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Crown rot of Strawberry caused *by Fusarium oxysporum* and its management

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Crown rot of strawberry caused by Fusarium oxysporum is one of the most important diseases limiting strawberry production worldwide. Samples of infected crown and roots were collected from Koviloor region of Idukki district of Kerala with 70 per cent incidence in March-April. Infected plants appeared stunted with reddish-orange discolouration of internal cortical tissues. Pathogen in culture produced white mycelium that turned light pinkish later and macroconidia and microconidia with 2-4 septate slightly curved apical cells and foot shaped basal cells. Molecular characterisation confirms the pathogen as *F. oxysporum*. On in vitro evaluation carbendazim 12% + mancozeb 63% (Saaf), cymoxanil 8% + mancozeb 64% (Curzate M8), copper hydroxide 77WP (Kocide) and carbendazim 50WP (Bavistin) showed 100 per cent inhibition compared with rest of the fungicides. Calphomil recorded up to 52.77 per cent inhibition and *Trichoderma asperellum* and *Pseudomonas flourescens* was 68 and 57.67 per cent efficient. Field trials recorded that carbendazim 12% + mancozeb 63% (Saaf), and copper hydroxide 77WP (Kocide) recorded disease suppression upto 75 per cent followed by T. asperellum.

Key words : Bio-control agents, crown rot, disease, *Fusarium*, fungicides, management, organic formulations, strawberry

INTRODUCTION

Fusarium wilt is considered as the most dreadful plant disease. It was first reported in strawberry from Australia in 1962 and then in Japan in. By then the disease has several to almost whole parts of the world. Almost 80 per cent of fresh strawberries produced in United States are infected by the pathogen (California Department of Agriculture, 2014 and USDA, 2015). The pathogen is soil-borne infecting the root tissues and succumb the plants to death. It has been reported that a high rate of root and crown decay of strawberries is seen in the Swan Coastal Plain north of Perth and it is noticed that Fusarium oxysporum f. sp. fragariae is reliably separated from crowns and hence considered as a noteworthy pathogen. Many works has been conducted nationally and internationally and no works have been carried out till now in Kerala to characterise the disease and to find best management strategies. Thus the study envisages characterising the crown rot pathogen infecting strawberry, epidemiology and its management.

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MATERIALS AND METHODS

Assessment of disease incidence and its relation with weather parameters

Two surveys were carried out during December-January and March-April in strawberry growing regions of Idukki district viz., Koviloor and Vattavada. Infected plant parts were collected for isolation and the climatic parameters were noted out. Per cent Disease incidence (PDI) was then calculated based on the formula given by Wheeler (1969). The number of plants infected among 100 plants was noted.

$$\text{Percentage disease incidence (PDI)} = \frac{\text{Number of plants infected}}{\text{Total number of plants observed}} \times 100$$

Symptomatology and pathogenicity testing

Infected crown and root portions were thoroughly examined and symptoms were recorded. Root bits

were cut into smaller pieces for isolation onto Potato Dextrose Agar (PDA) for culturing using standard protocol at laboratory of Department of Plant Pathology. Fungus starts growing within 2-3 days and the pure culture was maintained by single spore isolation and then stored at 4ºC for further studies. Mycelila suspension of the pathogen made up to 2 X 106 spores ml-1 was poured onto 3 week old strawberry plants after giving an injury to the roots to study the pathogenicity as suggested by (Urena-Padilla et al., 2002). Symptomatology of the disease was studied at natural conditions.

Characterisation of the pathogen

The isolate of the pathogen was studied for their cultural characters like colour of the colony, pattern of growth and morphological characters viz., colour of conidia, presence of septations in conidia and hyphae, size of the conidia etc by slide culture technique suggested by Riddle (1950). The culture was then sent to National Centre for Fungal Taxonomy (NCFT) for species level confirmation after identifying the pathogen up to genus level. Molecular characterisation was also carried out by ITS sequencing. Sequence analysis and nucleotide homology of pathogen were analysed through the BLASTn programme of NCBI (http:// ncbi.nlm.nhm.gov/blast).

