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Effect of Olive and Castor Bean Oil Cakes singly or combined with *Trichoderma* spp. on *Fusarium solani* and *Meloidogyne incognita* Infecting Eggplant

[1]Abd-El-Khair H., Wafaa M.A. El-Nagdi[2] and M. M. A. Hammam[1]

[1] Plant Pathology Department, National Research Centre, Dokki, Post Code 12622.
[2]Plant Pathology Department, Nematology Laboratory, National Research Centre, Dokki, Post Code 12622.

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ABSTRACT

Trichoderma harazianum (Th) and *Trichoderma vierns* (Tvr) as well as the oil cakes of olive (OC) and castor bean (CC) alone or in combination were applied for controlling *Fusarium solani* and *Meloidogyne incognita* on eggplants in pots experiment. Th or Tvr combined with OC or CC reduced *Fusarium* root rot disease incidence. They also reduced were nematode parameters (*viz.* J_2 of *M. incognita* in soil & roots and the numbers of galls & egg-masses) as well as increased the growth parameters of eggplants (*viz*. shoot length, fresh shoot weight, dry shoot weight, leaves number, fresh root weight and dry root weight), comparing with control.

Key words: Oil seed cakes, Combination, Eggplants, *Fusarium solani, Trichoderma spp., Meloidogyne incognita.*

Introduction

Eggplant (*Solanum melongena* L.) is an important vegetable crop in Egypt. Eggplant is highly susceptible to soil borne diseases. *Meloidogyne incognita* and *Fusarium solani* f. sp. *melongenae* were found to infect eggplants (Baloch *et al*., 2013) and cause losses in yield and fruit quality (El-Nagdi and Abd-El-Khair, 2008). Soil borne diseases can be managed effectively by chemical treatments, but the chemical pesticides are expensive, harmful to human health and environmental. Therefore, several methods are applied to manage the root-knot nematode and *Fusarium* root rot include organic amendments and bio-control agents that are environmentally friendly alternatives.

The organic amendments *viz.,* neem seed powder and farmyard manure alone and in combination reduced the root-knot wilt disease complex caused by *M. incognita* and *F. solani* on chilli (Kumar *et al*., 2009). The poultry manure significantly reduced the population of *M. incognita* in eggplant roots (Karmani *et al*., 2011). Also, the poultry manure and mustard oil cake reduced the root-knot infestation of bean (Faruk *et al*., 2012). Castor bean oil cake significantly reduced the galls and egg masses numbers of *M. incognita* on roots of tomato plants (El-Nagdi & Youssef, 2013 and Roldi *et al*., 2013). *T. harzianum* significantly reduced the nematode population in tomato roots, compared with their population in untreated plot (Olajide *et al*., 2015). The mustard oil cake was the most effective against wilt disease in chickpea (Kala *et al*., 2016). *Trichoderma harzianum*, *T. asperellum* and *T. virens* reduced disease incidence of *F. solani* and *F. oxysporum* on chickpea in greenhouse and fields (Akrami and Yousefi, 2015). They have a good protective effect against the two pathogens in cucumber (Akrami, 2015). *T. harzianum*, *T. koningii* and *T. viride* showed inhibitory effect on mycelial growth of *F. solani* f. sp. *melongenae* in dual culture, the maximum inhibition was recorded with *T. viride* (Bhadra *et al*., 2016).

The incorporation of neem leaves with *T .harzianum* had the highest suppressive effect against the nematode, decreasing the galling and egg mass production and leading to a significant increase in the dry matter production and yield in inoculated plants (Khan *et al*., 2012). *T. harzianum* in combinations with *Moringa oleifera* significantly reduced the reproduction, egg hatching and juvenile mortality of *M. javanica* on eggplant *in vitro, in vivo* and in greenhouse (Murslain *et al.,* 2014). *T. harzianum* combined with *L. camara* significantly reduced the root-knot nematode population, number of galls and egg masses per plant in pots .The highest mean of fruit weight and total yield

