Middle East Journal of Applied Sciences
ISSN 2077-4613

Volume : 08 | Issue :02 |April-June| 2018
Pages: 418-425

The allelopathic influence of *Sinapis alba* seed powder (white mustard) on the growth and yield of *Vicia faba* (faba bean) infected with *Orobanche crenata* (broomrape)

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Received: 26 Feb. 2018 / Accepted: 16 April 2018/ Publication date: 26 April 2018

ABSTRACT

Two pot experiments were performed in the greenhouse of National Research Centre, Dokki, Giza, Egypt, in the two successive seasons of 2015/2016 and 2016/2017 to study the allelopathic potentiality of *Sinapis alba* seed powder (Sasp) in comparison to the herbicidal effect of Basamid (Dazomet) treatments on the growth and yield of two *Vicia faba* cultivars infected with *Orobanche crenata.* Treatments were applied by incorporating different concentrations of (Sasp) to the soil at (0, 15, 30 and 45g/kg soil) and Basamid treatments at 0.2 and 0.4g/pot. All Sasp concentrations used as well as Basamid treatments minimized, to great extent, the dry weight of *O. crenata* tubercles/pot (g) infecting both *V. faba* cultivars at 90 days after sowing (DAS) and at harvest. The best treatments for controlling *O. crenata* infecting both *V. faba* cultivars were recorded with 45g/kg soil of Sasp concentration and 0.4g/pot Basamid treatments. Growth as well as yield and yield components of *V. faba* cultivars were significantly increased by all Sasp concentrations used and Basamid treatments when compared to their corresponding infected control. Sasp at 45g/kg soil and Basamid treatment at 0.4g/pot showed the highest increases which exceed than their corresponding healthy control. The allelopathic effect of Sasp due to the presence of allelochemicals, mainly glucosinolates and phenolic contents could play as a natural selective bioherbicide in controlling the parasitic weed *O. crenata* infecting *V. faba* cultivars and increasing the yield.

Key words: Allelopathy, *Sinapis alba,* Glucosinolates, phenolic contents*, Vicia faba, Orobanche crenata*

Introduction

Broomrape is an obligate root parasite belonging to Orobanchaceae. In Egypt, it is a major root parasite infesting faba bean and many other crops such as peas, lentils, chick pea and also several crops (Messiha *et al.,* 2004 and Hershenhorn *et al.,* 2009).
The extent of crop losses due to broomrape infestation depends on some factors such as the extent of infestation, crop sensitivity as well as the different prevailing environmental factors (El-Desoki *et al*., 2003).

Cultural and chemical methods are well known as an integrated approach to manage this parasite such as: A) Crop rotation with non-host crops such as wheat, barley, clover and many winter vegetables (Braun *et al*., 1984) B) Delayed sowing (Yadav *et al*., 2005) C) Two spraying treatments with glyphosate (Punia, 2014 and El-Rokiek *et al*., 2015) D) Hand pulling to the emerging broomrape shoots before flowering followed by burning to prevent the increase of seed bank of the parasite in the soil. (Krishnamurthy and Rao, 1976).

Our previous work at botany department of the National Research Centre showed the allelopathic efficiency of seed powder of some brassicaceae plants as (*Eruca sativa, Raphanus sativa, Brassica rapa* and *Sinapis alba*) in controlling some annual as well as perennial weeds (Messiha *et al*., 2013 ; Ahmed *et al*., 2014, 2016 ; El-Masry *et al*., 2015 and El-Rokiek *et al*., 2017).

The aim of the present investigation is to assess the potentiality of the seed powder of *Sinapis alba* as one of the Brassicaceae family in controlling broomrape infesting faba bean plants.

