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RESEARCH ARTICLE

Effect of Root Knot Nematode (*Meloidogyne incognita*) Infestation on Severity of Wilt (*Fusarium solani*) in Bitter Gourd and its Management

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ABSTRACT

A systematic field survey was conducted to document the wilt and nematode infestation on bitter gourd in Coimbatore district of Tamil Nadu. The higher disease severity and gall index were observed in Manakadavu village with 71.85 per cent wilt incidence and a mean gall index of 5.0. The pathogen, *Fusarium solani* was isolated and Koch's postulates were proved under glasshouse conditions, where Manakadavu isolate of *F. solani* was found to be virulent. The morphological and molecular characterization of *F. solani* were also carried out. The interaction of *F. solani* and *M. incognita* on bitter gourd was studied in order to know whether the root knot nematode acts as a predisposing factor for the soil borne pathogen *Fusarium* and aggrevates the wilt severity. The *in vitro* efficacy of the biocontrol agents *viz., Bacillus, Streptomyces, Pseudomonas* and *Trichoderma* were evaluated by dual plate method where *Streptomyces* (BG 01) recorded a higher mycelial inhibition of 80.38 per cent over control. The organic amendment neem cake was found to be highly effective at both 5 and 10 per cent concentrations. The efficacy of the biocontrol agents and neem cake against the egg hatching and juvenile mortality of root knot nematode, *Meloidogyne incognita* was also assessed. The neem cake followed by *Streptomyces* (BG 01) and *Bacillus* (PG 12) were found to be effective in inhibiting egg hatching and the mortality of *M. incognita*. A pot culture experiment was conducted with efficient biocontrol agents and organic amendments. The results revealed that soil application of neem cake @ 5 g / pot + *Streptomyces reticuli* (BG 01) @ 10 ml / lit / pot + *Pseudomonas fluorescens* (Pf 1) @ 2g / pot + *Trichoderma viride* (Tv 1) @ 2 g / pot decreased the *Fusarium* wilt incidence and *M. incognita* infestation significantly. The above treatment also resulted in increased plant growth and yield parameters. The field trial revealed that the soil application of neem cake @ 250 kg ha^{-1} + *Streptomyces reticuli* (BG 01) @ 10 ml/ lit + *Pseudomonas fluorescens* (Pf 1) @ 2.5 kg ha^{-1} + *Trichoderma viride* (Tv 1) @ 2.5 kg ha^{-1} recorded a significantly lower wilt incidence of 14.4 per cent as against 42.5 per cent in the control, which was found to be 66.1 per cent reduction over control and gall index was also significantly reduced to 1.6 as against 5.0 in control. The treatment recorded a higher fruit yield of 15.40 tonnes ha^{-1} as against 10.75 tonnes ha^{-1} in control.

Keywords: *Bitter gourd, Fusarium, Root knot nematode, Biocontrol, Organic amendments*

Bitter gourd (*Momordica charantia* L.) is one of the most popular vegetables in South East Asia. Bitter gourd plant gets affected due to several biotic stress caused by various fungi, bacteria, viruses, nematodes and insects. *Fusarium*, a soil borne fungus causes vascular wilt disease that affects large variety of economically important crops worldwide. The plant parasitic nematodes are considered as major agricultural constrain and attack number of crops. Among the nematodes, root knot nematode (*Meloidogyne* spp.) a sedentary endoparasite, is more devastating and has a wide host range of more than 3000 plants including cucurbits. The major problem with bitter gourd production in India is *Fusarium* wilt and root knot nematode complex. It is the most devastating soil borne disease complex and one of the major yield limiting constraint which cause profound economic losses ranging from 30 to 50 per cent under dry warm conditions (Tamilselvi and

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Pugalendhi, 2015). The synergistic interaction of *Fusarium* and root knot nematode has been recorded in cucurbitaceous crops *viz.,* muskmelon, summer squash, cucumber, watermelon, oriental melon, shintosa and cucumber. Oil cakes when applied to soil release some substances with fungicidal and nematicidal properties that incite the soil microflora.

The interest for biological control of the plant pathogens has increased over the past decade. Increasing the genetic diversity of rhizosphere region by application of compatible biocontrol agents and organic amendments in combination against several soil borne plant pathogens is considered to be eco-friendly. Compatible interactions are important pre requisite for successful development of an integrated approach for the control of plant diseases. Information is lacking on the complex pathogenic behaviour of the nematode and the pathogen parasitizing bitter gourd simultaneously. Similarly there is meager information on the management of such disease complex using biocontrol agents and organic amendments. This research was proposed to study the intensity of damage caused by fungus – nematode disease complex and its management.

Material and Methods

A systematic field survey was conducted in bitter gourd growing areas of Coimbatore district, Tamil Nadu during 2017 to document the occurrence of wilt and root-knot nematode disease complex. On an average, 5 farmers' fields were visited per village and per cent disease incidence and gall index were recorded. The plants showing typical wilt symptoms and galled roots were collected in separate paper bags and brought to the laboratory for further investigations.

Characterization and Pathogenicity of Fusarium

The isolates of *Fusarium* were isolated from the symptomatic vascular discoloured portions of bitter gourd. The infected portion of the stem were cut into small pieces and surface sterilized with 0.1 per cent mercuric chloride for 30 seconds and subsequently three washings with sterile distilled water. Then, they were placed in sterilized petri dishes containing Potato Dextrose Agar (PDA) medium and incubated at the laboratory conditions. The pure cultures were obtained by hyphal tip method and the cultures were transferred to PDA slants for maintanence. The length and breadth of micro conidia, macro conidia and chlamydospores were measured under phase contrast microscope. PCR amplification of ITS region of the five isolates of *Fusarium* was performed using the universal primer pair *viz.*, ITS 1 (5' – CTTGGTCATTTAGAGGAAGTAA - 3') and ITS 4 (5' – TCCTCCGTTATTGATATGC – 3').

