

ALLELOPATHIC EFFECT OF *Euphorbia helioscopia* ON *Avena fatua*, *Rumex dentatus*, *Helianthus annuus*, *Zea mays* AND *Triticum aestivum*

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ABSTRACT

Allelopathic compounds can inhibit or stimulate growth of nearby plants of the same or other species. In this connection, experiments were conducted to inspect the allelopathic effect of Euphorbia helioscopia L. aqueous extract and leaf dry powder on seeds and pre-germinated seeds of Avena fatua L., Rumex dentatus L., Triticum aestivum L., Helianthus annuus L. and Zea mays L. The aqueous extract of Euphorbia helioscopia significantly suppressed the germination and subsequent growth of R. dentatus and A. fatua. Radicle and plumule growth of H. annuus and Z. mays decreased when E. helioscopia aqueous extract and leaf dry powder were applied to seeds, while remained unaffected when applied to the pre-germinated seeds. The aqueous extract showed non-significant effect on radicle and plumule growth of wheat seedlings. In conclusion, aqueous extract of E. helioscopia has the potential to be used as a source of weed management.

Key words: Allelopathy, *A. fatua*, *E. helioscopia*, *H. annuus*, *R. dentatus*, suppression, *T. aestivum*, *Z. mays*.

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INTRODUCTION

Allelopathy is an environment friendly and economic technique in controlling weeds. Allelopathic plants are source of new herbicidal compounds, the necessity of which is due to the resistance development in weeds to synthetic compounds (Albuquerque *et al.*, 2011). Allelopathic crops restrain growth of weeds while seed germination is inhibited by phytotoxins released from their residues. Allelochemicals with herbicidal activity from different plant species have been identified (Bhadoria, 2011). Most of the allelochemicals are toxic thus can be used as herbicides (Troc *et al.*, 2011). It has been reported that weeds in rice fields cause yield loss of 10-40% that occasionally reaches upto 100%, 20% yield loss in sugarcane and 40% yield loss in soybean. Therefore, weeds are a big problem for farmers all over the world. There is an crucial need to discover new weed control technologies which will overcome limitations faced by synthetic chemicals and are eco-friendly. The discovery of bioherbicides has capacity to fulfill these limitations (Briggs, 1992; Arafat *et al.*, 2011; 2012). Bioherbicides are biocontrol agents applied to weeds in order to control them. These are the herbicides that contain biological components like fungi, bacteria, viruses and plants that can target very specific weeds (Khalid *et al.*, 2010). Almost all weeds have at least one naturally-occurring enemy that will reduce its population (Schroeder, 1983; Hasan and Ayers, 1990; Charudattan, 1991; Watson, 1991; Julien and Chan, 1992). The opportunity for bioherbicides is expanded by increasing herbicide resistant weeds (Riaz *et al.*, 2012; Sadia *et al.*, 2012). Due to the manifold mechanisms involved in pathogenesis, there is a low chance of bioherbicide resistance development (Auld and Morin, 1995).

Euphorbia helioscopia L. is common weed in Pakistan. It appears in November-December and has a strong invasive potential in winter crops, such as potato, wheat, chickpea, lentil and pea. Allelopathic inhibitory effect of weeds on chickpea and wheat has been reported (Mishra *et al.*, 2006; Singh *et al.*, 2005; Kadioglu *et al.*, 2005) but no research has yet been carried out on allelopathic effects of *Euphorbia helioscopia* specifically on maize, wheat and sunflower. The focus of present research was on the effect of *Euphorbia helioscopia* leaf dry powder and aqueous extract on seeds and pre-germinated seeds of *Avena fatua* L., *Rumex dentatus* L., *Triticum aestivum* L., *Helianthus annuus* L. and *Zea mays* L. on filter paper and in soil under laboratory conditions. This work encompasses the possible use of allelochemicals to regulate growth and control on *Avena fatua* L., *Rumex dentatus* L. (weeds) for sustainable agriculture.

MATERIALS AND METHODS

Collection and Mechanical Processing of Plant Material

Allelopathic activity of *Euphorbia helioscopia* was examined against the seed growth of five test species. Two screening methods, i.e., Aqueous Extract Method and Plant Sandwich Method were used to screen the allelopathic activity of *Euphorbia helioscopia* by using seeds and pre-germinated seeds (radicle protruded by at least 1 mm) of test species, on filter paper and soil. Fresh leaves of *Euphorbia helioscopia* were collected from different locations of district Rawalpindi, Punjab, Pakistan and thoroughly washed under running tap water. The dried plant material was crushed, separately ground to fine powder and kept separately at 4°C in air tight plastic zip lock bags for further use (Hegab *et al.*, 2008; Jafariehyazdi and Javidfar, 2011; Anwar *et al.*, 2012 a; b;c).

