

Research Article

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Allelopathic potential of *Euphorbia helioscopia* L. against wheat (*Triticum aestivum* L.), chickpea (*Cicer arietinum* L.) and lentil (*Lens culinaris* Medic.)

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Abstract: Studies investigating the allelopathic effect of root, stem, leaf, and fruit water extracts and infested soil of *Euphorbia helioscopia* L. on the seed germination and seedling growth of wheat, chickpea, and lentil were conducted in a completely randomized design with 4 replications. Water extracts of root, stem, leaf, and fruit were prepared by soaking dried plant parts of *E. helioscopia* in water (1:20 w/v) for a period of 24 h. Seedling emergence, seedling vigor index, and total dry weight of wheat, chickpea, and lentil seedlings were significantly reduced when these crops were grown in soil taken from an *E. helioscopia* infested field compared to soil collected from an area free of any vegetation. *E. helioscopia* infested soil also significantly decreased the root length of wheat and lentil, and shoot length of lentil compared to the control soil. Water extracts of various organs of *E. helioscopia* significantly decreased the seedling vigor index and growth of test crops. Leaf extract had a greater inhibitory effect than the other extracts. Water extracts from the root, stem, leaf, and fruit of *E. helioscopia* resulted in a reduction in the seed germination (chickpea and lentil only) and germination index but the leaf extract increased the mean germination time in all test crops.

Key words: Allelopathy, chickpea Euphorbia helioscopia, germination/seedling growth, lentil, wheat

Introduction

Besides competing for moisture, nutrients, and light, weeds can also affect a crop's growth by releasing allelochemicals into the growing environment (Rice 1984; Kim and Shin 1998; Kadioglue et al. 2005). All plant parts of the weed including leaf, stem, root, and fruit have allelopathic potential (Mahmood et al. 1999; Alam and Islam 2002; Tinnin and Muller 2006). However, various parts of weeds show different

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behavior in exerting their allelopathic effects on crops (Veenapani 2004). Weeds also exert allelopathic effects on crop seed germination and growth by releasing water-soluble compounds into the soil (Singh et al. 2005; Batish et al. 2007).

Euphorbia helioscopia (Sunspurge) is a common weed in Pakistan. It emerges from November to December and invades winter crops and vegetables, such as wheat, lentil, chickpea, potato, and pea. Allelopathic effects of different weeds on wheat and chickpea crops have been reported in the literature (Mishra et al. 2004; Shukla et al. 2003; Kadioglue et al. 2005; Singh et al. 2005) but no research has yet been conducted on the allelopathic effects of *E. helioscopia* specifically on wheat, chickpea, and lentil crops. The main objectives of this research were (a) to study the effects of root, stem, leaf, and fruit water extracts and soil infested with *E. helioscopia* on wheat, chickpea, and lentil germination and seedling growth, and b) to compare the allelopathic potential of various plant parts of *E. helioscopia*.

Materials and methods

Collection of *E. helioscopia* plants and *E. helioscopia* infested soil

Euphorbia helioscopia was collected from a selected area where no other vegetation was present on the campus of the University of Agriculture, Faisalabad, Pakistan. The plants were uprooted at maturity. After drying, roots, stems, leaves, and fruits were separated from the plants and chopped into small pieces with scissors. *E. helioscopia* infested soil was collected from a plot in which *E. helioscopia* had been present for the previous 3 years. Soil was collected from the upper 0–10 cm profile. Similarly, soil was also collected from a nearby area devoid of any kind of vegetation for many years, serving as a control. Collected soil was immediately put in polyethylene bags, tagged and brought to the laboratory, shade-dried, and sieved.

Preparation of water extracts of E. helioscopia

The dried pieces of the *E. helioscopia* plant (roots, stems, leaves, and fruits) were separated, weighed, and immersed in tap water at a ratio of 1:20 (w/v) at room temperature for 24 h. The water extracts of the different parts of *E. helioscopia* were obtained by filtering through 10- and 60-mesh sieves. The water extracts were individually bottled and tagged.

