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DIFFERENTIAL ALLELOPATHIC ACTIVITY OF *PARTHENIUM HYSTEROPHORUS* L. AGAINST CANARY GRASS AND WILD OAT

F. Aslam, A. Khaliq*, A. Matloob, R. N. Abbas, S. Hussain and F. Rasul

Department of Agronomy, University of Agriculture, Faisalabad-Pakistan
*Corresponding author email: khaliquaf@gmail.com

ABSTRACT

Allelopathic effects of invasive weed parthenium (*Parthenium hysterophorus* L.) were studied by using whole plant, leaf and root aqueous extracts at 0, 2.5, 5.0, 7.5 and 10% (w/v) concentrations against germination and early seedling growth of wild oat (*Avena fatua* L.) and canary grass (*Phalaris minor* Retz.). Studies were carried out in Petri plates using filter paper as substratum placed in controlled conditions and soil-filled plastic pots placed in open environments. Both the experiments were laid out in a completely randomized design using four replicates. Pronounced variation was noticed for allelopathic activity of different plant parts of parthenium, extract concentrations, test species, and bioassay techniques. Parthenium extracts either inhibited or delayed the germination and suppressed seedling growth of test species. Various germination and seedling growth attributes were diminished to a much greater extent in Petri plates than in soil. Soil application of these extracts failed to reproduce results identical to those achieved in Petri plates, suggesting variable allelopathic response under different bioassay techniques. Leaf extracts were more suppressive to germination of test species than whole plant and root extracts in both Petri plates and pot studies at all concentrations. Highest chlorophyll inhibition coupled with enhanced tissue phenolic contents was recorded by aqueous extracts of parthenium in both the test species. Canary grass appeared to be more susceptible than wild oat at all concentrations of aqueous extracts. It is concluded that bioassays conducted under controlled condition using filter paper as substratum may be misleading due to over estimation of allelopathic response and variation in potential of receiver and donor species. Hence, allelopathic bioassays must consider the components of natural settings in order to generate ecologically reliable information.

Key words: Allelopathic inhibition, aqueous extracts, bioassays, germination dynamics, phenolics, seedling growth

INTRODUCTION

Littleseed canary grass and wild oat are troublesome and highly competitive grassy weeds of wheat fields (Hassan *et al.*, 2005; Chhokar *et al*., 2008). *Green Revolution* in early 1960s characterized by advent of short duration, dwarf and fertilizer responsive wheat cultivars, led to unprecedented spread of weeds that became major threat to productivity of rice-wheat cropping system. Dwarf wheat cultivars favored the survival and spread of these weeds which was further triggered with increased availability of irrigation and fertilizer inputs. Moreover, prevalent crop rotation and associated management practices were also conducive to aggravated distribution of these weeds (Chhokar *et al*., 2008). At present, management of such obnoxious weeds depends largely on synthetic herbicides. Though efficient and prompt solution of many weed problems, fate of these herbicides remains a controversial issue and has posed serious ecological and health hazards. Indiscriminate herbicide usage is driving the agro-ecosystems towards declining species diversity and, in many situations, is leading to herbicide resistance (Powles and Yu, 2010). Resistance of canary grass and wild oat to a number of herbicides with contrasting mode of action has been well documented (Heap, 2012).

Given the increasing emphasis on sustainable agriculture, concern about the adverse effects of extensive use of synthetic herbicides, research attention is now focused to workout alternative strategies for weed management. Focused work on plant derived materials as an environment friendly alternative approach for weed control in field crops has been underway for the last two decades (Kuk *et al.*, 2001). Botanical derivatives being biodegradable, less persistent and exploiting new target sites, lacking halogens, and with greater structural diversity, can be used as nature's own herbicide or serve as lead for new herbicide molecules (Duke *et al*., 2000). Utilization of allelopathic properties of native plant species offers promising opportunities for this purpose (Khaliq *et al*., 2011). The allelopathy-based weed management systems are widely practiced in many low input farming systems of China and many other Asian countries (Xuan *et al*., 2004; Kong *et al*., 2010). Parthenium is an aggressive and troublesome weed with strong allelopathic potential (Javaid *et al*., 2011) infesting both cultivated and wastelands in Pakistan (Riaz and Javaid, 2010, 2011, 2012). Allelopathic interference by parthenium is well documented (Batish *et al*., 2002; Singh *et al*., 2003; Singh *et al*., 2005; Marwat *et al*., 2008) and almost all plant parts, including pollen and trichomes, are known to possess a number of water-

soluble allelochemicals predominantly as phenolic acids and sesquiterpene lactones-Parthenin (Kohli *et al*., 2006). Marwat *et al*. (2008) proposed that parthenium can be exploited as a potent source of bioherbicide and be used for weed management in field crops. Likewise, Kathiresan (2000) found that the dry powder of parthenium was effective against water hyacinth (*Eichhornia crassipes* (Mart.) Solms). Increasing concentration of aqueous parthenium extracts significantly reduced the germination percentage, seedling length and biomass of wild oat (Batish *et al*., 2002; Marwat *et al*., 2008).