Procurement of Seeds of Test Species

Seeds of *Avena fatua*, *Rumex dentatus*, *Helianthus annuus* (K.S.E 7777), *Zea mays* (Islamabad Gold 2010) and *Triticum aestivum* (Wafaq 2001) were procured from Department of Crop Science, National Agriculture and Research Centre, Islamabad.

Sterilization of Seeds of Test Species

Seeds of test species were sterilized with 1% sodium hypochlorite for 2 min, washed with distilled water and used for further bioassay studies.

Preparation of Aqueous Extract

A stock solution was prepared by soaking 10 gm of dried powder of each of plant in 200 ml water in a flask. It was agitated along an orbital shaker (150 RPM) for 24 hours at room temperature. The extract was strained through a muslin cloth and filtered through Whatman filter paper No. 1. The extract was stored in pre-sterilized flasks at 4°C. The extracts were used within 3-4 days to avoid prospective chemical alterations and contamination (Anwar *et al.*, 2012 d; e).

Bioassay Parameters

Three parameters included in allelopathic screening were Germination (%), Radicle length (cm) and Plumule length (cm).

Aqueous Extract Bioassay

This method was used to check the growth inhibition effects of leaf extracts of *Euphorbia helioscopia*. Five replicates were used in completely randomized designed (CRD). Ten surface sterilized seeds of each test species were placed to each Petri plate. The glass petri dishes (9cm) were tape sealed, covered with aluminum foil and incubated in the growth chamber at room temperature (25°C) for fifteen days (Anwar *et al.*, 2012 f). The germination was recorded daily. The results were analyzed by counting the number of

germinated seeds. Later on, this period, the dishes were observed and studied for parameters. The three parameters of the test species, i.e. germination percentage, radicle length (cm) and plumule length (cm) were recorded after 15 days with reference to the control (Nasir et al., 2005; Anwar et al., 2010; 2011 a). Screening of both seeds and pre-germinated seeds were carried out on filter paper and soil, separately.

Screening on Filter Paper

A filter paper was placed in a glass Petri dish (9cm). Five ml leaf extract of leaf extract of *Euphorbia helioscopia* was poured with the help of the pipette into petri dishes underlain with filter paper. Five ml distilled water was used in petri dishes as a control.

Screening on Soil

A measured quantity of 25g of soil was placed in a glass Petri dish (9cm). Fifteen ml leaf extract of *Euphorbia helioscopia* was poured with the help of pipette into petri dishes underlain with soil (Anwar et al., 2013). Fifteen ml distilled water was used in petri dishes as a control.

Plant Leaf Powder Bioassay

This method was used to inspect the growth inhibition effects of *Euphorbia helioscopia*. Five replicates were used in completely randomized designed (CRD). Ten surface sterilized seeds of test species were placed in each petri plate. Glass petri dishes were squash tape sealed, covered with aluminum foil and incubated in the growth chamber at room temperature for fifteen days. The germination was recorded on daily basis. The results were analyzed by counting the number of germinated seeds (in case of seed). After this period, the dishes were observed and studied for parameters. Three parameters of the test species i.e. germination percentage, plumule length (cm) and radicle length (cm) were recorded after 15 days with reference to control (Nasir et al., 2005). Screening of both seeds and pre-germinated seeds carried out on filter paper and soil, separately.

Screening on Filter Paper

A filter paper was placed in a glass Petri plate (9cm). A measured quantity 0.25g of *Euphorbia helioscopia* was added to filter paper along with 5ml distilled water was poured with the help of pipette into petri dishes per petri dish. 5ml distilled water was used in petri dishes as control without adding leaf powder on filter paper.

Screening on Soil

A filter paper was placed in a glass petri plate (9cm). A measured quantity 0.25g of *Euphorbia helioscopia* was added to 25g soil with 15ml distilled water by pipette into each petri plate. 15ml distilled water was used in petri dishes as control without adding leaf powder on soil (Anwar and Khalid, 2016).

Statistical Analysis

STATISTIX 9 software was used for analysis of results. Means were separated by using Fisher's protected LSD test (Steel and Torrie, 1997).

