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Effect of organic amendments and micronutrients on the incidence of Fusarium wilt (*Fusarium oxysporum* f.sp. *lycopersici* (Sacc.) (Snyder and Hansen) and root knot nematode (*Meloidogyne incognita* (Kofoid and White) Chitwood) disease complex of tomato (*Lycopersicon esculentum* Mill)

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ABSTRACT

The survival of native *P. fluorescens, B. cereus* and *P. lilacinus* isolates as influenced by the organic amendments was assessed and the results revealed that all the organic amendments supported the survival of the antagonists. The final population of the antagonists was the maximum in FYM amended soil, which also resulted in the least wilt incidence when compared with other organic amendments. All the micronutrients tested either individually or as mixture showed significant increase in the plant growth, reduction in the wilt incidence and population of *Fusarium* and root-knot nematode (*M. incognita*). Application of mixture of micronutrients effectively suppressed the wilt incidence and enhanced the plant growth and yield of tomato.

Keywords: organic amendments, micronutrients, *Fusarium* and root-knot nematode

Introduction

Tomato (*Lycopersicon esculentum* Mill) is one of the important vegetable crops in the world. It occupies a pride of place in view of its high nutritive value coupled with multivarious uses and it tops the list of processed vegetables. Tomato is a good source of vitamin-A, vitamin-C and various minerals. It is used directly as a raw vegetable in sandwiches, salads etc., and also processed into several food items like paste, puree, syrup, juice, ketchup, sauce, drinks, whole peeled tomato etc.

In tropical Asia, it is an important cash crop for small farmers (Villareal, 1980) [28]. Tomato is cultivated throughout the world in an area of 3.54 million ha with estimated annual production of 95.13 million tones. In India tomato is grown in 4.57 lakh ha and producing 74.27 lakh tones with a productivity of 16.3 tonnes/ha (Dharamveer *et al.*, 2005) [7].

In Tamil nadu, tomato is cultivated in about 21.055 ha in the districts of Theni, Madurai, Coimbatore, Dharmapuri and Erode (Anon, 1997) [2].

Tomato crop grown throughout the year is susceptible to several diseases and more than 200 pathogens affect the crop resulting in 75 – 95 per cent yield loss (Lukyanenko, 1991) [14]. Among these, *Fusarium oxysporum* f. sp. *lycopersici* considered as the major pathogen often results in 10-50 per cent crop losses around the world. The isolates of *F. oxysporum* f.sp. *Lycopersici* differed in their potentiality to induce wilt incidence in tomato (Padmodaya and Reddy, 1996) [20]. Besides fungal diseases, a number of plant parasitic nematodes affect tomato. Sasser (1979) [25] reported that the major nematode damaging tomato is the root-knot nematode Yield loss due to this nematode range from 15 to 71 per cent in tomato (Jain *et al*., 1994) [11].

Bora (1977) [3] reported about the association of *F. oxysporum* f. sp. *lycopersici* with *M. incognita*, which causes more damage to tomato than either of the pathogen alone. *Meloidogyne – Fusarium* wilt complex disease are import and widely distributed causing substantial yield losses on many important vegetable crops (Mai and Abawi, 1987) [15].

Thus, it is imperative that the management *Fusarium – Meloidogyne* wilt complex disease of tomato is of utmost importance for economic productivity of the crop. In modern agriculture, large quantities of several volatile and non-volatile pesticides are being used inevitably to control soilborne disease and nematodes. In India, pesticides are not indigenously manufactured and involve exorbitant cost. Besides, indiscriminate and improper use of these chemicals lead to environmental pollution, groundwater contamination, health hazards due to pesticide residues in the harvested produce, toxicity to non-target organisms and adverse effects on beneficial microflora and fauna of the soil. Hence, there is a need to develop an eco-friendly, economical and alternative method for effective management of *Fusarium – Meloidogyne* wilt complex disease in tomato.

Until today, no single method is found to be very effective and economical for the effective management of *fusarium* root-knot nematode wilt complex disease of tomato. Hence, an integrated approach would always ensure maximum suppression of the disease and higher yield without any deleterious effect on the ecosystem. In this context, it was thought that application of organic amendments could serve as the nutrient base for the antagonist and as well for the crop. Similarly, application of micronutrients like manganese sulphate, zinc sulphate and ferrous sulphate can improve the vigour of the crop, which may useful for the indirect suppression of the disease. With this background, the present develop integrated management practices using organic amendments, and micro nutrients for the effective management of Fusarium wilt and root-knot nematode wilt complex disease of tomato.

