Selection of Potential Antagonistic Bacillus and Trichoderma Isolates from Tomato Rhizospheric Soil Against *Fusarium oxysporum* f.sp. *lycoperscisi*

[1]D. Praveen Kumar, [2]R. Thenmozhi, [3] P.D. Anupama, [4]A. Nagasathya,
[5]N. Thajuddin and [6]A. Paneerselvam
[1, 2]P.G. and Research Department of Microbiology, J.J College of Arts and Science,
Namunasamudram, Sivapuram, Pudukkottai, Tamil Nadu, India
[3]Department of Energy and Environmental Biotechnology, TERI, New Delhi, India
[4]Department of Zoology, Govt. Arts College for Women, Pudukkottai, Tamil Nadu, India
[5]Department of Microbiology, Bharathidasan University, 24 Tiruchirapalli, Tamil Nadu, India
[6]P.G. and Research Department of Botany, Microbiology,
A.V.V.M. Sri Pushpam College, Poondi, Tamil Nadu, India

Abstract: The aim of the investigation was to obtain a potential indigenous biocontrol agent against *Fusarium oxysporum* f.sp. *lycopersici* on tomato from different agroclimatic zones in India. Wide collection of Trichoderma and Bacillus was conducted from tomato rhizospheric soil in IIVR farm (Varanasi), IIHR farm (Bangalore), IARI farm (Rajendra Nagar) and farm of APHU (T.P. Gudem) by using Trichoderma Specific Media (TSM) and Nutrient Agar (NA). As a result, total 65 strains were cultured and maintained in which 28 were Bacillus and 37 were Trichoderma, respectively. On the basis of *in vitro* bioassays viz., dual culture and poisoned food technique it was concluded that 3 most prominent Trichoderma isolates viz., DPNST-4, -8 and -29 and four most prominent of DPNSB-2, -11, -18 and -28 from Bacillus were selected.

Key words: Tomato wilt, Bacillus, Trichoderma, tomato rhizosphear, screening of antagonistic, India

INTRODUCTION

Fusarium wilt is an important disease of tomato in many warm regions in the world, caused by the fungus *Fusarium oxysporum* f.sp. *lycopersici* which is highly destructive both in greenhouses and field grown tomatoes destroying up to 10-50 % of yield (Kallo, 1991; Mao *et al.*, 1998). It is a very serious destructive disease of tomato crop occurring worldwide and poses a major threat in successful cultivation of tomatoes (Jones *et al.*, 1991).

Owing to which no fungicidal soil treatment at field scale is feasible. Therefore, there is a demand for new methods to supplement existing disease control strategies and to achieve better disease control. Moreover, alternatives to many of the synthetic pesticides currently in use are needed because they may lose their usefulness; due to revised safety regulations (Duke *et al.*, 1993) concern over non-target effects (Dernoeden and McIntosh, 1991; Elmholt, 1991) due to the development of resistance in pathogen populations (Russell, 1995). Hence, researcher have arrive at new solution for treating plant disease problems that provide effective control,

having minimal negative consequences for human health and upon the environment (Cook *et al.*,1996). The abuse of chemical pesticides or fungicides to cure or prevent plant diseases has caused huge soil pollution and detrimental effects in humans. It is desirable to replace chemical pesticides with substrates that possess the following three criteria: high specificity against the targeted plant pathogens; easy degradability after effective usage and low mass production cost (Strange, 1993).

Thus, it leads in alternative to control this disease such as biocontrol (De Boer *et al.*,1999; Shanmugam and Kanoujia, 2011).

Search for environmentally and toxicologically safe more selective and efficacious fungicides for the control of plant diseases is gaining momentum at present. In the light of the mentioned facts, present investigation was take up to examine the efficacy of native Bacillus and Trichoderma propagules against *Fusarium oxysporum* f.sp. *lycopersici* under *in vitro* conditions individually. To achieve this target following objectives were planned: Isolation of Trichoderma and Bacillus isolates from wilt suppressed tomato field, screening of potential isolates

Corresponding Author: D. Praveen Kumar, P.G. and Research Department of Microbiology, J.J. College of Arts and Science,
Namunasamudram, Sivapuram, Pudukkottai, 622422 Tamil Nadu, India

under *in vitro* conditions by using dual-culture assay and poisoned food assay and selection of prominent isolates based on the observations of both assays.

MATERIALS AND METHODS

Soil and sampling: Total 16 rhizospheric soil samples were collected from four sites of tomato fields which are located in different geographical regions of India viz. Farm of Indian Institute of Vegetable Research Varanasi, Uttar Pradesh, 82.52°E longitude and 25.10°N latitude. Farm of Indian Institute of Horticultural Research Bangalore, Karnataka, 78°E longitude and 13.58°N latitude. Farm of Indian Agricultural Research Institute, New Delhi,770°E longitudes and 28.38°N latitude and Farm of Andhra Pradesh Horticultural University, Tadepalligudem, West Godavari district, Andhra Pradesh, 16°E longitudes and 81.300°N latitude. Four composite soil samples were collected from each site and were marked accordingly.

