Variability in hydrolytic enzyme production and cultural characteristics of endophytic *Trichoderma* isolates associated with sugarcane

Upadhyay M.[1], Awasthi A.[1] and Joshi D.[2]*
1. Department of Environmental Science, Maharaja Agrasen University,Baddi,Solan -174103 (H.P.), INDIA
2. Division of Crop Protection ICAR- Indian Institute of Sugarcane Research, Lucknow -226002 (UP), INDIA
*14deeksha@gmail.com

Abstract

Endophytic microbes, especially Trichoderma spp., established from various crops, are being explored for their beneficial properties which include eco-friendly disease management, growth promotion and resistance induction in addition to being exploited as an environment friendly source of important hydrolytic enzymes. The present study was undertaken to isolate and characterize endophytic Trichoderma from 10 sugarcane varieties. Isolates were characterized for their colony characters, growth rates at different temperatures and the potential to produce hydrolytic enzymes chitinase and cellulase. Twenty nine endophytic Trichoderma isolates were established from different sugarcane plant parts (roots:14, stalk:13, leaf:2). There was considerable variability in growth of Trichoderma isolates at different temperatures with a general decline in growth rates observed at temperature beyond 30°C.

Cellulase production was recorded in 20 isolates with enzyme index ranging from 1.0 to 1.2. Chitinase production was observed in 17 isolates only. The results indicate presence of tissue specificity in endophytic Trichoderma with Trichoderma exhibiting preferential colonization of roots and stalks as compared to leaves. Based on variations in growth rate and colony characters, eight isolates were identified as T. longibrachiatum. Isolates exhibiting high chitinase and cellulase production can be explored further as biocontrol agents and for trash decomposition in the sugarcane agro-ecosystem.

Keyword: Endophyte, *Trichoderma*, cultural characters, chitinase, cellulase.

Introduction

Sugarcane (*Saccharum officinarum*) is an important cash crop cultivated in tropical and sub- tropical regions of the world. It is the second most important agro industrial crop in India, next only to cotton and is cultivated in an area of about 5 million hectares. Sugarcane is the primary source for manufacture of crystal sugar and also yields ethanol, bagasse, pressmud etc.as useful bye-products. Losses due to various diseases like red rot, smut, wilt etc. are major constraints in the profitable cultivation of sugarcane affecting its productivity world-wide.[22]Use of biological control agents like *Trichoderma* spp. presents a feasible, ecofriendly and sustainable option for disease management and is also highly amenable to the sugarcane agro-ecosystem which has been explored extensively in recent years.

Trichoderma spp. are ubiquitous fungi which are found in all types of soil and other natural habitats, both as free living fungi as well as in endophytic association with plants.[9]*Trichoderma* spp. are effective biocontrol agents of plant diseases and also provide benefits like plant growth promotion, solubilization of some plant nutrients [6,13,15]as well as induction of resistance in plants against biotic and abiotic stresses.[11]

Trichoderma strains also play an important role in the bioremediation of soil contaminated with pesticides and insecticide. They have the ability to degrade a wide range of insecticides: organochlorines, organophosphate and carbonates and are also highly resistant to a range of toxicants viz., heavy metals, organometallic compounds, tannery effluents and harmful chemicals like cyanide (CN).[7,10] The application of *Trichoderma* for treating polluted sites for cultivation may become a reality in the near future, as they can be produced cheaply in large quantities on an industrial scale and can be formulated for field applications.[19]

In particular, the production of diverse hydrolytic enzymes like chitinase and cellulase is an important attribute of *Trichoderma* spp.[20] The production of the enzyme chitinase has been implicated to play a major role in the antagonism of various pathogens by *Trichoderma* spp.[21]*Trichoderma* spp. are also effective producers of cellulase enzyme which helps in recycling of cellulosic biomass in nature [3,21] and some species of *Trichoderma* have even been successfully exploited for industrial scale production of cellulase. Sugarcane crop produces large quantities of trash, the disposal of which is a major problem. Identifying potent *Trichoderma* strains with potential for high production of these two hydrolytic enzymes will have multiple beneficial applications in the sugarcane agro-ecosystem.