Material and methods

Method of disease assessment

The per cent disease index was assessed by adopting 0-5 scale according to "Phytopathometry

Table 1

Index No.	Grades
1.	Symptom absent
2.	Vascular browning in root region and no plant mortality
3.	Vascular browning up to 25 per cent and no plant mortality
4.	Vascular browning>25 per cent leaves yellow and no plant mortality
5.	Dead plants

The percent disease incidence (PDI) was worked using the following formula,

$$\text{PDI} = \frac{\text{Number of diseased plants}}{\text{Number of plants observed}} \times 100$$

Biometric observations and yield

The morphological observations were taken periodically following standard procedures and recorded.

Shoot length

At the time of harvest, the shoot length from the base of the stem (at soil level) up to the tip of the shoot was measured and recorded as cm.

Root length

The root length was measured (cm) at the time of harvest from the base of the stem (soil level) up to the tip of the root and recorded.

Dry plant biomass

The sample plants were dried in an oven until constant weight and dry wt. was expressed as g/plant.

Fruit yield

The fruits were harvested at matured stage periodically and the fruit weight was recorded as Kg per plot.

Estimation of root-knot nematode population in soil

Soil sample weighing 250g was washed thoroughly and processed using combined "Cobb's sieving and Baerman funnel method" (Ayoub, 1977). Two hundred and fifty g of soil taken in a 1000 ml beaker was added with sufficient quantity of water to make a soil solution, stirred thoroughly and allowed to stand for the heavier particles to settle down. Then the soil solution was passed through a set of sieves of 100, 250, 325 and 400 mesh size, respectively. Residue from 325 and 400 mesh sieves were collected and poured over a tissue paper on a wire gauge placed on Baermann funnel. Level of water in the Baermann funnel was maintained to keep the tissue paper wet and left undisturbed for 48 h. After incubation for 48 h., the volume of suspension was made to 200 ml, out of which 10 ml was pipetted out and used for counting various plant parasite nematodes. Nematode population from this was finally calculated to 250 g of soil.

Estimation of root-knot nematode population in root samples

Root samples weighing five g were directly observed under stereo-binocular microscope for counting adult females of sedentary nematodes and the same was processed using root incubation method (Ayoub, 1977) for the extraction of active forms of sedentary nematodes along with migratory nematodes.

After incubation for 48 h. the volume of suspension was made to 200 ml, out of which 10 ml was pipetted out and used for counting various plant parasitic nematodes. Nematode count from this was finally estimated to five g of root same. The number of egg masses of root-knot nematode per root system was counted by exposing the infected roots to 0.25 per cent tryptan blue for three min. as per the standard procedure.

Estimation of *Fusarium* population in soil samples

Fusarium population was estimated from soil collected from pots and trial field at the time of harvest by dilution plate technique (Waksman, 1952) using *Fusarium* specific medium developed by Nash and Snyder (1962) [19].

Estimation of *P. fluorescens*, *B. cereus* and *P. lilacinus* population in soil rhizosphere

The rhizosphere population of the *P. fluorescens* and *B. cereus*

were assessed at 20, 40, 60 and 90 day after transplanting by dilution plating method as described by Papavizas Davey (1961) [22]. The population of *P. fluorescens* and *B. cereus* were expressed as cfu per g of soil.

Paecilomyces lilacinus

The rhisozphere population of *P. lilacinus* was assessed at 20, 40, 60 and 80 days after transplanting by dilution plating method on potato dextrose agar medium and expressed as cfu per g of soil.

Effect of organic amendments on the survivability of bio control agents

Two hundred g of garden land soil was filled in earthen pots (15 cm dia). The organic amendments viz., farm yard manure, poultry manure, green manure, pressmud and neem cake were incorporated in to soil at 1% level (W/W) (Ayyappan, 2005). The conidial suspensions of the antagonists were prepared with adequate CFU and added to soil @ two ml/100 g of soil and mixed thoroughly. The pots were maintained inside the glass house with judicious, uniform and regular watering. The population of the antagonist was assessed periodically at 0, 30, 60 and 90 days after incubation using serial dilution technique. Selective media viz., King's B, NA and PDA were used for assessing the population of *P. fluorescens, B. cereus* and *P. lilacinus*, respectively.

