JOURNAL OF PURE AND APPLIED MICROBIOLOGY, Sept. 2016. Vol. **10**(3), p. 2165-2179

Evaluation of Phosphate Solubilization, Hydrolytic Enzyme and Metabolite Production Ability of *Trichoderma* spp. Isolated from the Rhizospheric Soil of U.P.

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(Received: 09 December 2015; accepted: 20 January 2016)

Biocontrol agents are used as a alternative to chemical pesticides for the control of phytopathogens. *Trichoderma* species are well known biocontrol agents for a variety of soil borne pathogens. However, their efficient interaction with the host needs to be accompanied by production of secondary metabolites and cell wall degrading enzymes. Phosphate solubilizing microorganisms have a considerable positive impact on the growth and development of plants. In the present study ten *Trichoderma* species isolated from the various agro-climatic zones of Uttar Pradesh were tested for the phosphate solubilization, Volatile metabolite production and cell wall degrading enzyme production activity. All the species shows efficient production of all the tested parameters.

Keywords: *Trichoderma*, Phosphate, Volatile, Cell wall degrading Enzymes, Phytopathogens**.**

Soil borne pathogens have a broad host range and persist for longer periods in soil by resistant resting structures. Chemical control of soil borne pathogens provides certain degree of control but at the same time have adverse effects on environment affecting the beneficial soil microorganisms. Therefore, biological control of plant pathogens has been considered as a potential control strategy in recent years and search for these biological agents is increasing. *Trichoderma* is the most commonly used fungal biological control agent and have long been known as effective antagonists against plant pathogenic fungi (Harman, *et al* 2004 and Chet, *et al* 1981). In which, *Trichoderma harzianum* has been accepted as one of the most potent biocontrol agents against plant diseases and used as an antagonist against many soil-borne phytopathogenic fungi over the past few years (Samuels *et al*., 2004). Various strategies of biocontrol have been proposed. They include the creation of competition for nutrients or space, the production of antibiotics and lytic enzymes, the inactivation of the enzymes of phytopathogenic fungi, and parasitism. The cell wall-degrading enzymes (CWDEs), mostly chitinases, glucanases, and proteases, are major lytic enzymes that are secreted by biocontrol agents (Benitez T., 2004). CWDEs attack the cell wall of phytopathogenic fungi to cause cell lysis and subsequent death. Although the mechanism of mycoparasitism is not completely understood, this process has been assumed to involve the expression of extracellular CWDEs (Pandey S *et al* 2014).

Phosphorus is an important plant nutrient, playing a key role in the development and yield of crop plants. Phosphorus exists in nature in a variety of organic and inorganic forms. The majority of soils contain insoluble inorganic phosphates, which are of no use to plants unless they are solubilized. Soil contains organic

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phosphorus that can be used by plants only if it is mineralized. Phosphate solubilizing micro-organisms convert these insoluble phosphates into soluble form through the processes of acidification, chelation and exchange reaction (Earl *et al*. 1979, Starkanova *et al*. 1999, Narsian & Patel 2000, Reyes *et al*. 2002). A number of phosphate solubilizing bacteria, fungi and actinomycetes have been reported (Vassileva *et al*. 1998, Gaur 1990, Subba Rao 1999). The efficiency of phosphate solubilization depends on the phosphate source and the availability of different carbon and nitrogen sources. Altmore *et al* in 1999 demonstrate the phosphate solubilization capacity of *Trichoderma harzianum*. Oliveria *et al* 2012 confirmed the phosphate solubilization capacity of *Trichoderma.*

Trichoderma species have metabolite production capacity. *Trichoderma* species produce a wide range of volatile metabolites that have different effects on different fungi. The metabolites produced by *Trichoderma* species are harmful for the pathogens and have the ability to inhibit the pathogen growth. The potential use of the *Trichoderma* species as a biocontrol agent was suggested more than 70 years ago by Weindling, who was first to demonstrate the parasitic activity of member of this genus against soilborne fungal or bacterial pathogens.

Trichoderma spp. commonly live in association with plant roots. *T. harzianum* is a well known biocontrol agents responsible for the increase in plant growth rate and providing resistance against phytopathogenic microorganisms (Abdullah, F., *et al*., 2005). Due to these beneficial effects, some strains of *Trichoderma* such as *T. harzianum, T. atroviride* and *T. asperellum* are used as plant protection agents for the biocontrol of plant pathogens and plant growth promotion in agriculture.(Harman, G.E., *et al* 2004, Harman, G.E et 2006, Verma, M *et al* 2007). Reino *et al*. (Reino, J.L., *et al* 2008), found that *Trichoderma* spp. produce many volatile (e.g., pyrones, sesquiterpenes) and non-volatile secondary metabolites (e.g., peptabiols).Volatile secondary metabolites are responsible to play a key role in the mycoparasitic action (Vinale, F., *et al* 2008). Various volatiles metabolites play important signaling roles in the microbial kingdom. The 6-pentyl-a-pyrone (6-PAP) is a volatile product secondary metabolite of *Trichoderma*, is herbicidal and antimicrobial in action (Galindo, E., *et al* 2005). 1-octen-3-ol, 3-octanone, 3-octanol and 1-octen-3-one, all are are eight carbon volatiles metabolites which function as insect attractants and are fungi static and fungicidal in action (Combet, E., *et al* ., 2006, Chitarra, G.S., *et al* 2005– Okull, D.O., *et al* 2003, Siddiquee, S *et al* 2012**)**

The present work aimed to demonstrate the phosphate solubilization, metabolite and cell wall degrading enzyme production capacity of the *Trichoderma* species isolated from UP.

MATERIALS AND METHODS

Ten *Trichoderma* species used in this study were isolated from rhizosperic soils collected from the different locations of U.P.and maintained on potato dextrose agar (PDA) (Himedia, USA) at 28±2°C for 5 days. Ten *Trichoderma* species were isolated from rhizosphere samples according to soil dilution plate method described by Kucuk and Kivanc (2003) with some modification. One ml of each appropriate dilution (10^{-3} to 10^{-5}) was pipetted in petri dishes, then the sterilized and cooled at 45°C Rose Bengal Agar medium (RBA) was poured and left to solidified. All plates were incubated at 28°C for 7 days. The culture plates were examined daily and individual colonies were isolated and purified, then transferred to fresh potato dextrose Agar medium (PDA). Distinct morphological characteristics were observed for identification and the plates were stored at 4°C for further experiments.

