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INFLUENCE OF BIOFUMIGATION WITH MUSTARD OR CANOLA SEED MEAL IN CONTROLLING SOIL-BORNE PATHOGENIC FUNGI OF CHICKPEA

Sarhan, E.A.D.[1], Sahar A. El-Sayed.[1], H.M. Abdelmaksoud[1] and T.S. Elmarsafawy[2]

[1]*Plant Pathology Research Institute, Agricultural Research Center, Giza 12619, Egypt*

[2]*Field crops Research Institute, Agricultural Research Center, P.O. Box 12619, Giza, Egypt*

Correspondence: Sarhan, E.A.D. eh_sarhan@yahoo.com

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Abstract

Damping-off and root-rot are important diseases attacking chickpea (*Cicer arietinum* L.) in Egypt. The effect of biofumigation, using mustard (*Brassica juncea*) and canola (*Brassica napus*) seed meals were evaluated to control damping-off and root-rot diseases of chickpea *in vitro* and *in vivo* (under greenhouse and field conditions trials) conducted in Sers-Ellian Agricultural Research Station, Menoufia governorate, Egypt, in 2016/2017 and 2017/2018. Both mustard and canola seed meals significantly decreased the linear growth of the tested pathogenic fungi, *i.e.*, *Fusarium oxysporum*, *Sclerotinia sclerotiorum* and *Rhizoctonia solani*, this decrease was more important with the increasing of concentration when compared with the control. The fungicidal effect of mustard and canola seed meals against the tested fungi was demonstrated under greenhouse and field conditions. Obtained results indicated that mustard and canola seed meals significantly reduced the percentages of damping-off severity furthermore improved growth parameters, *i.e.,* plant height, pods number per plant and 100-seed weight. Mustard seed meal recorded the highest values of reduction in this respect as well as, significantly increased nodulation status of rhizobium (*Mesorhizobium ciceri*) *i.e*., number of nodules, nodules fresh and dry weights on chickpea roots in pots and field experiments. Mustard seed meal was superior. It could be concluded, that applying of such biofumigation procedure in the control of chickpea damping-off and root rot diseases on the field scale may offer a practical complement environmentally safe measure to control soil-borne pathogens, and may be combined in an integrated diseases management regime.

Keywords: Biofumigation, Mustard, Canola, Chickpea, *Fusarium, Sclerotinia, Rhizoctonia*.

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is a widely adapted and high-yielding legume crop grown in over 45 countries in 13.67 million hectares and produced about 13.1 million metric tons (Rawal and Navarro 2019). Cultivated for its protein-rich seeds as well as improve soil fertility by biological N_2 fixation (Jodha and Rao, 1987).

Soil-borne fungi such as *Fusarium eumartii*, *F. oxysporum* f. sp. *ciceris, F. solani, Pythium ultimum, Rhizoctonia solani*, *Sclerotium rolfsii, Sclerotinia sclerotiorum* and *Verticillium albo-atrum* are commonly pathogenic to chickpeas and are the cause for damping-off, root and/or stem rot and wilt diseases (Pande *et al.,* 2006 and Abera *et al.,* 2011). In Egypt, many investigators demonstrated that, soil-borne fungi, *i.e., F. oxysporum* f. sp. *ciceris*, *R. solani* and *S. sclerotiorum* were the most dangerous on chickpeas seeds, seedlings and roots causing damping-off, root-rot and wilt diseases which led to in serious economic losses limiting chickpeas growth and productivity (Ashour*et al.,* 2006; El-Blasy, 2006 and Abdel-Monaim, 2011).

Plant diseases are conventionally controlled by chemical pesticides. Fungicides are one of the principal tools for managing plant diseases (Reddy *et al.,* 2014 and Ozkara *et al.,* 2016). Management of crop health is essential for agricultural productivity which could be achieved by using of different fungicides and soil fumigants to control soil borne plant pathogens (Hansen *et al.,* 1990). Methyl bromide and chloropicrin were effective soil fumigants used in agriculture. However, most fumigants were recently phased out due for public health concerns and negative environmental impacts (McKenry *et al.,* 1994). Biofumigation is important alternative method to avoid the application of fungicides that achieved remarkable success in controlling plant disease and reduced the hazardous of side effects of fungicides (McKenry *et al.,* 1994; Matthiessen and Kirkegaard 2006 and Ozkara *et al.,* 2016).

