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ORIGINAL ARTICLE

Allelopathic efficiency of *Eruca sativa* in controlling two weeds associated with *Pisum sativum* plants

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Abstract

Allelopathy is a complex phenomenon which depends on allelochemical concentrations. So, two pot experiments were carried out to investigate the allelopathic effect of alcoholic fresh shoot extract of *Eruca sativa* (foliar spray) and *E. sativa* shoot powder (mixed with soil) on *Pisum sativum* plants and two associated weeds, *Phalaris minor* and *Beta vulgaris*. The experiments were conducted in the greenhouse of the National Research Centre, Giza, Egypt during two successive winter seasons (2016–2017 and 2017–2018). Ten treatments were applied in this study. Four treatments were applied before sowing, that *E. sativa* shoot powder was mixed with the soil at rates of 15, 30, 45 and 60 g · pot^{-1}. The other four treatments of *E. sativa* alcoholic fresh shoot extract were sprayed twice on both plants and weeds at 5, 10, 15 and 20% (w/v) concentrations. Additionally, two untreated treatments, healthy (*P. sativum* only) and unweeded (untreated infested *P. sativum* plants with weeds) were applied for comparison. The results indicated that both alcoholic extracts and powder reduced growth of both weeds. Moreover, there was a direct relationship between concentration and weed reduction. *Eruca sativa* alcoholic extracts increased yield parameters of *P. sativum* plants. The maximum yield attributes were recorded by spraying of *E. sativa* alcoholic extract at 20%. On the other hand, it was clearly noticed that the high powder rates affected negatively *P. sativum* yield parameters. But the lowest powder rate (15 g · pot^{-1}) stimulated *P. sativum* yield parameters as compared to unweeded treatment. Chemical analysis of *E. sativa* shoot powder ensured that the abundant amount of glucosinolates (9.6 µmol · g^{-1}) and phenolic compounds (46.5 mg · g^{-1}) may be responsible for its allelopathic effect. In conclusion, spraying of alcoholic fresh shoot extract of *E. sativa* at 20% (w/v) and mixing *E. sativa* shoot powder at 15 g · pot^{-1}can be applied as natural bioherbicides for controlling weeds.

Keywords: allelopathy, *Eruca sativa*, glucosinolates, phenolic compounds, *Pisum sativum*

Introduction

Pea (*Pisum sativum* L.) is a well-known vegetable and belongs to family Leguminosae. It is the major food ingredient of vegetarian diets and meets the food requirements of people all over the world. It also contains most of the essential nutrients like fiber and protein (Khan and Shakoor 1991). Many non-chemical and environmentally recommended weed management practices have been applied to increase *P. sativum* yield (Bakht *et al.* 2009; El-Rokiek and Saad El-Din 2017; El-Rokiek *et al.* 2018). Weed management aims to manipulate the competitive balance in favor of the crop and to keep undesirable weeds at manageable levels (Bond and Grundy 2000). Recently, allelopathy has become one of the eco-friendly approaches which can be used as an alternative safe method to control weeds. Allelopathy is a phenomenon involving either beneficial or harmful effects of a plant (including microorganisms) on another plant by releasing allelochemicals into the environment (Singh *et al.* 2001).

The Brassicaceae family has allelopathic potential on the growth of other plants (Martinez-Ballesta *et al.* 2013; Salisbury *et al.* 2018). Generally, *Brassica* species have been reported to have abundant amounts of glucosinolates (GSLs) especially in the seeds (Velasco *et al.*

2008; Messiha *et al*. 2013; Ahmed *et al.* 2014; El-Masry *et al*. 2015). GSLs are chemically stable and not biologically active under normal conditions. As Brassicaceae plant tissues are damaged, GSLs are hydrolyzed by the myrosinase enzyme to phytotoxic products such as isothiocyanates, nitriles, epithionitriles, thiocyanates and oxazolidines (Bones and Rossiter 2006). Among these products, special attention has been given to isothiocyanates which have achieved good results in controlling weeds (Zaji and Majd 2011; Martinez-Ballesta *et al.* 2013; Ahmed *et al.* 2014; Salim *et al.* 2017; Couedel *et al*. 2018). GSLs are mainly involved in many biological activities (Chen *et al*. 2012).