Bioassays are widely used to demonstrate allelopathy due to their usefulness as an early proof of allelopathic activity, low cost involved, easy execution and replication (Inderjit and Weston, 2000). However, bioassays conducted under controlled conditions using filter paper as substratum can be misleading due to over estimation of allelopathic response and potential of receiver and donor species. Inderjit and Weiner (2001) asserted that in order to achieve a better understanding of the subject matter, allelopathy should be conceptualized in terms of soil ecology. Without soil, any growth bioassay can be misleading with a little or no ecological relevance. Despite availability of volumetric literature on the allelopathic potential of this weed against associated crops, information regarding herbicidal potential of this weed against wild oat and canary grass remains feeble. The present work attempts to explore allelopathic potential of parthenium against weeds of economic significance. The objectives were to ascertain (1) whether aqueous extracts of different parts of parthenium affect the germination and growth of canary grass and wild oat, and to what extent under controlled conditions, (2) whether soil application of these extracts under open environments produces same results in pot experiments, and (3) to rank the different plant parts for their allelopathic potential and test species in terms of susceptibility.

MATERIALS AND METHODS

Aqueous extract preparation from different parthenium plant fractions: Field grown plants of parthenium were collected from Agronomic Research Area, University of Agriculture, Faisalabad, at flowering stage and respective fractions (whole plant, leaves and roots) were separately dried under shade. These were chopped into 2-3 cm pieces and the chopped material was oven dried at 50^{o}C for 48 h. These were separately ground and passed through a 40-mesh screen. Ground powder was soaked in distilled water at 10 g per 100 ml for 24 h at room temperature (25±2^{o}C). The filtrates of respective plant fractions were obtained after passing the mixture through a Whatman # 42 filter paper. These extracts were designated as 10% w/v stock solutions. These were used either as such or diluted with distilled water to prepare lower concentrations of 2.5, 5.0 and 7.5%.

Biochemical attributes of aqueous parthenium extracts: The pH and electrical conductivity (EC) of different concentrations of the aqueous extracts of different parts of parthenium were worked out using a digital pH and conductivity meter (HI-9811, Hannah, USA). The osmotic potential was determined using the following formula:

Osmotic potential (-MPa) = Ec (ds m^{-1}) × -0.036

Total water-soluble phenolics in aqueous parthenium extracts were quantified as per Swain and Hillis (1959) using Folin-cicalteu's reagent and expressed as ferullic acid equivalent because it has been reported to be a major allelochemical in parthenium tissues (Singh *et al*., 2005). Measurements were made by repeating the whole procedures twice, and average values are given in Table. 1.

***Lab experiment*: Influence of parthenium aqueous extracts on germination of wild oat and canary grass in Petri plates:** Aqueous extracts of whole plant, leaves and roots of parthenium at different concentrations viz., 0, 2.5, 5.0, 7.5 and 10.0% were evaluated for their effects on the germination of wild oat and canary grass. Fifteen surface sterilized seeds of test species were evenly placed between two layers of Whatman # 42 filter paper in sterilized 9-cm diameter Petri plates. Five ml extract of respective plant fraction and concentration was added to each Petri plate while same volume of distilled water was applied in control treatment. Half of the extract was used for moistening the filter paper receiving the seeds, while remaining was applied to the covering filter paper. Germination counts were recorded on daily basis according to AOSA (1990) until a constant count was achieved. Seeds were considered to be germinated when radicle and hypocotyl length was over 2 mm. Time taken to 50% germination of seedling (T_{50}) was calculated according to the modified formula of Farooq *et al.* (2005) as under:

$$T_{50} = t_i + \frac{\left(\frac{N}{2} - n_i\right)(t_j - t_i)}{n_j - n_i}$$

N is the final number of germinated seeds, and n_i and n_j are the cumulative number of seeds germinated by adjacent counts at times t_i and t_j where $n_i < N/2 < n_j$.

Mean germination time (MGT) was calculated according to Ellis and Robert (1981):

$$MGT = \frac{\sum Dn}{\sum n}$$

Where n is the number of seeds, which were germinated on day D, and D is the number of days

counted from the beginning of germination. Germination Index (GI) was calculated as described by AOSA (1983):

$$GI = \frac{\text{No.of germinatedseeds}}{\text{Daysof firstcount}} + \cdots + \frac{\text{No.of germinatedseeds}}{\text{Daysof finalcount}}$$

***Screen house experiment*: Influence of soil applied aqueous parthenium extracts on emergence, seedling growth and biochemical attributes of wild oat and canary grass**