The soil inoculation method was followed to prove the pathogenicity of *Fusarium*. The isolated wilt pathogen was multiplied in sand maize medium. Sand and ground maize were mixed at the ratio of 9:1, respectively. The mixture as slightly moistened with water and sterilized at 121°C at 15 psi for 2 hours. The fungus was inoculated into autoclaved medium and incubated for 15 days at room temperature for multiplication. Potting soil (red soil : sand : cow dung manure @ 1: 1: 1 ratio) was sterilized in autoclave at 121°C at 15 psi for 2 consecutive days. The inoculum on sand maize medium was incorporated into sterilized soil at the rate of 50 g kg^{-1} of the soil. Seedlings of bitter gourd cultivar Maya (25 days old) were planted at the rate of 4 seedlings per pot and maintained under glasshouse conditions. Four replications were maintained and monitored regularly. From the infected plants, pathogen as reisolated and Koch's postulates were proved.

Interaction of Fusarium and Meloidogyne

A pot culture experiment was carried out to examine the nematode infestation on wilt severity. The 25 days old seedlings of bitter gourd cultivar Maya were planted in pots. After the establishment of plants, the experiment was conducted to evaluate the effect of nematode and fungus individually and in combination i.e. simultaneous inoculation of nematode and fungus, nematode 15 days prior to fungus inoculation, nematode 15 days after fungus inoculation. Each treatment was replicated five times in completely randomized design. The second stage juveniles of root knot nematode @ 1 J_2/g of soil were inoculated in the soil. Soil inoculation of *Fusarium* at the rate of 50g kg^{-1} of soil. Per cent wilt incidence of the fungus infection was assessed at 60 DAS . The plant roots were observed for number of galls present per root and the gall index was assigned.

Evaluation of biocontrol agents and organic amendments

The rhizosphere soils of various cucurbitaceous crops were collected from Coimbatore district of Tamil Nadu and the serial dilution method was followed for the isolation of biocontrol agents from the rhizosphere soil. Ten isolates of *Bacillus* spp., nine isolates of *Pseudomonas* spp., seven isolates of *Streptomyces* spp. and three isolates of *Trichoderma* spp., were isolated through serial dilution method and eight isolates of *Bacillus* spp, three isolates of *Streptomyces* spp., one isolate of *Pseudomonas fluorescens* (Pf 1) and seven isolates of *Trichoderma* spp. were obtained from Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore.

Fusarium solani

The collected and isolated efficient isolates of *Bacillus* spp., *Pseudomonas* spp., *Streptomyces* spp., *Trichoderma* spp. were evaluated through dual culture technique to test their antagonistic activity (Dennis and Webster, 1971). For *Trichoderma,* the seven days old mycelial disc was placed on one side of the plate poured with PDA media. On the opposite side of the petri plate, the mycelia disc of the pathogen was placed perpendicularly. For bacterial biocontrol agents, the PDA was poured on the petri plates and mycelia disc of the pathogen was placed on one side. The antagonistic bacterial culture was streaked on the opposite side at 1 cm away from the edge of the plate. Three replications were maintained for each bio agent and control was also maintained. Per cent growth inhibition over control was measured after 8 days.

The oil cakes *viz.,* neem cake, pungam cake, coconut cake, sesame cake, groundnut cake, poultry manure, press mud, vermi compost were made into powder separately. It was then weighed as 5gram and 10 gram and soaked in 100 ml sterile distilled water kept overnight. It was then filtered through Whatman filter paper. The filtrate was then centrifuged at 10000 rpm for 15 minutes. The supernatant was collected. This extract solution was used for further studies (Dubey and Patel, 2000). The extracted oil cakes were tested for its efficacy against the pathogen using poisoned food technique. PDA medium was freshly prepared and 100 ml medium was evenly poured in 250 ml conical flasks. 5 ml and 10 ml of oilcake extracts were poured in conical flasks to obtain 5% and 10% and sterilized. The autoclaved PDA medium was poured at the rate of 15 ml per sterilized petri plates and allowed to solidify. A mycelial disc of actively growing culture of the pathogen was then transferred to the centre of the petri plates and incubated at room temperature. Control plate without the extract of oil cake was also maintained. The radial growth of the pathogen was recorded when the control plate was fully covered with the mycelia growth.

Meloidogyne incognita

Hatching and mortality test was carried out to determine the effect of different concentrations of efficient biocontrol agents namely *Trichoderma* sp., *Streptomyces* sp., *Bacillus* sp., *Pseudomonas* sp. and neem cake on hatching of *M. incognita* egg masses and mortality of J_2 juveniles under *in vitro* condition. Root-knot nematode infected bitter gourd plants from the pure culture pots were up-rooted and washed gently under running tap water. Egg masses of *M. incognita* were picked up from the root using dissecting needle and forceps. The egg masses were transferred to petri plates containing 25%, 50%, 75%, 100% concentrations of biocontrol agents and neem cake. Egg-masses in distilled water served as control. The experiment was laid out in completely randomized design with three replications. The results were observed for 4 consecutive days and egg hatching was calculated. The collected egg masses from glass house were placed in distilled water for hatching with periodical aeration. After 24-48 hours the freshly hatched juveniles were used for mortality studies. 25%, 50%, 75%, 100% concentrations of biocontrol agents and neem cake were poured into petri plate containing J_2 juveniles. The number of dead J_2 were recorded every 24 hours for three days. After 24, 48 and 72 hours, active and inactive J_2 were counted and sterilized distilled water was served as control. Juveniles were considered dead if they were not moving when probed with fine needle and body become straight. The per cent J_2 mortality was calculated.

