International Journal of Agricultural Research

ISSN 1816-4897

Academic Journals Inc.

www.academicjournals.com

International Journal of Agricultural Research 6 (8): 620-631, 2011
ISSN 1816-4897 / DOI: 10.3923/ijar.2011.620.631

In vitro Evaluation of *Trichoderma atroviride* against *Phomopsis theae* a Casual Agent of Collar Canker Disease in Tea Plants

S. Anita and P. Ponmurugan
Department of Biotechnology, K.S. Rangasamy College of Technology, Tiruchengode-637215, Namakkal District, Tamil Nadu, India

Corresponding Author: S. Anita, Department of Biotechnology, K.S. Rangasamy College of Technology, Tiruchengode-637215, Namakkal District, Tamil Nadu, India Tel: + 919486150579 Fax: +914288274745

ABSTRACT

An attempt was made to study the biocontrol activity of *Trichoderma atroviride* against *Phomopsis theae* Petch, a causative agent of Phomopsis canker disease in tea plants. Among 78 isolates enumerated, six strains, each representing an agroclimatic zone was chosen for further studies and later it was identified it as *Trichoderma atroviride*. A range of *in vitro* assessment such as growth parameters, antagonist activity against *P. theae* and compatibility with fungicides were evaluated for selected *T. atroviride* isolates. The correlation of various edaphic and environmental factors with population density of *Trichoderma* spp. showed positive results. The radial growth measurement and mycelium dry weight of Tv1 isolate obtained from Valparai showed the utmost growth on Potato Dextrose Agar. The highest antagonist activity of 65.77, 91.25 and 69.17% was observed for Tv1 isolates in dual culture test, antibiosis for volatile and non-volatile antifungal compounds. The compatibility of *T. atroviride* isolates tested against various contact and systematic fungicides clearly showed that Tv1 isolate can able to tolerate the concentration to higher extent. Hence the results revealed that Tv1 isolates proved to be eminent strains in controlling Phomopsis canker disease in tea plants.

Key words: Biocontrol agent, collar canker, *Phomopsis theae*, tea, *Trichoderma atroviride*, systematic fungicides, contact fungicides

INTRODUCTION

The quest for biological control of plant pests and pathogens continues to instigate research and development in numerous fields. This is especially the case in plantation crops like tea (*Camellia sinensis* (L.) O. Kunze). This foliage crop tends to endure severe stress both physically and physiologically owing to the frequent harvest of shoot (Thomas *et al*., 2009). Among the various diseases that afflict tea plants, collar canker disease caused by the fungus *Phomopsis theae* Petch is the most common one. The consequence of disease has led to the replanted of infected bushes. The decreases in yield have been estimated around 10-15% in Southern Indian tea plantation (Ponmurugan and Baby, 2008). Despite of its economic impact, effectual preventive measures are unavailable other than pruning of healthy wood and application of copper fungicides on prune cuts (Ponmurugan *et al*., 2002). The application of fungicides is mostly toxic and pollutes the atmosphere by spreading out in the air and accumulating in the soil. Random and extreme use of chemical fungicides for seed and soil management has led to the increase of pathogen resistance (Daghman *et al*., 2006). Using Biocontrol Agents (BCAs) could be an alternative to chemicals in the

management of fungal diseases. Several commercial BCAs including both bacteria and fungi have been registered and are available as commercial products for control of various diseases (Punja and Ukhtede, 2003).

According to Daami-Remadi *et al.* (2006), several soil borne plant pathogens, such as *Rhizoctonia solani, Sclerotium sclerotiorum, Pythium* spp., *Stereum purpureum, Botrytis cinerea, Phomopsis viticola* and *Fusarium* spp. are being successfully controlled by *Trichoderma* spp. Diverse mechanisms have been recommended for their biocontrol activity which comprises of antagonism for space and nutrients, secretion of enzymes, mycoparasitism and production of antimicrobial compounds. According to Jegathambigai *et al.* (2009), indigenous *Trichoderma* spp. has shown more effective, enhanced adaptability and antagonist activity in controlling the phytopathogen. Kucuk and Kivan (2004) had demonstrated the involvement of volatile metabolites secreted by *Trichoderma harzianum* in the inhibition of *G. graminis*, *F. culmorum* and *F. moniliforme*.

A biological agent, in addition to being competent, must also be adaptable to modern crop protection practices, including use of fungicides. Hence it has become essential to verify the compatibility of the bioagent with chemical fungicides. *Trichoderma* spp. has an intrinsic or stimulated resistance to many fungicides; however, the level of tolerance may varies with the fungicide (Khan and Shahzad, 2007). With this concept the intention of this present study was to screen the efficient indigenous isolate with prominent antagonist activity against *P. theae* and to ensure its compatibility with various fungicides.

MATERIALS AND METHODS

Sites of soil samples collected and soil analysis: The rhizosphere soil samples were collected from thirty commercial tea planting estates covering six major districts of southern India viz., Valparai, Coonoor, Munnar, Vandiperiyar, Gudalore and Koppa (Table 1). From each estate, soil samples were collected randomly, air dried in shade, gently alleted, sifted with 2 mm mesh sieves and stored at 4°C for further studies. These soil samples were subjected to various edaphic factors analysis such as pH (digital pH meter-Elico), EC (digital electrical conductivity meter-Elico), organic carbon Dichromate oxidation method by Walkely and Black (1934), total nitrogen Micro-kjeldahl and molybdenum blue methods by Jackson (1973), available phosphorus Molybdenum blue methods by Jackson (1973) and exchangeable K Digital flame photometer-Elico by Gammon (1951). The environmental particulars of the tea plantations such as elevation, rainfall, temperature, relative humidity and sunshine were also gathered from the respective estate to correlate the population density of *Trichoderma* spp. with edaphic and environmental factors and to analysis the seasonal influences over the population density of *Trichoderma* spp.