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Materials and Methods

Two pot experiments were carried out during two successive winter seasons of 2015/2016 and 2016/2017 in the greenhouse of National Research Centre, Dokki, Giza, Egypt. Two *Vicia faba* L. (faba bean) cultivars (Cv. Sakha 1 (a susceptible CV. To *Orobanche crenata* infection) and Giza 843 (a resistant CV. To *O. crenata* infestation) as well as *Sinapis alba* L. (mustard) were obtained from the Agriculture Research Centre, Giza, while parasitic weed seeds of *O.crenata* (broomrape) were obtained from the Weed Control section, Ministry of Agric., Giza, Egypt. Clean seeds of *S.alba* were grinded to fine powder and immediately incorporated to the soil surface before sowing *V. faba* seeds at rate of 15, 30 and 45g/kg soil. The experiment consisted of seven treatments for each *V. faba* cultivar, including two controls (healthy and infected). Each treatment represented by nine pots (30cm diameter) filled with 5kg Nile clay soil. All treatments, except healthy control were infected with *O. crenata* seeds (0.2g/pot) at 5cm depth from the soil surface. The experiment also includes two herbicidal treatments with Basamid (Dazomet) for comparison with the allelopathic effect of *S.alba* treatments. Basamid granules (Tetrahydro -3,5- dimethyl-2H-1,3,5-thiadiazine2-thione) mixed in the soil infected with *O. crenata* at the concentration 0.2 and 0.4g/pot ten days before planting the host seeds. *V. faba* seeds were sown (8 seeds/pot) at 3cm from the soil surface. Two weeks later, the *V. faba* plants were thinned to 4 plants/pot.

Characters studied

Weeds

Three replicates were collected from each treatment at 90 days after sowing (DAS) and at harvest. The number and fresh as well as the dry weight of *O. crenata* tubercles/pot were recorded at the two growth ages.

Vicia faba plants

Plant growth

In both seasons, samples of *V. faba* plants at 60 and 90 DAS were collected from each treatment to determine: plant height (cm), number of leaves/plant, number of branches/plant, fresh and dry weight of shoot/plant (g) as well as fresh and dry weight of root/plant (g).

Yield and yield components

At harvest, samples of *V. faba* plants cultivars were taken from each treatment to determine: number of pods/pot, pod length (cm), weight of pods/pot (g), number of seeds/pod, weight of seeds/ 10 pods (g) as well as weight of 100 seeds (g).

Chemical analysis

Total glucosinolates (µ mol/g DW)

Total glucosinolates were extracted from dry samples of seed powder of *S.alba*. 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 *S.alba* 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

Table (1) showed the potentiality of controlling *O. crenata* in the two *Vicia faba* cultivars (Sakha 1 and Giza 843) by different seed powder concentrations (15 to 45g/kg soil) of *Sinapis alba* (Sasp) as well as Basamid treatments (0.2 and 0.4 g/pot). The data recorded in Table (1) indicated that all Sasp concentrations (15 to 45g/kg soil) and Basamid treatments (0.2 and 0.4 g/pot) significantly reduced *O. crenata* infestation and decreased the number, fresh and dry weight of *O. crenata* tubercles/pot at the two ages of growth (90 DAS and at harvest) in both *V. faba* cultivars as compared to their corresponding infected control. The efficiency in controlling *O. crenata* increased by increasing the applied Sasp concentration used. The maximum reduction in number, fresh and dry weight of *O. crenata* was recorded with the highest Sasp concentration (45g/kg soil) and the higher Basamid treatment (0.4 g/pot) at the two ages of the growth with both *V. faba cultivars* as compared to their corresponding infected control. The maximum rate of reduction of *O. crenata* tubercles dry weight was recorded with the highest *Sasp* concentration (45g/kg soil) which reached to about 93.5 and 99.0% with both *V. faba cultivars* (Sakha 1 and Giza 843), respectively as compared to their corresponding infected control, while the reduction of the same character recorded with Basamid treatment (0.4 g/pot) reached to 98.1 and 97.9% with the two *V. faba cultivars* respectively when compared to their corresponding infected control at harvest.

The best results in controlling *O. crenata* in both *V.faba* cultivars (Sakha 1 and Giza 843) could be achieved by applying the highest Sasp concentration (45g/kg soil) as well as the higher Basamid concentration (0.4 g/pot).

