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Management of *Fusarium* Wilt (*Fusarium oxysporum* f.sp. *melongenae)* using Organic Soil Amendments in Eggplant

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Brinjal is one of the important vegetable crops in India. Many diseases affect its production. Among them, Fusarium wilt disease causes great losses in yield. Normally, this disease is controlled with fungicides, which not only develop resistance in disease-causing pathogens but also have deleterious effects on human health and the environment. So, in the present study, alternative methods of control that are eco-friendly, safe, easy, and affordable by using organic soil amendments were tested both *In vitro* and in pot culture studies. In the present study, aqueous

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extracts of different organic amendments were tested at 10 and 15 percent concentrations against the growth of the pathogen in vitro. Among them, neem cake showed highest percentages of mycelial inhibition (80.88% and 85.56%), followed by mustard cake (78.72% and 73.41%) against the test pathogen at 10 and 15 percent, respectively. However, the least mycelium growth inhibition was recorded with cotton cake extract (34.06% and 54.67%). Besides, pot culture studies also yielded similar results. Out of nine organic amendments tested against Fusarium wilt in pot conditions, neem seed kernel cake recorded the lowest wilt incidence (22.19% and 16.95%), followed by mustard cake (23.49% and 17.54%) at 100 and 150 g concentrations, respectively, whereas treatments with vermicompost, goat manure, and poultry manure showed lower inhibition against wilt incidence when compared to the remaining oil cake organic amendments at both concentrations tested. In the present study, it was found that aqueous extracts of organic amendments tested against the pathogen showed significant inhibition in-vitro. Similarly, incorporation of organic amendments into sick soil inoculated with pathogen showed a significant reduction in the incidence of wilt disease as compared to the untreated control.

Keywords: Egg plant; Fusarium wilt; organic amendments; disease management.

1. INTRODUCTION

Brinjal is commonly known as eggplant (*Solanum melongena* Linn.) which belongs to the family Solanaceae and is a widely grown vegetable crop in Asian countries, probably a native of South Asia. It is grown throughout India covering an area of 668.72 thousand ha with production of 123.99 thousand tonnes and productivity of 18.53 M. tonnes/ha. In Maharashtra, the area, production and productivity of Brinjal were 221.40 thousand ha, 433.28 thousand tonnes and 19.68 M. tones/ha, respectively during 2016-17 [1]. Fusarium species are the most important plant pathogens in the world and highly variable because of their genetic makeup and changes in environment in which they grow causing morphological changes [2]. *Fusarium* wilt of eggplant caused by *Fusarium oxysporum* f.sp.*melongenae* is an economically important soil borne disease limiting eggplant production worldwide. This pathogen was initially reported in Japan [3] and next in China [4]. It is extremely difficult to control soil-borne fungi through conventional strategies such as the use of synthetic fungicides, etc. Since their spores are able to survive for many years in the soil, biological control strategies for this pathogen should, therefore, be carefully selected and handled in an eco-friendly way instead of using chemical fungicides.

The incorporation of crop residues into the soil, organic wastes, composts and peats have been proposed to control diseases caused by soil borne pathogens. Many of these amendments reduced pathogen populations including wilt causing pathogens [5]. *Fusarium* wilt of tomato has been reported to be controlled by soil amendments with composts [6]. Organic soil amendments *viz.* neem cake, FYM, soyabean cake, cotton cake have been reported to stimulate the activities of microorganisms that are antagonistic to Plant pathogens and plant-parasitic nematodes [7-9]. Considering all the positive characteristics of organic soil amendments towards disease suppression, the present study was undertaken to evaluate various kinds of organic amendments by checking their efficacies against the wilt causing pathogen both invitro as well as under pot culture studies.

