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CODEN: IJRSFP (USA)

International Journal of Recent Scientific Research Vol. 9, Issue, 6(F), pp. 27566-27570, June, 2018 International Journal of Recent Scientific Re*r*earch

DOI: 10.24327/IJRSR

Research Article

ALLELOPATHIC ASSESSMENT OF AGERATUM CONYZOIDES WEED ON PISUM SATIVUM L

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DOI: http://dx.doi.org/10.24327/ijrsr.2018.0906.2286

ARTICLE INFO

ABSTRACT

Article History:Allelopathy isReceived 20th March, 2018through the reReceived in revised form 27thcompound andApril, 2018allelochemicalAccepted 5th May, 2018other plants.Published online 28th June, 2018allelochemicalKey Words:hydroxybenzoi

Weed, *Ageratum conyzoides, Pisum sativum,* Allelopathy, Nitrate and Nitrite reductase enzyme.

Allelopathy is defined as any direct or indirect, catastrophic effect of a plant on another plant through the release of chemicals into the soil environment. Plants produces some chemical compound and releases then in the environment which is called as allelochemicals. These allelochemicals shows beneficial or harmful effects on seed germination, growth, and progress of other plants. A number of studies have indicated that the *Ageratum conyzoides* have the allelochemicals potential. It releases various types of allelochemicals such as flavonoids, Alkaloids, saponins, tannins, cardiac glycosides, anthraquinones, gallic acid, ferulic acid, coumaric acid, hydroxybenzoic acid anisic acid and syringic acid. These allelochemicals may be harmful or helpful for the plant growth and the development. *Pisum sativum* (pea) is a valuable pulse crop cultivated in India. It is one of the major pulse crop of Uttar Pradesh grown in Rabi season. The present work has done to investigate allelopathic effects on some biochemical parameters such as chlorophyll, proline, protein, leghaemoglobin, nitrogen, phosphorous, and nitrate and nitrite reductase enzyme activity.

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INTRODUCTION

Grain legumes are particularly sensitive to biotic and abiotic stresses. Biological nitrogen fixation, specific to legumes, is highly sensitive to numerous abiotic factors like drought, heat, salinity etc. (Benezit, 2017). On the other hand, biotic factor such as weeds also affects the legumes growth and crop productivity. Pea (*Pisum sativum* L.) is a cool season legume crop. As the other legume crops it has also tolerate biotic and abiotic stresses at the vegetative stage but also experiences the yield loss at the reproductive stage.

In biotic stress allelopathy as an important form of plant interference (Putnam and Tang, 1986). It accounts for both disastrous and legatee biochemical relations amongst plants. Allelopathy studies have been done with many crops, shrubs, trees, and weeds under both laboratories as well as in field to determine their allelopathic potential. This phenomenon also used for weed control in many crops such as rice, soyabean etc. (Waseem Mushtaq, 2017). Allelopathy also affects crop growth by releasing allelochemicals into the growing environment (Kadioglue *et al*, 2005).

Plant *Ageratum conyzoides* (genus *Ageratum*) is a member of Asteraceae family tribe Eupatoriae has the allelopathic potential. The genus *Ageratum* consists of about 40 spp. found

in tropical and subtropical countries, however, only two spp. *Ageratum conyzoides* L. and *Ageratum houstonianum* are well known.

In India Ageratum conyzoides grown in mainly Himachal Pradesh, Uttar Pradesh, Arunachal Pradesh, West Bengal Assam, Orissa. Volatile oil and the aqueous extract of the Ageratum conyzoides have been shown allelopathic effects on a number of cultivated crops including radish, mung bean and various chemical compounds such as rvegrass. It has flavonoids. glycoside, Alkaloids, (agetochromene, demethoxyagerato-chromene and stigmast-5,22-diene-3β-ol) saponins, cardiac glycosides, anthraquinones, tannins. coumaric acid, gallic acid, ferulic acid, hydroxybenzoic acid, anisic acid and, syringic acid, j3-coumaric acid and phydroxybenzoic acid (Okunade, 2002).