Efficacy of organic amendment on plant growth, wilt incidence and nematode population of tomato

The pathogen *F. oxysporum* f.sp. *lycopersici* was multiplied in sand – maize medium and thoroughly mixed with the sterilized soil filled in earthen pots (30 cm dia) at 2 per cent level W/W. The organic amendments viz., FYM, poultry manure, green manure, press mud and neem cake were collected, powdered, sieved and incorporated in soil at one per cent level. Conidial suspension with adequate cfu from two days old culture of *P. fluorescens*, *B. cereus* and seven days old culture of *P. lilacinus* were prepared and added to soil and mixed (2 ml per 100 g of soil) well. The root-knot nematode was also inoculated @ 2 J2/g of soil. Three weeks old seedlings were transplanted into mud pots at three seedlings per pot. The observations on biometric characters, wilt incidence and nematode population were taken at periodical intervals as mentioned earlier.

Treatment details

T_1 : FYM
T_2 : Poultry menure
T_3 : Green menure
T_4 : Pressmud
T_5 : Neem cake
T_6 : Coirpith
T_7 : Control

Effect of application of certain micronutrients on wilt incidence, plant growth and nematode population of tomato

A pot culture experiment was conducted by incorporating various micronutrients following the standard blanket recommendations to the pathogen inoculated (at 5% level) sick soil to assess its effect on the management of tomato wilt pathogen. The micro nutrient mixture was prepared by mixing 5 kg each of $ZnSo_4$ + $MnSo_4$ + $FeSo_4$ and used @ 15 kg/ha. The following were the treatments.

T_1 : $ZnSo_4$ @ 5 kg/ha
T_2 : $FeSo_4$ @ 5 kg/ha
T_3 : $MnSo_4$ @ 5 kg/ha
T_4 : Micro nutrient mixture @ 1:1:1 ratio (15 kg/ha)
T_5 : Control

The experiment was conducted in a randomized block design was replicated thrice, where five pots per replication and three plants per pots were maintained. The wilt incidence (%), plant growth parameters and root knot nematode population were recorded periodically as mentioned earlier.

Results and Discussions

Efficacy of organic amendments on the survivability of antagonists

The survival of native *P. fluorescens, B. cereus* and *P. lilacinus* isolates as influenced by the organic amendments was assessed through periodical sampling and the results are presented in (Table 1). Generally all the organic amendments supported the survival of *P. fluorescens*, *B. cereus* and *P. lilacinus.* The population of the antagonist increased gradually in all the organic amendment treatments up to the maximum period tested except control. However, the final population of *P. fluorescens*, *Bacillus cereus* and *P. lilacinus* was the maximum in FYM amended soils followed by neem cake amended soils (34.31, 32.22, 32.54, 31.83 and 56.42, 55.61 cfu g^{-1} respectively). Other amendments *viz*., green manure, poultry manure, coir pith and pressmud recorded a final *P. fluorescens* population of 33.26, 31.61, 28.59 and 21.91 x 10^{-6} cfu g^{-1} soil, *B. cereus* 30.71, 31.41, 28.82 and 29.65 x 10^{-6} cfu g^{-1} soil and *P. lilacinus* 52.56, 49.45, 49.78 and 48.55 x 10^{-3} cfu g^{-1} of soil respectively.

Effect of organic amendments on wilt incidence, biometrics, *Fusarium* and root-knot nematode population in tomato

The data on the effect of various treatments on the biometrics, wilt incidence, *Fusarium* and root-knot nematode population were recorded in (Table 2) all the treatments had significantly increased growth parameters and reduced the wilt incidence and pathogen populations when compared to control. Among the treatments, FYM, the maximum shoot length (7.38 cm), root length (6.82 cm) and fruit yield (500.45 g/plant). Minimum disease incidence (35.47), soil root-knot population (1785.00) and soil fusarium population (1.43 x 10^{-3} cfu g^{-1} of soil) were observed in FYM application T_1. This was followed by T_5, T_3, T_2 and T_4. The untreated control recorded the maximum disease incidence (71.72) and minimum values in the biometrics of tomato.

Generally all the organic amendments supported the survival of *P.fluorescens*, *B.cereus* and *P.lilacinus* and the population of the antagonist increased gradually in all the organic amendments treatments up to the maximum period tested.

However, the final population of *P.fluorescens*, *B.cereus* and *P.lilacinus* was the maximum in FYM amended soil followed by neem cake amended soil. Number of workers has reported the control of *fusarium* wilt of different crops in the soil amended with organic amendments at varied conc. (0.5 to 5%) (Ramakrishnan and Jeyarajan, 1986; Lakshmi and Jeyarajan, 1987; Chakrabarthi *et al.*, 1991; Alice, 1996; Karthikeyan and Karunanithi, 1996; Cannayane and Rajendran, 2001) [24, 13, 1, 12, 5].