2. MATERIALS AND METHODS

2.1 *In-vitro* Evaluation of Aqueous Extracts of Organic Soil Amendments against Wilt Causing Pathogen in Egg Plant

As per the procedure given by Dhingani et al. [10], aqueous extracts of Neem seed cake, Castor cake, Cotton seed cake, vermicompost, Farm Yard Manure (FYM) etc., were prepared as mentioned in below treatments. Forty grams of each organic amendment was suspended in a conical flask in 150 ml of sterilized distilled water and kept for 15 days. Every day, the flasks were shaken to thoroughly mix and dissolve the content. The extract was strained through muslin cloth after 15 days and then filtered in 150 ml using Whatman filter paper No-41. Conical flasks were sterilized for 20 minutes in autoclave at 121^0C. The autoclaved extracts were tested against test pathogen using poisoned food technique at concentrations of 10 and 15 per cent [11]. For each treatment, three replications were maintained. According to the formula given by Dennis and Webster [12] per cent inhibition of mycelial growth was estimated.

$$I = C – T/ C X 100$$

Where,

I = Per cent inhibition of mycelial growth
C = Colony diameter in control (mm)
T = Colony diameter treatment (mm)

Experimental details:

Design : Completely Randomized Design (CRD)
Replications : Three
Treatments : Ten

List 1. Treatment details

Tr. No.	Treatments	Tr. No.	Treatments
T_1	Neem seed kernel cake	T_6	Cotton seed cake
T_2	Mustard cake	T_7	Vermicompost
T_3	Safflower cake	T_8	Goat Manure
T_4	Ground nut cake	T_9	Poultry Manure
T_5	Soya cake	T_{10}	Control (Untreated)

2.2 Evaluation of Organic Soil Amendments against Incidence of Wilt Disease under Pot Culture Studies

Earthen pots containing 4 kg of soil were amended with 100 and 150 g of amendments in separate treatments with a maximum of 10 organic amendments (as specified under treatments).These treatments were assessed against test pathogen in pot culture using soil application and sick soil system. All the test amendments were physically grinded to rough powder and applied in the pots. Unamended soil was used as control. Three replications were maintained in a fully randomized model for each treatment. Earthen pots (45 cm dia.) filled with potting soil mixture: sand (3:1) and multiplied to this mass (Sand: Maize medium) isolate culture FOM 22 was applied (@ 50 g / kg potting mixture), adequately watered and incubated for 10-12 days to proliferate the pathogen and make the potting mixture / soil sick with test pathogen.

The experimental amendments were added to the earthen pots containing sick soil / potting mixture (@ 50 g per kg mixture), thoroughly mixed, properly watered and kept in open space. There replications were maintained. After a week of application for amendments, the surface sterilized (1 per cent NaOCl) healthy Arka sirish seeds (10 seeds / pot) were sown, watered as and when required to maintain a water holding capacity of 50 per cent. The untreated control was maintained for the earthen pots containing sick soil (without any amendments) and sown with seeds.

Experimental details:

Design: Completely Randomized Design (CRD)
Replications: Three
Treatments: Ten

List 2. Treatment details

Tr. No.	Treatments	Tr. No.	Treatments
T_1	Neem seed kernel cake	T_6	Cotton Cake
T_2	Mustard cake	T_7	Vermicompost
T_3	Safflower cake	T_8	Goat Manure
T_4	Ground nut cake	T_9	Poultry Manure
T_5	Soya cake	T_{10}	Control (Untreated)

Observations were recorded on Pre-emergence mortality (PREM) after a week of sowing and post-emergency mortality (POEM) / wilting at 90 days after sowing. Per cent of pre-emergence mortality (PREM) and post-emergence mortality (POEM) / wilting were calculated using the following formulae:

PREM (%) = No. of seeds ungerminated / Total no. of seeds sown x 100

POEM (%) = No. of seedlings died / Total no. of seedlings x 100

3. RESULTS AND DISCUSSION

3.1 *In vitro* Efficacy of Aqueous Extracts of Organic Amendments against the Growth of Pathogen

The results (Table 1, Fig. 1, and Plate I and II) indicated that all the aqueous extracts of organic amendments had significant inhibitory effect on *Fusarium oxysporum* f.sp. *melongenae* at both the concentrations tested. It was observed the increase in concentration of extract there by decrease in mycelial growth of the pathogen.