Table 1: Effect of different concentrations of *Sinapis alba* seed powder and Basamid herbicide on *Orobanche crenata* developed on two *Vicia faba* L. cultivars at 90 days after sowing and at harvest. (Combined analysis of two seasons).

Treatments	At 90 days after sowing			At harvest		
	No. of *O. crenata* Tubercles/pot	Fresh wt. of *O. crenata* Tubercles/pot (g)	Dry wt. of *O. crenata* Tubercles/pot (g)	No. of *O. crenata* Tubercles/pot	Fresh wt. of *O. crenata* Tubercles/pot (g)	Dry wt. of *O. crenata* Tubercles/pot (g)
	Sakha 1					
Healthy control	00.00	00.00	00.00	00.00	00.00	00.00
Infected control (I) *	61.00	53.13	7.30	65.00	63.50	10.30
(I) + *Sinapis alba* 15g/kg soil	35.00	34.90	5.85	17.33	31.55	8.03
(I) + *Sinapis alba* 30g/kg soil	22.33	32.40	5.30	10.67	25.03	6.53
(I) + *Sinapis alba* 45g/kg soil	6.67	18.27	4.60	6.67	3.64	0.67
(I) +Basamid 0.2 g/pot	10.00	21.45	6.20	7.67	19.53	5.17
(I) +Basamid 0.4 g/pot	5.00	15.80	4.03	0.67	0.89	0.20
	Giza 843					
Healthy control	00.00	00.00	00.00	00.00	00.00	00.00
Infected control (I) *	51.33	37.22	5.97	55.30	47.25	8.15
(I) + *Sinapis alba* 15g/kg soil	30.00	25.57	4.33	13.00	20.50	3.83
(I) + *Sinapis alba* 30g/kg soil	6.13	9.07	1.73	2.23	2.53	1.00
(I) + *Sinapis alba* 45g/kg soil	1.33	1.43	0.77	0.33	0.21	0.07
(I) +Basamid 0.2 g/pot	9.00	9.83	1.23	4.70	7.95	1.01
(I) +Basamid 0.4 g/pot	2.00	2.55	0.50	0.43	0.27	0.17
LSD at 5%	0.96	0.72	0.67	0.94	1.22	0.53

** I = Infected control*

Vicia faba cultivars growth

The results in Tables (2 and 3) indicated that , most growth characters of the two *V. faba* cultivars (Sakha 1 and Giza 843) such as plant height (cm), number of leaves/plant, number of branches/plant, fresh and dry weight of shoot/plant (g) as well as fresh and dry weight of root/plant (g) at 60 and 90 DAS were significantly increased with all seed powder concentrations used (15 to 45g/kg soil) of *S.alba* as well as Basamid treatments (0.2 and 0.4 g/pot) when compared to their corresponding infected control. The highest significant increases in different growth parameters of the two *V.faba* cultivars were recorded with both 45g/kg soil of (Sasp) concentration and 0.4 g/pot Basamid treatment at the two growth ages when compared to the corresponding control. Not only

these treatments alleviated the harmful effect of *O. crenata* infestation, but also induced significant increases in most growth characters of both *V.faba* cultivars at the two growth ages when compared to their corresponding healthy control. At 90 DAS (Sasp) at 45g/kg soil concentration and Basamid treatment at 0.4 g/pot induced maximum increase in the total dry weight (shoot +root) of *V.faba* cultivar (Sakha 1) which reached to 97.0 and 74.4%, respectively over the corresponding healthy control. The second *V.faba* cultivar (Giza 843) recorded increases in the total dry weight by the same treatments and reached to 36.65 and 20.46%, respectively over the corresponding healthy control.

Table 2: Effect of different concentrations of *Sinapis alba* seed powder and Basamid herbicide on growth parameters of two *Vicia faba* L. cultivars at 60 days after sowing. (Combined analysis of two seasons).