In recent years, there has been an increasing focus on exploring endophytic microbes including *Trichoderma* spp. for various beneficial impacts like enzyme production, disease management, growth promotion etc.[2,14] To date several species of endophytic *Trichoderma* have been isolated and characterized from various crop plants.

However, in case of sugarcane, there have been limited studies on isolation and characterization of endophytic *Trichoderma* for their beneficial properties including enzyme producing potential, disease control etc., especially from India.

Since majority of economically important sugarcane diseases like red rot, smut etc. are set borne in nature damaging cane stalks; it is expected that the use of endophytic microbes as biocontrol agents may be more effective for sugarcane disease management compared to rhizospheric microbes since endophytes are already adapted to the niche where they later have to function as antagonists. The first step in successful identification of potent endophytic *Trichoderma* strains, is the isolation and characterization of indigenous isolates which will be better adapted to the specific agro-ecosystem where they later have to function.

In our previous investigation, we had conducted preliminary studies on isolation and characterization of endophytic *Trichoderma* and established 32 *Trichoderma* strains from root and leaf tissues of different sugarcane varieties.[12]However, to have a better chance of identifying highly potent endophytic *Trichoderma* strains, isolation and characterization of a larger set of strains is essential. As such, in the present study, we conducted further experiments on isolation of endophytic *Trichoderma* from different sugarcane varieties.

We attempted to isolate endophytic *Trichoderma* from root, leaf as well as stalk tissues of sugarcane and the established isolates were characterized for their colony characters and growth rates. The endophytic Trichoderma isolates were further assessed for production of the two major hydrolytic enzymes viz. chitinase and cellulase.

Material and Methods

Isolation of endophytic *Trichoderma* strains: For isolation of endophytic *Trichoderma* strains from sugarcane, apparently healthy clumps of 10 sugarcane varieties (Co 1148, CoJ 64, CoS 767, CoLk 94184, Co 0238, SES 594, CoS 8436, BO 91, Co 7701, Baragua) were collected from the experimental farm of the ICAR-Indian Institute of Sugarcane Research, Lucknow, India (26°56′ N, 80°52′ E and 111 m above mean sea level). The sampling was done during the months of January-February, 2017.

For isolation of endophytes, healthy clumps of selected varieties were randomly selected in field and the whole clump was uprooted, placed in sterile zip lock pouches and brought to the laboratory. The root, stalk and leaf samples were separated from the uprooted clumps, washed under running tap water to remove all soil and then air dried.

The root, stalk and leaf samples were cut in approximately 0.5-1 cm long segments with help of a sterile blade. The cut segments were surface-sterilized by immersing them in 70% ethanol for 30 sec followed by dipping in sodium hypochlorite (4% chlorine) for 3 min. The surface-sterilized samples were rinsed thrice with sterile water and then blotted dry on sterile filter paper. A total of 60 sterilized segments for each part (leaf/ stalk or root) were then placed on Petri dishes @ 3 segments/ plate, containing *Trichoderma* selective media (TSM). The inoculated Petri plates were incubated at 28±1°C for 15 days and observed regularly for emergence of typical *Trichoderma* colonies. Typical *Trichoderma* colonies emerging from the segments were isolated, purified and subsequently maintained on potato dextrose agar (PDA) slants at 4°C.

Cultural characterization of *Trichoderma* isolates: The colony characteristics and growth rates of the isolated endophytic *Trichoderma* isolates were determined on PDA (HiMedia) following the protocol of Samuels et al.[18] The growth rate of the isolates was evaluated at four different temperatures *viz*. 25°C, 30°C, 35°C and 40°C. For the study, Petri plates (dia. 90mm) containing PDA were inoculated approximately 10 mm from the edge of the plate with a mycelial disc (5-6 mm dia.) of the isolate cut from the edge of a 3-4 day old culture.

