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Controlling the Grassy weed *Avena fatua* associating wheat plants with the seed powder of two brassicaceae *plants Brassica rapa* and *Sinapis alba*.

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ABSTRACT

The allelopathic efficiency of the seed powder of two Brassicaceae plants *Brassica rapa* and *Sinapis alba* associating wheat plants cv. Giza168 was investigated in the two successive seasons of the years 2014/2015 and 2015/2016 under greenhouse conditions at National Research Centre. The powder of the two Brassicaceae seeds was applied to the soil at 0, 10, 20, 30 and 40g/kg soil. The results indicated reduction in *Avena fatua* by the two Brassicaceae seeds at all concentrations. The reduction increased with the increase in concentration up to 40g/kg soil. The reduction caused by *S. alba* was the higher. On the other hand, this reduction in weed growth was concomitant with increase in growth as well as yield components of wheat specially weight of 1000grains (g) and yield/plant (g). Analysis of the extract of the seed powder of *B. rapa* and *S. alba* indicated that the contents of glucosinolates and total phenols were higher in *S. alba* seed extract than their correspondence in *B. rapa* seed extract. From the results, it can be concluded that seed powder of *B. rapa* or *S. alba* can be used as a promising bioherbicide to control the grassy weed *Avena fatua* associating wheat plants.

Key words: Glucosinolates, Wheat, Brassica rapa, Sinapis alba, Phenolic acids.

Introduction

Allelopathy was explained as any direct or indirect harmful or beneficial effect of one plant on another through the production of chemical compounds (allelochemicals) that are released to the environment (Rice 1984). The allelochemical compounds, glucosinolates occur in many plant species, but are widely produced by species of the Brassicaceae family (Malik *et al*., 2008). Glucosinolate under special conditions, released to environment and affects plant growth (Brown and Morra 1996). The enzyme myrosinase degraded glucosinolates to various hydrolysis products such as isothiocyanates, nitriles and others (Mithen, 2001; Price *et al*. 2005 and Hopkins *et al*., 2009). These specific plant metabolites determine various interactions with other organisms (Halkier and Gershenzon, 2006) and might be one of the key factors including the invasion success of Brassicaceae species. Previous work using extracts from glucosinolate-producing plant species have indicated inhibition of other species through reduced germination, reduced seedling emergence and reduced size, as well as delayed seed germination (Brown and Morra 1996; Al-Khatib *et al*., 1997; Norsworthy *et al*. 2007 and Malik *et al*. 2008). *Brassica spp*. has been reported as allelopathic or weed suppressive, mustards form can be successful invaders of native grasslands (Siemens *et al*., 2002). Water extracts of black mustard (*Brassica nigra* L.) plant parts such as leaf, stem, flower and root inhibited seed germination and seedling growth of alfalfa, lentil, wild oat and radish (Turk and Tawaha 2002&2003 and Turk *et al*., 2005). Extracts from fresh mustard plant leaves and stems solutions showed inhibitory effects on seed germination and growth of wheat (Saeedipour, 2010). In other work, the number of annual bluegrass (*Poa annua* L.) seedlings was reduced to 98%, as well as the number of common chickweed (*Stellaria media* L.) seedlings reduced to 74%, by using mustard seed meal up to 450 gm^{-2} (Boydston and Anderson, 2008).

In the previous work from our Institute National Research Centre of Egypt, seed powder of *Eruca sativa*, *Brassia rapa*, *Raphanus sativus* were used as selective bioherbicides to control the perennial troublesome weed *Cyperus rotundus* associating *Zea mays*, *Beta vulgaris* associating *Pisum*

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sativus, *Portulaca oleracea* and *Corchorus olitorius* associated *Phaseolus vulgaris* and *Echinochloa crus-galli* associated sunflower (Messiha *et al*., 2013; Ahmed *et al*., 2014 & 2016 and El-Masry *et al*., 2015).

The objective of this work is to study the allelopathic effect of the two Brassicaceae seeds *Sinapis alba* and *Brassica rapa* on controlling *Avena fatua* and the reflection of this effect on wheat growth.

