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Impact of Phenolic Compounds and Glucosinolates of Two Brassicaceae Seeds Powder as natural herbicides.

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Abstract

Phenolic compounds and glucosinolates have been improved as natural bioherbicides for controlling weeds. So, two pot experiments were conducted. Both experiments aimed to study the allelopathic potentiality of *Eruca sativa* (ESSP) and *Sinapis alba* seed powder (SASP) on *Lupinus albus* and associated weeds *Phalaris minor* and *Malva parviflora*. Treatments were applied as follow: Six treatments were applied by sole incorporation of ESSP (10, 20, 30 and 40g/pot) and (SASP) at (5 and 10 g/pot). Other eight treatments were applied by mix incorporation of ESSP at the aforementioned successive concentrations with SASP at (5 and 10 g/pot). Additionally, two control treatments of healthy and infested plants were applied for comparison. Although the recorded results proved that all allelopathic treatments minimized dry weight of the two investigated weeds, but mix incorporation is more effective than sole incorporation. Concerning to *Lupinus albus* mix incorporation treatments also scored the highest growth and yield parameters after the healthy control treatment. However, the chemical analysis of both investigated allelopathic plant materials ensured the presence of glucosinolates and phenolic compounds which have a bioherbicidal inhibitory effect on weeds.

Keywords: *Eruca sativa, Sinapis alba, Lupinus albus, weeds,* Glucosinolates content, Phenolic compounds, Allelopathy.

Introduction

Continuous and repeated use of herbicides cause toxicological or economic problems [1], thus, alternative natural herbicide become important to reduce the continuous use of synthetic herbicides and for the development of safe and alternative crop protectants [2]. The recent agricultural approaches aim to achieve safe management strategies in controlling pests i.e. weeds, insects and nematodes. Safe management strategies not only aim to decrease the harmful side effects of chemical pesticides but also aim to increase crop production with high quality.

Allelopathy as a modern approach for controlling weeds depends on releasing allelochemicals from different plant parts affecting other plants [3]. These allelochemicals are released to the surrounding through root exudation or leaf leaching or plant residues decomposition [4]. The neighboring receptor plants may be positively or negatively affected [5]. Flavonoids, terpenoids, alkaloids, phenolic acids, glucosinolates and amino acids are the common known allelochemicals in allelopathic plants [6,7]. Allelochemicals in brassica plants have been recorded to have inhibitory allelopathic effect on weeds. Hence, it can be applied as a safe natural herbicide [7-10]. The allelochemicals in brassica tissues are known as glucosinolates. By disrupting brassica tissue myrosinase enzyme is released to hydrolyze glucosinolates other simple products such as nitriles, isothiocyanates, thiocyanates, oxazoliolines and epithionitriles [11]. Isothiocyanates are the main phytotoxic pesticidal product [6-9]. Many scientists ensured that brassica seeds glucosinolates contents is higher than other plant parts [6,7].

In Eruca sativa Mill. (Rocket salad), glucoerucin (4-methyl-thiobutylglucosinolate) and low levels of glucoraphanin (4-methyl-sulphinylbutyl glucosinolate) are the identified glucosinolates [12]. Glucoerucin is enzymatically hydrolyzed to erucin (4-methyl-thiobutylisothiocyanate) [13]. Moreover, glucoraphanin is also enzymatically hydrolyzed to sulforaphane (4-methyl-sulfi-nylbutylisothiocyanate) which is one of the most known natural anti-cancer isothiocyanates [14,15]. Whereas, the main glucosinolate of Sinapis alba (yellow mustard) is

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sinalbin which is hydrolyzed yielding p-hydroxy benzyl isothiocyanate [16].

Lupinus albus (Lupine) plant was well established in classical Greek and Roman times and practiced in Egypt as early as 2000B.C. lupines are much used for green manuring on the light lands. Also, the seeds after boiling and prolonged steeping to get rid of their bitter and poisonous alkaloids are used as stock feed and for human consumption, an important source of dietary protein. The germ meal of white lupine seeds contained about 33-40% crude protein [17]. The quality of protein in lupine seed is similar to soybean protein [18]. Increasing yield could be achieved through integrated pests management of pests such as weeds, pests and diseases. Weeds are considered to be serious pests that can damage most crops, since they compete the crop on all natural resources which is negatively reflected on crop growth and yield [19,20]. Weed scientists ensured that weed competition cause million dollars losses all over the world ranges from 18-36% [21,22]. So, weeds reduce the quality and yield of crops and increase the cost of production [23,24,25,26]. The high L. albus production remands successful weed management strategy.