Three replications were maintained for each isolate at each temperature and the inoculated Petri plates were incubated in darkness at 25°C, 30°C, 35°C and 40°C. The colonies were examined regularly at 24 h intervals and thecolony radius was measured from the edge of the inoculum plug after 72 h at all temperatures. Besides growth at different temperatures, observations were also recorded on colony appearance and production of pigmentation in agar.

Enzymatic assay for cellulase and chitinase production by *Trichoderma* isolates: The cellulase producing potential of the 29 *Trichoderma* isolates was assessed using the carboxymethyl cellulose plate assay[5]. A basal medium containing g L^{-1} amended with carboxymethyl cellulose was prepared, sterilized and poured into 90 mm Petri plates. Petri plates were then inoculated in the center with *Trichoderma* isolates and the inoculated plates were incubated for 7 days at 28±1°C with three replications for each isolate. The diametrical growth of the different *Trichoderma* isolates was measured after 7 days.

A 10 ml aliquot of congo red dye (1% solution) was then added to each plate. After 30-45 min, the dye was removed and the plates were de-stained by washing with 10 ml of 1M sodium chloride for 15-20 min. Cellulase production was indicated by the appearance of a pale halo with orange edges indicative of areas of hydrolysis. The diameter of the halo zone was measured and the enzymatic index (EI) was calculated as:

EI = diameter of hydrolysis zone/ diameter of colony

For chitinase assay, 34 *Trichoderma* isolates were selected representing 12 isolates established in the present study and

22 isolates established in previous study. The selected isolates were evaluated for chitinase production following the method of Agrawal and Kotasthane.[1] A chitinase detection medium, amended with bromocresol purple, was prepared, sterilized and poured into 90 mm Petri plates. After solidification, plates were inoculated in the center with a 5 mm diameter plug of *Trichoderma* isolates with three replications for each isolate.

Inoculated plates were incubated at 28±1°C and observed at 24 h intervals for formation of purple coloured zone[1]. The diameter and intensity of the purple zone after 7 days were recorded and the isolates were categorized as (i) high chitinase activity: colour change to dark purple and >50 mm zone diameter after 5 days (ii) low chitinase activity: colour change to light purple and <50 mm zone diameter after 5 days and (iii) no chitinase activity: no colour change visible.

Results and Discussion

In the present study, a total of 29 endophytic *Trichoderma* isolates were established from root, leaf and stalk tissues of 10 different sugarcane varieties using Trichoderma selective medium (TSM). The isolates were designated as SER-31 to SER 44 (for isolates established from root tissue), SES-1 to SES-13 (for isolates established from stalk tissue) and SEL-6 and SEL-7 (for isolates established from leaf tissue). The results clearly revealed that there was considerable variability in recovery of endophytic *Trichoderma* from different plant parts as well as across different varieties (Table 1).

Overall, 14 isolates were established from root tissues of the different varieties followed by 13 isolates recovered from stalk tissues and two isolates from leaf tissues. In case of root tissues, endophytic *Trichoderma* was isolated from only four out of the 10 varieties with maximum isolates recovered from roots of variety Co 1148 (11 no.). One leaf isolate each was established from varieties CoS 767 and Co 7701. Stalk isolates were recovered from four varieties with the highest recovery again recorded from variety Co 1148 (6 no.) followed by CoJ 64 (4 no.). Overall, maximum number of endophytic *Trichoderma* isolates was established from the variety Co 1148 (17 isolates).

In our previous study we had attempted to isolate endophytic *Trichoderma* from root and leaf tissue of nine of the same varieties, as taken in the current study, from samples collected during the months of July to September, 2016.[12] In general, our current findings on *Trichoderma* recovery from different varieties and plant parts are in accordance with observations made in our previous study in which a total of 32 isolates were established. In both years, highest number of *Trichoderma* isolates were established from root tissues while recovery from leaf tissue was very poor (Table 1).

