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Exploring biocontrol efficacy of *Trichoderma* spp. against *Fusarium sacchari*, the causal agent of sugarcane wilt

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ABSTRACT
Wilt caused by *Fusarium sacchari*, is one of the major diseases of sugarcane (*Saccharum* spp.) causing considerable economic loss. *Trichoderma* spp. are important biocontrol agents which have been exploited effectively for the management of several plant diseases including wilt in various crops. The present study was undertaken to evaluate the antagonistic potential of *Trichoderma* spp. against sugarcane wilt pathogen. A total of 103 *Trichoderma* isolates comprising of 43 isolates established from sugarcane rhizosphere soils as well as 60 endophytic isolates established from root, leaf and stalk tissue of sugarcane were assessed for their antagonistic activity against *F. sacchari* by dual culture technique. We observed considerable variability in the inhibitory activity of the 103 isolates against the pathogen with inhibition ranging from 1.4% to 27.2% (SER42). In general, the endophytic *Trichoderma* isolates were more effective in inhibiting *F. sacchari* as compared to rhizospheric isolates in dual culture studies. Twelve most promising endophytic isolates were selected and assessed further for production of soluble inhibitory metabolites against *F. sacchari* by poison food technique. Variability was observed among the *Trichoderma* isolates in inhibition of *F. sacchari* growth with inhibition ranging between 1.4% (SER 43) to 44.2% (SER10). Overall, based on the *in vitro* assays, *Trichoderma* isolate SER 10, isolated from root tissue of sugarcane, was found most promising showing 44.2% inhibition of *F. sacchari* growth in poison food and 26.3% inhibition in dual culture studies. This is the first study reporting biocontrol potential of endophytic *Trichoderma* spp. against *F. sacchari*, a causative agent of wilt in sugarcane

Keywords: endophytes, dual culture, rhizospheric

Explorando la eficacia del biocontrol de *Trichoderma* spp. contra *Fusarium sacchari*, el agente causal de la marchitez de la caña de azúcar

RESUMEN
La marchitez causada por *Fusarium sacchari*, es una de las principales enfermedades de la caña de azúcar (*Saccharum* spp.) que causa considerables pérdidas económicas. *Trichoderma* spp. es un importante agente de biocontrol que se ha explotado eficazmente para el manejo de varias enfermedades de las plantas, incluida la marchitez en varios cultivos. El presente estudio se realizó para evaluar el potencial antagonista de *Trichoderma* spp. contra el patógeno de la marchitez de la caña de azúcar. Un total de 103 aislamientos de *Trichoderma* que comprenden 43 aislamientos establecidos a partir de suelos de la rizosfera de la caña de azúcar, así como 60 aislamientos endofíticos establecidos a partir del tejido de las raíces, hojas y tallos de la caña de azúcar, fueron evaluados por su actividad antagonista contra *F. sacchari* mediante la técnica de cultivo dual. Se observó una considerable variabilidad en la

actividad inhibidora de los 103 aislamientos contra el organismo patógeno con una inhibición que osciló entre 1.4% y el 27.2% (SER42). En general, los aislados endofíticos de *Trichoderma* fueron más efectivos en la inhibición de *F. sacchari* en comparación con los aislados rizosféricos en estudios de cultivo dual y se seleccionaron 12 aislados endofíticos prometedores que se evaluaron más a fondo para la producción de metabolitos inhibidores solubles contra *F. sacchari* mediante la técnica de medios de cultivo envenenado. Se observó variabilidad entre los aislados de *Trichoderma* en la inhibición del crecimiento de *F. sacchari* con valores que oscilaban entre el 1.4% (SER 43) y el 44.2% (SER10). En general, según los ensayos *in vitro*, *Trichoderma* SER 10, aislado del tejido de la raíz de la caña de azúcar, resultó ser el más prometedor que mostró una inhibición del 44.2% del crecimiento de *F. sacchari* en el método de medios de cultivo envenenado y un 26.3% de inhibición en los estudios de cultivo dual. Este es el primer estudio informa sobre el potencial de control biológico de *Trichoderma* spp. contra *F. sacchari*, agente causal de la marchitez en la caña de azúcar.

