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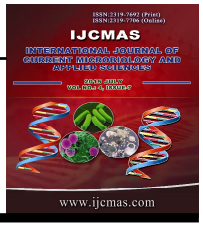


Original Research Article

Biological and chemical control of *Fusarium solani*, causing dieback disease of tea *Camellia sinensis* (L): An in vitro study

Kishor Chand Kumhar*, Azariah Babu, Mitali Bordoloi, Priyanka Banerjee and Tanima Dey

Tea Research Association, North Bengal Regional Research & Development Centre, Nagrakata, District - Jalpaiguri, West Bengal – 735 225, India
**Corresponding author*

Keywords

Gliocladium virens, dieback disease, *Fusarium solani*, dual culture, Fungicide compatibility

A B S T R A C T

A local strain of *Gliocladium virens* (= *Trichoderma virens*) was isolated at Tea Research Association, North Bengal Regional Research and Development Centre Nagrakata, West Bengal, India and its *in vitro* evaluation was done against dieback pathogen (*Fusarium solani*). To prove the pathogenicity of *F. solani*, two different methods were employed and in both methods pathogen could express peculiar disease symptoms. Among three tested fungicides, copper oxychloride was the most effective (85.0% inhibition) against this pathogen, followed by copper hydroxide (80.1%) and hexaconazole (68.2%) at recommended doses. The local antagonist, *G. virens* inhibited pathogen's growth by 77.6 per cent in dual culture. Antagonist adversely affected conidiation of pathogen and therefore, pathogens could produce lesser number of conidia (2.7 to 30.0) in comparison to un-treated control (46.3). The local antagonist showed 44.2 per cent growth inhibition of pathogen in comparative *in vitro* bioefficacy study. Results of the compatibility study revealed that, all three fungicides significantly inhibited the growth of *G. virens* (71.3 - 89.4%) and hence none of them was found to be compatible with this antagonist.

Introduction

Tea "dieback disease" caused by *F. solani* is one of the most destructive diseases in North East India as well as West Bengal (Barthakur and Dutta, 2011), responsible for huge crop loss, because it infects tender pluckable shoots. The pathogen, first of all, infects leaf petioles and then infection progresses upward and downward, resulting in chlorosis followed by shoot mortality, hence the named as "die back". Various fungicides have been used to manage this disease; however, their injudicious use may certainly lead to numerous ill effects like, deterioration of soil as well as plant health, environment pollution, ecological imbalance etc. (Tu, 1997; Chattopadhyay *et al*., 2002). To minimise the fungicide generated problems and achieve successful long term disease control strategy, integrated disease management (IDM) approach by intensifying the use of biological control agents (BCAs) is of immense importance

and need of hour. *G. virens* (= *T. virens)* is one of the most potential biocontrol agents which could effectively manage several plant pathogens (Papavizas, 1985) under lab, green house and field conditions (Mukhopadhyay, 1995). It produces extracellular enzymes, secondary metabolites (Elad et al., 1982; Howell and Stipanovic, 1983; Papavizas, 1985; Taylor, 1986; Ridout et al., 1988) and antibiotic *i.e.* gliotoxin (Lumsden, et al., 1992). Various formulations of antagonistic fungi especially, *Trichoderma* spp have been used in tea plantation to protect it from different fungal pathogens and promote vegetative growth but the use of indigenous isolates is almost negligible, in Dooars region, therefore, our present study is aimed to develop and evaluate the bio-efficacy of indigenous *G. virens* against *F. solani* to judge its suitability as a component of integrated disease management strategy.