At 10 per cent concentration of aqueous extracts of Neem cake and Mustard cake were highly significant by inhibiting the mycelial growth of pathogen at 5 days after inoculation (11.29 mm and 14.39 mm) and 7 days after inoculation (18.85 mm and 23.92 mm) with a mean growths o 15.07 mm and 19.16 mm respectively. Moreover, the growth inhibitions were also reflected inversely and highest level of growth inhibition at 5^{th} and 7^{th} DAI with Neem cake (83.31% and 79.05 %) and Mustard cake (78.72% and 73.41 %) with a mean growth inhibition (80S.88% and 75.70%) as compared to control.

The third and fourth superior treatments were safflower cake (19.14 mm and 30.87 mm) and groundnut cake (22.60 mm and 31.97 mm) with a mean mycelia growths of 25.01mm and 27.29 mm respectively at 5 and 7 DAI. Similar trend in growth inhibition was observed with Safflower cake (71.70% and 65.72%) and Groundnut cake (66.59% and 64.52%) with a mean inhibition of 68.28 per cent and 65.39 per cent respectively at 5 and 7 DAI.

The remaining treatments that showed substantial inhibition effect against the pathogen were Soya cake (24.37 mm and 31.12 mm), Vermicompost (29.87 mm and 42.08 mm),Goat manure (30.67 mm and 42.19 mm),Poultry manure (31.57 mm and 41.81 mm) and Cotton cake (44.81 mm and 59.14 mm) which were on par with each other showing mean growth inhibitions of 27.75 mm,35.98 mm,36.43 mm,36.69 mm, 51.98 mm respectively over control (90 mm) at 5^{th} and 7^{th} DAI. Similarly, these treatments were shown the significant growth inhibition as in Soya cake (63.98% and 65.34%), Vermicompost (55.88% and 53.24%), Goat manure (54.66% and 53.11%), poultry manure (53.34% and 53.54%) and cotton cake (33.76% and 34.28%) respectively at 5 and 7 DAI with a mean growth inhibition of 64.80 per cent, 54.36 per cent, 53.78 per cent, 53.45 per cent and 34.08 per cent.

In other dosage at 15 per cent concentration of aqueous extracts of Neem cake and Mustard cake were highly significant by inhibiting the mycelial growth of pathogen at 5 days after inoculation (9.29 mm and 12.39 mm) and 7 days after inoculation (13.47 mm and 15.23 mm) with a mean of 11.38mm and 13.81mm respectively . Moreover, Neem cake and Mustard cake shown greatest level of growth inhibition at 5 DAI (86.26 % and 81.68%) and 7 DAI (85.02% and 83.07%) with a mean inhibition of 85.56 per cent and 82.48 per cent compared to control. The third and fourth superior treatments at 15 per cent concentration of aqueous extracts were safflower cake (15.23 mm and 16.59 mm) and Groundnut cake (15.34mm and 21.63 mm) with a mean of 15.91 mm and 18.49 mm respectively at 5 and 7 DAI. Similarly, growth inhibition showed by Safflower cake (77.48% and 81.55%) and Groundnut cake (77.33% and 75.96%) were significant with a mean inhibition of 79.82 per cent and 76.55 per cent respectively at 5 and 7 DAI (Table 1, Fig. 1 and Plates I and II).

Though, the remaining treatments were shown substantial inhibition effect against the pathogen, they were on par with each other as in the order of Soya cake (17.85 mm and 21.17 mm),Vermicompost (20.81 mm and 31.33 mm), Goat manure (21.27 mm and 32.33 mm), Poultry manure (22.03 mm and 33.33 mm) and Cotton cake (29.14 mm and 42.33 mm) with a mean inhibition of 19.51 mm,26.07 mm,26.80 mm,27.70 mm and 35.74 mm respectively over control (90 mm) at 5 and 7 DAI. Similarly, these treatments were shown the significant growth inhibition as in soya cake (73.62% and 76.48%), vermicompost (69.24% and 65.18%), goat manure (68.56% and 64.07%), poultry manure (67.43% and 62.92%) and cotton cake (56.92% and 52.96%) respectively at 5 and 7 DAI with a

mean growth inhibition of 75.25 per cent, 66.93 per cent, 66.00 per cent, 64.86 per cent and 54.67 per cent, respectively.

