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Control of Soil-Borne Pathogenic Fungi of Soybean by Biofumigation with Mustard Seed Meal

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Abstract: *Fusarium oxysporum*, *Rhizoctonia solani*, *Macrophomina phaseolina* and *Sclerotium rolfsii* are common fungal pathogens to soybean causing damping off, root rot and wilt diseases resulting in serious economic losses. Biofumigation is used as a means to control many diseases by biocidal compounds (mainly isothiocyanates) released from glucosinolates in mustard seed meal which is hydrolyzed during incorporation in the soil. Laboratory, greenhouse and field experiments were conducted to evaluate the efficacy of mustard (*Brassica juncea*) seed meal against the soil-borne pathogens. Mustard seed meal decreased the linear growth of the tested fungi as compared with the control. The fungicidal effect of mustard seed meal against the tested fungi was demonstrated in pot experiments where diseases were suppressed and plant growth was increased compared to the untreated control treatment. Results of the field experiment (conducted in Ismailia Agricultural Research Station) indicated that mustard seed meal reduced the Disease Incidence (DI) over the control by 69.7% four months after planting. As a reference, the DI reduction was 74.4% over the control when Rhizolex® was used.

Key words: Soybean, biofumigation, mustard, *Brassica juncea*, isothiocyanates *Fusarium*, *Rhizoctonia solani*, *Macrophomina phaseolina*, *Sclerotium rolfsii*

INTRODUCTION

Soybean (*Glycine max* (L.) Merr.) has many benefits for human and animal nutrition. It can be considered as a friendly crop to the environment related to its efficient nitrogen fixation system, in addition to its improvement to the traditional cereal rotation and protein supply in low input farming systems (Nassiuma and Wasike, 2002; Akande *et al.*, 2007).

Soil-borne fungal diseases are among the most important factors limiting the yield production of grain legumes in many countries, resulting in serious economic losses. Pathogens such as *Fusarium oxysporum*, *Rhizoctonia solani*, *Macrophomina phaseolina* and *Sclerotium rolfsii* can have negative significant effects on the growth of soybean plants (Amer, 2005; Hashem, 2004; Luiz *et al.*, 2006; Wrather *et al.*, 2007; Haikal Nahed, 2008; Sweets, 2008). These pathogens are difficult to control because of their persistence in the soil and wide host range. Some chemicals are effective in controlling these diseases but, these chemicals are expensive and not environmental friendly. Therefore, alternative control methods are needed for managing these pathogens. Several alternative measures are being tested, including biofumigation. The suppressive effects of biofumigation are generally attributed to biocidal compounds, principally isothiocyanates (ITCs), released when the glucosinolates in *Brassica* species tissues are hydrolyzed in the soil (Sarwar *et al.*, 1998; Olivier *et al.*, 1999). These isothiocyanates have been shown previously to be fungicidal (Mazzola, 2003; Kirkegaard *et al.*, 2006). Several studies have shown that amending the soil with *Brassica* sp. as seed meal suppressed many plant fungal pathogens (Charron and Sams, 1999; Mawar and Lodha, 2002; Lodha and Sharma, 2002). Mazzola and Abi Ghanem (2006) evaluated the efficacy of Brassicaceae seed meal amendments for control of the soil-borne pathogen/parasite complex inciting apple replant disease and the capacity of such treatments to enhance tree growth and yield. Van Os *et al.* (2004) and Van Os and Lazzeri (2006) reported that in soil infested with *R. solani*, application of mustard seed meal resulted in significant control of stem infection in lily. Also, they proved that its efficacy was comparable to the chemical treatment with azoxystrobine. Robert Larkin and Griffin (2007) have shown that *Brassica* crops used as green manures have been associated with reductions in soil-borne pathogens. These reductions have been attributed to the production of volatile compounds through a process known as biofumigation.

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The objective of the present study was to evaluate the efficacy of mustard seed meal as a biofumigant for controlling damping off, root rot and wilt diseases of soybean plants under laboratory, greenhouse and field conditions.