Phosphate solubilization activity of *Trichoderma*

A modified phosphate-growth medium, the National Botanical Research Institute's phosphate growth medium (NBRIP), was used to detect the phosphate-solubilizing abilities of *Trichoderma* isolates . NBRIP (1 L) included 10 g Glucose , 0.5 g Ammonium sulfate, 0.2 g Sodium chloride, 0.1 g Magnesium sulfate, 0.2 g Potassium chloride, 0.5 g Yeast extract, 0.002 g Manganous sulfate, and 0.002 g Ferrous sulfate, with 2g Calcium phosphate (or aluminum phosphate or iron phosphate) as phosphate source. Finally, 0.4 % B romophenol blue (m/v, pH=6.7) was added to the media as a pH indicator for acidification (Nautiyal 1999 &Vazquez *et al*. 2000). A disc of Trichoderma was placed on NBRIP plate and incubated at 28 °C for 36 h. If the color of the

medium changed from purple to yellow in zones of acidification, this indicated that the strain had the ability to utilize calcium phosphate

Volatile Metabolites

The effect of volatile metabolites produced by *Trichoderma* spp were evaluated by the method of Dennis and Webster (1971) and Goyal *et al*. (1994). The mycelia disc (5mm) of *Trichoderma* species as well as test pathogens were centrally placed on separate PDA plates and incubated at 28 ± 2°C for 1 day. After the completion of incubation period, the top of each plate was replaced with the bottom of PDA plate inoculated centrally by the test pathogen so as test pathogen was directly exposed to the antagonistic environment created by *Trichoderma*. Two plates were sealed together with paraffin tape and further incubated at28 ± 2°C for seven days . In control treatment, *T.spp* replaced with inoculums of 5 mm of sterile PDA medium only. Colonies diameter of the pathogen was measured at 5 and 7 days after incubation and the inhibition of mycelia growth was calculated. The percent growth inhibition was calculated by using equation 1

$$I = (C-T)/C \times 100,$$

Where C is mycelial growth in control plate, T is mycelial growth in test organism inoculated plate and I is inhibition of mycelial growth.

Induction of chitinolytic enzymes

Chitinolytic activity

Chitinolytic activity was assayed by measuring the release of reducing saccharides from colloidal chitin. A reaction mixture containing 1ml of culture supernatant, 0.3ml of 1 M sodium acetate buffer (SA-buffer), pH 4.6 and 0.2ml of colloidal chitin was incubated at 40°C for 20h and then centrifuged at 13,000 rpm for 5 min at 6°C. After centrifugation, an aliquot of 0.75ml of the supernatant, 0.25ml of 1% solution of dinitrosalycilic acid in 0.7 M NaOH and 0.1ml of 10M NaOH were mixed in 1.5ml microcentrifuge tubes and heated at 100°C for 5min. Absorbance of the reaction mixture at A_{582} was measured after cooling to room temperature. Calibration curve with N-acetyl-β–D-glucosamine (NAGA) was used as a standard to determine reducing saccharide concentration. Chitinolytic activity was estimated in terms of the concentration (mg/ml) of NAGA released.

Exochitinase activity

N-acetyl-β-D-glucosaminidase (exochitinase) activity was measured and monitored spectrophotometrically as the release of p-nitrophenol (pNP) from p-nitrophenyl-Nacetyl- β-D-glucosaminide (pNPg). A mixture of 25µl of culture filtrate, 0.2ml of pNPg solution (1mg pNPg ml-1), and 1ml of 0.1 M SA-buffer (pH 4.6) was incubated at 40^0C for 20h and then centrifuged at 13,000 rpm. An aliquot of 0.3ml of 0.125 M Sodium tetraborate–NaOH buffer (pH 10.7) was added to 0.6ml of supernatant, absorbance at 400nm (A_{400}) was measured immediately after mixing and pNP concentration.

Induction of β-1-3 Glucanase enzyme taken from *Trichoderma* sp. on different carbon sources

Two different carbon sources were selected for the induction of glucanase enzyme *viz*- CMC and Wood Dust. These carbon sources were added in Czapek Dox medium at the rate of 1%. Cultures were incubated for 10 days at 28°C on orbital shaker at 150rpm. At the end of the incubation time, wood dust residues were removed and filtrate was centrifuged at 5000rpm for 10min. The clear supernatant was considered as a source of crude enzyme. This obtained supernatant was used for measuring enzyme activity. Glucanase enzyme activity was assayed using 1% (w/v) CMC as a substrate. Enzyme activity is expressed as U/mg.

Determination of Enzyme activity

The enzyme solution 1ml in appropriate dilution was added to 1ml of 1% carboxymethyl cellulose dissolved in 50m M sodium acetate buffer, pH 5.0. After incubation at 50^0C for 60min. the reaction was stopped by the addition of 3ml DNS reagent. After incubating for 10 min. in a boiling water bath enzymatic activity was determined at 540nm. One unit of CMCase activity was expressed as the amount of protein that liberate reducing sugar equivalent to glucose per minute under assay conditions.

Induction of xylanase enzyme taken from *Trichoderma* sp. on different carbon sources

Effect of different carbon sources viz. Birch Wood Xylan, Wheat Bran, Corn Cob and CMC were studied on xylanase expression. These carbon sources were added in Vogel's medium at the rate of 1%. After 5-7 days the culture mixture was taken out of orbital shaker. Supernatant

obtained after centrifugation was tested for xylanase activity. Xylanase activity was assayed using 1 % (w/v) of birch wood xylan as a substrate. Reaction mixture contained 1ml of 1% xylan solution (in 0.1 M, pH 5 sodium citrate buffer) and 2ml of enzyme were added to the reaction tubes and incubated at 40°C and the amount of reducing sugar in the reaction tubes was measured using the Dinitrosalicylic Acid. After heating for 5min in a boiling water bath and cooling, the absorbance was read at 550nm using UV spectrophotometer. The amount of reducing sugar was calculated from the standard curve based on the equivalent xylose. One unit of xylanase activity is defined as 1ìmol of xylose equivalent produced/min under the assay conditions.

Induction of cellulase enzyme from *Trichoderma* sp. on different carbon sources

Five different carbon sources were selected for the induction of cellulose enzyme viz. maltose, corn cob, wheat bran, sucrose and filter paper. These carbon sources were added in the cellulose production media @ 1 %. Cultures were incubated for 7 days at 28°C on orbital shaker at 150rpm. The crude enzyme were filtered and centrifuged at 11000 x g for 10 min. The clear supernatant was considered as the source of crude enzyme. The obtained supernatant was used for enzymatic activity determination. Different species of *Trichoderma* was analysed by DNS (Di Nitro Salicylic acid) method for cellulase production against different carbon sources.

Filter Paper Assay (FPA)

FPA expressed in international units (IU/ml/min) was recommended by the International Union of Pure and Applied Chemistry (IUPAC), the most commonly used analytical method for determination of total cellulase activity. One unit is the amount of enzyme required to release 1µ mole glucose equivalents in 1ml of enzyme solution in one minute.