Corresponding Author: M. M. A. Hammam, Plant Pathology Department, Nematology Laboratory, National Research Centre, Dokki, Post Code 12622, Cairo, Egypt.

were observed in *T. harzianum* combined with *L. camara* treatment (Feyisa *et al*., 2015). Application of mixture of *T. harzianum* and neem oil cake suppressed *F. solani* f.sp. *melongenae* (Brinjal wilt disease) as well as increased the growth character and fruit yield (Bhadauria *et al*., 2012). *T. harzianum* or mixed with poultry manure suppressed *Fusarium* wilt incidence and increasing the plant growth and fruit yield of eggplant (Faruq *et al*., 2014). *T. viride* and *T. harzianum* and organic amendments *viz*; farm yard manure; vermicompost and mustard oil cake were applied for controlling *F. oxysporum* f. sp. *ciceri* in chickpea (Kala *et al*., 2016). Using tactics such as antagonistic fungi and organic amendment as an integrated method controlled the *Fusarium* root rot pathogen in *Lupinus termis* (Ibrahim and Abdelaziz, 2017).

Therefore this work is aimed to study the inhibitory effects of *T. harziamum* or *T. vierns* as well as soil amendments *viz*. olive pomace (*Olea europaea*) and castor bean seeds (*Ricinus communis*) alone or in combination on root-knot (*M .incognita)* and *Fusarium* root-rot (*F. solani*) in eggplants under greenhouse conditions.

Materials and Methods

The tested materials:

Two of *Trichoderma* spp. namely *T. harziamum* (Th) and *T. viride* (Tvr) as well as the oil cakes of olive (OC) and castor beans (CC) as soil amendments were applied.

Inhibitory effect of soil amendments against *Trichoderma* spp.:

The potential antagonistic activity of OC and CC against Th and Tvr was tested by the poisoned food technique described by Schmitz (1930) and Borum & Sinclair (1968).The experiment was conducted in Completely Randomized Design. The aqueous extracts of OC and CC were separately prepared as follow; 10 g of dried material were added to 100 ml of distilled water and then extracted in a boiling water bath at 80°C for 15 min (Abd-El-Khair and El-Gamal, 2011). Then, 15 ml of each plant extract were mixed with potato dextrose agar medium and poured in sterilized Petri plates (9 cm - diameter). The poisoned medium was allowed to solidify. Then, 0.5 cm fungal mycelia disc of each *Trichoderma* spp. was picked from 7-days-old purified culture with the help of a sterilized cork borer and then the disc was put in the center of each plate. Three Petri plates were used as replicates for each treatment as well as untreated control. The mycelial diameter of *Trichoderma* spp. was recorded.

Fusarium root-rot pathogen:

Fusarium solani (Mart.) Appel *et* Wollenw. emend. Snyd. *et* Has., was isolated from naturally infected eggplants in pervious study was done in Plant Pathology Department , NRC, (El-Nagdi and Abd El-Khair, 2008).

Identification and inoculum of *M. incognita*:

Egg-masses of *M. incognita* were isolated from infected eggplant roots collected from naturally infected field. A single egg- mass culture of this nematode was established and reared on eggplant cv. Pusa Purple Long in a greenhouse. Adult females were used to identify the nematode species by the morphological characteristics of the female perineal pattern (Taylor and Sasser, 1978).

Preparation of *Trichoderma* spp. and *F. solani* inoculums:

The inoculums of *T. harziamum, T. viride* as well as *F. solani* was separately prepared using corn: sand: water (2:2:1V/V/V) medium. The sterilized medium was individually inoculated with each fungus using fungal disc (1cm-diameter) obtained from 7 days-old-culture. The inoculated media were incubated at 30 ± 2°C for 15 days. The resulting fungal inoculums were applied in pots experiment (Abd-El-Khair *et al*., 2016).