Materials and Methods

Two pot experiments were carried out during two successive seasons of the years 2014/2015 and 2015/2016 in the greenhouse of the National Research Centre, Dokki, Giza, Egypt. The two experiments for studying the possibility of controlling aggressive weed, *Avena fatua* growing in wheat using seeds of *Sinapis alba* or *Brassica rapa*. Wheat (*Triticum aestivum* var. Giza 168) grains, seeds of *Sinapis alba* and *Brassica rapa* were obtained from Agricultural Research Centre, Giza, Egypt. Seeds of *Sinapis alba* and *Brassica rapa* were grinded to fine powder, after that the powder was immediately mixed with the soil surface before sowing at the rate of 0, 10, 20, 30 and 40g/kg soil. Wheat grains were sown 2cm deep in pots filled with 7kg of soil. The pots were infested with a constant number of seeds of *Avena fatua*. Weed seeds were sown simultaneously and mixed thoroughly at a depth of 2 cm in the soil. Thinning of wheat seedlings was done after 2 weeks so that 3 homogeneous seedlings were left per pot. The experiment consisted of 10 treatments including control; each treatment consisted of 9 pots. All pots were distributed in a complete randomized design. Three replicates were collected from each treatment 45 and 60 days after sowing and at harvest.

Weeds and Wheat data

Weeds

In each season, weed samples were taken from each of the three pots at the vegetative stage and at heading (all weed samples in each pot were pulled up). The fresh weight of *Avena fatua* was recorded then were oven dried at 60°C for determination of dry weight (g/pot).

Wheat data

Three plants in each pot that were taken for recording plant height, number of leaves, as well as dry weight (g/plant) were recorded 45 and 60 days after sowing. At harvest, spike length, number of spikelets/spike, weight of grains/spike, grain yield (g/plant) and 1000- grain weight (g) were determined.

Chemical analysis

Total glucosinolates (µ mol/g DW)

Total glucosinolates were extracted from dry samples of seed powder of both *Sinapis alba* and *Brassica rapa,* Glucosinolates were measured by determining the liberated glucose which released during hydrolysis by myrosinase enzyme defined by Nasirullah and Krishnamurthy (1996).

Total phenolic contents (mg/g DW)

Total phenolic contents of both *Sinapis alba* and *Brassica rapa* seed 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 the least significant difference (LSD) at 5% significant level.

Results

Effect of seed powder on *Avena fatua*

Table 1 shows significant reduction in the fresh and dry weight of the weed, *Avena fatua* due to using seed powder of *Brassica rapa* or *Sinapis alba* up to 40g/kg soil. The reduction increased with increasing concentration. The most significant reduction in both fresh and dry weight of *A. fatua* was recorded by using 40g/kg soil of either of the two materials. In general, using *S. alba* seed powder was more inhibitory on weed growth than of *B. rapa* as compared to the infected control. The rate of reduction in dry weight of the weed (growth) increased with the experimental period. At the first stage (wheat vegetative growth) the reduction in the weed reduced to 38.2% and 55.3% of infected control by using 40g/kg soil of *B. rapa* and *S. alba* respectively. The corresponding results at the second stage (heading stage) were 46.1 and 60.6%.

Table 1: Effect of seeds powder of *Sinapis alba* and *Brassica rapa* on the growth of *Avena fatua* associated wheat growth 45 and 60 days after sowing.

Treatments			Weed growth			
			45 days after sowing		60 days after sowing	
Plants	Seed powder	Rate (g/kg soil)	Fresh Weight (g)	Dry weight (g)	Fresh weight (g)	Dry weight (g)
Triticum aestivum only	……	…….	……	……	……	……
T. aestivum + A. fatua	……	…….	16.466	8.033	97.667	22.333
T. aestivum + A. fatua	*B. rapa*	10	14.766	7.016	90.133	20.710
		20	12.633	6.833	74.867	16.966
		30	8.900	6.333	56.266	13.450
		40	6.500	4.967	43.366	12.033
T. aestivum + A. fatua	*S. alba*	10	13.933	7.000	79.300	18.883
		20	9.900	6.396	71.800	16.133
		30	8.166	5.266	54.466	12.200
		40	5.033	3.593	41.833	8.800
LSD at 5 %			0.779	0.302	2.851	1.121

Effect of seed powder on wheat growth

The data in Table 2 reveal significant increases in different growth characters in wheat plants due to incorporation of seed powder of *B. rapa* or *S. alba* as compared to the infected control. The increase in wheat growth was concentration dependent. The increase in concentration of the added materials increased wheat growth. *B. rapa* or *S. alba* increased plant height, number of tillers, number of leaves, as well as fresh and dry weight. In comparison to the infected control, the increase in growth was persistent during the experimental period. Maximum significant increase in wheat growth (dry weight) was realized by using 40g of *S. alba* followed by 40g/kg soil of *B. rapa* at both vegetative and heading stages. These results were 76.8, 70.4, 49.1 and 60.2%, respectively over the infected control.