Materials and Methods

Isolation of *F. solani* and *G. virens*

Dieback diseased tender tea shoots were collected from different tea gardens including, experimental plot of our centre (Table 1) and used for isolation of fungi of interest. For isolation of pathogen, samples were cut in to small pieces, followed by surface sterilization with mercuric chloride (0.1%) and subsequent two washing with distilled water. Then these pieces were inoculated in to PDA plates. For isolation of *Gliocladium virens* leaf washing technique (Bhuvaneswari, 2006) was employed with minor modifications. Plates were then incubated at room temperature for 3-4 days. Colonies developed in plates were transferred in to another PDA plate and again incubated. After colony development they were identified on the basis of morphological characteristics. Identity of antagonist was further, get reconfirmed from Agharkar Research Institute, Pune. The isolates were preserved in refrigerator at 4 ± 1^0C and used for further studies.

Pathogenicity of *F. solani*

From the *Fusarium solani* isolates, isolate of North Bengal Regional Research and Development Centre Nagrakata, was chosen for pathogenicity test by adopting two different methods i.e. (1) direct contact and (2) direct inoculation, as used by Rajput *et al* (2008) with slight modifications. In first method, a linear mechanical injury on healthy shoot was made by rubbing diseased shoot and then tying it using cotton thread, in the field. In second method, 2 mL spore suspension ($2x10^6$ cfu/ ml) was injected using surgical syringe in to the healthy leaves near petiole. Ten shoots were used for each method. Disease development was observed till 15 days.

In vitro bioassay of fungicides and *G. virens*

Bioefficacy of common fungicides against *F. solani* was carried out by poisoned food technique (Nene and Thapliyal, 1993) using potato dextrose agar medium (Hi-Media). Different concentrations like 500, 1000, 1500, 2000, 2500 and 3000 ppm, were made by adding required amount of fungicidal in to medium at its lukewarm state and medium was poured in to plates. After solidification, 5 mm diameter discs of pathogen's mycelia were inoculated in the centre of plates followed by incubation at room temperature for two weeks.

Dual culture technique (Stack et al., 1986) was applied to assess the bioefficacy of antagonist. 5 mm discs of fungi (*G. virens* and *F. solani)* were inoculated at variable distances followed by incubation for two

weeks. Pathogen's mycelial growth was recorded after 6 days. The effect of antagonist on pathogen's sporulation was also assessed after two weeks using compound microscope and haemocytometer.

Further, bioefficacy of *G. virens* was compared with three local isolates of *Trichoderma* sp (developed and maintained at the centre). Five millimetre discs of pathogen as well as antagonists were inoculated at 3 cm apart in the centre of plates in four replications. Plates were incubated at room temperature for one week and radial growth was measured after one week. Growth inhibition was calculated by following formula:

Growth inhibition (%) =

$$\frac{\text{Colony dia. in control} - \text{Colony dia. in treatment}}{\text{Colony dia. in control}} \times 100$$

Compatibility of *G. virens* with fungicides

Recommended doses of fungicides were evaluated by poisoned food technique of Nene and Thapliyal (1993) for this purpose. According, appropriate quantity of each fungicide was added and mixed thoroughly in PDA medium before pouring it in to plates of 90 mm diameter. After solidification, 5 mm diameter mycelial discs of *G. virens* were placed in the centre of plates followed by incubation at room temperature. Colony diameter was measured followed by calculation of per cent growth inhibition using formula described in previous method.

Data analysis

Statistical analysis of data was done by using online statistical package "OPSTAT" of Chaudhary Charan Singh Haryana Agricultural University, Hisar, Haryana, India (www.hau.ernet.in/opstat).

Results and Discussion

Isolation of *F. solani* and *G. virens*

All ten samples of different gardens showed the presence of pathogen and only one sample showed the presence of both pathogenic as well as antagonistic fungi (table 1). Morphologically, isolates were identified as *F. solani* and *G. virens.* Pathogen produced creamish colonies with pinkish pigmentation on PDA. It produced sickle shaped macro-conidia (mostly mono, bi, tri, rarely tetra septate) and aseptate micro-conidia. *G. virens* produced greenish coloured colonies.