Greenhouse experiment:

The antagonistic effects of *Trichoderma* spp. (Th and Tvr) and efficacy of soil amendments (OC and CC) and their combination were tested angnist *M. incognita* and *F. solani* in separately pots under greenhouse conditions. Eighty-eight pots containing 2 kg of sterilized sandy loam soil (1:1 W/W) were used. The treatments were as follows; Th+OC, Th+CC, Tvr+OC, Tvr+CC, OC, CC, OC+CC, Th, Tvr, Th+Tvr and untreated control (treated with either *M. incognita* or *F. solani* only). The pots were divided into two groups; each one contains 44pots, the first group for *M. incognita* and the second for *F. solani*. Four pots were used as replicated for each treatment.

Effect on Fusarium solani:

Fourty four pots were inoculated with 7- days-old cultures *F. solani* adjusted to 10^8 CFU/g at the rate of 3% soil weight (W: W). Then, sterilized sandy loam soil was inoculated with both *Trichoderma* spp. and soil amendments at the rate of 3% soil weight (W: W). The untreated control was treated with *F. solani* only. The pots were watered and left for one week. Then, four weeks old eggplants seedling cv. Pusa Purple Long was transplanted in each pot .The pots were arranged according to a completely randomized design on a bench in the glasshouse. After one week of antagonistic materials. After three months of treatments, Feeder root of eggplants were taken, from treated and untreated plants, to examine the presence of *F. solani* infection using the standard isolation method, where the pathogen was detected according to cultural characters (Nelson *et al.*, 1983 and Dhingra & Sinclair, 1985). The root rot disease incidence of *F. solani* was recorded according the following formula:

Fsarium root-rot incidence % = No. of infected root pieces / Total root pieces ×100

Effect on Meloidogyne incognita:

Before sowing, pots were firstly inoculated with *Trichoderma* spp. and soil amendments each at the rate of 3% soil weight (w: w). Then the soil was mixed thoroughly to ensure equal distribution of materials with soil. Then, four weeks old eggplants seedling cv. Pusa Purple Long was transplanted in each pot. Each pot was inoculated with 1,000 newly hatched J_2 of *M. incognita* (in four holes made around the plant). The untreated control was treated with *M. incognita* only. The pots were watered and left for one week. The pots were arranged according to a completely randomized design on a bench in the glasshouse. Three months after inoculation, the numbers J_2 in the soil and roots as well as the number of galls and egg-masses on the root were counted**.**

Effect on growth parameters:

Three months after inoculation, the growth parameters of eggplants *viz*. lengths of shoot, fresh and dry weights of shoot, leaves number and fresh & dry weight of roots were recorded.

Statistical analysis

The means were compared by Duncan's Multiple Range Test at $P = 0.05$, using analysis of variance by Computer Statistical Package (CO-STATE) User Manual Version 3.03, Barkley Co., USA. Nematode data were normalized before analysis by log transformation (Snedecor and Cochran, 1980).

Results

Inhibitory effect of soil amendments:

Results of *in vitro* test revealed that the soil amendments *viz*. OC and CC had no inhibitory effect against two applied *Trichoderma* spp. (Th and Tvr), where mycelial growth reduction was recorded.

Effects on *Fusarium* root rot incidence:

Data presented in Figure (1) showed that *Fusarium* root rot incidence was affected by tested treatments. Lowest disease incidence (8.4%) was obtained by Tvr + CC treatments , followed by Tvr+ OC (12.5%) , Th+CC (12.5%) , Th +OC (16.7%) , Th+Tvr (16.7%) , Tvr (25.0%) and both Th & OC+CC (29.2%). Relatively high disease incidence 38.0 and 42.0% were occurred at OC and CC treatments, respectively. High disease incidence (70.9%) was found at control treatment.