Wheat (*Triticum aestivum* L.), lentil (*Lens culinaris* Medic.), and chickpea (*Cicer arietinum*) crops were used to test the effect of *E. helioscopia* on their germination and early seedling growth. The study was carried out in the Laboratory, Department of Agronomy, University of Agriculture, Faisalabad, Pakistan, during 2007. A completely randomized

design (CRD) with 4 replications was used to conduct 3 experiments.

Experiment: 1 Soil residual toxicity

Petri dishes of 9 cm diameter were filled with control soil and E. helioscopia infested soil. Ten seeds of each crop were placed in each petri dish separately. Distilled water was added to each. Petri dishes were then placed in a germinator at 15 °C for 16 days. This temperature was used because in Pakistan average soil temperature is around 15 °C at the time of wheat, chickpea, and lentil germination. During this period the petri dishes were observed daily and an equal amount (72 mL) of water was added to each petri dish as needed to prevent seeds or seedlings from drying out. After 16 days the seedlings were uprooted and washed with water. The length of roots and shoots was measured in centimeters and they were weighed separately after oven drying, with the help of an electric balance.

Experiment: 2 Sand culture bioassay

In this experiment, seeds sown in sand-filled petri dishes were treated with root, stem, leaf, and fruit extract and distilled water as a control. Ten seeds of each crop were sown in sand-filled petri dishes separately. In each petri dish 72 mL of extract or distilled water was added according to the treatment to avoid the drying out of seedlings throughout the growth period. Petri dishes were placed in a germinator at 15 °C for 16 days. During this period the petri dishes were observed daily and extracts/distilled water were added when needed. After 16 days, the seedlings were uprooted and washed with water. The length of roots and shoots was measured. Roots and shoots were oven dried at 65 °C for 48 h until a constant weight was obtained.

Experiment: 3 Germination bioassay

In this experiment, seeds were treated with root, stem, leaf, and fruit extracts and distilled water as a control. Ten seeds of each crop were placed on filter paper in petri dishes. In each petri dish, 10 mL of extract or distilled water was added according to the treatment to avoid the drying out of seeds throughout the incubation period. Petri dishes were placed in a germinator at 15 °C. During this period the petri dishes were observed daily and water or extract was added to each petri dish as needed.

Procedure for recording the data

Seed germination

Germinated seeds were counted daily according to the seedling evaluation procedure in the Handbook of Association of Official Seed Analysts (AOSA 1990). The seeds were considered as germinated when the radical size was 2 mm. The number of germinated seeds was recorded every 24 h, and these seeds were discarded. Ten days after sowing, and germination, the percentage was calculated using the formula (Germinated seed/Total seed \times 100) for each replication of the treatment.

Mean germination time (MGT) was calculated according to the equation of Ellis and Roberts (1981):

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

where *n* is the number of seeds that emerged on day D, and D is the number of days counted from the beginning of germination.

The germination index (GI) was calculated as described by the Association of Official Seed Analysts (AOSA 1983) by using the following formula:

$$GI = \frac{No. of germinated seeds}{Days of first count} + -----$$

 $+ \frac{\text{No. of germinated seeds}}{\text{Days of final count}}$

Seedling growth

All the emerged seedlings from each replication were counted and the percentage of emergence was calculated by using the following formula:

Emergence % =
$$\frac{\text{Emerged seed}}{\text{Total seed}} \times 100$$

The length of roots and shoots was measured in centimeters from the point where the root and shoot joins together at the end of the root and to the top of the shoot. Roots and shoots of all the seedlings were separated, oven dried at 65 °C for 48 h until they reached a constant weight and then they were weighed separately. Seedling vigor index (SVI) was calculated according to the following formula of Abdul-baki and Anderson (1973) SVI = Germination / Emergence $\% \times$

Radical length (cm)

Statistical analysis

The data collected were analyzed statistically using Fisher's analysis of variance and treatment means were compared using the least significant difference (LSD) at a probability level of 0.05 (Steel et al. 1997).

