Lake of the Cochin backwaters. By studying the effect of leaf extracts of these aquatic weeds on treated seeds on germination and determination of growth indices, it is possible to evaluate the positive or negative influence or toxicity of the chemical compounds. The objective of this study is a preliminary attempt to determine the impact of the leaf extracts of *Pistia stratiotes* and *Eichhornia crassipes* for stimulation of seed germination and phenolic content in *Cicer arietinum* L.

II. MATERIALSAND METHODS

The seeds of *Cicer arietinum* L. purchased from Ernakulam District Agri-horticultural society, were taken for this study. Whole mature plants of selected weeds viz. *Eichhornia crassipes* (Mart.) Solms and *Pistia stratiotes* L. were uprooted randomly from the thickly populated area of the Vembanad Lake at Palluruthy in Ernakulam during November 2014. To test allelopathic effects of aqueous extracts of weeds, these weeds were washed in distilled water for removing dust and soil particles and then were dried in shade for a week at 25^{o}C. These pieces were put in oven for 24 hours at 70^{o}C. After oven drying, whole plants of each species were chopped into small pieces. The dried pieces of the weeds plants were weighed and aqueous leaf extracts of the following concentrations viz., 0.01ppm, 0. 1ppm, 1ppm, 4 ppm and 10 ppm were prepared .The leachates were collected in separate bottles and tagged. The extract obtained after filtering was used for study. The seeds of *Cicer arietinum* were surface sterilized with 0.1% mercuric chloride and washed thoroughly with distilled water 3 times.A hundred seeds each were soaked in the respective concentrations of the leaf extracts for 6 hrs. Distilled water was used in place of extracts in control. Twenty seeds were uniformly spread on each Petri plate and were allowed to germinate at room temperature. The filter papers lined in Petri plate were moistened with respective concentration of the extracts. Petri plates containing moistened filter paper soaked different concentrations of 0.01ppm, 0.1ppm, 1ppm, 4ppm and 10ppmand with distilled water (control). Three replicates for each of the five treatments including control were used. The soaked seeds were incubated in Petri plates lined with moist blotter at 28 ± 2^{o}C in dark. The percentage of germination was calculated from the second day itself by counting the number of seeds germinated in each Petri plate. On the 2nd day the seedlings were taken out, and the radicle and plumule length were measured. The chlorophyll was estimated in the 15 day old seedlings (Arnon1949).The fresh and dry weights of 15 day old seedlings were measured. For dry weight seedlings were kept in a oven for 48h at 60°C.The data was average of three replicates. Total water-soluble phenolics in the weeds were estimated as per the method of Swain and Hillis (1959) using Folin-ciocalteu reagent. Their amounts were determined spectrophotometrically at 700 nm against the standard of ferulic acid.

III. RESULTS AND DISCUSSION

Different concentrations of the extracts of *Eichhornia crassipes* and *Pistia stratiotes* revealed different influences on the seed germination of *Cicer arietinum* L. (Fig.1 &2).Control recorded 100% germination after 48 hours of treatment. The effect of the extracts of *E.crassipes* on seed germination recorded a maximum delay of 60% (10 ppm) and 50% (4ppm) in the highest concentration studied. In the treatment with 10% concentrations of the leaf extracts of *P.stratiotes* the lowest of 50% in 10ppm and 35% in treatment with 4 ppm was recorded after 48 hours of treatment. The seeds treated with different concentrations of leaf extracts of *E.crassipes* and *P.stratiotes* showed a remarkable decrease in germination percentage with increasing concentrations of the treatment. The decrease in germinability was well correlated with increased membrane deterioration, assayed as electrical conductivity and enhanced lipid peroxidation, detected as increased malondialdehyde content (Bogatek et al. 2006). The allelopathic effect of the exudate secreted by *Cistus ladanifer* leaves on different plant species clearly inhibited seed germination(Chaves and Escudero2003). Fahmy et al. (2012) observed that the roots and leaf extracts of *Pluchea dioscoridis* had inhibitory effects upon the seed germination and seedling growth of itself and the test species *Corchorus olitorius*.

Results displaying the effect of the extracts of *Eichhornia crassipes* and *Pistia stratiotes* on the radicle elongation of of *Cicer arietinum* L. (Fig.3&4) recorded its maximum in the control (24.5cms) on the second day of germination. The radicle length in .01 ppm treatments in both the plants did not record any decrease when compared to the control. A decrease in length of the radicle was recorded with increasing concentrations of the extract, treatments with extracts of *E.crassipes*,on the tenth day showed a decrease of 28% while it was only 16% over the control in treatments with *P.stratiotes*.The radicle emergence recorded an increase from the fourth day onwards. The allelopathic inhibitory effect on seed germination, radicle (root) and coleoptile development and root axis production have been affected adversely (Tasawer et al.2014).