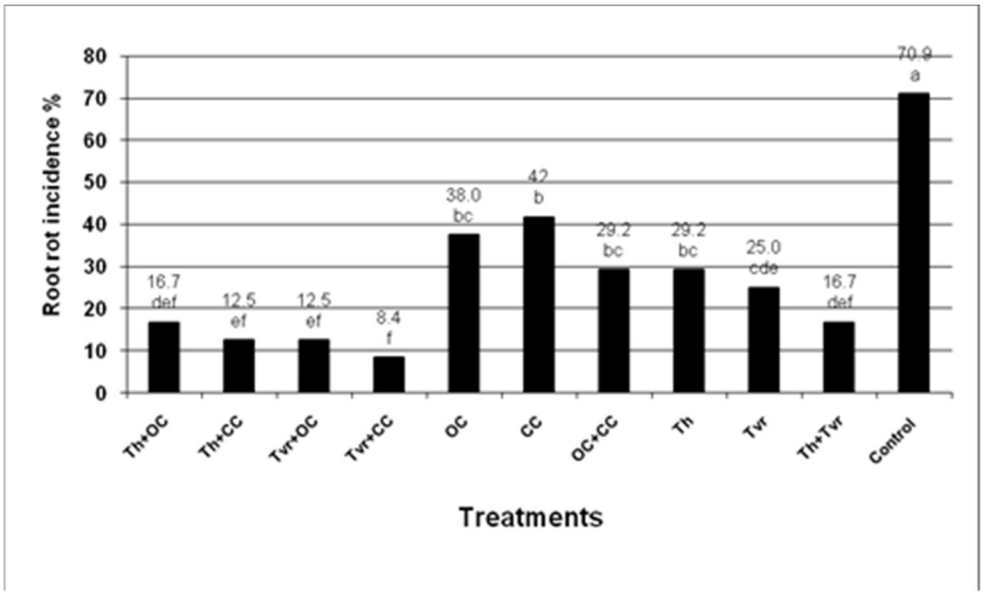

Fig. 1: Effects of *Trichoderma harzianum* (Th), *Trichoderma vierns* (Tvr), olive cake (OC) and castor bean cake (CC) alone or in combination on root rot disease incidence in eggplants , under artificial infection conditions with *Fusarium solani*.

Effect on *M. incognita* parameters:

Results showed that the pots treated with Th or Tvr and then amended with Oc or CC highly reduced the J_2 of *M. incognita* in soil and roots of eggplants as well as the numbers of galls and egg-masses in the ranges of 86-90; 92-95; 85-86 and 89-90%, respectively. Details of inhibitory effect of *Trichoderma* spp. combined with OC or CC against nematode parameters are listed in Table (1). The soil amendments viz. OC and CC alone or in combination reduced the above *M. incognita* parameters in eggplants in the ranges of 74-84; 88-93; 79-81 and 72-76%, respectively. The treatment of OC + CC highly reduced the tested nematode parameters, than individual treatments. Th + Tvr alone or in combination also reduced the same parameters of *M. incognita* in the ranges of 76-84; 89-93; 72-81 and 73-76%, respectively. Th+Tvr as combined treatment also highly reduced the J_2 in soil & roots, galls and egg-masses, than individual treatments (Table 1).

Table 1: Effect of *Trichoderma harzianum* (Th), *Trichoderma vierns* (Tvr), olive oil cake (OC) and castor bean oil cake (CC) alone or in combination on *Meloidogyne incognita* parameters in eggplants, under artificial infection conditions.

Treatments[1]	*M. incognita* parameters							
	J_2 in /200g soil		J_2 in /5g roots		Galls /5g roots		Egg-masses/5g roots	
	Log_{10}	**Red. %**	Log_{10}	**Red. %**	Log_{10}	**Red. %**	Log_{10}	**Red. %**
Th+OC	1.80bc[2]	**87**	1.20c	**95**	0.86c	**85**	0.53c	**89**
Th+CC	1.82bc	**86**	1.42bc	**92**	0.84c	**86**	0.55c	**89**
Tvr+OC	1.63c	**90**	1.32bc	**93**	0.82c	**86**	0.49c	**90**
Tvr+CC	1.70c	**89**	1.20c	**95**	0.82c	**86**	0.50c	**90**
OC	2.01b	**79**	1.46bc	**91**	1.01bc	**79**	0.96b	**72**
CC	2.10b	**74**	1.57b	**88**	0.97bc	**81**	0.95b	**74**
OC + CC	1.89bc	**84**	1.49bc	**90**	0.92bc	**83**	0.91b	**76**
Th	2.05b	**76**	1.50bc	**90**	1.14b	**72**	0.96b	**73**
Tvr	2.00b	**78**	1.53b	**89**	0.99bc	**80**	0.95b	**74**
Th+ Tvr	1.87bc	**84**	1.36bc	**93**	0.95bc	**81**	0.91b	**76**
Nematode only	2.68a	**-**	2.49a	**-**	1.69a	**-**	1.53a	**-**

