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HERBICIDAL POTENTIAL OF SELECTED SPECIES TO OVERCOME WEED INFESTATION IN *Triticum aestivum, Zea mays* AND *Helianthus annuus*

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ABSTRACT

Filter paper and soil bioassays were performed to study the allelopathic effect of aqueous extracts of Carica papaya L., Parthenium hysterophorus L., Euphorbia helioscopia L. and Rumex dentatus L. on intact and pre-germinated seeds of Rumex dentatus, Avena fatua, Helianthus annuus, Zea mays and Triticum aestivum. Experiments were designed in CRD with five replications. The parameters studied included germination percentage, radicle length (cm) and plumule length (cm) of test species. C. papaya and P. hysterophorus leaf aqueous extracts decreased the emergence of R. dentatus and A. fatua in soil bioassay while on filter paper, all extracts inhibited the germination of R. dentatus and A. fatua. In direct seeding, filter paper bioassays, radicle growth of R. dentatus and A. fatua were decreased by all the extracts. In some experiments, plumule of A. fatua was significantly repressed by E. helioscopia extract. In direct seeding soil bioassays, E. Helioscopia reduced the radicle length of R. dentatus and A. fatua. R. dentatus radicle growth was also significantly inhibited by P. hysterophorus. All aqueous extracts in soil inhibited the plumule length of R. dentatus and A. fatua. In seedling filter paper and soil bioassays, P. hysterophorus significantly inhibited radicle as well as the plumule of all test species. P. hysterophorus and E. helioscopia aqueous extracts were evaluated as good weed suppressants. Weed suppressive effects of these extracts can be attributed to allelopathicsecondary metabolites that are further needed to be explored.

Key words: Allelopathy, Eco-friendly, Bioassay, Germination parameters, Seedling inhibition, Weed management.

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INTRODUCTION

Allelopathy is "injurious or beneficial effects of plants on each other". Allelopathic interactions have been observed in agricultural lands for centuries. It has been accepted as a novel approach for weed management and thereby increase crop yields (Putnam *et al.,* 1983). Allelochemicals offer great potential for weed control, directly or their chemistry could be used as a template to develop new herbicides (Putnam *et al.,* 1990).

In Pakistan, the agricultural sector contributes about 25% of the whole economic structure. During 2004-2008, a decrease in the mean annual growth rate of agriculture is noticed as production of main crops was reduced (Jabeen and Ahmad, 2009). Irrigation water scarcity, costly inputs, conventional farming, poor-quality seed, non-availability of fertilizers, lack of farm automation and reduced weed control are among the major causes for this decline (Nasir and Sultan, 2004). Weeds consume space, nutrients, moisture and sunlight vital for crop plants. Thus, weed management is one of the substantive requisites to increase crop yield (Norris, 1982). In different crops, 20%-30% losses are estimated to be caused by weeds in Pakistan (Anonymous, 2005). To some extent, herbicides cope the problem but due to constant use and the remnant toxic elements of these chemicals, they affect the environment by making pollution (Chung *et al*., 2003). Therefore, environment friendly methods for controlling weeds are need of time (Hussain *et al*., 2007). Use of plant-based chemicals (allelochemicals) is eco-friendly (Sharma *et al*., 2000; Khan *et al.*, 2016) and useful in increase in crop productivity and weed control (Ridenour and Callaway, 2001). In crop defense against weeds, allelopathy is an important component. Previous reports documented decrease in growth of *Avena fatua*, *Coronopus didymus*, *Chenopodium album* and *Phalaris minor* by sunflower extract with no effect on wheat (Naseem *et al*., 2009). Fungal growth was suppressed by *Rumex dentatus* n-hexane extracts (Fatima *et al*., 2009). Rye was inhibited by Roshan cultivar of wheat due to its allelochemical nature than Tabasi, Niknejad and Shiraz (Labbafy *et al*., 2009). Seed germination and seedling growth of *Lactuca sativa* was moderately affected by *Lactuca dissecta*, highly affected by *Inula koelzii* and *Inula falconeri*, while showed stimulatory effect by *Anthemis nobilis* (Khan *et al*., 2009).

The current study was aimed to study the effect of selected plants on seeds and seedlings of weeds *R. dentatus, A. fatua* and

associated crops *T. aestivum*, *H. annuus*, *Z. mays* in soil as well as on filter paper in laboratory conditions.

