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Evaluating the Allelopathic Efficiency of the Seed Powder of *Raphanus Sativus* L. in Controlling Some Weeds Associating *Phaseolus Vulgaris* L.

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Keywords: corchorus olitorius, abelmoschus esculentus, portulaca oleracea, common bean, allelopathy, raphanus sativus, glucosinolates content, phenolic content.

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Ahmed, S.A.A. [α]; R.R. El-Masry [σ], Nadia K. Messiha [ρ] & Kowther G. El-Rokiek [Ѡ]

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I. Introduction

Phaseolus vulgaris is one of the major grain legumes mostly grown in the world as a source of proteins for the human. Like many other grain legumes and crop plants, weeds grown associating these crop plants caused high percentage of yield loss (El-Rokiek *et al*., 2013 and 2016 and Ahmed *et al*., 2014). Due to the competition of weeds for nutrients, water, light, etc. different herbicides were used for weed control to decrease its competition and consequently increase the crop yield (Abdelhamid and El-Metwally, 2008 and El-Rokiek *et al*., 2013).

However, the continuous use of herbicides caused problems because of environmental, toxicological or economic purposes associated with their use (Duke *et al.,* 1999). So, the need for alternative natural herbicides becomes important to reduce the continuous use of synthetic herbicides and for the development of safer, alternative crop protectants (Mahmood and Cheema, 2004). Many crop, trees and weed species have been reported to possess allelopathic activity on the growth of other plant species (Jabran *et al.,* 2010). Allelopathic substances (secondary plant metabolites) are present in many plant tissues e.g., leaves, stems, flowers, fruits, seeds and roots (Mahmood *et al*., 2010 and Ahmed *et al*., 2014). Allelochemicals are released to the environment from plants through degradation, volatilization, leaching from plant leaves, and from root exudation (Petersen *et al*., 2001 and Price *et al*., 2005).

The allelopathic potentiality of Brassicaceae plants in suppressing the growth of different types of weeds (annual, perennial and parasitic) have been recently reported from the Botany department of the National Research Centre of Egypt (Messiha *et al.* 2013& 2018; Ahmed *et al.*2014& 2016; El-Masry *et al.* 2015 and El-Rokiek *et al.* 2017).

Brassicaceae plant tissues are known to contain considerable amounts of glucosinolates in their tissues which could be easily hydrolyzed in the soil to phytotoxic products such as isothiocyanates, nitriles, thiocyanates, epithionitriles and oxazolidines (Bones and Rossiter 2006). Isothiocyanates is the main phytotoxic product (Fahey *et al*., 2001; Zaji and Majd, 2011 and Martinez-Ballesta *et al*., 2013) and have distinct pesticidal activities (Velasco *et al*., 2008).

The aim of the present work is to continue our strategy to evaluate the allelopathic efficiency of another member in the Brassicaceae family (*Raphanus sativus*) in controlling weeds associating *Phaseolus vulgaris* plants.

II. Materials And Methods

Two pot experiments were carried out during two successive seasons of (2016) and (2017) in the greenhouse of the National Research Centre, Dokki, Giza, Egypt. Common bean (*Phaseolus vulgaris*) cv.Giza 4 seeds as well as seeds of Radish (*Raphanus sativus)*, Jew's mallow(*Corchorus olitorius*), Okra (*Abelmoschus esculentus*) and Purslane (*Portulaca oleracea*) were obtained from Agriculture Research Centre, Giza, Egypt.

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Clean seeds of *R. sativus* were ground to a fine powder after that the powder was immediately incorporated in the soil surface before sowing *P. vulgaris* seeds at rate of 0. 15, 30, 45 and 60 g/kg soil. In the same time, the seeds of *P. vulgaris, C. olitorius*, *A. esculentus* and *P. oleracea* were sown 2cm deep in plastic pots filled with 2kg of soil. The experiment consisted of 18 treatments including control; each treatment consisted of 8 replicates. All pots were distributed in a complete randomized design. The normal cultural practices of growing *P. vulgaris* plants were followed especially fertilization and irrigation.

a) Characters studied

Weeds

Three replicates were collected from each treatment at 40 days after sowing (DAS) and at harvest. The dry weight of each weed species was recorded (g /pot) at the two growth ages and the percentage of reduction was calculated as compared to control.

b) Phaseolus vulgaris plants

Plant growth

Samples of *P. vulgaris* plants at 40DAS and at harvest were collected from each treatment to determine: plant height (cm), number of leaves/plant and dry weight of plant (g).

c) Yield and yield components

At harvest, samples of *P. vulgaris*plants were taken from each treatment to determine: A- Green yield, i.e. number of pods/plant, and weight of pods/plant (g) B- Dry yield, i.e. number of pods/plant, weight of seeds/plod (g), weight of 100 seeds (g) and weight of seeds/plant (g).

d) Chemical analysis

Total glucosinolates (µ mol/g DW)

Total glucosinolates were extracted from dry samples of seed powder of *R. sativus*. 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).*

e) Total phenolic contents (mg/g DW)

Total phenolic contents of *R. sativus* seeds were determined colorimetrically using Folin and Ciocalteu phenol reagent according to the method defined by *Snell and Snell (1953).*

f) 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.