MATERIALS AND METHODS

Samples collection, isolation and identification: Soybean plants showing root rot and wilt symptoms were collected from different localities of Ismailia Governorate. Ten plants (two from the center and two near each corner) were removed from each field. Root-rot samples were first washed in running tap water to remove the adhering soil particles and then surface sterilized in 5% sodium hypochlorite solution for 2 min. The sterilized plant parts were rinsed several times in sterilized distilled water and dried between sterilized filter paper. Then, cut into small pieces and directly placed on Potato Dextrose Agar (PDA) medium in Petri-dishes. The Petri dishes were incubated at 25°C for 5-7 days. The hyphal tips of the growing hyphae were taken from the growing colonies and transferred to PDA plates for culture purification. Pure cultures were identified according to their morphological characters according to Booth (1971), Nelson *et al.* (1983) and Barnett and Hunter (1986).

Effect of mustard (*Brassica juncea*) seed meal on linear growth of soybean soil-borne pathogens: Four soil-borne fungal pathogens (*F. oxysporum*, *R. solani*, *M. phaseolina* and *Sclerotium rolfsii*) isolated as mention before from soybean diseased plants were used in this study. Five millimeter diameter discs of actively growing mycelium were taken from the margins of fungal growth and transferred to Petri dishes (9 cm in diameter) containing PDA supplemented with 150 ppm streptomycin sulfate. Fresh seeds of mustard were ground in a food processor for about 2 min. The ground seed meal was placed in 5 cm Petri dish onto the upturned lid of the plates with the inverted bottom containing the fungal plug as the lid. Sterile distilled water at a rate 1:2 (w/v) was added to the meal to induce releasing the isothiocyanates (ITCs) and the plate was immediately sealed with Parafilm. The rates of seed meal used were 0 (control 1), 5, 10 and 25 mg per plate and duplicate plates of each rate were prepared. Seed-meal-free PDA medium was used as control treatment. The plates were incubated at 25°C for 5 days, after which the colony diameter was measured in each treatment while the pathogenic fungi almost covered the medium surface in control treatment.

Greenhouse studies

Effect of mustard seed meal on soybean germination and growth: Soil collected from Ismailia Agricultural Research Station was packed into plastic pots (12 cm in diameter and 15 cm high). Mustard seed meal was mixed with the upper layer of potted soil at rates of 0 (control) , 0.113, 0.226, 0.565, 1.130 g pot^{-1}. Five replicate pots were used. Soybean seeds were sown in the pots (10 seeds per pot) at a depth of 1 cm immediately following the application of mustard meal. The pots were watered throughout the experiment. Percentage of seed germination and the time to emergence (days) were recorded. Thirty days after seeding, the plants were harvested for measuring the plant height and shoot weight.

Effect of mustard seed meal compared to Rhizolex® on soil-borne fungal pathogens: Pots sterilized sorghum-seed medium was inoculated with the tested fungi (*F. oxysporum*, *R. solani*, *M. phaseolina* and *S. rolfsii*) and incubated at 25°C for 15 days. Pots (30 cm in diam.) containing unsterilized soil were infested with each of the tested fungi incorporated in its solid substrate at the rate of 3% (w/w) of soil weight. The infested soil was mixed thoroughly, watered and kept for one week to insure even distribution of the inoculum. Four treatments were made in four replicates including:

- Non-infested soil (control 1)
- Infested soil with a pathogen (control 2)
- Infested soil with a pathogen + mustard seed meal (the meal (3.5 g pot^{-1}) was mixed through the pot soil at depth of 2 cm and watered)
- Infested soil with a pathogen + Rhizolex® (50 mL pot^{-1} at a rate of 1.5 g L^{-1}, mixed through the soil prior to planting)

Four pots for each treatment were cultivated with 10 soybean seeds (cv. Giza 35) pot^{-1}. Percentages of damping off and survived plants (infected and healthy plants, respectively) were recorded 30 and 90 days after planting. Healthy survival plants were those which have no visual evidence of disease. Infected but survival plants were evaluated 3 months after seeding by cutting longitudinally through each plant (stem and root) and any discoloration of internal tissue was recorded. Plant growth parameters (plant height and shoot weight plant^{-1} as average of 5 plants) were recorded 4 months after planting. Disease Severity Indexing (DSI) of root rot and any discoloration of tissue were recorded according to Haware and Nene (1980) based on 0-4 scale, where 0 = healthy (no disease), 1 = 1-33% root damage rot^{-1}, 2 = 34-66% root damage rot^{-1}, 3 = 67-100% root damage rot^{-1} and 4 = dead plant.