Present results were in agreement with the earlier workers [13,14]. A high reduction of enzymatic activities of pathogen by aqueous extract of neem cake might be due to the presence of biological active constituents such as gedunin (tetranortriterpenoid) or Azadirachtin [15,16]. Compost extracts reported significant inhibition of test pathogen mycelial growth. probably due to the toxic substances and nutrient content ratio in the culture media. Thus the suppressive effect of compost would be a combination of biotic and abiotic factors [17,19].

3.2 Effect of Organic Soil Amendments against the Incidence of Wilt Disease of Eggplant under Pot Culture Studies

The results showed that all the organic amendments significantly inhibited the disease. The results were presented in the Table 2, Plate III and Fig. 2.

At 100 gram concentration, the disease incidence was recorded with all the test amendments ranged from 22.19 to 36.52 per cent, as against 72.57 per cent in untreated control. However, significantly lowest *Fusarium* wilt disease incidence was recorded with neem seed cake (22.19 %) with reduction of 69.42 per cent over control. While, Mustard cake and Safflower cake recorded 23.49 and 28.34 per cent disease incidence, respectively with reduction of 67.62 and 60.95 per cent over control. These were followed by the amendments *viz.*, groundnut cake (31.97%), soybean seed cake (32.85%), Vermicompost (35.03%), goat manure (34.39%), poultry manure (35.04%) and cotton seed cake (36.32%), with reduction of 55.94, 54, 51.72, 52.60, 51.70 and 49.67 per cent over control respectively. All these were at par with each other.

At 150 gram concentration, the disease incidence was recorded with all the test amendments ranged from 16.95 to 33.15 per cent, as against 72.75 per cent in untreated control. However, significantly lowest *Fusarium* wilt disease incidence was recorded with neem seed cake (16.95 %) with reduction of 76.64 per cent over control. While, Mustard cake and Safflower cake recorded 17.54 and 20.29 per cent disease incidence, respectively with reduction of 75.82 and 72.04 per cent over control. These were followed by the amendments *viz*., groundnut cake (23.48%), soybean seed cake (24.51%), vermicompost (23.33%), goat manure (25.92%) , poultry manure (27.16%) and cotton seed cake (33.15%), with reduction of 67.67,66.21,67.84,64.28,62.56 and 54.31 per cent over control respectively, but these were at par with each other (Table 2, Plate III and Fig. 2).

The research findings were in agreement with several earlier experimental findings. Organic amendments were found to be effective in reducing the incidence of wilt and also improving seed germination. The highest percentage of plant survival was recorded when neem cake followed by mustard cake [20].

Organic amendments have the capability to modify soil characteristics such as concentration of nutrients (e.g., P, K, and Fe), pH, NO_3 content, organic material, and structure. Since these traits were decisively shaping the structure of the soil-resident microbiota, there was no doubt that organic amendments addition into soil will eventually affect microbial contents and their activity. A positive results for growth inhibition against fusarium wilt with soybean cake (32.96%), followed by groundnut cake (29.63%) has already been reported. Similarly, the highest per cent growth inhibition in neem cake and the lowest per cent growth inhibition in groundnut cake has been already reported [21]. The decline in the pathogen population in the organic amendments can be predicted to some extent based on the heat generation activity of soil and its ammonium ion content. Some residual levels of suppressiveness were believed to be attributed to abiotic factors such as pHand carbon sourses [22, 23].

The composts treatments *viz.,* vermicompost, goat manure and poultry manure were performed next to the some of the organic amendment treatments because usually they do not provide complete control of disease and therefore control of pathogens requires successful application of biocontrol agents at the right time and place. The competitive exclusion of deleterious pathogens were directly related to the ability of the bacteria in the rhizosphere to grow successfully [24].