As the defined glucosinolates in Eruca sativa differ from glucosinolates in Sinapis alba seed powder. So, the present investigation is applied to evaluate the sole or mix potentiality of using the seed powder of these two Brassicaceae plants in controlling two weeds (Phalaris minor and Malva parviflora) associating Lupinus albus plant.

Materials and Methods

In the greenhouse of the National Research Centre, Dokki, Giza, Egypt, two pot experiments were conducted during two successive winter seasons of 2019/2020 and 2020/2021, for studying the possibility of controlling both cheese weed (*Malva parviflora*) and lesser canary grass (*Phalaris minor*) growing with lupine (*Lupinus albus*). The experimental procedures were applied by sole or mixing application of white mustard (*Sinapis alba)* and Rocket salad (*Eruca sativa).* Lupine (*L. albus*) cv. Giza 2 seeds, *Malva parviflora* and *Phalaris minor* seeds were obtained from Agriculture Research Centre, Giza, Egypt. Clean seeds of *S. alba* and *E. sativa* were grinded to fine powder using an electric mill.

Both experiments were applied in complete randomized block design with nine replicates. In plastic pots filled with 2kg soil five seeds of *L. albus* were sown 2 cm in depth. All pots except healthy treatment were infested with equal weight (0.5 g) of *M. parviflora* and *P. minor* simultaneously and mixed thoroughly. Each experiment includes 16 treatments that applied simultaneously as follows: two treatments investigated the sole incorporation of *S. alba* in powder form that was mixed with the soil surface at rates of 5and 10 g/pot. Other four treatments investigated the sole application of *E. sativa* at rates of 10, 20, 30 and 40 g/pot. The corresponding eight treatments investigated the mix incorporation of 5 and 10 g *S. alba* with *E. sativa* at rates of 10, 20, 30 and 40 g/pot. Additionally, two control treatments healthy and infested untreated treatment were applied for comparison.

All the cultural practices of growing *L. albus* plants were applied especially fertilization and irrigation.

Parameters studied

Weed parameters studied

At vegetative and flowering ages (50 and 90 days after sowing (DAS)) three replicates were collected. Fresh and dry weights of the two weeds under investigation were recorded (g /pot).

Lupinus albus plants

Plant growth parameters

At 50 and 90 DAS samples of *L. albus* plants were collected from each treatment to determine: plant height (cm), number of leaves/plant, fresh and dry weight / plant (g) as well as SPAD value (at 50 DAS only).

Yield and yield components

Three plants from each replicate of *L. albus* were taken at harvest from three replicates of each treatment to determine: number of pods/plant, pods dry weight/plant (g), number of seeds/pod, number of seeds/plant, 100 -seed weight (g), seed yield / plant (g) and straw yield/plant (g).

Chemical analysis

Total glucosinolates (µmol/g dry weight)

Total glucosinolates were extracted from dry samples of both *E. sativa* and *S. alba* seeds. Glucosinolates were measured by determining the liberated glucose which release during hydrolysis by myrosinase enzyme [27]. The resulting glucose was determined coloriemetrically according to methods defined by [28].

Total phenolic contents (mg/g dry weight)

Total phenolic contents of both *E. sativa* and *S. alba* seeds were determined coloriemetrically using Folin and Ciocalteu phenol reagent to the methods defined by [29].

Statistical analysis

All data were statistically analyzed according to [30] and the treatment means were compared by using LSD at 5% probability.

Results

Weed growth parameters

Results recorded in Tables (1 and 2) and Fig. (1) show that, sole or mixed incorporation of the investigated plant materials i.e. *E. sativa* (ESSP) and *S. alba* (SASP) significantly reduced the dry weight of both weeds *M. parviflora* and *P. minor* at the two recorded ages of growth 50 and 90 DAS as compared to control. Moreover, mix incorporation of both investigated seed powders was more effective in inhibiting weeds than sole incorporation of the seed powders. The rate of reduction in dry weight of each weed was directly proportional with the rate of the investigated powder plant materials. At 90 DAS, the maximum reductions in weed dry weight were recorded with ESSP at 40g+SASP at 10g,ESSP at 30g+SASP at 10g,ESSP at 40g+SASP at 5g,ESSP at 40g and ESSP at 30g+SASP at 5g/pot which reached to 95.26, 86.81, 86.37, 84.30 and 82.81% with *M. parviflora* and to 87.94, 68.22, 55.66, 53.85 and 52.30%, with *P. minor* respectively as compared to the corresponding infested control.