RESULTS AND DISCUSSION

Germination percentage

Euphorbia helioscopia significantly reduced the germination of all tested plant species in soil as well as on filter paper (Table-1). Reduction in the emergence and length of radicle and plumule, weight of test crops and vigor index indicates that soil treated with *Euphorbia helioscopia* extract contain leached phytotoxins from the leaf residues, which reduced the growth of the test species (Anwar and Khalid, 2016 c, d). Findings of Hussain *et al.* (1992) are correlated with present results.

Radicle Length

Effect of powder extract on radicle length of test species

Powdered extract of *Euphorbia* showed inhibitory effect on radicle length of test plant species. Significant reduction of 16.7%, 21.35% and 24.7% was recorded in radicle length of wheat, maize and sunflower respectively as compared to their respective control (Anwar *et al.*, 2016). More drastic decrease of 62% and 78% were observed in radicle length of *Avena fatua* and *Rumex dentatus*, respectively. Similar effect of powdered extract was observed on radicle length of plants in soil as compared to their respective controls (Table-2). There was a detectable impact on the growth by the weed (Shaukat and Siddiqui, 2001) which explained that inhibitory compounds in soil cause marked suppression or inhibit growth of plant. Present trial also showed the same effect.

Effect of aqueous extract on radicle length of test species

Considerable reduction in radicle length was observed in tested plant species. Results of Petri plate experiment has shown 9.7%, 19.4% and 23.67% decrease in radicle length of wheat, maize and sunflower respectively as compared to their respective control. Aqueous extract of *Euphorbia* caused drastic reduction of 78% and 56.7% in radicle length of *Avena fatua* and *Rumex dentatus*, respectively (Table-3). Similar results of aqueous extract were observed on radicle length of plants in soil as compared to respective control. These results are also correlated to the findings of Channappagoudar *et al.* (2005). Several research reports have documented that many plant secondary metabolites release in environment as exudation or by plant materials decomposition under certain circumstances (El-Rokiek *et al.*, 2010; Rice *et al.*, 1984). These chemicals like alkaloids, terpenoids and phenolics are suppressors of

seed germination and seedling growth (Muller, 1966; Gniazowska and Bogatek, 2005; Qasem and Hill, 1989).

Plumule Length

Effect of powder extract on plumule length of tested plant species

Significant effect of powdered extract on plumule length was recorded in tested plant species. A remarkable decrease of 28.09% and 35.5% on plumule length of maize and sunflower were observed in petri plate experiment respectively. Same trend was followed in soil. No significant effect of aqueous extract was observed on wheat plant in petriplate as well as in soil. Plumule length of *Avenafatua* and *Rumex dentatus* was significantly affected (69% and 61% respectively) (Table-4). The same results were observed in soil experiment. The significant suppression of seedling (except *Triticum aestivum*), radicle, plumule length, seedling vigor index, and dry weight of chickpea, wheat and lentil by leaf extracts of *E. helioscopia* reflect water soluble inhibitors in these tissues (Tanveer et al., 2010).

Effect of aqueous extract on plumule length of tested plant species

A remarkable decrease of 10.5% and 32.7% was recorded in plumule length of wheat and maize compared to their respective control plant in petri plate experiment. While plumule length of *Avena fatua* is more drastically affected resulted 63.6% decrease followed by sunflower 43.17% and 42.2% of *Rumaxdentatus* as compared to their respective control plant (Table-5). Similar effect of aqueous extract was observed in soil of tested plant species. Different toxic levels of *Euphorbia helioscopia* can be credited to variable phytotoxic compounds in various plant parts leached under different conditions. Modern studies exploring the allelopathic effect of aqueous extracts of weeds include: *Euphorbia helioscopia* (Batish et al., 2002), *Parthenium hysterophorus* (Singh et al., 2003), *Brassica nigra* (Tawaha and Turk, 2003) and *Raphanus raphanistrum* (Norsworthy, 2003). All these reports documented the release of allelopathic chemicals in their aqueous extracts. Leaf, stem and root water extracts of *Euphorbia helioscopia* significantly suppressed the germination, dry weight and vigor of chickpea, lentil and wheat (Tanveer et al., 2010).

CONCLUSION

The experiments indicate that aqueous extract of *Euphorbia helioscopia* at different concentration levels suppress the germination percentage, radicle length and plumule length of tested plant species. Its efficiency to suppress seed germination and seedling growth suggests that leaves of *Euphorbia helioscopia* have the potential to act as a source of inhibitory allelochemicals. There is a need to inform

farmers about weed suppressive potential of *Euphorbia helioscopia*. Further inquiries are recommended to investigate the possible physiological mechanism of allelopathic effect of these allelochemicals on plants.