Available alternatives to replace synthetic organic pesticides are being tested including biofumigation. Biofumigation refers to the release of compounds which are toxic to many soil pathogens, specifically thioglucoside, glucosinolates compounds principally isothiocyanates (ITCs) (Sarwar *et al.,* 1998; Olivier *et al.,* 1999 and Matthiessen and Kirkegaard, 2006). These isothiocyanates previously showed fungicidal effect and a promising approach to inhibiting the growth of diseases caused by soil borne pathogens (Kirkegaard *et al.,* 2006 and Mazzola *et al.,* 2007). Larkin and Griffin (2007) showed reductions in soil-borne pathogens had been associated with used *Brassica* crops as green manures. As well Abera *et al.,* (2011) demonstrated reduction in the incidence of chickpea Fusarium wilt in soil amended with *Brassica* cultivars green manure and dried plant residue. In Egypt, Fayzalla *et al.,* (2009) and Shaban, *et al.,* (2011) found that mustard and canola seed meal amendments inhibited the mycelial growth of *Rhizoctonia solani*, *Macrophomina phaseolina, Sclerotium rolfsii* and *Fusarium oxysporum in vitro,* in addition, fungicidal effect against soil-borne pathogens of soybean and lupine in pot and field experiments was recorded. Meanwhile, plant growth parameters were increased when compared to the control treatment.

However, because biofumigation may adversely affect non-target microorganisms in soil rhizosphere as well as root nodulation status increased attention had been given to isothiocyanates and related compounds, those may affect bacterial and eukaryotic community structure (Muehlchen *et al.,* 1990; Scott and Knudsen, 1999; Autumn *et al.,* 2012).

The objective of this study was to evaluate the seed meal of mustard and canola biofumigation in controlling damping-off and wilt diseases of chickpea plants under laboratory, greenhouse and field conditions, also to determine the biofumigation effect on *Rhizobium* quantitatively or qualitatively on chickpea roots.

MATERIALS AND METHODS

1. Isolation of the causal pathogens

Chickpea plants displaying root-rot disease typical symptoms were collected from different locations in Menoufia governorate, isolated and tagged for identification. The infected roots were cleaned from adhering soil and washed several times in running tap water, cutting each root into small pieces (1 cm) and disinfected for 2 min. with 2% sodium hypochlorite. Small pieces were rinsed three times in sterilized water then dried, placed in sterilized Petri-dishes containing potato dextrose agar medium (PDA), incubated at 28±1°C for five days and scanned daily for fungal development.

Isolated fungi were purified by hyphal tip or single spore techniques and identified based on morphological characters as described by (Sinclair and Dhingra, 1995; Barnett and Hunter, 1998 and Leslie and Summerell, 2006). The isolates were kept for short term storage on corn meal agar till used.

2. Preparation of mustard and canola seed meals

Fresh seeds of mustard (*Brassica juncea*) or canola (*Brassica napus*) were ground for about 2min. in a food processor.

3. Effect of mustard and canola seed meal on tested pathogens *in vitro*

The study focuses on three soil-borne fungal pathogens (*F. oxysporum, S. sclerotiorum* and *R. solani*) isolated from chickpea diseased plants. Mycelial discs (5 mm) was taken from the previously prepared cultures of the fungal isolates, each was transferred onto PDA medium in Petri-dishes (9 cm in diameter) supplemented with 100 µg ml^{-1} streptomycin sulfate. The seed meals were placed in the Petri-dishes with the rates of 5, 10 and 25 mg per plate. Sterile distilled water was added to the meal with a ratio of 1:2 (w/v) to induce the release of isothiocyanates (ITCs) into the upturned plates cover and the inverted bottom containing the fungal disc. Seed meal-free Petri-dishes were used as control treatment. Plates were immediately sealed with Parafilm. Three replicates were used for each treatment in addition to the control and incubated at 28±1°C. The averages of the linear growth were recorded when the check treatment plates were covered with the fungal growth. The percentage of growth inhibition (GI %) for each treatment was calculated as related to its growth in check treatment using the following formula:

$$GI\ \% = (C - T) / C \times 100.$$

Where, GI % = Percentage of growth inhibition, T = Linear growth of the pathogen in treated plate (cm) and C = Linear growth of the pathogen in control plates (cm).

4. Preparation of Fungal inocula:

Inocula of each fungus *F. oxysporum, S. sclerotiorum* or *R. solani* were prepared by growing on sorghum sand medium (1:1w/w and water 40%) at 25±1°C for two weeks according to (Filonow *et al.,* 1988).

