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Bioherbicidal activity of *Eruca sativa* fresh shoot aqueous extract for the management of two annual weeds associating *Pisum sativum* plants

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Abstract

Background: Brassica species have been established to have very high concentrations of glucosinolates, flavonols, and other secondary metabolites that achieved good results in weed management strategy. So, this study highlights how to investigate the allelopathic potential of *Eruca sativa* fresh shoot aqueous extract as a natural bioherbicide to control *Phalaris minor* and *Beta vulgaris* weeds beside its effect on *Pisum sativum* growth as well as yield traits. Two pot experiments were conducted in the greenhouse of the National Research Centre, Dokki, Giza, Egypt, in the two successive winter seasons of (2016–2017, 2017–2018). Treatments were applied by spraying *E. sativa* fresh shoot aqueous extract once at 14 days after sowing and twice at 14 and 21 days after sowing at rates of 20, 40, 60, and 80% w/v.

Results: *E. sativa* fresh shoot aqueous extract at 80% achieved the maximum inhibition effect on the growth of both weeds. This in turn was reflected on *P. sativum* plant and gave the observable highest growth and yield parameters. Chemical analysis of *E. sativa* shoot powder approved the presence glucosinolates (9.6 $\mu\text{mol/g}$) and phenolic compounds (46.5 mg/g) which may be responsible for the allelopathic effect.

Conclusion: Spraying of aqueous fresh shoot extract of *E. sativa* at 80% (w/v) can be applied as natural selective bioherbicide in controlling the two annual grassy and broad-leaved weeds associated with *P. sativum* plants.

Keywords: Allelopathy, *Eruca sativa*, *Pisum sativum*, Glucosinolates, Phenolic compounds

Background

Pea (*Pisum sativum*) is one of the important grain legumes that grow in various parts of the world. Several types of weeds are associated with *P. sativum*. Weeds can reduce grain yield as well as quality through direct competition on nutrients, moisture, space, and light (Wu et al. 2000). On an average, weeds cause a depression in crop productivity that reaches to 34% (Oerke 2006). Many cultural, mechanical, chemical, and biological methods were applied for controlling weeds. Hand weeding is a useful method, but is time-consuming and costs. Although the application of chemical herbicides is effective in controlling weeds, it results to a negative impact on human and

animal (Vyvyan 2002). Moreover, widespread use of herbicides causes soil and groundwater pollution, and toxic residues that accumulate in agricultural products and weeds become resistant to these herbicides (Jabran et al. 2015). More than 471 weed species are documented to have a resistance to commonly used chemical herbicides such as those in triazolopyrimidine sulfonanilide, sulfonylurea, and theimidazolinone families. Recently, allelopathic potential of plants are getting much interest to face all these problems in controlling weeds (Jabran et al. 2015; El-Rokiek et al. 2018 and El-Dabaa et al. 2019).

Allelopathy is a biological phenomenon by which the plant (including microorganisms) produces biochemicals that influence the germination and growth of other plant. These biochemicals (allelochemicals) may have beneficial or harmful effect on the target plant (Reigosa

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et al. 2006). Modern phytochemical methods of extraction, isolation, purification, and identification have contributed to identify these allelochemicals which can be classified in various ways (Ferreira and Áquila 2000). Manipulation of natural products extracted from plants is a healthy and eco-friendly approach to control the weeds (Khan et al. 2007).

Eruca sativa Mill. (commonly known as Rocket salad) belongs to family Brassicaceae. *E. sativa* is a good source of vitamin C and antioxidants such as phenolic compounds, carotenoids, glucosinolates, and degradation products as isothiocyanates (Martinez-Sanchez et al. 2008; Villatoro-Pulido et al. 2012; Martinez-Ballesta et al. 2013; Messiha et al. 2013; Ahmed et al. 2014; El-Masry et al. 2015). Glucosinolates (GSLs) are sequestered in the vacuoles of Brassicaceae plants only (Daxenbichler et al. 1991). As Brassicaceae plant tissues are damaged, GSLs are hydrolyzed by myrosinase enzyme (present at high levels in myrosin cells) (Bones and Rossiter 2006). GSLs are hydrolyzed to glucose and unstable intermediate. This intermediate degrades to various products including thiocyanates, isothiocyanates, and nitriles. The produced hydrolyzed products are dependent on the glucosinolate itself and the conditions of the reaction (Fahey et al. 2001).