Results displaying the effects of the varying concentrations of the extracts on the growth of the seedlings did not show any inhibition with 0.01 % concentrations of both the extracts treated. With increase in concentration of the leaf extracts of *E.crassipes* and *P.stratiotes*, the elongation of the hypocotyl showed a decrease. Data from Fig. 3 &4 displayed maximum inhibition of 63.6 % (treatment with *E.crassipes*) and 32.5% (treatment with *Pistia stratiotes*) in the hypocotyls of *C.arietinum* on the 10th day over the control. The plumule elongation was found to decrease with increasing concentrations of the extracts which was more pronounced in treatments with *Eichhornia crassipes* than in *Pistia stratiotes*. Similar results were obtained by Daniel and Oliver (2003) in a study of the Garlic mustard (*Alliaria petiolata*, Brassicaceae) and attributed it to the allelopathic interference by garlic mustard. Extracts and residues from leaves of lettuce (*Lactuca sativa* L.) cultivar "Cheongchima", showed Coleoptile growth to be significantly lowered, when assayed for their allelopathic effects on seed germination and early seedling growth.A strong reciprocal correlation exist between the concentration and the seedling growth or the water content of the crops under study supports the dose linked allelopathic phenomenon. (Ramanathan et al. 2006).

Results displaying the effect of extracts of *Eichhornia crassipes* and *Pistia stratiotes* on the chlorophyll content of *Cicer arietinum* is given in Fig.7 & 8. The total chlorophyll, chlorophyll *a* and the chlorophyll *b* content recorded the highest in control and .01ppm treated seeds in both the treatments. The total chlorophyll and the chlorophyll *a* content of seeds treated with extracts of *E.crassipes* and *P.stratiotes* showed a marginal decrease with increase in the extract concentrations.The 4ppm treatment with *E.crassipes* showed a decrease of 46.82 % while *P.stratiotes* recorded only a 23.44% decrease over the control. In seeds treated with extracts of *E.crassipes* and *P.stratiotes* chlorophyll *b* content showed a marginal decrease with increase in extract concentration with a maximum reduction of 18.64 % recorded in 10ppm dilution of *E.crassipes*. On the contrary in seeds treated with 0.1 ppm concentration of *P.stratiotes*, ten day old seedlings of *Cicer arietinum* recorded a marginal increase (8.5%) in the concentration of the chlorophyll *b*. Low concentration of extracts of *E.crassipes* and *P.stratiotes* enhanced leaf growth components at all the growth stages studied. The major water-soluble allelochemicals also caused reductions in leaf chlorophyll content. They may be responsible for negative allelopathic effects of quackgrass on soybean by inhibiting root growth, by altering ion uptake and transport, and by reducing chlorophyll content (Baziramakenga et al.1994).The chloroplast block by allelochemicals is in the photosystem II complex. Cinnamic and benzoic acid derivatives alter membrane potential and have several physiological effects that suggest membrane perturbations are their initial site of action.

The Fresh and dry weights of 15 day old seedlings for both the extracts of *Eichhornia crassipes* and *Pistia stratiotes* treatment showed the maximum value in the control and in 0.01% concentrations (Fig. 9). The effect of the extracts of *E.crassipes* and *P.stratiotes* on the biomass of 15 day old seedlings recorded a 37% decrease in the fresh and dry weight in treatment with extracts of *P.stratiotes* with 4 ppm concentration, while a marginal decrease of 2% was observed in treatment with extracts of *E.crassipes* in the above concentration. Results showed by Benyas et al. (2010) that low concentrations of *Xanthium strumarium* shoot had no significant effect on the germination percentage, radicle length, plumule and radicle dry weight, total chlorophyll content and chlorophylls *a* and *b* content. However, treatments with higher concentrations had negative effects on mean rate of germination, plumule length and seedling dry weight. In the present study 0.01ppm concentration of both the extracts did not show any inhibition on the biomass of *C.arietinum*. The inhibition of seedling growth, singly or in combinations,correlate with impairment of plant-water relationships. These phenolic compounds also alter mineral uptake, chlorophyll content, photosynthesis, carbon flow, and phytohormone activities (Einhellig1995).The different responses of chlorophyllase *a* and *b* activities to the same concentrations of allelochemical phenolics suggest that they may be two different enzymes. It is apparent that the three phenolics may enhance the activities of enzymes, such as chlorophyllase and Mg-dechelatase, responsible for the Chldegradative pathway (Yang et al. 2004).