Pathogenicity of *F. solani*

In both the methods, pathogen successfully established and caused infection in every artificially inoculated shoots. All the treated shoots showed peculiar disease symptoms. In physical contact method (rubbing of diseased shoots with healthy shoots), tissue necrosis was noticed after four days, which gradually increased and covered the entire shoot area within two weeks. In case of inoculation method, infection started at inoculation site after one week and prominent symptoms were developed on shoots after 15 days (Fig. 1).

***In vitro* bioassay of fungicides and antagonists**

All three fungicides influenced the growth of *F. solani* to a variable degree. Copper oxychloride showed the maximum inhibition (80.0 – 88.1%) followed by copper hydroxide and Hexaconazole (table 2 & fig. 2). At recommended doses, copper

oxychloride, copper hydroxide and Hexaconazole inhibited 85.0, 72.9 and 58.6 per cent, respectively.

Indigenous antagonist *G. virens* inhibited 53.0 to 77.6 per cent growth of *F. solani* after 6 days. It also adversely affected pathogen's sporulation. Pathogen could produce comparatively less conidia (3.75 to 30.25) in dual culture as compared to control (47.0) as given in table 3.

Its comparative bioefficacy with other local antagonists indicated that this strain inhibited 44.2 growth of *F. solani*. However, the maximum growth inhibition (50.0%) was noted in case of *T. viride* (KBN 24) followed by both *Trichoderma sp, i.e.* KBN-33 and KBN-35. All the antagonists were statistically at par with respect to their performance (table 4).

Compatibility of *G. virens* with fungicides

Result of this study revealed that all three tested fungicides inhibited the growth at recommended concentrations (figure 3 & 4). However, the maximum inhibition (89.4%) was noted in case of copper hydroxide followed by copper oxychloride (88.2%) and Hexaconazole (71.3%).

In present study *F. solani* was isolated from ten diseased samples. All ten isolates were identified on the basis of colony characters and conidial shape. Similar morphological characteristics of *F. solani* have already been reported by earlier workers (Demicri and Maden, 2006; Leslie and Summerell, 2006, Mirzaee et al., 2011 & Albores et al., 2014) which are in support of our findings.

In addition to infecting tea plantation, this pathogen is capable of infecting some other host plant like ber, *Ziziphus jujuba* (Mirzaee et al, 2011), plum (Salleh et al., 2011), shisham (Rajput et al., 2008), mango (Khanzda et al, 2004), date palm (Maitlo et al., 2013) etc.

In present study, we tested copper oxychloride, copper hydroxide and hexaconazole against this challenging pathogen and found that all fungicides were effective in controlling it under lab conditions. Similarly, Bashar and Chakma (2014) tested *in vitro* efficacy of some fungicides against *F. solani* and reported that vitavax 200B was the most effective against it.

It has also been noted by Maitlo et al (2013) that bavistin provided better control of this pathogen under lab conditions, however, Topsin-M, Aliette, Ridomil, mancozeb and copper oxychloride exhibited less inhibitory effect in comparison to bavistin.

Our local strain of *G. virens* showed very good inhibitory effect against this pathogen and successfully controlled it within a short span of time. The findings of Hamid et al (2012) are in close conformity to our present investigation wherein it was reported that *G. virens*, *T. viride* and *T. harzianum* had strong mycoparasitism and rapidly covered the colonies *F. solani* f.sp. *pisi*. Jagtap et al (2013) reported that *G. virens* and other *Trichoderma* spp. were effective biocontrol agents for the management of *Colletotrichum capsici*, which supports the results of our study.

In this study, all fungicides, copper oxychloride (2500 ppm), copper hydroxide (2500 ppm) and hexaconazole (1000 ppm) inhibited antagonist's growth significantly and it was concluded that the tested antagonist was highly susceptible to these fungicides.