The identified GSLs in Rocket seeds are glucoerucin (4-methyl-thiobutylglucosinolate) and low levels of glucoraphanin (4-methyl-sulphanylbutylglucosinolate) (Ori et al. 1999; Bennett et al. 2006), whereas the major identified GSL in Rocket leaves is glucosativin (4-mercaptobutylglucosinolate) (Bennett et al. 2002). Glucoerucin is the precursor of erucin (4-methyl-thiobutylisothiocyanate) (Bennett et al. 2007). Moreover, sulforaphane (4-methyl-sulfi-nylbutylisothiocyanate) is derived from glucoraphanin. Sulforaphane is one of the most known natural anti-cancer isothiocyanate compounds and is identified in Rocket species (Bonnessen et al. 2001; Smith 2001 and Zhang 2004). Additionally, sativin (4-mercaptobutyl-isothiocyanate) is derived by hydrolysis of glucosativin. Sativin is a pungent volatile compound which may be responsible for *E. sativa* distinct odor (Bennett et al. 2002). Bennett et al. 2007 found that erucin and sativin are significantly biologically active isothiocyanates in Rocket species.

Material and methods

Preparation of water extract

Eruca sativa (Rocket salad) shoots were collected from Egyptian fields and washed with tap water then cut into small particles. Stock solution (80% w/v) was prepared according to Fuentes et al. 2012. Eight hundred grams of *E. sativa* fresh shoots was soaked in 1 L of distilled water then mixed well using an electric ground blender. The produced mixture 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). Three concentrations 20, 40, and 60% (w/v) were prepared by dilution of 80% crude extract using distilled water. The method of extraction was repeated according to need that the extracts were always fresh.

Experimental procedure

Two pot experiments were applied in November during two successive winter seasons (2016–2017) and (2017–2018) in the greenhouse of the National Research Centre (NRC). Both experiments were performed in a completely randomized design with nine replicates. Pottery pots (30 cm in diameter and 0.07m² in area) were filled with equal amounts of sieved sandy-loam soil (4Kg soil/pot). Seeds of *Pisum sativum* (Pea) (cv. MasterB) 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 [*P. sativum* only]) were infested with the same weight (0.5 g/pot) of *Phalaris minor* (littleseed canarygrass) and *Beta vulgaris* (chard) weed seeds and mixed well. Ten treatments were applied in this study. Four treatments were sprayed once at 14 days after sowing (DAS) with *E. sativa* fresh shoot aqueous extracts (20, 40, 60, and 80 (crude extract)). The corresponding four treatments were sprayed twice at 14 and 21 DAS (seedlings were at 4 leaf stage) with the *E. sativa* fresh shoot aqueous extracts at the same concentrations. Additionally, healthy and infested untreated control treatments were sprayed with tap water for comparison. Both extracts and tap water were sprayed using a hand sprayer at the rate of 50 ml/pot on foliage part of *P. sativum* and its associated weeds (*P. minor* and *B. vulgaris*). All treatments were maintained under greenhouse condition, and all cultural practices of irrigation and fertilization were applied.

Studied parameters

Weeds

In both seasons, weeds under study were collected from three replicates of each applied treatment at 45 and 70 DAS. The dry weight (g/pot) of separated weeds was determined after drying in a forced draft oven at 70 °C for 48 h.

Pisum sativum plants

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

number of leaves/plant, number of branches/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 number of pods/plant, dry weight of pods/plant (g), number of seeds/plant, and dry weight of seeds/plant.

Chemical analysis of *E. sativa* shoots powder

Total glucosinolates (GSLs) ($\mu\text{mol/g DW}$) 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 colorimetrically 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 DW) were determined in *E. sativa* dry shoot powder colorimetrically using Folin and Ciocalteu phenol reagent according to the method defined by Snell and Snell (1953) (Table 1).

Statistical analysis

Since the homogeneity test proved the homogeneity and normality of the data of the two seasons, combined analysis was performed. All obtained data were subjected to proper statistical of variance according to Snedecor and Cochran (1980). The mean values were compared using Duncan's multiple range test (Duncan 1955) at 5% level of probability.

Results

Weed growth parameters

Results presented in Table 2 revealed that the dry weights of both weeds, i.e., *P. minor* and *B. vulgaris*, were significantly reduced by spraying of *E. sativa* fresh shoot aqueous extract. Once or twice spraying of *E. sativa* fresh shoot aqueous extract at different concentrations (20, 40, 60, and 80%) affected on dry weight of both weeds as compared to the unweeded treatment at 45 and 70 DAS.