Table 1. *In vitro* efficacy of aqueous extracts of organic amendments against mycelial growth of *Fusarium oxysporum* f.sp.*melongenae*

Tr. No.	Treatments	Mycelial Growth of *Fusarium oxysporum* (mm)											
		10 % Concentration						15 % Concentration					
		5 DAI (mm)	Growth inhibition (%)	7 DAI (mm)	Growth inhibition (%)	Mean	Mean Growth inhibition (%)	5 DAI (mm)	Growth inhibition (%)	7 DAI (mm)	Growth inhibition (%)	Mean	Mean Growth inhibition (%)
T1	Neem Cake	11.29	83.31 (65.86)	18.85	79.05 (62.74)	15.07	80.88	9.29	86.26 (68.22)	13.47	85.02 (67.22)	11.38	85.56
T2	Mustard cake	14.39	78.72 (62.50)	23.92	73.41 (58.94)	19.16	75.70	12.39	81.68 (64.63)	15.23	83.07 (65.68)	13.81	82.48
T3	Safflower cake	19.14	71.70 (57.85)	30.87	65.72 (54.15)	25.01	68.28	15.23	77.48 (61.65)	16.59	81.55 (64.56)	15.91	79.82
T4	Ground nut cake	22.60	66.59 (54.67)	31.97	64.52 (53.42)	27.29	65.39	15.34	77.33 (61.54)	21.63	75.96 (60.62)	18.49	76.55
T5	Soya cake	24.37	63.98 (53.10)	31.12	65.34 (53.91)	27.75	64.80	17.85	73.62 (59.13)	21.17	76.48 (60.96)	19.51	75.25
T6	Cotton Cake	44.81	33.76 (35.38)	59.14	34.28 (35.82)	51.98	34.06	29.14	56.92 (48.99)	42.33	52.96 (46.68)	35.74	54.67
T7	Vermicompost	29.87	55.88 (48.35)	42.08	53.24 (46.84)	35.98	54.36	20.81	69.24 (56.3)	31.33	65.18 (53.82)	26.07	66.93
T8	Goat Manure	30.67	54.66 (47.65)	42.19	53.11 (46.78)	36.43	53.78	21.27	68.56 (55.87)	32.33	64.07 (53.15)	26.80	66.00
T9	Poultry Manure	31.57	53.34 (46.89)	41.81	53.54 (47.02)	36.69	53.45	22.03	67.43 (55.21)	33.37	62.92 (65.56)	27.70	64.86
T10	Control	67.65	0.00	90.00	0.00 (0.00)	78.83	0.00	67.65	0.00	90.00	0.00 (0.00)	78.83	0.00
	SE(m)±	**1.04**	**1.05**	**1.51**	**0.97**			**1.37**	**1.23**	**0.79**	**0.57**		
	C.D.(P=0.01)	**3.09**	**3.14**	**4.50**	**2.90**			**4.09**	**3.67**	**2.35**	**1.70**		

Table 2. Effect of organic amendments on *Fusarium* wilt (Pot culture)

Tr. No.	Treatments	Per cent disease incidence at 100 DAS			
		100 gm Concentration	Reduction over control	150 gm concentration	Reduction over control
T_1	Neem Cake	22.19 (28.08)	69.42	16.95 (24.29)	76.64
T_2	Mustard Cake	23.49 (28.97)	67.62	17.54 (24.74)	75.82
T_3	Safflower Cake	28.34 (32.14)	60.95	20.29 (26.74)	72.04
T_4	Ground nut Cake	31.97 (34.41)	55.94	23.48 (28.97)	67.64
T_5	Soya Cake	32.85 (34.95)	54.72	24.51 (29.65)	66.21
T_6	Cotton Cake	36.52 (36.34)	49.67	33.15 (35.13)	54.31
T_7	Vermicompost	35.03 (36.27)	51.72	23.33 (28.87)	67.84
T_8	Goat Manure	34.39 (35.89)	52.60	25.92 (31.39)	64.28
T_9	Poultry Manure	35.04 (36.28)	51.70	27.16 (30.58)	62.56
T_{10}	Control	72.57 (58.42)	0.00	72.57 (58.42)	0.00
	SE(m)±	**0.63**		**0.739**	
	C.D.(P=0.01)	**1.88**		**2.196**	

