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The Efficiency of Allelochemicals in The Seed Powder of *Eruca sativa* in Controlling Weeds in *Pisum sativum*

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ABSTRACT

Two pot experiments were performed in the greenhouse of National Research Centre in the two successive winter seasons of 2009 / 2010 and 2010 / 2011 to determine allelopathic potential of *Eruca sativa* seed powder (Essp) on *Pisum sativum* seed yield and associated broad-leaved weeds, *Beta vulgaris* and *Malva parviflora.* Treatments were applied by incorporation of *E. sativa* seed powder (Essp) into the soil, in four levels (15, 30, 45 and 60g/kg soil). *E. sativa* seed powder showed allelopathic effects at which fresh and dry weight of both weeds were significantly reduced till the complete inhibition of *Beta vulgaris* by the higher concentrations (45 and 60 g/kg soil Essp). On the other hand, *P.sativum* yield as well as yield components were significantly increased with both 15 and 30 g/kg soil of ESSP. The presence of glucosinolates and phenolic compound in the seed powder of *E. sativa* could play an important role in its natural selective bioherbicide properties in controlling the two annual weeds associated *P. sativum*.

Key words: Allelopathy, *Eruca sativa*, Glucosinolates, *Pisum sativum, Beta vulgaris*, *Malva parviflora*

Introduction

Allelopathy is one of the approaches which can be used as an alternative method to control weeds. Different plant species/families have been reported to possess this allelopathic activity and could be used in this respect. Among the families, Brassicaceae has had great attention. Brassicaceae family has allelopathic potential on the growth of other plants **(**Fenwick *et al*., 1983; Velasco *et al*., 2008; Martinez- Ballesta *et al*., 2013). Generally, Brassica species have been reported to have higher glucosinolate levels especially in the seeds, Fahey *et al*., 2001; Einhellig 2002; Velasco *et al.*, 2008, they mainly produce glucosinolates that are not biologically active under normal conditions. When Brassicaceae plants tissues are disrupted glucosinolates are hydrolyzed to a number of products such as isothiocyanates which are phytotoxic and achieved good result in controlling weeds (Fenwick *et al*., 1983; Borek *et al*., 1994; Fahey *et al*., 2001; Bennett *et al*., 2002; Bones and Rossiter, 2006; Kim and Ishii, 2006; Velasco *et al*., 2008; Zaji and Majd, 2011; Martinez-Ballesta *et al*., 2013). One of the famous plants which belong to Brassicaceae family is *E. sativa.* The crude extract of this plant at 25 and 50%, increased the percentage of germination as well as radical length of *Zea mays*, while 100% crude extract suppressed radical length of *Z.mays* compared to control (Bashen, 2014).

In the previous work from our Institute National Research Centre of Egypt, Messiha *et al.*, 2013, showed clearly that both *Eruca sativa* and *Brassia rapa* seed powder can be used as a selective bioherbicide to control the perennial troublesome weed *Cyperus rotundus* infesting *Zea mays* plants in the summer season.
The objective of this work is to determine the capability of *E. sativa* seed powder in controlling two annual broad-leaved weeds, *Beta vulgaris* and *Malva parviflora* associating *Pisum sativum* plants as well as the yield of *Pisum sativum* during the winter season.

Materials and Methods

Two pot experiments were carried out during two successive winter seasons of 2009/2010 and 2010/2011 in the greenhouse of National Research Centre, Dokki, Giza, Egypt. Peas (*Pisum sativum* L.) seeds cv. Master as well as the seeds of watercress (*Eruca sativa*), mallow (*Malva parviflora*) and chard (*Beta vulgaris*) were obtained from Agricultural Research Centre, Giza Egypt. Clean seeds of *E.sativa* were grinded to fine powder, after that the powder was immediately incorporated in the soil surface before sowing at the rate of 0, 15, 30, 45 and 60g/kg soil. The seeds of *P. sativum*, *M. parviflora* and *B. vulgaris* were sown 2cm deep in plastic pots filled with 2 kg of soil. The experiment consisted of 11 treatments including control; each treatment consisted of 8 replicates.