For conducting pot culture experiments, the potting mixture was sterilized for 1 h at 121^{0}C at 15-18 psi for two consecutive days before use. The seeds were sown in pots and soil application of biocontrol agents *Streptomyces reticuli* (BG 01) @ 10 ml /lit /pot, *Pseudomonas fluorescens* (Pf 1) @ 2g / pot, *Trichoderma viride* (Tv 1) @ 2g / pot and organic amendments neem cake @ 5g / pot was done in different combinations. In field trial also the same treatments were test verified. The observations on wilt incidence and gall index were recorded. The growth parameters and fruit yield were also recorded. The data were analyzed using statistical tool AGRES software. The treatment means were compared by Duncan's Multiple Range Test (DMRT).

Results and Discussion

The data on occurrence and distribution of *Fusarium* wilt and root knot nematode on bitter gourd in Coimbatore district of Tamil Nadu have been furnished in Table 1. The survey revealed that the maximum wilt disease incidence and nematode severity was recorded in Manakkadavu village with 71.85 per cent of wilt disease incidence and mean gall index of 5.0. Cumagun *et al.* (2008) assessed the disease severity of *Fusarium* wilt on bitter gourd in Philippines. Among the areas surveyed around Philippines, and the results revealed that the bitter gourd recorded 70.97% of *Fusarium* wilt while bottle gourd recorded 42.31% of *Fusarium* wilt severity.

In the present study, five isolates of *Fusarium* were isolated from the wilt infected bitter gourd plants. The pure culture produced white mycelium with pink or orange pigmentations. The length and breadth of macroconidia, microconidia and chlamydospores ranged between 29.17 to 37.02 µm x 3.93 to 6.51 µm, 11.62 to 17.06 µm x 3.92 to 5.71 µm and 8.42 to 11.84 µm x 7.54 to 11.80 µm, respectively. Sun and Huang (1982) isolated

Table 1. Occurrence and distribution of *Fusarium* wilt and root knot nematode on bittergourd in Coimbatore district of Tamil Nadu

Location	GPS co-ordinates		Wilt disease incidence (%)	Gall index
	Latitude (^{0}N)	Longitude (^{0}E)		
Anaimalai	10.5826	76.9528	60.38	4.8
Manakkadavu	12.2180	75.5015	71.85	5.0
T.K.Pudur	10.9016	76.9765	49.85	4.0
Sarkarpathy	10.4681	76.8561	10.64	2.8
Semanampathy	11.0045	76.9616	16.38	3.3
Sethumadai	10.5107	76.8807	22.69	1.8
Vettaikaranpudur	10.5629	76.9168	19.64	3.8
Pappampatti	10.9595	77.1018	53.47	0.0
Ramapatinam	10.7103	76.9178	60.69	2.8
Thimmanguthu	10.6572	77.0106	30.15	3.5

the pathogen, *Fusarium oxysporum* f. sp. *momordicae* from infected bitter gourd plants on potato – dextrose medium. The length and breadth of macroconidia, microconidia and chlamydospores ranged between 12.5 to 38.75 x 2.5 to 5 µm, 6.25 to 22.5 x 2.5 to 5µm and 6.3 to 12.2 µm, respectively.

Table 2. Interaction of *Fusarium solani* and *Meloidogyne incognita* on bitter gourd

Treatments	Wilt incidence (%)*	Gall index
F. solani alone	52.33^{d} (46.33)	0.00
Meloidogyneincognita alone	8.33^{e} (16.77)	4.83
F. solani first and 15 days later *M. incognita*	66.66^{c} (54.73)	1.66
M. incognita first and 15 days later *F. solani*	83.33^{a} (65.90)	3.33
Concomitant inoculation of *F. solani* and *M. incognita*	75.00^{b} (60.00)	2.50
Uninoculated control	0.00^{f} (0.76)	0.00
CD (p= 0.05)	0.16	

*Values are mean of three replications

Values in parentheses are arcsine transformed values

In a column, means followed by a common letter are not significantly different at the 5% level by DMRT

The artificially inoculated bitter gourd plants with Manakkadavu isolate exhibited yellowing, drooping / abscission of leaves, vascular discolouration and cracking of collar region of the stem and disease incidence was as high as 75.00%.