Fusarium oxysporum **f.sp.** *lycoperscisi* **and their pathogenicity:** The isolate was obtained from Indian Type Culture Collection with accession No. of ITCC-0223. For pathogenicity assay inoculum of *Fusarium oxysporum* f.sp. *lycoperscisi* was inoculated in 100 mL potato-dextrose broth followed by shake culture at 25°C for 3-4 days for microspore production. After incubation, microspore suspension filtered through autoclaved musclin cloth and final concentrations were adjusted to 2×10^7 spores mL. Tomato wilt resistant varieties P48024, P48025, P28026 and P48027, Bejo Zadeb b.v., Netherlands were used. Firstly, the roots of 20-30 days old tomato seedlings were steam-sterilized in greenhouse soil were carefully uprooted and freed from soil by immersion in distilled water. They were dipped into microspore suspension for 10-30 min and then replanted to sterilized soil contained pots (9 cm in diameter). Inoculated plants were maintained at incubation chamber at 22-25°C. After 3 weeks of incubation, they were uprooted and the lower stem and tap root were longitudinally sectioned for examination of internal tissues. Each plant was rated on a scale of 0-4 as follows: 0 = healthy plants; 1 = <25% vascular discoloration; 2 = 26-50% vascular discoloration; 3 = wilting with 51-75% vascular discoloration and 4 = 76-100% vascular discoloration or death (Jarvis and Thorpe, 1976; Sato and Araki, 1974; Sonoda, 1976; Yamamoto *et al.*, 1974, Thippeswamy *et al.*, 2010).

Isolation of biocontrol agents and determination of colony forming units: About 1 g of each soil sample was taken and added to 10 mL of Sterilized Distilled Water (SDW) to make a dilution of 10^{-1}. About 1 mL of suspension was subjected to serial dilutions up to 10^{-8} by using 9 mL of SDW. From the dilutions viz., 10^{-3}-10^{-6} was later poured on to Trichoderma Specific Medium (TSM) contained in petri plates and spread uniformly by spread plate method (Thatcher and Clark, 1968). The petri plates were then incubated at 24±2°C for isolation of *Trichoderma* sp. up to 7 days. The single colonies were picked and maintained in PDA, the isolates were stored in 10% glycerol at -70°C. Pure colonies were confirmed as *Trichoderma* sp., according to the identification key based on the branching of conidiophores, shape of the phialides, emergence of phialophores and phialospores (Rifai, 1969). For isolation of *Bacillus* sp., heat treatment was used to bias this selection towards spore-forming bacteria (Walker *et al.*, 1998).

About 1 mL of each dilutions from 10^{-2}-10^{-4} was heat treated in water bath at 80°C for 10 min and of the suspension was spread on nutrient agar medium plates enriched with 1% dextrose. The plates were incubated at 28±2°C for 24-48 h. The colonies that appeared on the media were streaked on to fresh plates of the same media to obtain pure colonies. The isolates were stored in nutrient broth with 15% glycerol at -70°C. The Bacillus isolates were confirmed based on the instructions of Berger's manual.

Screening of isolates for antagonistic activity towards *Fusarium oxysporum* **f.sp.** *lycoperscisi* **via dual-culture assay:** The initial screening under *in vitro* for antagonism against pathogen was performed according to the method described by Morton and Stroube (1955). A pathogenic fungal disc of five day old mycelia of 5 mm diameter was inoculated at centre of the petriplate (90 mm) containing Czapek-dox agar for Bacillus and end of the petriplate containing PDA for Trichoderma screening to facilitate the growth. Inoculate same size of 5 days old Trichoderma mycelial disc placed opposite side of pathogen as well streak a loop full of 24 h old Bacillus culture suspension into four corners, approximately 3 cm from the edge of the mycelial disc. The plates were incubated at 28°C and observed after every 24 h. The termination of experiment was done up to the full growth pathogen obtained in control plate. The percentage of Inhibition calculated as described by Edington *et al.* (1971):

$$L = [(C-T)/C] \times 100$$

Where:
L = Inhibition of radial mycelial growth
C = Radial growth measurement of the pathogen in control
T = Radial growth of the pathogen in the presence of Trichoderma/Bacillus isolates

Screening of isolates for antagonistic activity towards *Fusarium oxysporum* **f.sp.** *lycoperscisi* **via poisoned food technique:** To determine the effect of the non-volatile metabolites on mycelia growth of pathogen poisoned food technique was used. For the production of non-volatiles, three discs of mycelial agar plugs (5 mm diameter) of Trichoderma isolates were inoculated in 100 mL sterilized Potato Dextrose Broth (PDB) in 250 mL conical flasks and incubated at $25\pm1\,^{\circ}C$ on a rotary shaker at 100 rpm for 14 days. The control conical flasks were inoculated with sterile PDA plugs, respectively. After incubation, the culture was filtered through Millipore filter for removing spores and then sterilized through 0.2 im pore biological membrane filter (FP30/0.2 CA-S, Schleeicher and Schuell MicroScience GmbH) for collecting non-volatile metabolites from Trichoderma isolates. For non-volatiles from Bacillus, a loopfull of fresh culture was inoculated in to 100 mL of Nutrient Broth (NB) in 250 mL conical flasks and incubated at $28\pm2\,^{\circ}C$ on a rotary shaker at 120 rpm for 3 days. After incubation, cenntrifuged it at 12000 rpm for 10 min, collect the transparent supernatant containing non-volatile metabolites. For poisoned food assay the liquid formed non volatile was added to molten PDA medium (at $40\pm5\,^{\circ}C$) to obtain a final concentration of 10% (v/v). The medium was poured in petri dishes at 20 mL per plate and inoculated with 5 mm mycelial plugs of the pathogens in the centre of the plates and incubated at $25\pm2\,^{\circ}C$ for 7 days or until the colony reached the plate edge in control plate (Dennis and Webster, 1971). Triplicates was maintained for each treatment and radial growth of the pathogen was recorded.

Statistical analysis: Data was statistically analyzed using one-way ANOVA (Analysis of Variance) by SPSS. Differences among treatments were determined using Duncan's Multiple Range Tests (DMRT) at a significant level of p = 0.05. Data are presented as means±Standard Errors (SE).