Effect of seed powder on wheat yield

The results in Table 3 indicate that yield and yield components of wheat increased significantly over infected control by using seed powder of *B. rapa* or *S. alba* at different concentrations up to 40g/kg soil of either of the two materials. Adding seed powder of *B. rapa* or *S. alba* to the soil at all concentrations (10-40g/kg soil) caused significant increase in spike length, number of spikes/ plant as well as number of spikelets/spike recording observable significant increase over infected control by using 40g/kg soil of seed powder of *S. alba*. The results of weight grains/spike, weight of 1000 grains as well as grain yield /plant follow the same trend. Maximum increase in grain yield/plant exceeded that of infected control by 85.2 and 64.6%. These results were obtained by application of 40g of *S. alba* and40g /kg soil of *B. rapa,* respectively.

Table 2: Effect of seeds powder of *Sinapis alba* and *Brassica rapa* on some growth characters of wheat plants cv. Giza 168.

Treatments			Wheat growth				
			45 days after sowing				
Plants	Seed powder	Rate (g/kg soil)	Plant height (cm)	No. of tillers/plant	No. of leaves/plant	Fresh weight (g)	Dry weight (g)
Triticum aestivum only	………	……	51.00	2.50	12.00	3.50	1.633
T. aestivum + *A. fatua*	…….....	……	41.83	1.38	6.00	2.07	0.822
T. aestivum + *A. fatua*	*B. rapa*	10	45.33	1.50	6.66	2.12	0.843
		20	45.33	1.55	6.83	2.33	0.973
		30	46.00	1.86	7.30	2.68	1.106
		40	48.33	2. 16	8.66	2.89	1.266
T. aestivum + *A. fatua*	*S. alba*	10	45.33	1.53	6.77	2.27	0.917
		20	46.00	1.63	7.00	2.40	1.033
		30	46.00	2.06	8.27	2.76	1.163
		40	51.00	2.13	10.00	3.21	1.453
LSD at 5%			1.71	0.28	0.46	0.31	0.057
			60 days after sowing				
Triticum aestivum only	………	……	70.33	4.66	16.66	30.50	6.453
T. aestivum + *A. fatua*	…….....	……	52.50	1.93	8.66	14.33	3.413
T. aestivum + *A. fatua*	*B. rapa*	10	55.00	2.50	8.86	16.63	3.816
		20	56.66	3.16	12.16	19.16	4.233
		30	59.33	3.50	12.26	21.96	4.943
		40	61.33	3.66	13.83	25.46	5.466
T. aestivum + *A. fatua*	*S. alba*	10	55.66	2.66	10.00	17.83	4.106
		20	57.33	3.33	12.23	21.13	4.530
		30	60.00	3.60	12.50	23.50	5.086
		40	63.66	4.16	16.03	27.96	5.816
LSD at 5%			2.14	0.47	0.61	1.61	0.223

Table 3: Effect of seeds powder of *Sinapis alba* or *Brassica rapa* on yield and yield components of wheat cv. Giza 168

Treatments			Wheat yield and yield components					
Plants	Seed powder	Rate (g/kg soil)	Spike length	No. of spike/plant	No .of spikelets /spike	Weight of grains/ spike (g)	Weight of grains/ plant (g)	Weight of 1000 grains (g)
Triticum aestivum only		……	12.50	4.00	16.23	1.926	5.69	41.500
T. aestivum + *A. fatua*		……	6.77	2.50	11.03	0.592	2.57	23.533
T. aestivum + *A. fatua*	*B. rapa*	10	8.43	2.57	11.40	0.801	2.71	25.600
		20	9.36	2.66	12.56	0.926	2.83	26.666
		30	10.63	3.00	12.75	1.108	3.32	28.400
		40	11.20	3.33	13.50	1.338	4.23	32.667
T. aestivum + *A. fatua*	*S. alba*	10	9.33	2.65	11.46	0.906	2.75	26.133
		20	10.50	3.00	12.73	1.105	3.02	27.966
		30	11.03	3.17	13.00	1.309	3.35	31.500
		40	11.60	3.50	14.00	1.554	4.76	36.083
LSD at 5%			0.42	0.31	0.41	0.066	0.21	1.309

Changes in Total Glucosinolates and Total Phenolic Contents in B. rapa and S. alba

Analysis of the seed powder of *B. rapa and S. alba* reveal that the content of total glucosinolates in *S. alba* seed extract was higher than that of *B. rapa* seed powder. Moreover, the total phenolic content of *S. alba* seed water extract was more than one and half of its correspondence in *B. rapa* seed water extract (Table 4).