Pot bioassays: Fifteen surface sterilized seeds of both test species were sown in separate thermocol trays measuring 18 × 14 cm filled with air dried and well mixed field soil (450 g). Soil belonged to Lyallpur soil series (Aridisol-fine-silty, mixed, hyperthermic Ustalfic, Haplargid in USDA classification, and Haplic Yermosols in FAO classification). The pH of saturated soil paste and EC of the saturation extract were 7.6 and 0.41 dS m^{-1}, respectively. Aqueous extracts (10 ml) of different parthenium fractions (whole plant, leaves and roots) at 2.5, 5.0, 7.5 and 10.0% concentration (v/v) were applied just after sowing of seeds. The pots with distilled water application were maintained as control. All pots were placed in a screen house at Agronomic Research area, University of Agriculture, Faisalabad (latitude 31.25° N, longitude 73.09° E and 184 m asl) during November, 2011 (11/13 h light/dark period). The pots were irrigated subsequently as and when required to keep the soil moist.

The emergence data were collected on daily basis and were used to compute attributes of emergence as time to start emergence, time taken to 50% emergence, mean emergence time, emergence index and final emergence percentage as described in previous section. Two weeks after the emergence, seedlings were uprooted and root and shoot length determined with a measuring tape. Seedling roots and shoots from each pot were oven dried separately at 70°C for 48 h and weighed thereafter. Total seedling biomass was calculated as the sum of biomass of root and shoot.

Biochemical analyses: Seedling fresh tissue (leaf) was used for determination of total soluble phenolics (Randhir and Shetty, 2005), that are expressed as gallic acid equivalents. Photosynthetic pigments were extracted in ice cold acetone (80%) and read out at 663 and 645 nm wavelength in a UV-spectrophotometer (UV-4000, ORI, Germany). These are expressed as mg g^{-1} fresh leaf weight (Lichtenthaler, 1987). Determinations were made as per treatment and replication.

Experimental design and statistical analyses: Both the experiments were conducted using a completely randomized design with four replications and repeated in time. Results of both repeats were similar; hence the data were pooled and averaged. Fisher's analysis of variance was run on the data (Steel *et al*., 1997) and mean values were separated using HSD Tukey's test at $p \leq 0.05$.

RESULTS AND DISCUSSION

***Lab experiment*: Influence of parthenium aqueous extracts on germination of wild oat and canary grass in Petri plates:** Test species responded significantly ($p \leq 0.05$) for all germination attributes except final germination percentage. Time to start germination (TSG) and mean germination time (MGT) was delayed by 1-2 days over control at higher extract concentrations (7.5-10%) of all extract fractions (Fig. 1). Time taken to 50% germination (T_{50}) was also significantly affected but delay was more pronounced at higher extract concentration (7.5-10%). Lower germination indices were also associated with these concentrations. Nonetheless maximum inhibition in germination index of wild oat (47-67%) and canary grass (32-65%) was recorded for leaf extract (Fig. 1). Final germination inhibition was insignificant at lower extract concentration (2.5%) but rouse to significant level ($p \leq 0.05$) beyond this concentration. Final germination of wild oat was dropped by 22-52% (whole plant extract), 43-56% (leaf)) and 50-56% (root) extract at 7.5 and 10% concentration. The corresponding inhibition of canary grass ranged from 25-55%, 46-55% and 48-55%, respectively for these extract fractions (Fig. 1).

Alterations in germination patterns may be due to the presence of suppressive allelochemicals in these extracts. Imbibition of such allelochemicals leads to either the death of embryo or lead to changes in the permeability of cell membranes, respiration, conformation of enzymes (Zeng *et al.,* 2001). Batish *et al*. (2002) reported that extracts prepared from parthenium residues were rich in allelochemicals (phenolics) and exhibited phytotoxicity to the crops. These allelochemicals are reported to be present in almost all plant parts including stems, leaves, flowers, buds, pollen grains, seeds, fruits, roots and rhizomes (Singh *et al*., 2003). However, differences are observed among species regarding their allelopathic potential and in their abilities to produce toxins in various parts (Qasem and Foy, 2001). Our data revealed that leaf extract had the strongest allelopathic effect on seed germination. Tefera (2002) also found that the germination of *Eragrostis* was suppressed more by the application of parthenium leaf extract than other plant parts. Suppression of germination in terms of relative susceptibility of test species and activity of various extract sources varied as a function of extract concentration. Difference in the activity of aqueous parthenium extracts prepared from different fractions in suppressing germination of test species can be attributed to quantitative and qualitative differences in allelochemicals present in these extracts. More inhibition by application of higher extract concentration may be due to presence of greater fraction of alleochemicals in concentrated extracts (Turk and Tawaha, 2003). Chon and Kim (2004) also reported that higher extract

concentration contains more quantity of allelochemicals, which enhance suppressive activity of an extract.