Growth of *Lupinus albus*

The results in Tables (3 and 4) illustrate that different *L. albus* growth parameters, as plant height (cm), number of leaves/plant, fresh and dry weight / plant (g) (at 50 and 90 DAS) as well as SPAD value (only at 50 DAS). were significantly increased with all applied seed powder concentrations of *E. sativa* (ESSP) or *S. alba* (SASP) (sole or mixed incorporation) except ESSP at 40g+SASP at 10g/pot when compared to the corresponding infested control. Whereas, SPAD value significantly increased with most applied treatments comparing with untreated pot. At 50 DAS, the highest significant *L. albus* growth parameters were recorded with ESSP at 20g+SASP at 5g and ESSP at 20g /pot comparing with other treatments. At 90 DAS, treatments of *L. albus* alone (free control), ESSP at 10 g + SASP at 5 g, ESSP at30 g, ESSP at10 g + SASP at 10g and ESSP at 20g + SASP at 5g/pot induced maximum increase in dry weight of *L. albus* associated with *M. parviflora* and *P. minor*, that reached to 448.06, 334.11, 247.29, 212.40 and 181.40%, respectively as compared to infested control.

***Lupinus albus* yield and yield components**

L. albus yield and yield components results at harvest such as number of pods/plant, pods dry weight/ plant (g), number of seeds/pod, 100-seed weight (g), seed yield/plant (g) and straw yield/plant (g) are recorded in Table (5) and Fig. (2) show that, the effect of all applied treatments of ESSP or SASP (sole or mixed incorporation) on the plant associated with *M. parviflora* and *P. minor* significantly increased *L. albus* yield parameters as compared to the corresponding controls, except, ESSP at 40g+ SASP at 10g and ESSP at 30g + SASP at 10g /pot. The best results of *L. albus* yield parameters resulted with *L. albus* alone (free control), ESSP at 10 g + SASP at 5 g, ESSP at30 g, ESSP at10 g + SASP at 10g and ESSP at 20g + SASP at 5g/pot, respectively. The previous treatments increased 100-seed weight by 100.60, 94.17, 81.02, 77.28 and 71.06% and increased seed yield/plant by 203.38, 186.46, 163.38, 113.54 and 108.31%, respectively as compared to infested control.

Quantitative estimation of total glucosinolates and total phenolic compounds in the investigated plant materials

As shown in Table 6, total glucosinolates quantity is higher in *E. sativa* seed extract than *S. alba* seed extract. Whereas, total phenolic compounds is higher in *S. alba* seed extract than *E. sativa* seed extract

Table (1): Effect of *Eruca sativa* and *Sinapis alba* seed powder on dry weight of *Malva parviflora* and *Phalaris minor* (g/pot) associated *Lupinus albus* at 50 DAS (Average of the two seasons)

Treatments				*Malva parviflora*		*Phalaris minor*	
				Fresh weight (g)	Dry weight (g)	Fresh weight (g)	Dry weight (g)
Healthy control				……..	……..	……..	……..
infested control				10.16	1.65	29.36	4.19
Sole incorporation	SASP	5g		7.09	1.39	21.51	2.95
		10g		5.24	0.95	19.59	2.65
	ESSP	10g		6.47	1.17	27.38	3.39
		20g		4.05	0.73	21.11	2.87
		30g		2.84	0.56	17.83	2.35
		40g		2.35	0.43	14.73	1.96
Mix incorporation	SASP 5g	ESSP	10g	4.67	0.85	24.57	3.11
			20g	2.82	0.54	19.43	2.52
			30g	2.57	0.47	16.58	2.04

			40g	1.98	0.36	14.27	1.85
	SASP 10g	**ESSP**	**10g**	3.45	0.63	24.17	2.99
			20g	2.71	0.51	19.32	2.50
			30g	1.88	0.34	9.09	1.33
			40g	0.64	0.08	7.76	0.85
LSD at 0.05				0.76	0.11	1.38	0.33

.**Table (2): Effect of *Eruca sativa* and *Sinapis alba* seed powder on dry weight of *Malva parviflora* and *Phalaris minor* (g/pot) associated *Lupinus albus* at 90 DAS (Average of the two seasons)**