Eruca sativa (Rocket salad) belongs to the family Brassicaceae. *Eruca sativa* like other Brassica vegetables is known to contain various phytochemical metabolites such as polyphenols, vitamin C and GSLs (Lazzeri *et al*. 2003; Kim *et al*. 2006; Martınez-Ballesta *et al*. 2013; Ahmed *et al.* 2014). Bennett *et al.* (2006) reported that glucosativin was the dominant GSL in the *Eruca* species. Additionally, *E. sativa* leaves were found to contain 67 volatile essential oil components, representing 96.52% of the oil. 4-methyl thiobutylisothiocyanate (60.13%) and 5-methyl thiopentanonitrile (11.25%) were the major constituents (Mitsuo *et al*. 2002).

The objectives of this study were to:

1. Evaluate the allelopathic effect of alcoholic fresh shoot extract of *E. sativa* as well as *E. sativa* shoot powder on the growth and yield of *P. sativum* plants and its associated weeds i.e. *Phalaris minor* and *Beta vulgaris*.
2. Study the possibility of using either alcoholic fresh shoot extract of *E. sativa* or *E. sativa* shoot powder as a natural bioherbicide to control *P. minor* and *B. vulgaris* weeds.

Materials and Methods

Preparation of dry plant material

Shoot parts of *E. sativa* were collected from fields and washed with tap water, then dried at room temperature in the shade for several days. Dried plant tissues were ground separately into a fine powder using an electric mill.

Preparation of alcoholic fresh shoot extract of *Eruca sativa*

Fresh shoots of (*E. sativa*) were collected and washed with tap water, then cut into small particles. Stock solution (20% w/v) was prepared according to Mekonnen (1999) by soaking 200 g of *E. sativa* fresh shoots in 1 l of 80% ethanol, then mixed well using an electric ground blender. The produced mixture was transferred to a 2 l beaker and covered with parafilm. The beaker was placed on a shaker (200 revolution/min) for 48 h at room temperature. The mixture was filtered through four layers of cheesecloth to remove debris and centrifuged for 30 min. The supernatant was then filtered through one layer of filter paper (Whatman No. 1). After filtration, ethanol was evaporated using a rotary evaporator device. Three concentrations, 5, 10 and 15% (w/v), were prepared from 20% stock solution using distilled water. The method of extraction was repeated according to need to ensure that the extracts were always fresh.

Pot experiments

Two pot experiments were carried out in November during two successive winter seasons (2016–2017 and 2017–2018) in the greenhouse of the National Research Centre (NRC). Both experiments were conducted in a completely randomized design with nine replicates. Pottery pots (30 cm in diameter and 0.07 m^2) were filled with equal amounts of sieved sandy-loam soil. Seeds of *P. sativum* (cv. Master B) were obtained from the Agricultural Research Centre, Egypt. Five seeds of *P. sativum* were sown 2 cm deep from the soil surface. All pots (except the healthy treatment) were infested with the same number of *B. vulgaris* and *P. minor* weed seeds and mixed thoroughly. Ten treatments were applied in this investigation. Four treatments were treated with *E. sativa* shoot in powder form which was mixed with the soil surface before sowing at rates of 15, 30, 45 and 60 g · pot^{-1}. After sowing of pots, the corresponding four treatments of *E. sativa* alcoholic extracts were sprayed at 5, 10, 15 and 20% (w/v). Extracts were sprayed twice using a hand sprayer at the rate of 50 ml · pot^{-1} 2 and 3 weeks after sowing (plants were at four leaf stage) on the foliage part of *P. sativum* and its associated weeds (*P. minor* and *B. vulgaris*). Additionally, two control treatments i.e. healthy and untreated were sprayed with distilled water for comparison. All treatments were maintained under greenhouse conditions and all cultural practices were applied especially irrigation and fertilization.

Studied parameters

Weeds

Three replicates were collected from each treatment at 45 and 70 days after sowing (DAS) and the dry weights of both *P. minor* and *B. vulgaris* (g · pot^{-1}) were recorded.

Pisum sativum plants

Growth parameters

In both seasons at 45 and 70 DAS, three replicates of *P. sativum* plants were collected from each treatment to determine shoot height/plant (cm), number of leaves/plant and dry weight of plant (g).

Yield and yield attributes

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

Chemical analysis of *Erucca sativa* shoot powder

Total glucosinolates GSLs (μmol · g^{-1} DW) were extracted from *E. sativa* dry shoot powder. GSLs were measured by determining the liberated glucose which was released during hydrolysis by the myrosinase enzyme (Rauchberger *et al.* 1979). The resulting glucose was determined using aspectrophotometer device at wave length 490 nm according to the methods defined by Nasirullah and Krishnamurthy (1996). The GSLs value was obtained by multiplying the factor 2.1 for glucose.

Total phenolic contents (mg · g^{-1} DW) were determined in *E. sativa* dry shoot powder with aspectrophotometer device at wave length 520 nm using Folin 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 0.05 probability.