Also, the maximum number of isolates was established from the variety Co 1148 in both studies.

Table 1
Endophytic *Trichoderma* isolates established from root, leaf and stalk tissue of different sugarcane varieties.

S.N.	Variety	Root		Leaf		Stalk		Total
		Number*	Isolates	Number	Isolates	Number	Isolates	
1.	Co1148	11	SER 31, SER32, SER33, SER34, SER35, SER36, SER37, SER38, SER39, SER40, SER41	0	-	6	SES1, SES2, SES3, SES4, SES5, SES6	17
2.	CoJ 64	1	SER42	--	-	4	SES7, SES8, SES9,SES 10	5
3.	CoS 767	-	-	1	SEL6	-	-	1
4.	SES 594	-	-	-	-	-	-	-
5.	CoS 8436	-	-	-	-	-	-	-
6.	Bo 91	-	-	-	-	-	-	-
7.	Co 0238	1	SER43	-	-	-	-	1
8.	CoLk 94184	1	SER44	-	-	1	SES13	2
9.	Co 7701	--	-	1	SEL7	--	-	1
10	Baragua	--	-	--	-	2	SES11, SES12	2
	Total	14		2		13		29

However, in the previous study, *Trichoderma* isolates were recovered from root tissue of all nine varieties but in the current study, we could establish isolates from roots of only five of the ten varieties. Similarly, in isolations from leaf tissue, we recovered *Trichoderma* from only two varieties as compared to three varieties in previous study.

Moreover, the recovery of *Trichoderma* from both root as well as leaf tissue was higher in the previous study (27 root isolates + 5 leaf isolates) as compared to the present study (14 root isolates + 2 leaf isolates). In the previous study, isolations were attempted from samples collected during the months of July to September, while in the current study isolations were made from samples collected during January to February. These findings indicate that population of endophytic *Trichoderma* may be influenced by the sampling time.

Rather et al[16] had made similar observations on recovery of fungal endophytes from *Asparagus racemosus* and *Hemidesmus indicus* and reported that endophyte recovery from plant tissue exhibited seasonal variations with highest recovery recorded during wet periods. In our studies also, higher isolation rates were observed from samples collected during July to September (wet months) as compared to January. Our findings further validate the observations made in our previous study that *Trichoderma* spp. exhibit tissue specificity and show preferential colonization of root tissues as compared to other parts as indicated by the higher recovery of Trichoderma from roots in both studies.

The colony characters and growth rates of 29 endophytic *Trichoderma* isolates established in the present study were assessed on PDA after 72 h. We observed considerable variability across the endophytic *Trichoderma* isolates with respect to their growth at different temperatures (Table 2). At 25°C, the growth ranged from <20 mm to almost 60 mm in different isolates. Most of the isolates showed faster growth with an increase in temperature to 30°C which ranged from 30 mm to > 60 mm. Only two isolates viz. SES1 and SES-7 showed a slight decline in growth with increase in temperature from 25°C to 30°C.

In general, for most isolates, a decline in growth was observed with increase in temperature beyond 30°C. At 35°C, 18 out of the 29 isolates did not show any growth while 9 isolates exhibited growth of > 50 mm after 72 h. With further increase in temperature to 40°C, there was a considerable decline in growth; even the isolates were exhibiting good growth at 35°C. At this temperature, 21 isolates failed to grow completely while for remaining eight isolates, the growth recorded was quite low ranging from 20 to 40 mm.

Overall, the results indicated that optimum growth temperature for most isolates was around 30°C, the exceptions being isolates SER 33, 36, 37 39 and SES-5, 6, 8 and 12 which showed similar growth at 35°C also indicating that their optimum growth temperature was above 30°C. There was not much variability in the colony characters among the isolates except in the production of pigmentation in agar. In general, the conidial colour change was observed from white to varying shades of green and sometimes from white to yellow green with most isolates showing conidia formation by 48 h which turned green within 72 h.