5. Preparation of *Rhizobium* inoculum

Rhizobium (*Mesorhizobium ciceri*) was kindly provided by Biofertilizers Production Unit, Soils, Water and Environ. Res. Inst., Agric. Res. Centre., Giza (Egypt). Rhizobium was prepared according to (Vincent, 1970).

6. Greenhouse trials

This trial was carried out in the greenhouse of the Plant Pathol. Res. Inst., Agric. Res. Cent., Giza, Egypt. Pots (30 cm in diameter) filled with steam sterilized sandy clay soil 1:2 (V/V). In this experiment, chickpea cv. Giza-3 was used to study the effect of mustard or canola on damping-off diseases in chickpea. Each previously prepared fungus (*F. oxysporum, S. sclerotiorum* or *R. solani*) were used as inocula at the rate of 5% by weight and mixed thoroughly with the sterilized soil in pots (Abdel-Monaim 2011). Pots were irrigated and kept for a week to insure distribution of the inocula. Mustard or canola meal was mixed with the soil at rates of 0 (control), 0.5, 1.٠ and ٢.5 g/pot.

Chickpea seeds were coated by the previously prepared rhizobium (*M. ciceri*) at the rate of 10g/kg seeds and mixed thoroughly. Five drops of Arabic gum solution were added as a sticker. Coated seeds left for air drying then sown at the rate of 5 seeds/ pot and covered with thin layer of dry meal/soil mixture. Five pots were used as replicates for each treatment in addition to a control check (uninfected soil).

Disease assessment was recorded as percentages damping-off during the 45 days of sowing (Mahmoud *et al.,* 2013). Percentages of the survived plants were counted and recorded 60 days after sowing. Percentages of damping-off as well as the survived plants were calculated using the following formulas:

$$\text{Damping-off (\%)} = \frac{\text{Number of dead seedlings}}{\text{Number of sown seeds}} \times 100$$

$$\text{Survival plant (\%)} = \frac{\text{Number of survived healthy plants}}{\text{Number of sown seeds}} \times 100$$

For nodulation status, plants were uprooted 75 days after the sowing date to estimate nodules number/plant and nodules fresh and dry weight per plant (mg).

7. Field trials

Field trials were conducted at Sers-Ellian Agricultural Research Station, Menoufia governorate, Egypt, during the seasons 2016/2017 and 2017/2018. The area of each plot was 10.5 m^2 divided into five rows (3.5 m length and 0.6 m width). One seed was sown in each hill 20 cm apart on both row sides with four replicates for each treatment. Mustard or canola seed meals were applied at sowing at rate of 5 g/m^2 of the plot soil and untreated plots were left as the control. Treatments were arranged in a complete randomized block design. The field trials were treated by Rhizobium (*M. ciceri*) as previously applied in the greenhouse experiments. All agricultural practices were received according to Egyptian Ministry of Agriculture & Land Reclamation recommendations.

Five chickpea plants from each treatment were uprooted 75 days after sowing to assay the nodulation status (*i.e.*, number of nodules and nodules dry weight (mg) per plant). At harvest, yield components, *i.e*. plant height (cm), pods number per plant and 100-seed weight (gm) were also estimated. The damping-off incidence percentage was calculated 75 days after sowing.

$$\text{Disease incidence (\%)} = \frac{\text{Number of dead plants}}{\text{Number of sown seeds in a plot}} \times 100$$

8. Statistical Analysis

The collected data were subjected to one-way ANOVA as outlined by Gomez and Gomez (1984). Least significant difference (LSD) and Duncan multiple range test were automated to compare treatments means at 0.05 and 0.01 probability levels.