Endoglucanase assay

0.5 ml of the enzyme solution was added into test tubes. The enzyme solution and substrate solution were equilibrated at 50°C. 0.5 ml of the CMC solution was taken into the test tubes and mixed well. Incubated at 50°C for 30 min. 3.0 ml of DNS solution was added and mixed well, boiled for exactly 5.0 min in vigorously boiling water. The tubes were placed in an ice-cooled water bath to quench the reaction. 20 ml of distilled water was added to it, which was mixed by inverting the tubes several times. Absorbance was taken at 540 nm. Enzyme activity is expressed as IU/ml/min.

Induction of lipase enzyme from *Trichoderma* sp. on different carbon sources

Already reported three carbon sources (Tween 80, coconut and soybean oil) were tested for extracellular lipase synthesis. These carbon sources were supplemented in lipase production media @ 1% .The crude enzyme extract was stored at –20°C until used. Lipase activity in the synthetic media was determined titrimatrically on the basis of olive oil hydrolysis. The end point was light pink in colour. One unit of lipase is defined as the amount of enzyme which releases one micro mole fatty acid per minute under specified assay conditions.

Lipase unit calculation

$$Lipase\ Activity = \frac{\Delta V \times N}{V\ sample} \times \frac{1000}{60}$$

$\Delta V =$ V2 – V1

V1 = Volume of NaOH used against control flask

V2 = Volume of NaOH used against experimental flask

N = Normality of NaOH

V (Sample) = Volume of enzyme extract

Units of extracellular lipase activity were units per ml (U mL^{-1}) while intracellular activity was calculated in units per gram (U g^{-1})

RESULTS AND DISCUSSION

Isolation and morphological identification of *Trichoderma* isolates

Genus and species level identification of *Trichooderma* species isolated from the rhizosphere soil of different locations of U.P.were done based on morphological and microscopic observation. The identification of *Trichoderma* isolates were confirmed both by morphological and molecular characters (ITS) and deposited in Indian Type Culture Collection (ITCC), IARI, Pusa, New Delhi. The size of conidia and some morphological characteristics of the isolated *Trichoderma* species are summarized in Table (1).

All the *Trichodemra* species shows the phosphate solubilization capacity (Figure 2). Kapri and Tewari 2010 isolated 14 *Trichoderma* strains from the rhizospheric soil and found that all the

Table 1. Sequences of potential strains of *Trichoderma* deposited at NCBI

Strain No.	Name of Bioagent	Strain code	Source	GPS location	ITCC Accession No.	NCBI GenBank Accession No. with ITS marker	EMBL Data base Accession No. with *tef* marker	NBAIM, Mau Accession No
T_1	*T. aggressivum*	T.agg(CSAU)	Pratapgarh, UP	Longitude: 81° 59' ELatitude: 25° 35' N	7277	KT315919	LN897318 F-03193	NAIMCC-
T_2	*T. aureoviride*	T.avi(CSAU)	Hamirpur, UP Latitude: 25° 58' N	Longitude: 80° 12' E	6131	KT337463	LN897319 -F-03194	NAIMCC
T_3	*T.citrinoviride*	T.cvi (CSAU)	New Dairy Farm, CSA Kanpur	Longitude: 81° 24' 11.414" Latitude: 25° 21' 39.794"	7283	KT315921	LN897320	NAIMCC-F-03195
T_4	*T. erinaceum*	T. eri(CSAU)	New Dairy Farm, CSA Kanpur	Longitude: 81° 24' 11.414" Latitude: 25° 21' 39.794"	7287	KT315922	LN897321	NAIMCC-F-03192
T_5	*T. koningiopsis*	T. kop(CSAU)	Raibareilly, UP	Longitude: 81°16' E Latitude: 26° 14' N	7291	KT337462	LN897322	NAIMCC-F-03191
T_6	*T. tomentosum*	T. tos(CSAU)	Legume Research Farm, CSAU Kanpur	Longitude: 81° 24' 11.414" Latitude: 25° 21' 39.794"	7269	KT315920	LN897323	NAIMCC-F-03186
T_7	*T.mintisporum*	T. mip(CSAU)	Vegetable Farm, CSAU Kanpur	Longitude: 81° 24' 11.414" Latitude: 25° 21' 39.794"	7280	KT626565	LN897312	NAIMCC-F-03187
T_8	*T. pubscenes*	T. sce (CSAU)	Student Instruction Farm,CSAU Kanpur	Longitude: 81° 24' 11.414" Latitude: 25° 21' 39.794"	7268	KT337461	LN897315	NAIMCC-F-03188
T_9	*T. saturnisporum*	T. ssp (CSAU)	Allahabad, UP	Longitude: 81° 54' E Latitude: 25° 25' N	7274	KT626566	LN897313	NAIMCC-F-03189
T_{10}	*T. spirale*	T. sp. (CSAU)	Nawabganj Farm, CSAU Kanpur	Longitude: 81° 24' 11.414" Latitude: 25° 21' 39.794"	7276	KT626567	LN897314	NAIMCC-

isolated spcies have the ability to solubilize phosphates in different proportions. The phosphate solubilization ability of *Trichodemra* species depends upon the ability of *Trichodemra* strain to colonize the plant roots. John *et al* 2010 found that when soyabean plants are inoculated with *T.viride* it shows greater shoot and root length. the use of phosphate solubilizing species can effectively replace or reduce the use of chemical fertilizers (Mamta *et al* 2010). Badwai showed that *T.harzianum* had a greater ability to solubilize phosphate as compared to *Bradyrhizobium* and *Serratia marscens*. Carvraj in 2009 reports the phosphate solubilization ability of *Trichoderma* species.

Table 2. Spectrophotometric determination of chitinolytic and exochitinase activity of *Trichoderma* isolates in media supplemented with colloidal chitin.

Isolates	Chitinolytic activity mg/ml		Exochitinase Activity(U/ml ×10^{-3})	
	Colloidal	Seashell	Colloidal	Seashell
T.aggressivum	7.76	6.6	0.0167	0.00709
T.aureoviride	6.06	5.32	0.0145	0.00605
T.citrinoviride	5.9	4.07	0.0120	0.00607
T.erinaceum	5.4	4.12	0.0132	0.00508
T.koningiopsis	6.1	5.08	0.0142	0.00603
T.tomentosum	6.38	4.34	0.0153	0.00703
T.ministisporum	5.89	3.6	0.0132	0.00408
T.pubscens	4.76	3.2	0.0090	0.00504
T.saturnisporum	5.27	4.0	0.0130	0.00607
T.spirale	6.09	6.04	0.0165	0.00701

Table 3. Showing glucanase activity produced by *Trichoderma* spp. grown on different carbon sources

Strain name	CMC	Wood Dust
T.aggressivum	2.86	0.83
T.aureoviride	2.08	0.8
T.citrinoviride	2.31	0.72
T.erinaceum	1.25	0.88
T.koningiopsis	1.67	0.88
T.tomentosum	1.44	0.51
T.ministisporum	1.23	0.56
T.pubscens	1.67	0.36
T.saturnisporum	1.16	0.59
T.spirale	2.18	0.77