Characters studied
Weeds

Four replicates were collected from each treatment 55 and 90 days after sowing (DAS) and the following growth characters were taken: Fresh and Dry weight of both *M. parviflora* and *B. vulgaris* (g/pot) were recorded.

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Pisum sativum plants

Plant growth

In both seasons 55 and 90 DAS, samples of *Pisum sativum* plants were collected from each treatment to determine plant height (cm), number of leaves/plant, fresh and dry weight of plant (g) and number of flowers/plant, 55 DAS only.

Yield and yield components

At harvest, samples of *P.sativum* plants were taken from each treatment to determine number of pods/plant, fresh and dry weight of pods/ plant (g) and number of seeds/pod.

Chemical analysis

Total glucosinolates (µmol/g DW)

Total glucosinolates were extracted from dry samples of seeds powder of *E. sativa*. Glucosinolates were measured by determining the liberated glucose which released during hydrolysis by myrosinase enzyme (Rauchberger *et al*., 1979). The resulting glucose was determined colorimetrically according to the methods defined by Nasirullah and Krishnamurthy, 1996.

Total phenolic contents (mg/g DW)

Total phenolic contents of *E.sativa* seeds were determined colorimetrically using Folin and Ciocalteu phenol reagent according to the method defined by Snell and Snell, 1953.

Statistical analysis

All data were statistically analyzed according to Snedecor and Cochran ,1980 and the treatment means were compared by using LSD at 5% probability.

Results

Weed growth characters

The results in Table (1) show that different concentrations (15-60g/kg soil) of *Eruca sativa* seed powder (Essp) incorporated to the soil significantly suppress both the fresh and dry weight of *Malva parviflora* when compared to the untreated weed. The reduction in growth of *M. parviflora* was concentration dependent. Maximum reduction in the dry weight of *M. parviflora* was recorded with the highest concentration (60g/kg soil) of Essp that reached to about 96%, 55 days after sowing (DAS) and 76%, 90 DAS. Moreover, the rate of reduction of *Beta vulgaris* growth increased with increasing age (Table 1). The dry weight reduction of *B. vulgaris* reached to about 96% by incorporation of the highest concentration (60g/kg soil) of Essp when compared to the control 55 DAS, while complete inhibition was recorded 90 DAS with both higher concentrations (45 and 60g/kg soil) of Essp .

Table 1: Effect of different concentrations of *Eruca sativa* seed powder on fresh and dry weight of *Malva parviflora* and *Beta vulgaris* (g/pot). (Average of the two seasons).

Treatments		Fresh weight (g/pot)		Dry weight (g/pot)	
Plants	*E.sativa* (g/kg soil)	55 DAS	90 DAS	55 DAS	90 DAS
Malva parviflora (M)	…..	16.23	21.7	2.60	6.6
Pisum sativum (P) + (M)	…..	9.03	13.8	1.63	4.2
P. + M. + *Eruca sativa*	15	6.87	10.3	1.20	3.0
	30	3.27	6.3	0.53	2.0
	45	2.27	5.1	0.40	1.8
	60	1.07	3.6	0.10	1.6
Beta vulgaris (B)	…...	29.80	57.6	2.70	12.8
P. + *Beta vulgaris* (B)	…..	11.20	26.5	1.60	5.2
P. + B.+ *Eruca sativa*	15	8.80	10.6	1.20	2.8
	30	5.00	5.8	0.70	1.9
	45	3.50	0.0	0.40	0.0
	60	2.30	0.0	0.11	0.0
LSD at 5%		0.94	1.4	0.46	0.58

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Pisum sativum growth

The results in Table (2) show that all growth characters of *P. sativum* as plant height, number of leaves/plant, fresh and dry weight as well as number of flowers / plant were significantly affected by different concentrations of Essp used. Although the reduction in weed growth was recorded with all concentrations of Essp used (Table 1), yet the incorporation of 15 and 30g/kg soil concentrations of Essp induced significant increase in all growth characters of *P. sativum* 55 DAS (Table 2) as compared with the corresponding controls. Maximum increase in the dry weight of *P. sativum* plant was recorded with 30g/kg soil concentration of Essp that reached to about 36 and 46 %, with *M. parviflora* and *B. vulgaris*, respectively as compared with corresponding control. The highest concentration (60g/kg soil) of Essp induced significant reduction in *P. sativum* growth characters when grown with *M. parviflora* or *B. vulgaris* comparing to corresponding controls.