Table-1: Germination percentage of seeds of test species in aqueous extract and leaf drypowder of *Euphorbia helioscopia* on filter papers and in soil

Treatments		Test species				
		<i>Triticum aestivum</i>	<i>Helianthus annuus</i>	<i>Zea mays</i>	<i>Avena fatua</i>	<i>Rumex dentatus</i>
Aqueous extract	Filter Paper	92a	77b	89a	42b	57b
	Control	99a	100a	92a	86a	80a
	¹ LSD	17.902	17.604	19.778	14.197	14.541
	² F-value	12.13*	14.67*	13.52*	42*	43.98*
	Soil	90a	76b	88a	50b	35b
	Control	94a	96a	93a	90a	89a
	¹ LSD	13.554	14.744	14.08	17.510	16.580
² F-value	13.63*	21.04*	38.81*	22.36*	51.38*	
Plant powder	Filter Paper	93a	77b	78a	40b	66b
	Control	98a	97a	82a	95a	86a
	¹ LSD	14.722	16.974	16.974	17.66	17.529
	² F-value	27.64*	13.78*	13.29*	26.08*	21.73*
	Soil	91a	76b	39b	46b	70b
	Control	96a	99a	84a	99a	89a
	¹ LSD	18.608	18.60	17.435	18.890	17.67
² F-value	10.89**	33.19*	21.97*	17.19*	32.71*	

¹Means followed within one column by different alphabets differ significantly at ($P < 5\%$); ²*=Significant at ($P < 1\%$)

Table-2. Radicle length (cm) of seeds and pre-germinated seeds of test species in leaf drypowder of *Euphorbia helioscopia* in filter papers and soil

Treatments		Test species				
		<i>Triticum aestivum</i>	<i>Zea mays</i>	<i>Helianthus annuus</i>	<i>Rumex dentatus</i>	<i>Avena fatua</i>
Seeds	Filter Paper	11.3a	8.10b	2.45b	3.05b	7.13b
	Control	13.15a	10.3a	11.2a	8.03a	9.48a
	¹ LSD	3.0879	0.6573	1.0971	1.0027	1.0360
	² F-value	18.78*	309.12*	126.95*	92.48*	128.48*
	Soil	10.6a	6.14b	3.12b	2.06b	7.02b
	Control	11.4a	8.45a	10.45a	7.17a	8.39a
	¹ LSD	1.8106	1.0912	1.1918	0.8063	1.0634
	² F-value	52.46*	83.01*	98.97*	126.70*	74.89*
Pre-germinated seeds	Filter Paper	15.3a	6.14a	4.07b	2.13b	9.88a
	Control	16.2a	7.27a	10.2a	5.33a	10.1a
	¹ LSD	3.4976	1.5567	0.4135	0.7263	2.0401
	² F-value	18.78*	22.96*	740.81*	64.28*	30.76*
	Soil	11.8a	5.45a	1.17b	1.00b	7.69a
	Control	13.4a	6.42a	9.11a	4.11a	8.49a
	¹ LSD	2.5542	1.2569	0.8095	0.9043	1.9073
	² F-value	23.58*	39.32*	206.09*	20.88*	27.90*

¹Means followed within one column by different alphabets differ significantly at ($P < 5\%$)

²*=Significant at ($P < 1\%$)

Table-3. Radicle length (cm) of seeds and pre-germinated seeds of test species in aqueous extract of *Euphorbia helioscopia* in filter papers and soil

Treatments		Test species				
		<i>Triticum aestivum</i>	<i>Zea mays</i>	<i>Helianthus annuus</i>	<i>Rumex dentatus</i>	<i>Avena fatua</i>
Seeds	FilterPaper	12.9a	7.26b	6.48b	3.06b	2.03b
	Control	14.3a	9.01a	8.49a	7.07a	9.34a
	¹ LSD	3.2679	0.6136	1.4388	1.203	0.5692
	² F-value	22.22*	279.70*	56.00*	56.71*	485.76*
	Soil	9.45a	9.28b	7.31b	3.19b	3.22b
	Control	9.49a	11.1a	9.30a	8.46a	8.05a
	¹ LSD	1.540	1.1125	1.0662	1.320	0.999
	² F-value	45.47*	111.84*	95.32*	56.72*	66.33*
Pre-germinated seeds	FilterPaper	12.1a	7.41a	9.03a	2.44a	5.17b
	Control	13.4a	8.07a	9.45a	6.07a	12.3a
	¹ LSD	2.4770	1.4150	1.1352	0.9713	0.9870
	² F-value	18.01*	41.84*	110.25*	49.22*	209.06*
	Soil	11.3a	7.00a	7.49a	1.10b	4.24b
	Control	13.4a	8.14a	8.00a	5.21a	10.05a
	¹ LSD	3.1490	1.7841	1.2929	0.8953	0.8763
	² F-value	22.59*	34.17*	66.06*	50.17*	129.81*