There are many mechanisms which are employed by *Trichoderma* against pathogens. During antibiosis the anatgonistic fungus produce antibiotics, compete for nutrients, while in case of mycoparasitism *Trichoderma* directly attacks the pathogen by secreting CWDEs such as chitinase xylanase, glucanase protease etc. CWDEs play a crucial role in the anatagonistic mechanism as they degrade the cell wall of pathogens. These enzymes lyseed the pathogen cell wall. Present findings

Table 4. Xylanase activity produced by *Trichoderma* spp. grown on different carbon sources

Strain name	Birch Wood Xylan	Wheat Bran	Corn Cob	Carboxy Methyl Cellulose
T.aggressivum	9.18	4.2	6.77	5.34
T.aureoviride	5.42	3.38	5.19	4.89
T.citrinoviride	5.79	3.24	5.12	4.96
T.erinaceum	6.7	3.64	5.79	5.27
T.koningiopsis	7.002	3.51	3.4	3.72
T.tomentosum	7.6	3.28	5.87	3.76
T.ministisporum	5.12	3.08	3.64	3.12
T.pubscens	6.09	3.06	4.42	2.25
T.saturnisporum	5.19	2.4	4.59	4.6
T.spirale	6.09	3.34	4.06	4.42

showed higher specific activity of enzymes-chitinase and β-1, 3 glucanase in *Trichoderma* spp. Activity of these enzymes varied among the *Trichoderma* species. This may be due to the expression of certain gene in *Trichoderma* spp. Marco *et al* showed that Two isolates of *T.harzianum* secretes chitinolytic enzymes and it increased within 72 h.

Trichodemra speices are efficient producers of hydrolytic enzymes. The fungal cell wall ia a complex structure consisting of 80-90% carbohydrate (glucanase chitin cellulose mannns

Table 5. Effect of different carbon sources on FP activity

Strain Name	Wheat Bran	Maltose	Corn Cob	Sucrose	Filter Paper
T.aggressivum	2.16	1.18	2.27	1.03	1.83
T.aureoviride	1.13	0.95	1.4	0.77	0.82
T.citrinoviride	0.56	0.72	0.92	0.77	0.59
T.erinaceum	0.43	0.72	1.16	0.87	0.87
T.koningiopsis	0.93	1	1.1	1	0.98
T.tomentosum	0.75	0.7	1.05	1.01	1.01
T.ministisporum	0.53	0.8	1.03	0.87	0.69
T.pubscens	0.26	0.69	1.16	0.56	0.74
T.saturnisporum	0.36	0.78	0.92	0.82	0.9
T.spirale	1	0.9	1.08	1.03	0.87

Table 6. Effect of different carbon sources on endoglucanase activity

Isolate name	Wheat Bran	Maltose	Corn Cob	Sucrose	Filter Paper
T.aggressivum	0.89	0.86	2.9	0.72	0.89
T.aureoviride	0.79	0.55	2.69	0.58	0.5
T.citrinoviride	0.65	0.29	0.86	0.52	0.25
T.erinaceum	0.55	0.35	2.23	0.73	0.44
T.koningiopsis	0.8	0.37	1.73	0.68	0.65
T.tomentosum	0.81	0.46	1.79	0.66	0.72
T.ministisporum	0.71	0.3	0.8	0.72	0.47
T.pubscens	0.5	0.38	0.76	0.61	0.59
T.saturnisporum	0.52	0.38	0.84	0.69	0.39
T.spirale	0.59	0.91	1.69	0.62	0.5

Table 7. Effect of different carbon sources on lipase activity

Strain name	Coconut oil	Sunflower oil	Tween-80
T.aggressivum	12.69	31.1	36.25
T.aureoviride	9.63	13.9	14.8
T.citrinoviride	10	8.8	11.3
T.erinaceum	6.85	11.9	13.8
T.koningiopsis	12.6	10.2	13.7
T.tomentosum	14.2	11.3	14.4
T.ministisporum	9.1	10.1	15.8
T.pubscens	10.15	12.15	13
T.saturnisporum	11.5	11.2	13.1
T.spirale	16.6	11.45	31.8

and others and 10-20% proteins and lipid). Chitin can be cleaved by Chitinase and N -acetyl-glucosaminidase (Hartl *et al* 2012). In the present investigation *T.aggersivum* showed the highest chitinase enzyme production capacity (Table 2). *Trichoderma* species utilize chitinase and NAGAse to degdrade the plant pathogen cell walls(Gruber and Seidl -Seiboth 2012).

Beside s chitinase glucanase is the second most abundant fibrilar polymer in fungal cell walls (Feofila 2010). β-glucanase hydrolyse the o-glycosidic linkages of β-glucan chains. In our study highest β glucanase activity was reported by *T.aggresivum* followed by *T.spirale* (Table 3 and Figure 2). A number of fungal β-1,3-glucanases

Table 8. Effect of volatile metabolites produced by *Trichoderma* species on the mycelia growth inhibition of phytopathogens

Name of Bioagent	Strain code	ITCC Accession No.	Average growth in (cm)	% Inhibition growth (cm)	Average growth in (cm)	% Inhibition growth (cm)	Average growth in (cm)	% Inhibition growth (cm)	Average growth in (cm)	% Inhibition growth (cm)	Average growth in (cm)	% Inhibition growth (cm)
			Sclerotium rolfsii		*R. bataticola*		*FOC*		*FU*		*CC*	
T. *aggressivum*	T.agg(CSAU)	7277	5.95	27.82	5.7	24	3.9	18.73	3.25	54.54	4.6	8
T.aureoviride	T.avi(CSAU)	6131	6.8	17.57	6.25	16.6	5.05	19.84	3.1	56.64	3.2	36
T.citrinoviride	T.cvi (CSAU)	7283	5.4	34.54	5.7	24	4.1	34.92	3.2	55.24	3.95	21
T.erinaceum	T. eri(CSAU)	7287	4.95	40	5.15	31.33	4.0	36.50	3.25	54.54	3.15	37
T.koningiopsis	T. kop(CSAU)	7291	5.5	33.33	6.15	18.	5.25	16.16	3.05	57.34	2.45	51
T.tomentosum	T. tos(CSAU)	7269	7.2	12.72	6	20	4.8	23.80	3.2	55.24	4.3	14
T.ministisporum	T. mip(CSAU)	7280	7.15	13.33	5.95	20.66	5.15	18.25	3.15	55.94	3.6	28
T.pubscens	T. sce (CSAU)	7268	5.9	28.48	5.7	24	5.95	5.5	2.9	59.44	4.7	6
T.saturnisporum	T. ssp (CSAU)	7274	5.35	35.15	5.35	26.66	3.9	50.79	3.2	55.24	2.55	49
T.spirale	T. sp. (CSAU)	7276	5.95	27.8	5.4	28	4.1	5.55	3.95	44.75	3.55	29
T Control			8.25		7.5		6.3		7.15		5	
CD at % 5				.3224		.4362		.4827		.3603		.2845
SE (d				.1591		.2159		.2380		.1770		.1414