In vitro evaluation of fungicides, organic formulations and bioagents

Fungicides like copper hydroxide 77WP (Kocide), cymoxanil 8% + mancozeb 64% (Curzate M8), propineb 70WP (Antracol), copper oxychloride 50 WP (Fytolan), carbendazim 12% + mancozeb 63% (Saaf), difenoconazole 25 EC (Score), Bordeaux mixture, carbendazim 50WP (Bavistin) and Potassium phosphonate (Akomin 40), four organic formulations (calphomil, neem oil, panchagavya and baking powder + vegetable oil mixture) at three different concentrations was evaluated by poison food technique (Zentmeyer, 1955). Efficacy of the reference bio control agent from Kerala Agricultural University (KAU) viz., *T. asperellum* and *Pseudomonas fluorescens* (KAU isolates) against isolated fungal pathogens by tested by dual culture method suggested by Skidmore and Dickinson (1976). The per cent inhibition of mycelial growth in each treatment was calculated using the formula given by Vincent (1947).

Per cent inhibition of growth = C-T x100.
C= Growth of fungus in control (mm)
T= Growth of fungus in treatment (mm)

The nature of reaction of the antagonist T. asperellum against the pathogen was assessed by following the method of Purkayastha and Bhattacharya (1982).

Homogenous -Free intermingling of hyphae; Overgrowth -Pathogen overgrown by test pathogen; Cessation of growth -Cessation of growth at line of contact; Aversion - Development of clear zone of inhibition

Pot culture studies for the management of pathogen

For *in vivo* evaluation promising treatments were selected based on the results of the previous in vitro studies on the efficacy of fungicides and biocontrol agents. A pot culture experiment was laid out with strawberry variety Winter dawn to study the management of the predominant fungal pathogens observed during the survey period based on the intensity of the disease. The experiment was carried out during December 2016 - January 2017 at College of Horticulture, Vellanikkara.

RESULTS AND DISCUSSION

Assessment of disease incidence and its relation with weather parameters

Crown and root rot of strawberry plants infected by *Fusarium* sp. was recorded from Idukki. Plants were found infected when grown under open field conditions with a maximum incidence of 70 per cent during March-April in Koviloor, where the RH was 95.8 per cent with lower rainfall of 22.7 cm and a temperature of 24.25°C. Lower disease incidence of 30 per cent was observed in December-January at a temperature of 27.92°C, a RH of 94.85 per cent and rainfall of 169 mm. It was opined that black root rot of strawberry caused by *Fusarium culmorum* is severe in summer on heavy and wet soils, where the plants are grown in succession for several years. Recent evidences of Mina and Dubey (2010) interpreted the role of soil temperature and ambient atmospheric temperature in development of Fusarium oxysporum in chickpea. Similar findings on the

progression of disease in relation to climatic factors have been elucidated by Fang et al. (2011) where they observed optimum temperature of 27°C for disease development in strawberry caused by *Fusarium oxysporum*.

Symptomatology and pathogenicity testing

An attempt was made to study the crown root rot symptoms caused by *Fusarium* pathogen of strawberry where it appeared as reddish-orange discolouration of internal cortical tissues with poor growth and stunting of plants, which gradually lead to complete death and collapse (Fig. 1 and 2). The description of symptoms detailed above on strawberry plants was in congruence with the findings putforth by Fang et al. (2011a), Fang et al. (2011 b), Fang et al. (2013) and Koike and Gordon (2015). Pathogenicity of the isolate when carried out, symptoms initiated as wilting and drooping of plants, 13 days after inoculation where the roots became black and crown tissue appeared brown in colour.