Treatments	Growth parameters						
	Plant height (cm)	No. of leaves/ plant	No. of branches/ plant	F.W. of shoot/plant (g)	F.W. of root/plant (g)	D.W. of shoot/plant (g)	D.W. of Root/plant (g)
	Sakha 1						
Healthy control	39.70	18.83	1.78	18.28	7.83	2.52	1.20
Infected control (I) *	37.00	14.25	1.36	13.00	6.07	1.93	1.13
(I) + *Sinapis alba* 15g/kg soil	38.60	16.80	1.72	15.22	6.83	2.34	1.19
(I) + *Sinapis alba* 30g/kg soil	43.00	19.40	2.00	21.49	9.35	3.40	1.29
(I) + *Sinapis alba* 45g/kg soil	45.50	21.86	2.43	26.14	11.78	3.70	1.50
(I) +Basamid 0.2 g/pot	42.92	19.13	1.83	19.74	8.06	3.04	1.25
(I) +Basamid 0.4 g/pot	44.38	20.33	2.10	23.75	11.30	3.53	1.30
	Giza 843						
Healthy control	45.42	20.50	1.90	21.29	8.65	3.71	1.64
Infected control (I) *	41.75	15.50	1.43	14.85	6.54	2.20	1.35
(I) + *Sinapis alba* 15g/kg soil	43.90	17.90	1.80	17.13	7.19	3.00	1.42
(I) + *Sinapis alba* 30g/kg soil	45.58	20.88	2.10	22.82	9.91	3.72	1.75
(I) + *Sinapis alba* 45g/kg soil	47.50	23.90	2.50	32.23	15.65	3.92	2.01
(I) +Basamid 0.2 g/pot	45.38	19.58	1.87	20.10	8.35	3.58	1.45
(I) +Basamid 0.4 g/pot	46.81	21.33	2.30	28.23	12.42	3.74	1.86
LSD at 5%	1.41	1.49	0.18	1.84	0.84	0.21	0.17

** I = Infected control*

Table 3: Effect of different concentrations of *Sinapis alba* seed powder and Basamid herbicide on growth parameters of two *Vicia faba* L. cultivars at 90 days after sowing. (Combined analysis of two seasons).

Treatments	Growth parameters						
	Plant height (cm)	No. of leaves/ plant	No. of branches/ plant	F.W. of shoot/plant (g)	F.W. of root/plant (g)	D.W. of shoot/ plant (g)	D.W. of Root/ plant (g)
	Sakha 1						
Healthy control	58.30	19.27	1.73	19.09	3.29	3.03	1.19
Infected control (I) *	46.50	17.00	1.58	16.00	2.88	2.60	1.00
(I) + *Sinapis alba* 15g/kg soil	57.91	19.00	1.64	17.91	3.00	2.91	1.17
(I) + *Sinapis alba* 30g/kg soil	58.64	21.64	1.88	35.27	3.73	5.30	1.43
(I) + *Sinapis alba* 45g/kg soil	61.00	22.91	2.50	40.70	5.20	6.56	1.78
(I) +Basamid 0.2 g/pot	58.40	19.64	1.82	21.40	3.36	3.54	1.36
(I) +Basamid 0.4 g/pot	59.18	22.82	1.91	35.64	4.00	5.81	1.55
	Giza 843						
Healthy control	75.50	22.33	2.25	42.58	4.75	6.17	1.55
Infected control (I) *	68.08	19.42	1.61	36.83	3.00	4.57	1.15
(I) + *Sinapis alba* 15g/kg soil	70.45	21.55	1.75	40.19	3.33	5.39	1.36
(I) + *Sinapis alba* 30g/kg soil	80.83	37.80	2.36	43.18	5.25	6.23	2.20
(I) + *Sinapis alba* 45g/kg soil	83.58	45.10	2.72	50.00	6.78	7.77	2.78
(I) +Basamid 0.2 g/pot	70.75	21.83	1.92	41.67	3.83	5.41	1.41
(I) +Basamid 0.4 g/pot	80.70	33.25	2.40	49.50	5.67	7.50	1.80
LSD at 5%	1.90	1.51	0.17	1.81	0.34	0.21	0.17