have been the subject of basic and applied research, as they seem to have different functions during cell development and differentiation. β-1,3-Glucanases may also play a nutritional role in saprophytes and mycoparasites (Lorito *et al*. 2010). Expression of the majority of β-1,3-glucanase genes during mycoparasitism is well-documented. Marcello *et al*. (2010), using RT-qPCR, showed that the expression of an exob- 1,3-glucanase from *T. asperellum* (tag83) issignificantly increased during in vivo assay of *T. asperellum* against *R. solani*. Steindorff *et al*. (2012) analyzed the expression of four β-1,3-glucanase genes: (bgn13.4), β-1,6-glucanase (P1), chitinase (chi33) and β-1,4-N-acetylglucosaminidase (nag1) using RT-qPCR during an *in vivo* interaction assay between *T. harzianum* and *F. solani*, transcripts encoding these enzymes were highly expressed mainly after contact between *T. harzianum* and plant-pathogenic fungi.

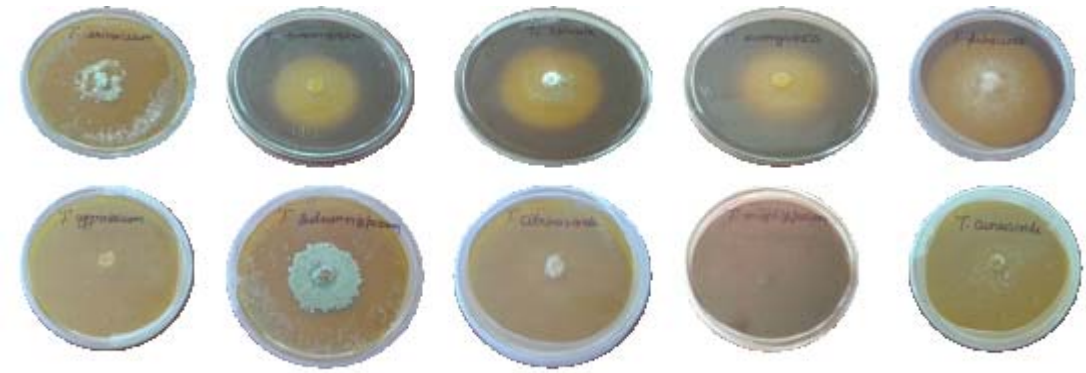

Fig. 1. Phosphate solubilization activity of *Trichoderma* species.

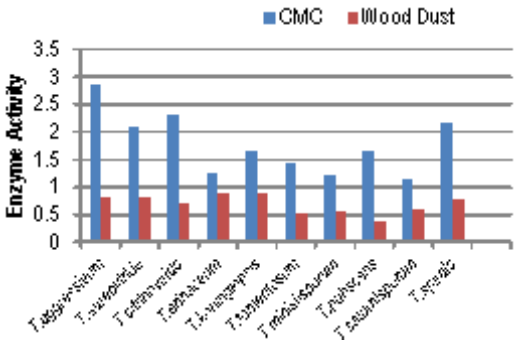

Fig. 2. Effect of carbon sources on glucanase enzyme induction

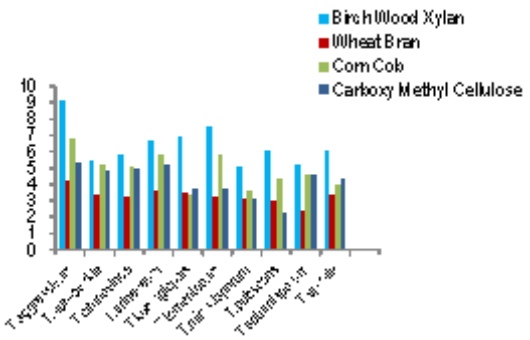

Fig. 3. Showing effect of carbon source on xylanase production by *Trichoderma* sp.

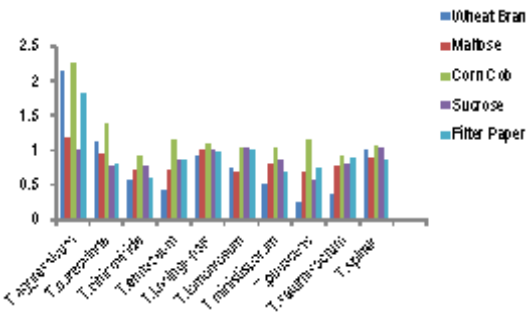

Fig. 4. Effect of different carbon sources on FP- activity

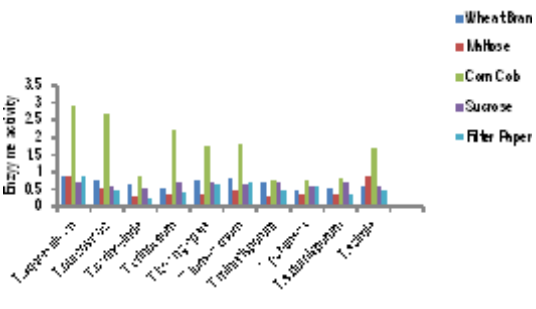

Fig. 5. Effect of different carbon sources on endoglucanase activity

Xylanases (β-1,4-xylan xylanohydrolase, E.C.3.2.1.8) is the major component of xylanolytic enxymes and randomly cleaves the β-1,4-glycosidic bond of xylan backbone. Xylan is the second most abundant natural polysaccharide. Xylan is a heteropolysaccharide consisting of β -1,4-linked D-xylose monomers in connection with side branches of arabinosyl, glucuronosyl, acetyl, uronyl, and mannosyl residues (Tsujibo *et al*., 1997).

Data presented in Table 5, Figure 4, showed that cellulase production by *Trichoderma* species was significantly influenced by the type of carbon source in the basal salt medium. Corn cob was the most effective carbon source for cellulase enzyme production followed by wheat bran, filter paper, sucrose and maltose Table 5 and Figure 4.