RESULTS

1. Effect of mustard or canola seed meals on linear growth of the tested fungi

Data presented in Table (1) show that the three tested fungi *F. oxysporum*, *S. sclerotiorum* and *R. solani* were sensitive to either mustard or canola seed meal. However, the mustard seed meal was significantly effective than canola seed meal at all concentrations (5, 10 and 25 mg plate^{-1}). Increasing concentration of mustard and canola seed meal proportionally increased mycelial growth inhibition. Significant differences were observed with different amounts (5, 10 and 25 mg plate^{-1}) of mustard or canola seed meal for each fungus. *R. solani* was the most sensitive to mustard seed meal as recorded the lowest values of linear growth (4.4 and 1.07 cm) followed by *S. sclerotiorum* (5.57 and 2.87 cm) and *F. oxysporum* (5.13 and 1.6 cm) at 10 and 25 mg plate^{-1} of mustard seed meal, respectively, While at 5 mg plate^{-1} of mustard meal sensitivity of the tested pathogens was *S. sclerotiorum* (8.0 cm), *F. oxysporum* (8.1 cm) and *R. solani* (8.5 cm) respectively. *S. sclerotiorum* was the most sensitive to canola seed meal recording the lowest values of linear growth (7.77, 5.07 and 2.0 cm) followed by *F. oxysporum* (8.33, 4.97 and 1.93 cm) and *R. solani* (8.77, 6.03 and 1.93 cm) at 5, 10 and 25 mg of canola seed meal, respectively.

Table (1): Effect of mustard and canola seed meals on mycelia growth of selected pathogenic fungi of chickpea

Treatments	Concentrations	*Fusarium oxysporum*		*Sclerotinia sclerotiorum*		*Rhizoctonia solani*	
		Linear growth (cm)	Reduction (%)	Linear growth (cm)	Reduction (%)	Linear growth (cm)	Reduction (%)
Mustard seed meal	5 mg	8.10	10.00c	8.00	11.11c	8.50	5.56c
	10 mg	5.13	43.00b	5.57	38.11b	4.40	51.11b
	25 mg	1.60	82.22a	2.87	68.11a	1.07	88.11a
	(Control)	9.00	-	9.00	-	9.00	-
Canola seed meal	5 mg	8.33	7.44c	7.77	13.67c	8.77	2.56c
	10 mg	4.97	44.78b	5.07	43.67b	6.03	33.00b
	25 mg	1.93	78.56a	2.00	77.78a	1.93	78.56a
	(Control)	9.00	-	9.00	-	9.00	-
L.S.D at 0.05 and 0.01							
Treatments (A)		Ns		**		**	
Concentration (B)		**		**		**	
Interaction (AB)		Ns		**		**	

Means followed by the same letters in the same column are not statistically differed in the same treatment.

Ns, * and ** indicate insignificant, significant at 0.05 and 0.01 levels of probability, respectively

2. Pots trials

2.1. Effect of mustard or canola seed meals on the tested pathogenic fungi under greenhouse conditions

Soil amendments with mustard or canola seed meal significantly reduced damping-off incidence caused by *F. oxysporum*, *S. sclerotiorum* and *R. solani* under greenhouse conditions. Data in (Table 2) show means of effect of treatments whereas, the healthy chickpea plants number was increased in comparison with the untreated control treatment. No differences were observed between the mean effect of mustard and canola seed meal on the tested fungi. *Fusarium oxysporum* showed the highest occurrence of damping-off (84 %) followed by *S. sclerotiorum* 80 % and *R. solani* 80 % in control treatment. Data also reveal significant increases to the efficacy of either treatments with mustard or canola seed meals to reduce the damping-off incidence and increasing number of healthy survival percentage of chickpea plants based on increasing concentrations (0.5, 1.0 and 2.5 g pot^{-1}). *R. solani* was the most sensitive to mustard seed meal treatment followed by *F. oxysporum* and *S. sclerotiorum* with recorded average of damping-off 34.7, 38.7 and 38.7 %, respectively and an average of survival plants were 65.3, 61.3 and 61.3 % respectively. Similar results were obtained in case of the canola seed meal treatment. *R. solani* was the most sensitive to canola seed meal treatment followed by *F. oxysporum* and *S. sclerotiorum* with recorded average damping-off 36.0, 38.7 and 50.7 % respectively and an average of survival plants of 64.0, 61.3 and 49.3%, respectively.