Characterisation of the pathogen

Fusarium crown and root rot infecting strawberry plants identified from Idukki district produced white fluffy mycelium which subsequently turned light pinkish. Hyphae were hyaline, septate and branched (Fig. 3 and 4). Colonies produced macroconidia and microconidia, 2-4 septate with slightly curved apical cells and foot shaped basal cells measuring 8.50 to 18.71 ìm long and 2.1 to 4.9 ìm wide, whereas microconidia was one celled, oval to ellipsoid, borne abundantly of size 1.64 to 3.92 x 4.12 to 9.34 ìm. The above descriptions were in line with those studied by Ignjatov et al. (2015), Joshi et al. (2013), Liu et al. (2014) Wright et al. (2014) and Dinler et al. (2016). Thus, the pathogen was confirmed as *Fusarium oxysporum*.

Comparison of nucleotide sequence of the isolate *Fusarium* with other sequences showed cent per cent identity and query coverage with a maximum score of 861 with different isolates of *Fusarium oxysporum. Fusarium oxysporum* isolate F2 (Accession KY810793.1), *Fusarium oxysporum* isolate F1 (Accession KY810792.1), Fusarium oxysporum culture collection MUT:2205 (Accession KX551959.1), *Fusarium oxysporum* isolate ELRF 8 (Accession KX786247.1), *Fusarium oxysporum* isolate A549 (Accession KX463005.1) and *Fusarium oxysporum* isolate F. ox4bal (Accession KX058546.1) are some of strains of that showed similarity with the isolate from

Table 1: *In vitro* evaluation of fungicides against *Fusarium oxysporum*

Fungicide	Conc (%)	Per cent Inhibition over control *Fusarium oxysporum*
Carbendazim 12% + Mancozeb 63% (Saaf)	0.15	100(10)[a]
	0.20	100(10)[a]
	0.25	100(10)[a]
Cymoxanil 8% + Mancozeb 64% (Curzate M8)	0.15	100(10)[a]
	0.20	100(10)[a]
	0.25	100(10)[a]
Copper hydroxide 77WP (Kocide)	0.10	100(10)[a]
	0.15	100(10)[a]
	0.20	100(10)[a]
Copper oxychloride 50WP (Fytolan)	0.20	36.1(6.05)[k]
	0.25	42.7(6.55)[j]
	0.30	52.7(7.27)[i]
Propineb 70WP (Antracol)	0.25	73.3(8.56)[g]
	0.30	75.5(8.70)[f]
	0.35	76.1(8.75)[e]
Carbendazim 50WP (Bavistin)	0.05	100(10)[a]
	0.10	100(10)[a]
	0.15	100(10)[a]
Difenoconazole 25EC (score)	0.05	83.5(8.4)[h]
	0.10	86.2(8.67)[f]
	0.15	88.3(8.97)[d]
Potassium phosphonate	0.25	28.3(5.33)[n]
	0.30	28.8(5.39)[m]
Potassium phosphonate 40% (Akomin 40)	0.25	28.3(5.33)[n]
	0.30	28.8(5.39)[m]
	0.35	31.6(5.62)[l]
Bordeaux Mixture	0.50	86.1(9.28)[c]
	1.0	93.3(9.66)[b]
	1.50	100(10)[a]
CD (0.05)		0.029

Table 2: Effect of treatments on per cent incidence of *Fusarium oxysporum*(pot culture)

Treatment No.	Treatments (drenching)	Conc (%)	15 days after inoculation *PDI	10 days after first drench *PDI	10 days after second drench		
					Per cent disease reduction over control	*PDI	Per cent disease reduction over control
T_1	Control	-	100	100	-	100	-
T_2	Cymoxanil 8% + mancozeb 64% (Curzate M8)	0.2	87.5	75	25	62.5	37.5
T_3	Carbendazim 12% + mancozeb 63% (Saaf)	0.2	75	50	50	25	75
T_4	Copper hydroxide 77WP (Kocide)	0.2	75	50	50	25	75
T_5	Propineb 70 WP(Antracol)	0.3	75	75	25	62.5	37.5
T_6	*Trichoderma asperellum*	2	62.5	50	50	50	50

*Mean of the eight replications
PDI-Per cent disease incidence

strawberry. Hence, the result confirmed the identity of the fungus as *Fusarium oxysporum* based on the cultural and morphological characters of the isolate.