Field study

Effect of mustard seed meal and the fungicide (Rhizolex®) on root rot and wilt diseases complex of soybean under field conditions: This experiment was conducted at Ismailia Agricultural Research Station. Treatments were arranged in a complete randomized block design with four replicates. The field plot was 3×3 m with 5 rows. The treatments were as follows: (1) untreated control, (2) mustard seed meal (the meal was applied to the plot soil at rate of 5 g m^{-2} at sowing) and (3) Rhizolex®, mixed through the soil prior to planting at rate of 3 kg/feddan as recommended. Disease incidence (%) (number of dead plants/total number of plants in a plot × 100) was calculated 4 months after planting.

Statistical analysis: All the data were statistically processed by the analysis of variance and by determining the significance threshold using Duncan's test (Duncan, 1955).

RESULTS

Isolation and identification of the causal pathogens: Isolation trails from rotted and wilted soybean plants collected from different localities in Ismailia Governorate yielded some fungi which were identified as *F. oxysporum*, *R. solani*, *M. phaseolina* and *S. rolfsii*.

Effect of mustard seed meal on linear growth of soybean root-rot and wilt fungal pathogens: There were differences in the sensitivity of the pathogens to the seed meal at all levels with *R. solani* being the most sensitive fungus. The lowest values of linear growth (3.8, 1.2 and 0.7 cm) were recorded with *R. solani* at 5, 10 and 25 mg of seed meal, respectively. The highest values of linear growth (5.2, 4 and 3.3 cm) were obtained from *S. rolfsii* at 5, 10 and 25 mg of seed meal, respectively. Data also reveal that the effect of mustard seed meal on mycelial growth of the pathogens was proportional to the quantity of ground mustard seeds. Significant differences were observed with the different amount of seed meal for each fungus (Table 1).

Greenhouse studies

Effect of mustard seed meal on soybean seed germination and plant growth: The effect of mustard seed meal mixed with pot soil at rates of 0.113, 0.226, 0.565 and 1.130 g pot^{-1} (12 cm diam.) on seed germination and plant growth of soybean was shown in Table 2. Data reveal that mustard meal banded with soybean seed had no effect on seed germination at the rates of 0.113 and 0.226 g pot^{-1} compared with the untreated control. At rates of 0.226 and 0.565 g pot^{-1} emergence was delayed by 1 and 2.5 days, respectively but shoot weight and plant height were not reduced. The highest rate 1.13 g pot^{-1} delayed emergence by 4 days. Seed germination, shoot weight and plant height at this rate (1.13 g pot^{-1}) were 61.2%, 4.3 g and 15.25 cm, respectively with reductions than the untreated control by 64, 54 and 71.6%, respectively) (Table 2). Deleterious effects were experienced at higher doses into all parameters tested.

Effect of mustard seed meal and Rhizolex® fungicide on soil-borne diseases of soybean: Mustard seed meal and Rhizolex® fungicide significantly reduced the disease incidence by *F. oxysporum*, *R. solani*, *M. phaseolina* and *S. rolfsii* (Table 3, Fig. 1) while the number of healthy soybean plants increased in comparison with the

Table 1: Effect of mustard seed meal on mycelial growth of selected pathogenic fungi of soybean