Table (2): Effect of soil amendment with mustard and canola seed meals with different concentrations against *Fusarium oxysporum Sclerotinia sclerotiorum* and *Rhizoctonia solani* under greenhouse conditions

Treatments	Concentrations	***Fusarium oxysporum***		***Sclerotinia sclerotiorum***		***Rhizoctonia solani***	
		Damping-off %	Survival plants %	Damping-off %	Survival plants %	Damping-off %	Survival plants %
Mustard seed meal	Control	84a	16c	80a	20c	80a	20c
	0.5 g/pot	60b	40b	64b	36b	56b	44b
	1.0 g/pot	32c	68a	32c	68a	28c	72a
	2.5 g/pot	24c	76a	20c	80a	20c	80a
	Mean	**38.7**	**61.3**	**38.7**	**61.3**	**34.7**	**65.3**
Canola seed meal	Control	84a	16c	80a	20d	80a	20d
	0.5 g/pot	56b	44b	76b	24c	56b	44c
	1.0 g/pot	36c	64a	44c	56b	32c	68b
	2.5 g/pot	24c	76a	32d	68a	20d	80a
	Mean	**38.7**	**61.3**	**50.7**	**49.3**	**36.0**	**64.0**
L.S.D at 0.05 and 0.01							
Treatments (A)		Ns		Ns		Ns	
Concentrations (B)		**		**		**	
Interaction (AB)		Ns		Ns		Ns	

Means followed by the same letters in the same column are not statistically differed in the same treatment.

Ns, * and ** indicate insignificant, significant at 0.05 and 0.01 levels of probability, respectively

2.2. Effect of mustard or canola seed meals on chickpea plants nodulation under greenhouse conditions

Data in (Table 3) show means of effect of soil amendments with mustard or canola seed meal on chickpea plants nodulation status, *i.e.* number of nodules per plant, nodules fresh and dry weight (mg/plant) under greenhouse conditions. Nodulation status of chickpea plants was significantly decreased than the control (non-infected soil) when affected by the tested soil-borne fungi, *F. oxysporum* as the most effective one followed by *S. sclerotiorum* and *R. solani*. Meanwhile, mustard or canola seed meal treatments significantly increased the nodulation as mustard seed meal was more effective than canola seed meal in the case of nodules per plant and nodules dry weight (mg) per plant while there was no significance between the efficiency of either mustard or canola seed meal treatments for the nodules fresh weight (mg) per plant. No significant difference of mustard or canola seed meal treatments on the tested fungi, number of nodules per plant and/or nodules fresh weight (mg) per plant; however, mustard seed meal treatment was slightly higher than canola seed meal for nodules dry weight (mg) per plant. While it was clear that the increase in the concentrations used *i.e.* 0.5, 1.0 and 2.5 g plot^{-1} of each of the two treatments gave clear and significant effects on number of nodules per plant, nodules fresh and dry weights (mg) per plant.

Table (3): Effect of different concentrations of mustard or canola seed meals on chickpea plants nodulation under greenhouse conditions

Concentrations	Mustard seed meal				Canola seed meal			
	#N.I.S	***F.o.***	***S.s.***	***R.s.***	**N.I.S**	***F.o.***	***S.s.***	***R.s.***
				Number of nodules plant^{-1}				
0.0 g/pot	10.0c	5.8d	6.0c	5.60c	11.0a	6.4c	6.8c	5.6c
0.5 g/pot	12.4b	8.6c	9.2b	10.2b	11.8b	7.8ab	8.2b	8.0b
1.0 g/pot	14.6b	9.8b	10.2b	11.6b	13.8c	8.2a	8.2b	9.6b
2.5 g/pot	18.4a	14.6a	15.2a	18.8a	14.2d	9.8a	10.2a	11.2a
Mean	**15.1**	**11.0**	**11.5**	**15.2**	**8.6**	**8.9**	**9.6**	**15.1**
L.S.D. at 0.05 and 0.01								
Soil Infestation (A)**; Treatments (B)**; (AB) NS; Concentration (C)**; (AC)*; (BC)**; (ABC) NS								
				Fresh weight of nodules plant^{-1} (mg)				
0.0 g/pot	197d	161c	183c	155d	195c	169d	186d	162c
0.5 g/pot	300c	221c	232b	230c	296b	212c	206c	230b
1.0 g/pot	305b	245b	268a	258b	302b	251b	261b	238b
2.5 g/pot	322a	268a	283a	270a	325a	277a	303a	278a
Mean	**309.0**	**244.7**	**261.0**	**252.7**	**246.7**	**256.7**	**248.7**	**309.0**
L.S.D. at 0.05 and 0.01								
Soil Infestation (A)**; Treatments (B)NS; (AB)NS; Concentration (C)**; (AC)**; (BC)**; (ABC)NS								
				Dry weight of nodules plant^{-1} (mg)				
0.0 g/pot	57.4c	37.0d	42.0d	32.0d	53.2d	34.2d	38.2d	32.8c
0.5 g/pot	76.8b	51.2c	51.2c	53.4c	71.2c	44.2c	46.2c	52.2b
1.0 g/pot	82.2ab	57.6abc	68.8b	60.6b	82.2b	56.8ab	61.6b	56.2b
2.5 g/pot	80.8a	63.8a	73.6a	65.6a	91.6a	63.4a	66.8a	66.2a
Mean	**79.9**	**57.5**	**64.5**	**59.9**	**54.8**	**58.2**	**58.2**	**79.9**
L.S.D. at 0.05 and 0.01								
Soil Infestation (A)**; Treatments (B)**; (AB)*; Concentration (C)**; (AC)**; (BC)**; (ABC)**								