Allelochemicals has the potential to be used as growth regulators, insecticides, herbicides, and antimicrobial crop protection products. Many aspects of plant physiological and biochemical processes have been proved to be affected by allelochemicals (Gniazdowska and Bogatek, 2005).

The involvement of Allelopathy in negative interactions among plants relatively more is known about its action at the different part level but need it to study its positive aspects. To ensure sustainable agricultural development, it is important to exploit

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cultivation systems that take advantage of the stimulatory/inhibitory influence of allelopathy to regulate plant growth and development and to avoid allelopathic auto toxicity.

Thus the present work was undertaken to study the allelopathic effects of *Ageratum conyzoides on* nitrogen -fixing ability and other important biochemical parameters such as chlorophyll, proline, protein, leghaemoglobin, nitrogen, phosphorous, and nitrate and nitrite reductase enzyme activities *of Pisum sativum*.

MATERIALS AND METHODS

An experiment was conducted at Botany department of C.C.S. University campus, Meerut during the month of November (2017-2018). Different amount of Ageratum conyzoides (10, 20, and 30 g) was soaked in 1000 ml of double distilled water and filtered after 96 hours with triple layer Masson cloth and Whatman paper. Three plots of (equal size of $1 \times 1m^2$) irrigated with different concentrations of Ageratum leachate after sowing the pea seeds (Pusa pargati). Fourth plot (untreated) of same size irrigated with tap water at the same time. Biochemical parameters of pea plant such as chlorophyll, protein and proline concentration were calculated following Arnon's (1949), Bradford (1976) and Bates (1973) method. Nitrogen fixation related parameters such as leghaemoglobin content, nitrate, and nitrite reductase enzyme activity were measured by Bergersen (1980), Hageman (1980), and Guerrero method (1982) and phosphorous and nitrogen content was measured by the Olsen (1954) Snell and Snell (1967) methods respectively.

RESULT AND DISCUSSION

Chlorophyll



Fig 1 Allelopathic effects of *Ageratum conyzoides* on total chlorophyll content of *Pisum sativum*.

Use of *Ageratum* plant leachate at lower concentration (10%) enhances the chlorophyll contents more than the control in pea plant leaves. But at higher concentrations of the extract, less chlorophyll content was recorded. As the leachate concentration increases the amount of chlorophyll content decreases (Fig. 1). It may be due to the volatile allelochemicals, which are present in *Ageratum conyzoides* leachate irrigated soil and disrupt the synthesis of porphyrin precursors of chlorophyll biosynthesis (Rice, 1984). The reduction of chlorophyll contents in leachate treated plants could be attributed to the inhibition of chlorophyll biosynthesis or the

stimulation of chlorophyll degradation (yang, 2014). The reason may be the allelochemicals such as pcoumaric acid, ferulic acid, gallic acid, anisic acid, and p-hydroxybenzoic acid present in Ageratum conyzoides leachate (Batish et al, 2008) inhibit the photosystem I reaction as an inhibitor compounds, which reduces chlorophyll content in the test crop species by interfering with the biosynthesis of photosynthetic pigments or enhance chlorophyll degradation (Huang et al, 2010). Changes in chlorophyll contents in the present study are also supported by the findings of Inderjit and Dakshini (1992) who cited allelochemicals-mediated reduction in seedling photosynthetic pigments primarily due to phenolic acids and phytotoxic compounds which are present in varying concentrations in different parts of the Ageratum weed. Similarly, Kaur et al. (2016) were also reported the reduction of total chlorophyll (chlorophyll a and chlorophyll b) in Vigna radiata plants due to the aqueous extracts/leachate of Ageratum convzoides weed.





Fig 2 Allelopathic effects of *Ageratum conyzoides* on proline content of *Pisum sativum*.