Table 3. Effect of biocontrol agents and organic amendments on hatching of *M.incognita* eggs

Per cent egg hatch at different time of exposure															
Concentration (%)	*Bacillus* (PG 12)			*Streptomyces* (BG 01)			*Pseudomonas* (Pf 1)			*Trichoderma* (Tv 1)			Neem cake		
	24hrs	48hrs	72hrs	24hrs	48hrs	72hrs	24hrs	48hrs	72hrs	24hrs	48hrs	72hrs	24hrs	48hrs	72hrs
25	19.10^{d} (25.91)	20.00^{c} (26.56)	22.21^{c} (28.11)	18.12^{d} (25.19)	19.37^{d} (26.11)	20.62^{d} (27.00)	20.31^{d} (31.02)	21.15^{d} (31.05)	21.87^{d} (21.40)	19.37^{d} (31.55)	20.00^{d} (31.87)	20.06^{c} (30.73)	5.31^{d} (13.32)	6.25^{d} (14.47)	6.56^{d} (14.84)
50	16.56^{c} (24.01)	19.42^{c} (26.14)	21.31^{c} (27.49)	15.93^{c} (23.52)	17.81^{c} (24.96)	19.06^{c} (25.88)	17.18^{c} (30.28)	17.50^{c} (30.73)	18.12^{c} (21.06)	17.81^{c} (29.82)	18.43^{c} (30.12)	19.37^{c} (29.97)	5.00^{c} (12.92)	5.62^{c} (13.71)	5.93^{c} (14.09)
75	13.12^{b} (21.23)	15.00^{b} (22.78)	16.62^{b} (24.05)	12.81^{b} (20.97)	14.37^{b} (22.27)	15.31^{b} (23.03)	13.75^{b} (28.34)	15.00^{b} (28.57)	15.62^{b} (20.70)	14.37^{b} (28.51)	14.69^{b} (28.84)	15.12^{b} (28.16)	4.68^{b} (12.49)	5.31^{b} (13.32)	5.62^{b} (13.71)
100	9.68^{a} (18.12)	11.26^{a} (19.60)	13.43^{a} (21.49)	9.06^{a} (17.51)	11.56^{a} (19.87)	13.12^{a} (21.23)	10.93^{a} (26.47)	11.56^{a} (26.75)	14.06^{a} (28.52)	10.62^{a} (26.47)	11.56^{a} (27.98)	12.00^{a} (28.16)	5.93^{a} (.52)	0.00^{a} (14.09)	0.00^{a} (0.52)
Control	25.93^{e} (30.61)	42.50^{d} (40.68)	55.93^{d} (48.40)	25.9^{e} (30.59)	42.50^{e} (40.68)	55.93^{e} (48.40)	27.81^{e} (40.60)	45.37^{e} (45.23)	59.37^{e} (33.84)	27.81^{e} (40.59)	45.39^{e} (45.23)	59.37^{e} (34.34)	26.56^{e} (31.02)	47.50^{e} (43.56)	55.00^{e} (47.87)
CD (p=0.05)	0.14	0.13	0.15	0.13	0.14	0.19	0.14	0.03	0.13	0.09	0.09	0.13	0.12	0.04	0.12

*Values are mean of three replications

Values in parentheses are arcsine transformed values

In a column, means followed by a common letter are not significantly different at the 5% level by DMRT

The re-isolated culture was similar to the original culture and Koch's postulates were proved. Asma *et al.* (2018) recovered seventeen isolates of *Fusarium* from infected cucumber plants. All *Fusarium* isolates were

tested for pathogenicity. The most virulent was recorded by *F. oxysporum* isolate D2505C with disease severity of 50%. *F. solani* and *F. oxysporum* showed significant effect on the vascular tissues compared to the control. The pathogenicity and aggressiveness of *Fusarium* isolated from bitter gourd and bottle gourd were assessed for 11 isolates of *F. oxysporum* f. sp. *momordicae* and *F.oxysporum* f. sp. *lagenariae* (Cumugan *et al.,* 2008).

Table 4. Effect of biocontrol agents and organic amendments on the mortality of juveniles of *M. incognita*

Per cent mortality of juveniles at different time of exposure															
Concentration (%)	*Bacillus* (PG 12)			*Streptomyces* (BG 01)			*Pseudomonas* (Pf 1)			*Trichoderma* (Tv 1)			Neem cake		
	24hrs	48hrs	72hrs	24hrs	48hrs	72hrs	24hrs	48hrs	72hrs	24hrs	48hrs	72hrs	24hrs	48hrs	72hrs
25	15.33 d (23.05)	26.00 d (30.65)	39.66 d (39.03)	16.71 d 24.12)	21.00 d (27.27)	45.66 d (42.51)	14.00 d 21.97)	28.32 d (32.15)	36.66 c (37.26)	16.00 d (23.57)	26.33 d (30.87)	44.00 d (41.55)	18.33 d 25.35)	29.20 d (32.70)	41.06 d (39.85)
50	18.33 c 25.35)	29.66 c (32.99)	48.33 c (44.04)	19.33 c 26.08)	36.66 c (37.26)	59.33 c (50.37)	17.71 c (24.88)	39.20 c (38.76)	51.00 b (45.57)	18.66 c 25.59)	27.30 c (31.50)	58.66 c (49.98)	29.66 c 32.99)	40.33 c (39.42)	66.66 c (54.73)
75	21.71 b (27.77)	33.33 b (35.26)	52.00 b (46.14)	22.33 b (28.20)	41.33 b (40.00)	63.00 b (52.53)	23.71 b (29.13)	33.33 b (35.26)	62.66b (52.33)	20.33 b (26.80)	34.66 b (36.06)	66.33 b (54.53)	34.66 b (36.06)	54.33 b (47.48)	81.33 b (45.76)
100	29.33 a (32.79)	48.00 a (43.85)	75.66 a (60.44)	31.00 a (33.83)	58.66 a (49.98)	80.33 a (52.53)	30.33 a (33.41)	45.66 a (42.51)	73.33a (58.90)	28.66 a (32.36)	36.33 a (37.06)	73.66 a (59.12)	38.00 a (38.05)	62.71 a (52.36)	97.33 a (80.59)
Control	0.00e (0.52)	0.00e (0.52)	0.00e (0.52)	0.00e (0.52)	0.00e (0.52)	0.00e (0.52)	0.00e (0.52)	0.00e (0.52)	0.00e (0.52)	0.00e (0.52)	0.00e (0.52)	0.00e (0.52)	0.00e (0.52)	0.00e (0.52)	0.00e (0.52)
CD (p=0.05)	0.16	0.16	0.15	0.42	0.15	0.12	0.13	0.15	0.16	0.13	0.18	0.19	0.12	0.12	0.14