MATERIALS AND METHODS

Plant Collection and Processing

Allelopathic activity of *Carica papaya* L., *Euphorbia helioscopia* L*., Parthenium hysterophorus* L*.* and *Rumex dentatus* L*.* was tested against the growth and germination of selected test species.

Fresh leaves of *C. papaya*, *E. helioscopia, P. hysterophorus.* and *R. dentatus* were collected from different locations of District Rawalpindi (73° 02' E longitude and 33° 36' N latitude 508 m above sea level), Punjab, Pakistan and thoroughly splashed under tap water to remove dust and dried under shade at room temperature 25°C because shade-drying can prevent loss of heat-labile and readily oxidized nutrients (Ramsumair *et al*., 2014). The dried plant material was crushed, separately ground to fine powder and saved in air tight plastic bags separately at 4°C. 'Aqueous Extract Method' was used to screen the allelopathic activity of *C. papaya*, *E. helioscopia, P. hysterophorus* and *R. dentatus*, by using seeds and pre-germinated seeds (radicle protruded by at least 1 mm) of test species, on filter paper and soil.

Procurement of Seeds of Test Species

Seeds of *Avena fatua, Rumex dentatus*, *Helianthus annuus*, *Zea mays* and *Triticum aestivum* were procured from Department of Crop Science, National Agriculture and Research Centre, Islamabad.

Sterilization of Seeds of Test Species

Sodium hypochlorite (1% NaClO) was used to sterilize the test species seeds for 2 min. Seeds were then washed with distilled water and used for further bioassay studies.

Aqueous Extract Preparation

A stock solution was prepared by soaking 10 g of dried powder of each of selected 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 stored in pre-sterilized flasks at 4^{o}C. The extracts were used within 3-4 days to avoid any chemical alterations and contamination.

Bioassay Parameters

Following three parameters were used in allelopathic screening methodologies (a) Germination (%), (b) Radicle length (cm), and (c) Plumule length (cm).

Aqueous Extract Bioassay

This method was used to study the growth inhibition effects of leaf extracts of selected plants. Five replicates were used in completely randomized designed (CRD). Ten surface sterilized seeds of each test species were placed to each Petri plate (Arafat *et al.,* 2011; 2012). The glass petri dishes (9 cm) were tape sealed, covered with aluminum foil and incubated in the growth chamber at room temperature (25^{o}C) for fifteen days. The germination was recorded on a daily basis. The results were analyzed by counting the number of germinated seeds. Later on this period, the dishes were observed and studied for parameters (Khalid *et al.,* 2010). 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 (Anwar *et al.,* 2012 a). Screening of both seeds and pre-germinated seeds were carried out on filter paper and soil, separately (Riaz *et al.,* 2012; Sadia *et al.,* 2012).

Screening on Filter Paper

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

Statistical Analysis

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

RESULTS AND DISCUSSION

1. Germination Percentage

Wheat germination (%) was reduced significantly by *P. hysterophorus* followed by *R. dentatus.* Maize and sunflower germination was reduced in common by *C. papaya* followed by *R. dentatus*. The Results were correlated with previous analysis. Sajjan and Pawa (2005) and Dhole *et al.* (2011) stated that *P. hysterophorus* extract significantly affected germination and growth of *T. aestivum*. *R. dentatus, P. hysterophorus, Sisymbrium irio* and *Oxalis corniculata* were reported (Umer *et al.,* 2010). Germination and seedling growth of *Triticum aestivum* was significantly affected by *R. dentatus* extract (Hussain *et al.*, 1997). All extracts inhibited the germination (%) of *R. dentatus* and *A. fatua.* Germination (%) of *Z. mays* was not affected by *E. helioscopia* extract. However, auto-toxicity was exhibited by *R.*

dentatus (Fig. 1). Emergence of *H. annuus* and *Z. mays* was not affected by *P. hysterophorus* and *E. helioscopia* extracts, respectively. *P. hysterophorus* and *C. papaya* reduced the emergence of *R. dentatus* and *A. fatua.* Germination percentage in *T. aestivum* was significantly decreased by *P. hysterophorus* and *R. dentatus* while remained unaffected by *C. papaya* and *E. helioscopia* extracts (Fig. 2). Suppressive effect of *P. hysterophorus* on germination of *A. fatua* is reported earlier (Batish *et al.,* 2002; Marwat *et al.,* 2008).