Table 4: Total glucosinolates (µmol/g dry weight) and Total phenolic contents (mg/g dry weight) in the seed extract of *Brassica rapa* and *Sinapis alba*

Materials	Total glucosinolates (µmol/g dry weight)	Total phenolic contents (mg/g dry weight)
Brassica rapa seed extract	252.70	28.41
Sinapis alba seed extract	288.59	43.62

Discussion

Allelopathic potentiality is found in Brassicaceae family. All the members of Brassicaceae family are known to be allelopathic that have been reported as weed suppressive (Siemens *et al*., 2002). The members of this family are glucosinolates producing plants. Glucosinolates are secondary metabolites containing sulfur and nitrogen, are enzymatically hydrolyzed by myrosinase in the presence of water to form isothiocyanates, the active allelochemicals (Petersen *et al*. 2001 and Price *et al*. 2005).

The two Brassicaceae seeds powder *B. rapa* or *S. alba* were suppressive to the grass weed *Avena fatua* with varying degree according to the concentration and species (Table 1). The seeds of *S. alba* were more suppressive as compared to the infected control. The increase in the concentrations of either of the two materials increased the rate of reduction which reached to 60.6% in pots treated with 40g/kg of *S. alba* seeds as compared to the infected control. Previous work on extracts from glucosinolate-producing plant species have indicted inhibition of other species through reduced germination, reduced seedling emergence as well as delayed seed germination (Al-Khatib *et al*. 1997, Norsworthy *et al*. 2007 and Malik *et al*. 2008). Boydston *et al.* (2008) cited that application of mustard seed meal to the soil surface at 113, 225 and 450 $g \cdot m^{-2}$ reduced the number of annual bluegrass (*Poa annua* L.) seedlings by 60%, 86% and 98%, respectively and the number of common chickweed (*Stellaria media* L.) seedlings by 61%, 74% and 73%, respectively, at 8 weeks after treatment. Similar results were obtained by Messiha *et al*., 2013 on *Cyperus rotundus*, Ahmed *et al*., 2014 and El-Masry *et al*., 2015. These results were confirmed by Ahmed *et al*. (2016). Glucosinolates play a key role for weed suppression as they can be converted to the corresponding isothiocyanates by the enzyme myrosinase (Petersen *et al*., 2001). Hence, the phytotoxicity on weeds may be arised from thiocyanate ion that released from glucosinolates in the presence of myrosinase enzyme and water (Borek and Morra 2005). Confirming this conclusion the pesticide basamid that breaks down in the soil to methyl isothiocyanate has a broad spectrum of effectiveness against weeds (Messiha *et al*., 1993, Khalaf *et al*., 1996 and Sharara *et al*., 2011). Subsequently, The higher level of suppression of *Avena fatua* by *S. alba* may be resulted from higher contents of glucosinolates (Uremis *et al*., 2009 and Ahmed *et al.,* 2016) and herein in our work (Table 4). Furthermore, determination of internal contents of total phenols indicated that phenol contents in seeds of *S. alba* was higher than its correspondence in *B. rapa*. This may be added reason for weed suppression (El-Rokiek *et al*., 2014).

However, the reduction in growth of *Avena fatua* was accompanied by enhancement of wheat growth as have been reported by many workers, weed suppression decreased the competitive ability of weeds against the main crop (El-Metwally *et al*. 2010, El-Rokiek *et al*., 2014 and 2015). Moreover, controlling *Avena fatua* in wheat increased wheat growth and yield (El-Rokiek *et al*., 2012&2014). So, in accordance with El-Rokiek *et al*. (2012) the increase in wheat growth was coupled with increase in yield and its components. El-Rokiek *et al*. (2014) confirmed this conclusion. So, the results indicated the increase in spike length, number of spikes /plant, number of spikelets /spike, weight of 1000grains as well as wheat yield/plant (Table 3).

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