Table 2
Growth of endophytic *Trichoderma i*solates at different temperatures on PDA after 72 h

S.N.	Growth Range	Growth of *Trichoderma* isolates at different temperatures			
		25°C	**30°C**	**35°C**	**40°C**
1	>60 mm	----	SER-32, 33, 36, 37, 38, 39, 40, 44 SES-5, 8, 10 SEL-6	SER-33, 36, 37, 39 SES-5, 6, 8, 12	---
2	50-59.9 mm	SER-39 SES-1, 2, 3, 4, 5, 7, 10 SEL-6, 7	SER-31, 34, 35, 41, 42, 43 SES-2, 3, 4, 6, 9, 11, 12 SEL-7	SER-44	----
3	40-49.9 mm	SER-32, 34, 35, 36, 37, 38, 43, 44 SES-6, 8, 12	SES-13	---	----
4	30-39.9 mm	SER-33, 40, 41 SES-9, 11, 13	SES-1, 7	---	SER-39 SES-12
5	20-29.9 mm	SER-31	---	---	SER-36, 37, 44 SES-5, 6, 8
6	< 20 mm	SER-42	---	SER-31, 35	----
7	No Growth	----	---	Remaining 18 isolates	Remaining 21 isolates

Table 3
Colony characters of *Trichoderma* isolates at 30°C

S.N.	Pigmentation in PDA	Production of diffusible pigments by endophytic *Trichoderma* isolates on PDA at 30°C					
		Roots		Leaves		Stalks	
		Isolates	No.	Isolates	No.	Isolates	No.
1	Yellow Pigmentation	SER-31, SER-32, SER-33, SER-34, SER-35, SER-38, SER-40, SER-41, SER-42, SER-43	10	SEL- 6	1	SES-1, SES-2, SES- 3, SES-7, SES- 9, SES-10, SES-11, SES- 13	8
2	Bright Yellow-Green Pigmentation	SER-36, SER-37, SER-39, SER-44	4	--	0	SES-5, SES-6, SES-8, SES-12	4

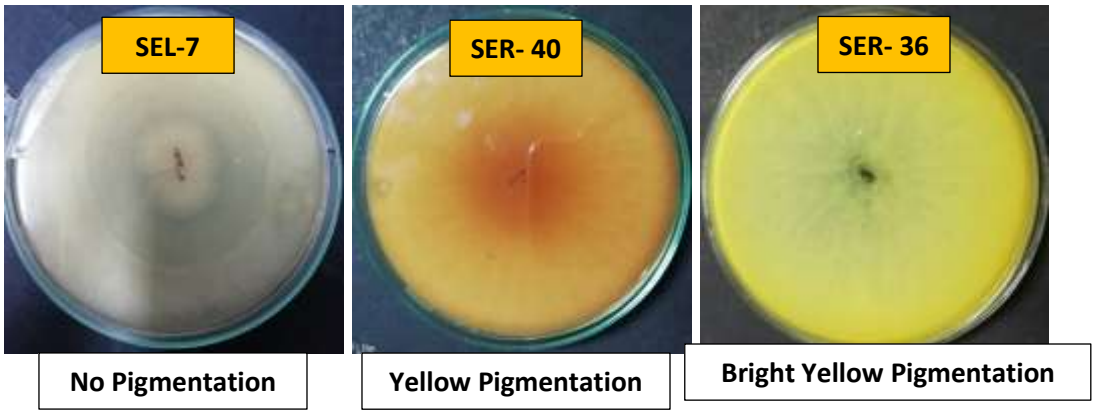

Fig. 1: Production of diffusible pigment in agar by *Trichoderma* isolates

The production of diffusing pigment in agar was recorded in colonies grown on PDA at 30°C (Table 3). Twenty seven out of the 29 isolates showed production of diffusing pigment in agar in varying shades of yellow (Fig. 1). Out of the 27 isolates, 19 isolates showed production of light to dark yellow pigmenting agar, while rest eight isolates showed production of distinctive bright yellow-green pigmentation in agar (Fig. 1).