***Screen house experiment*: Influence of soil applied parthenium aqueous extracts on emergence and seedling growth attributes of wild oat and canary grass:** Soil application of aqueous parthenium extract revealed significant ($p \leq 0.05$) differences for emergence attributes among different extract fractions, their concentrations and test species. Such differences were also significant ($p \leq 0.05$) for some of the interactions between these factors. Time to start emergence (TSE) was delayed by one and a half day as compared to control in both test species. Time taken to 50% emergence (E_{50}) by wild oat was not affected by different extract fractions at any concentration; nevertheless, it was delayed by 2-3 days at higher concentrations (7.5-10%) in canary grass (Fig. 2). At these higher concentrations, mean emergence time (MET) was increased by more than 2-3 days and emergence index was dropped in canary grass (Fig. 2). Final emergence percentage (FEP) of canary grass was significantly ($p \leq 0.05$) inhibited by various extract fractions of parthenium and their concentrations, while FEP of wild oat remained almost unaffected even at higher extract concentrations of these fractions (Fig. 2). Leaf extract at highest concentration (10%) scored maximum (34%) inhibition of canary grass FEP that was far higher than that realized with other extract fractions (whole plant and roots). Interestingly, the FEP of wild oat dropped only to a slight extent (8%) even at highest concentration of whole plant extract.

Significant suppression in root and shoot length of wild oat and canary grass was forced by different extract sources at higher concentrations (Fig. 3). However, the root and shoot length of canary grass was more suppressed as compared to wild oat. Root and shoot length of canary grass was reduced by 80% and 71% by whole plant extracts, 82% and 74% by leaf extracts and 81% and 71% by root extracts, respectively. The corresponding inhibition for wild oat was 17% and 31%, 29% and 22% and 17% and 15%, respectively for these extract fractions. Ultimately dry matter accumulation in root, shoot and cumulative seedling dry weight of canary grass was more suppressed than wild oat by the application of parthenium whole plant, leaf and root extracts and inhibition being the highest at higher extract concentration (Fig. 3). Root and shoot dry weight of canary grass was inhibited by 72% and 71% by whole plant extracts, 73% and 77% by leaf extracts and 68% and 60% by root extracts of parthenium. The respective suppression for wild oat was 50% and 38%, 36% and 23% and 41% and 27%.

Leaf chlorophyll content in both test species declined under the influence of different extract sources and their increasing concentrations (Fig. 4). Species specificity was manifested for chlorophyll contents in receiver species that varied as a function of extract concentration and wild oat appeared as the more susceptible one. A reduction of 70-78% (whole plant), 77-82% (leaf) and 46-50% (root) by extracts of parthenium was recorded in wild oat, and that amounted to 59-60%, 64-70% and 44-62%, respectively for canary grass (Fig. 4). Phenolic content was significantly affected by extract concentration and varied between test species and their interactions (Fig. 4). Phenolic content in wild oat recorded an increase of 85-137% (whole plant), 87-188% (leaf) and 40-66% (root) with the increasing extract concentration over control. The corresponding increase in phenolic content of canary grass was 20-53%, 32-57% and 24-45%, respectively.

Parthenium aqueous extracts demonstrated inhibitory effect on the emergence and seedling growth of wild oat and canary grass in soil medium. Such inhibition is attributed to the presence of suppressive allelochemicals in parthenium aqueous extracts. Suppression in emergence of test species upon exposure to allelochemicals has been explained as a secondary expression of induced physiological and biochemical changes in cell ultra-structures, membrane integrity and permeability, *de novo* synthesis of certain compounds and enzymatic activity during germination (Weir *et al*., 2004; Gniazdowska and Bogatek, 2005).

Suppression in seedling growth of both species might be due to the inhibitory action of allelochemicals, either by creating physiological drought, prevention of cell division and elongation, or by reduction of the stimulatory growth (Al-Wakeel *et al*., 2007). Furthermore, these allelochemicals may cause alterations in the cell membranes, which provoke several other cross-stresses due to secondary effects like ROS damage to cell ultra-structures (Khaliq *et al.,* 2012) and lipid peroxiadation (Zeng *et al*., 2001). Canary grass remained more sensitive as compared to wild oat. Such differences in species have been observed earlier. Maharjan *et al*. (2007) reported that germination inhibition of the crucifer species (*Raphanus sativus* L., *Brassica campestris* L. and *B. oleracea* L.) was more pronounced as compared to rice (*Oryza sativa*), wheat (*Triticum aestivum*) crofton weed (*Ageratina adenophora*) and *Artemisia dubia* even at low concentration. Species also varied considerably in their sensitivity to aqueous extracts of parthenium (Belz *et al*., 2007; Rashid *et al*., 2008). Differential suppression of test species due to allelopathy is also in line with the findings of Khaliq *et al*. (2011).

Soil application of aqueous extracts of different parthenium fractions reduced the leaf chlorophyll content in both the test species (Figure 4a). Decrease in chlorophyll content may be due to exposure of growing seedlings to phytotoxic compounds released by parthenium, which are mostly phenolic in nature. Phenolic compounds are reported to decrease leaf expansion, chlorophyll content, photosynthesis and

electron transport (Leu *et al*., 2002; Colpas *et al*., 2003; Norman *et al*., 2004). Phytochemical-mediated reduction in seedling photosynthetic pigments primarily due to phenolic acids has also been reported by Khaliq *et al*. (2012).