Results displaying the effect of extracts of *Eichhornia crassipes* and *Pistia stratiotes* on the total phenol content of *Cicer arietinum* in 15 day old seedlingsis given in Fig.10 & 11. The total phenolic content recorded the highest in 10 ppm treated seeds in both the plant extracts treated. The total phenol content of seeds treated with extracts of *E.crassipes* and *P.stratiotes* showed a increase with increase in the extract concentrations, the 10 ppm treatment with *E.crassipes* recorded a threefold increase in the phenol content(0.5mg/g),while *Pistia stratiotes* recorded only a two fold increase over the control. In seeds treated with extracts of *E.crassipes* and *P.stratiotes,*0.01 ppm concentration of the extract showed the minimum increase of 16% in*P.stratiotes and* 33 % in *E.crassipes*. Phenolic compounds are a class of the most important and common plant allelochemicals in the ecosystem (Li et al. 2010). The inhibitory action of the aqueous extracts or mulch of *P. dioscoridis* is possibly attributed to the presence of phenolic compounds. (Kim et al. 2003) .The role of some identified phenolics as allelopathic agents present in walnut tissues, also reduced the germination of dandelion seeds and the catechin was most strongest inhibitor. (Matok et al. 2009).The highest concentration of the phenolic compounds inhibited the germination of all these weeds, but lower concentrations had no effect or were stimulatory. (Reigosa1999)

IV: Figures & Tables

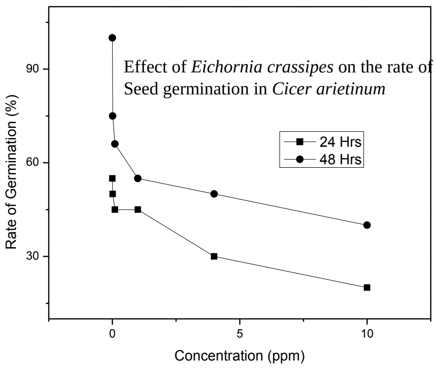


Fig. 1

Fig.1: Effect of *Eichornia crassipes* on the rate of seed germination in *Cicer arietinum*

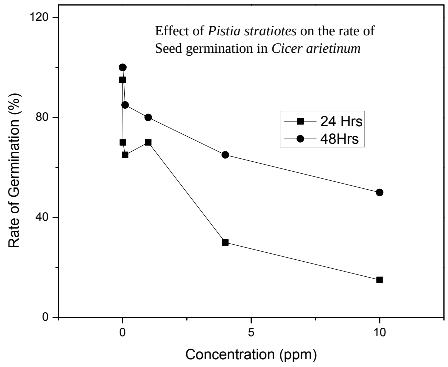


Fig. 2

Fig.2: Effect of *Pistia stratiotes* on the rate of seed germination in *Cicer arietinum.*

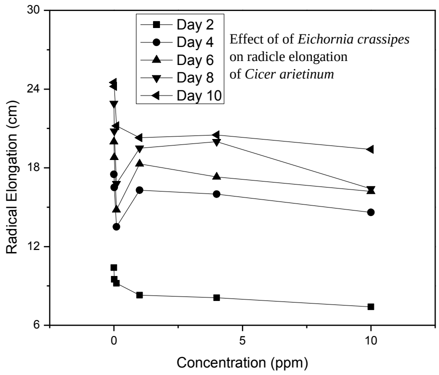


Fig. 3

Fig. 3. Effect of *Eichornia crassipes* on radicle elongation of*Cicer arietinum*

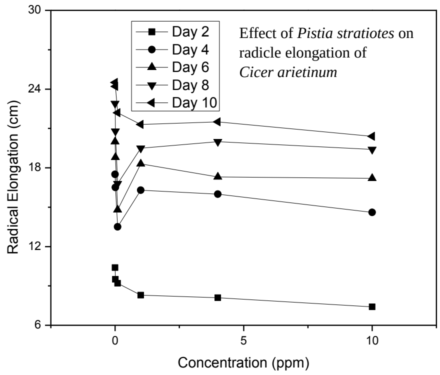


Fig. 4

Fig. 4. Effect of *Pistia stratiotes* on radicle elongation of*Cicer arietinum*

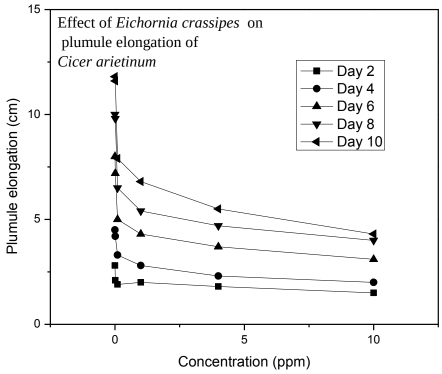


Fig. 5
Fig. 5. Effect of *Eichornia crassipes* on plumule elongation of*Cicer arietinum.*