*Values are mean of three replications

Values in parentheses are arcsine transformed values

In a column, means followed by a common letter are not significantly different at the 5% level by DMRT

PCR amplification of ITS region in the five isolates of *Fusarium* was performed using the universal primers ITS1 and ITS 4. The isolates of *Fusarium* culture amplified a fragment of 560bp corresponding to the region of the 18S-28S rRNA intervening sequence of *Fusarium* sp (Plate 1). The PCR product was cloned and sequenced. The sequence analysis revealed that the isolate had 100 per cent homology with *Fusarium solani*. The pathogen causing wilt disease in bitter gourd was confirmed as *F. solani.* A PCR product from each isolate of the three *Fusarium* species was amplified by using primer pairs, ITS1 and ITS4. *F. oxysporum*, *F. equiseti* and *F. semitectum* produced approximately 550 bp band, *F. solani* species complex produced about 570 bp amplicon and *F. proliferatum* resulted in approximately 560 bp bands (Chehri *et al.*, 2010).

Table 5. Effect of biocontrol agents and neem cake on the wilt incidence and severity of root-knot nematode in bitter gourd under pot culture / glasshouse conditions

Treatment (s)	Fusarium wilt		Root-knot nematode	
	Incidence (%)	Per cent reduction over control	Gall index	Per cent reduction over control
Soil application of neem cake alone @ 5g / pot	31.8e(34.32)	55.46e	1.8e	40.00
Soil application of neem cake @ 5g / pot + *Streptomyces reticuli* (BG 01) @ 10 ml / lit/ pot	22.0b (27.97)	69.18b	1.6d	46.67
Soil application of neem cake @ 5g / pot + *Pseudomonas fluorescens* (Pf 1) @ 2g / pot	27.9d (31.88)	60.92d	2.2f	26.67
Soil application of neem cake @ 5g / pot + *Trichoderma viride* (Tv 1) @2g / pot	24.7c (27.06)	65.40c	1.5c	50.00
Soil application of neem cake @ 5g / pot + Streptomyces reticuli (BG 01) @ + 10 ml / lit/ pot + *Pseudomonas fluorescens* (Pf 1) 2g / pot + *Trichoderma viride* (Tv 1) @ 2g / pot	20.7b (25.69)	71.40ab	1.0a	66.67
Carbendazim 0.1% soil drenching + carbofuran 3G soil application @ 0.1 g / pot	18.8a (24.50)	73.66a	1.2b	60.00
Inoculated control	71.4g (69.35)	0.00g	3.0g	-
Uninoculated control	39.6f (38.99)	44.41f	0.0	-

Values are mean of three replications

Values in parentheses are arcsine transformed values

In a column, means followed by a common letter are not significantly different at the 5% level by DMRT

Interaction of Fusarium and root knot nematode

The data revealed that per cent wilt incidence has significantly increased in all treatments over control. Maximum wilt incidence was recorded in the treatment where *M. incognita* was inoculated 15 days prior to the

pathogen followed by concomitant inoculation of *F.solani* and *M. incognita.* A maximum of 83.33 per cent wilt incidence was recorded in the treatment where *M. incognita* was inoculated 15 days prior to the inoculation of *F. solani.* The gall index in the above treatment was found to be 3.33 (Table 2). The inoculation of nematode prior to the inoculation of the pathogen recorded the maximum wilt incidence confirming the role of nematode as a pre disposing factor for the entry of soil borne pathogen, *F. solani,* the incitant of bittergourd wilt. Seo and Kim (2017) examined the pathological inter relationship of *Fusarium* and *M. incognita* in four cucurbits namely watermelon, oriental melon, shintosa and cucumber. *F. proliferatum* and *M. incognita* infecting watermelon and oriental melon had higher infection with increased disease severity.

Table 6. Effect of biocontrol agents and neem cake on the wilt incidence and severity of root-knot nematode in bitter gourd under field conditions

Treatments	*Fusarium* wilt		Root knot nematode	
	Incidence (%)	Per cent reduction over control	Gall index	Per cent reduction over control
Soil application of neem cake alone @ 250 kg ha^{-1}	21.0^{e}(27.27)	50.58^{e}	1.8^{b}	64.00
Soil application of neem cake @ 250 kg ha^{-1}+ *Streptomyces reticuli* (BG 01) @ 10ml/ lit	17.2^{c}(24.50)	59.52^{c}	2.1^{c}	58.00
Soil application of neem cake @ 250 kg ha^{-1}+ *Pseudomonas fluorescens* (Pf 1) @ 2.5 kg ha^{-1}	20.4^{e}(26.85)	52.00^{e}	2.8^{e}	44.00
Soil application of neem cake @ 250 kg ha^{-1}+ *Trichoderma viride* (Tv 1) @ 2.5 kg ha^{-1}	19.1^{d}(25.91)	55.05^{d}	3.2^{f}	36.00
Soil application of neem cake @ 250 kg ha^{-1}+ *Streptomyces reticuli* @ 10 ml / lit + *Pseudomonas fluorescens (*Pf 1) 2.5 kg ha^{-1} + *Trichoderma viride* (Tv 1) @ 2.5 kg ha^{-1}	14.4^{b}(22.30)	66.1^{b}	1.6^{a}	68.00
Carbendazim 0.1% soil drenching + carbofuran 3G soil application @ 1kg a.i/ha.	13.1^{a}(21.21)	69.1^{a}	2.2^{d}	56.00
Control	42.5^{f}(40.68)	0.00^{f}	5.0^{g}	-