Results

Weeds

As shown in Table 1 different concentrations of alcoholic fresh shoot extract of *E. sativa* (foliar spray) (5–20% w/v) and *E. sativa* shoot powder (mixed with soil) (15–60 g · pot^{-1}) significantly suppressed the dry weight of both *P. minor* and *B. vulgaris* weeds.

The reduction in the dry weight of both weeds was concentration dependent. The highest concentration of *E. sativa* shoot powder (60 g · pot^{-1}) was followed by alcoholic fresh shoot extract of *E. sativa* (20%) and scored the maximum reduction in both weeds. These ideal treatments caused a reduction in *P. minor* up to 90.27 and 84.63% and reduction in *B. vulgaris* reached up to 87.20 and 78.02%, respectively at 45 DAS. Whereas, at 70 DAS alcoholic fresh shoot extract of *E. sativa* (20%) controlled both weeds the most effectively, followed by *E. sativa* shoot powder (60 g · pot^{-1}). *Phalaris minor* reduction was 79.44 and 76.74% and *B. vulgaris* reduction amounted to 84.82 and 84.67%, respectively, as compared to unweeded control treatment.

Pisum sativum plants

Growth parameters

Results in Table 2 indicated that all growth parameters (shoot height, number of leaves/plant and plant dry weight) of *P. sativum* were affected by fresh shoot alcoholic extract of *E. sativa* and *E. sativa* shoot powder at different concentrations. At 45 DAS, the low concentrations of alcoholic extracts at 10 and 15% significantly increased shoot height from 22.8 to 37.8 and 35.5 cm, respectively, which was higher than the healthy value of 34.0 cm. At 70 DAS, 15 and 10% concentrations induced shoot height from 30.3 to 58.3 and 53.0 cm, respectively, followed by healthy plants (52.0 cm). By increasing the extract concentration to 20%, the rates of increasing in shoot height decreased (46.7 cm) but were still higher than unweeded treatment. The lowest

Table 1. Effect of different concentrations of alcoholic fresh shoot extracts of *Eruca sativa* and *E. sativa* shoot powder on the dry weight of *Phalaris minor* and *Beta vulgaris* (g · pot^{-1}) (average of the two seasons)

Treatments		Dry weight [g · pot^{-1}]			
		45 days after sowing		70 days after sowing	
		P. minor	*B. vulgaris*	*P. minor*	*B. vulgaris*
P. sativum only (healthy)		0.00	0.00	0.00	0.00
Unweeded		7.61	4.14	11.09	7.18
Alcoholic fresh shoot extract of *E. sativa* [w/v]	5%	2.40	2.58	6.00	3.23
	10%	1.72	1.90	5.60	2.54
	15%	1.40	1.12	3.80	1.82
	20%	1.17	0.91	2.28	1.09
E. sativa shoot powder [g · pot^{-1}]	15	2.36	2.06	8.34	3.11
	30	1.36	1.09	6.37	2.43
	45	1.03	0.81	5.31	1.63
	60	0.74	0.53	2.58	1.1
LSD at 0.05		3.70	1.58	4.09	1.49

Table 2. Effect of different concentrations of alcoholic fresh shoot extract of *Eruca sativa* and *E. sativa* shoot powder on growth parameters of *Pisum sativum* L. plants at 45 and 70 days after sowing (average of the two seasons)

Treatments		45 days after sowing			70 days after sowing		
		shoot height/plant [cm]	no. of leaves/ plant	dry weight/ plant [g]	shoot height/plant [cm]	no. of leaves/ plant	dry weight/ plant [g]
P. sativum only (healthy)		34.0	11.5	1.45	52.0	18.00	4.76
Unweeded		22.8	9.5	0.83	30.3	13.33	1.97
Alcoholic fresh shoot extract of *E. sativa* [w/v]	5%	32.8	10.2	0.91	51.0	16.00	2.07
	10%	37.8	10.8	0.99	53.0	16.33	2.38
	15%	35.5	10.9	1.55	58.3	16.67	3.80
	20%	33.0	10.3	1.84	46.7	14.67	4.80
E. sativa shoot powder [g · pot^{-1}]	15	28.5	12.3	1.11	46.0	17.67	4.16
	30	23.5	10.0	1.03	33.7	16.67	3.65
	45	22.5	9.7	0.81	25.7	16.33	1.95
	60	16.5	7.5	0.73	22.3	16.00	1.39
LSD at 0.05		3.28	1.18	0.21	6.68	2.11	0.98

rate of *E. sativa* shoot powder (15 g · pot^{-1}) significantly increased *P. sativum* shoot height more than unweeded treatment (28.5 and 46.0 cm, respectively, at 45 and 70 DAS). Higher powder rate (30 g · pot^{-1}) slightly increased shoot height (23.5 and 33.7 cm, respectively, at 45 and 70 DAS) with no significant difference with unweeded treatment. It was noticeable that at the highest concentration of *E. sativa* shoot powder (60 g · pot^{-1}), *P. sativum* shoot height was negatively affected and was significantly lower than unweeded treatment (16.5 and 22.3 cm, respectively, at 45 and 70 DAS).