The increase in the rhizosphere population of the antagonists could be attributed to the reason that the organic amendments might have served as a ideal food base of the growth and multiplication of antagonists as reported by Hoitink and Boehm (1999) [10]. Enhanced growth of bio agent's viz., *T. harzianum* and *P.fluorescens* in FYM was observed by Najam Waris Zaidi and Singh (2003) [16]. Which might also be a reason for the enhanced survival of bio inoculants in organic amendment applied soil.

Suppression of soil-borne plant pathogens by organic amendments operates through several mechanisms. Organic matter influences soil physical characters such as pore size, aeration, and temp. And water retention capacity etc, which helps in better solubiliation of minerals together with the nutrients released by decomposing organic matter. This in turn facilitates rapid expansion of the root system, better uptake of nutrients and finally improved vigor of the plants. Decomposition of organic amendments, being a biological process stimulates microbial activity both quantitatively and qualitatively antagonistic to soil-borne plant pathogens.

The amendments are also attributed to inhibit soil-borne disease either by antibiosis or enhancing the population of selective antagonistic rhizosphere micro flora which ultimately result in reduction of population of soil bone plant pathogens (Papavizas and Davey, 1963; Mixo and Curl, 1967 [17], Zakaria and Lock wood, 1980 [29]; Bouhot, 1981 [4]; Prakash *et al*., 1985 [23]. The organic amendments have also been reported to provide adequate nutrients to the seedling and reduce their predisposal to soil borne plant diseases (Muhammad *et al*., 2001) [18]. Which might also be a reason for the enhanced survival of bio inoculants in organic amendment applied soil.

From the available evidences, it can be concluded that the organic amendments added to the soil would have acted as relatively permanent food bases. However, they may not have acted as food base to native isolates alone. The efficient colonization of the substrate might be made possible amidst other pathogenic organisms through the possession and exhibition of good competitive saprophytic ability, enzymatic antagonistic potential and other features of antagonists (Sivan *et al*., 1984) [26].

Effect of application of micronutrients on the plant growth, wilt incidence, fusarium and root-knot nematode population in tomato

The results depicted in (Table 3) revealed that all the micro nutrients tested either individually or as mixture showed significant increase in the plant growth, reduction in the wilt incidence and *Fusarium* and root-knot nematode population when compared to control. Among the treatments application of mixture of micronutrients resulted in the maximum plant growth with 10.74 cm of shoot length, 7.99 cm of root length and yield 560 g/plants and minimum wilt incidence (31.45), Fusarium population 1.23 x 10^{-3} cfu g^{-1} soil and soil root-knot nematode population (1400.32). This was followed by the treatment with $MnSo_4$, $ZnSo_4$ and $FeSo_4$ in the decreasing order of merit. The nutrients *viz*., $ZnSo_4$ and $MnSo_4$ produced statistically at par results in reducing the disease incidence. In the present study, the mixture of micronutrients consisting Mnso4, Zns04 and F2 S04 @ 15kg/ha, significantly reduced the disease incidence and increased the biometrics of tomato. Manganese could indirectly affect host susceptibility through its effect on root exudation. Increased concentration of manganese at the root surface and consequent lignin deposition in the roots has been implicated as a barrier to infection by *Fusarium* spp. (Eavans and Stephen, 1989) [9]. Likewise, it would be reasonable to assume that application of MnSo4 would have helped in faster accumulation of lignin in the roots, which might have offered resistance to infection by *F. oxysporum* f.sp. *Lycopersici.* Similarly, zinc significantly improved the bio control activity of *P. fluorescens* against *F. oxysporum* f. sp. *radicis lycopersici* (Duffy and Defago, 1999) [8]. Zinc and other trace minerals stimulate the production of phenazine type antibiotics and stabilized regulator genes critical for antibiotic production in fluorescent *Pseudomonas* (Duffy and Defago, 1997). The dry matter from both shoots and roots of wheat showed a marked response to the addition of Zinc (Sparrow and Graham, 1998). Similarly in the present study also the combination of micronutrients might have helped for the increased growth parameters of tomato. These reports lend support to the present findings