Isolation and characteristic features of *Trichoderma* spp.: *Trichoderma* spp. was isolated from the above soil samples using *Trichoderma* Selective Media (TSM). After incubation, the fungal colonies were identified as *Trichoderma* spp. by morphological characterization traits (Ponmurugan and Deepa, 2010). Depending upon on radial growth measurements, antagonist activity and cultural characterization (Grondona *et al.*, 1997) six isolates from each agroclimatic zones were chosen for the present study. Isolated *Trichoderma* spp. was sent to Microbial Type Culture Collection Center (MTCC), Chandigarh, India for identification at species level and further identified as *Trichoderma atroviride* (MTCC 9461). The standard culture T MTCC (*T. atroviride* isolate - MTCC 2461) was also acquired from MTCC for comparison.

***In vitro* evaluation selected *T. atroviride* isolates:** Radial growth measurements and mycelial dry weight of *T. atroviride* isolates was studied using Potato Dextrose Agar (PDA) and Potato Dextrose Broth (PDB), respectively. Petri dishes (90 mm diameter) containing PDA were centrally inoculated with a 5 mm of agar plugs from 7 days old cultures of *T. atroviride* isolates to determine the radial growth measurement. Similarly, dry weight of the mycelium was determined by transferring 5 mm actively growing cultures into 100 mL of PDB and incubated at 25°C. The mycelial mats were harvested at different intervals and the dry weight of the mycelium was recorded. In order to study the growth habit of *T. atroviride* isolate in the tea ecosystem, tea plant extract agars and tea soil agar medium were prepared by the method outlined by Ponmurugan and Baby (2007a). Different parts of tea plants such as root, root-bark, root wood, stem bark, stem wood and leaf extracts were used for the assay. Soil agar medium was prepared separately for each district and the radial growth of the antagonist was measured.

Bioefficacy between indigenous *T. atroviride* and *P. theae* was studied by following the method of dual culture test (Huang and Hoes, 1976) and antibiosis of volatile (Fiddaman and Rossall, 1993) and non-volatile antifungal compounds (Dennis and Webster, 1971). The *P. theae* (IMI No.-384005) strain was obtained from Plant Pathology Division, UPASI Tea Research Institute, Valparai, India. For dual culture experiments mycelial disks (5 mm in diameter) of *P. theae* and *T. atroviride* were placed at diametrically opposite points on a petri dish containing PDA. After 48 h of incubation time, the percentage inhibition of the pathogen by the antagonist was determined. To study the antagonist effect of volatile metabolites produced by *T. atroviride* isolates, each antagonistic isolate was grown on a sterile cellophane disk lying on PDA for 48 h. The cellophane with the mycelium was removed in the same position in which the pathogen was made to grow. Radial growth of the pathogen was determined after 72 h and was compared with the control. In order to study the efficacy of non-volatile compounds, the bottom lids of two PDA petri plates were inoculated with mycelial discs of *T. atroviride* isolates and *P. theae*, separately. The two lids were then reversed, placing one above the other and sealed air-tight through parafilm. After 96 h, the colony diameter of *P. theae* was measured.

The sensitivity of the different *T. atroviride* isolates to various contact and systemic fungicides was evaluated by food poisoned technique (Adams and Wong, 1991). The contact fungicide such as Blitox (Copper Oxychloride), Kocide (copper hydroxide), Mancozeb (Dithane M-45) and Bordeaux mixture and systemic fungicide such as Bavistin (carbendazim), Contaf (hexaconzole), Calixin (tridemorph) and Baycor (bitertanol) were tested against *T. atroviride* isolates.

Statistical analysis: The data obtained were subjected to Analysis Of Variance (ANOVA) and the significant means were segregated by Critical Difference (CD) at various levels and Standard Error (SE) was also calculated (Gomez and Gomez, 1984).

RESULTS

Population density, morphological and physiological features of *Trichoderma* spp.: The results of the edaphic factors showed that the rhizosphere soils taken from different tea plantations were acidic in nature (Table 1). Values of pH and EC were almost similar to all the regions were *Trichoderma* spp. were isolated. Organic carbon, phosphorus and potassium levels continued to be higher in Valparai when compared with other districts and showed significant difference when compared with Vandiperiyar, Gudalur and Koppa regions. The population density of *Trichoderma* spp. enumerated from the six districts clearly showed that the Valparai region has the highest count of 14.30×10^{-3} cfu g^{-1} soil dry wt when compared with other regions. No significant

difference was observed for population density of *Trichoderma* spp. within the districts, however, the significant difference was observed between the Valparai and Koppa regions. The correlation coefficients of edaphic and environment factors which influence the population density of *Trichoderma* spp. are presented in Table 2. Positive correlation was observed among the environmental factors such as rainfall, temperature minimum, Relative humidity and sunshine, with the population density of *Trichoderma* spp. Correlation of population density with potassium gave positive results ($r = 0.910$, $p<0.01$). Other parameters such as available phosphorus and total Nitrogen content also gave positive results with population density by the correlation values of $r = 0.686, 0.602$ at $p<0.05$, respectively. The above results justify that edaphic and environmental factors were favorable for the growth of *Trichoderma* spp. This might be the reason for increased population density at Valparai regions than compared to other districts. However, negative correlation was observed for minimum temperature ($r = -0.372$, $p<0.05$).