Treatments				***Malva parviflora***		***Phalaris minor***	
				Fresh weight (g)	**Dry weight (g)**	**Fresh weight (g)**	**Dry weight (g)**
Healthy control				……..	……..	……..	……..
Infested control				22.65	6.75	86.58	19.98
Sole incorporation	**SASP**	**5g**		14.12	4.17	65.79	12.98
		10g		7.90	2.48	62.91	11.21
	ESSP	**10g**		9.73	2.95	82.12	18.96
		20g		6.12	1.89	65.79	12.66
		30g		4.28	1.31	49.92	9.63
		40g		3.55	1.06	44.87	9.22
Mix incorporation	**SASP 5g**	**ESSP**	**10g**	7.04	2.13	76.11	18.66
			20g	4.25	1.27	61.19	9.80
			30g	3.88	1.16	47.21	9.53
			40g	2.99	0.92	44.68	8.86
	SASP 10g	**ESSP**	**10g**	5.21	1.56	74.49	17.30
			20g	4.10	1.23	56.88	9.78
			30g	2.84	0.89	33.68	6.35
			40g	0.99	0.32	22.11	2.41
LSD at 0.05				0.81	0.37	1.49	0.94

Table (3): Effect of *Eruca sativa* and *Sinapis alba* seed powder on some growth parameters of *Lupinus albus* plants at 50 DAS (Average of the two seasons)

Treatments				**Growth parameters of *Lupinus albus***				
				Plant height (cm)	**No. of leaves/ plant**	**F. W. of plant (g)**	**D.W. of plant (g)**	**SPAD value**
Healthy control				40.0	12.0	10.47	1.84	56.6
Infested control				27.0	8.0	4.66	0.93	46.4
Sole incorporation	**SASP**	**5g**		37.0	11.5	7.62	1.31	2.43
		10g		41.1	12.5	7.79	1.42	2.46
	ESSP	**10g**		39.3	11.2	8.31	1.45	3.03
		20g		47.7	13.0	11.50	1.87	3.41
		30g		35.5	11.0	7.53	1.29	4.48
		40g		34.0	10.0	7.05	1.25	1.80
Mix incorporation	**SASP 5g**	**ESSP**	**10g**	47.0	10.3	8.24	1.54	5.60
			20g	48.8	13.7	13.35	2.21	3.63
			30g	31.2	9.5	6.24	1.22	2.81
			40g	30.0	9.3	5.72	1.09	2.34
	SASP 10g	**ESSP**	**10g**	45.2	12.2	9.92	1.72	4.03
			20g	33.3	9.7	9.10	1.55	2.38
			30g	30.3	9.2	5.76	1.19	1.73
			40g	28.3	9.0	5.43	1.03	1.55
LSD at 0.05				1.5	0.99	0.90	0.14	1.3