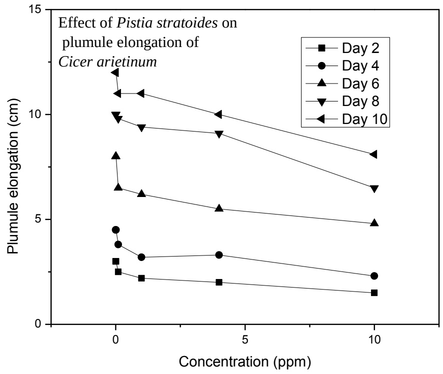


Fig. 6
Fig. 6 Effect of *Pistia stratoides* on plumule elongation of*Cicer arietinum.*

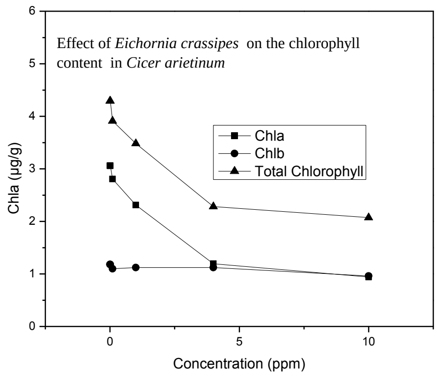


Fig. 7
Fig.7. Effect of *Eichornia crassipes* on the chlorophyll content in *Cicer arietinum.*

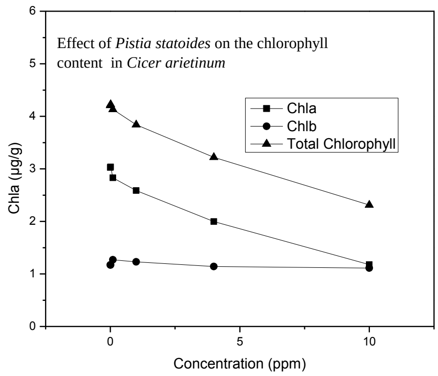


Fig. 8
Fig.8. Effect of *Pistia stratoides* on the chlorophyll content in *Cicer arietinum.*

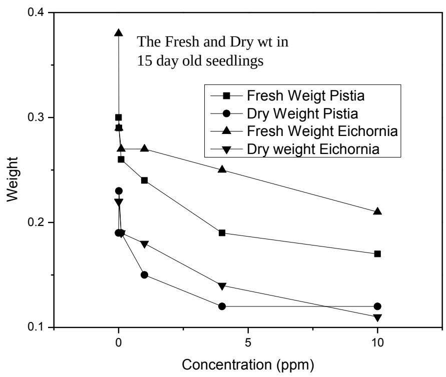
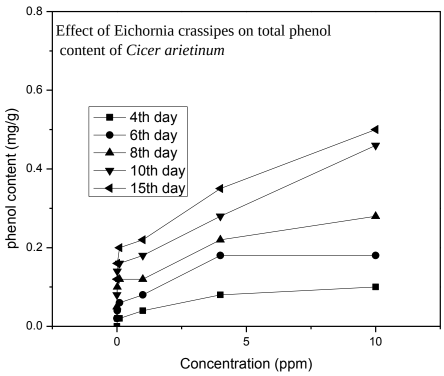


Fig. 9
Fig. 9. The Fresh and Dry wt (mg) in 15 day old seedlings



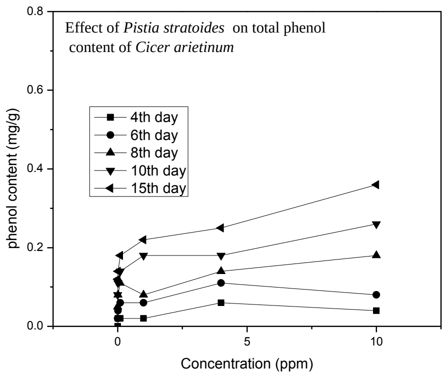


Fig. 10

Fig. 10.Effect of *Eichornia crassipes* on total phenolic content of*Cicer arietinum.*

Fig. 11

Fig. 11. Effect of *Pistia stratoides* on total phenolic content of*Cicer arietinum.*

IV. CONCLUSION

Invasive Aquatic plant species acquire spreading advantage in new water bodies through use of their allelochemicals to inhibit germination and growth of other hydrophytes including algae. The results revealed that the inhibitory effect was proportional to the concentrations of the extracts. Higher concentrations of extracts displayed stronger inhibitory effect of radicle emergence, seedling growth and reduction of chlorophyll content in the growing seedlings. Phenols recorded higher values in higher concentrations of the extract treatments. Accumulation of chlorophyll contents were more inhibited as the phenolic concentrations increased. *Eichhornia crassipes* extracts showed higher inhibition rate than *Pistia stratiotes*.The identification of weeds with potential pool of allelochemicals and characterization of their adverse effects on germination of seeds of crops during early growth stages and finally on the commercial yield can be exploited in sustainable agriculture programmes.

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