RESULTS AND DISCUSSION

Pathogenicity of pathogenic strain: The culture ITCC-0223 showed highest pathogenicity range from 3.8-4.0 on P48025 and P28026. In other cultivars showed pathogenicity was comparatively lesser. Accordingly, based on the pathogenicity observations, the isolate was identified as FOL race (Table 1). Yamamoto *et al.* (1974) proved that *F. oxysporum* isolated from wilting tomato plants in greenhouses was carried out to differentiate races and forma specialis of *F. oxysporum* by using four tomato cultivars.

Table 1: Pathogenicity range of *Fusarium oxysporum* f.sp. *lycopersici* on resistant tomato cultivars

	Disease index (Tomato cultivar)			
Isolates	1	2	3	4
F. oxysporum	(P48024)	(P48025)	(P28026)	(P48027)
f.sp. *lycopersici*	1.2	4.0	3.8	1.4

Isolation of microbes: Culture media is more efficient and useful tool than non-culturable methods for the isolation, quantification and functional study of soil microorganisms (Sorheim *et al.*, 1989; Tabacchioni *et al.*, 2000; Vieira and Nahas, 2005). Thus, selecting an adequate media is a highly necessary step which indicates the importance of the present study. Differences in microorganism development are based on nutritional aspects and on the inhibition of contamination (Tabacchioni *et al.*, 2000; Mohamed *et al.*, 2005). A total 65 isolates out of them 28 No. of Bacillus and 37 No. of Trichoderma were isolated from tomato rhizosphere soil of different locations in India viz., IIVR farm (Varanasi), IIHR Farm (Bangalore), IARI farm (Rajendra nagar) and farm of APHU (T.P. Gudem) by using Trichoderma Specific Media (TSM) and Nutrient Agar (NA). Out of 28 Bacillus isolates 7 were obtained from IIVR, 8 from IIHR farm, 5 from IARI farm and 8 from APHU farm, respectively. The Trichoderma population was quite higher is each site except IIHR farm, i.e., 13 from IIVR farm, 9 from IARI, APHU farm each and only 6 from IIHR farm. Plate dilution has proved to be a useful method, however it has some limitations: the methodology is slow and laborious and requires a large volume of material (Taylor *et al.*, 2002; Gamalero *et al.*, 2003). From the site of IARI farm most higher ratio of colonies were observed, i.e., 22 colonies from the range of 10^3-10^5 on Trichoderm Specific Media (TSM) and 25 colonies from the range of 10^2 and 10^3 on Nutrient Agar (NA), respectively (Table 2).

Screening of Trichoderma isolates through dual-culture assay: Under the experiment conducted between Trichoderma isolates with respect to pathogen, the growth inhibition of *Fusarium oxysporum* f.sp. *lycopercisi* observed after 5 days incubation (Fig. 1) revealed that IIVR isolate of DPNST-8 caused maximum growth inhibition of 84.8% followed by APHU isolate of DPNST-29 which exhibit 81.7%. Coiling of antagonistic hyphae around hyphae of Fusarium and lysis was studied (Elad *et al.*, 1980; Morshed, 1985; Padmodaya and Reddy, 1996; Kumar and Dubey, 2001). *Trichoderma viride* and *T. harzianum* were reported by several researchers as the best antagonists for growth inhibition of several soil and seed borne plant pathogens (Dubey, 2002, 2003; Poddar *et al.*, 2004). Other isolates in order of superiority

Table 2: Population of Bacillus and Trichoderma isolates obtained from different soil samples

			Population of isolates			
Sample sited	Soil character	Sample code	Bacillus (CFU mL^{-1})	No. of pure isolates	Trichoderma (CFU mL^{-1})	No. of pure isolates
IIVR farm (Varanasi)	Alluvial soil	IIVRF-S1	5×103	DPNSB-1	3×104	DPNST-1
		IIVRF-S2	4×104	DPNSB-2	5×104	DPNST-2
		IIVRF-S3	6×103	DPNSB-3	4×105	DPNST-3
		IIVRF-S4	4×103	DPNSB-4	6×104	DPNST-4
				DPNSB-5		DPNST-5
				DPNSB-6		DPNST-6
				DPNSB-7		DPNST-7
						DPNST-8
						DPNST-9
						DPNST-10
						DPNST-11
						DPNST-12
						DPNST-13
IIHR farm (Bangalore)	Loamy to clayey soils	IIHRF-S5	4×103	DPNSB-8	7×104	DPNST-14
		IIHRF-S6	5×104	DPNSB-9	5×105	DPNST-15
		IIHRF-S7	6×104	DPNSB-10	4×104	DPNST-16
		IIHRF-S8	5×103	DPNSB-11	5×105	DPNST-17
				DPNSB-12		DPNST-18
				DPNSB-13		DPNST-19
				DPNSB-14		
				DPNSB-15		
				DPNSB-16		
IARI farm (Rajendra Nagar)	Alluvial soil	IARIF-S9	6×103	DPNSB-17	8×104	DPNST-20
		IARIF-S10	7×102	DPNSB-18	4×103	DPNST-21
		IARIF-S11	8×103	DPNSB-19	5×104	DPNST-22
		IARIF-S12	4×103	DPNSB-20		DPNST-23
				DPNSB-21		DPNST-24
						DPNST-25
						DPNST-26
						DPNST-27
						DPNST-28
APHU farm (T.P. Gudem)	Red sandy to sandy loam	APHUF-S13	5×103	DPNSB-22	8×104	DPNST-29
		APHUF-S14	6×103	DPNSB-23	5×103	DPNST-30
		APHUF-S15	6×104	DPNSB-24	4×105	DPNST-31
		APHUF-S16	5×103	DPNSB-25	2×104	DPNST-32
				DPNSB-26		DPNST-33
				DPNSB-27		DPNST-34
				DPNSB-28		DPNST-35
						DPNST-36
						DPNST-37

