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Management of *Fusarium* wilt of tomato caused by *Fusarium oxysporum* f sp. *lycopersici*

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Abstract
Fusarium wilt of tomato (*Lycopersicum esculentum* Miller) caused by *Fusarium oxysporum* f sp.*lycopersici* is one of the most destructive diseases in tomato throughout the world. Effective and efficient management of the crop disease is generally achieved by the use of synthetic pesticides. These pesticides cause deleterious effects on human health and biosphere. Amendments (Neem cake, oil cake, cow dung, rabbit manure and chicken manure) were used in tomato seedlings to observe growth promotion increase in healthy and treated tomato seedlings of two varieties, Shrijana and Param. Results revealed that growth of the tomato seedlings was significantly increased following amendment with neem cake and oil cake in the treated *Fusarium oxysporum* f sp.*lycopersici* inoculated plants than in untreated uninoculated plants as recorded. Oil cake had better effect than neem cake manure. It has been observed that the growth of tomato seedlings increased in untreated inoculated than treated inoculated tomato seedlings. Among cow dung, rabbit manure and chicken manure, chicken manure gave better growth of tomato seedlings than that of rabbit manure and cow dung. Similarly effective integrated management practices against *Fusarium oxysporum* f sp.*lycopersici* were also developed using neem cake, oil cake, aqueous bulb extract of *Allium sativum* , bio-control agent like *Trichoderma harzianum* and calixin (0.0125%) in vivo. Combination with cow dung, neem cake, oil cake, chicken manure and rabbit manure, disease reduction were insignificant. However, combination with neem cake and oil cake showed 64.4% disease incidence, whereas in oil cake, neem cake and *Allium sativum* in combination disease incidence were recorded 10.1%. Under pot culture conditions *T. harzianum* alone and in combination with neem cake, oil cake and *Allium sativum* provided best effective management practices of *Fusarium* wilt in all the three modes of application viz ., simultaneous, repeated and post infection.

Key words: *Fusarium oxysporum f sp.lycopersici, Fungicides,* Plant extracts, *Lycopersicum esculentum* Organic amendment, Pathogen, Manure.

Tomato (*Lycopersium esculentum* Miller) is one of the second most popular vegetable crops of world. It is attacked by a large number of fungal, viral and bacterial pathogens. *Fusarium oxysporum f sp. lycopersici* is one of the important fungal pathogens that cause wilt resulting in substantial yield losses. *Fusarium* wilt is one of the most prevalent, serious diseases of tomato (Reis et al., 2005; Sudhamoy et al., 2009). This disease appears in the nursery grown tomato seedlings. The fungus is a soil borne rotting pathogen of very aggressive nature and causes considerable damage to young tomato seedlings in the nursery which is very common in the plains but rare in the hills. Effective integrated management practices against *Fusarium oxysporum f sp.lycopersici* were developed using neem cake, oil cake, aqueous bulb extract of *Allium sativum,* bio-control agent like *Trichoderma harzianum* and calixin (0.0125%) *in vivo.* Combination with cow dung, neem cake, oil cake, chicken manure and rabbit manure, disease reduction were insignificant. However, combination with neem cake and oil cake showed 64.4% disease incidence, whereas in oil cake, neem cake and *Allium sativum* in combination disease incidence were recorded 10.1%. Under pot culture conditions *T. harzianum* alone and in combination with neem cake, oil cake and *Allium sativum* provided best effective management practices of Fusarium wilt in all the three modes of application *viz.*, simultaneous, repeated and post infection. In the present investigation an attempt was made to develop an effective management strategy against Fusarium wilt of disease of tomato.

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Materials and methods

Plant material

Three tomato varieties which include Param, Shrijana and Manisha were grown in pots as well as fields and were used for experimental purpose.

Fungal cultures

Source of cultures

Virulent culture of Fusarium oxsporum f.sp. lycopersici was obtained from Immuno-Phytopathology Laboratory, Department of Botany, North Bengal University. Trichoderma harzianum (bio-control agent) was also obtained from the laboratory mentioned above.

Inoculation technique

Fungal pathogen

F.oxysporum f.sp. lycopersici was mass culture on PDB for 9 d. The mycelial mats was harvested and macerated in a homogenizer in a known proportion of distilled water. Fifty ml of fungus suspension containing 10g mycelia was added to each pot containing 1 kg sterilized soil and incubated for 48 h in shade. Each pot containing one month old tomato seedling variety and was kept for development of disease reaction.