2. Radicle Length (cm) in Different Plant Extracts

2.1. Radicle length (cm) in direct seeding on filter paper

Radicle length of *T. aestivum* was significantly inhibited by *R. dentatus* and *P.hysterophorus* while it was not affected by *C. papaya* and *E. helioscopia* extracts. Radicle growth of *Z. mays*, *R. dentatus, A. fatua* and *H. annuus* was suppressed by all the extracts. *R. dentatus* exhibited auto-toxicity and was significantly ihibited by all the extracts (Table-1).

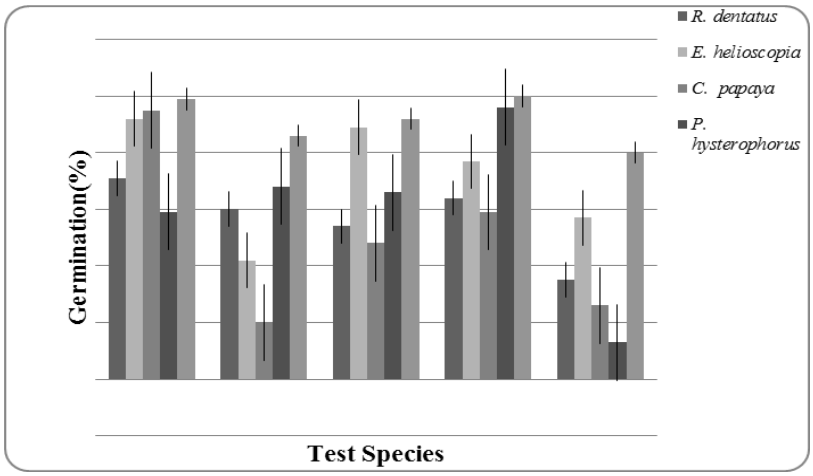

Figure 1. Seed germination (%) of test species treated with the aqueous extracts of selected plants and grown on filter papers under room temperature 25°C

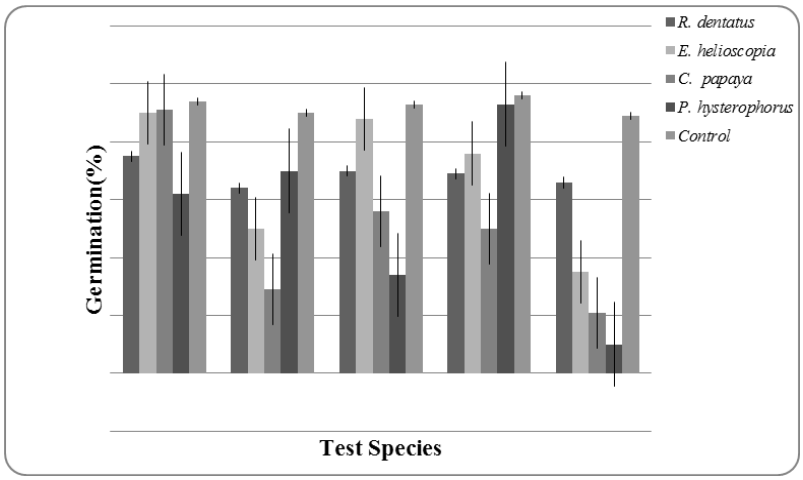

Figure 2. Seed emergence (%) of test species treated with the aqueous extracts of selected plants and grown in soil under room temperature 25°C

2.2. Radicle length (cm) of direct seeding in soil

P. hysterophorus and *R. dentatus* inhibited radicle length of all test species. Auto-toxicity was exhibited by *R. dentatus* and it was significantly inhibited by *P. hysterophorus* (Anwar *et al.,* 2012 b, c). *E. helioscopia* suppressed the radicle growth of *R.dentatus* and *A. fatua.* Radicle length of *T. aestivum* was significantly inhibited by *P. hysterophorus* and *R. dentatus* while was not affected by *C. papaya* and *E. helioscopia* extracts (Table-2).