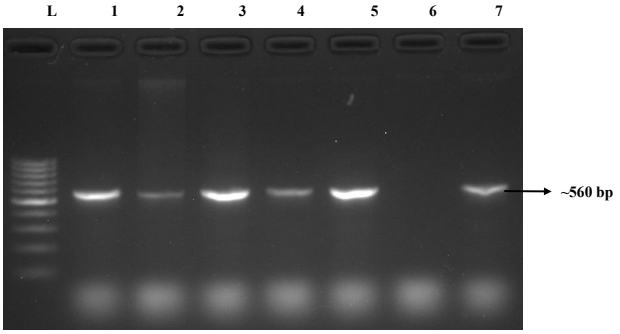
Values are mean of three replications

Values in parentheses are arcsine transformed values

In a column, means followed by a common letter are not significantly different at the 5% level by DMRT

Screening of antagonists and organic amendments against wilt pathogen and nematode

In the present study, among the seventeen *Bacillus* isolates screened, the isolate PG 12 recorded a higher mycelial inhibition of 72.6 per cent over control (Fig 1). *F. oxysporum* f. sp. *cucumerinum* was successfully



1. - Manakadavu 2 - Anaimalai 3- Ramapattinam
4 – Semanampathy 5 - V. K. Pudur 6 - Negative Control
7 - Positive Control *(F. oxysporum* f. sp. *cubense)* L - 100 bp ladder

Plate 1. Molecular confirmation of Fusarium isolates

controlled by a newly isolated strain of *Bacillus subtilis,* SQR 9 with 61 per cent mycelia inhibition under *in vitro* conditions (Zhang *et al.*, 2008). The dual culture assay results revealed that among the ten *Streptomyces* isolates screened, BG 01 recorded a higher mycelial inhibition of 80.38 per cent over control (Fig. 2). Wang *et al.* (2016) reported that *Streptomyces albospinus* strain CT205 was very effective in controlling *Fusarium*

wilt of cucurbits under *in vitro* conditions. Hyphal deformation and enlarged cytoplasmic vacuoles of *F. oxysporum* were observed for the treatment with CT205.

Plate 2. *In vitro* efficacy of organic amendments @ 5% concentration against *Fusarium solani*

The results of dual culture assay with *Pseudomonas* revealed that among the ten *Pseudomonas* isolates screened, BG 01 recorded 77.43 per cent mycelial inhibition over control (Fig. 3). Avinash and Ravishanker (2013) isolated various *Fusarium* sp. of cucurbits *viz.*, *F. solani, F. oxysporum, F. verticillodes* and *F. semitectem.*

Plate 2. *In vitro* efficacy of organic amendments @ 10% concentration against *Fusarium solani*

In vitro evaluation of these *Fusarium* isolates was done with various biocontrol agents *viz., Pseudomonas, Serratia* and *Bacillus*. *P. aeruginosa* strain MIC2 inhibited all types of *Fusarium* spp. to the maximum extent. *P. aeruginosa* strain MTCC2581 inhibited mycelial growth of *F. oxysporum* to 55.53%. *F. acuminatum* causing wilt disease of cucumber and other cucurbits was tested in dual plate assay with biocontrol agents *viz.*, *Pseudomonas chlororaphis* Q16 and *Pseudomonas* sp. K24, K27, K35, N3 and *Bacillus* sp. Q10. *P. chlororaphis* Q16 inhibited in vitro mycelial growth of *F. acuminatum* by 45-50%. The *Pseudomonas* strain K35 and K24 were almost equally effective in controlling the pathogen with inhibition values from 68 - 80%.

The dual plate assay of twelve isolates of *Trichoderma* revealed that BiG 01 registered a per cent mycelial inhibition of 79.63 over control (Fig. 4). Al-Tuwaijri (2015) tested *T. harzianum, T. ressei, T. viride, T. hamatum* and *T. glaucum* for their efficacy against *Fusarium oxysporum* f. sp. *cucumerinum*. *T. reesei* was found to be most effective with 60.55 per cent mycelial inhibition followed by *T. viride* with 52.22 per cent inhibition, while the lowest growth reduction was recorded by *T. glaucum* with 31.66 per cent inhibition.