Whole plant and leaf extracts were more suppressive than root extract to both the test species at all concentrations. It may be due to presence of more phenolic contents in leaf than whole plant and root extracts (Table 1). Phytotoxicity of foliar parts of parthenium weed is well documented (Belz *et al*., 2007; Li and Jin, 2010) presumably due to greater biomass and metabolic activities of leaves (Xuan *et al.*, 2004). Parthenin isolated and purified from the aqueous extract of parthenium leaves under laboratory conditions was significantly phytotoxic (Belz *et al*., 2007), and reduced the germination and seedling growth of wheat (Patil and Hedge, 1988). The adverse effects of foliar leachates of parthenium against goat weed (*Ageratum conyzoides*) (Belz *et al*., 2007), wild oat (*Avena fatua*), hairy beggarticks (*Bidens pilosa*) (Batish *et al*., 2002) are well documented.

It may be argued that inhibitory effects of aqueous extracts might have originated due changes in pH and osmotic potential, and hence, raise concerns about allelopathy and its ecological existence and relevance (Conway *et al*., 2002). In the present study, pH of extract fractions (whole plant, leaf and root) ranged from 6.6 to 7.8 (Table 1) and osmotic potential ranged from 0.04 to 0.20 -MPa, which are unlikely to cause inhibitory effect on plant growth (Mersie and Singh, 1987). Any growth reduction was presumably due to presence of inhibitors in the growth media. The amount of phenolics was also quantified in extracts from different fractions of parthenium and was in the order of leaves>whole plant>root extracts (Table 1). A 10% leaf extract concentration recorded about 1345 µg ml^{-1} soluble phenolics.

Parthenium extracts suppressed the germination of test species to greater extent when evaluated in Petri plates than when soil was used as a growth medium. Even mortality of seedlings was observed in Petri plates but none in soil filled pots and all extract fractions came up with almost similar level of germination inhibition. However, soil application of these extracts failed to reproduce results identical to those achieved in Petri plates. In soil, allelopathic compounds are likely to undergo various transformations such as utilization by soil microorganisms (Blum *et al*., 1999), chemical transformation (Okumura *et al*., 1999), and polymerization (Inderjit, 2001) among others acting either simultaneously or sequentially. These modify persistence, concentration, availability and biological activities of allelochemicals. Allelochemicals are also known to affect the physcio-chemical soil properties and are qualitatively and quantitatively affected by these factors (Inderjit, 2001). Such transformations are unlikely to occur in the Petri plates, and hence serve as a base for differential allelopathic response under different media.

The present study concluded that the bioassay conducted under controlled condition using filter paper as substratum can lead to over estimation of allelopathic response and potential of receiver and donor species. Nevertheless, leaf extracts of parthenium were most phytotoxic fraction while canary grass was most susceptible test species.

Table 1: Chemical properties of different concentrations of aqueous parthenium extracts

Plant part bio-assayed	Concentration (%)	pH	EC (ds m^{-1})	Osmotic potential (-MPa)	Total soluble phenol (µg ml^{-1})
Whole plant	25	6.8	1.43	0.05	74.21
	50	6.7	2.63	0.09	220.53
	75	6.6	3.69	0.13	312.11
	100	6.6	4.69	0.17	510
Leaf	25	7.8	1.55	0.06	503.68
	50	7.5	3.07	0.11	817.37
	75	7.5	4.43	0.16	1047.89
	100	7.1	5.63	0.20	1345.76
Root	25	7.7	1.05	0.04	0.53
	50	7.5	2.07	0.07	33.16
	75	7.5	3.15	0.11	39.47
	100	7.2	4.06	0.15	125.79