Fig. 1 : Natural symptoms

Fig. 2 : Pathogenicity testing

In vitro evaluation of fungicides, organic formulations and bioagents

A complete inhibition in radial growth of the pathogen, *Fusarium oxysporum* was observed with chemicals viz., carbendazim 12% + mancozeb 63% (Saaf), cymoxanil 8% + mancozeb 64% (Curzate M8), copper hydroxide 77WP (Kocide) and carbendazim 50WP (Bavistin) at all concentrations (Fig. 5a, 5b, 5c and 5d). Several workers reviewed cent per cent efficacy with Saaf Kumari *et al.,* 2014 and Rajan *et al.*, 2013) and with Curzate M8 and Bavistin Dar *et al.*, 2013 and Somu *et al.*, 2014) whereas, Madhavi and Bhattiprolu (2011) observed more than 90 per cent efficacy with Curzate M8 and carbendazim against *Fusarium* sp. isolated from gladiolus, chickpea, banana, fir and chilli which is in accordance with that of the results of present study. Bordeaux mixture at 1.5 per cent recorded 100 per cent inhibition of the pathogen whereas, difenoconazole

25 EC (Score) at all concentrations showed 70 to 80 per cent efficacy over control. Similar results were also reported by Somu et al. (2014) against

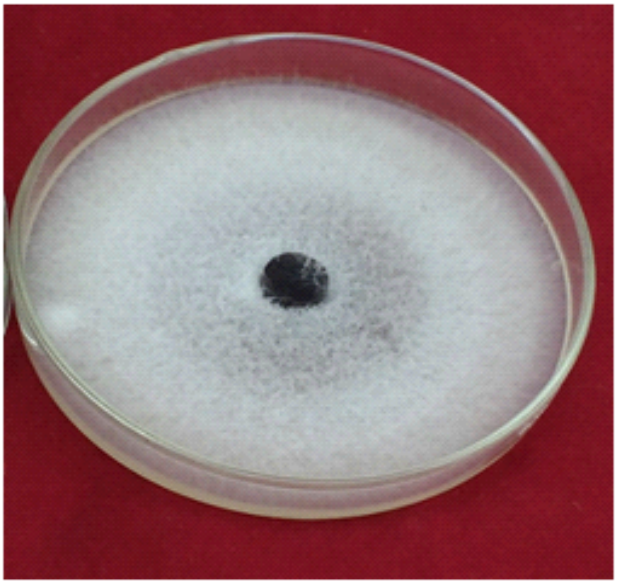


Fig. 3 : Culture growth of pathogen on PDA

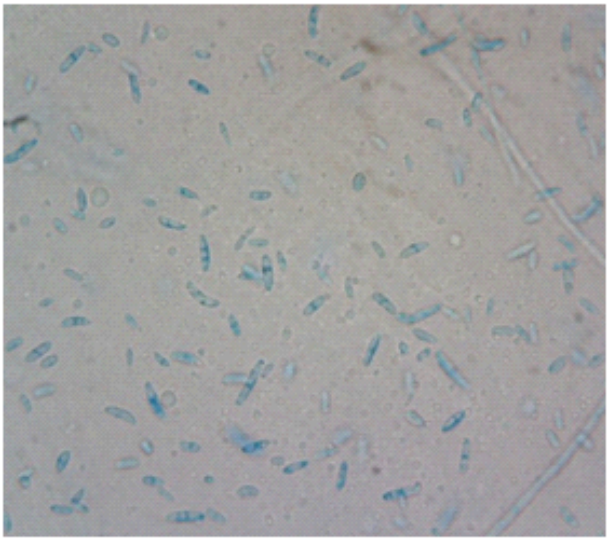
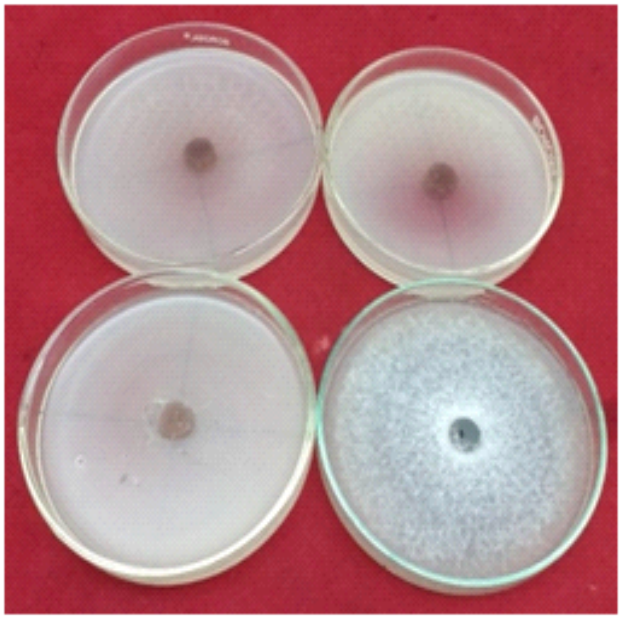