Table 1: Effect of edaphic and environmental parameters on the population density of *Trichoderma* spp. in Southern Indian tea plantations

		Edaphic parameters						Environmental parameters[y]						
										Temp (°C)		RH%		
Districts	Isolates[x]	pH	EC ds m^{-1}	OC (%)	N (ppm)	P (ppm)	K (ppm)	Rainfall (mm)	Sunshine (h day^{-1})	Max	Min	Max	Min	Population density[z]
Valparai (1193)	Tv1	4.93^{a}	0.25^{a}	3.38^{a}	3346.3^{a}	16.60^{a}	74.70^{a}	300.3^{a}	4.28^{b}	25.3^{b}	13.7^{a}	89.60^{a}	79.28^{a}	14.30^{a}
Coonoor (1502)	Tc3	4.97^{a}	0.18^{a}	3.20^{a}	2857.3^{ab}	15.47^{ab}	53.72^{cd}	190.2^{b}	5.71^{a}	21.0^{b}	13.3^{a}	80.44^{b}	76.07^{a}	12.33^{ab}
Munnar (2300)	Tm3	4.67^{b}	0.25^{a}	3.28^{a}	3548.3^{a}	16.30^{a}	62.57^{b}	286.8^{ab}	3.12^{b}	23.7^{b}	14.0^{a}	87.18^{a}	76.27^{a}	13.33^{ab}
Vandiperiyar (836)	Tvan4	4.60^{c}	0.20^{a}	2.72^{b}	2790.6^{ab}	12.27^{bc}	50.07^{d}	151.7^{c}	4.36^{b}	25.0^{ab}	13.7^{a}	85.32^{ab}	78.88^{a}	12.33^{ab}
Gudalur (1072)	Tg2	4.63^{c}	0.18^{a}	3.24^{a}	2275.3^{b}	11.07^{c}	43.53^{d}	161.3^{c}	4.30^{b}	24.3^{b}	13.3^{a}	85.75^{ab}	75.89^{a}	12.67^{ab}
Koppa (530)	Tk2	4.70^{b}	0.23^{a}	3.28^{a}	2828.0^{ab}	12.23^{bc}	43.53^{d}	265.0^{ab}	5.00^{ab}	32.3^{a}	13.7^{a}	84.98^{a}	61.56^{b}	11.67^{b}

Values in the parentheses indicate the elevation of particular place in meter above mean sea level. [x] Isolates were designated based on name of the place; T: represents *Trichoderma*; Number denotes field number of the tea estate. OC: Total Organic Carbon (%); N: Total Nitrogen content (ppm); P: Available Phosphorus (ppm); K: Exchangeable Potassium (ppm); Temp: Temperature (°C), RH: Relative humidity (%); Sun shine-Mean Sunshine period (h day^{-1}); [z] Population density of *Trichoderma* spp. (cfu x 10^{-3} g/ soil dry wt); [y]Average values for four years (2006-2010). Means within a column followed by the same superscript letter(s) are not significant

Table 2: Correlation coefficient of edaphic and environmental parameters on the population density of *Trichoderma* spp. in Valparai tea plantations

	Edaphic parameters						Environmental parameters[c]						
Parameters	pH	EC (dS m^{-1})	OC (%)	N (ppm)	P (ppm)	v (ppm)	Rainfall (mm)	Temp Max (°C)	Temp Min (°C)	Sunshine (h day^{-1})	RH Max (%)	RH Min (%)	Population density[b]
pH	-												
EC (dS m^{-1})	0.058												
OC (%)	0.478	0.419											
N(ppm)	0.318	0.843*	0.298										
P (ppm)	0.683	0.564	0.433	0.873**									
K (ppm)	0.555	0.657	0.359	0.818*	0.887**								
Rainfall (mm)	0.460	0.809*	0.704	0.861**	0.810*	0.666							
Temp max (°C)	0.591	0.245	-0.033	0.617	0.803*	0.853**	0.328						
Temp min (°C)	-0.334	0.387	0.120	-0.081	-0.418	-0.324	0.099	-0.656					
Sunshine (h day^{-1})	-0.296	0.867**	-0.001	0.787*	0.389	0.439	0.582	0.175	0.317				
RH max (%)	-0.125	0.751*	0.300	0.484	0.280	0.632	0.376	0.262	0.216	0.584			
RH min (%)	0.169	-0.125	-0.267	0.186	0.380	0.543	-0.161	0.786*	-0.828**	-0.064	0.251		
Pop. density[b]	0.363	0.544	0.406	0.602	0.686	0.910**	0.479	0.723	-0.372	0.307	0.775*	0.651	1.000*

*Correlation is significant at the 0.05 level (2 tailed). **Correlation is significant at the 0.01 level (2 tailed). OC: Total Organic Carbon (%); N: Total Nitrogen content (ppm): P: Available Phosphorus (ppm); K: Exchangeable Potassium (ppm); Temp: Temperature, RH: Relative humidity; Sun shine: Mean Sunshine period (h day^{-1}), [b]Population density of *Trichoderma* spp. (cfu x 10^{3} g/ soil dry wt). [c]Average values for four years (2006-2010)

Seasonal variations seemed to persuade the density of *Trichoderma* spp. population to a large extent (Fig. 1). The rainy season resulted in higher population, followed by the winter and summer seasons. The southwest monsoon is highly beneficial for Valparai, Munnar, Coonoor and Gudalur. The remaining regions were benefited from the northeast monsoon. In these areas *Trichoderma* spp. attains its maximum population density during the months of June to September and reaches lower level in the post monsoon season. Also, there was a drastic reduction in the population density in the winter, with the decline continuing in the summer.

