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Root-mediated allelopathic interference of bhringraj (*Eclipta alba* L.) Hassk. on peanut (*Arachis hypogaea*) and mung bean (*Vigna radiata*)

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ABSTRACT

The root mediated allelopathic interference of *Eclipta* alba infested soil on growth, physiological parameters and antioxidant enzyme activity was conducted on *Arachis hypogaea* L. and *vigna radiata* L. It was found that rhizosphere soil significantly reduced the germination percentage, seedling growth and dry biomass depending upon the species sensitivity. The germination inhibition was correlated with membrane deterioration as proved by a strong electrolyte leakage, increase in malondialdehyde (MDA) and H_2O_2 content. The physiological parameters like chlorophyll content, photosynthetic rate (P_n), intercellular CO_2 concentration (C_i), stomatal conductance (G_s), and transpiration (E) also showed significant reduction in *E. alba* infested soil and non-significant increase in leaf temperature (L_t) of two test species. The test seedlings have circumvented the allelochemicals stress, by both significant decrease and non-significant increase in the antioxidant activities in *E. alba* infested soil in contrast to control soil. Rhizosphere soil contained significantly higher amount of water-soluble phenolics as the putative allelochemicals, which were vanillic acid, benzoic acid, ferulic acid, and *p*-coumaric acid. The study concluded that rhizosphere soil exerts an allelopathic influence on peanut and mung bean by releasing water soluble phenolic acids as putative allelochemicals in soil.

1. Introduction

Allelopathy is an interference mechanism in which donor plant interferes with the growth of nearby plant (receiver) by means of allelochemicals and play an important role in natural and managed ecosystems. The chemicals involved in allelopathic interactions are present in all plant parts such as leaves, roots, stem, inflorescence and even pollen grains (Rice, 1984). These are released upon foliar leachation, residue decomposition, root exudation or even volatile emissions (Rice, 1984). Among these modes, the role of roots is significantly more as these are in direct contact with soil and contribute allelochemicals into the growth medium (Batish et al., 2007). However, recent studies have shown that roots can synthesize, accumulate, and actively secrete several compounds into the rhizosphere soil (Bertin et al., 2003; Bais et al., 2004; Prithiviraj et al., 2007). Synthesis and release of allelochemicals from root cells into the soil involve the cellular transport system, therefore depending on localized soil conditions, especially stress factors (Weston et al., 2012). Furthermore, the activities of allelochemicals in the soil are strongly linked with physical, chemical, biological, and physicochemical properties of the soil, which in turn affect their adsorption and degradation. Different phytotoxins in root exudates interfere with the basic processes of receiver plants as retarded germination, root growth, shoot growth, (PSII) efficiency, respiration, membrane transport, ATP synthesis, cell cycle, phytohormone metabolism, gene expression etc and cell mortality in susceptible plants (Weir et al., 2004; Einhellig, 1995; Inderjit and Duke, 2003). Another important effect of the allelochemicals is the activation of the cellular antioxidant system in response to uncontrolled production and accumulation of reactive oxygen species (Bogatek and Gniazdowska, 2007). Thus, allelopathy can indirectly activate other forms of stresses, most possibly an oxidative burst. Due to these interactions, rhizosphere must be considered the main site in studies of the allelopathic potential of a plant (Inderjit et al., 2010).

Eclipta alba belonging to the family Asteraceae, is a serious weed in Aligarh district of Uttar Pradesh, India. It is native of Asia and has a general distribution in areas of Gangetic plains, in pasture lands, roadsides, in marshes, rivers, lakes and on the foothills of the Himalayas (Jadhav et al., 2009; Mithun and Shashidhara, 2011) and

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is worldwide spreading in ecological terms as invasive species. It usually invades cultivated fields, particularly lowland rice and spinach in India (Kiran and Rao, 2013; Khan et al., 2008). The area, fully invested by weed along with their close-up is shown in Fig. 2. Various biotic features of the weeds are described in Table 1. Its invasive nature is due to its fast growth rate, high reproductive and vegetative potential, adaptability to changing environmental conditions, wide ecological amplitude and allelopathy. *E. alba* has long been suspected of using an allelopathic mechanism to interfere with other plant species (Pawinde et al., 2008; Yonli et al., 2010; Gulzar and Siddiqui, 2014a,b). The plant produces and releases several types of secondary metabolites including carbohydrates, flavonoids, phytosterols, tannins, coumestans, saponins, alkaloids, etc. (Dalal et al., 2010). The nature of allelopathic interference of this weed, especially through rhizosphere soil however, is lacking. Therefore, the present study was conducted to assess phytotoxicity and to identify key allelochemicals in the rhizosphere soil, which provide evidence of *E. alba* interference with crop plants via allelopathy. For the study, we selected two target species, namely *Arachis hypogaea* and *Vigna radiata*, both crops whose fields are mainly infested by *E. alba* during active period of their growth.