Latifian *et al*., (2007) obtained the maximum activity (1.1635 U/g) using rice bran like substrate and in same conditions for incubation. Yang *et al*. (2004) found similar results for a microbial consortium of *T. reesei* (AS3.3711), *Aspergillus niger* (3.316 U/g) and *Saccharomyces cerevisiaes* (AS2.399) on rice chaff in SSF. Similar

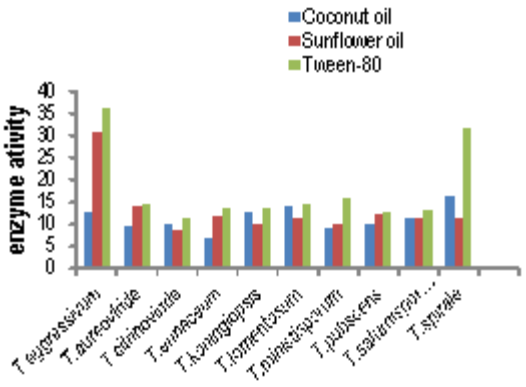

Fig. 6. Effect of different carbon sources on lipase activity

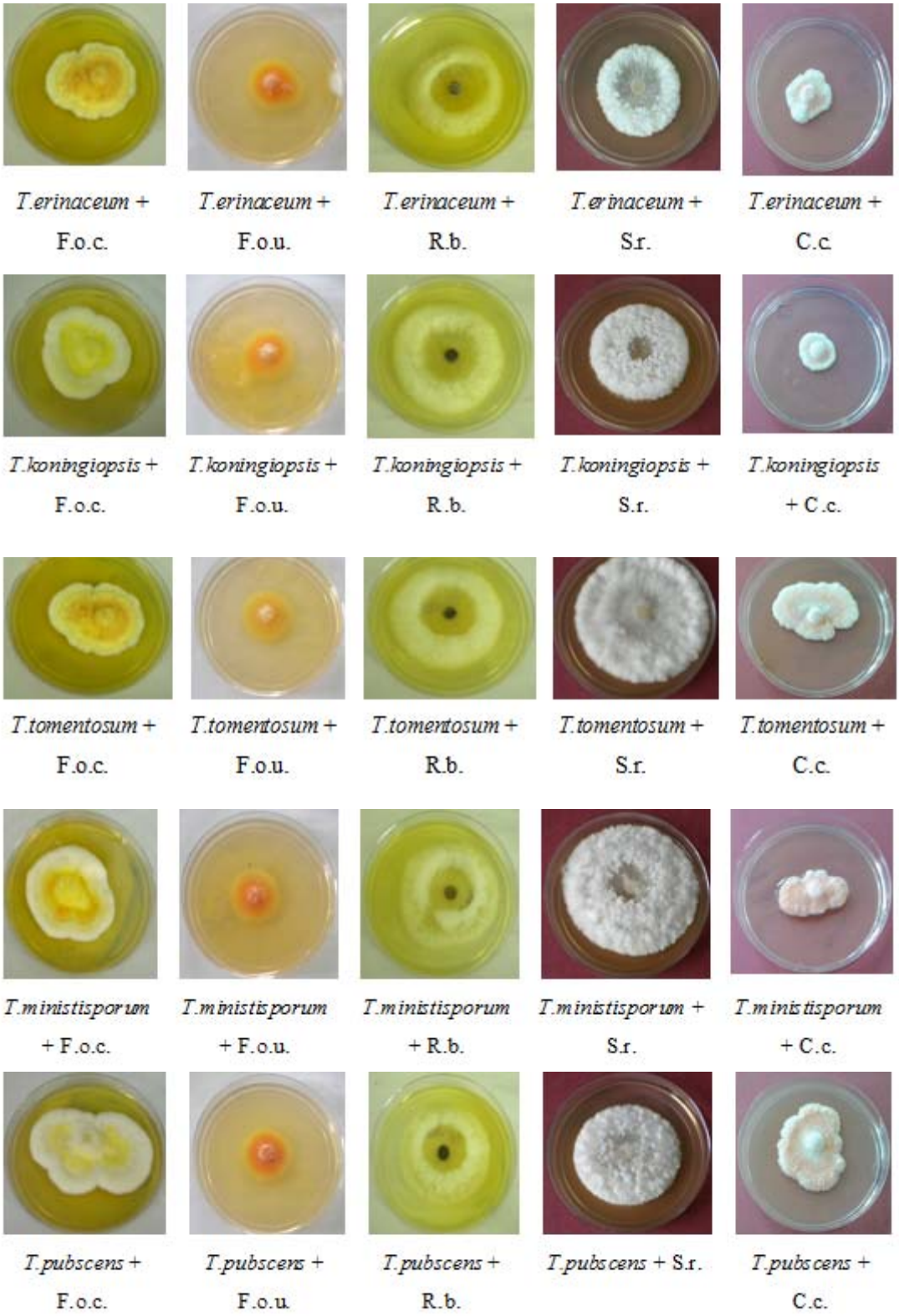
T.erinaceum + F.o.c.
T.erinaceum + F.o.u.
T.erinaceum + R.b.
T.erinaceum + S.r.
T.erinaceum + C.c.
T.koningiopsis + F.o.c.
T.koningiopsis + F.o.u.
T.koningiopsis + R.b.
T.koningiopsis + S.r.
T.koningiopsis + C.c.
T.tomentosum + F.o.c.
T.tomentosum + F.o.u.
T.tomentosum + R.b.
T.tomentosum + S.r.
T.tomentosum + C.c.
T.ministisporum + F.o.c.
T.ministisporum + F.o.u.
T.ministisporum + R.b.
T.ministisporum + S.r.
T.ministisporum + C.c.
T.pubscens + F.o.c.
T.pubscens + F.o.u.
T.pubscens + R.b.
T.pubscens + S.r.
T.pubscens + C.c.

Fig. 7. Mycelial growth inhibition of phytopathogens by the volatile metabolites produced by *Trichoderma* species

results were also obtained using municipal solid waste residue and *Aspergillus niger*, with the maximum activity of exoglucanase (1.64U/g) and endoglucanase (1.84U/g) after 4 days (Gautam *et al*., 2011).

The production medium supplemented with different carbon sources like sunflower oil, coconut oil and tween-80 was estimated for lipase activity and the results are depicted in figure 6. All strains shows maximum lipase activity in media supplemented with Tween 80. Minimum lipase activity occurs in coconut supplemented media. Among all the tested strains *T.harzianum* shows maximum lipase activity on media supplemented with tween-80. The maximum lipase enzyme production was achieved with Tween- 80 supplemented media (Figure 6 and Table 7).

Ohnishi *et al*., 1994 reporetd that lipase activity tended to increase as the glucose concentration increased from 0% to 4%. In another study Iftikhar *et al*., 2002, found that there was no difference between glucose and olive oil for lipase production. Moreover, the lipase activities of many other fungi, such as *Aspergillus wentii* (Chander *et al* 1980) *Mucor hiemalis* (Akhtar *et al*., 1980 and Rathi *et al* 2001) are also stimulated by the addition of glucose to the production medium.