In the present investigation, maximum (0.0229) proline accumulation was observed in 30% Ageratum conyzoides leachate amended soil. While minimum proline (0.0106) accumulation was found in control. However, the 10% and 20% Ageratum convzoides leachate amended treated plots shows moderate levels (0.0180, 0.0190) of proline content (Fig 2). The detrimental effects are more pronounced in high strength leachate along with high values of proline. A concentration dependent increase in proline content of pea leaves was observed with increasing levels of leachate. This may be due to the breakdown of proteins or de novo synthesis of proline (Inderjit et at., 2004). Stress also increases the proline contents (Ghoulam et at., 2002). At the lower amount of Ageratum convzoides leachate may provide a better chemical environment to a plant which reduces the stress conditions (proline is an amino acid which plays an important role in plants exposed too many stress conditions). Similar kinds of finding with explanations have been also submitted by Rawat (2015).

Protein 0.71 0.708 Protein (mg/gm) 0.706 0 704 0.702 0.7 0.698 0.696 0% leachate 10 % 20% 30% amended leachate leachate leachate amended amended soil amended soil soil soil Kinds of leachate %

Fig 3 Allelopathic effects of *Ageratum conyzoides* on protein content of *Pisum sativum*.

Maximum (0.708) protein content was observed in 10% Ageratum conyzoides leachate and in control plants seed. However, minimum amount of protein was found in 30% Ageratum conyzoides treated plots (Fig 3). Le *et al*, (2010) were also suggested that phenolics like ferulic acid and cinnamic acid can decline protein synthesis. Phenolic allelochemicals of *Ageratum conyzoides* inhibit amino acids transport and synthesis of proteins and the subsequent growth of treated plants. All phenolics allelochemicals could decrease the integrity of DNA and RNA (Zeng *et al*, 2001). Kaur *et al*, (2015) were also found that the *Ageratum conyzoides* may naturally contain phytotoxic compounds which inhibit the protein content in *Vigna radiata* plants by decreasing their biosynthesis or increasing their degradation level of protein.

Legheamoglobin



Fig 4 Allelopathic effects of Ageratum conyzoides on total leghaemoglobin content of Pisum sativum.

In the present study, the maximum leghaemoglobin accumulation was found in control (0.0182), while a minimum amount of leghaemoglobin content was found in 30% *Ageratum conyzoides* leachate (0.0146) treated plot (Fig 4). With the increasing concentrations of *Ageratum conyzoides* leachate, responsible fordecreases leghaemoglobin amount proportionally in the plant nodules due to the availability of allelochemicals and secondary metabolites. This may be due to the presence of a number of chemical compounds (Alkaloids, flavonoids saponins, tannins) that are released by roots or other parts of *Ageratum conyzoides* into the soil rhizosphere, which under natural conditions bring significant ecological effects

(Serafimov and Dimitrova, 2007). In rhizosphere soil, however, roots are the major source of interactions, which bring about the phytotoxic effect in rhizosphere soil (Zhimei *et al*, 2007). On the other hand accumulation of phenolics-the most common water-soluble allelochemicals also plays a significant role in plant-plant and plant-microbe interactions which affect the nodules formation and synthesis of leghaemoglobin in plants root nodules. Wardle *et al*, (1994), Mallik (1999), Batish *et al*, (2006) also recorded similar kinds of observations in their study.





Fig 5 Allelopathic effects of Ageratum conyzoides on nitrogen content of Pisum sativum.

Nitrogen concentration of the leaves of pea is influenced by the application of Ageratum conyzoides leachate clearly shown in the (Fig 5). Maximum nitrogen content (0.585) was obtained with the application of Ageratum conyzoides leachate amended plots as compared to 0.0% (0.459), 20% (0.420), 30% (0.420) treatments respectively. These results show a positive impact of Ageratum conyzoides leachate on nitrogen content of leguminous crops at a lower amount. Contrarily, the higher amount of Ageratum conyzoides leachate which presents allelochemicals such as gallic acid, ferulic acid, coumaric acid, hydroxybenzoic acid anisic acid and syringic acid inhibit the biological nitrogen fixation process. Mallik (1999) also reported that allelochemicals inhibit the biological nitrogen fixation mechanism thus uptake of nitrogen and its metabolism were also affected. Almost same types of results were also made Wardle et al, 1994; Batish et al, (2004); Batish et al, (2007).