IIVRF = Indian Institute of Vegetable Research Farm, IIHRF = Indian Institute of Horticulture Farm, IARIF = Indian Agricultural Research Institute Farm, APHUF = Andhra Pradesh 11 Horticultural University Farm
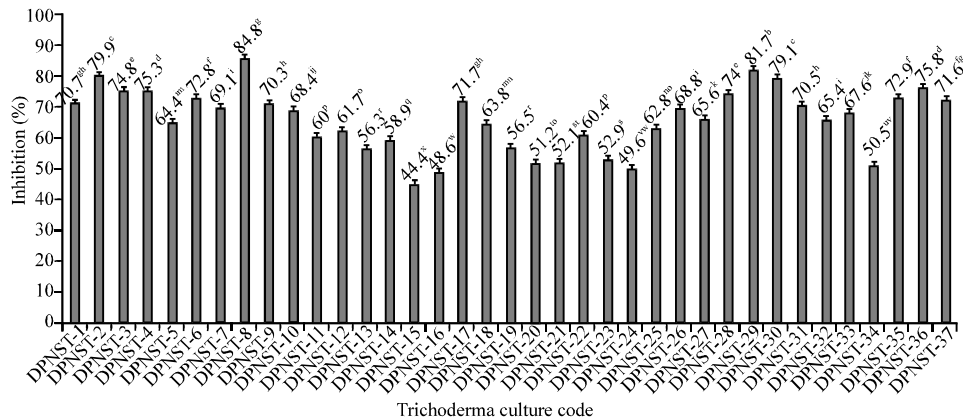

Fig. 1: Trichoderma isolates pecentage of inhibition against *Fusarium oxysporum* f.sp. *lycopercisi*
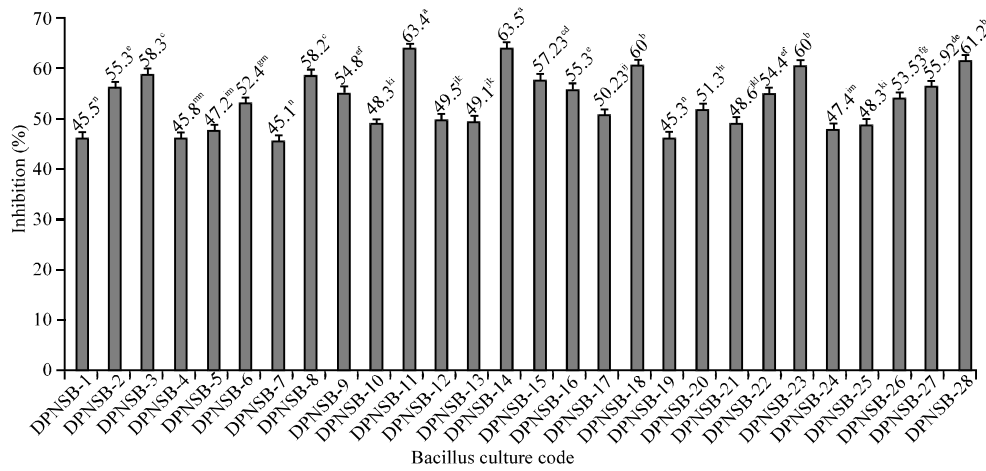
Fig. 2: Bacillus isolates percentage of inhibition against *Fusarium oxysporum* f.sp. *lycopercisi*

shows inhibition in the range of 70-80% were IIVR isolate of DPNST-1, -2, -3, -4, -6 and -9, IIHR isolate of DPNST-17, IARI isolate of DPNST-28, APHU isolate of DPNST-30, -31, -35, -36 and -37 with respect to mycelia growth inhibition were statistically near (Fig. 1). In the dual culture tests, hyphal contact between *Trichoderma* sp. and the wood decay fungi was observed for all host-pathogen combinations (Mark *et al.*, 2008). The remaining isolates showed inhibition from the inhibition range of 44.4-69.1, respectively. In this investigation out of 37 isolates three isolates viz., IIHR isolate DPNST-15, -16, IARI isolate DPNST-24 displayed minimum growth inhibition of <50% with respect to pathogenic fungi. Hajieghrari *et al.* (2008) evaluate that *in vitro* potential of six selected Iranian isolates of three species of Trichoderma (*Trichoderma hamatum* T614, *T. hamatum* T612, *Trichoderma harzianum* T447, *T. harzianum* T969, *Trichoderma virens* T523 and *Trichoderma* sp., T.) against five isolates of soil borne phytopathogenic fungi (*Fusarium graminearum, Rhizoctonia solani* (AG4 and AG5), *Macrophomina phaseoli* and *Phytophtora cacturum*) in dual culture techniques. However, the total no of potential isolates which shows the percentage of inhibition range >70 were obtained isolates of IIVR followed by APHU, IIHR and IARI. Sanchez *et al.* (2007) investigated on 79 Trichoderma strains were isolated from soil taken from 28 commercial plantations of *Agave tequilana* cv. Azul in the state of Jalisco, Mexico. Nine of these isolates completely inhibited the growth of *Thielaviopsis paradoxa* on potato dextrose agar plates. Observations of hyphal interaction indicated that antagonistic hyphae coiled around the hyphae of pathogen and killed them. Occasionally, Trichoderma hyphae formed hook or bunch like structures

around the hyphae of the pathogen from where penetration took place. Hyphae of antagonist either coiled around the hyphae of *Fusarium oxysporum* f.sp. *lycopercisi* before penetration or entered directly. The antagonistic mycelium of isolates of DPNST-8, -29 overgrew on the mycelium of pathogen whereas rest only checked its growth.