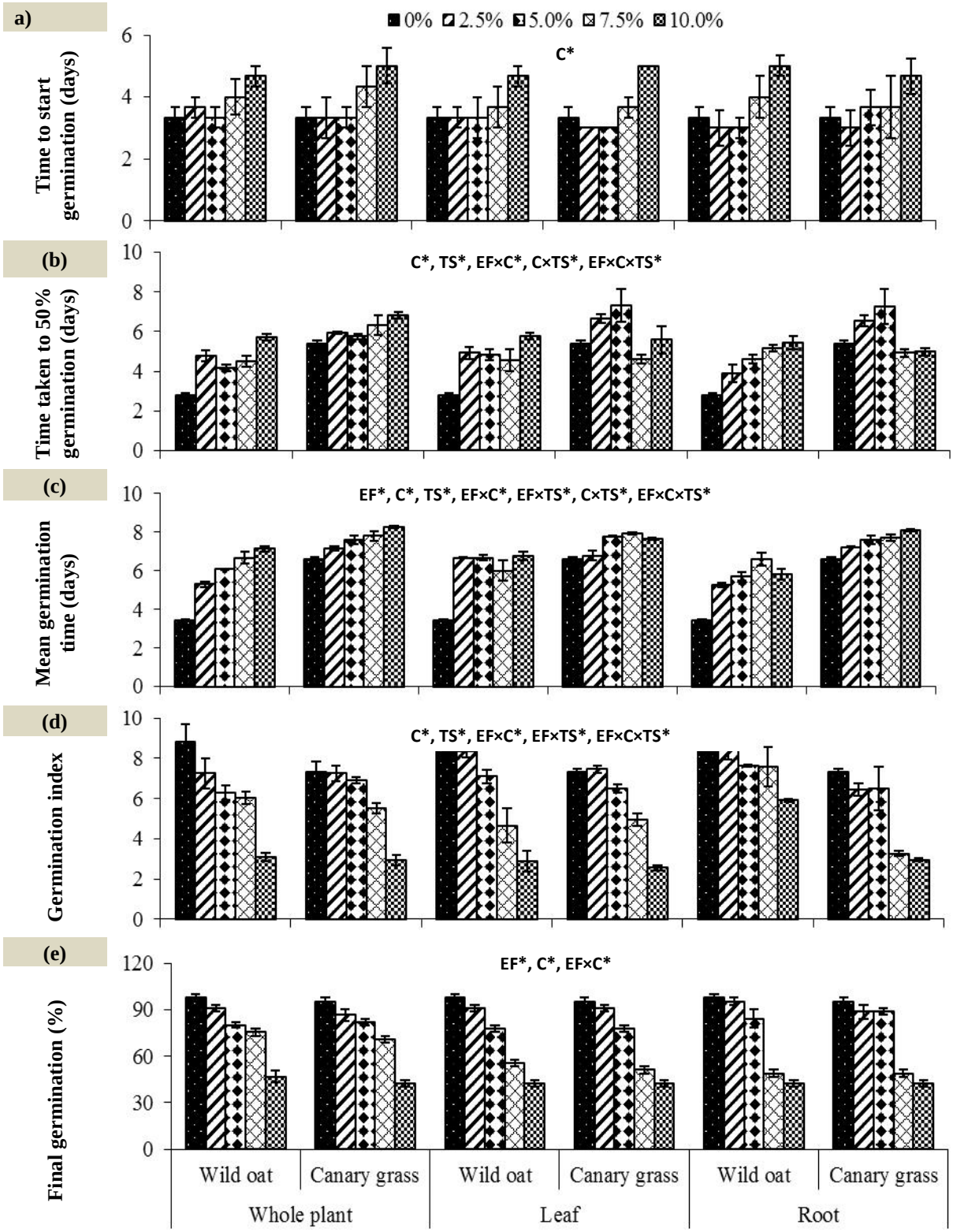

Figure 1. Effect of different concentrations of parthenium extracts on germination dynamics of wild oat and canary grass in Petri plates. Vertical bars above the mean denote the standard error of four replicates. *represent statistical significance ($P \leq 0.05$) between EF (extract fractions), TS (test species), C (extract concentrations) and their interactions, if any.