III. Results

a) Weeds growth parameters

The results in Table (1) showed the effect of incorporating different rates from the seed powder of *Raphanus sativus* (0. 15, 30, 45 and 60 g per pot) on the dry weight of the three different weeds, i.e. *Corchorus olitorius*, *Abelmoschus esculentus* as well as *Portulaca oleracea* associating the growth of *Phaseolus vulgaris* after 40 DAS and at harvest. The results show that the competition between *P. vulgaris* and each weed caused a significant decrease in the dry weight of weeds after 40 DAS and lasted till harvest. It is worthy to mention that incorporating the seed powder of *R. sativus* at the rate of 30g/kg soil showed its bioherbicidal efficiency in controlling the different three weeds which reached more than 90% control. Higher amounts of (RSSP) also showed a higher bioherbicidal effect which reached to 100% of the three weeds with the highest rate (60g/kg soil).

b) Phaseolus vulgaris growth

The results in Table (2) indicated the effect of incorporating different amountsof the seed powder of *R. sativus* on different growth parameters of *P. vulgaris* as Plant height (cm), No. of leaves/plant and Dry weight of plant (g) at 40 DAS and at harvest. The results indicated that all growth parameters of *P. vulgaris* were significantly increased by different concentrations of*R. sativus* seed powder (Rssp). Not only, all treatments of Rsspconcentrations (15 to 60 g/kg soil) alleviated the harmful effect of the three weeds (*C. olitorius*, *A. esculentus* and *P. oleracea*) associating *P. vulgaris* plants, but also induced significant increase in all *P. vulgaris* growth parameters at the two ages of growth (40 DAS and at harvest) as compared to the corresponding untreated controls. The maximum increase in all *P. vulgaris* growth parameters at 40 DAS and at harvest was recorded with 30g/kg soil Rssp concentration when compared to corresponding controls. The increase in the dry weight of *P. vulgaris* associated with *C. olitorius* or *A. esculentus* or *P. oleracea* at 40 DAS reached to about 10.0, 15,7 and 4.8 %, while at harvest were about 22.6, 31.4 and 16.2 %, respectively over the dry weight of *P. vulgaris* free from weeds as shown from the results in Table (2).

c) Phaseolus vulgaris yield

A- Green yield

The results of the green yield of *P. vulgaris* associated with *C. olitorius* or *A. esculentus* or *P. oleracea* recorded in Table (3) cleared that different concentrations of Rssp (15 to 60 g/kg soil) induced a significant increase in the number of green pods/plant and weight of pods/plant of *P. vulgaris* when compared to their corresponding controls. Maximum increase in number and weight of green pods/plant of *P. vulgaris*

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was recorded with 30 g/kg soil Rssp treatment as compared to their corresponding controls. Not only this treatment (RSSP at 30 g/kg soil) alleviated the reduction caused by the effect of *C. olitorius* or *A. esculentus* or *P. oleracea* on the weight of green pods/plant that reached to about 72.2, 68.3 and 74.8%, respectively, but also increased this character to about 7.6, 9.9 and 3.7%, respectively over their corresponding healthy control.

B- Dry yield

Dry yield and yield components of *P. vulgaris* associated with *C. olitorius* or *A. esculentus* or *P. oleracea* cleared that all Rssp concentrations (15 to 60 g/kg soil) induced significant increase in the different yield parameters (number of dry pods/plant, weight of seeds/plod, weight of 100 seeds as well as weight of seeds/plant) as compared to corresponding untreated controls (Table 3).The best treatment was recorded with 30g/kg soil Rssp concentration that achieved the highest increase in all *P. vulgaris* plant yield parameters. The increase in weight of seeds/plant of *P. vulgaris* associated with *C. olitorius* or *A. esculentus* or *P. oleracea* reached to about 25.9, 29.4 and 19.6 %, respectively over their corresponding control free from weeds.