It was observed that there was a direct relationship between the alcoholic extract concentration and the number of leaves. The alcoholic fresh shoot extracts increased the number of leaves/plant at all concentrations especially at 15%. An adverse relationship was observed between *E. sativa* shoot powder and the number of leaves. The lowest concentration of *E. sativa* shoot powder (15 g · pot^{-1}) scored the highest number of leaves at 45 DAS (12.3 leaves/plant). At 70 DAS, healthy plants had the highest number of leaves (18.0 leaves/plant) followed by *E. sativa* shoot powder at 15 g · pot^{-1} (17.67 leaves/plant) with no significant difference between them as compared to unweeded treatment.

As alcoholic shoot extract of *E. sativa* concentration increased, *P. sativum* dry weight reached the highest dry weight values (1.84 and 4.80 g, respectively at 45 and 70 DAS) using *E. sativa* alcoholic extract at 20%. But in the case of *E. sativa* powder, a negative response was recorded by increasing concentration. The highest concentration (60 g · pot^{-1}) gave values (0.73 and 1.39 g, respectively, at 45 and 70 DAS) lower than unweeded treatment with no significant difference between them.

Yield and yield attributes

From the recorded results in Table 3 it is clear that most of the applied treatments i.e. alcoholic fresh shoot extract of *E. sativa* and low concentrations of *E. sativa* shoot powder increased yield and the attributes of *P. sativum* plants (number of pods/plant, dry weight of pods/plant, number of seeds/plant and dry weight of seeds/plant). Healthy treatment, *E. sativa* alcoholic extract at 20% and *E. sativa* powder treatment at 15 g · pot^{-1} gave the highest number of pods/plant (1.86, 1.50 and 1.49 pods/plant), dry weight of pods/plant (2.36, 2.19 and 2.15 g) and dry weight of seeds/plant (2.007, 1.977 and 1.973 g), successively. The number of seeds/plant did not respond with the same trend to the applied treatment. The *E. sativa* alcoholic extract at 20, 15 and 10% gave the highest number of seeds/plant (14.33, 13.67 and 12.0 seed/plant, respectively, with no significant difference between them.

A direct relationship was noticed between increasing the concentration of the applied alcoholic extract of *E. sativa* fresh shoot and yield increment. In contrast, although *E. sativa* powder reduced the dry weight of both investigated weeds (Table 1), it was clear that as the powder concentration increased the yield and yield attributes decreased to the maximum reduction at 60 g · pot^{-1}. Particularly, *E. sativa* shoot powder at 15 and 30 g · pot^{-1} increased yield and its attributes as compared to unweeded treatment. On the contrary, *E. sativa* shoot powder at 45 and 60 g · pot^{-1} affected negatively yield and yield attributes of *P. sativum* which decreased lower than unweeded treatment. In conclusion, alcoholic fresh shoot extract of *E. sativa* at 20% and *E. sativa* powder at 15 g · pot^{-1} gave the highest yield increment, about 128.0 and 127.6%, respectively.

Table 3. Effect of different concentrations of alcoholic fresh shoot extracts of *Eruca sativa* and *E. sativa* shoot powder on *Pisum sativum* L. yield and its attributes (average of the two seasons)

Treatments		No. of pods/ plant [g]	Dry weight of pods/plant [g]	No. of seeds/ plant	Dry weight of seeds/plant [g]	% of yield increment
P. sativum only (healthy)		1.86	2.36	8.67	2.007	56.8
Unweeded		1.03	1.14	5.33	0.867	0.0
Alcoholic fresh shoot extract of *E. sativa* [w/v]	5%	1.42	1.36	9.33	1.228	41.6
	10%	1.47	1.84	12.00	1.563	80.3
	15%	1.47	2.12	13.67	1.937	123.4
	20%	1.50	2.19	14.33	1.977	128.0
E. sativa shoot powder [g · pot^{-1}]	15	1.49	2.15	9.67	1.973	127.6
	30	1.33	1.50	7.67	1.510	74.2
	45	1.00	1.01	5.00	0.777	–10.4
	60	0.87	0.87	4.67	0.663	–23.5
LSD at 0.05		ns*	0.45	2.88	0.34	–

*not significant

Chemical analysis of *Eruca sativa* shoot powder

The results (Table 4) demonstrate the abundant amount of GSLs (9.55 µmol · g^{-1} DW) and phenolic compounds (46.5 mg · g^{-1} DW) in *E. sativa* shoot powder which could be considered as the main tools of safe weed management strategy.