[1] All treatments were done with artificial infection by *M. incognita*
[2] Means are averages of four replicates followed by same small letter(s) are not significant according to Duncan's Multiple Range Test at $p \leq 0.05$

Effects on growth parameters:

Data presented in Table (2) showed the effect of tested treatments on the growth parameters of eggplant infected with *F. solani*. High increase percentage of shoot length (67%) was occurred at Th+Tvr treatment followed by Th+CC (62%) , Tvr+CC (61%) , Th+OC and Tvr+OC (58%) , Th (57%) and Tvr (56%) ,while the low increase percentage were obtained by OC , CC and OC+CC treatments. Same trends were occurred in fresh and dry weight of shoot, number of leaves and root fresh weight. Large increase % of root dry weight were obtained at Tvr+CC , Tvr+OC , Th+CC and Th+OC treatments as they were 127, 118 ,109, 1ne 100%, respectively. The other treatments had less increase % (Table 2).

Data presented in Table (3) showed the effect of tested treatments on the growth parameters of eggplant infected with *M. incognita*. Results showed that Th or Tvr combined with OC or CC increased the growth parmeters of eggplants *viz*. shoot length, fresh shoot weight, dry shoot weight, leaves number, fresh root weight and dry root weight in the ranges of 62-82; 86-107; 98-102; 102-116 ; 40-65% and 118-136`%, respectively. The OC and CC treatments alone or in together increased the growth parmeters of eggplants in the ranges of 24-39; 42-47; 21-33; 20-36; 6-14 and 18-55%, respectively. Th and Tvr also alone or in together also increased the above growth parameters in the ranges of 65-70; 89-113; 67-74; 75-102; 38-63 and 64-109`%, respectively (Table 3).

Table 2: Effect of *Trichoderma harzianum* (Th), *Trichoderma vierns* (Tvr), olive oil cake (OC) and castor bean oil cake (CC) alone or in combination on the vegetative growth parameters of eggplants, under artificial infection conditions with *Fusarium solani*, in greenhouse conditions.

Treatments[1]	Vegetative growth parameters											
	Shoot parameters								Root parameters			
	Length		Fresh weight		Dry weight		Leaves no.		Fresh weight		Dry weight	
	Cm.	**Incr. %**	g	**Incr. %**	g	**Incr. %**	Count	**Incr. %**	g	**Incr. %**	g	**Incr. %**
Th+OC	40.7ab[2]	**58**	34.3a	**112**	8.5b	**85**	25.7a	**99**	6.9c	**61**	2.2cd	**100**
Th+CC	41.7ab	**62**	35.4a	**119**	8.6b	**87**	25.0ab	**92**	7.1bc	**65**	2.3bc	**109**
Tvr+OC	40.7ab	**58**	35.9a	**122**	8.6b	**87**	24.3ab	**87**	7.9a	**84**	2.4ab	**118**
Tvr+CC	41.3ab	**61**	36.8a	**127**	9.1a	**99**	25.7a	**99**	8.1a	**88**	2.5a	**127**
OC	35.0abc	**36**	26.8a	**65**	5.0f	**9**	20.7ab	**56**	5.6ef	**30**	1.3f	**18**
CC	30.3bc	**18**	27.4a	**69**	5.1f	**11**	17.7bc	**36**	5.5f	**28**	1.3f	**18**
OC + CC	36.7ab	**43**	29.1a	**80**	5.4e	**17**	19.3abc	**48**	5.8de	**35**	1.7e	**55**
Th	40.3ab	**57**	32.1a	**98**	7.0d	**50**	24.0ab	**85**	5.9d	**37**	1.8e	**64**
Tvr	40.0ab	**56**	33.3a	**106**	7.8c	**70**	22.0ab	**69**	7.0c	**63**	1.8e	**64**
Th+Tvr	43.0a	**63**	34.8a	**115**	8.5b	**85**	23.7ab	**82**	7.3b	**70**	2.1d	**91**
F. solani only	25.7c	-	16.2b	-	4.6g	-	13.0c	-	4.3g	-	1.1g	-