The results in (Table 3) show that different growth characters 90 DAS reveal to great extent the same trend obtained in Table 2. Maximum increase in the dry weight of *P. sativum* plant was recorded with 30g/kg soil Essp that reached to about 53 and 75%, with *M. parviflora* and *B. vulgaris*, respectively comparing to *P. sativum* alone, while when comparing to corresponding control, the increase of the same character reached to about 109 and 129 %, respectively.

P. sativum yield

The results of yield and yield components on *P. sativum* recorded in Table (4) show the effect of different concentrations of Essp on the plant associated with *M. parviflora or B. vulgaris*. Both 15 and 30g/kg soil of Essp induced high significant increase in yield and yield components as compared to corresponding controls. Maximum fresh weight of pods / plant was recorded by treatment with 30g/kg soil of Essp of *P. sativum* infested with *M. parviflora* and *B. vulgaris* recording 70.7 and 73% over corresponding controls. The corresponding results in dry weight of pods/plant reached to 130.7 and 142.9%.

Table 2: Effect of different concentrations of *Eruca sativa* seed powder on growth characters of *Pisum sativum* L. plants 55 days after sowing. (Average of the two seasons).

Treatments		Growth characters				
Plants	*E.sativa* (g/kg soil)	Plant height (cm)	No. of leaves/ plant	Fresh weight /plant (g)	Dry weight/plant (g)	No. of flowers/ plant
Pisum sativum alone (P))	…	39.33	7.33	12.67	2.57	1.5
P.+ *Malva parviflora* (M)	…..	35.33	6.00	11.30	2.20	1.0
P. + M. + *Eruca sativa*	15	42.00	8.00	14.47	2.74	1.9
	30	49.00	8.67	15.47	2.99	2.1
	45	30.67	6.33	12.73	2.40	1.1
	60	30.33	6.00	11.37	2.13	0.8
P. + *Beta vulgaris* (B)	…...	34.67	6.00	11.23	2.10	0.9
P. + B.+ *Eruca sativa*	15	44.00	8.33	14.63	2.78	2.0
	30	50.56	9.00	16.20	3.07	2.2
	45	32.00	7.00	13.10	2.47	1.2
	60	26.33	5.67	10.00	1.90	0.7
LSD at 5%		2.25	1.23	0.99	0.16	0.29

Table 3: Effect of different concentrations of *Eruca sativa* seed powder on growth characters of *Pisum sativum* L. plants at 90 days after sowing. (Average of the two seasons).

Treatments		Growth characters			
Plants	*E.sativa* (g/kg soil)	Plant height (cm)	No. of leaves/ plant	Fresh weight /plant (g)	Dry weight/plant (g)
Pisum sativum alone (P)	.....	42.0	9.0	9.00	2.23
P.+ *Malva parviflora* (M)	…..	38.3	7.5	6.87	1.63
P. + M. + *Eruca sativa*	15	44.5	9.3	10.33	2.76
	30	47.5	10.3	12.33	3.40
	45	34.7	8.7	7.67	1.90
	60	31.5	8.3	7.33	1.70
P. + *Beta vulgaris* (B)	…..	39.2	7.7	7.67	1.70
P. + B.+ *Eruca sativa*	15	46.7	9.7	10.67	2.90
	30	49.9	10.7	13.67	3.90
	45	36.2	8.7	8.33	2.10
	60	30.6	8.0	5.90	1.40
LSD at 5%		2.35	1.08	1.02	0.51

Table 4: Effect of different concentrations of *Eruca sativa* seed powder on yield and yield components of *Pisum sativum* L. plants at harvest. (Average of the two seasons).