Rate of seed-meal (mg)	*Fusarium oxysporum*		*Rhizoctonia solani*		*Macrophomina phaseolina*		*Sclerotium rolfsii*	
	Linear growth (cm)	R	Linear growth (cm)	R	Linear growth (cm)	R	Linear growth (cm)	R
5	4.1b[a]	54.4	3.8b	57.7	4.50b	50.0	5.2b	42.2
10	3.8c	57.7	1.2c	86.6	2.13c	76.6	4.0c	55.5
25	2.1c	76.6	0.7d	92.2	1.30d	85.5	3.3d	63.3
0 (Control)	9.0a	-	9.0a	-	9.00a	-	9.0a	-
LSD	1.18		0.79		0.79		1.03	

[a]Values in the same column followed by the same letter(s) are not significantly different (p>0.05) based on Duncan's multiple range test. R: Reduction over the control (%)

Table 2: Effect of mustard seed meal on Days to Emergence (DTE), seed germination, shoot weight and shoot height of soybean seedlings

	Growth parameter			
Rate of seed-meal (g pot^{-1})	Time to emergence (day)	Seed germination (%)	Shoot weight (g)	Shoot height (cm)
0.0 (Control)	6.0	95.0a[a]	7.9ab	21.30b
0.113	6.0	95.0a	7.1b	18.20c
0.226	7.0	95.0a	8.9a	24.50a
0.565	8.5	87.5a	7.2b	19.25c
1.13	10.0	61.2b	4.3c	15.25d
LSD		16.5	1.57	2.80

[a]Values in the same column followed by the same letter(s) are not significantly different (p>0.05) based on Duncan's multiple range test

Table 3: Effect of mustard seed meal and Rhizolex® fungicide (as a reference) on disease parameters on soybean planted in soil artificially infested with selected soil-born pathogens

	Fusarium oxysporum				*Rhizoctonia solani*				*Macrophomina phaseolina*				*Sclerotium rolfsii*			
	Damping off (%)		Survival plants (%)		Damping off (%)		Survival plants (%)		Damping off (%)		Survival plants (%)		Damping off (%)		Survival plants (%)	
Treatments	pre	post	I[a]	H[b]	pre	post	I	H	pre	post	I	H	pre	post	I	H
Infested soil+Mustard seed meal	17.5b[c]	7.5a	12.50b	62.5b	30.0b	2.50bc	2.5a	65.0b	35.0b	7.5a	5.0a	52.5c	35.0b	12.5a	7.5a	45.0b
Infested soil+Rhizolex®	22.5b	5.0ab	12.50b	60.0b	30.0b	7.50ab	5.0a	57.5b	30.0b	2.5a	10.0a	57.5b	32.5b	10.0a	2.5a	55.0b
Infested soil (control 1)	42.5a	10.0a	22.50a	25.0c	72.5a	12.50a	0.0a	15.0c	60.0a	15.0a	7.5a	17.5d	65.0a	5.0a	5.0a	25.0d
Non-infested soil (control 2)	5.0c	0.0b	5.00ab	90.0a	5.0c	0.00c	5.0a	90.0a	5.0c	0.0a	5.0a	90.0a	2.5c	0.0a	7.5a	90.0a
LSD	11.9	6.6	8.61	8.61	13.5	6.67	7.37	11.55	14.8	12.33	11.5	4.16	15.4	13.8	10.91	11.26

[a]I: Infected but survived plants (not totally killed); [b]H: Healthy plants, [c]Values within the same column followed by the same letter(s) are not significantly different (p>0.05) based on Duncan's multiple range test

Fig. 1: Effect of mustard seed meal on selected soil-borne pathogenic fungi of soybean plants. F: Infested soil with *F. oxysporum*, F1: Infested soil with *F. oxysporum* + mustard seed meal, R: Infested soil with *R. solani*, R1: Infested soil with *R. solani* + mustard seed meal, M: Infested soil with *M. phaseolina*, M1: Infested soil with *M. phaseolina* + mustard seed meal, S: Infested soil with *S. rolfsii*, S1: Infested soil with *S. rolfsii* + mustard seed meal

control 1 (untreated infested soil). No significant differences were noted between the effect of mustard and the fungicide on the tested fungal pathogens (Table 3).