Screening of Bacillus isolates through dual-culture assay: The proliferation of the Bacillus isolates was observed for 6 days after inoculation. Several reports have described Bacillus strains as one of the potential biocontrol agents for plant diseases (Shoda, 2000). The biocontrol efficiency of *B. subtilis, B. cereus, B. amyloliquifaciens, B. licheniformis* and *B. pumilis* has been mentioned in various studies (Marten *et al.*, 2000; Siddiqui *et al.*, 2005; Li *et al.*, 2007). The isolates were able to parasitize the host fungi with different levels of growth inhibition depending upon the isolate. Of the 28 (DPNSB-1 to -28) Bacillus isolates evaluated, 5 isolates viz., DPNSB-11, -14, -18, -22 and -28 had a significant antifungal activity, i.e., inhibitory range from 60-63.5% against *Fusarium oxysporum* f.sp. *lycopercisi* (Fig. 2). Inhibition was clearly discerned by limited growth or the complete absence of fungal mycelium in the inhibition zones surrounding the streak of the isolates tested. Annouschka *et al.* (2003) studied the isolate *Pseudomonas fluorescens* WCS365 and *P. chlororaphis* PCL1391 were controlled foot and root rot on tomato which caused by *Fusarium oxysporum* f.sp. *radicis lycopersici*. Overall the total proportion of antagonistic bacteria isolated from four different site was clearly observed where from IIVR isolates of 7 showed the percentage of inhibition from 45.1-58.3 range followed by
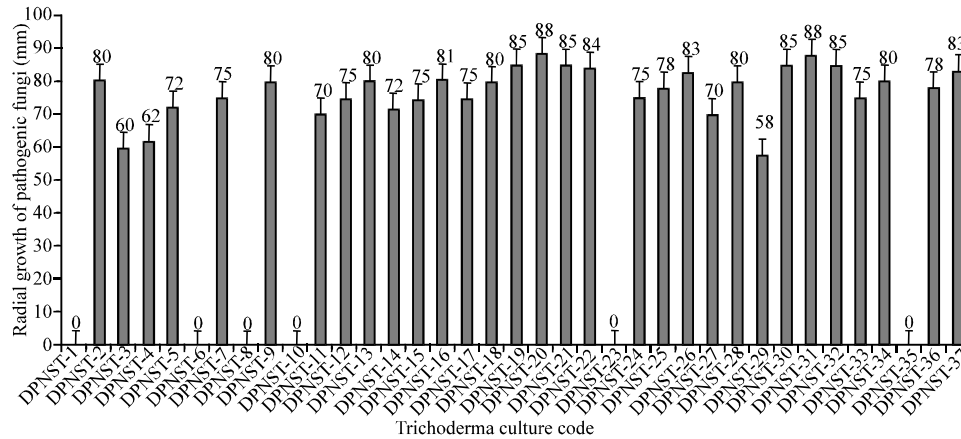
Fig. 3: Effect of non volatiles of Trichoderma isolates against *Fusarium oxysporum* f.sp. *lycopercisi*

9 isolates from IIHR the range was 48.3-63.5, 45.3-60% was maintain by 5 isolates of IARI with respect to APHU isolates; all the seven isolates sustain 47.4-61.2%. Recently in the year, 2011 Shanmugham V and Kanoujia V reported the biological management of vascular wilt of tomato caused by *Fusarium oxysporum* f.sp. *lycopercisi* by plant growth prooting rhizobacterial mixture. Maximum range of inhibition was found in IIHR isolates followed by APHU, IARI and IIVR. Nevertheless, the proportion of antagonists was clearly higher in all suppressive soils. The isolates viz., DPNSB-1, -7 and -19 showed <50% inhibition, these isolates statistically as par. *Bacillus subtilis* from cotton rhizosphere and their evaluation as biocontrol agent against *Fusarium oxysporum* (Archana *et al.*, 2010).

Screening of Trichoderm isolates through Poisoned food assay: The results of effect of non-volatile compounds revealed that among the different isolates of *Trichoderma* sp., evaluated against *Fusarium oxysporum* f.sp. *radicis-lycopersici*. Some attempts have been done in order to find a correlation between the structures of the Trichoderma secondary metabolites and the species that produce them (Ghisalberti and Sivasithamparam, 1991; Sivasithamparam and Ghisalberti, 1998; Christopher *et al.*, 2010). Secretions of IIVR isolates viz., DPNST-6, -1, -8 and -10 caused repression on mycelial growth of the pathogen followed by IARI isolate of DPNST-23, APHU isolate DPNST-35 express similar observation (Fig. 3).

The isolate of *T. virens* has been found effective and inhibited maximum growth of *F. solani* f.sp. *pisi* by the production of volatile compounds (Kumar and Dubey, 2001). The volatile compounds produced by *T. viride* proved inhibitory against *F. oxysporum* f.sp. *lycopersici* (Padmodaya and Reddy, 1996) and *Thanatephorus*

cucumeris (Dubey and Patel, 2001). The secretions of remaining isolates were allowed to grow pathogen superiorly (Fig. 3). Josie *et al.* (2003) examined the mycoparasitic and saprotrophic behavior of isolates representing groups of *Trichoderma harzianum* to establish a mechanism for the aggressiveness towards *Agaricus bisporus* in infested commercial compost. Few isolates viz., DPNST-3, -4 and -29 samples were allowed to grown up to the range of 60, 62 and 58 mm, respectively.

However, maximum samples allowed to grow >70 mm, i.e., similar to control. Vizcai-no *et al.* (2005) studied that Methanol extracts from 24 Trichoderma isolates, selected as biocontrol agents and representating different species and genotypes from three of the four taxonomic sections of this genus (*T. sect.* Trichoderma, *T. sect. Pachybasium* and *T. sect. Longibrachiatum*) were screened for antibacterial, anti-yeast and antifungal activities against a panel of seven bacteria, seven yeasts and six filamentous fungi previously used in similar studies.