Treatments		Yield and yield components			
Plants	*E.sativa* (g/kg soil)	No.of pods/plant	Fresh weight of pods/plant	Dry weight of pods/plant	No. of seeds/pod
Pisum sativum alone (P)	.....	2.50	11.5	2.30	2.81
P.+ *Malva parviflora* (M)	…..	1.80	9.2	1.40	2.00
P. + M. + *Eruca sativa*	15	3.10	14.9	3.01	3.66
	30	3.33	15.7	3.23	4.00
	45	2.80	10.3	2.12	2.67
	60	2.60	8.8	1.75	2.63
P. + *Beta vulgaris* (B)	…..	1.80	9.3	1.40	2.00
P. + B.+ *Eruca sativa*	15	3.17	15.2	3.13	3.67
	30	3.50	16.1	3.40	4.33
	45	2.83	10.5	2.24	3.00
	60	2.33	8.3	1.72	2.33
LSD at 5%		0.56	1.37	0.54	0.78

Table 5. Total glucosinolates (µmol/g DW) and Total phenolic contents (mg/g DW) in *Eruca sativa* seeds*

Materials	Total glucosinolates (µmol/g DW)	Total phenolic contents (mg/g DW)
Eruca sativa seeds	313.03	35.62

**Messiha et al. (2013)*

Discussion

The allelopathic potential of residues of some Brassicaceae plants were investigated under both laboratory and field conditions to determine the possibility of using them as natural herbicide for controlling weeds (Borek *et al*., 1994; Malik *et al*., 2008; Velasco *et al.,* 2008; Uremis *et al*., 2009; Zaji and Majd, 2011; Messiha *et al*., 2013).

As previously mentioned in the introduction, when the Brassicaceae plant tissues are disrupted, glucosinolates are hydrolyzed to a number of products. The main breakdown is Isothiocyanates, which are phytotoxic and achieved good results in controlling weeds (Borek *et al*., 1994; Cerny *et al*., 1996; Fahey *et al*., 2001; Petersen *et al*., 2001; Weckerle *et al*., 2001; Bennett *et al*., 2002; Jirovetz *et al*., 2002; Miyazawa *et al*., 2002; Kim and Ishii, 2006; Uremis *et al*., 2009; Zaji and Majd, 2011; Martinez-Ballesta, *et al*., 2013). In this connection, it is worthy to mention that Messiha *et al*., 2013, recorded that *Eruca sativa* seed powder could be used as a natural and selective bioherbicide similar to the chemically synthetic herbicide Basamid, since the mode of action of both is their capability to produce isothiocyanates (Messiha *et al*., 1993; Khalaf *et al*., 1996; Sharara *et al*., 2011).), which effectively control the propagative capacity of the troublesome perennial weed *Cyperus rotundus.*

The results of the present investigation reveal significant reduction in the two broad-leaved weeds *M. parviflora* and *B. vulgaris* growth after the incorporation of Essp to the soil (Table 1). Complete inhibition of *B. vulgaris* growth occurred by the high concentrations of Essp (45& 60g/kg soil) (Table 1). The previous results showed that Brassicaceae family has allelopathic potential on the growth of other plants (Petersen *et al*., 2001; Norsworthy 2003; Turk and Tawaha 2003; Messiha *et al*., 2013; Baeshen, 2014). Messiha *et al*., 2013 attributed the effect of *E. sativa* seed powder on the reduction of weed growth to the effect of alellochemicals, glucosinolates and phenols (Table 5).

On the other hand, the reduction in weed growth was accompanied by the increase in *P. sativum* growth (Tables 2&3) as shown in plant height, number of leaves/plant, fresh and dry weight and consequently increase in the yield. Several workers found that inhibition of weed growth increased the competitive ability of crop plants and consequently increased growth and yield (Abdelhamid and El-Metwally, 2008; Ahmed, *et al*., 2012; El-Rokiek *et al*., 2012 and 2013). It is worthy to mention that Essp at 15 and 30g / kg soil not only controlled the two broad- leaved weeds, but also increased the growth and yield of *P. sativum* as compared to the yield of *P. sativum* alone that may be due to its stimulatory effect (Messiha *et al*., 2013; Baeshen, 2014).

The result of the present work as well as the previous work showed clearly that the seed powder of *Eruca sativa* is capable to control annual and perennial weeds in summer as well as winter seasons.

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