¹Means followed within one column by different alphabets differ significantly at ($P < 5\%$)

²*=Significant at ($P < 1\%$)

Table-4. Plumule length (cm) of seeds and pre-germinated seeds of test species in leaf drypowder of *Euphorbia helioscopia* in filter papers and soil

Treatments		Test species				
		<i>Triticum aestivum</i>	<i>Helianthus annuus</i>	<i>Zea mays</i>	<i>Avena fatua</i>	<i>Rumex dentatus</i>
Seeds	Filter Paper	7.45a	4.12b	6.08b	3.12b	3.07b
	Control	8.16a	6.39a	8.45a	10.15a	8.06a
	¹ LSD	1.3210	0.9468	1.0678	0.6647	0.6294
	² F-value	45.63*	37.04*	97.24*	238.96*	197.36*
	Soil	7.10a	6.05b	5.12b	3.07b	2.02b
	Control	8.49a	8.37a	7.00a	7.40a	7.45a
	¹ LSD	2.3502	1.0434	0.7620	0.8978	1.0019
	² F-value	9.64**	73.9*	101.44*	71.85*	87.41*
Pre-germinated seeds	Filter Paper	9.65a	9.27a	11.2a	4.12b	7.08b
	Control	11.8a	10.05a	11.45a	9.08a	9.20a
	¹ LSD	2.5454	1.5861	1.7866	0.3036	0.8317
	² F-value	14.38*	47.89*	80.15*	825.69*	152.73*
	Soil	9.27a	8.24a	12.2a	3.33a	5.12b
	Control	10.1a	9.08a	13.2a	8.48a	7.44a
	¹ LSD	2.3413	1.2301	1.2891	1.2227	0.9318
	² F-value	20.36*	56.75*	88.02*	54.40*	69.05*

¹Means followed within one column by different alphabets differ significantly at ($P < 5\%$)
²*=Significant at ($P < 1\%$) **=Significant at ($P < 5\%$)

Table-5. Plumule length (cm) of seeds and pre-germinated seeds of test species in aqueous extract of *Euphorbia helioscopia* in filter papers and soil

Treatments		Test species				
		<i>Triticum aestivum</i>	<i>Helianthus annuus</i>	<i>Zea mays</i>	<i>Avena fatua</i>	<i>Rumex dentatus</i>
Direct Seeding	Filter Paper	8.48a	3.08b	5.02b	3.09b	4.60b
	Control	9.48a	5.42a	7.46a	8.49a	7.96a
	¹ LSD	2.7846	0.8718	0.9640	1.2971	0.0182
	² F-value	6.44**	55.81*	57.31*	46.74*	14.11*
	Soil	10.9a	4.05b	6.35b	4.32b	2.09b
	Control	12.15a	6.40a	8.07a	7.44a	9.07a
	¹ LSD	2.2332	1.1428	0.9679	1.4091	0.7297
	² F-value	28.95*	29.95*	83.03*	26.83*	221.41*
Pre-germinated seeds	Filter Paper	8.19a	8.32a	10.2a	3.11b	6.32b
	Control	9.35a	9.45a	11.4a	9.45a	8.12a
	¹ LSD	1.8694	1.2510	2.1870	0.9834	0.6111
	² F-value	17.04*	57.71*	37.65*	124.10*	216.40*
	Soil	7.48a	7.00a	9.30a	4.33b	5.48b
	Control	8.45a	8.06a	10.05a	8.07a	7.10a
	¹ LSD	2.1252	1.1964	0.8164	1.0523	0.9823
	² F-value	70.87*	66.81*	280.93*	48.12*	66.95*

¹Means within one column followed by different alphabets differ significantly at ($P < 5\%$)
²*=Significant at ($P < 1\%$) **=Significant at ($P < 5\%$)

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