Characteristic features of *T. atroviride* were described in Table 3. Individual variations were not observed between the isolates. The appearance of the colonies was similar with young whitish conidia with restricted concentric rings which turn to greenish with compact conidiophores throughout when it becomes older. Formations of chlamydospores were abundant within seven days and *T. atroviride* can be easily distinguished by the presence of coconut odor. The diffusing pigment in culture medium was not observed. None of the isolates studied showed any change in the pH of the medium with glucose as the carbon source. Growth and sporulation were seen on citric acid, lactic acid, urea and nitrite amended medium. However, there was no growth observed with ammonium oxalate as the carbon source. The isolates showed positive response at 37°C but were unable to grow over 4 and 40°C temperature which coincided with the nature of environmental factors. This clearly verifies that all isolates obtained from different agro climatic regions and selected for the present study belongs to similar species of *T. atroviride*.

Efficacy of *T. atroviride* isolates over *P. theae*: The results of radial growth and dry weight of mycelium of *T. atroviride* isolates are presented in Table 4. The mycelium of Tv1 isolate had covered the entire plate within five days of incubation. The radial growth measurement for isolates show vast divergence ranged and the lowest value ranged from 30 mm day^{-1} for T MTCC to the highest value of 38.20 mm for Tv1 isolates on 3rd of incubation. Significant difference was observed between Tv1 and other isolates at $p \leq 0.05$. However, there was no significant difference observed between Tc3, Tm3, Tvan4, Tg2 and Tk2 isolates. The percentage of dry weight of *T. atroviride* isolates between fifth and seventh days did not show much difference for all the isolates. The Tv1 isolate had a higher percentage of 95.02 and the standard culture obtained from MTCC showed around 85.32% in its dry weight. The above results illustrate that significant difference was not observed between Tv1, Tc3, Tm3 and Tg2 isolates but however increased in biomass for Tv1 isolates on 7th day of incubation showed significant difference between Tvan4, Tk2 and T MTCC isolates.

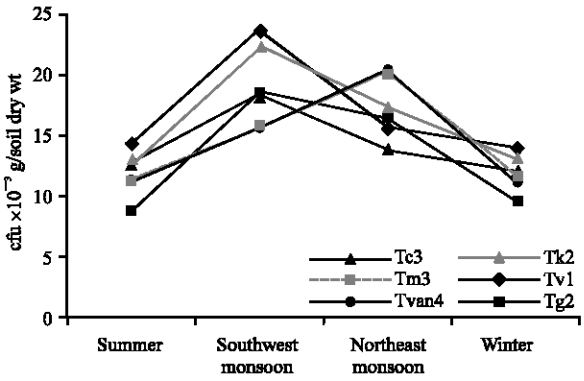

Fig. 1: Seasonal variation of population density of *Trichoderma* spp. in Southern Indian tea plantations

Table 3: Characteristic features of *T. atroviride* isolates

	Isolates of *T. atroviride*						
Features	Tv1	Tc3	Tm3	Tvan4	Tg2	Tk2	T MTCC *
Production of chlamydospores	+	+	+	+	+	+	+
Conidial diameter > 2 μm	+	+	+	+	+	+	+
Growth on glucose	+	+	+	+	+	+	+
Growth on citric acid	+	+	+	+	+	+	+
Sporulation on citric acid	+	+	+	+	+	+	+
Purple coloration on citric acid	+	+	+	+	+	+	+
Growth on lactic acid	+	+	+	+	+	+	+
Sporulation on lactic acid	+	+	+	+	+	+	+
Purple coloration on lactic acid	+	+	+	+	+	+	+
Growth on ammonium oxalate	-	-	-	-	-	-	-
Sporulation on ammonium oxalate	-	-	-	-	-	-	-
Purple coloration on ammonium oxalate	-	-	-	-	-	-	-
Growth on urea	+	+	+	+	+	+	+
Sporulation on creatine	+	+	+	+	+	+	+
Growth on crystal violet (0.01 g L^{-1})	-	-	-	-	-	-	-
Hydrolysis of gelatin	+	+	+	+	+	+	+
Yellow pigment on MEA	-	-	-	-	-	-	-
Aerial mycelium on Czapek-ammonium agar	+	+	+	+	+	+	+
Sporulation on Czapek-ammonium agar	+	+	+	+	+	+	+
Hydrolysis of gelatin	+	+	+	+	+	+	+
Sporulation on gelatin	+	+	+	+	+	+	+
Orange pigment on gelatin	+	+	+	+	+	+	+
Growth on nitrite agar	+	+	+	+	+	+	+
Sporulation on nitrite agar	+	+	+	+	+	+	+
Spore resistance to heating (75°C for 5 min)	-	-	-	-	-	-	-
4°C	-	-	-	-	-	-	-
37°C	+	+	+	+	+	+	+
40°C	-	-	-	-	-	-	-
pH 2	-	-	-	-	-	-	-
pH 7	+	+	+	+	+	+	+
pH 12	-	-	-	-	-	-	-
Coconut odour	+	+	+	+	+	+	+