Results

Euphorbia helioscopia infested soil significantly inhibited the seedling emergence, seedling vigor index, root/shoot dry weight, and total dry weight of wheat, chickpea, and lentil seedlings as compared to the control soil. The results indicated that *E. helioscopia* infested soil did not affect the shoot length of wheat and chickpea, or the root dry weight of chickpea and lentil seedlings (Tables 1 and 2).

Root, stem, leaf, and fruit extracts of E. helioscopia caused a reduction in seedling vigour index, root length, shoot length, root dry weight, and total dry weight of wheat, chickpea, and lentil seedlings, compared with the seedlings grown with distilled water. The leaf extract of E. helioscopia inhibited plant growth more than root, stem, and fruit extracts. Data presented in Tables 3 and 4 reveal that all the traits of chickpea seedlings were significantly reduced by different water extracts from E. helioscopia compared with the control soil. The leaf extract showed a more inhibitory effect on the emergence percentage, seedling vigor index, and seedling growth of chickpea as compared to other extracts. Water extracts of root, stem, leaf, and fruit of E. helioscopia significantly reduced the emergence, seedling vigor index, length of roots and shoots, their weights and total dry weight of lentil seedlings (Tables 3 and 4) and this reduction was 100% with the leaf extract. The root extract caused a slight reduction in the emergence and seedling growth of crops compared with the other extracts.

The germination percentage of wheat was not significantly reduced by any extract; however, mean germination time was significantly increased and the germination index of wheat seeds was significantly reduced. A significant increase was observed (except for the leaf extract) on lentil mean germination time, Table 1. Allelopathic effect of *E. helioscopia* infested soil on seedling emergence %, seedling vigor index, root and shoot length (cm) of wheat, chickpea, and lentil seedlings.

	Seedling emergence %			Seedling vigor index			Root length (cm)			Shoot length (cm).		
Treatments	Wheat	Chickpea	Lentil	Wheat	Chickpea	Lentil	Wheat	Chickpea	Lentil	Wheat	Chickpea	Lentil
Non-weedy soil (Control)	97.50 a	95.00 a	50.00 a	1358.17 a	855 a	237.50 a	13.93 a	9.00	4.75 a	12.35	11.86	13.10 a
Weedy soil	32.50 b	80.00 b	45.00 b	313.3 b	656 b	131.85 b	9.64 b	8.20	2.93b	10.78	9.39	9.04 b
LSD value	16.57	2.244	1.122	0.371	1.586	1.117	0.031	NS	1.53	NS	NS	2.626

Means having different letters in the same column differ significantly from each other at a 5% probability level

Table 2. Allelopathic effect of *E. helioscopia* infested soil on root dry weight, shoot dry weight and total dry weight (mg) of wheat, chickpea, and lentil seedlings.

Treatments	Root dry weight (mg)			Shoo	ot dry weigh	t (mg)	Total dry weight (mg)			
	Wheat	Chickpea	Lentil	Wheat	Chickpea	Lentil	Wheat	Chickpea	Lentil	
Non-weedy soil (Control)	12.75 a	36.87	5.01	12.75 a	42.00 a	6.50 a	25.50 a	78.87 a	11.51 a	
Weedy soil	7.75 b	31.56	4.16	7.00 b	35.20 b	4.90 b	15.00 b	66.76 b	9.06 b	
LSD value	2.95	NS	NS	3.369	2.02	1.28	6.993	0.624	2.02	

Means having different letters in the same column differ significantly from each other at a 5% probability level

Table 3. Allelopathic effect of water extracts of *E. helioscopia* on seedling emergence %, seedling vigor index, root and shoot length (cm) of wheat, chickpea, and lentil seedlings.