Fig 6 Allelopathic effects of *Ageratum conyzoides* on total phosphorous content of *Pisum sativum*.

In present investigation minimum (0.390) phosphorous content was observed in control in respect of all *Ageratum conyzoides* leachate amended soil. However, 10%, 20%, 30% *Ageratum conyzoides* leachate treated plots shows almost similar kinds of values 0.447, 0.445, 0.445 respectively (Fig 6). It may be the reason, phenolics compounds changes the soil pH, electric conductivity, organic matter which affects the available nutrients e.g. nitrogen (N), phosphorus (P), potassium (K), sodium (Na), calcium (Ca), magnesium (Mg) and chloride (Cl). Such similar kinds of result were also reported by Batish *et al*, (2004) and Dogra (2009).

Nitrate reductase activity



Fig 7 Allelopathic effects of *Ageratum conyzoides* on nitrate reductase activity of *Pisum sativum*.

In the present study activity of nitrate reductase (at 65 days) was significantly decreased in all *Ageratum conyzoides* leachate irrigated plots as compared to control (Fig 7). It may be due to the presence of allelochemicals in *Ageratum conyzoides* leachate inhibited the nitrate reductase activity in pea plants. Presence of allelochemicals in soil declined carbon-skeleton, energy, electron donors which are important for nitrate reductase activity and also declined the transport of nitrate from roots to leaves consequently decreased the foliar nitrate (Singh *et al*, 2013).

Nitrite reductase activity



Fig 8 Allelopathic effects of Ageratum conyzoides on nitrite reductase activity of Pisum sativum.

Nitrite reductase is an enzyme that reduces nitrate (NO_2) to ammonia (NH_4^+) and plays a critical role in the production of proteins in most of the crop plants. In present investigation

maximum, nitrite reductase activity (0.840) was observed in control while minimum nitrite reductase activity (0.625) was found in 30% Ageratum conyzoides leachate treated plots (Fig. 8). Nitrite reductase enzyme activity decreases when a relatively high amount of Ageratum conyzoides leachate is used. Availability of nitrite (which is a product of nitrate enzyme product) enhances the nitrite reductase activity in the present tested plants. Nitrite reductase activity ultimately depends on nitrate reductase activity. As the nitrate reductase activity decreases vice versa nitrite reductase activity decreases. Nitrite (NO_2^{-}) is a highly reactive, potentially toxic ion. Plant cells immediately transport the nitrite generated by nitrate from the cytosol into chloroplasts of the leaves and plastids in roots. In these sites, the enzyme nitrite reductase reduces nitrite to ammonium $(NO_2^- \rightarrow NH_4^+)$ (Siegel *et al*, 1989). Minimum amount of nitrate and immediately conversion of nitrite to ammonium ion may be the reason of decline nitrite reeducates activity in treated plants.

CONCLUSION

The present study concludes that *Ageratum conyzoides* L. (Billygoat-weed) interferes with the biochemical pathways of the pea plants. The harmful effects at a higher concentration of *Ageratum conyzoides* leachate are due to leaching of phytotoxic substances into the soil, shift in microbial population and adverse change in soil chemistry. But at lower concentration (10%) shows a positive response in relation to many biochemical parameters. So, allelopathy is not only the detrimental factors but also has the positive aspects in relation to cope with environmental stress such as biotic (weed) stress.

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How to cite this article:

Ashok Kumar et al.2018, Allelopathic Assessment of Ageratum Conyzoides Weed on Pisum Sativum L. Int J Recent Sci Res. 9(6), pp. 27566-27570. DOI: http://dx.doi.org/10.24327/ijrsr.2018.0906.2286