* Standard culture used for comparison purpose, +: Presence, -: Absence

Table 4: Assessment of growth parameters of different isolates of *T. atroviride*

	Radial growth (mm)		Dry weight of mycelium (%)		
T. atroviride isolates	3rd day	5th day	3rd day	5th day	7th day
Tv1	38.20^{a}	45.00^{a}	86.83^{a}	94.76^{a}	95.02^{a}
Tc3	33.00^{b}	42.00^{b}	85.34^{ab}	91.73^{a}	92.67^{ab}
Tm3	32.40^{b}	40.00^{b}	83.69^{b}	91.97^{a}	92.79^{ab}
Tvan4	32.80^{b}	41.00^{b}	84.21^{b}	90.72^{ab}	91.27^{b}
Tg2	32.40^{b}	40.00^{b}	84.69^{b}	91.02^{a}	92.39^{ab}
Tk2	34.00^{b}	40.00^{b}	83.91^{b}	90.02^{b}	91.39^{b}
T MTCC *	30.00^{c}	37.00^{c}	79.62^{c}	84.34^{c}	85.32^{c}
SE ±	1.75	1.98	1.41	2.06	1.93

*Standard culture used for comparison purpose, Means within a column followed by the same superscript letter (s) are not significant; Each value is the mean of five replicates

The radial growth of *T. atroviride* isolate on media amended with various extracts of tea root, stem, leaf and soil was clearly specified in Table 5. Vast significant difference was observed between the isolates. Among the tea plant extract agar medium tested, tea root and stem extract agar supported the maximal growth for Tv1 isolate of 42.33 mm followed by the soil extract agar medium of 41.67 mm with five days of incubation. Tc3 isolate also showed better results of 40.33 mm radial growth in tea stem bark, tea stem wood and tea soil extract agar medium. Indigenous isolates obtained from the tea soil sample showed vast divergence in radial growth when compared with standard culture obtained from MTCC.

All the six indigenous *T. atroviride* isolates and T MTCC produced different percentages of inhibition on *P. theae*, ranging from 51.11 to 65.77% (Table 6) in dual culture test. Strain Tv1 showed the highest linear growth of 74.33 mm which was statistically significant when compared to all other isolates. Tk2 and T MTCC isolate showed the least measurement of 44.33 and 41 mm followed by Tg2 isolates. No significant difference was observed between Tvan4, Tg2 and Tk2 isolates. Hence the dual culture experiment clearly demonstrated that Tv1 *T. atroviride* isolates completely inhibited the growth of *P. theae* with colony degradation ranging from 7 to 9 days, it achieved this by visible penetration with the formation of small tufts thereby crumpling and distorting the pathogen hyphae.

The results of antagonists, Tv1 isolate registered higher antibiosis for non-volatile compounds of 91.25% of inhibition than other isolates which was followed by Tc3 and Tm3 of 81.08 and 76.25%, respectively (Table 6). The isolates studied here varied greatly in their abilities to produce antimicrobial compounds against *P. theae* and their capability to parasitize the pathogen. Tv1 isolate showed vast significant difference between other isolates but however no significant difference was observed between Tc3 and Tm3 isolates. Inhibition of radial growth of *P. theae* in the presence of volatile compounds ranged from 30.83 to 69.17%. The Tv1 isolate showed the highest percentage of inhibition which was statistically significant when compared to other isolates. T MTCC and Tg2 correspondingly showed least inhibitory result on *P. theae*, at 30.83 and 37.50%.

The average growth of *T. atroviride* isolates against different contact and systemic fungicides in various concentrations was described in Table 7. The response of *T. atroviride* isolates varied with the fungicides used. The Tv1 isolate showed better compatibility than compared to other isolates and it was able to tolerate the Blitox, Kocide and Bordeaux mixture to a large extent, when compared with Calixin, Carbendazim and Dithane M-45. The inhibitory effect of all fungicides on

Table 5: Effect of tea plant extract and tea soil extract agar medium on the radial growth of *T. atroviride*

	Radial growth on 5th day (mm)							
Media	Tv1	Tc3	Tm3	Tvan4	Tg2	Tk2	TMTCC *	SE ±
Tea root extract agar	42.33^{a}	39.67^{ab}	40.67^{ab}	39.00^{bc}	38.67^{bc}	37.33^{c}	29.33^{d}	0.75
Tea root-bark extract agar	40.00^{a}	38.67^{ab}	37.00^{bc}	37.33^{bc}	36.67^{bc}	35.00^{c}	31.33^{d}	0.94
Tea root wood extract agar	39.67^{a}	38.67^{a}	37.33^{ab}	35.67^{bc}	37.33^{bc}	36.00^{bc}	28.00^{d}	0.85
Tea stem bark extract agar	41.00^{a}	40.33^{a}	38.67^{b}	38.00^{b}	35.00^{c}	38.67^{b}	31.00^{d}	0.69
Tea stem wood extract agar	42.33^{a}	40.33^{b}	39.67^{bc}	38.67^{c}	38.33^{c}	36.33^{d}	29.67^{e}	0.56
Tea leaf extract agar	37.67^{a}	36.00^{a}	35.67^{a}	36.67^{a}	34.33^{ab}	32.00^{b}	27.33^{c}	1.22
Tea soil extract agar	41.67^{a}	40.33^{ab}	38.33^{bc}	35.33^{d}	36.00^{cd}	31.00^{e}	24.67^{f}	0.78
TSM **	45.00^{a}	41.00^{b}	40.00^{bc}	40.33^{bc}	39.00^{bc}	39.00^{bc}	37.00^{c}	2.56