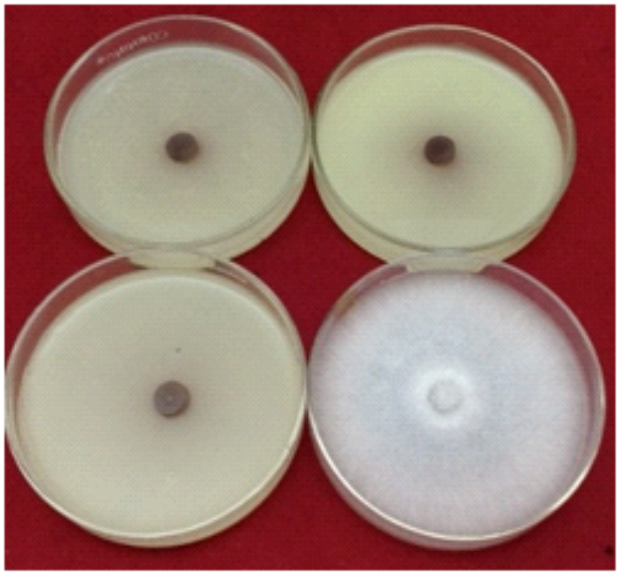
Fig. 4 : Hyphae of *F. oxysporum* (400x)

Fusarium oxysporum f. sp. *cubense* in banana. Propineb 70WP (Antracol) showed an inhibition of more than 70 per cent at all concentrations tested. However, comparatively less inhibition was noticed by Dar *et al*. (2013) with copper oxychloride 50 WP (Fytolan) which is in congruence with the present findings in *Fusarium oxysporum* isolated from fir.

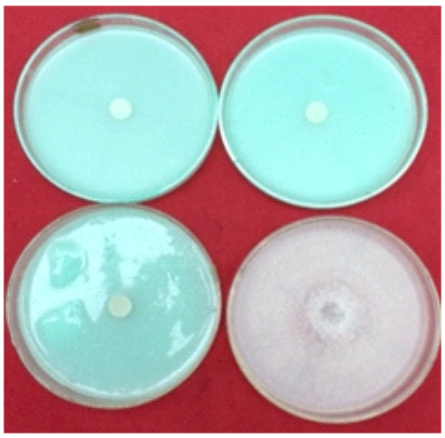
In vitro evaluation of organic preparations against *Fusarium oxysporum* revealed that Calphomil at different concentrations exhibited 20 to 55 per cent control (Fig. 5e). Neem oil recorded only 11 per