Treatment of spraying 80% twice recorded the highest reduction in dry weight of the grassy weed followed by 60% twice and 80% once in both samples. The reduction amounted to 80.80, 69.95, and 65.59 at the first sample, whereas 68.27, 60.41 and 59.48% at the second sample, respectively, when compared to unweeded treatment (control). With regard to broad-leaved weed, the results

in Table 2 also cleared that *B. vulgaris* was affected in the same trend as grass weed. The reduction amounted to 84.66, 76.43, and 75.41% at the first sample and reached to 72.73, 68.87 and 61.39%, respectively at the second sample as compared to control treatment.

***Pisum sativum* plants**

Growth parameters

As shown in Table 3, most of the applied concentrations of *E. sativa* fresh shoot aqueous extract, sprayed either once or twice, had a significant effect on most growth parameters under study, i.e., shoot height, number of leaves/plan, number of branches/plant, and dry weight of plant. At 45 and 70 DAS, healthy plants and spraying 80% twice recorded the highest growth parameters, with no significant difference between them in most parameters. Treatments of spraying 60% twice and 80% once followed these ideal treatments, also with no significant difference between them in most parameters, as compared to the other treatments. The increases in plant dry weight at the first sample reached to 112.09, 109.89, 108.79, and 83.52%. Whereas in the second sample, the increment percentage reached to 235.19, 201.85, 182.10, and 153.70%, respectively, as compared to untreated plants (control). It is clearly observed that the twice spray of *E. sativa* water extract induced the growth parameters of *P. sativum* than once spray treatments. Additionally, there is a direct relationship between concentration of extract and increment in growth parameters. Conversely, the unweeded treatment recorded the lowest values in all growth parameters of *P. sativum* plants at both ages.

Yield and yield attributes

Results in Table 4 revealed that most of the applied weed control treatments caused a significant progress in yield and its attributes, i.e., number of pods/plant, dry weight of pods/plant, number of seeds/plant, and dry weight of seeds/plant. Weed free, 80 and 60% twice spray treatments provided the maximum values of yield and its attributes. These mentioned superior treatments were followed by 80% once spray except in the case of number of pods/plants that 40% twice spray came in the fourth rank. Generally, it is worthy to mention that twice spray of *E. sativa* fresh aqueous extract provided a higher yield progress than once spray. Moreover, the increment in extract concentration is accompanied with high yield production. So, 80% twice spray of *E. sativa* fresh aqueous extract

Table 1 Total glucosinolates ($\mu\text{mol/g DW}$) and total phenolic contents (mg/g DW) in *E. sativa* shoot powder

Material	Total GSLs ($\mu\text{mol/g DW}$)	Total phenolic compounds (mg/g DW)
<i>Eruca sativa</i> shoot powder	9.55	46.5

Table 2 Effect of spraying different concentrations of *E. sativa* fresh aqueous extract on dry weight of *P. minor* and *B. vulgaris* (g/pot). (Combined analysis of the two seasons)

Treatments	<i>E. sativa</i> fresh aqueous extract concentration (%)		First sample		Second sample	
			Dry weight (g/pot)			
			<i>P. minor</i>	<i>B. vulgaris</i>	<i>P. minor</i>	<i>B. vulgaris</i>
<i>P. sativum</i> only	0		0.00 a	0.00 a	0.00 a	0.00 a
<i>P. sativum</i> + <i>P. minor</i> + <i>B. vulgaris</i>	0		8.02 h	7.89 f	10.81 f	8.03 f
<i>P. sativum</i> + <i>P. minor</i> + <i>B. vulgaris</i> + <i>E. sativa</i>	Once spray	20	6.78 g	6.72 f	9.24 e	7.64 ef
		40	5.74 f	3.35 de	8.37 de	5.30 cd
		60	3.65de	2.52 cd	6.70 c	3.88 bc
		80	2.76 cd	1.94 bc	4.38 b	3.10 b
<i>P. sativum</i> + <i>P. minor</i> + <i>B. vulgaris</i> + <i>E. sativa</i>	Twice	20	4.62 e	4.36 e	7.25 cd	5.93 de
		40	3.44 cd	2.67 cd	6.14 c	3.62 bc
		60	2.41 bc	1.86 bc	4.28 b	2.50 b
		80	1.54 b	1.21 ab	3.43 b	2.19 b

scored the maximum yield increment (155.17%) after healthy plant yield increment (185.06%).