** I = Infected control*

***Vicia faba* cultivars yield**

The results of yield and yield components of the two *V.faba* cultivars (Sakha 1 and Giza 843) such as number of pods/pot, pod length (cm), weight of pods/pot (g), number of seeds/pod, weight of seeds/10 pods (g) and weight of 100 seeds (g) recorded in Table (4) revealed that, all (Sasp) concentrations used (15 to 45g/kg soil) as well as Basamid treatments (0.2 and 0.4 g/pot) significantly increased most yield parameters of the both *V.faba* cultivars as compared to their corresponding infected control. The best results of the two *V.faba* cultivars yield components recorded with 45g/kg soil (Sasp) and 0.4 g/pot Basamid treatment. Not only, Both applied treatments 45g/kg soil (Sasp) and 0.4 g/pot Basamid alleviated the harmful effect of *O. crenata* infestation, but also increased the plant yield over than their corresponding healthy control. The sensitive *V.faba* cultivar (Sakha 1) achieved maximum increase in the weight of seeds/10 pods (g) and weight of 100 seeds (g) with the highest (Sasp) seed powder concentration (45g/kg soil), reached to 57.0 and 50.0%, respectively, while with 0.4 g/pot Basamid treatment, the increases of the previous yield characters reached to 40.50 and 47.49 %, respectively over their corresponding healthy control. The same trend was obvious with the second *V.faba* cultivar Giza 843, which also showed maximum increases in the same yield characters and reached to 31.28 and 31.30% with 45g/kg soil (Sasp) concentration, while with 0.4 g/pot Basamid treatment, these increases reached to 26.50 and 26.29%, respectively over the corresponding healthy control.

Generally, it is obvious from the data recorded in Tables (2, 3 and 4) that, the growth parameters as well as yield and yield components of *V.faba* cultivar Giza 843 is better than that of *V.faba* cultivar Sakha 1, these results could be attributed to its partial tolerance to *O. crenata* infestation.

Table 4: Effect of different concentrations of *Sinapis alba* seed powder and Basamid herbicide on yield and yield components of two *Vicia faba* L. cultivars at harvest. (Combined analysis of two seasons).

Treatments	Yield and yield components					
	No. of pods/pot	Pod length (cm)	Wt. of pods/pot (g)	No. of seeds/pod	Wt. of seeds/10 pods (g)	Wt. of 100 seeds (g)
	Sakha 1					
Healthy control	4.00	4.60	3.48	2.40	9.28	41.90
Infected control (I) *	2.00	3.50	1.95	1.50	4.50	21.40
(I) + *Sinapis alba* 15g/kg soil	3.00	4.25	2.73	2.35	6.60	36.00
(I) + *Sinapis alba* 30g/kg soil	4.85	5.30	5.83	3.00	9.80	57.10
(I))+ *Sinapis alba* 45g/kg soil	5.70	6.20	6.51	3.60	14.58	62.90
(I) +Basamid 0.2 g/pot	4.50	5.00	4.70	2.75	9.70	48.00
(I) +Basamid 0.4 g/pot	5.00	5.60	6.41	3.34	11.04	61.80
	Giza 843					
Healthy control	5.20	8.10	5.14	3.40	13.84	65.40
Infected control (I) *	3.10	4.60	3.56	1.93	5.27	33.16
(I) + *Sinapis alba* 15g/kg soil	4.60	7.80	5.03	3.25	12.08	54.40
(I) + *Sinapis alba* 30g/kg soil	5.40	8.40	5.89	3.81	16.43	75.00
(I) + *Sinapis alba* 45g/kg soil	7.70	9.00	8.79	4.26	18.17	85.90
(I) +Basamid 0.2 g/pot	4.70	7.60	5.06	3.39	13.61	61.00
(I) +Basamid 0.4 g/pot	6.30	8.90	6.43	3.92	17.51	79.60
LSD at 5%	1.02	1.01	1.00	0.99	1.03	1.86

** I = Infected control*

Table 5: Total glucosinolates (µmol/g dry weight) and Total phenolic contents (mg/g dry weight) in the seed powder of *Sinapis alba*