*Standard culture used for comparison purpose. **Trichoderma selective mediaused for comparison purpose; Means within a row followed by the same superscript letter(s) are not significant; Each value is the mean of three replicates

Table 6: Hyperparasitism of different isolates of *T. atroviride* on the growth of *P. theae*

		Antibiosis[y]	
T. atroviride isolates×*P. theae*	Hyperparasitism x	Non-volatile compounds	Volatile compounds
Tv1	74.33[a] (65.77)	3.50[e] (91.25)	12.33[b] (69.17)
Tc3	66.33[b] (56.00)	7.57[b] (81.08)	22.33[ab] (44.17)
Tm3	54.33[c] (53.78)	10.40[ab] (76.25)	15.00[b] (62.50)
Tvan4	47.67[d] (52.00)	11.67[a] (74.00)	22.67[ab] (43.33)
Tg2	46.00[d] (52.89)	12.60[a] (70.83)	25.00[ab] (37.50)
Tk2	44.33[de] (52.44)	12.63[a] (68.43)	22.67[ab] (43.33)
T MTCC *	41.00[e] (51.11)	12.50[a] (68.75)	27.67 [a](30.83)
SE ±	2.47	2.44	4.87

* Standard culture used for comparison purpose, Values in parentheses indicate the percentage of inhibition of *P. theae* by *T. atroviride* isolates; [x] Linear growth of *T. atroviride* isolates on 5th day (mm); [y] Radial growth of *P. theae* on 5th day (mm); Means within a column followed by the same superscript letter (s) are not significant; Each value is the mean of three replicates

Table 7: Effect of various fungicides on the growth of *T. atroviride* isolates

	Radial growth of *T. atroviride* isolates on 5th day (mm)[y]							
Treatments	Tv1	Tc3	Tm3	Tvan4	Tg2	Tk2	T MTCC *	SE ±
Carbendazim	17.00[a]	15.55[ab]	14.89[ab]	14.11[bc]	13.00[cd]	12.11[d]	6.78[e]	0.83
Contaf	22.44[a]	19.56[b]	16.67[cd]	14.67[d]	12.22[ef]	11.78[f]	8.78[g]	0.84
Calixin	14.78[a]	12.00[bc]	11.11[cd]	9.67[d]	9.00[d]	8.67[de]	7.44[e]	0.53
Blitox [x]	23.44[a]	22.22[ab]	20.22[bc]	18.22[c]	15.89[de]	14.22[ef]	12.56[f]	0.69
Kocide[x]	31.11[a]	28.11[b]	24.66[c]	21.56[de]	21.44[de]	20.89[e]	17.11[f]	0.73
Dithane[x]M-45	16.89[a]	14.89[ab]	15.78[ab]	13.67[bc]	12.11[cd]	11.89[d]	10.45[d]	0.79
Bordeaux mixture	28.78[a]	26.56[b]	25.56[bc]	25.00[bc]	23.78[cd]	22.67[d]	19.44[e]	0.85
Baycor	23.32[a]	22.55[ab]	19.67[bc]	15.22[de]	13.89[e]	16.22[cd]	7.67[f]	1.03

* Standard culture used for comparison purpose; [x] Contact fungicides; other fungicides are systemic in nature. [y]Average values obtained for 10, 50 and 100 ppm concentration of different fungicides used; Means within a row followed by the same superscript letter (s) are not significant; Each value is the mean of three replicates

mycelium growth increases with an increase in the concentration. The significant difference was observed between indigenous and standard isolate used. However, the growth of the mycelium colony obtained when treated with fungicide was meager and scanty when compared with the control.

DISCUSSION

It is best to limit or avoid the use of chemicals in agriculture, particularly in plantation crops. The most capable way to accomplish this is to use of biocontrol agents. These not only control the phytopathogen but also could easily provide growth enhancement for crops. The biocontrol agent in crop protection and pest management will have the prospective to enhance crop yield quality and quantity (Oyekanmi *et al*., 2008).

Soil biodiversity plays a key role in the sustainability of agriculture systems and indicates the level of health of the soil. This is especially so when we consider the richness of microorganisms that is involved in biological control of soilborne diseases (Gil *et al*., 2009). The present study clearly indicates that edaphic and environmental factors highly favor the *Trichoderma* spp. population obtained from tea soil samples. Tea soils are usually acidic due to the prolonged use of nitrogenous

fertilizers such as urea and ammonium sulphate to increase crop production (Nioh *et al*., 1995). Although, similarity was observed for pH and EC values, other essential nutrient showed variation between each agro climatic zones. Mineral nutrition is essential for growth and, within a narrower range, for stimulating fungal secondary metabolism (Duffy *et al*., 1997). High total Nitrogen availability increased sporulation, production of antifungal anthroquinone pigments and hyphal growth rate (Fargasova, 1992). This highly correlated with Valparai soil samples, where edaphic factors were favorable for *Trichoderma* spp. and this strain showed more antagonists when compared to other isolates. The population density of *Trichoderma* spp. isolated from soil samples during different seasons evidently shows that rainy season favors the growth of *Trichoderma* spp., whereas summer influences a reduction in its populations. This relates with the study done by Panda *et al*. (2009) which concluded that higher moisture content of the soil in the rainy season and higher soil temperature in the summer might be the reason for causing such fluctuation.