[1]All treatments were done with artificial infection by *F. solani*
[2]Means are averages of four replicates followed by same small letter(s) are not significant according to Duncan's Multiple Range Test at $p \leq 0.05$

Table 3: Effects of *Trichoderma harzianum* (Th), *Trichoderma vierns* (Tvr), olive oil cake (OC) and castor bean oil cake (CC) alone or in combination on vegetative growth parameters in eggplants , under artificial infection conditions with *M. incognita*.

Treatments[1]	Vegetative growth parameters											
	Shoot parameters								Root parameters			
	Length		Fresh weight		Dry weight		Leaves no.		Fresh weight		Dry weight	
	Cm.	**Incr. %**	g	**Incr. %**	g	**Incr. %**	Count	**Incr. %**	g	**Incr. %**	g	**Incr. %**
Th+OC	45.0a[2]	**82**	34.3a	**86**	8.5b	**98**	30.7a	**108**	7.3d	**40**	2.5b	**127**
Th+CC	41.3ab	**67**	35.5s	**93**	8.7a	**102**	31.0a	**110**	8.6a	**65**	2.6a	**136**
Tvr+OC	44.0a	**78**	36.1a	**96**	8.7a	**102**	31.7a	**116**	8.0b	**54**	2.4c	**118**
Tvr+CC	40.0ab	**62**	38.0a	**107**	8.8a	**102**	29.7a	**102**	8.1b	**56**	2.5b	**127**
OC	31.0cd	**26**	26.3ab	**43**	5.2g	**21**	19.3bc	**31**	5.7f	**10**	1.3g	**18**
CC	30.7cd	**24**	26.1ab	**42**	5.4f	**26**	17.7c	**20**	5.5g	**6**	1.3g	**18**
OC + CC	34.3bc	**39**	27.1ab	**47**	5.7e	**33**	20.0bc	**36**	5.9e	**14**	1.7f	**55**
Th	40.7ab	**65**	34.7a	**89**	7.5c	**74**	25.7ab	**75**	7.2d	**38**	1.8e	**64**
Tvr	41.3ab	**67**	34.8a	**89**	7.2d	**67**	27.7a	**88**	7.8c	**50**	1.8e	**64**
Th +Tvr	42.0ab	**70**	39.2a	**113**	7.4c	**72**	29.7a	**102**	8.5a	**63**	2.3d	**109**
Nematode only	24.7d	-	18.4b	-	4.3h	-	14.7c	-	5.2h	-	1.1h	-

[1]All treatments were done with artificial infection by M. incognita
[2]Means are averages of four replicates followed by same small letter(s) are not significant according to Duncan's Multiple Range Test at $p \leq 0.05$

Discussion

Several methods such as bio-control agents and soil amendments can be used to protect crops against soil-borne pathogens (Feyisa *et al*., 2015 and Shafique *et al*., 2016). In our study, we aimed to apply *T. harazianum* (Th) and *T. vierns* (Tvr) combined with olive oil cake (OC) and castor bean oil cake (CC) for controlling *M. incognita* and *F. solani*. Especially the extracts of OC or CC had no inhibition effect against mycelium growth of Th or Tvr *in vitro* tests.