Table 1: Effect of organic amendments on survivability of antagonistic organisms

T. No.	Organic amendments	Pf (10^{-6} cfu g^{-1})				Bc (10^{-6} cfu g^{-1})				Pl (10^{-3} cfu g^{-1})			
		0	30	60	90	0	30	60	90	0	30	60	90
T_1	FYM	27.45	29.22	32.17	34.31	26.05	28.39	30.53	32.54	48.41	51.43	54.44	56.42
T_2	Poultry manure	26.72	27.71	30.63	31.61	26.76	27.79	29.63	31.41	45.47	46.55	48.49	49.45
T_3	Green manure	25.25	27.72	30.35	33.26	25.65	26.67	31.04	30.71	46.32	48.69	50.46	52.56
T_4	Pressmud	25.65	24.70	23.63	21.91	24.66	25.96	27.52	29.65	44.54	44.60	46.66	48.55
T_5	Neem cake	26.40	28.53	30.54	32.22	25.73	27.89	29.64	31.83	47.64	49.68	53.44	55.61
T_6	Coir pith	24.31	26.27	28.64	28.59	24.55	24.81	26.51	28.82	45.36	46.35	47.52	49.78
T_7	Control	25.49	26.36	27.37	29.46	25.27	26.28	27.92	29.30	43.40	42.77	42.46	41.05

	O	D	T	OD	DT	OT	ODT
SE (d)	0.51877	0.59903	0.79244	1.03754	1.58488	1.37254	2.74508
CD (1%)	1.35164	1.56074	2.06467	2.70329	4.12934	3.57612	7.15223

Table 2: Effect of organic amendments on wilt incidence, biometrics Fusarium and Root Knot Nematode population in Tomato

T. No.	Organic amendments	*Wilt incidence (%)	Decrease over control	*Shoot length (cm)	*Root length (cm)	*Soil RKN population (250g/soil)	*Soil Fusarium population	*Fruit yield (g/plant)
T_1	FYM	35.47 (36.55)	44.95	7.38	6.88	1785.00	1.43	500.45
T_2	Poultry manure	48.31 (44.03)	32.64	6.43	6.23	1789.81	1.99	482.48
T_3	Green manure	43.71 (41.39)	39.05	7.18	6.47	1786.33	1.89	490.00
T_4	Pressmud	50.43 (45.25)	29.68	7.37	6.21	1876.33	2.15	450.20
T_5	Neem cake	39.48 (38.92)	50.54	7.97	6.28	1945.04	1.93	480.75
T_6	Coir pith	52.32 (46.33)	27.05	7.07	6.21	1880.69	2.00	445.25
T_7	Control	71.72 (57.89)		4.38	3.28	2896.63	4.57	322.05
Mean	48.78 (44.34)		6.83	5.94	1994.26	2.28	453.02	
SE (d)	0.9964		0.6178	0.5629	87.7970	0.4494	8.4563	SE (d)
CD (1%)	2.9662		1.8392	1.6757	261.3760	1.3378	25.1748	CD (1%)

* Mean of three replications
* Data in the parentheses are angular transformed value
* Fusarium population (cfux10 $^{-3}$ g^{-1} soil)

Table 3: Effect of micronutrients on wilt incidence, biometrics Fusarium and Root Knot Nematode population in tomato

T. No.	Treatments	*Wilt incidence (%)	Decrease over control	*Shoot length (cm)	*Root length (cm)	*Soil RKN populatio (250g/soil)	*Soil Fusarium population	*Fruit yield (g/plant)
T_1	$ZnSo_4$ @ 5 kg/ha	37.47 (37.72)	32.46	6.43	5.65	1836.28	1.82	480.38
T_2	$FeSo_4$ @ 5 kg/ha	48.63 (44.21)	12.35	5.64	4.34	1920.30	1.89	390.52
T_3	$MnSo_4$ @ 5 kg/ha	35.71 (36.67)	35.63	6.72	5.50	1795.26	1.50	501.46
T_4	Mixture @ 15 kg/ha	31.45 (34.08)	43.31	10.74	7.99	1400.32	1.23	560.61
T_5	Control	55.48 (48.16)	-	5.38	3.54	2800.35	3.63	330.56
	Mean	41.75 (40.17)		6.98	5.40	1950.50	2.02	452.70
	SE (d)	2.6382		1.5514	1.2352	43.7086	0.5574	14.8880
	CD (1%)	8.3616		4.9171	3.9148	138.5314	1.7667	47.1866

* Mean of three replications
* Data in the parentheses are angular transformed value
* Fusarium population (cfux10 $^{-3}$ g^{-1} soil)

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