Material	Total glucosinolates(µmol/g dry weight)	Total phenolic contents(mg/g dry weight)
Sinapis alba seed extract	288.59	43.62

Discussion

Among Orobanche spp. *Orobanche crenata,* is one of the most important parasitic weeds that cause severe yield losses in legume crops (e.g. faba bean, lentil, pea and common vetch) (Aksoy *et al*.,2016). The control of *Orobanche* is exceptionally very difficult due to its underground location as

well as to the complete association with the host plant roots. Moreover when the parasitic shoots become visible on the above ground surface, most of the damage occurred and the usual methods of weed control would often have no effect (Punia, 2014 and El-Rokiek *et al*., 2015). Several approaches were suggested to control *Orobanche* infestation such as A- Inhibiting or preventing the parasitic seeds from germination. B- Stimulating the parasitic seeds to germinate in the absence of the host. C- Preventing the parasite propagules from further growth and development on the host. (Rubiales *et al*., 2009).

Recently, several researches showed the potentiality of using the allelopathic technique as a component of integrated weed management as bioherbicides to suppress weeds in crops (Zaji and Majd, 2011; Ahmed *et al*., 2012, 2016; Messiha *et al*., 2013 ; El-Masry *et al*., 2015 and El-Rokiek *et al*., 2017).

The results of the present study showed that one of the Brassicaceae plant seeds (*S.alba*) possess a useful allelopathic effects in controlling *O. crenata* infected *V.faba* cultivars when added to the soil. Incorporating Sasp to the soil at a rate from 15 to 45g/kg soil reduced the number of *Orobanche* tubercles as well as their fresh and dry weight especially with the highest concentration (45g/kg soil). These results were similar to the effect of synthetic herbicide Basamid treatment at 0.4g/pot (Table 1), since the mode of action of both is the production of isothiocyanates, which effectively could control the growth of parasitic and non-parasitic weeds (Messiha, *et al*., 1993; Khalaf, *et al*., 1994 and Sharara, *et al*., 2011).

In this connection, it is worthy to mention that the allelopathic effects of Brassicaceae plants were attributed to its natural allelochemicals mainly glucosinolates and phenolic compounds (Table 5). Glucosinolates hydrolyzed by endogenous enzyme myrosinase to a number of products. The main breakdown products are isothiocyanates, which are phytotoxic and achieved good results in controlling weeds (Bangarwa *et al.,*2011; Zaji and Majd, 2011; Auger *et al*., 2012; Martinez-Ballesta *et al*.,2013; Messiha *et al*., 2013; Ahmed *et al*., 2014, 2016 ; El-Masry *et al*., 2015 and El-Rokiek *et al*., 2017). Moreover, the results of present research reveal that most growth characters and yield components of both *V.faba* cultivars significantly increased by different Sasp concentrations as well as Basamid treatments (Tables 2, 3 and 4). The best treatments were recorded with both 45g/kg soil Sasp and Basamid treatment at 0.4g/pot. The inhibition of weed growth by chemical or biological means increased the competitive ability of the plant and consequently improved the growth and yield (Ahmed *et al*., 2012, 2014; Messiha *et al*., 2013 ; El-Masry *et al*. ,2015 ; Jursik *et al*., 2015 and Seshadri *et al*., 2015). Moreover, it is worthy to mention also that improving the growth and yield of *V.faba* cultivars may be due to the selectivity of the allelochemicals in their action and the plants in their responses (Einhellig, 1995). Allelochemicals which inhibit the growth of some species at certain concentration may stimulate the growth of same or different species at different concentrations (Ahmed *et al*., 2012, 2014 and 2016; Messiha *et al*., 2013; Baeshen, 2014 and El-Masry *et al*. 2015).

The results of this study as well as our previous work indicate clearly the possibility of using the allelopathic activity of Brassicaceae plant seed powder such as *Eruca sativa*, *Raphanus sativus*, *Brassica rapa* and *Sinapis alba*, as a selective bioherbicide for controlling annual, perennial as well as parasitic weeds accompanied different crops.

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