In case of *Trichoderma*, colony character like production of diffusing pigment in agar along with growth rate at different temperatures (especially at 35°C and 40°C) has been effectively used for preliminary identification of somespecies.[4,17] Very bright yellow pigmentation in media along with the ability to grow at 40°C is reported as a distinctive character of *Trichoderma* strains belonging to the species under group *Longibrachiatum*[22].

In the present study also, we observed that eight isolates could grow at 40°C and the same eight isolates also produced bright yellow pigmentation on PDA indicating that these isolates (SER-39, SES-12, SER-36, SER-37, SER-44, SES-5,SES- 6, SES-8) belong to *T. longibrachiatum*. Several species of *Trichoderma* belonging to the group *Longibrachiatum* are reported to be highly effective producers of cellulase enzymes.

In the cellulase assays, out of the 29 isolates evaluated, cellulase production, as indicated by formation of a halo zone was recorded in 20 isolates (Table 4, Fig. 2). The enzymatic index of the isolates ranged from 1.0 to 1.2. *Trichoderma* spp. are very effective producers of cellulase[21]. Members of this genus have been exploited industrially for their cellulose producing potential and additionally they play a major role in the ecosystem by recycling cellulose. Sugarcane produces large quantities of dry leaves (trash) annually and it was observed by Yadav et al[24] that trash mulching along with inoculation of a biocontrol strain of *T. viride* in sugarcane ratoon crop was more effective than trash mulching alone in increasing soil organic matter and nutrient status.

The application of *Trichoderma* strains having potential to produce cellulase may give an added advantage for trash decomposition as opposed to the application of biocontrol strains, which may or may not be effective cellulase producers. Based on the results of the chitinase assay, the isolates were categorized as high, low or non-chitinase producing isolates.

We observed considerable variability in the chitinase producing potential of the *Trichoderma* isolates (Table 4, Fig. 3). Out of the 34 isolates evaluated, high chitinase production was observed in 11 isolates (9 root endophytes, one each of leaf and stalk endophyte), low chitinase production observed in 6 isolates (2 root endophyte, 3 stalk endophyte, 1 leaf isolate) while 17 isolates showed no chitinase activity (Table 4). Our results are in accordance

with previous studies reporting that the chitinase producing potential of *Trichoderma* varies considerably among different strains[1,8].

The production of chitinase enzyme is attributed to be a major factor determining the mycoparasitic activity and biocontrol potential of *Trichoderma* strains. In case of sugarcane also, previous studies have reported the involvement of *Trichoderma* chitinolytic enzymes in the suppression of *Colletotrichum falcatum* Went, the causal agent of red rot disease of sugarcane[23].

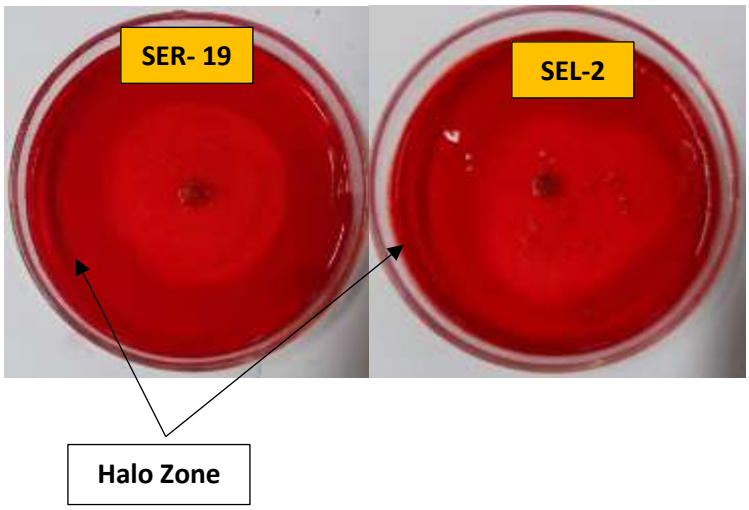

Fig. 2: Cellulase production by *Trichoderma* isolates as indicated by halo zone

Fig. 3: Chitinase production by *Trichoderma* isolates

Table 4
Cellulase and Chitinase production by endophytic *Trichoderma* isolates.