For the soil infested with *F. oxysporum*, the control 1 treatment (artificially infested with *F. oxysporum* but no seed-meal or fungicide added), showed the highest occurrence of pre emergence damping off (42.5%) followed by infested soil treated with Rhizolex® and mustard seed meal (22.5 and 17.5%, respectively). No significant difference was observed between Rhizolex® and mustard seed meal treatments (Table 3).

In soil infested with *R. solani* (control 1 treatment), the percentage of pre emergence damping off, averaged 72.5% compared to 30% in artificially infested soil treated with either Rhizolex®, or mustard seed meal. For post emergence damping off, the control 1 recorded the highest percentage of infected plants (12.5) while in mustard seed meal treatment, the lowest rate of post emergence damping-off (2.5%) was obtained followed by Rhizolex® (7.5%) with no significant difference between the last two treatments. Concerning the percentage of healthy plants, the most effective treatments were mustard seed meal (65%) and Rhizolex® (57.5%) while the control 1 showed only 15% survival (Table 3).

Also, when mustard seed meal and Rhizolex® used to control *M. phaseolina*, both of them provided the highest percentage of healthy plants (57.5 and 52.5%, respectively) comparing with the lowest percentage (17.5%) obtained from the control 1 treatment (Table 3). For soil infested with *S. rolfsii*, the lowest rate of healthy plants (25%) was obtained in control 1 treatment. while, Rhizolex® and mustard seed meal treatments provided the highest rate of healthy plants (55 and 45%, respectively).

Data in Table 4 revealed that soil infested with *R. solani* used as control 1 show the highest level of disease severity (4.1) followed by, *M. phaseolina* (3.8), *S. rolfsii* (3.6) and *F. oxysporum* (2.9) (Table 4). For *F. oxysporum* and *M. phaseolina*, mustard seed-meal and Rhizolex® had same suppressive effect on the disease with no significant difference between them but both treatments significantly differed from the control 1 treatment (Table 4). For *R. solani*, Rhizolex® was significantly more suppressive to the disease than mustard seed-meal while the latter was more effective against *S. rolfsii* than Rhizolex® (Table 4).

Data presented in Table 5 revealed that the lowest values of shoot weight and plant height were observed in the control 1 treatment, while the greatest shoot weight of soybean plants (37.5 g plant^{-1}) was recorded for soybean seedlings grown in *R. solani* infested soil that was treated with mustard seed meal. The same trend was noticed with the other fungi (Table 5) where mustard seed meal treatment induced the highest values of shoot weight with all tested fungi. Data in Table 5 also reveal that mustard seed meal treatment provided the highest values of plant height with all tested fungi.

Field study

Effect of mustard seed meal and the fungicide (Rhizolex®) on root rot and wilt diseases complex of soybean under field conditions: The disease incidence on soybean plants decreased by using mustard seed meal and Rhizolex® (23.3 and 15%, respectively) compared with the untreated control (58.7%), 4 months after sowing (Table 6). There were no significant difference between the mustard seed meal and Rhizolex® but both of them were significantly different from the control (Table 6).

Table 4: Effect of mustard seed meal and Rhizolex® fungicide (as a reference) on disease severity on soybean seedling grown in soil artificially infested with selected soil-borne pathogens

	Disease severity[a]			
Treatments	*F. oxysporum*	*R. solani*	*M. phaseolina*	*S. rolfsii*
Mustard seed meal	2.2b	2.0b	2.0b	2.1c
Rhizolex®	2.2b	1.6c	2.5b	2.6b
Infested soil (control 1)	2.9a	4.1a	3.8a	3.6a
Non-infested soil (control 2)	1.07a	1.07d	1.07c	1.07d
LSD	0.3	0.34	0.55	0.46

[a]Disease severity of root rot and any discoloration of tissue were recorded according to Haware and Nene (1980) based on 0-4 scale according to percentage of foliage yellowing or necrosis (0 = 0%, 1 = 1-33%, 2 = 34-66%, 3 = 67-l00% and 4 = dead plant); [b]Values within the same column followed by the same letter(s) are not significantly different (p>0.05) based on Duncan's multiple range test