Discussion

Allelopathy is one of the modern applied strategies for controlling weeds which aim to minimize the use of chemical herbicides. Allelopathy phenomenon depends on the biochemical interaction between plants (Cheng and Cheng 2016). The results in Table 1 revealed the presence of GSLs (9.55 μmol/g DW) and phenolic compounds (46.5 mg/g DW) in *E. sativa* shoot powder which could be responsible for the allelopathic inhibitory effect on both weeds under investigation. Many researchers such as Messiha et al. 2013, Ahmed et al. 2014, and El-Dabaa et al. 2019 attributed the reduction in dry weight of weeds to the allelopathic effect of GSLs or

phenolic compounds in *E. sativa* seed powder. Al-qasomi et al. 2009 revealed that *E. sativa* and other Brassica vegetables contain GSLs compounds which exert an antioxidant activity. Bell and Wagstaff (2014) ensured that 12 GLS compounds were identified in *E. sativa*. Additionally, 4-mercaptobutyl GSL (glucosativin), 4-methylthiobutyl GSL (glucoerucin), and 4-methyl sulfanylbutyl GSL (glucoraphanin) are the most abundant GSLs in *E. sativa*. These GSLs are hydrolyzed by myrosinase enzyme to create isothiocyanates, nitriles, thiocyanates, epithionitriles, indoles, oxazolidine-2-thiones, ascorbigens, goitrogens, cyanopithioalkanes, epithioalkanes, and flavonols (Hecht 1999; Bones and Rossiter 2006; Bell and Wagstaff 2014). Isothiocyanates is the main produced phytotoxic compound which achieved good results in weed management strategy (Ebrahimi et

Table 3 Effect of spraying different concentrations of *E. sativa* fresh aqueous extract on growth parameters of *P. sativum* plants. (Combined analysis of the two seasons)

Treatments	<i>E. sativa</i> fresh aqueous extract concentration (%)	First sample				Second sample				
		Shoot height (cm)	No. of leaves/plant	No. of branches/plant	Dry weight of plant (g)	Shoot height (cm)	No. of leaves/plant	No. of branches/plant	Dry weight of plant (g)	
<i>P. sativum</i> only	0	34.0 a	18 a	1.4 a	1.93 a	58.0 a	24 a	1.6 a	5.43 a	
<i>P. sativum</i> + <i>P. minor</i> + <i>B. vulgaris</i>	0	20.0 e	13 d	1.0 a	0.91 e	31.0 g	16 b	1.1 b	1.62 h	
<i>P. sativum</i> + <i>P. minor</i> + <i>B. vulgaris</i> + <i>E. sativa</i>	Once spray	20	22.0 e	15ab	1.0 a	1.16 d	36.7 f	22 a	1.2 b	2.43 g
		40	23.0 de	14 cd	1.0 a	1.38 c	42.0 e	22 a	1.2 b	2.95 f
		60	27.3 c	16ab	1.0 a	1.49 c	46.5 de	22 a	1.2 b	3.25 ef
		80	29.5bc	16ab	1.3 a	1.67 b	50.2 bc	22 a	1.3 ab	4.11 d
<i>P. sativum</i> + <i>P. minor</i> + <i>B. vulgaris</i> + <i>E. sativa</i>	Twice spray	20	26.5 cd	16ab	1.1 a	1.42 c	45.5 de	22 a	1.2 ab	3.20 ef
		40	28.0bc	16ab	1.2 a	1.52 bc	48.8 cd	22 a	1.3 ab	3.34 e
		60	30.0bc	17ab	1.3 a	1.90 a	52.5 bc	22 a	1.4 ab	4.57 c
		80	31.5ab	17ab	1.3 a	1.91 a	54.8 ab	24 a	1.4 ab	4.89 b

Table 4 Effect of spraying different concentrations of *E. sativa* fresh aqueous extract on yield and its attributes of *P. sativum* plants. (Combined analysis of the two seasons)

Treatments	<i>E. sativa</i> fresh aqueous extract concentration (%)	No. of pods/plant	Dry weight of pods/plant (g)	No. of seeds/plant	Dry weight of seeds/plant (g)	% of yield increment /plant	
<i>P. sativum</i> only	0	5.60 a	2.81 a	11.0 a	2.48 a	185.06	
<i>P. sativum</i> + <i>P. minor</i> + <i>B. vulgaris</i>	0	2.80 e	0.99 i	5.0 e	0.87 h	0.00	
<i>P. sativum</i> + <i>P. minor</i> + <i>B. vulgaris</i> + <i>E. sativa</i>	Once spray	20	3.50 de	1.35 h	5.97 de	1.53 g	75.86
		40	3.84 cd	1.43 gh	6.47 de	1.58 fg	81.61
		60	3.95 bc	1.65 f	7.58 bc	1.83 e	110.35
		80	4.20 bc	1.91 d	8.1 bc	2.04 cd	134.48
<i>P. sativum</i> + <i>P. minor</i> + <i>B. vulgaris</i> + <i>E. sativa</i>	Twice spray	20	3.84 cd	1.53 g	7.0 cd	1.67 f	91.95
		40	4.68 ab	1.79 e	7.7 bc	1.98 d	127.59
		60	4.87 ab	2.37 c	9.0 bc	2.13 bc	144.83
		80	5.21 a	2.62 b	9.4 ab	2.22 b	155.17