Screening of Bacillus isolates through poisoned food assay: Among 28 isolates only five isolates viz., DPNSB-2, -11, -18, -23 and -28 was found to be effective against pathogenic mycelial growth inhibition (Fig. 4). Cho *et al.* (2003) detected and characterised an iturin A-like compound produced by *B. subtilis* KSO_3 which inhibits growth of the phytopathogenic fungus *Gloeosporium gloeosporioides* (Chitarra *et al.*, 2003) detected an antifungal compound produced by a strain of *B. subtilis* that permeabilises fungal spores and blocks germination of *Penicillium roqueforti*. Maximum growth inhibition of *Fusarium oxysporum* f.sp. *lycopercisi* was observed by these five isolates non-volatile metabolites, i.e., 0.0 mm. Remaining isolates did not show any type of growth inhibition. The antifungal activity of *B. subtilis* grown
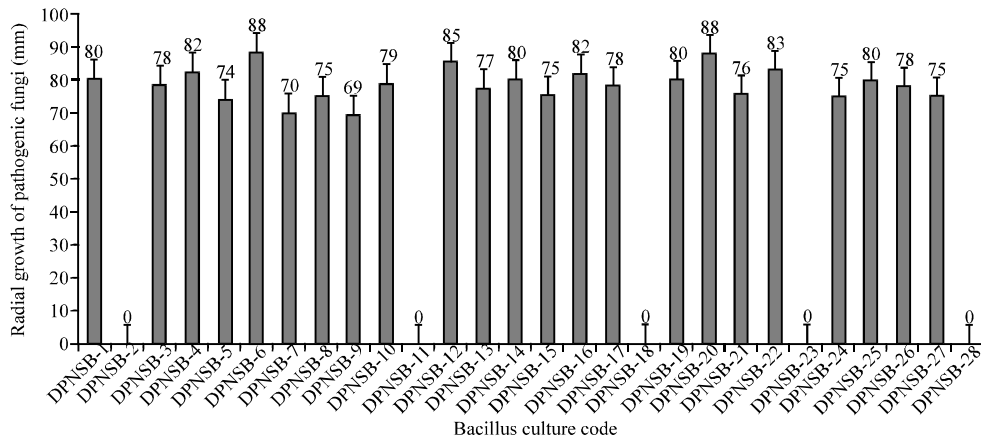
Fig. 4: Effect of non volatiles of Bacillus isolates against *Fusarium oxysporum* f.sp. *lycopercisi*

on five different media was evaluated towards phytopathogenic and wood surface contaminant fungi under the incubation of 11-14 days respectively. Out of the five isolates two were distributed from APHU farm isolate and remaining three from each site, i.e., farm of IIVR, farm of IIHR and farm of IARI.

Selection of potential Trichoderma and Bacillus isolates:
One of the most promising properties of *Bacillus* and *Trichoderma* sp., contributing to the biocontrol mechanism is the amazing battery of antibiotic compounds synthesized that exhibit a wide antimicrobial spectrum, the ability to modify attachment of other microorganisms to different surfaces and to contribute to the survival of the Bacillus cells in their habitat (Stein, 2005). The selection of prospective isolates was done based on the excellent criterion of the isolates those produce greatest inhibition zone under dual-culture assay, growth inhibition of pathogenic mycelia under poisoned food technique and those shows optimistic level of performance in both assays commonly. As per the surveyed literature and observations the optimistic levels were selected for both types of isolates under both experiments individually. In the case of Trichoderma dual culture assay optimistic level preferred as >75% and 0 mm for poisoned food technique as well for Bacillus the level is >55% followed by 0 mm, respectively. However, as per the optimistic levels out of 37 No. of Trichoderma isolates three isolates viz., DPNST-4, -8 and -29, among 28 No. of Bacillus isolates four isolates viz., DPNSB-2, -11, -18 and -28 were observed as most prominent as compare to remaining isolates for the reason that of their observations in both *in vitro* bioassays. As per the interpretation on dual-culture assay individually total six isolates viz., DPNST-2, -4, -8, -29, -30 and -36 from Trichoderma and eight isolates viz., DPNSB-2, -3, -8, -11, -14, -18, -23 and -28 were selected and chosen as lead strains from Bacillus. Followed by the observation of

poisoned food technique among the Trichoderma isolates, six viz., DPNST-1, -6, -8, -10, -23 and -35 exhibited promising antagosnistcs. In the case of Bacillus the prominent isolates viz., DPNSB-2, -11, -18, -23 and -28 were observed, respectively.

CONCLUSION

Non-volatile compounds produced by different Trichoderma and Bacillus isolates drastically reduced the mycelia growth of test pathogens which is helpful in disease reduction by checking the survival and spread by pathogen.

REFERENCES

Annouschka, B., L. Anastasia, H.M.W. Andre, E.M.L. Gerda, F.C. Thomas, A.W. Chin and J.L. Ben*et al.*, 2003. Interactions in the tomato rhizosphere of two Pseudomonas biocontrol strains with the phytopathogenic fungus *Fusarium oxysporum* f.sp. radicis-lycopercisi. Mol. Plant Microb Interactions, 16: 983-993.

Archana, G., R.R. Alok, U.M. Sudhir and A.B. Dongre, 2010. Isolation, evaluation and characterization of *Bacillus subtilis* from cotton rhizospheric soil with biocontrol activity against *Fusarium oxysporum*. World J. Microbiol Biotech., 26: 1187-1194.