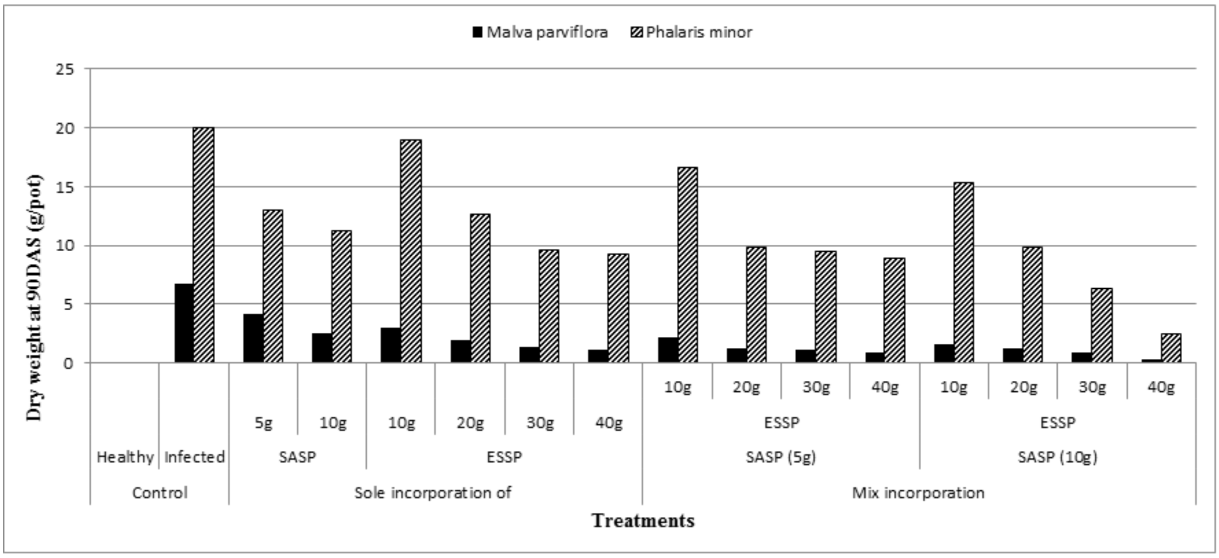

Fig. (1): Effect of *Eruca sativa* and *Sinapis alba* seed powder on dry weight of *Malva parviflora* and *Phalaris minor* (g/pot) associated *Lupinus albus* at 50 and 90 days after sowing**.**

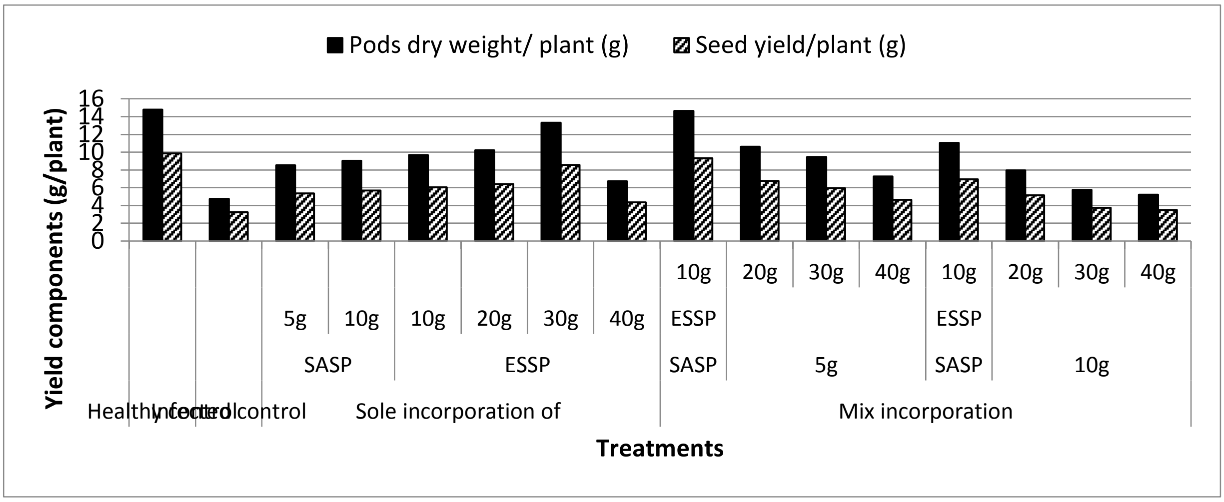

Fig. (2): Effect of *Eruca sativa* and *Sinapis alba* seed powder on yield and yield components of *Lupinus albus* plants associated with *Malva parviflora* and *Phalaris minor* at harvest

Table (4): Effect of *Eruca sativa* and *Sinapis alba* seed powder on some growth parameters of *Lupinus albus* plants at 90 DAS (Average of the two seasons)

Treatments				Growth parameters of *Lupinus albus*			
				Plant height (cm)	No. of leaves	F. W. of plant (g)	D.W. of plant (g)
Healthy control				77.2	29.5	33.04	7.07
Infested control				43.0	12.6	6.39	1.29
Sole incorporation	SASP	5g		61.2	19.8	10.79	2.43
		10g		62.8	20.7	11.22	2.46
	ESSP	10g		63.8	22.7	13.96	3.03
		20g		65.3	23.5	15.06	3.41
		30g		72.3	26.3	20.31	4.48
		40g		51.8	17.0	8.24	1.80
Mix incorporation	SASP 5g	ESSP	10g	74.5	28.2	25.63	5.60
			20g	68.7	24.3	16.73	3.63
			30g	63.3	22.2	12.19	2.81

			40g	53.0	18.2	8.95	2.34
	SASP 10g	**ESSP**	**10g**	70.7	26.2	17.54	4.03
			20g	54.5	18.4	10.44	2.38
			30g	47.0	16.5	7.54	1.73
			40g	44.5	15.0	6.85	1.55
LSD at 0.05				1.6	1.3	0.99	0.42

Table (5): Effect of *Eruca sativa* and *Sinapis alba* seed powder on yield and yield components of *Lupinus albus* plants associated with *Malva parviflora* and *Phalaris minor* at harvest (Average of the two seasons).