Table 5: Effect of mustard seed meal and Rhizolex® fungicide on shoot weight and plant height of soybean plants grown in soil artificially infested with selected soil-borne pathogens

	F. oxysporum		*R. solani*		*M. phaseolina*		*S. rolfsii*	
Treatments	Shoot weight (g plant^{-1})	Plant height (cm)	Shoot weight (g plant^{-1})	Plant height (cm)	Shoot weight (g plant^{-1})	Plant height (cm)	Shoot weight (g plant^{-1})	Plant height (cm)
Mustard seed meal	34.2b[a]	33.8b	37.5a	26.0b	31.2b	24.4b	35.4b	32.0b
Rhizolex®	22.8 c	17.0c	33.6a	21.6b	32.6	23.6b	31.4b	18.4c
Infested soil (control 1)	18.0d	12.0b	9.6	9.8c	8.8c	10.4c	12.0c	11.5d
Non-infested soil (control 2)	39.0a	37.4a	39.0a	37.4a	39.0a	37.4a	39.0a	37.4a
LSD	2.21	5.9	2.13	5.6	2.2	5.5	2.3	4.64

[a]Values within the same column followed by the same letter(s) are not significantly different (p>0.05) based on Duncan's multiple range test

Table 6: Effect of mustard seed meal and Rhizolex® fungicide (as a reference) on root rot and wilt diseases complex of soybean plants under field conditions, 4 months after sowing

Treatment	Disease incidence (%)	Reduction over the control (%)
Mustard seed meal	23.20b[a]	69.5
Rhizolex®	15.00b	74.4
Control	58.70a	-
LSD	11.15	

[a]Values within the same column followed by the same letter(s) are not significantly different (p>0.05) based on Duncan's multiple range test

DISCUSSION

Isolation trails from rotted and wilted soybean plants yielded *F. oxysporum*, *Fusarium solani*, *R. solani*, *M. phaseolina* and *S. rolfsii* conforming with other reports by Amer (2005), Bahaa El-Din (2005), Luiz *et al.* (2006), Pabon *et al.* (2006), Wrather *et al.* (2007) and Sweets (2008) who stated that these pathogens cause serious economic losses.

Fumigation of soil before planting to control soil-borne diseases and pests of economically important crops has been used in agriculture for many years. However, recently the use of chemical fumigants has been reduced for several reasons, including pollution of the environment, particularly ground water and food supplies. Recently, an increasing desire to reduce the use of pesticides is seen through the attempts to develop integrated pest management approaches, where natural resources are put to maximum use. However, the use of pesticides will continue but at lower rates, wherever it is necessary. Therefore, it was thought to be of value to use biofumigation in comparison to fungicides to be included in the protection of the crops.

Biofumigation refers to the suppression of soil-borne pathogens by volatile biocidal compounds (mainly isothiocyanates (ITCs), released when the glucosinolates in *Brassica* species as seed meal are hydrolyzed during breakdown in the soil (Cole, 1980; Angua *et al.*, 1994; Noble *et al.*, 2002; Harvey *et al.*, 2002; Kirkegaard *et al.*, 2006).

The effect of mustard as a biofumigant was evaluated in laboratory, in the greenhouse and in the field against *F. oxysporum*, *R. solani*, *M. Phaseolina* and *S. rolfsii* infecting soybean plants.

In the present investigation, mustard seed meal proved to be effective for controlling the causal pathogens of root rot and wilt of soybean plants. It had resulted in decreasing the linear growth of the tested fungi as compared with the control. There were differences in the sensitivity of the pathogens to the seed meal at all levels, *R. solani* was the most sensitive fungus, it was noticed that the seed meal reduced the linear growth of *R. solani* at all levels (5, 10 and 25 mg plate^{-1}) over the control. Also, it showed reduction in the liner growth of *M. phaseolina*, *S. rolfsii* and *F. oxysporum* at all levels. The results are in conformity with those of Noble *et al.* (2002). Seed meal of *Brassica* species suppresses the growth of *Pythium ultimum*, *R. solani* (Charron and Sams, 1999) and *Fusarium sambucinum* (Mayton *et al.*, 1996). Chung *et al.* (2002) proved that the volatile substances in the ground seed of mustard showed the strongest fungicidal effect on *R. solani* through comparing three *Brassica* species for volatile compounds in hydrated ground seeds. Kirkegaard *et al.* (2006) found that seed meal of mustard was fungicidal to five soil-borne pathogens.