Biocontrol agent

Trichoderma harzianum prepared in several media *viz.*, wheat bran media (wheat-bran: sand 1:1, and 25 ml of water for 150 g of inoculum in each polythene packet); Saw dust media (saw dust and water), Tea waste media (Tea waste and water). Media were autoclaved and inoculated above.

Inoculation of healthy tomato seedling in pot

One-month-old tomato seedlings was planted in earthen pots containing 1kg soil and allowed to be established. Regular watering was done for two weeks and then fifty ml of fungus suspension containing 10g mycelia was added carefully in the rhizosphere of each plant. Fungal pathogen suspension was also sprayed in whole plant. Disease assessment was done after two days-intervals and up to 15 days of inoculation.

Inducing agent and their application

In vivo test

Mature leaves (500 g) each of *Allium sativum* was harvested, was thoroughly with running tap water, rinsed with distilled water, air dried and macerated separately homogenized in a electric blender. The leaf extract was filtered through double-layered muslin cloth and centrifuged at 10,000g for 30 minutes. The supernatant was collected and filtered through what man No.1 filter paper. Each filtrate will be further filter sterilized and preserved as stock (100%) solution aseptically in bottles at 5°C for further use. Leaf extracts was diluted (1:10) with distilled water; drops of Tween-80 was mixed and spayed on tomato plants with the help of sprayer. The control plants were sprayed with distilled water mixed with Tween 80. Spray was done four times at 7-day intervals. Both treated and untreated plants was inoculated with *Fusarium oxysporum* f.sp. *lycopersici* and disease assessment was made.

Mustard oil cakes and neem cakes were allowed to decompose separately for a week in a clay pot covered with polythene. After decomposition, 100 ml of decomposed oil cake solution was added in each tomato seedlings pots. The pots were then inoculated with *F. oxysporum* f.sp. *lycopersici*. Untreated control was kept for comparison. Growth behavior also observed up to two months. Organic additives (cow dung, goat and chicken manure), 100 gm of each was taken separately and mixed in 1 kg of soil. These soil mixtures were separately kept in each pot. Tomato seedlings will be planted in each pot containing different organic components. After one week, 10g of pathogen (*F. oxysporum* f.sp. *lycopersici*) inoculums was added in the rhizosphere of each tomato seedling.

Mass cultures of *T. harzianum* were prepared on carrier medium comprising of wheat bran and sawdust (WBSD) in 3:1 ratio. Five hundred grams of the contents of carrier medium moistened with 20 percent (w/w) distilled water will be filled in each bag. These polythene bags were sterilized at 15 lb pressure for 1 h for 2 consecutive days. Each polythene bag was then inoculated with 4-6 days old bits (0.3 cm) of pure culture either of *T.*

harzianum and incubated at 25±1°C. During incubation, these bags were gently hand shaken to promote uniform sporulation over the carrier medium and to avoid clusters. Addition of biocontrol agents in soil was done 10 days prior to inoculation with *F. oxysporum* f.sp. *lycopersici.*

Results

In vivo evaluation

Growth promotion in tomato seedlings

Tomato seedlings of three varieties (Shrijana, Manisha and Param) were grown in soil amended with neem cake and oil cake separately. Each treatment consisted of 10 plants, in triplicate and the values are an average of 30 plants. Results were recorded after one-month interval and up to two months following the treatment of neem cake and oil cake and after inoculation with *F.oxysporum* sp. *lycopersici.* Results (Table 1) revealed that the growth of the tomato seedlings was significantly increased following amendment with neem cake and oil cake in the treated *F.oxysporum* sp. *lycopersici* inoculated seedlings than in untreated inoculated seedlings as recorded after two months. Oil cake had better effect than neem cake.