Chitarra, G.S., P. Breeuwer, M.J. Nout, A.C. van Aelst, F.M. Rombouts and T. Abee, 2003. An antifungal compound produced by *Bacillus subtilis* YM 10-20 inhibits germination of *Penicillium roqueforti* conidiospores. J. Applied Microbiol., 94: 159-166.

Cho, S.J., S.K. Lee, B.J. Cha, Y. H. Kim and K. Shin, 2003. Detection and characterization of the *Gloeosporium gloeosporioides* growth inhibitory compound iturin A from *Bacillus subtilis* strain KS03. FEMS Microbiol. Lett., 223: 47-51.

Christopher, D.J., T.S. Raj, S.U. Rani, R. Udhayakumar, 2010.. Role of defense enzymes activity in tomato as induced by *Trichoderma virens* against *Fusarium* wilt caused by *Fusarium oxysporum* f sp. lycopersici. J. Biopesticides, 3: 158-162.

Cook, R.J., W.L. Bruckart, J.R. Coulson, M.S. Goettel and R.A. Humber *et al.*, 1996. Safety of microorganisms intended for pest and plant disease control: A framework for scientific evaluation. Biol. Control, 7: 333-351.

De Boer, M., I. van der Sluis, L.C. van Loon and P.A.H.M. Bakker, 1999. Combining fluorescent *Pseudomonas* spp. strains to enhance suppression of *Fusarium* wilt of radish. Eur. J. Plant. Pathol., 105: 201-210.

Dennis, C. and J. Webster, 1971. Antagonistic properties of species groups of *Trichoderma*: I. Production of non-volatile antibiotics. Trans. Br. Mycol. Soc., 57: 25-39.

Dernoeden, P.H. and M.S. McIntosh, 1991. Disease enhancement and *Kentucky bluegrass* quality as influenced by fungicides. Agron. J., 83: 322-326.

Dubey, S.C. and B. Patel, 2001. Evaluation of fungal antagonists against *Thanatephorus cucumeris* causing web blight of urd and mung bean. Ind. Phytopathol., 54: 206-209.

Dubey, S.C., 2002. Bioagent based integrated management of collar rot of French bean. Indian Phytopathol., 55: 230-231.

Dubey, S.C., 2003. Integrated management of web blight of urd and mungbean. Indian Phytopathol., 56: 413-417.

Duke, S.O., J.J. Menn and J.R. Plimmer, 1993. Challenges of Pest Control with Enhanced Toxicological and Environmental Safety, an overview. In: Pest Control with Enhanced Environmental Safety: ACS Symp. Ser., Duke, S.O., J.J. Menn and J.R. Plimmer (Eds.). Am. Chem. Soc., Washington, DC., pp: 1-13.

Edington, L.V., K.L. Khew and G.I. Barron, 1971. Fungitoxic spectrum of benzimidazole compounds. Phytopathology, 61: 42-44.

Elad, T., J. Chet and J. Katan, 1980. *Trichoderma harzianum* a biocontrol effective against Sclerotium rolfsii and *Rhizoctonia solani*. Phytopathol., 70: 119-121.

Elmholt, S., 1991. Side effects of propiconazole (Tilt 250 EC) on non-target soil fungi in a field trial compared with natural stress effects. Microb. Ecol., 22: 99-108.

Gamalero, E., G. Lingua, G. Berta and P. Lemanceau, 2003. Methods for studying root colonization by introduced beneficial bacteria. Agronomie, 23: 407-418.

Ghisalberti, E.L. and K. Sivasithamparam, 1991. Antifungal antibiotics produced by *Trichoderma* spp. Soil Biol. Biochem., 23: 1011-1020.

Hajieghrari, B., M. Torabi-Giglou, M.R. Mohammadi and M. Davari, 2008. Biological potential of some Iranian *Trichoderma* isolates in control of soil borne plant pathogenic fungi. Afr. J. Biotechnol., 7: 967-972.

Jarvis, W.R. and H.J. Thorpe, 1976. Susceptibility of Lycopersicon species and hybrids to the foot and root rot pathogen *Fusarium oxysporum*. Plant Dis. Rep., 60: 1027-1031.

Jones, J.B., J.P. Jones, R.E. Stall and T.A. Zitter, 1991. Compendium of Tomato Disease. 1st (Edn). The American Phytopathological Society, New Yark, Pp:100-100.

Josie, W., M.C. John, R.M. Peter and M.C. Richard, 2003. Saprotrophic and Mycoparasitic components of Aggressiveness of *Trichoderma harzianum* groups toward the Commercial Mushroom *Agaricus bisporus*. Applied Environ. Microbiol., 69: 4192-1499.

Kallo, G., 1991. Genetic Improvement of Tomato. Springer-Verlag Berlin Heidelberg, Germany, Pp: 99-99.

Kumar, D. and S.C. Dubey, 2001. Management of collar rot of pea by the integration of biological and chemical methods. Indian Phytopathol., 54: 62-66.

Li, D., F. Nie, L. Wei, B. Wei and Z. Chen, 2007. Screening of high-yielding biocontrol bacterium Bs-916 mutant by ion implantation. Appl Microbiol Biotechnol, 75: 1401-1408.

Mao, W., J.A. Lewis, R.D. Lumsden and K.P. Hebbar, 1998. Biocontrol of selected soil borne diseases of tomato and pepper plants. Crop Protection, 17: 535-542.

Mark, S., F. Siegfried and W.M.R. Francis, 2008. Evaluation of Trichoderma spp. as a biocontrol agent against wood decay fungi in urban trees. Biological Control, 45: 111-123.

Marten, P., K. Smella and G. Berge, 2000. Genotypic and phenotypic differentiation of an antifungal biocontrol strains belonging to *Bacillus subtilis*. J. Appl Microbiol, 89: 463-471.