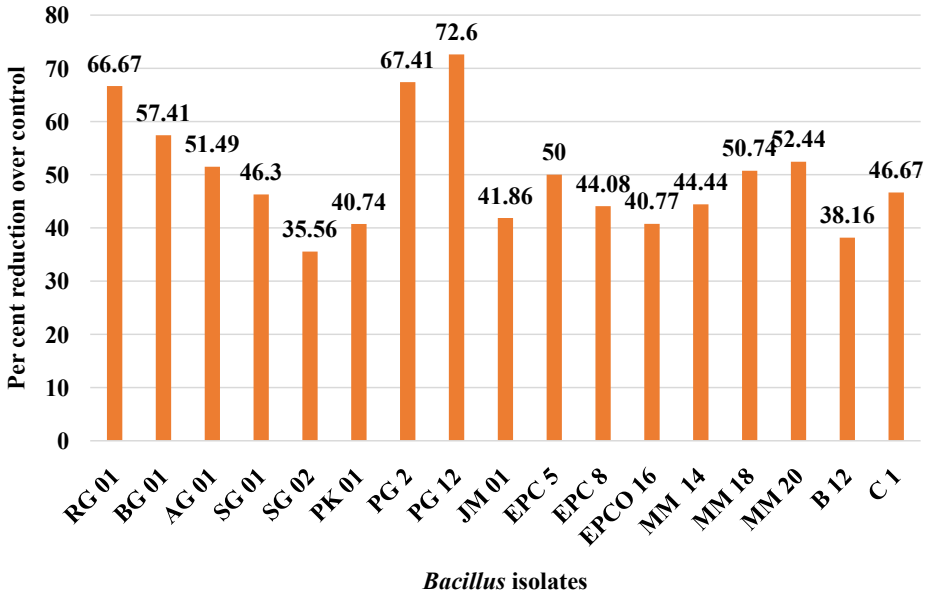


Fig. 1. *In vitro* efficacy of *Bacillus* isolates against *F. solani*

At 5% concentration, neem cake and press mud were found to be on par with each other in inhibiting the mycelial growth of the pathogen with 67.77 and 67.04 per cent inhibition over control, respectively. At 10% concentration of organic amendments, press mud recorded 86.30 per cent inhibition of mycelial growth over

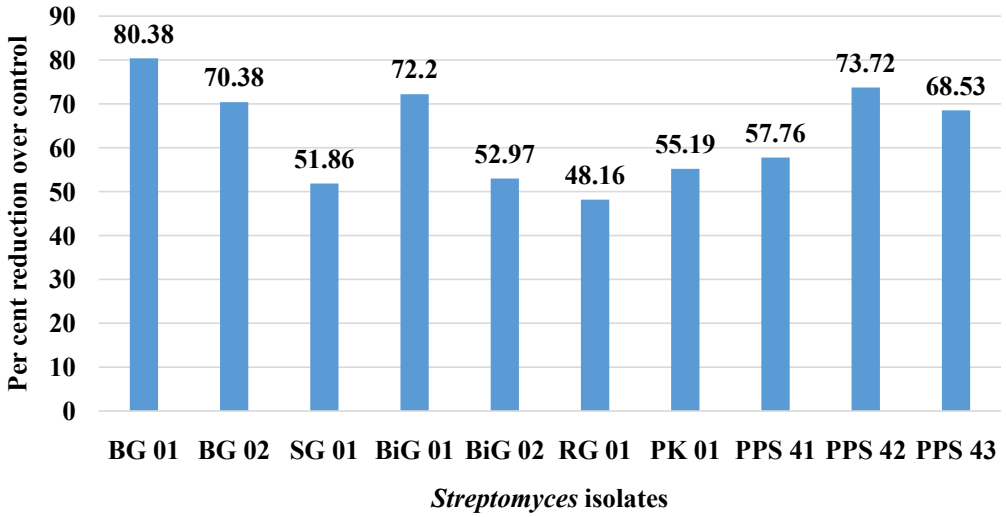


Fig. 2. *In vitro* efficacy of *Streptomyces* isolates against *F. solani*

control followed by neem cake with 82.60 per cent growth inhibition over control (Plate 2). Sultana and Ghaffar (2010) studied *in vitro* and *in vivo* effect of fungicides, microbial antagonists and oilcakes in the control of *F. solani* the cause of seed rot, seedling and root infection on bottle gourd, bitter gourd and cucumber. *F. solani* infected seeds of bottle gourd, cucumber and bitter gourd were applied with different oil cakes. Reduced seedling mortality and root infections were observed when sown in mustard and neem cake amended soil. Mustard cake was found most effective at all ratios followed by neem and castor cake.

In egg hatching test, the culture filtrates of *Bacillus, Streptomyces, Pseudomonas, Trichoderma* and the filtrate of neem cake significantly inhibited the egg hatching of *M. incognita* at 72 hours of exposure. It is inferred, that the per cent inhibition of egg hatch was directly proportional to the time of exposure. The results revealed that at 72 hours, there was only 13.12 per cent egg hatching in *Streptomyces* (BG 01) followed by *Bacillus* (PG 12) with13.43 per cent egg hatch, while control recorded 55.93 per cent as maximum. There was cent per cent inhibition of egg hatching by neem cake at 72 hrs of treatment (Table 3). The crude extract from *Streptomyces* sp. (CMU-MH021) significantly reduced root-knot nematode hatch and increased J_2 mortality

in vitro. The egg hatch was 14.6 per cent after incubation for 7 days and J_2 mortality was 69.2 per cent after incubation for 96 hours (Ruanpanun *et al.*, 2011).