Similarly seedlings of two tomato varieties (Shrijana & Param) were grown in soil amended separately with cow dung, rabbit manure and chicken manure. Each treatment consisted of 10 plants, in triplicate and the values are an average of 30 plants. Results were recorded after one month interval up to two months following the treatment of organic components and after inoculation with *F.oxysporum* sp. *lycopersici.* It has been observed that the growth of tomato seedlings had been increased in treated uninoculated than treated inoculated tea seedlings (Table 2). Among the three treatments with organic components, chicken manure gave very good and healthy growth of tomato seedlings than rabbit manure and cow dung. Under field and pot culture conditions *T. harzianum* alone and in combination with neem cake, oil cake and *Allium sativum* provided best effective management practices of *Fusarium* wilt in all the three modes of application *viz.*, simultaneous, repeated and pot infection. Combination with neem cake and oil cake showed 64.4% disease incidence where as in oil cake, neem cake and *Allium sativum* in combination disease incidence were recorded 10.1%. But in combination with cowdung, neem

Table 1. Growth promotion in tomato seedlings following soil amendment with neem cake and oil cake in *F.oxysporum* sp. *lycopersici.*

Tomato variety	One month				Two months			
	Healthy		Infected		Healthy		Infected	
	Increase in height (cm)	Increase no. of compound leaves	Increase in height (cm)	Increase no. of compound leaves	Increase in height (cm)	Increase no. of compound leaves	Increase in height cm	Increase no. of compound leaves
Shrijana Untreateded	34±0.8	11±.09	22±1.02	10±.03	41±.07	12±.06	38±.05	18±.02
Treated Neem cake	32±.04	11±.02	38±.08	9±.02	44±.03	14±.06	70±.05	22±.04
Oil cake	45±.07	13±.05	44±.02	12±.08	80±.04	22±.03	77±.08	21±.02
Param Untreated	15±.03	7±.05	14±.06	6±.07	21±.09	10±.04	18±.03	8±.06
Treated Neem cake	20±.02	7±.08	19±.05	6±.04	32±.07	11±.05	30±.03	10±.05
Oil cake	21±.02	9±.06	23±.05	10±.04	55±.06	17±.06	45±.04	15±.03

± Stand for standard deviation, Average of three replicates.

Table 2. Growth promotion in tomato seedlings by different organic components after inoculation with *F.oxysporum* f.sp.*lycopersici.*

Tomato variety	One month				Two months			
	Healthy		Infected		Healthy		Infected	
	Increase in height (cm)	Increase no. of compound leaves	Increase in height (cm)	Increase no. of compound leaves	Increase in height (cm)	Increase no. of compound leaves	Increase in height (cm)	Increase no. of compound leaves
Shrijana Untreated	34±1.02	18±1.02	32±1.02	17±1.02	43±1.02	20±1.02	36±1.02	19±1.02
Treated Cow dung	60±1.02	23±1.02	52±1.02	20±1.02	76±1.02	26±1.02	68±1.02	28±1.02
Rabbit manure	45±1.02	18±1.02	46±1.02	20±1.02	57±1.02	27±1.02	53±1.02	25±1.02
Chicken manure	62±1.02	26±1.02	60±1.02	23±1.02	108±.02	33±1.02	80±1.02	30±1.02
Param Untreated	31±1.02	14±1.02	32±1.02	12±1.02	40±1.02	16±1.02	39±1.02	17±1.02
Treated Cow dung	42±1.02	16±1.02	32±1.02	10±1.02	69±1.02	18±1.02	46±1.02	15±1.02
Rabbit manure	31±1.02	12±1.02	35±1.02	15±1.02	51±1.02	15±1.02	48±1.02	22±1.02
Chicken manure	55±1.02	13±1.02	47±1.02	21±1.02	91±1.02	26±1.02	61±1.02	23±1.02

± Stand for standard deviation, Average of three replicates.

Table 3. Effect of simultaneous treatments with biocontrol, fungicide, organic amendments and plant extract on development of collar rot of tomato following inoculation with *F.oxysporum* sp. *lycopersici.*

Treatment	Disease incidence (%)	Disease control (%)
Trichoderma harzianum	0	100
Oil cake with Neem cake	64.4	35.6
Oil cake. Neem cake and *Allium sativum* bulb (aqueous extract)	10.1	89.9
T. harzianum, Allium sativum bulb (aqueous extract) Oil Cake and Neem cake	0	100
Cowdung. Neem cake and Oil cake	52.6	47.4
Chicken manure. Neem cake and Oil cake	57.5	42.5
Rabbit manure. Neem cake and Oil cake	56.6	43.4
T. harzianum, Calixin (0.0125%) and *Allium sativum* , bulb (aqueous extract)	0	100
Untreated Control	100	0