Treatments	Seedling emergence %			Seedling vigor index			Root length (cm)			Shoot length (cm).		
	Wheat	Chickpea	Lentil	Wheat	Chickpea	Lentil	Wheat	Chickpea	Lentil	Wheat	Chickpea	Lentil
Distilled Water (Control)	100.00	92.50 a	90.00 a	1248.00 a	1547.52 a	269.1 a	12.48 a	16.73 a	2.99 a	16.17 a	18.24 a	16.83 a
Root Extract	97.50	90.00 b	85.00 b	992.50 b	914.4 b	60.35 b	10.18 b	10.16 b	0.71 b	15.80 a	14.59 b	12.67 b
Stem Extract	95.00	87.50 c	77.50 c	902.50 c	337.75 с	30.22 c	9.50 bc	3.86 c	0.39 c	14.90 a	10.37 c	8.57 c
Leaf Extract	85.00	72.50 e	0.00 e	663.0 d	215.32 c	0.00 e	7.80 d	2.97 c	0.00 e	13.24 b	6.80 c	0.00 e
Fruit Extract	95.00	77.50 d	77.50 d	855.0 c	240.5 c	12.38 d	9.00 c	3.10 c	0.160 d	14.99 a	8.96 c	7.50 d
LSD	NS	0.953	1.231	89.1	315.3	0.40	0.96	2.23	0.143	1.56	3.62	0.477

Means having different letters in the same column differ significantly from each other at a 5% probability level

Table 4. Allelopathic effect of water extracts of *E. helioscopia* on root dry weight, shoot dry weight, and total dry weight (mg) of wheat, chickpea, and lentil seedlings.

	Root dry weight (mg)			Sho	ot dry weigh	t (mg)	Total dry weight (mg)			
Treatments	Wheat	Chickpea	Lentil	Wheat	Chickpea	Lentil	Wheat	Chickpea	Lentil	
Distilled Water (Control)	8.175 a	55.25 a	2.36 a	14.05	56.53 a	5.07 a	22.22 a	111.78 a	7.43 a	
Root Extract	7.325 ab	35.57 b	1.94 b	13.12	55.43 a	4.27 b	20.45 ab	91.00 b	6.21 b	
Stem Extract	7.670 a	30.88 bc	1.61 c	14.33	47.47 ab	3.72 c	22.00 a	78.35 c	5.33 c	
Leaf Extract	6.063 b	23.84 d	0.00 e	12.78	34.67 c	0.00 e	19.0 b	58.51 d	0.00 e	
Fruit Extract	7.050 ab	26.51 cd	1.24 d	13.95	37.63 bc	3.52 d	21.0 ab	64.14 cd	4.76 d	
LSD	1.332	6.440	0.067	NS	12.33	0.48	1.984	17.01	0.048	

Means having different letters in the same column differ significantly from each other at a 5% probability level

while a reduction in the germination index and germination percentage was recorded in chickpea and lentil seeds treated with different extracts (Table 5).

Dicussion

A reduction in the emergence, vigor index, and weight of test crops indicates that soil infested with E. helioscopia may contain phytotoxins leached from the residues of E. helioscopia, which affected the growth of test species compared with the controlled soil. These results are supported by the findings of Hussain et al. (1992). They reported that soil infested with Imperata cylindrica reduced the early growth, fresh and dry weight of lentil. Reduction in dry weight of wheat grown in soil infested with E. helioscopia is also supported by the findings published by Singh et al. (2003) and Batish et al. (2007), who recorded the same but with different weeds (Ageratum conyzoides and Chenopodium murale). E. helioscopia infested soil did not inhibit the root length, shoot length, or root dry weight of chickpea seedlings but significantly inhibited seedling emergence and seedling vigor index. These results are supported by the findings of Mohammadi et al. (2004), who reported that the soil from the root zone of Glycyrrhiza glabra and Polygonum aviculare did not affect seedling growth of chickpea compared to the control soil. A nonsignificant decrease in the length of root, shoot and dry weight of chickpea seedlings grown in soil previously infested with E. helioscopia is in contradiction with the results of Singh et al. (2003), who observed a significant reduction in wheat seedling growth in soil infested with A. conyzoides compared with the control soil. It could be due to

differential responses of different crops to different allelochemicals released by different weeds in soil.