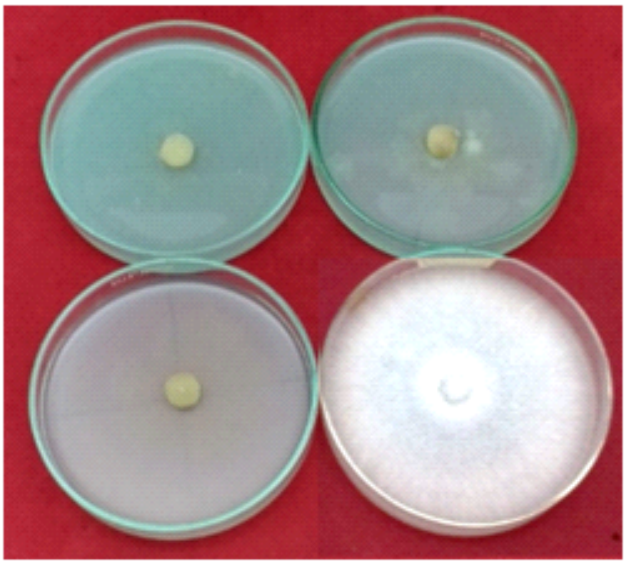
a. Carbendazim 50WP



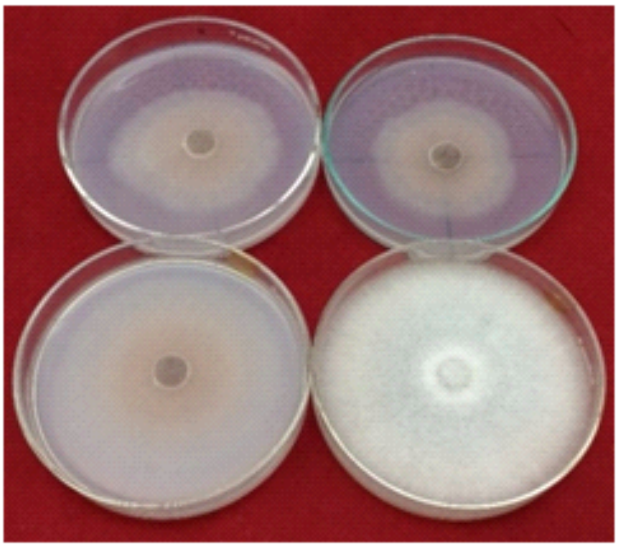
b. Cymoxanil 8% + Mancozeb 64%



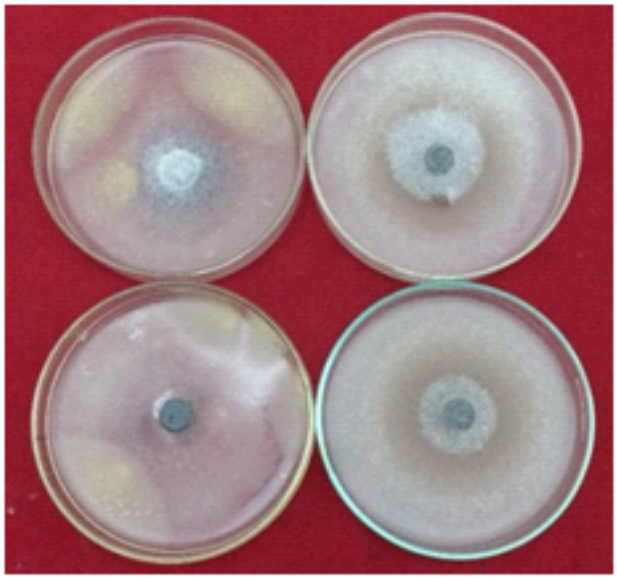
c. Carbendazim 12% + Mancozeb 63%



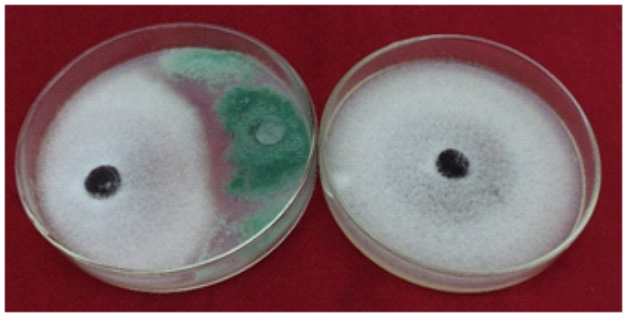
d) Copper hydroxide 77WP



e) Calphomil



f) Baking powder + Vegetable oil



g) *T. asperellum*

h) *P. fluorescences*

Fig. 5 : *In vitro* evaluation of fungicides, organic formulations and bio-control agents against *F.oxysporum*