Evaluation of volatile metabolite production by 10 *Trichoderma* species against five species of pathogens was done. Volatile metabolites produced by *T.saturnisporum* shows maximum growth inhibition of *Sclerotium rolfsii* (35.15). *T. erinaceum* showed maximum inhibition percentage of mycelia growth was recorded against *Rhizoctonia bataticola* (31.33). *T. saturnisporm* shows maximum growth inhibition of *Foc* (50.79) and CC (49). and *T. pubscens* shows maximum mycelia growth inhibition of *Fu* (59.44). (Figure7 and table 8)

Our results are in agreement with those

reported previously for different *Trichoderma* spp. The effect of secondary metabolites produced by *Trichoderma* on the development of plant pathogens has been extensively studied. Kapil and Kapoor (2005) reported that culture filtrates of *T. viride* inhibited mycelial growth of *S. sclerotiorum* due to production of an antibiotic like substance, Vinale *et al*. (2008) reported that volatile secondary metabolites play a key role not only in mycoparasitism, but also in their interactions with tomato and canola seedlings. Ajith and Lakshmidevi (2010) reported the effect of volatile and non-volatile compounds from *Trichoderma* spp. against *Colletotrichum capsici*, a fungus responsible for anthracnose disease in bell peppers.

CONCLUSION

Present study highlights the phosphate solubilization volatile metabolite production and cell wall degrading enzyme production capacity of Trichoderma species isolated from the Rhizospheric soils of Uttar Pradesh. Thus, suggesting their applicability for crop improvement.

ACKNOWLEDGEMENTS

The authors are grateful for the financial support granted by the Indian Council of Agricultural Research (ICAR), under Niche Area of Excellence of "Exploration and Exploitation of *Trichoderma* as antagonist against soil borne pathogen" programme running in Biocontrol Lab, Department of Plant Pathology, C.S. Azad University of Agriculture and Technology, Kanpur.

REFERENCES

1. Abdullah, F., Nagappan, J., and Sebran, N.H. Biomass production of *Trichoderma harzianum* Rifai in palm oil mill effluents (pome). *International Journal of Biology and Biotechnology*., 2005, **2**: 571–575.
2. Ajith P.S, Lakshmidevi N. Effect of volatile and nonvolatile compounds from *Trichoderma* spp. against *Colletotrichum capsici* incitant of anthracnose on Bell peppers. *Nat Sci*., 2010, **8**(9): 265-269.
3. Akhtar M.W, Mirza A.Q, Chughtai M.I.D. Lipase induction in *Mucor hiemalis*. *Appl Environ Microbiol*.,1980, **44**: 257-63.
4. Benitez T, Rincon M.A., Limon M.C., Codon C.A.Biocontrol mechanisms of *Trichoderma* strains. *Inter microbial*., 2004, **7**: 249-260
5. Carvraj L.H., Orduz S, Bissett J. Growth stimulation in bean (*Phaseolus vulgaris* L.) by *Trichoderma*. *Boil*., 2009, **51**: 409-416
6. Chander H., Batish V.K., Sannabhadti S.S. Factors affecting lipase production in *Aspergillus wentii*. *J Food Sci*.,1980, **45**: 598-600.
7. Chet I., Harman G.E., Bake R.*Trichoderma hamatum*, its hyphal interactions with *Rhizoctonia solani* and *Phytium* spp. *Microbial Ecolog*.,1981, **7**(1): 29–38.
8. Chitarra, G.S., Abee, T., Rombouts, F.M., Dijksterhuis J.1-Octen-3-ol inhibits conidia germination of *Penicillium paneum* despite of mild effects on membrane permeability, respiration, intracellular pH, and changes the protein composition, *FEMS Microbiology Ecology*., 2005, **54**: 67–75
9. Combet, E., Henderson, J., Eastwood, D.C., Burton, K.S. Eight-carbon volatiles in mushrooms and fungi: Properties, analysis, and biosynthesis. *Mycoscience*, 2006; **47**(6): 317-326
10. Dennis C. Webster J.Antagonistic properties of species group of *Trichoderma* III. Hyphal interaction, Trnas. *British Mycological Soceity*., 1971, **57**: 363-369
11. Earl K., Syers J. McLaughlin R.M.Origin of the effect of citrate, tartrate, and acetate on phosphate sorption by soils and synthetic gels. *Soil Science Society of America Journal*., 1979, **43**: 674-678
12. Fabiano G.S. Phoshpate solubilization and phytohormone production by endophytic and rhizospheric *Trichoderma* isolates of guanandi (*Calophyllum brasiliense* Cambess). *African journal of microbiology research*., 2014,8 (27).
13. Galindo, E., Flores, C., Larralde-Corona, P., Corkidi-Blanco, G., Rocha-Valadez, J.A., Serrano-Carreo´ N. L. Production of 6-pentyl-alpha-pyrone by *Trichoderma harzianum* cultured in unbaffled and baffled shake flasks. *Biochemical Engineering Journal*, 2004,**18**: 1-8
14. Gaur, A.C. Phosphate solubilizing Microorganisms as Biofertilizers. Omega Scientific publishers, 1990,New Delhi. pp. 176
15. Gautam S. P., BundelaP. S. , Pandey A. K., Jamaluddin K,. Awasthi M. K,and Sarsaiya S.Optimization for the Production of Cellulase Enzyme from Municipal Solid Waste Residue by Two Novel Cellulolytic Fungi. *Biotechnol Res In*., 2011, PMC 3042683.
16. Goyal S.P., Jandiak C.L.,Sharma V.P. Effect of weed fungi metabolites on the mycelial growth