In vitro assays, Indian mustard resulted in nearly complete inhibition (80-100%) of growth of soil-borne pathogens of potato, including *R. solani*, *Phytophthora erythroseptica*, *P. ultimum*, *Sclerotinia sclerotiorum* and *F. sambucinam* (Robert Larkin and Griffin, 2007).

Effects of mustard seed meal on soybean germination and growth were studied. The obtained results indicated that up to 40 kg/feddan could be applied with the seed at the time of sowing without reducing germination or seedling growth. There are many researchers supporting these results such as Kirkegaard *et al.* (2006) who reported that mustard seed meal was applied with the wheat. It had no effect on the germination or growth of wheat when used at a rate of 40 kg/feddan or below although at the lowest level (25 kg/feddan), there was a trend towards increased shoot growth.

Effect of mustard seed meal and Rhizolex® fungicide on soybean seedling grown in pots containing soil infested with some pathogenic fungi was studied. The data from this study showed that soil amendments with mustard seed meal have shown promising results for controlling soil borne pathogens. It has been found that sufficient control of damping off was obtained by using mustard seed meal and fungicide. Rhizolex® showed a good effect but mustard seed meal was superior to all treatments. In the non-infested soil, 90% of the plants were healthy. Treatment with mustard seed meal or Rhizolex® fungicide showed better effect on plant health than the control when the pathogen was involved. In soil infested with *F. solani*, *R. solani*, *M. phaseolina* and *S. rolfsii*, application of mustard seed meal resulted in significant reduction of damping off and increasing of healthy plants compared to the control (pathogen-infested but not treated with mustard seed meal or Rhizolex®). The reduction in diseases reflected on soybean growth parameters. Increases in shoot weight and heights of plants grown in pathogen-infested and meal-treated pot were obtained compared with pathogen-infested and non-meal-treated ones. Effects of mustard seed meal on the tested fungi may be due to the biocidal effect principally isothiocyanates (ITCs), released when the glucosinolates in mustard seed meal are hydrolyzed during their breakdown in the soil (Angua *et al.*, 1994; Noble *et al.*, 2002; Kirkegaard *et al.*, 2006). Earlier reports supporting these results such as Chung *et al.* (2002) reported that coating cabbage seeds with MBF mixture (40% mustard ground seed in 60% Biolan peat B3) significantly reduced incidence of *Rhizoctonia* damping-off and enhanced seedling growth of cabbage. Van Os and Lazzeri (2006) reported that in soil infested with *R. solani*, application of mustard seed meal resulted in control of stem infection in lily. And they also found that percentage of healthy plants was 20% in the pathogen-infested control treatment and 60% in the pathogen-infested and mustard seed meal treatment. The efficacy of seed meal was equal to the chemical treatment with azoxystrobine. Brassicaceae seed meal amendments effectively control Rhizoctonia root rot of apple and suppress weed growth in the soil (Mazzola *et al*., 2006).

Robert Larkin and Griffin (2007) revealed in greenhouse tests that Indian mustard reduced potato seedling diseases by 40-83%.

Results of the field experiment indicated that mustard seed meal and Rhizolex® treatments reduced the disease incidence by 69.5 and 74.4%, respectively 4 months after planting. There are many reports supporting these results such as Mazzola and Abi Ghanem (2006) who established field trials to evaluate the efficacy of Brassicaceae seed meal amendments for control of the soil borne pathogen/parasite complex inciting apple replant disease and capacity of such treatments to enhance tree growth and yield. They found also that tree growth in *B. juncea* seed meal treated soils has been superior to all other treatments, including pre-plant soil fumigation.

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