Mohamed, M., L. Ranjard, C. Catroux, G. Catroux and A. Hartmann, 2005. Effect of natamycin on the enumeration, genetic structure and composition of bacterial community isolated from soils and soybean rhizosphere. J Microb. Meth, 60: 31-40.

Morshed, M.S., 1985. *In vitro* antagonism of diVerent species of *Trichoderma* on some seed borne fungi of bean (*Phasiolus vulgaris* L.). Bangladesh Jr. Botany, 14: 119-126.

Morton, D.T. and N.H. Stroube, 1955. Antagonistic and stimulatory effects of microorganism upon *Sclerotium rolfsii*. Phytopathol., 45: 419-420.

Padmodaya, B. and H.R. Reddy, 1996. Screening of *Trichoderma* spp. against *Fusarium oxysporum* f. sp. lycopersici causing wilt in tomato. J. Mycol. Plant Pathol., 26: 266-270.

Poddar, R.K., D.V. Singh and S.C. Dubey, 2004. Integrated application of *Trichoderma harzianum* mutants and carbendazim to manage chickpea wilt (*Fusarium oxysporum* f. sp. ciceri). Ind. J. Agric. Sci., 74: 346-348.

Rifai, M.A., 1969. A revision of the genus *Trichoderma*. Mycol. Pap. Commonwealth Mycol. Inst., Kew, Surrey, England, pp: 116.

Russell, P.E., 1995. Fungicide resistance: occurrence and management. J. Agric. Sci., 124: 317-323.

Sanchez, V., O. Rebolledo, R.M. Picaso, E. Cardenas, J. Cordova, O. Gonzalez and G.J. Samuels, 2007. *In vitro* antagonism of *Thielaviopsis paradoxa* by *Trichoderma longibrachiatum*. Mycopathologia, 163: 49-58.

Sato, R. and T. Araki, 1974. On the tomato root-rot disease occurring under vinyl-house conditions in southern Hokkaido. Ann. Rep. Soc. Plant Prot. North Jpn, 25: 5-13.

Shanmugam, V. and N. Kanoujia, 2011. Biological management of vascular wilt of tomato caused by *Fusarium oxysporum* f.sp. *lycospersici* by plant growth-promoting rhizobacterial mixture. Bio. Control, 57: 85-93.

Shoda, M., 2000. Bacterial control of plant diseases. J. Biosci. Bioeng, 89: 515-521.

Siddiqui, S., Z.A. Siddiqui and I. Ahmad, 2005. Evaluation of fluorescent pseudomonads and *Bacillus* isolates for the biocontrol of wilt disease complex of pigeonpea. World J. Microbiol. Biotechnol., 21: 729-732.

Sivasithamparam, K. and E.L. Ghisalberti, 1998. Secondary Metabolism in *Trichoderma* and *Gliocladium*. In: *Trichoderma* and Gliocladium. Basic Biology, Taxonomy and Genetics, Kubicek, C.P. and G.E. Harman (Eds.), Taylor and Francis, London, UK., pp: 139-191.

Sonoda, R.M., 1976. The occurrence of fusarium root rot of tomatoes in South Florida. Plant Dis. Rep., 60: 221-274.

Sorheim, R., V.L. Torsvik and J. Goksoyr, 1989. Phenotypical divergences between population of soil bacteria isolated on different media. Microb Ecol, 40: 169-176.

Stein, T., 2005. *Bacillus subtilis* antibiotics: Structures, syntheses and specific functions. Mol. Microbiol., 56: 845-857.

Strange, R.N., 1993. Plant Disease Control: Towards Environmentally Acceptable Methods. Chapman and Hall Publishing, New York, pp: 354.

Tabacchioni, S., L. Chiarini, A. Bevivino, C. Cantale and C. Dalmastri, 2000. Bias caused by using different isolation media for assessing the genetic diversity of a natural microbial population. Microb Ecol, 40: 169-176.

Taylor, J.P., B. Wilson, M.S. Mills and R.G. Burns, 2002. Comparison of microbial numbers and enzymatic activities in surface soils and subsoils using various techniques. Soil Biol. Biochem., 34: 387-401.

Thatcher, P.S. and D.S. Clark, 1968. Microorganisms in Foods: Their Significance and Methods of Enumeration. Vol. 1, University of Toronto Press, Toronto, ISBN: 0-8020-2293-6.

Thippeswamy, B., H.V. Sowmya and M. Krishnappa, 2010. Pathogenicity and management of early blight and wilt in tomato caused by *Alternaria solani* and *Fusarium oxysporum*. Asian J. Microb., Biotechnol. Environ. Sci., 12: 739-744.

Vieira, F.C.S. and E. Nahas, 2005. Comparison of microbial numbers in soils by using various culture media and temperatures. Microb Res., 160: 197-202.

Vizcai-no, J.A., L. Sanz, A. Basilio, F. Vicente, S. Gutierrez, M.R. Hermosa and E. Monte, 2005. Screening of antimicrobial activities in *Trichoderma* isolates representing three *Trichoderma* sections. Mycol. Res., 109: 1397-1406.

Walker, R., A.A. Powell and B. Seddon, 1998. *Bacillus* isolates from the spermosphere of peas and dwarf French beans with antifungal activity against *Botrytis cinerea* and *Pythium* species. J. Applied Microbiol., 84: 791-801.

Yamamoto, I., H. Komada, K. Kuniyasu, M. Satio and A. Ezuka, 1974. A new race of *Fusarium oxysporum* f. sp. lycopersici inducing root rot of tomato. Proc. Kansai Plant Prot. Soc., 16: 17-29.