Treatments				No. of pods/plant	Pods dry weight / plant (g)	No. of seeds/ pod	No. of seeds/ plant	100-seed weight (g)	Seed yield/ plant (g)	Straw yield/ plant (g)
Healthy control				7.50	14.79	4.11	30.83	47.14	9.86	30.65
Infested control				2.50	4.73	2.00	5.00	23.50	3.25	6.10
Sole incorporation	**SASP**	**5g**		4.10	8.52	2.88	11.81	31.20	5.34	16.01
		10g		4.30	9.03	3.00	12.90	34.50	5.68	16.33
	ESSP	**10g**		4.60	9.68	3.20	14.72	37.30	6.05	18.04
		20g		4.80	10.21	3.22	15.46	38.90	6.39	18.56
		30g		6.40	13.32	3.79	24.26	42.54	8.56	20.38
		40g		3.40	6.74	2.25	7.65	24.80	4.35	7.46
Mix incorporation	**SASP 5g**	**ESSP**	**10g**	7.00	14.65	4.00	28.00	45.63	9.31	25.32
			20g	5.14	10.63	3.24	16.65	40.20	6.77	19.32
			30g	4.50	9.47	3.15	14.18	35.70	5.93	17.43
			40g	3.60	7.28	2.34	8.42	28.57	4.62	9.66
	SASP 10g	**ESSP**	**10g**	5.20	11.06	3.35	17.42	41.66	6.94	19.91
			20g	4.00	7.95	2.50	10.00	30.02	5.15	13.42
			30g	2.90	5.75	2.18	6.32	24.30	3.72	6.99
			40g	2.70	5.21	2.14	5.78	24.00	3.48	6.72
LSD at 0.05				0.36	1.15	0.78	0.79	1.28	0.96	1.21

Table (6): Total glucosinolates (µmol/g dry weight) and total phenolic contents (mg/g dry weight) in the seed powder of both *Eruca sativa* and *Sinapis alba*

Materials	**Total glucosinolates (µmol/g dry weight)**	**Total phenolic compounds (mg/g dry weight)**
***Eruca sativa* seed extract**	316.03	35.62
***Sinapis alba* seed extract**	288.59	43.62

Discussion

Non chemical integrated weed management strategies aim to apply safe methods for suppressing competitor weeds. Our previous studies ensured the bioherbicidal effect of some brassica plants in controlling annual and perennial weeds [23,31,32]. Additionally, brassica plants have been improved to have allelopathic inhibitory effect on Orobanche species (*O. crenata* and *O. ramosa*) which parasite on faba bean and tomato plants [25,26].

The present investigation reported that either sole or mix incorporation of ESSP and SASP with the soil as a pre emergence bioherbicide for controlling *P. minor* and *M. parviflora* weeds. Moreover, mix incorporation is highly effective than sole incorporation. However, many studies proved the potential allelopathic effect of brassica plants on weeds [31,33,34,35]. The allelopathic effect of two plant materials under investigation (ESSP and SASP) may be related to glucosinolates or phenolic compounds. By the action of myrosinase enzyme, glucosinolates are hydrolyzed to isothiocyanates [36]. Isothiyanates were not only improved to have allelopathic properties, but also they were reported to have anti- fungal, anti- oxidative, anti- cancer and anti- bacterial [37-38-39]. So, it can be applied as an alternative to synthetic pesticides for pest control [40] and as bioherbicides for weed control [31].

Good manageable effect of both sole or mixed incorporation is reflected on *L. albus* growth and yield traits, the same results were reported by many scientists [31,41,42]. Additionally, this may be due to the selective response of *L. albus* and two weeds to the allelopathic effect of the investigated plant materials [43]. The selective response is improved by positive growth of some species to the allelopathic compounds at certain concentrations and negative growth response of other species at the same concentration [25,26,31,35].

Conclusion

The present study revealed that the Brassicaceae plants seed powder as *E. sativa* and *S. alba* have allelopathic inhibitory effect on annual weeds under investigation. However, this inhibitory effect is directly proportional with the rate of the allelopathic seed powder. Moreover, Mix incorporation of ESSP and SASP achieved inhibitory effect on investigated weeds higher than sole incorporation. So, the mix incorporation of ESSP and SASP is a selective natural herbicide to control annual weeds accompanied *L. albus* crop.

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