2. Materials and methods

2.1. Collection of soil

E. alba infested site were selected from the campus of Aligarh Muslim University, Aligarh (27°, 29° to 28°, 100° N.L and 77°, 29° to 78°, 38° E.L). The map of Aligarh district shown in Fig. 1. Aligarh district experiences tropical monsoon climate characterized by two extreme conditions of severe cold in winter and oppressive heat in summer with a rainy season in between. Samples of non-rhizosphere soil infested *E. alba* or control soil were randomly collected from different locations in the treatment or control sites, respectively. Several soil cores from each location were collected from 0 to 10 cm depth, bulked, air-dried and sieved (2 mm mesh) to remove debris and root tissues. Samples of rhizosphere soils of *E. alba* was collected by pulling plants from the soil and shaking soil off from plants (Ibekwe and Kennedy, 1999; Kong et al., 2007). Collected soil was immediately put in polyethylene bags, tagged and brought to the laboratory, shade-dried, and sieved. One thousand grams of rhizosphere soils were obtained from approximately 4500 roots of *E. alba* seedlings or 2000 flowering or 1500 mature *E. alba*, respectively. Rhizosphere soil was sampled during the months of August 2012 using root sampling (Fig. 2).

Table 1
Characteristic of *E.* alba (L.) Hassk. collected from study site.[a]

Growth features	Pre flowering stage	Post flowering stage
Rhizosphere area (cm^2)	12.43 ± 3.17	43.24 ± 1.19
Basal area (cm^2)	5.87 ± 0.53	12.43 ± 0.77
Aerial spread (m^2)	0.02 ± 0.002	0.038 ± 0.03
Dry weight of whole plant	8.85 ± 2.14	29.47 ± 4.29
Root depth(cm)	19.20 ± 1.15	28.44 ± 2.21
Dry biomass of roots	0.78 ± 1.82	4.14 ± 0.99
Average length of primary root	5.28 ± 1.13	14.17 ± 4.16
Average length of secondary root	6.23 ± 1.19	12.11 ± 3.26
Average length of tertiary root	3.11 ± 1.22	7.23 ± 1.34
Average no. of branches/plant	7.49 ± 3.46	14.10 ± 5.60
Average no. of leaves/plant	540.2 ± 17.13	1998 ± 20.21
No. of inflorescences/plant	–	42.2 ± 11.6
Average no. of seeds/plant	–	2838.76 ± 8.77

[a] The data between the pre-and post-flowering stage were significantly different applying two sample *t*-tests ± represent standard deviation, for measurement of any of the feature, 100 plant samples were used.

2.2. Soil chemical analysis

The collected soil was analyzed for various physical–chemical properties in an effort to separate an allelopathic interference from resource competition. The pH of saturated soil paste and electrical conductivity of the saturation extract were determined with the help of digital pH and conductivity meter (HI-9811, Hannah, USA). Organic matter and organic carbon determined by rapid titration method (Walkey and Black, 1934). Available nitrogen (Kjeldahl's method), available phosphorus (molybdenum blue method) and potassium (ammonium acetate extract, pH 7) were measured as per Allen (1989). The total carbonate content was determined by the volumetric method, sulfate was determined by the gravimetric method, and mineral ions were determined by the atomic absorption spectrophotometer.

2.3. Growth studies in soil

The first experiment was performed to determine whether *E. alba* infested soil caused phytotoxicity to peanut and mung bean growth. Seeds of peanut (*A. hypogaea* var. JL-24) and mung bean (*V. radiata* var. PS-16) were procured from the Indian Agricultural Research Institute, New Delhi, India. Ten seeds of each test species were sown in thermocol glasses (250 ml capacity) filled with *E. alba* infested soil and control soil. The experiment was conducted in a completely randomized block design in the net house, department of botany, Aligarh Muslim University with an average temperature of (22/14 ± 3 °C), constant supply of photosynthetically active radiation (PAR) (400–700 nm) and relative humidity maintained at (62 ± 5%.). The daily observation was done and an equal amount (30 ml) of water was added to each thermocol glass as needed to prevent seeds or seedlings from drying out. After 20 days, the seedlings of test species were clipped from each thermocol glass and their germination percentage = (germinated seed/total seed × 100) determined, shoot length and root length were measured by using a meter scale. The samples were dried in oven at 72 °C followed by dry biomass determination on a four digit digital balance of Scientech, Model ZSA 120, Colorado (USA). The total chlorophyll content from leaves of treated or control plants were extracted in Di-methyl sulphoxide (DMSO) following the method of Hiscox and Israelstan (1979).

2.4. Gas exchange parameters

From each seedling, the second fully developed leaves after 30 days after sowing was used for measurement of the net photosynthetic rate (P_{net}), leaf transpiration (E), stomatal conductance (G_s), intercellular CO_2 concentration (C_i) and leaf temperature (L_t) using a portable infrared gas analyzer (LCA-4 and PLC, Analytical Development Corporation, Hoddesdon, UK) (Yu et al., 2003).

2.5. Determination of lipid, membrane peroxidation and antioxidant enzyme activity

The leaves were harvested after 30 days after sowing and then freeze dried for biochemical analysis. The lipid peroxidation was analyzed as per the method of Zhou et al. (2004) using thiobarbituric acid (TBA) which determines malondialdehyde (MDA) as an end-product of lipid peroxidation. The Sullivan and Ross (1979) method were used for the determination of the total inorganic ions leaked out of the leaves. H_2O_2 content was determined using the method given by Velikova et al. (2000). Glutathione reductase (GR) activity was assayed by as per the method of Smith et al. (1988). Superoxide dismutase was measured by the photochemical method as described by Giannopolitis and