Table-1. Radicle length (cm) of seeds of test species in aqueous extracts of selected plants in filter papers

Treatments	Test species				
	T. aestivum	*H. annuus*	*Z. mays*	*A. fatua*	*R. dentatus*
C. papaya	12.6 a	5.11 c	4.12 d	4.32 d	6.21 b
E. helioscopia	11.3 a	7.13 b	8.10 b	2.45 e	3.05 c
P. hysterophorus	5.49 b	1.17 d	2.19 e	8.21 b	1.31 d
R. dentatus	6.01 b	3.34 d	6.14 c	6.26 c	5.49 b
Control	13.15 a	9.48 a	10.3 a	11.2 a	8.03 a
LSD	3.0879	1.0360	0.6573	1.0971	1.0027
F-value	18.78*	128.48*	309.12*	126.95*	92.48*

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

Table-2. Radicel length (cm) of seeds of test species in aqueous extracts of selected plants in soil

Treatments	Test species				
	T. aestivum	*H. annuus*	*Z. mays*	*A. fatua*	*R. dentatus*
C. papaya	11.2 a	2.48 d	3.29 c	2.48 d	5.46 b
E. helioscopia	10.6 a	7.02 b	6.14 b	3.12 d	2.06 d
P. hysterophorus	4.02 c	3.02 d	1.30 d	7.29 b	1.01 e
R. dentatus	6.11 b	5.21 c	4.23 c	5.06 c	4.01 c
Control	11.4 a	8.39 a	8.45 a	10.45 a	7.17 a
LSD	1.8106	1.0634	1.0912	1.1918	0.8063
F-value	52.46*	74.89*	83.01*	98.97*	126.70*

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

2.3. Radicle length (cm) of seedlings on filter paper

P. hysterophorus significantly inhibited the growth of all the test species. *T. aestivum* was not affected by *E. helioscopia* and *C. papaya* while suppressed *A. fatua* (Anwar *et al.,* 2013). Radicle length of both *H. annuus* and *Z.mays* was not affected by *E. helioscopia* extract (Table-3).

2.4. Radicle length (cm) of seedlings in soil

P. hysterophorus extract significantly inhibited all the test species. There was no effect of *E. helioscopia* and *C. papaya* on seedlings of wheat. Radicle length of *R. dentatus* and *A. fatua* was suppressed by *E. helioscopia* extract (Anwar *et al.,* 2012 d, e). Radicle length of both *H. annuus* and *Z.mays* was not affected by *E. helioscopia* extract (Table-4).

Table-3. Radicle length (cm) of pre-germinated seeds of test species in aqueous extracts of selected plants in filter papers

Treatments	Test species				
	T. aestivum	*H. annuus*	*Z. mays*	*A. fatua*	*R. dentatus*
C. papaya	14.9 a	3.48 c	2.37 c	2.09 e	1.24 e
E. helioscopia	15.3 a	9.88 a	6.14 a	4.07d	2.13 d
P. hysterophorus	6.32 c	6.07 b	4.05 b	5.01 c	3.09 c
R. dentatus	10.45 b	4.12 bc	3.17 bc	7.10 b	4.05 b
Control	16.2 a	10.1 a	7.27 a	10.2 a	5.33 a
LSD	3.4976	2.0401	1.5567	0.4135	0.7263
F-value	18.78*	30.76*	22.96*	740.81*	64.28*

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

Table-4. Radicle length (cm) of seeds of test species in aqueous extracts of selected plants in soil

Treatments	Test species				
	T. aestivum	*H. annuus*	*Z. mays*	*A. fatua*	*R. dentatus*
C. papaya	12.1 a	2.17c	2.12 bc	2.28 d	3.07 b
E. helioscopia	11.8 a	7.69 a	5.45 a	1.17 e	1.00 c
P. hysterophorus	5.04 c	3.05c	1.32 c	4.07 c	2.43 b
R. dentatus	8.30 b	5.14 b	3.30 b	6.35 b	2.32 b
Control	13.4 a	8.49 a	6.42 a	9.11 a	4.11 a
LSD	2.5542	1.9073	1.2569	0.8095	0.9043
F-value	23.58*	27.90*	39.32*	206.09*	20.88*