#NIS. Non-infested soil; F.o. Fusarium oxysporum; S.s. Sclerotinia sclerotiorum. R.s. Rhizoctonia solani
Means followed by the same letters in the same column are not statistically differed in the same treatment.
Ns, * and ** indicate insignificant, significant at 0.05 and 0.01 levels of probability, respectively

3. Field trials:

3.1. Effect of mustard or canola seed meals on the damping-off disease of chickpea under field condition

Soil amendments with mustard or canola seed meals significantly decreased damping-off compared to the untreated control (Table 4) in the two successive seasons. Mustard seed meal was proven to be the most effective to control damping-off in both seasons. Mustard or canola seed meals decreased the disease incidence % on chickpea plants by 6.60 and 13.4%, respectively when compared with the untreated control (26.0%) causing a reduction of the incidence (74.61 and 48.46%) than the control respectively at the first season 2016/17 and disease incidence % (9.8 and 16.8%, respectively) compared with the untreated control (28.8%) at the second season 2017/18, causing (65.97 and 41.67%) a reduction of the incidence than the control respectively.

Table (4) Effect of soil amendments with mustard or canola seed meals on damping-off incidence (%) on chickpea plant under field condition

Seasons	Treatment	Damping-off %	Reduction %
2016-2017	Mustard seed meal	6.60c	74.61
	Canola seed meal	13.40b	48.46
	Control	26.00a	0.00
2017-2018	Mustard seed meal	9.80c	65.97
	Canola seed meal	16.80b	41.67
	Control	28.80a	0.00
L.S.D at 0.05 and 0.01	**Seasons (A)**	**	
	Treatments (B)	**	
	Interaction (AB)	**Ns**	

Means followed by the same letters in the same column are not statistically differed in the same treatment.
Ns, * and ** indicate, significant at 0.05 and 0.01 levels of probability, respectively

3.2. Effect of soil amendments with mustard or canola seeds meals on chickpea plant nodulation under field condition.

Data in (Table 5) show the effects of soil amendments with mustard or canola seed meals on chickpea plants nodulation status, *i.e.*, number of nodules per plant and nodules dry weight (mg) per plant under field conditions. Soil amendments with mustard or canola seed meal treatments significantly increased the nodulation status 24.60 and 22.40 nodule/plant and (22.20 and 23.80 nodule/plant) compared to the untreated control (15.80 and 14.80) during both of the successive seasons (2016/17 and 2017/18) respectively. There was no significant difference observed between both treatments in the two successive seasons. In addition, soil amendments with mustard or canola seed meal treatments had significant increases in dry weight (mg) of nodules per plant (280 and 240 mg/plant) and (280 and 310 mg/plant) compared to the untreated control (220 and 200 mg/plant) during both of the successive seasons respectively.

Table (5): Effect of soil amendments with mustard or canola seeds meals on chickpea plant nodulation under field condition

Seasons	Treatments	Number of nodules plant^{-1}	Dry weight of nodules plant^{-1}(mg)
2016-2017	Mustard seed meal	24.60a	280a
	Canola seed meal	22.40a	240ab
	Control	15.80b	220b
2017-2018	Mustard seed meal	22.20a	280a
	Canola seed meal	23.80a	310a
	Control	14.80b	200b
L.S.D at 0.05 and 0.01	Seasons (A)	Ns	Ns
	Treatments (B)	**	**
	Interaction AB	Ns	**

Means followed by the same letters in the same column are not statistically differed in the same treatment. Ns, * and ** indicate, significant at 0.05 and 0.01 levels of probability, respectively.