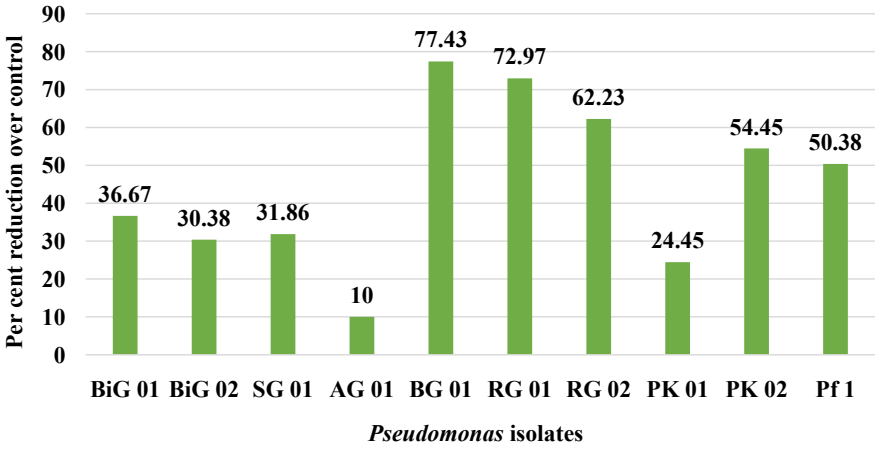


Fig. 3. *In vitro* efficacy of *Pseudomonas* isolates against *F.solani*

In the juvenile mortality study, the culture filtrates of *Bacillus, Streptomyces, Pseudomonas, Trichoderma* and the filtrate of neem cake, recorded significant percentage of mortality in the juveniles of *M. incognita* at 72 hours of exposure. It is inferred, that the per cent mortality of juveniles was positively correlated with the

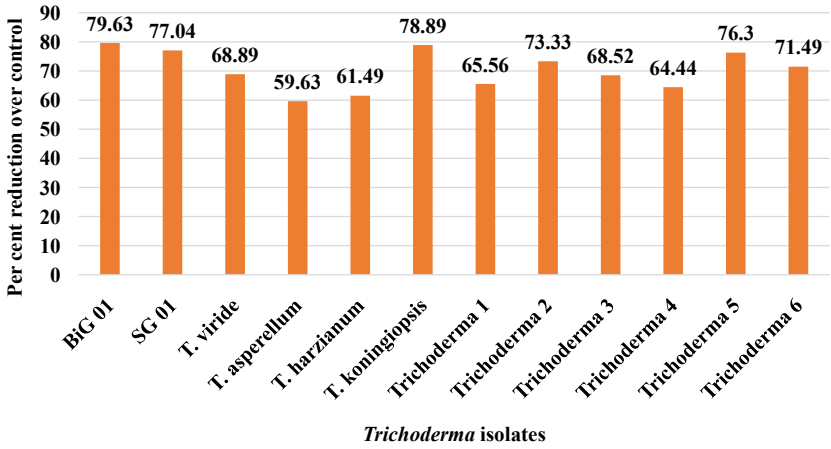


Fig. 4. *In vitro* efficacy of *Trichoderma* isolates against *F. solani*

time of exposure. At 72 hours of exposure, among the biocontrol agents a higher mortality of second stage juveniles of 80.33 per cent was observed in *Streptomyces* (BG 01) followed by *Bacillus* sp. (PG 12) with 75.66 per cent mortality. In treatment with neem cake, the highest J_2 mortality rate of 97.33 per cent was observed (Table 4). The efficacy of three oil cakes *viz.,* neem, castor and mahua were evaluated against root knot nematode, *Meloidogyne incognita* through mortality test. Among the oilcakes, neemcake was the best with 97% mortality at 48 hours (Jothi and Poornima, 2017).

The different treatments were imposed on bitter gourd cultivar CO 1 under glasshouse conditions. Among the different treatments soil application of neem cake @ 5 g per pot + *Streptomyces reticuli* (BG 01) @ 10 ml / lit / pot + *Pseudomonas fluorescens* (Pf 1) @ 2g per pot + *Trichoderma viride* (Tv 1) @ 2 g per pot recorded the least wilt incidence of 20.7 per cent as against 71.4 per cent in inoculated control with 71.01 per cent disease reduction of wilt over inoculated control. The nematode gall index was also significantly reduced to 1.0 as against 3.0 in inoculated control (Table 5). Results of the field experiment revealed that the combined application of neem cake @ 250 kg ha^{-1} + *Streptomyces reticuli* (BG 01) @ 10 ml/ lit + *Pseudomonas fluorescens* (Pf 1) @ 2.5 kg ha^{-1} + *Trichoderma viride* (Tv 1) @ 2.5 kg ha^{-1} recorded the lower wilt incidence of 14.4 per cent as against 42.5 per cent in the control, which was found to be 66.1 per cent reduction over control and gall index was also significantly reduced to 1.6 as against 5.0 in control. The growth and yield parameters *viz.,* vine

length, root length, fruit length, number of fruits per plant and the fruit yield were also found to be increased when compared to control. The treatment recorded a higher fruit yield of 15.40 tonnes ha^{-1} as against 10.75 tonnes ha^{-1} in control (Table 6).

Application of neem cake @ 1 t / ha was effective in suppression of nematode and increased the growth parameters and yield in cucumber (Sunitha Devi and Debanand Das, 2016). Manikandan *et al*. (2010) reported that liquid formulation of Pf 1 as seedling dip @ 500 ml / ha + soil drenching @ 500 ml / ha was found to be effective in controlling the *Fusarium* wilt of tomato. Similar results were found in the combination of seed and substrate treatment using the combination of *T. viride, P. lilacinus* and *P. flourescens* enriched by mixing of neem cake was more effective than treatments with individual bio-agents in reducing *M. incognita* population and disease incidence caused by *F. oxysporum* (Sowmya *et al.,* 2012).

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