**Figures in the parenthesis are angular transformed values DAS: Days after sowing*

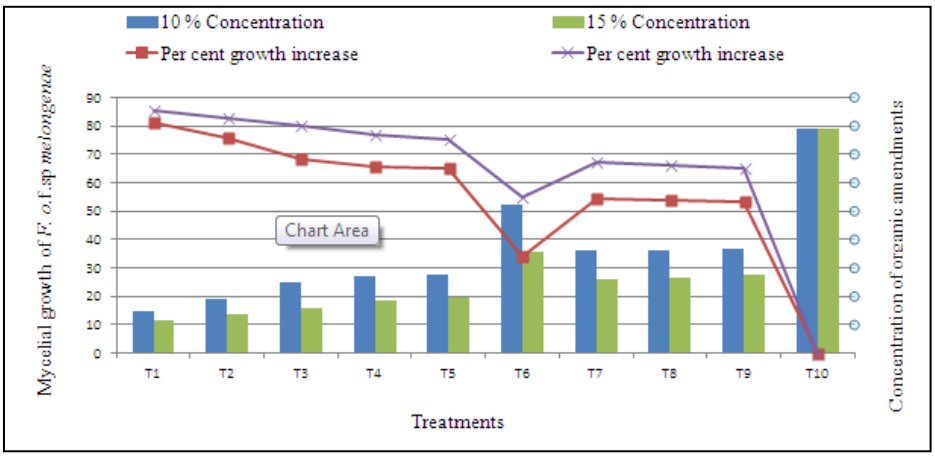
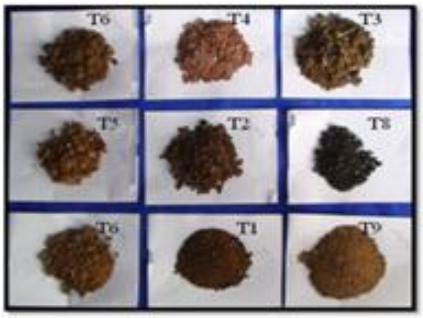


Fig. 1. *In vitro* efficacy of aqueous extracts of organic amendments against mycelial growth of *Fusarium oxysporum* f.sp.*melongenae* in eggplant

Aqueous extracts of organic amendments used against *Fusarium oxysporum* f.sp.*melongenae* under *in vitro* conditions



Tr.No	Treatment	Tr.No	Treatment
1	Neem cake	6	Cotton cake
2	Mustord cake	7	Vermicompost
3	Sawflower cake	8	Goatmanure
4	Ground nut cake	9	Poultry manure
5	Soya cake	10	Control

PLATE I

Effect of aqueous extracts of organic amendments against *Fusarium oxysporum* f.sp.*melongenae* under *in vitro* conditions at 5th and 7thday after inoculation

10 % Concentration

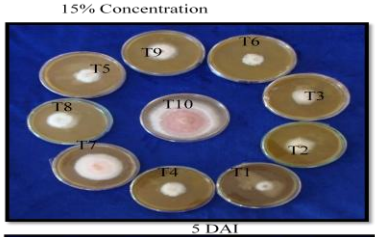
5 DAI

7 DAI

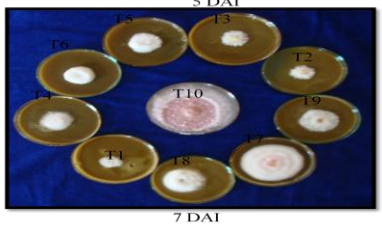
T1	Neem Cake
T2	Musturd cake
T3	Sawflower cake
T4	Ground nut cake
T5	Soya cake
T6	Cotton Cake
T7	Vermicompost
T8	Goat Manure
T9	Poultry Manure
T10	Control