4. Effect of mustard or canola seed meals on chickpea yield components under field conditions

Data in (Table 6) show the effects of soil amendments with mustard or canola seed meals on chickpea yield components under field condition, *i.e.* plant height, pods number per plant and 100-seed weight. Soil amendments with mustard or canola seed meal on chickpea significantly increased plant height, pods number per plant and 100-seed weight than the untreated control plants during both successive seasons (2016/17 and 2017/18). Mustard or canola seed meal treatment increased the plant height, (89.6 and 85.4 cm) and (83.4 and 81.2 cm) compared to the untreated control (79.4 and 76.2 cm) during both of the successive seasons (2016/17 and 2017/18), respectively, as well as mustard or canola seed meal treatment increased number of pods per plant (50.8 and 47.4) and (48.6 and 45.2) compared to the untreated control (40.4 and 38.2) during both successive seasons of (2016/17 and 2017/18), respectively, the same trend was obtained as the treatment increased 100-seed weight (24.3 and 22.1 gm) and (22.3 and 21.1 gm) compared to the untreated control (19.5 and 16.1 gm) during both of the successive seasons (2016/17 and 2017/18), respectively.

Table (6): Effect of soil amendments with mustard or canola seed meals on chickpea yield components (plant height, number of pods per plant and 100 seed weight) under field conditions

Seasons	Treatments	Plant Height (cm)	Number of pods/plant	100-seed weight (gm)
2016-2017	Mustard seed meal	89.6a	50.8a	24.3a
	Canola seed meal	83.4b	48.6a	22.3b
	Control	79. 4b	40.4b	19.5c
2017-2018	Mustard seed meal	85.4a	47.4a	22.1a
	Canola seed meal	81.2b	45.2a	21.1a
	Control	76.2c	38.2b	16.1b
L.S.D at 0.05 and 0.01	Seasons (A	**	**	**
	Treatments (B)	**	**	**
	Interaction (AB)	Ns	Ns	Ns

Means followed by the same letters in the same column are not statistically differed in the same treatment. Ns, * and ** indicate, significant at 0.05 and 0.01 levels of probability, respectively

DISCUSSION

Biofumigation using *Brassica* spp. as seed meal, green manure amendment, or in crop rotation, is an approach to control multiple soil-borne pathogens when the glucosinolates in *Brassica* spp. are hydrolyzed in the soil resulting in the release of volatile biocidal compounds isothiocyanates (ITCs) (Matthiessen and Kirkegaard, 2006; Mazzola *et al.,* 2007; Fayzalla *et al.,* 2009; Shaban *et al.,* 2011 and Hassan *et al.,* 2016).

Using mustard and canola seed meals as biofumigation was evaluated against the soil-borne pathogens *F. oxysporum*, *S. sclerotiorum* and *R. solani* infecting chickpea plants *In Vitro*, greenhouse and field conditions. Results indicated decrease in the linear growth of the tested fungi *F. oxysporum*, *S. sclerotiorum* and *R. solani* as compared with the control. Current results could be correlated with the (Fayzalla *et al.,* 2009 and Shaban *et al.,* 2011) seed meal from the *Brassica* species suppresses and/or inhibiting the linear growth of soil-borne pathogens compared with the control. Earlier reports in this respect (Mayton *et al.,* 1996; Charron and Sams, 1999 and Smolinska *et al.,* 2003) stated that the volatile substances released from the ground mustard seed *In vitro* assays showed a strongest fungicidal effect, toward soil-borne pathogens. Likewise, Chung *et al.,* (2002) showed a strong fungicidal effect on *R. solani* because of the volatile substances of ground seed of mustard.

The obtained data in pots and field experiments showed promising results for controlling the soil-borne pathogenic fungi. Significant control of damping-off had been found by using mustard or canola seed meal, whereas mustard meal was the most effective treatment. The reduction in damping-off was significantly reflected on the chickpea yield components under field conditions as increased plant height, pods number per plant and weight of 100-seed than untreated control plants. Many similar reports are supporting these results using *Brassica* spp. as seed meal or as green manure suppresses the soil-borne pathogenic fungi which enhanced plant growth and yield by the released volatile biocidal compounds mainly isothiocyanates (ITCs), resulted from the hydrolyzed *Brassica* spp. in the soil (Matthiessen and Kirkegaard, 2006 and Mazzola *et al.,* 2007 and Smolinska *et al.,* 2003). Results of the field experiment conducted in Agric. Res. Station, Menoufia Gov. (Egypt) during the seasons 2016/17 and 2017/18, indicated reduced in the damping-off incidence over the control whereas mustard seed meal treatment was the superior effect in the greenhouse and field conditions for controlling damping-off diseases of chickpea. Similar results in Egypt, biofumigation using mustard and canola seed meal against the soil-borne pathogenic fungi of soybean and lupine, showed the fungicidal effect against the tested fungi in pot and field experiments, meanwhile plant growth was increased compared to the control treatment (Fayzalla *et al.,* 2009 and Shaban *et al.,* 2011).