In the present study *T. atroviride* was selected to evaluate the performance against the Phomopsis canker disease. Various studies have reported that *T. atroviride* acts as a biocontrol agent for wide range of aerial and soilborne plant pathogens (Brunner *et al*., 2005). Jakubikova *et al*. (2006) have reported that *T. atroviride* was found to be effective against *Polymyxa betae*. According to Vinale *et al*. (2006) the ability of *T. harzianum* and *T. atroviride* to improve the growth of lettuce, tomato and pepper plants under field conditions was investigated. These findings evidently showed the unrivaled activity of *T. atroviride* against different pathogens which perhaps also acts as a successful biocontrol agent against *P. theae*. From recent findings carried out by Osman *et al*. (2011) revealed that *T. harzianum* was found efficient antagonist in controlling of root rot disease in soy bean caused by *R. solani*. Above studies goes along with the present research that indigenous *T. atroviride* isolates were highly effective in controlling stem canker diseases. Apart from enhance biocontrol activity *T. harzianum* was also found to be an efficient mobilize of organic phosphorous when compared to other fungi (Yadav *et al*., 2011). Hence, usage of *Trichoderma* spp. for biocontrol of *P. theae* in tea field will surely enhance the tea productivity also.

The main objective of the present study was to screen the efficient indigenous isolates for controlling Phomopsis canker diseases in tea plants. Out of 78 isolates obtained, six were chosen based on rapid growth rate, antagonist activity and morphological similarity for further studies. Subsequently it was identified as *T. atroviride* by MTCC. Present study, the Tv1 isolate obtained from tea soil sample of Valparai district proved to be remarkably competent in growth rate and biomass production than other indigenous isolates and standard culture. Additionally the capacity of this isolate to utilize the various tea plant extract medium clearly showed its ability to survive in tea soils and act as the best antagonist against tea phytopathogen *P. theae*. The antagonist activity of Tv1 isolate against *P. theae* in dual culture and antibiosis clearly shows its competent nature and proved to be a pre-eminent strain in controlling the Phomopsis canker disease. This highlights the finding of Ponmurugan and Baby (2007b) that *Trichoderma* spp. could act as an efficient biocontrol agents against root diseases and collar canker in tea plantations.

To check the compatibility of biocontrol agent with frequently used fungicides in tea plants by *in vitro* is quiet essential. This helps to ensure the survival of the antagonist in the soil after the application in tea fields. Hence, with this approach the compatibility of both systemic and contact fungicides tested gave positive results and proved the efficacy of Tv1 as the best antagonist to be applied in the field. This highly correlates with the work done by Bagwan (2010), which shows the capable nature of *Trichoderma* spp. in resisting to the fungicides used. According to

Ayed *et al.* (2007), various biological fungicides such as Biocont-T, Funga stop and Polyversum has proved to effectual in control of vascular wilt disease in potato caused by *F. oxysporum*.

CONCLUSION

The current study highlights with the findings that Tv1 *T. atroviride* isolated from Valparai tea soil samples showed higher efficiency in growth parameters and antagonist activities. Moreover, this isolate has proved to be a competent strain with common fungicides used in tea plantation. The present study endows researches with primary data. Further research is being conducted to analysis the integrated approach of this species as biocontrol agents along with common fungicides in the tea field against *P. theae*.

REFERENCES

Adams, P.B. and J.A.L. Wong, 1991. The effect of chemical pesticide on the infection of sclerotia of *Sclerotinia minor* by the biocontrol agent *Sporidesmium sclerotivorum*. Phytopathol., 81: 1340-1343.

Ayed, F., M. Daami-Remadi, H. Jabnoun-Khiareddine and M. El Mahjoub, 2007. *In vitro* and *in vivo* evaluation of some biofungicides for potato fusarium wilt biocontrol. Int. J. Agric. Res., 2: 282-288.

Bagwan, N.B., 2010. Evaluation of Trichoderma compatibility with fungicides, pesticides, organic cakes and botanicals for integrated management of soil borne diseases of soybean (Glycine max (L.) Merrill). Int. J. Plant Prot., 3: 206-209.

Brunner, K., S. Zeilinger, R. Ciliento, S.L. Woo, M. Lorito, C.P. Kubicek and R.L. Mach, 2005. Improvement of the fungal biocontrol agent *Trichoderma atroviride* to enhance both antagonism and induction of plant systemic disease resistance. Applied Environ. Microbiol., 7: 3959-3965.

Daami-Remadi, M., K. Hibar, H. Jabnoun-Khiareddine, F. Ayed and M. El Mahjoub, 2006. Effect of two *Trichoderma* species on severity of potato tuber dry rot caused by tunisian fusarium complex. Int. J. Agric. Res., 1: 432-441.

Daghman, I.M., M. Sariah, J., Kadir, M.A. Zainal Abidin and A.B. Rosenani, 2006. Dry preparation of *Trichoderma harzianum* for Controlling *Rhizoctonia damping*-off in *Brassica rapa*. Int. J. Agric. Res., 1: 590-596.

Dennis, C. and J. Webster, 1971. Antagonistic properties of species groups of *Trichoderma*: III. Hyphal interaction. Trans. Br. Mycol. Soc., 57: 363-369.

Duffy, B.K., B.H. Ownley and D.M. Weller, 1997. Soil chemical and physical properties associated with suppression of take all of wheat by *Trichoderma koningii*. Phytopathology, 87: 1118-1124.

Fargasova, A., 1992. The influence of various nitrogen sources on the growth, conidiation and pigmentation production of the brown mutant of *Trichoderma viridae* M-108. Biol., 47: 453-464.