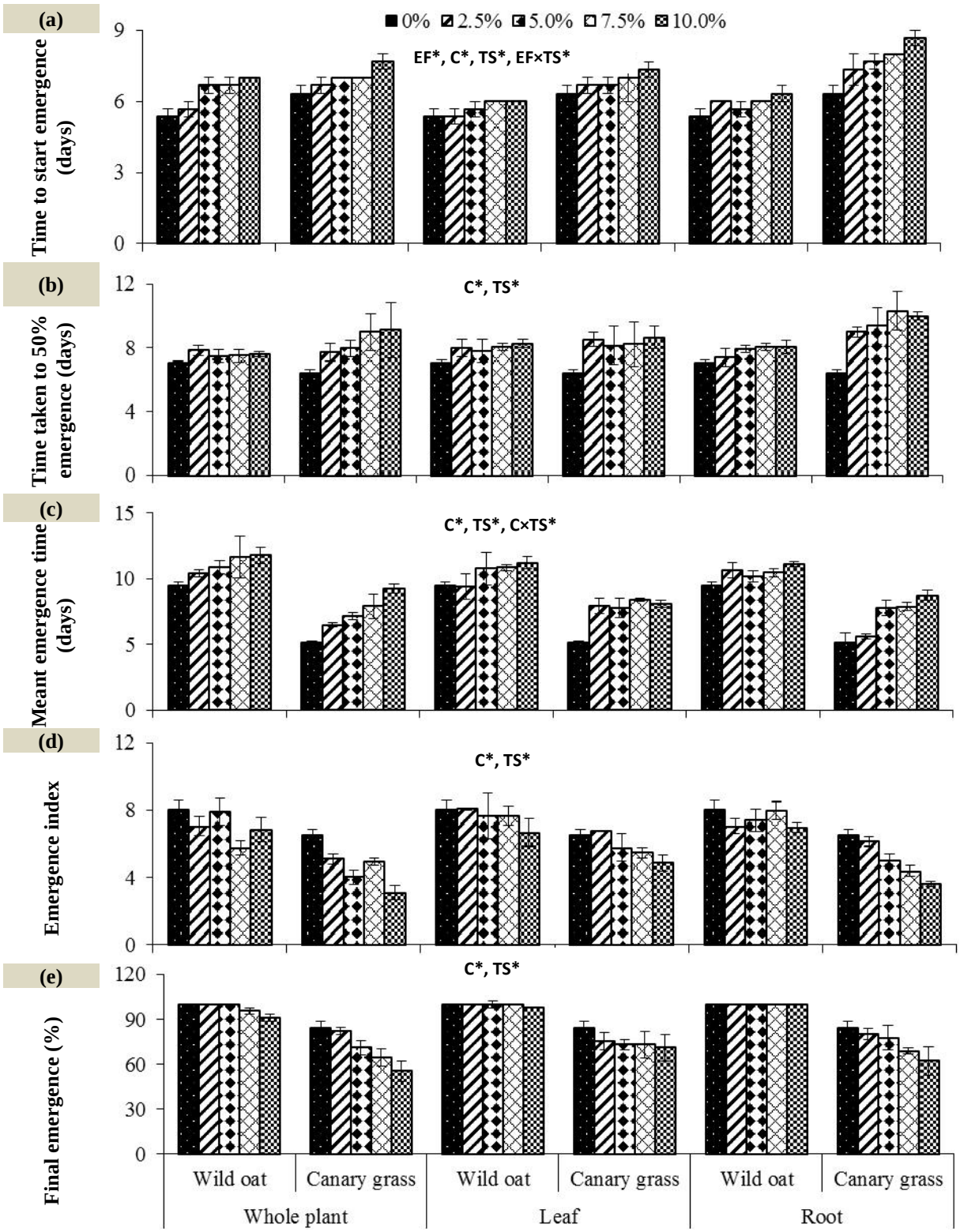

Figure 2. Effect of different concentrations of parthenium extracts on emergence attributes of wild oat and canary grass of four replicates grown in soil. Vertical bars above the mean denote the standard error of four replicates. *represent statistical significance ($P \leq 0.05$) between EF (extract fractions), TS (test species), C (extract concentrations) and their interactions, if any.