Table.1 Isolation of *F. solani* and *G. virens*

SN	Location (Tea Estate)	Number of sample collected	Isolation frequency	
			***F.* solani**	***G. virens***
1	TRA, NBRRDC	3	3	1
2	Indon TE	3	3	0
3	Nagrakata TE	3	3	0
4	Bhogotpur TE	3	3	0
5	Nageshwari TE	3	3	0
6	Ibheel TE	3	3	0
7	Lakhipara TE	3	3	0
8	Bhatpara TE	3	3	0
9	Hope TE	3	3	0
10	Lankapara TE	3	3	0
	Total	**30**	**30**	**01**

Table.2 Effect of fungicides on growth of *F. solani* under lab conditions

Fungicide	Fungicide conc. (ppm)	Per cent growth inhibition over control*
Copper oxychloride 50 WP	2000	80.0 (63.7 ± 3.3)
	2500	85.0 (67.2 ± 1.5)
	3000	88.1 (69.8 ± 0.3)
Hexaconazole 5 EC	500	51.6 (45.9 ± 0.5)
	1000	58.6 (49.9 ± 1.9)
	1500	68.2 (55.7 ± 0.7)
Copper hydroxide 77 WP	2000	61.9 (51.9 ± 1.4)
	2500	72.9 (58.7 ± 1.6)
	3000	80.1 (63.5 ± 0.9)
C.D.		4.5
C.V.		5.0

Table.3 Effect of *G. virens on* mycelial growth and sporulation of *F. solani*

Dual culture distance between antagonist and pathogen in PDA plate	Per cent growth inhibition over control after 6 days*	Number of conidia of *F. solani* per haemocytometer field*
T1-0 mm	77.6 (61.01 ± 0.74)	3.75 ± 1.37
T2-10 mm	61.2 (50.96 ± 0.61)	8.0 ± 1.82
T3- 20 mm	59.0 (49.35 ±0.97	14.25 ± 4.23
T4- 30 mm	53.0 (46.08 ±0.93)	30.25 ± 1.88
T5- Control (*F. solani* alone)	-	47.0 ± 1.78
C.D.	2.24	7.43
C.V.	3.56	23.66

*Mean of 4 replications

Table.4 Effect of *G. virens* and *Trichoderma* sp on mycelial growth of *F.* solani under lab conditions

Antagonist	**Growth inhibition of *F.* solani after one week***
Trichoderma sp (KBN-33)	43.0 (41.7 ±3.2)
Trichoderma sp (KBN-35)	49.9 (44.4 ± 3.6)
T. viride (KBN 24)	50.0 (45.2±2.3)
G. virens (KBN-3)	44.2 (41.5±1.9)
C.D.	7.8
C.V.	14.8