IV. Discussion

The allelopathic compounds (allelochemicals) released from plants into the environment, as a result of secondary metabolites, include a variety of compounds, often attract or repel, nourish or poison to other organisms. Allelochemicals like phenolic compounds, flavonoids, terpenoids, alkaloids, amino acids and glucosinolates were found in allelopathic plants *(Fahey et al., 2001; Velasco et al., 2008 and Ahmed et al., 2012).*

Recently, several researches showed the potentiality of using the allelopathic technique as a component of integrated weed management as bioherbicide to suppress weeds in crops *(Zaji and Majd, 2011; Ahmed et al., 2012, 2016; Messiha et al., 2013 & 2018; El-Masry et al., 2015 and El-Rokiek et al., 2017).* Also, weed management systems seek biological solutions to minimize the harmful effects resulted from the use of herbicides in agricultural systems. Therefore, allelochemicals could be considered as an important tool for sustainableweed control management *(El-Metwally et al., 2014 and El-Wakeel, 2015).*

The results of the present investigation reveal to a great extent a significant reduction in the three weeds, i.e. *Corchorus olitorius*, *Abelmoschus esculentus* and *Portulaca oleracea*growth after the incorporation of (Rssp) to the soil till 40 (DAS). Complete reduction of all weeds recorded by the higher concentration (60g/kg soil) of (Rssp) at harvest (Table 1). The previous results showed that Brassicaceae family has allelopathic potential on the growth of other plants *(Petersen et al., 2001; Messiha et al., 2013 & 2018; Ahmed et al., 2014 & 2016; Bashen, 2014 and El-Masry et al., 2015).* 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 4). 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 *(Zaji and Majd, 2011; Martinez-Ballesta et al., 2013; Messiha et al., 2013 & 2018; Ahmed et al., 2014, & 2016; El-Masry et al., 2015 and El-Rokiek et al., 2017).* Moreover*, Petersen et al., 2001 and Uremis et al., 2009* reported that the allelopathic effect of *Raphanus sativus L.* could also be due to the presence of p-hydroxy benzoic acid in addition to is othiocyanates.

On the other side (Rssp) treatments not only achieved to great extent good results in controlling the three weeds, i.e. *C. olitorius*(C_3 plant), *A. esculentus* (C_3 plant) and *P. oleracea* (C_4plant) but also increased *P. vulgaris* growth (Table 2) and consequently improved its green and dry yield (Table 3). Several workers found that the inhibition of weed growth increased the competitive ability of the crop plant and consequently improved growth and yield *(Abdelhamid and El-Metwally, 2008; Ahmed et al., 2012 & 2014; El-Rokiek et al., 2013 and El-Masry et al., 2015).*

It is worthy to mention that (Rssp) at 30g/kg soil was the best treatment in controlling the three weeds (*C. olitorius*, *A. esculentus* and *P. oleracea*) and consequently increased the growth and yield of *P. vulgaris* as compared to corresponding controls, this may be due to the selectivity of allelochemicals in their action and plants in their responses *(Einhellig, 1995).* Allelochemicals which inhibit the growth of some species at certain concentrations may stimulate the growth of same or different species at different concentrations *(Ahmed et al., 2012 & 2014; Messiha et al., 2013 & 2018 and Bashen, 2014).*

The results of the present work indicate clearly the possibility of using allelopathic activity of *Raphanus sativus* seed powder as a selective bioherbicide for controlling annual weeds.

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Table (1): Effect of incorporating different concentrationsof the seed powder of *Raphanus sativus* on dry weight of *Corchorus olitorius*, *Hibiscus esculentus* as well as *Portulaca oleracea* (g/pot). (Combined analysis of the two seasons).

Treatments		At 40 days after sowing		At harvest	
Plants	Concentrationsof *Raphanus sativus* (g/kg soil)	Dry weight of weed (g/pot)	% of reduction	Dry weight of weed (g/pot)	% of reduction
Corchorus olitorius	………	11.96	00.0	22.85	00.0
Corchorus olitorius + *Phaseolus vulgaris*	………	7.45	37.7	19.34	15.4
Corchorus olitorius + *Phaseolus vulgaris*	15 g	2.41	79.8	3.62	84.2
	30 g	1.63	86.4	1.95	91.5
	45 g	0.82	93.1	1.10	95.2
	60 g	0.43	96.4	0.00	100
Abelmoschus esculentus	………	13.42	00.0	25.16	00.0
Abelmoschus esculentus + *Phaseolus vulgaris*	………	9.69	27.8	21.04	16.4
Hibiscus esculentus + *Phaseolus vulgaris*	15 g	3.62	73.0	4.15	83.5
	30 g	1.91	85.8	2.27	91.0
	45 g	0.90	93.3	1.41	94.4
	60 g	0.62	95.4	0.00	100
Portulaca oleracea	………	16.54	00.0	29.40	00.0
Portulaca oleracea + *Phaseolus vulgaris*	………	12.37	25.2	24.90	15.3
Portulaca oleracea + *Phaseolus vulgaris*	15 g	3.95	76.1	8.30	71.8
	30 g	2.06	87.5	6.50	77.9
	45 g	1.08	93.5	1.82	93.8
	60 g	0.86	94.8	0.00	100
LSD at 5%		0.78		1.21	

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Table (2): Effect of incorporating different concentrationsof the seed powder of *Raphanus sativus* on different growth parameters of *Phaseolus vulgaris.*(Combined analysis of the two seasons).