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

The suppressive effect of *P. hysterophorus* on wheat radicle length was also reported by Mishra *et al.* (2011). *P. hysterophorus* also suppressed *R. dentatus* significantly. The radicle length *A. fatua* and *R. dentatus* were suppressed by *E. helioscopia* extract. Radicle length of wheat was significantly repressed by *P. hysterophorus* and *R. dentatus* while remained unaffected by *C. papaya* and *E. helioscopia* extrcts as compared to control. Non-toxic effects of *E. helioscopia* on *T. aestivum* are reported previously (Tanveer *et al.*, 2007). The work of Mahbarjan *et al*. (2007) and Oudhi (2001) also support the above results. In seedlings, when *E. helioscopia* extract was applied, the radicle length of both *H. annuus* and *Z. mays* remained unaffected. Hussain *et al.* (1997) and Batish *et al.* (2002) reported growth reduction in wheat seedling by *P. hysterophorus.*

3. Plumule Length (cm) in Different Plant Extracts

3.1. Plumule length (cm) in direct seeding on filter paper

Plumule growth of *Triticum aestivum* was significantly inhibited by *P. hysterophorus* and *R. dentatus* while it was not affected by *C. papaya* and *E. helioscopia* extracts as compared to control (Anwar *et al.,* 2010). *A. fatua* plumule length (cm) was repressed in *E. helioscopia* extract. *C. papaya* extract reduced plumule growth of *H. annuus, Z. mays* and *R. dentatus* (Table-5).

3.2. Plumule length (cm) in direct seeding in soil

P. hysterophorus and *R. dentatus* extracts repressed plumule length of all the test species (Anwar *et al.,* 2011).. In soil, aqueous extracts inhibited the plumule length of *R. dentatus, H. annuus, A. fatua* and *Z. mays* (Anwar *et al.,* 2012 f). Plumule length of wheat was significantly inhibited by *R. dentatus* and *P. hysterophorus* while was not affected by *C. papaya* and *E. helioscopia* extracts (Table-6).

Table-5. Plumule length (cm) of seeds of test species in aqueous extracts of selected plants in filter papers

Treatments	Test species				
	T. aestivum	*H. annuus*	*Z. mays*	*A. fatua*	*R. dentatus*
C. papaya	7.05 a	2.22 d	4.22 c	4.34 d	2.12 e
E. helioscopia	7.45 a	4.12 b	6.08 b	3.12 e	3.07 d
P. hysterophorus	2.02 c	3.05 cd	1.39 d	6.09 c	4.08 c
R. dentatus	5.34 b	3.34 bc	2.12 d	8.07 b	6.34 b
Control	8.16 a	6.39 a	8.45 a	10.15 a	8.06 a
LSD	1.3210	0.9468	1.0678	0.6647	0.6294
F-value	45.63*	37.04*	97.24*	238.96*	197.36*

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

Table-6. Plumule length (cm) of seeds of test species in aqueous extracts of selected plants in soil

Treatments	Test species				
	T. aestivum	*H. annuus*	*Z. mays*	*A. fatua*	*R. dentatus*
C. papaya	7.32 a	2.00 d	3.09 d	2.34 d	1.69 d
E. helioscopia	7.10 a	6.05 b	5.12 b	3.07 d	2.02 d
P. hysterophorus	3.47 c	3.39 c	1.39 e	6.08 b	3.21 c
R. dentatus	5.02 b	4.33 c	4.21 c	5.09 c	5.34 b
Control	8.49 a	8.37 a	7.00 a	7.40 a	7.45 a
LSD	2.3502	1.0434	0.7620	0.8978	1.0019
F-value	9.64**	73.9*	101.44*	71.85*	87.41*

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

3.3. Plumule length (cm) of seedlings on filter paper

P. hysterophorus extract significantly inhibited plumule length (cm) of all the test species. *T. aestivum* growth was unaffected by *E. helioscopia* and *C. papaya* extracts while these were suppressive for *A. fatua.* Plumule length of seedlings of both *H. annuus* and *Z. mays* was not affected by the extract of *E. helioscopia* indicating that it was not inhibitory towards plumule length of seedlings (Table-7).

3.4. Plumule length (cm) of seedlings in soil

P. hysterophorus extract significantly inhibited plumule length of all the test species. *E. helioscopia* and *C. papaya* extracts do not affectwheat growth. The plumule length of crop seedlings in this experiment was not reduced by *P.hysterophorus*. *E. helioscopia* extract was most suppressive to crop seedlings. *C. papaya* extract equally

inhibited the plumule length of *R. dentatus* and *A. fatua* in soil and on filter paper (Table-8).