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). Erucin and sativin compounds are the most biologically active isothiocyanate in *E. sativa* (Bennett et al. 2007). Also, in medicine, erucin and erucolin compounds have medicinal and therapeutic properties in *E. sativa* extract (Lamy et al. 2008). Hanafi et al. 2010 attributed the antifungal activity of *E. sativa* to the presence of antioxidant constituents: glucosinolate, flavonoids, carotenoids, and volatile oils. Moreover, *E. sativa* leaves contain kaempferol, quercetin, and isorhamnetin-3,4-diglucoside derivatives as a major group of phenolics. Kaempferol representing 77%–88% of total phenolics is followed by quercetin and isorhamnetin-3,4-diglucoside, representing 9% and 16.3% of the total phenolics, respectively (Weckerle et al. 2001; Pasini et al. 2011).

All these allelochemicals directly affect the physiological processes of plant, i.e., mitotic activity, photosynthesis, nutrient uptake, permeability of cell membrane, and respiration as well as enzyme activity inhibition and protein formation (Rice 1984; Wu et al. 2000; Xuan et al. 2004). Allelochemicals also affect photosynthetic area or assimilation rate which may be in turn cause plant dry matter reduction (Dadkhah 2012). As shown in Table 1, *E. sativa* fresh shoot aqueous extract negatively affected the dry weight of both weeds and this reduction increased by increasing concentration. These findings are in agreement with Hegab et al. (2008) who ensured the direct relationship between the high response to the inhibitory effect of the applied allelopathic extract and the increment in allelochemicals concentration. Additionally, twice spray of the extract was more effective than once and this in accordance with El-Wakeel (2015).

So, the twice spray of *E. sativa* water extract at the highest concentration (80%) achieved the maximum reduction in weeds dry weight.

Tables 2 and 3 show that *P. sativum* growth and yield traits are increased by spraying *E. sativa* water extract. A direct relationship was observed between the concentration of the extract and the positive response of *P. sativum* plants. So, the twice spray of *E. sativa* water extract at the highest concentration (80%) scored the highest *P. sativum* growth and yield parameters and also may be related to reduction of weed competition 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). To this date, few studies have been carried out using the plant material of *E. sativa* to be used as a bioherbicide. Further studies must be explored on the mechanical action of these allelopathic compounds in controlling weeds and is hoped to be applied in the future as a source for natural herbicides under field conditions.

Conclusion

The presence of allelochemicals either GSLs or phenolic compounds in *E. sativa* fresh aqueous extract can be applied as a natural selective bioherbicide to control *P. minor* grassy weed and *B. vulgaris* broad-leaved weed infecting *P. sativum* crop. Twice spray of 80% fresh shoot aqueous extract was the most effective treatment in controlling both weeds under investigation. The efficiency of 80% fresh shoot aqueous extract in controlling weeds reflected in turn on *P. sativum* plants scoring the maximum yield traits following healthy treatment. So, 80% fresh shoot aqueous extract can be tested under field condition as a natural selective bioherbicide.

Abbreviations

B. vulgaris: *Beta vulgaris*; DAS: Days after sowing; *E. sativa*: *Eruca sativa*; GSls: Glucosinolates; *P. minor*: *Phalaris minor*; *P. sativum*: *Pisum sativum*

Acknowledgements

This research was supported by the project of National Research Centre " (Egypt)" Some safe strategies to improve weed control efficiency in some export crops" (No11040202).

Funding

This work was supported and funded by National Research Centre through the project entitled: (Some safe strategies to improve weed control efficiency in some export crops). Project No. (11040202), during in-house projects strategy 2016–2019.

Availability of data and materials

The datasets generated during and/or analyzed during the current study are included in this published.

Authors' contributions

All authors share in every step of this work, and all of them contribute in writing the manuscript. All authors read and approved the final manuscript.

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Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Received: 26 March 2019 Accepted: 10 May 2019

Published online: 06 June 2019

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