PLATE II

15% Concentration



5 DAI



7 DAI

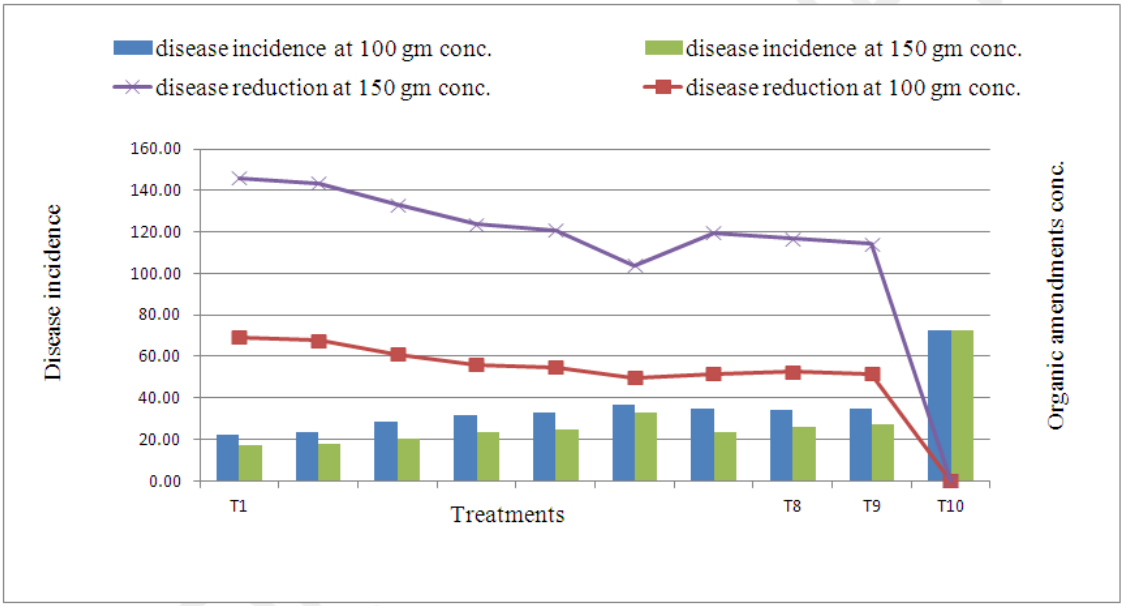
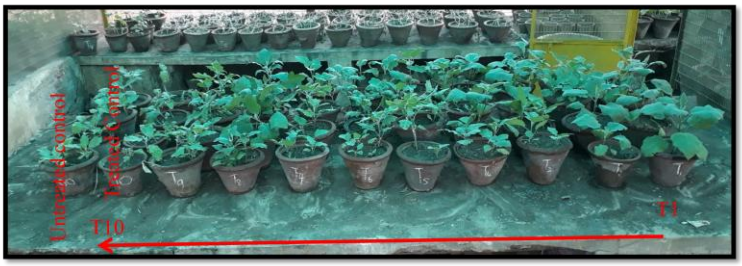


Fig. 2. Effect of organic amendments in the management of *Fusarium* wilt in brinjal under glasshouse conditions (Pot culture)

Effect of organic amendments on *Fsarium* wilt in brinjal under glasshouse conditions (pot culture)

Organic amendments @100 gm/4 kg soil



Organic amendments @150 gm/4 kg soil

PLATE III

Several researchers noticed that compost induced suppressiveness operated by several mechanisms including induced resistance (ISR) associated with certain native strains of PGPR *viz.*, *Pseudomonas* and *Bacillus* [25]. The suppression of disease accompanied by reduced root and stem colonization by pathogens and induced resistance in the plant or from the both [18]. As the antagonistic microbial biomass colonizes the organic amendments, various biotic mechanisms such as competition, antibiosis, and hyperparasitism emerge. Composting could have a direct effect on a pathogen [26,27]. When a circular association of microorganisms forms around a particle of manure, solid manure and manure extract directly prevent Fusarium wilt [28-35].

4. CONCLUSION

Among nine organic amendments evaluated against *F. oxysporum* f.sp *melongenae* under laboratory and pot culture studies, neem seed kernel cake was found highly effective in reducing the disease incidence followed by mustard cake. The other organic amendments, like safflower cake, groundnut cake, vermicompost, also yielded positive results.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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