Table 4. Comparative efficacy of application of organic amendments and formulation against *F.oxysporum* sp. *lycopersici.*

Treatment	Disease incidence (%)		
	Simultaneous	Repetitive	Post infection
Trichoderma harzianum	0	0	0
Oil cake. Neem cake and *Allium sativum* bulb (aqueous extract)	14.8	0	46.6
T. harzianum, Allium sativum bulb (aqueous extract) Oil Cake and Neem cake	0	0	0
Cowdung. Neem cake and Oil cake	42.6	31.5	77.7
Rabbit manure. Neem cake and Oil cake	46.3	30.0	85.8
Chicken manure. Neem cake and Oil cake	47.5	34.5	88.2
T. harzianum, Calixin (0.0125%), *Allium sativum* , bulb (aqueous extract)	0	0	0
Untreated Control	100	100	100

cake, oil cake, chicken manure and rabbit manure, results were insignificant as shown in (Tables 3 and 4).

Discussion

This result was supported by Bhagawati et al (2000) who reported that mustard cake was found effective in reducing the incidence of *Fusarium oxysporum* f. sp. *lycopersici*. Neem cake was found as effective for the control of *F. oxysporum* f. sp. *cubense* in banana (Karthikeyan & Karunanithi, 1996); Saravanan et al (2004).

Organic amendments increases the availability of nutrients besides improving physical condition of soil, increase the yield and reduce the soil-borne diseases (Ramarethinam & Rajagopal, 1999). Organic soil amendments have also been reported to be effective in controlling the pathogen (Linderman 1989; Hadar & Golodeeki 1991; Kulkarni & Kulkarni 1995. The superiority of this amendment may be due to release of some inhibitory substances like nimbicidin, nimbin or azadirachtin on the decomposition, effecting the population of pathogen. Besides the nutrient content of these amendments may have a possible role in enhancing the host growth and vigor, increasing antagonistic microbial activity and enabling them to resist the attack of pathogen.

Similarly, *in vivo* trials demonstrated that *Trichoderma harzianum* alone as well as in combination with neem cake, oil cake, aqueous extract of *Allium sativum* and calixin (0.0125%) provided a total control of sclerotial blight disease. Similar results were obtained by Sonali and Gupta (2004) when *T. viride* alone and in combination with neem oil, neem cake and deodar needles used in radial growth of *S. rolfsii* resulted in a total control of the disease. But repeated application of neem cake, oil cake with various combinations of cow dung, rabbit manure and chicken manure were found to be less significant. Finally it was observed that *T. harzianum* and in combination with neem cake, oil cake, neem extract and calixin (0.0125%) were found most effective in reducing disease incidence on tomato seedling plants *in vivo*. There are several reports on the management of disease by Integrated Disease Management (IDM). Management of chickpea root rot and collar rot against *S. rolfsii* by integration of biological and chemical seed treatment was reported by Tiwari and Mukhopadhyay (2003). They observed that application of carboxymethyl cellulose (CMC) with *G virens* powder (10^9 spores per g) in combination with vitavax provided maximum protection (81.9%) to the crop against chickpea root rot and collar rot pathogens in glasshouse. Chickpea seeds treated with GV powder + CMC + vitavax significantly increased seedling emergence (47.9%); final plant stand (85.8%) and grain yield (79.7%) which was statistically at par with the treatment GV powder + vitavax and GV suspension + vitavax in a sick plot. Upamanyu et al., (2002) reported the management of root rot and web blight caused by *Rhizoctonia solani*. They obtained that *T. viride* showed the maximum tolerance to carboxin, tebuconazole and carbendazim followed by *T. virens*, *T. harzianum* and *A. niger* when used in integrated disease management along with fungicides and oil cakes both under glass house and field conditions. Soil amendment (cotton cake) + *T. virens* and carboxin (ST), mustard cake + *T. virens* + tebuconazole and soil amendement (mustard cake) + carbendazim (ST) were found effective in containing the root rot under glass house conditions while soil amendment (mustard cake) + carbendazim (ST) + carbendazim (FS) were found highly effective in reducing pre and post-emergence root rot and web blight. Severity was best contained by soil amendement (mustard cake) + carbendazim (ST+FS) followed by tebuconazole + *T. virens* (ST) + carbendazim (FS).

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