Table-7. Plumule length (cm) of pre-germinated seeds of test species in aqueous extracts of selected plants in filter papers.

Treatments	Test species				
	T. aestivum	*H. annuus*	*Z. mays*	*A. fatua*	*R. dentatus*
C. papaya	10.1 a	4.49 c	4.01c	3.01 e	2.12 e
E. helioscopia	9.65 ab	9.27 a	11.2 a	4.12 d	7.08 b
P. hysterophorus	5.05 c	3.00 c	2.32 c	7.09 b	3.42 d
R. dentatus	7.13 bc	7.06 b	8.12 b	6.60 c	5.13 c
Control	11.8 a	10.05 a	11.45 a	9.08 a	9.20 a
LSD	2.5454	1.5861	1.7866	0.3036	0.8317
F-value	14.38*	47.89*	80.15*	825.69*	152.73*

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

Table-8. Plumule length (cm) of pre-germinated seeds of test species in aqueous extracts of selected plants in soil

Treatments	Test species				
	T. aestivum	*H. annuus*	*Z. mays*	*A. fatua*	*R. dentatus*
C. papaya	8.96 a	4.16 c	7.13 c	2.30 d	2.01 d
E. helioscopia	9.27 a	8.24 a	12.2 a	3.33 cd	5.12 b
P. hysterophorus	3.06 c	3.14 c	5.32 d	4.14 c	3.09 c
R. dentatus	6.05 b	6.31 b	10.5 b	6.33 b	3.32 c
Control	10.1 a	9.08 a	13.2 a	8.48 a	7.44 a
LSD	2.3413	1.2301	1.2891	1.2227	0.9318
F-value	20.36*	56.75*	88.02*	54.40*	69.05*

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

The plumule growth of *T.aestivum* seeds was significantly inhibited by *P. hysterophorus* and *R. dentatus* when treated with their aqueous extracts as compared to control while remained unaffected by *C. papaya* and *E. helioscopia* extracts. *P. hysterophorus* and *R. dentatus* inhibited plumule growth of all the test species. Dhole *et al.* (2011) and Mishra *et al.* (2011) reported suppressive effect of *P. hysterophorus* on maize growth. The work of Singh and Sangeeta (1991) and Sajjan and Pawa (2005) are in agreement with the above given results. *C. papaya* extract likewise inhibited plumule length of *R. dentatus* and *A. fatua* seedlings in soil and on filter paper. *P. hysterophorus* significantly inhibited the plumule length of all the test species. *E. helioscopia* and *C. papaya* did not suppress wheat growth. The radicle and plumule length (cm) of the crops in this experiment was not reduced by *E. helioscopia*.

Weed suppressive activity of plants is attributed to diverse nature of allelochemicals. *E. helioscopia* (Euphorbiaceae) contains a series of jatrophanes, euphoscopin, euphornins, diterpenes, lathyrane euphohelioscopin and euphoscopin (Barile *et al*., 2008). *P. hysterophorus* (Asteraceae) has secondary metabolites like kaempferol, parthenin, coronopilin, caffeic acid, p-coumaric acid (Patil and Hegde, 1988). The sesquiterpene lactones, coronopilin and parthenin have phytotoxicity for other plants including aquatic species (Batish *et al*., 2002). *Rumex dentatus* (Plygonaceae) contains significant phytochemicals including emodin, aloe-emodin, chrysophanol, physocin, chrysophanol, parietin, nepodine and anthraquinones (Choi *et al.,* 2004; Liu *et al.,* 1997). Compounds such as carotenoids, papain, pectin, dehydrocarpines, chymopapain, carpaine, carposide, pseudocarpaine, cis-violaxanthin, cryptoglavine and antheraxanthin (Ortega and Pino, 1997) have been isolated from *C. papaya*.

CONCLUSION

Application of aqueous extracts of allelopathic plants showed a pronounced inhibitory impact for controlling weeds and can be utilized to increase the yield result of wheat, maize and sunflower. Moreover, *P. hysterophorus* and *E. heliosccopia* are more efficient in weed growth supression. Active compounds from these allelopathic plants are needed to be explored to support organic agriculture.

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