The obtained data also revealed a significant increase in nodulation *i.e*., number of nodules per plant, nodules fresh and dry weight (mg) per plant under greenhouse conditions and field conditions. Further report confirming these findings (Ramirez-Villapudua and Munnecke, 1988) who reported that the soil microbial communities composition had been changed and total bacterial counts increased 16-fold after exposing the soil to volatile compounds released from the cabbage. As well microbial activity increased 115% in first week when added cabbage residues to field micro plots (Gamliel and Stapleton, 1993). Brassicaceae plant residues amended with pea roots significantly increased the number of nodules formed on pea roots by *Rhizobium leguminosarum* (Muehlchen *et al.,* 1990 and Scott and Knudsen, 1999).

In conclusion, The present study indicated that the application of biofumigation with mustard or canola seed meals significantly protected chickpea plants against the soil-borne pathogenic fungi, mainly through the release of volatile biocidal compounds isothiocyanates (ITCs), resulting from the hydrolyzed of the glucosinolates in *Brassica* spp. in the soil. Also, the application of such biofumigants for the control of chickpea damping-off diseases on the field scale may provide a practical environmentally friendly disease management against soil-borne diseases, can be used through integrated disease management.

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تأثير التبخير الحيوي بدقيق بذور الخردل أوالكانولا في مكافحة امراض التربه في الحمص

إيهاب علي ضياء سرحان[١] ، سحر عباس السيد[١] ، هشام عبد المقصود محمدين[١] ،و تامر سلامة المرصفاوي[٢]

[١] *معهد بحوث أمراض النباتات – مركز البحوث الزراعيه – الجيزه – مصر*

[٢] *معهد بحوث المحاصيل الحقليه – مركز البحوث الزراعيه – الجيزه – مصر*

يعتبر مرض موت البادرات واعفان الجذور من اهم الأمراض التي تصيب نبات الحمص (*Cicer arietinum* L.) في مصر. تم تقييم تأثير التبخير الحيوي بدقيق بذور الخردل (*Brassica juncea*) والكانولا (*Brassica napus*) في المعمل وتحت ظروف الصوبه والحقل في محطة البحوث الزراعيه بسرس الليان محافظة المنوفيه في موسمي النمو ٢٠١٦/2017، و ٢٠١٧/2018 لمكافحة موت البادرات واعفان الجذور في الحمص. وقد تبين ان دقيق بذور الخردل والكانولا ثبطا معنويا النمو الخيطي للفطريات الممرضه المختبره *Fusarium oxysporum, Sclerotinia sclerotiorum*، و *Rhizoctonia solani*، ويزداد هذا التأثير بزيادة التركيز مقارنه بالمقارنه الغير معامله. أثبتت النتائج المتحصل عليها تأثير دقيق بذور الخردل والكانولا كمبيد فطري في تجارب الأُصص والحقل حيث قللا معنويا نسبة موت بادرات الحمص بالاضافه الي تحسين الصفات المحصوليه مثل اطوال النباتات، عدد القرون للنبات الواحد ووزن ال ١٠٠ بذره، وقد سجلت المعاملة بدقيق بذور الخردل اعلي القيم متبوعا بدقيق الكانولا. كما اوضحت النتائج المتحصل عليها زيادة معنوية في العقد الجذريه للبكتيريا (*Mesorhizobium ciceri*) على جذور النبات وكذلك الوزن الجاف والرطب للعقد البكتيريه بنباتات الحمص النامية في تربه معامله بدقيق بذور الخردل اوالكانولا تحت ظروف الصوبة او الحقل. وكانت المعاملة بدقيق بذور الخردل هي الاكثر تأثيرا. ونتيجة لهذه الدراسه فان المعاملة بالتبخير الحيوي لمكافحة أمراض موت البادرات واعفان الجذور في الحمص قد تكون أحد الحلول العملية الصديقه للبيئة والتي يمكن دمجها مع المكافحه المتكاملة للأمراض.