The significant inhibition of seedling emergence (except wheat), seedling vigor index, root, shoot length, and dry weight of wheat, chickpea, and lentil by extracts of E. helioscopia may reflect a presence of water soluble inhibitors. Similar results were observed by Rehman et al. (1991) when wheat was grown with water extracts of roots, shoots, and leaves of Sisymbrium irio. These results are also similar to the findings of Channappagoudar et al. (2005). They reported that extracts of Cyperus rotundus and Commelina benghalensis had an inhibitory effect on the germination, seedling length, and seedling vigor index of wheat, sorghum, green gram, and soybean. Agarwal et al. (2002), Stavrianakou et al. (2004), and Dongre and Yadav (2005) also reported inhibition in the length of plumule and radicle, a reduction in their dry weights and total seedling weight in wheat, pea and lentil with water extracts of various weeds. The presence of inhibitory chemicals in higher concentrations in the leaves might be the reason for differential behavior of the extracts and maximum reduction in seedling growth (Shukla et al. 2003).

More delay in seed germination and lower germination index with leaf extracts of *E. helioscopia* compared with other plant part extracts could be attributed to a more inhibitory effect of allelochemicals present in leaves. Leaf extract completely inhibited the germination of lentil. Complete inhibition of germination/emergence in lentil and less inhibition in wheat and chickpea with leaf extract compared with extract from other parts of *E. helioscopia* indicate differential sensitivity behavior

Table 5. Allelopathic effect of water extract of *E. helioscopia* on germination %, mean germination time (MGT), time taken for 50%
germination, and germination index for wheat, chickpea, and lentil seeds.

Treatments	Germination %			Mean ger	mination tir	ne (days)	Germination index			
	Wheat	Chickpea	Lentil	Wheat	Chickpea	Lentil	Wheat	Chickpea	Lentil	
Distilled Water (Control)	100.00	97.50 a	92.50 a	2.00 c	2.95 c	3.58 a	5.00 a	3.99 a	3.82 a	
Root Extract	100.00	97.50 a	80.00 a	2.83 b	3.56 b	3.82 a	4.53 b	3.38 b	3.21 at	
Stem Extract	97.50	92.50 a	72.50 a	2.84 b	4.87 a	4.20 a	4.37 bc	2.72 c	2.79 b	
Leaf Extract	95.00	37.50 b	0.00 b	4.81 a	4.75 a	0.00 b	2.26 d	0.64 d	0.00 c	
Fruit Extract	95.00	97.50 a	77.50 a	2.63 b	4.56 a	4.34 a	4.12 c	2.45 c	2.45 b	
LSD value	NS	9.331	20.68	0.418	0.490	0.834	0.393	0.294	0.799	

Means having different letters in the same column differ significantly from each other at a 5% probability level.

or response of different crops to the same extract. The non-toxic effect of *E. helioscopia* extracts on the germination of wheat seeds is in line with the findings of Mishra et al. (2004). Inhibition of germination (except wheat) and germination index and increase in germination time of all test crops are supported by Stavrianakou et al. (2004), Dongre and Yadav (2005), and Kadioglue et al. (2005). They reported inhibition in the germination rate and final germination of lentil, chickpea, and wheat with different plant part extracts of different broad and narrow leaf weeds. Chickpea seeds treated with the *E. helioscopia* leaf extract had the lowest germination, which is supported by the

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findings of Oudhia (2001) and Veenapani (2004). They recorded the lowest germination of wheat and rice with the leaf extracts of 9 different weeds. Furthermore, 100% inhibition of the seed germination of lentil with the leaf extract of *E. helioscopia* could be due to a more toxic effect of allelochemicals on the lentil seed than on other crops (Deka et al. 2004). On the basis of these results we can propose that *E. heliscopia* phytotoxic has biomolecules in its organs in various concentrations; therefore it is necessary to keep this weed under check at the emergence stage so that its allelopathic based crop growth suppression may be avoided.

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