of *A. bisporus* (Lang.), *Imbach. Mushroom Research.*, 1994, **3**: 69-74
17. Gruber S., Seidl-Seiboth V. Self and non-self: fungal cell wall degradation in *Trichoderma*. *Microbiology*., 2012,**158**:26–34
18. Harman, G.E. Overview of mechanisms and uses of *Trichoderma* spp, *Phytopathology*, 2006, **96**: 190–194
19. Harman, G.E., Howell, C.R., Viterbo, A., Chet, I., and Lorito, M., *Trichoderma* species-Opportunistic, avirulent plant symbionts. *Nature Reviews Microbiology*., 2004: **2**: 43–56.
20. Hartl L, Zach S, Seidl-Seiboth V. Fungal chitinases: diversity, mechanistic properties and biotechnological potential. *Appl Microbiol Biotechnol* 2012, **93**:533–543
21. Iftikhar T, Hussain A. Effects of nutrients on the extracellular lipase production by mutant strain of *Rhizopus oligosporus* TUV-31. *Biotechnology.* 2002, **1**: 15-20
22. John R.P., Tyagi R.D., Prevost D., Bar S.K., Pouleur S., Surampalli R.Y. Mycoparasitic *Trichoderma viride* as a biocotntrol agent against *Fusarium oxysoprum* f.sp. adzuki and *Pythium arrhenomanes* and as a growth promoter of soyabean. *Crop Prot*., 2010, **29**: 1452-1459.
23. Kapil R., Kapoor A.S. Management of white rot of pea incited by *Sclerotinia sclerotiorum* using *Trichoderma* spp. and biopesticides. *Indian Phytopathol*., 2005, **58**(1):10–16.
24. Kapri A, Tewari L.Phosphate solubilization potential and phosphatese activity of rhizispheric *Trichoderma* spp. *Braz. J. Microbiol*., 2010, **41**:787-795.
25. Kucuk C., Kivanc M. Isolation of *Trichoderma* spp. and determination of their antifungal, biochemical and physiological features. *Turkish J of Biol*., 2003, **27**: 247-53.
26. Latifian, M. Z., Hamidi-Esfahani and Barzegar M. Evaluation of culture conditions for cellulase production by two *Trichoderma reesei* mutants under solid-state fermentation conditions. Short communication. *Bioresource technology*. 2007,**98**:3634–3637.
27. Lorito M., Woo S.L., Harman G.E., Monte E.Translational research on *Trichoderma*: from 'omics to the field. *Annu Rev Phytopathol*., 2010, **48**: 395-417.
28. Mamta R.P., Pathania V., Gulati A., Singh B., Bhanwara R.K., Tewari R. Stimulatoryeffect of phosphate solubilizing bacteria on plant growth, stevioside and rebauside - A contents of *Stevia rebudiana* Bertoni. *Appl. soil Ecol*.,2010, **46**:222-229
29. Marcello C.M., Steindorff A.S., Silva S.P., Silva R.N., Bataus L.A.M., Ulhoa C.J. Expression analysis of the exo-b-1,3- glucanase from the mycoparasitic fungus *Trichoderma asperellum*. Res Microbiol., 2010, **165**:75–81.
30. Narsian V.,Patel H.H. *Aspergillus aculeatus* as rock phosphate solubilizers. *Soil Biology and Biochemistry*.,2000,**32**, 559 565.
31. Nautiyal, C.S. An efficient microbiological growth medium for screening phosphorus solubilizing microorganisms. *FEMS Microbiol.Lett*., 1999,**170**, 2017-2021.
32. Ohnishi K., Yohko Y., Sekiguchi J. Purification and characterization of a novel lipolytic enzyme from *Aspergillus oryzae*. *J Ferment Bioeng*., 1994,**77**: 490-495
33. Okull, D.O., Beelman, R.B., and Gourama, H. Antifungal activity of 10-oxo-trans-8-decenoic acid and 1-octen-3-ol against *Penicillium expansum* in potato dextrose agar medium. *Journal of Food Protection*., 2003, **66**: 1503–1505
34. Olviria A.G., Junior A.F.C., Santos G.R., Miller L.O.,Chagas L.F.B. Potential de solubilizacao de fosfato e producao de aia por *Trichoderma* spp. *Rev. Verde*., 2012, **7**: 149-155.
35. Pandey S, Shahid M, Srivastava M, Singh A, Sharma A, Kumar V. Chitinolytic assay *Trichoderma* Strains isolated from different geographical locations of Uttar Pradesh. *Afr J Biotechnol*., 2014, **13**: 4246-4250
36. Rathi P., Saxena R.K., Gupta R. A. Novel alkaline lipase from Burkholderia cepacia for detergent formulation. *Process Biochem*., 2001, **37**: 187-92.
37. Reino, J.L., Guerriero, R.F., Herna'ndez-Gala' R., Collado, I.G.Secondary metabolites from species of the biocontrol agent *Trichoderma*. *Phytochemistry Reviews*., 2008, **7**: 89-123.
38. Reyes I., Bernier L. & Antoun H. Rock phosphate solubilization and colonization of maize rhizosphere by wild and genetically modified strains of *Penicillium rugulosum*. *Microbial Ecology*., 2002, **44**: 3948
39. Samuels G.J., Chaverri P., Farr D.F., McCray E.B.*Trichoderma* Online. Systematic Botany & Mycology Laboratory, ARS, USDA.,2004,from http://nt.ars-grin.gov/taxadescriptions/ keys/ *Trichoderma* Index.cfm.
40. Siddiquee S., Cheong B. E., Taslima, K., Kausar H., and Hasan, M. M. Separation and Identification of Volatile Compounds from Liquid Cultures of *Trichoderma harzianum* by GC-MS using Three Different Capillary Columns. *Journal of Chromatographic Science*., 2012, **50**: 358–367.

41. Starkanova G., Vorisek K., Mikanova O. & Randova D. 1999. P solubilisation activity of *Rhizobium* species strains. *Rostlinna Vyroba*., 1999, 45, 403406.
42. Steindorff A.S., Silva R.N., Coelho A.S.G., Noronha E.F., Ulhoa C.J. *Trichoderma harzianum* expressed sequence tags for identification of genes with putative roles in mycoparasitism against *F. solani*. *Biol Control.*,2012, **61**(2):134–140.
43. Subba R,N.S.. In Soil Microbiology. Fourth Edition of Soil Microorganisms and Plant Growth. Oxford & IBH Publishing Co. Pvt. Ltd., New Delhi., 1999
44. Tsujibo H., Ohtsuki T., Iio T., Yamazaki I., Miyamoto K., Sugiyama M., Inamori Y.Cloning and sequence analysis of genes encoding xylanases and acetyl xylan esterase from *Streptomyces thermomviolaceus* OPC-520. *Appl. Environ Microbiol*.,1997, **63**: 661-664.
45. Vassilev N., Vasileva, M.A., Nikolaeva L., Simultaneous P solubilizing and biocontrol activity of microorganisms: potentials and future trends. *Appl. Microbiol. Biotechnol.*, 2006,**71**(2), 137-144
46. Verma, M., Brar, S.K., Tyagi, R.D., Surampalli, R.Y., and Vale´ ro, J.R. Antagonistic fungi, *Trichoderma* spp.: Panoply of biological control. *Biochemical Engineering Journal*., 2007, **37**: 1–20.
47. Vesquez, P., Holiguin, G., Puente, M.E.,Lopez Cortes, A., Bashan, Y. Phosphate solubilizing microorganisms associated with the rhizosphere of mangroves in semi & coastal lagoon. *Biol. Fert. Soils*., 2000, 460-468.
48. Vinale, F., Sivasithamparam, K., Ghisalberti, E.L., Marra, R., Barbetti, M.J., Li, H., *et al*. A novel role for *Trichoderma* secondary metabolites in the interactions with plants. *Physiological and Molecular Plant Pathology*., 2008, **72**: 80–86.
49. Weindling R. *Trichoderma lignorum* as a parasite of other soil fungi. *Phytopathology*.,1932:**22**: 837-845.
50. Yang Y.H., B.C. Wang Q.H. Wang L.J. ,Xiang C.R. Duan Research on solidstate fermentation on rice chaff with a microbial consortium. Colloids and Surfaces B: *Biointerfaces*., 2004, **34**:1-6.