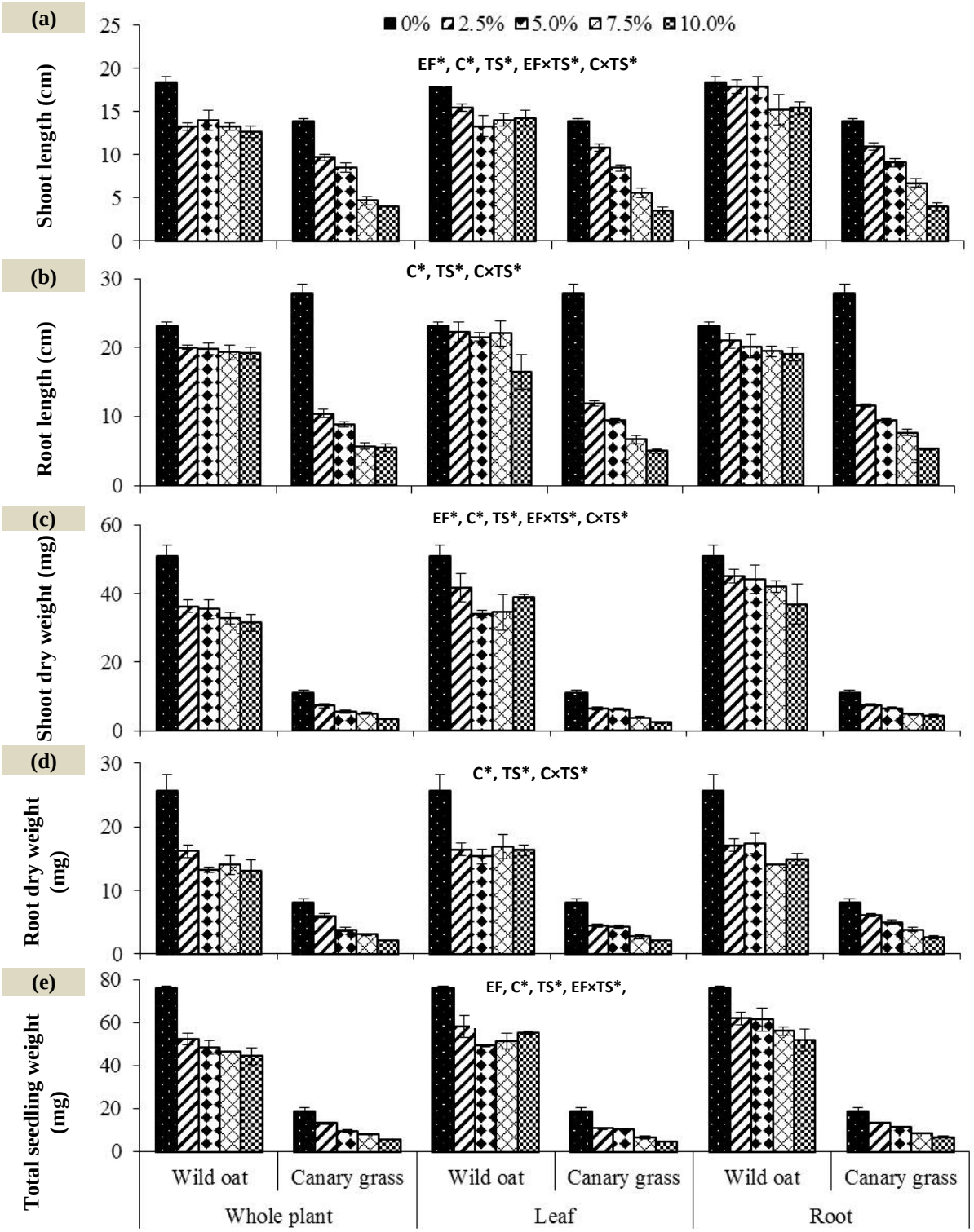

Figure 3. Effect of different concentrations of parthenium extracts on seedling growth of wild oat and canary grass of four replicates grown in soil. Vertical bars above the mean denote the standard error of four replicates. *represent statistical significance ($P \leq 0.05$) between EF (extract fractions), TS (test species), C (extract concentrations) and their interactions, if any.

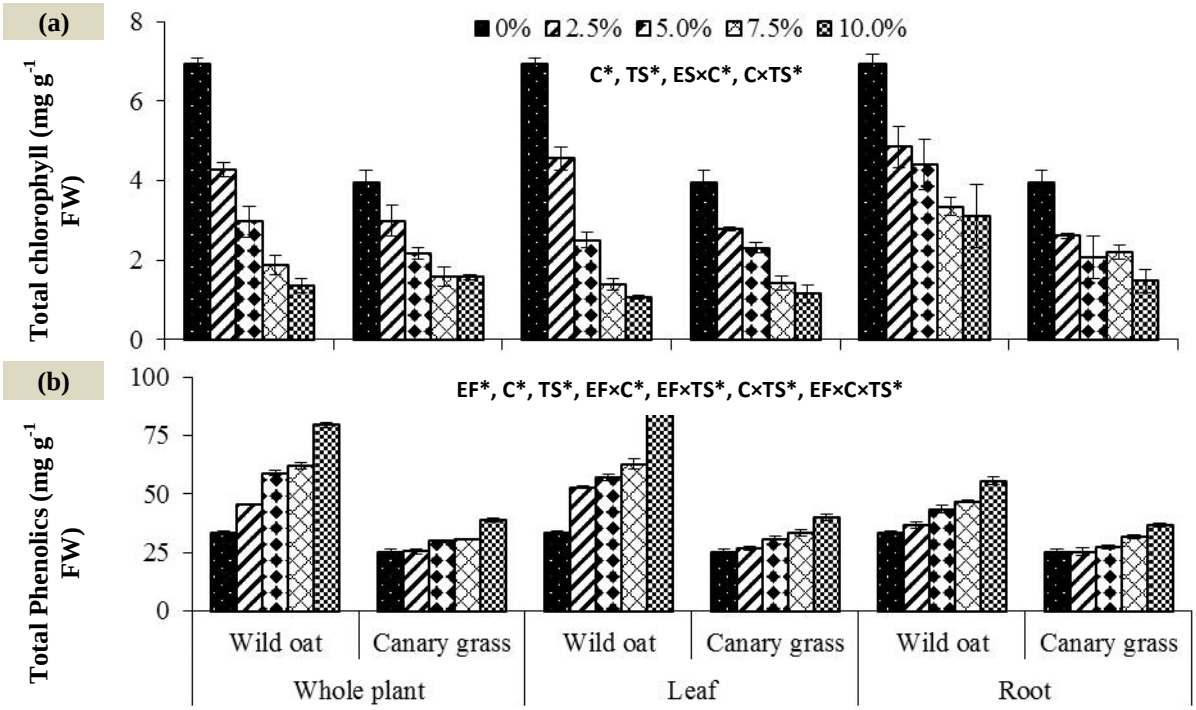

Figure 4. Effect of different concentration of parthenium extracts on chlorophyll and phenolic contents of wild oat and canary grass grown in soil. Vertical bars above the mean denote the standard error of four replicates. *represent statistical significance ($P \leq 0.05$) between EF (extract fractions), TS (test species), C (extract concentrations) and their interactions, if any.

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