		Isolates	Numbers
Cellulase production	EI= 1.0	SER31, SER35, SER37, SER40, SER41, SER42, SER43, SER44, SES1, SES 9, SES 10, SES 11, SEL6, SEL7	14
	EI= 1.1	SER39, SES4, SES7, SES8, SES13	5
	EI= 1.2	SER33	1
	No production	SER32, SER34, SER36, SER38, SES2, SES3, SES5, SES6, SES12	9
Chitinase production	High chitinase activity	SER4, SER6, SER8, SER17, SER18, SER19, SER30, SER40, SER41, SES4, SEL6	11
	Low chitinase activity	SER2, SER39, SES6, SES11, SES13, SEL5	6
	No chitinase activity	SER1, SER5, SER10, SER11, SER13, SER16, SER21, SER24, SER25 SES5, SES7, SES12, SEL1, SEL2, SEL3, SEL4, SEL7	17

Conclusion

The promising isolates identified for chitinase and cellulase production in this study can be further exploited for disease management and *in situ* trash decomposition under the sugarcane agro-ecosystem.

Since most economically important sugarcane diseases are set borne in nature, use of endophytic strains may provide added advantage as these strains are already adapted to the niche where they have to survive and target the pathogen.

Acknowledgement

Authors wish to thank the Director, ICAR-Indian Institute of Sugarcane Research, Lucknow for the encouragement and the facilities extended for this work.

References

1. Agrawal T. and Kotasthane A.S., Chitinolytic assay of indigenous *Trichoderma* isolates collected from different geographical locations of Chattisgarh in central India, *Springer Plus*, **1**, 73 **(2012)**

2. Bailey B.A., Bae H., Strem M.D., Crozier J., Thomas S.E., Samuels G.J., Vinyard B.T. and Holmes K.A., Antibiosis, mycoparasitism and colonization success for endophytic *Trichoderma* isolates with biological control potential in *Theobroma cacao*, *Biol. Control*, **46**, 24-35 **(2008)**

3. Benoliel B., Torres F.A.G. and De Moraes L.M.P., A novel promising *Trichoderma harzianum* strain for the production of a cellulolytic complex using sugarcane bagasse in natura, *Springer Plus*, doi:10.1186/2193-1801-2-656, **2**, 656 **(2013)**

4. Boureghda H., Bouznad Z. and Decock C., Cultural and molecular characterizations of some isolates of *Trichoderma* spp., *Arab. J. Plant Prot.*, **26**, 75-80 **(2008)**

5. Bradner J.R., Gillings M. and Nevalainen K.M.H., Qualitative assessment of hydrolytic activities in Antarctic microfungi grown at different temperatures on solid media, *World J. Microbiol. Biotechnol.*, **15**, 131–132 **(1999)**

6. Chagas L.F.B., Chagas A.F., De Carvalho M.R., Miller L., De O. and Orozco Colonia B.S., Evaluation of the phosphate solubilization potential of *Trichoderma* strains (Trichoplus JCO) and effects on rice biomass, *J. Soil Sci*., **15(3),** 794-804 **(2015)**

7. Ezzi M.I. and Lynch J.M., Biodegradation of cyanide by *Trichoderma* spp. and *Fusarium* spp. Enzyme, *Microb. Technol*., **36**, 849–854 **(2005)**

8. Gajera H.P. and Vakharia D.N., Molecular and biochemical characterization of *Trichoderma* isolates inhibiting a phytopathogenic fungi *Aspergillus niger* Van Tieghem, *Physiol. Molec. Plant Pathol.*, **74**, 274-282 **(2010)**