Fiddaman, P.J. and S. Rossall, 1993. The production of antifungal volatile by *Bacillus subtilis*. J. Applied Bacteriol., 74: 119-126.

Gammon, N. Jr., 1951. Determination of total potassium and sodium in sandy soils by flame photometer. Soil Sci., 74: 211-214.

Gil, S.V., S. Pastor and G.J. March, 2009. Quantitative isolation of biocontrol agents *Trichoderma* spp, *Gliocladium* spp. and actinomycetes from soil with culture media. Microbiol. Res., 164: 196-205.

Gomez, K.A. and A.A. Gomez, 1984. Statistical Procedure for Agricultural Research. IRRI, Manila, Philippines, pp: 139-207.

Grondona, I., R. Hermosa, M. Tejada, M.D. Gomis and P. Mateos *et al*., 1997. Physiological and biochemical characterization of *Trichoderma harzianum*. A biological control agent against soilborne fungal plant pathogens. Applied Environ. Microbiol., 63: 3189-3198.

Huang, H.C. and J.A. Hoes, 1976. Penetration and infection of *Sclerotinia sclerotiorum* by *Coniothyrium minitans*. Can. J. Bot., 54: 406-410.

Jackson, M.L., 1973. Soil Chemical Analysis. 1st Edn., Prentice Hall of India Pvt. Ltd., New Delhi, India.

Jakubikova, L., V. Farkas, N. Kolarova and M. Nemcovic, 2006. Conidiation of *Trichoderma atroviride* isolate during submerged cultivation in a laboratory stirred tank fermentor. Folia Microbiol., 51: 209-213.

Jegathambigai, V., R.S.W. Wijeratnam and R.L.C. Wijesundera, 2009. Control of *Fusarium oxysporum* wilts disease of *Crossandra infundibuliformis* var. Danica by *Trichoderma viride* and *Trichoderma harzianum*. Asian J. Plant Pathol., 3: 50-60.

Khan, M.O. and S. Shahzad, 2007. Screening of *Trichoderma* species for tolerance to fungicides. Pak. J. Bot., 39: 945-951.

Kucuk, C. and M. Kivan, 2004. *In vitro* antifungal activity of strains of *Trichoderma harzianum*. Turk. J. Biol., 28: 111-115.

Nioh, I., M. Osada, T. Yamamura and K. Maramatsu, 1995. Acidophilus and acid tolerant actionomycetes in acid tea field soil. J. Gen. Appl. Microbiol., 41: 175-180.

Osman, M.E.H., M.M. El-Sheekh, M.A. Metwally, A.E.A. Ismail and M.M. Ismail, 2011. Antagonistic activity of some fungi and *Cyanobacteria* species against *Rhizoctonia solani*. Int. J. Plant Pathol., 2: 101-114.

Oyekanmi, E.O., D.L. Coyne and B. Fawole, 2008. Utilization of the potentials of selected microorganisms as biocontrol and biofertilizer for enhanced crop improvement. J. Boil. Sci., 8: 746-752.

Panda, T., B. Panda, B.K. Prasad and N. Mishra, 2009. Influence of soil environment and surface vegetation on soil micro flora in a coastal sandy belt of orissa, India. Hum. Ecol., 27: 69-73.

Ponmurugan, P. and M.A. Deepa, 2010. Implications of *Trichoderma* spp. as a biocontrol agent in plant disease management. Scitech Publication, Chennai, Pages: 136.

Ponmurugan, P. and U.I. Baby, 2007a. Evaluation of fungicides and biocontrol agents against *Phomopsis canker* of tea under field conditions. Australasian J. Plant Pathol., 36: 68-72.

Ponmurugan, P. and U.I. Baby, 2007b. Utilization of different nutrient sources by *Phomopsis theae* and their influence on its growth and reproduction. J. Mycol. Pl. Pathol., 37: 499-504.

Ponmurugan, P. and U.I. Baby, 2008. Estimation of different cellular compounds from the mycelium of *Phomopsis theae*, causal agent of Phomopsis canker disease of tea. J. Phytopathol., 156: 358-361.

Ponmurugan, P., U.I. Baby, R. Premkumar and B. Radhakrishnan, 2002. Integrated control of Phomopsis canker disease of tea. Proceeding of the Placrosym XV, Dec. 10-13, Mysore, pp: 95-95.

Punja, Z.K. and R.S. Ukhtede, 2003. Using fungi and yeasts to manage vegetable crop diseases. Trends Biotechnol., 21: 400-407.

Thomas, J., A.K.A. Mandal, R. Raj Kumar and A. Chordia, 2009. Role of biologically active amino acid formulations on quality and crop productivity of tea (*Camellia* sp.). Int. J. Agric. Res., 4: 228-236.

Vinale, F., G. Ambrosio, K. Abadi, F. Scala, R. Marra, D. Turra, S.L. Woo and M. Lorito, 2006. Application of *Trichoderma harzianum* (T22) *Trichoderma atroviride* (P1) as plant growth promoters and their compatibility with copper oxychloride. J. Zhejiang Univ., 30: 425-425.

Walkely, A. and I.A. Black, 1934. An examination of Degtjareff method for determining soil organic matter and a proposed modification of the chromic acid titration method. Soil Sci., 37: 29-38.

Yadav, J., J.P. Verma and K.N. Tiwari, 2011. Plant growth promoting activities of fungi and their effect on chickpea plant growth. Asian J. Biol. Sci., 4: 291-299.