Discussion

Allelopathic potential of Brassicaceae plants is one of many strategies recently applied to minimize the use of chemical herbicides in agriculture. Kimberly *et al.* (2002) reported that *E. sativa* contains GSLs derived from a group of amino acids, including methionine, phenylalanine, alanine, leucine and tryptophan which may be responsible for this suppressive effect on weeds (Table 1). Bennett *et al.* (2007) found that *E. sativa* sprouts contain abundant amounts of GSLs which are the precursors of erucin and sativin biologically active isothiocyanates. Messiha *et al.* (2013) and Ahmed *et al.* (2014) attributed the suppressive effect of *E. sativa* seed powder on weeds to GSLs and phenolic compounds. Moreover, many scientists found that Brassicaceae plants contain GSLs which hydrolyzed under stress to a number of phytotoxic products. Isothiocyanate is the main created phytotoxic compound which achieved good results in controlling weeds (Ebrahimi *et al.* 2011; Cerdeira *et al.* 2012; Messiha *et al.* 2013; Ahmed *et al.* 2014; El-Masry *et al.*2015; Salim *et al.* 2017; Salisbury *et al.* 2018). Moreover, *E. sativa* also has antifungal activity which may be accompanied with the presence of antioxidants such as glucosinolates, flavonoids, carotenoids in addition to the volatile fractions (Hanafi *et al.* 2010). Weckerle *et al.* (2001) and Pasini *et al.* (2011) reported that kaempferol derivatives are considered to be the major group of phenolic compounds present in *E. sativa* leaves (77–88% of total phenolic compounds), followed by isorhamnetin-3,4-diglucoside and quercetin, representing 16.3% and 9%, respectively, of the total phenolic compounds. El-Rokiek *et al.* (2018) revealed that phenolic compounds, flavonoids as well as essential oils may be responsible for the allelopathic inhibition effect on weeds associated with *P. sativum* plants. Allelochemicals directly affect the physiological processes in plants such as mitotic activity, photosynthesis, nutrient absorption, permeability of cell membranes, respirationas well as enzyme action inhibition and protein formation (Rice 1984; Wu *et al.* 2000; Xuan *et al.* 2004). Allelochemicals also affect the photosynthetic area or assimilation rate which may in turn cause plant dry matter reduction (Dadkhah 2012). Additionally, as shown in Table 1, *E. sativa* fresh shoot alcoholic extract and shoot powder reduced the dry weight of both weeds and this reduction was increased by increasing concentration. These findings are in accordance with Hegab *et al.* (2008) and Ahmed *et al.* (2014) who showed a direct relationship between the high response to the inhibition effect of the applied allelopathic extract or powder and the increment in allelochemicals concentration.

The recorded inhibition of weeds by spraying alcoholic extract of *E. sativa* of fresh shoot at 20% or by mixing of *E. sativa* shoot powder at 15 g · pot^{-1} (Table 1) was reflected on *P. sativum* growth and yield parameters (Tables 2 and 3). This may be related to the stimulatory effect of *E. sativa* secondary metabolites on

Table 4. Total glucosinolates (GSLs) and total phenolic content in *Eruca sativa* shoot powder

Material	Total GSLs [μmol · g^{-1} DW]	Total phenolic compounds [mg · g^{-1} DW]
Eruca sativa shoot powder	9.55	46.5

P. sativum plants (Kimberly *et al.* 2002). Also, it may be related to the reduction of competitor agents with *P. sativum* plants as recorded by several researchers (Bakht *et al.* 2009; El-Rokiek and Saad El-Din 2017; El-Rokiek *et al.* 2018).

Conclusions

Alcoholic extract of *E. sativa* fresh shoot and *E. sativa* shoot powder reduced the dry weight of both weeds. This reduction increased with increasing concentrations. *Eruca sativa* shoot powder at high concentrations affected negatively *P. sativum* plants. So, *E. sativa* alcoholic extract at 20% (w/v) and shoot powder at 15 g · pot^{-1} can be tested under field conditions in controlling *P. minor* and *B. vulgaris,* associated with *P. sativum* plants, as natural eco-friendly herbicides.

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