9. Harman G.E., Multifunctional fungal plant symbionts: new tools to enhance plant growth and productivity, *New Phytol.*, **189,** 647-649 **(2011)**

10. Harman G.E., Lorito M. and Lynch J.M., Uses of *Trichoderma* spp. to remediate soil and water pollution, *Adv. Appl. Microbiol*., **56**, 313–330 **(2004)**

11. Hidangmayum A. and Dwivedi P., Plant Responses to *Trichoderma spp*. and their Tolerance to Abiotic Stresses, *J. Pharmacogn. Phytochem.*, **7(1),** 758-766 **(2018)**

12. Joshi D., Gupta J., Mishra A., Upadhyay M., Holkar S.K. and Singh P., Distribution, composition and bioactivity of endophytic *Trichoderma* spp. associated with sugarcane, Proc. Natl. Acad. Sci., India, Sect. B Biol. Sci. https://doi.org/10.1007/s40011-018-1036-3 **(2018)**

13. Li R.X., Cai F., Pang G., Shen Q.R., Li R. and Chen W., Solubilisation of phosphate and micronutrients by *Trichoderma harzianum* and its relationship with the promotion of tomato plant growth, *PLoS One*, **10(6),** e0130081 **(2015)**

14. Mulaw T.B., Kubicek C.P. and Druzhinina I.S., The rhizosphere of *Coffeaarabica* in its native highland forests of Ethiopia provides a niche for a distinguished diversity of *Trichoderma*, *Diversity*, **2**, 527-549 **(2010)**

15. Pawar A., Ismail S., Mundhe S. and Patil V.D., Solubilization of insoluble zinc compounds by different microbial isolates in vitro condition, *Int. J. Trop. Agric*., **33(2) (2015)**

16. Rather R.A., Srinivasan V. and Awar M., Seasonal deviation effects foliar endophyte assemblage and diversity in *Asparagus racemosus* and *Hemidesmus indicus*, *BMC Ecol.*, **18**, 52 **(2018)**

17. Samuels G.J., *Trichoderma*: Systematics, the sexual state and ecology, *Phytopathol*, **96,** 195-206 **(2006)**

18. Samuels G.J., Dodd S.L., Gams W., Castlebury L.A. and Petrini O., *Trichoderma* species associated with the green mold epidemic of commercially grown *Agaricusbisporus*, *Mycologia*, **94**,146–170 **(2002)**

19. Singh P.C. and Nautiyal C.S., A novel method to prepare concentrated conidial biomass formulation of *Trichoderma harzianum* for seed application, *J. Appl. Microbiol*., DOI: 10.1111/j.1365-2672.2012.05426.x **(2012)**

20. Smitha C., Finosh G.T., Rajesh R. and Abraham P.K., Induction of hydrolytic enzymes of phytopathogenic fungi in response to *Trichoderma viride* influence biocontrol activity, *Int. J. Curr. Microbiol. App. Sci*, **3(9),**1207-1217 **(2014)**

21. Vinale F., Sivasithamparam K., Ghisalberti E.L., Marra R., Woo S.L. and Lorito M., *Trichoderma*-plant–pathogen interactions, *Soil Biol. Biochem.*, **40**, 1-10 **(2008)**

22. Viswanathan R. et al, Disease scenario and management of major sugarcane diseases in India, *Sugar Tech*, **13**, 336–353 **(2011)**

23. Viswanathan R., Ramesh Sundar A. and Kumari S.M., Mycolytic effect of extracellular enzymes of antagonistic microbes to *Colletotrichum falcatum*, red rot pathogen of sugarcane, *World J. Microbiol. Biotechnol.*, **19**, 953–959 **(2003)**

24. Yadav R.L. et al, Trichoderma inoculation and trash management effects on soil microbial biomass, soil respiration, nutrient uptake and yield of ratoon sugarcane under subtropical conditions, *Biol. Fertil. Soils*, **45**, 461-468 **(2009)**.

(Received 28th February 2020, accepted 29th April 2020)