Our results indicated that combination between Th or Tvr with OC or CC highly reduced the disease incidence caused by *F. solani*, compared to control treatment. These results are agreement with those recorded by many workers. The seed treatment with oil seed cakes like Cotton cake, Mustard cake (Black and yellow) and Taramera cake alone or in combination with *T. harzianum* and *T. resei* significantly reduced the colonization of roots by charcoal rot fungus (*Macrophomina phaseolina*) in sunflower plants (Anis *et al*., 2010). Soil amended with vermin-compost and neem cake with or without *Trichoderma harzianum* suppressed *F. solani* f.sp. *melongenae* in eggplants as well as increased the growth parameters and fruit yield (Bhadauria *et al*., 2012). *T. harzianum*, *T. asperellum*, and *T. virens* reduced the severity of wilt in roots, where *T. harzianum, T. asperellum* and *T. viride* could control *of each F. solani*, *F. oxysporum* f. sp. *ciceri* and *Rhizoctonia solani* in tomato (Akrami and Yousefi, 2015). *T. harzianum*, *T. koningii*, *T. viride* (green strain) and *T. viride* (yellow strain) had inhibitory effect against *F. solani* f. sp. *melongenae* in dual culture. The pathogen was most susceptible to the volatile inhibitors produced by *T. koningii* and *T. viride* (Bhadra *et al*., 2016).

Our results showed that the Th or Tvr combined with OC or CC had the highest nematicdal effect against *M. incognita* parameters *viz*.J_2 in soil and roots; galls and egg-masses in eggplants. These results are agreement with those recorded by Olabiyi and Gbadamosi (2013) .They mentioned that *T. harzanium* combined with composted materials mixture reduced nematode population and gall index in Sesame. *T. vierns* combined with the plant debris of oak forests also significantly decreased the number of galls of *M. javanica* on tomato roots (Moradi *et al*., 2015). *Paecilomyces lilacinus* or *Cladosporium oxysporum* combined with the oil cakes of castor, linseed, groundnut, mahua and neem suppressed the root knot nematode, *M. javanica* infecting eggplant under glasshouse conditions (Ashraf and Khan, 2010).

The soil amended with rape seed, lantana, African marigold and neem significantly inhibited root-knot nematode in tomato (Radwan *et al*., 2009 and Feyisa *et al*., 2015). The culture filtrates of *Trichoderma* spp. (*T. harazianum*, *T. viride*, *T. koningii*, *T. reesei* and *T. hamatum*) significantly controlled the *M. javanica in vitro* and on eggplant cv. Black beauty seedlings (Bokhari , 2009). The combined of *T. harzianum* and *T. viride* had significantly nematicidal effect against *M. incognita* in cowpea in pot experiment (Nama *et al*., 2015). Treatments of Th or Tvr alone or in combination with OC or CC increased the growth parameters of eggplants *viz*. shoot length, fresh shoot weight, dry shoot weight, leaves number, fresh root weight and dry root weight compared to control treatment (El-Nagdi and Abd-El-Khair, 2017). Our results were in harmony with results recorded by Anis *et al*. (2010). They showed that oil seed cakes (cotton cake, mustard cake and taramera cake) alone or in combination with *T. harzianum* and *T. resei*, as seed treatment, significantly increased the growth of sunflower plants. *T. harzanium* with composted materials mixture have better performances on the growth and yield of Sesame (Olabiyi and Gbadamosi, 2013). Application of *T. harzianum* increased the plant height, the number of branches and yield of soybean plants in field (Izuogu and Abiri, 2015). The combination of *T. harzianum* and *T. viride* improved the plant growth in cowpea in pot experiment (Nama *et al*., 2015). *T. harzianum* treatment resulted in significant higher plant height, number of leaves, length and breadth of leaves, number of flowers and fruit weight of tomato in field experiment (Olajide *et al*., 2015). The pots amended with plant debris of oak forests with *T. vierns* highly increased the dry weight of root in tomato (Moradi *et al*., 2015). Therefore, our results suggest that the oil cakes of olive or castor bean combined with *T. harzianum T. harzianum* and *T. vierns* can be play an important role in controlling *M. incoginta* and *F. solani* in eggplants. The soil amendments and bio-control agents have become focus of attention today for facing the nematode problems and fungal root rot in an eco-friendly manner (Singh and Prasad, 2014).

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