Treatments		Growth parameters					
		At 40 days after sowing			At harvest		
Plants	Concentrationsof *Raphanus sativus* (g/kg soil)	Plant height (cm)	No. of leaves/ plant	Dry weight of plant (g)	Plant height (cm)	No. of leaves/p lant	Dry weight of plant (g)
Phaseolus vulgaris only	………..	33.2	6.2	2.10	42.5	11.0	4.21
Phaseolus vulgaris+*Corchorus olitorius*	………..	24.0	3.3	1.10	33.2	5.5	1.79
Phaseolus vulgaris +*Corchorus olitorius*	15 g	32.0	6.0	1.73	39.2	10.3	3.76
	30 g	35.0	7.8	2.31	49.6	12.6	5.16
	45 g	31.0	5.7	1.61	38.9	10.0	3.57
	60 g	28.0	5.0	1.33	35.8	7.4	2.36
Phaseolus vulgaris +*Abelmoschus esculentus*	………..	26.0	3.8	1.18	34.0	6.0	1.95
Phaseolus vulgaris + *Abelmoschus esculentus*	15 g	33.0	6.4	1.84	41.8	10.7	4.13
	30 g	36.0	8.0	2.43	51.5	13.5	5.53
	45 g	33.5	6.7	1.80	44.0	11.5	4.58
	60 g	29.7	4.4	1.50	37.2	9.0	3.29
Phaseolus vulgaris + *Portulaca oleracea*	………..	22.0	2.7	1.04	32.6	5.2	1.55
Phaseolus vulgaris + *Portulaca oleracea*	15 g	30.0	5.5	1.56	38.0	9.6	3.40
	30 g	34.0	7.3	2.20	47.3	12.0	4.89
	45 g	29.0	4.6	1.45	36.4	8.0	3.07
	60 g	26.5	4.1	1.24	35.0	7.0	2.15
LSD at 5%		1.91	0.88	0.81	1.93	1.18	1.08

Table (3): Effect of incorporating different concentrations of the seed powder of *Raphanus sativus* on yield and yield components of *Phaseolus vulgaris.*(Combined analysis of the two seasons).

Treatments		Yield and yield components					
		Green yield		Dry yield			
Plants	Concentrations of *Raphanus sativus* (g/kg soil)	No.of green pods/ plant	Weight of green pods/plant (g)	No.of dry pods/ plant	Weight of seeds/pod (g)	Weight of 100 seeds (g)	Weight of seeds /plant (g)
Phaseolus vulgaris only	……..	7.20	13.74	7.36	1.64	29.7	10.12
Phaseolus vulgaris + *Corchorus olitorius*	……..	3.30	3.82	3.16	0.86	21.0	3.45
Phaseolus vulgaris + *Corchorus olitorius*	15 g	6.30	10.10	6.14	1.49	28.9	9.51
	30 g	8.32	14.79	8.01	1.89	33.2	12.74
	45 g	6.00	10.30	5.95	1.38	28.0	9.36
	60 g	4.36	6.50	3.99	1.10	23.8	5.25
Phaseolus vulgaris + *Abelmoschus esculentus*	……..	3.52	4.36	3.23	0.95	21.8	3.97
Phaseolus vulgaris + *Abelmoschus esculentus*	15 g	6.90	11.25	6.61	1.56	31.1	10.60
	30 g	8.65	15.10	8.20	1.93	34.3	13.10
	45 g	7.60	12.32	6.95	1.70	31.6	11.42
	60 g	5.51	7.42	4.97	1.22	25.3	7.83
Phaseolus vulgaris + *Portulaca oleracea*	……..	2.95	3.46	2.96	0.79	20.0	2.91
Phaseolus vulgaris + *Portulaca oleracea*	15 g	5.63	8.80	5.72	1.26	27.0	8.74
	30 g	8.00	14.25	7.84	1.77	32.7	12.10
	45 g	5.10	8.40	4.19	1.19	24.3	7.53
	60 g	4.20	5.80	3.62	1.00	23.0	4.06
LSD at 5%			1.03	1.16	1.15	0.65	2.08

Table (4): Total glucosinolates (μmol/g dry weight) and Total phenolic contents (mg/g dry weight) in the seed powder of *Raphanus sativus.*

Material	Total glucosinolates (μmol/g dry weight)	Total phenolic contents (mg/g dry weight)
Seed powder of *Raphanus sativus*	688.54	69.50