a. Challenge inoculation

b. Symptom appearance

cent efficacy over control. Adepoju *et al.* (2014) pointed out the antifungal activity of neem oil in inhibiting the pathogen up to 7.37 per cent against *Fusarium* spp. in banana. Likewise, panchagavya and baking powder + vegetable oil mixture also recorded a per cent inhibition of below 20.3 per cent only (Fig. 5f). Joseph and Sankarganesh (2011) and Ashlesha and Paul (2014) observed the antimicrobial property of panchagavya which exhibited 67.25 per cent control over *Fusarium solani* and 79.05 per cent against *Fusarium oxysporum* in capsicum when used at 4 per cent. Similarly, Jandaik and Sharma (2016) reported 82.62 per cent control with panchagavya at 15 per cent against *Fusarium oxysporum* in capsicum.

Fusarium oxysporum when evaluated against *T. asperellum* and *P. fluorescens*, indicated relatively less efficacy of the pathogen where antagonist restricted the growth upto 65.67 and 57.67 per cent respectively (Fig. 5g and h). Rajan et al. (2013), Madhavi and Bhattiprolu (2011), Ragab et al. (2012) and Bashar and Chakma (2014) reviewed 63 per cent inhibition with *Trichoderma viride* and 57.14 per cent with *Pseudomonas fluorescens* against *Fusarium oxysporum* and *Fusarium solani* infecting chickpea, chilli and brinjal. Similar findings were reported by Bardia and Rai (2011) and Kumar and Naik (2015) against *F. oxysporum* isolated from cumin and castor. Contrary to the above results, a higher inhibition of 92.5 per cent and 86.5 per cent was noticed by Dar *et al.* (2013) against *Fusarium oxysporum* infecting fir. However, Tapwal *et al.* (2015) could observe only 15.80 and 27.04 per cent inhibition with *T. viride* and *T. harzianum.*

Pot culture management of Fusarium oxysporum

An *in vivo* experiment on *Fusarium oxysporum* in strawberry plants were carried out with the best treatments screened under lab study. Soon after challenge inoculation of the pathogen, an incidence of 62.5 to 100 per cent was obtained in varying treatments. However, plants after second treatment application showed a significant reduction in the disease incidence, where, T3 (carbendazim 12% + mancozeb 63%) (0.2%) and T4 (copper hydroxide 77WP) (0.2%) recorded only 25 per cent of the disease incidence followed byT6 (*Trichoderma asperellum*) (2%) with 50 per cent incidence. Highest percentage of disease incidence of 62.5 was noticed with the treatments T2 (cymoxanil 8% + mancozeb 64) (0.2%) and T5 (propineb 70WP) (0.3%). The less efficacy of *Trichoderma* under field conditions can be due to the presence of higher potential amount of pathogen in soil and due to the lack of sufficient time for multiplication under the root zone (Fig. 6). Also, the inefficacy of the antagonist in the field may be related to low supply of organic material since it is the organic matter on which antagonist multiplies and becomes more competitive against microorganisms. Sumana et al. (2012) and Akhtar *et al.* (2017) observed the efficacy of copper hydroxide and propineb against Fusarium in tobacco and tomato. Amini and Sidovich (2010) and Narayanan et al. (2015) observed that carbendazim could alone control the disease from 83.9 to 100 per cent in infected tomato plants and 59.6 per cent in blueberry. However, Narayanan et al. (2015) observed that the combination fungicide, Saaf could reduce the disease incidence of *Fusarium* only upto 28.8 per cent in blueberry. Akrami and Yousefi (2015) and Narayanan et al. (2015) recorded upto 85 to 87 per cent disease control with the combination of *T. harzianum*, *T. asperellum*, and *T. virens* against *Fusarium solani* and *Fusarium oxysporum* in tomato and upto 57.6 per cent with *T. viride* in blueberry.

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