*Mean of 4 replications

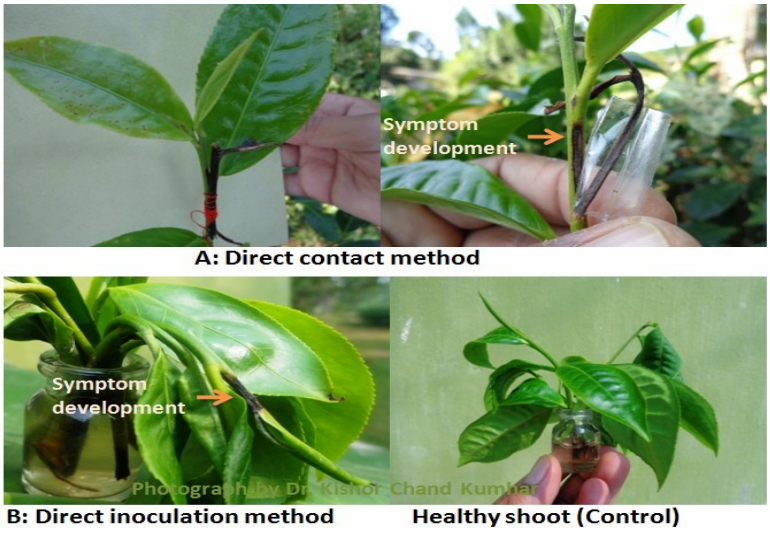


Fig. 1: Pathogenicity of *F. solani* on tea shoots

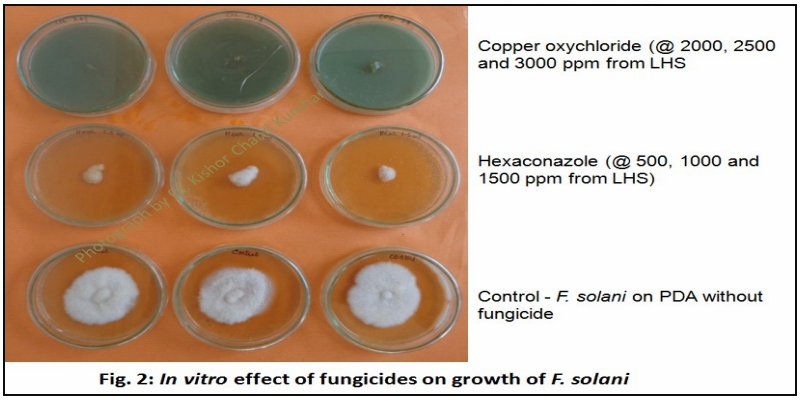
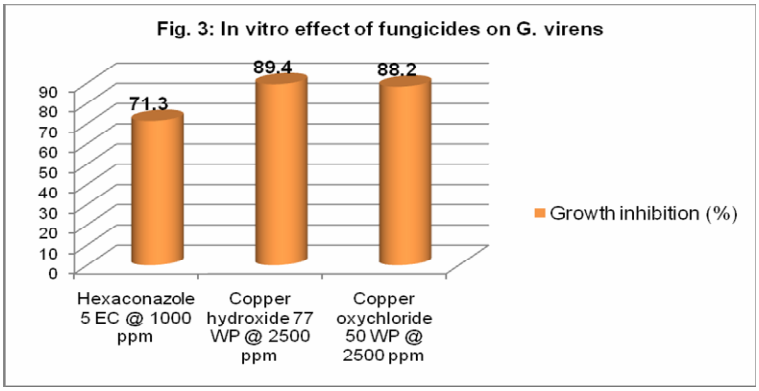


Fig. 2: *In vitro* effect of fungicides on growth of *F. solani*



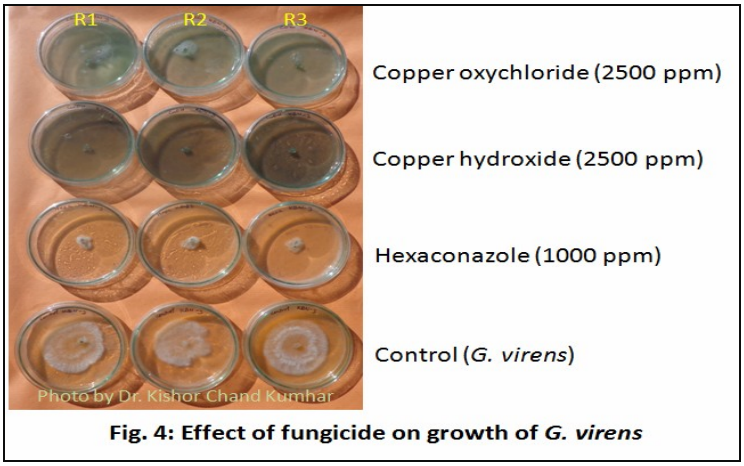


Fig. 4: Effect of fungicide on growth of *G. virens*

On this aspect, Mukherjee and Tripathi, (2000) reported that propineb had no inhibitory effect on *G. virens* but Narayana and Srivastava (2003) concluded that triazole fungicides (hexaconazole, propiconazole and penconazole) were highly inhibitory to *T. harzianum* at various concentrations. Similarly, Johnson (2001) found that hexaconazole was highly inhibitory to *T. viride*, *T. harzianum* and *G. virens* even at low (0.5 ppm) concentration. Hence, *G. virens* has been found to be an effective biological control agent for dieback pathogen of tea, it should be evaluated under field conditions after developing suitable formulation.

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