Palabras clave: cultivo dual, endofíticos, rizosféricos

INTRODUCTION

Sugarcane, *Saccharum officinarum* L., is a leading cash crop cultivated in many parts of the world for commercial purposes, especially in sub-tropical and tropical regions of the world (Mirajkar *et al.,* 2019). Worldwide, sugarcane is cultivated over a zone of 20.10 million hectares with a production of 1 318.10 million tones and productivity of 65.5 tons per hectare. Sugarcane area and profitability contrast broadly from country to country. Brazil commands the highest area (5.34 million hectares) while Australia has the highest productivity (85.1 t ha^{-1}).

India positions second among the sugarcane developing nations of the world in both area and productivity after Brazil with an area of 4.94 million hectares under cultivation and average yield of 78 tons per hectare (Verma and Solanki, 2020). However, diseases caused by various fungi, bacteria, phytoplasma and viruses pose a serious threat to sugarcane cultivation in all the sugarcane growing regions of India (Rao *et al.*, 2002; Vishwanathan and Rao, 2011). Among the various fungal diseases; wilt, smut and red rot are of major economic importance imposing severe direct and indirect limitations to sugarcane cultivation throughout the country (Viswanathan and Rao, 2011).

Wilt of sugarcane was first reported in 1913 (Butler and Khan, 2013). Over the years various researchers have reported different fungal species to be associated with sugarcane wilt like *Fusarium sacchari, F. proliferatum* and *F. moniliforme*. However, Vishwanathan *et al*. (2011) identified *F. sacchari* as the fungal species most frequently associated with wilt based on molecular studies. The predominant mode of transmission of this disease is through infected setts at time of planting, but the inoculum can also survive in soil for up to 3 years. Sugarcane wilt causes reduction in germination and in severe cases, total loss of cane yield may also occur due to drying up and wilting of the stalks. Agnihotri and Rao (2002) observed wilt as one of the major fungal diseases of sugarcane next to red rot in causing economic losses. Studies have reported that economic losses due to wilt can go up to 65% (Sharma, 1976). Besides reduction in cane yield, this disease also results in reduced juice extraction (15-30%) and sugar recovery (20%). Moreover, it has been observed that combined infection of red rot and wilt can cause much higher damage to crop than the individual infection of these diseases (Viswanathan *et al*., 2012; Bhat *et al*., 2016).

To date, the management of sugarcane wilt has primarily been done by replacing and/ or removing susceptible sugarcane varieties with new resistant varieties for cultivation. However, due to frequent breakdown of resistance, the varieties have to be replaced continuously. Use of fungicides has also not yielded desired results for wilt management besides being deleterious to environment and human health (Jahanshir and Dzhalilov, 2010).

In this scenario, use of biocontrol agents like *Trichoderma* offers a viable and ecofriendly option for disease management. *Trichoderma* spp. are cosmopolitan in occurrence. They are commonly found in all types of soil both as free living fungi as well as in endophytic association with crops (Bae *et al.,* 2011;

Harman *et al.,* 2004). To date, these fungi have been explored extensively for management of several diseases in diverse crops (Sharma *et al.,* 2014). In sugarcane, this fungus has been successfully explored for management of red rot of sugarcane caused by *Colletotrichum falcatum* Went (Joshi *et al*., 2016; Joshi *et al*., 2019). Besides, several strains of *Trichoderma* have been identified against *Fusarium* wilt of various other crops like eggplant (*Solanum melongena* L.) (Abdel-Moraim *et al.,* 2014), chilli (*Capsicum annum* L.) (Bhat *et al.,* 2016), tomato (*Solanum lycopersicum* L.) (Sundarmoorty and Balabaskar, 2013), etc.

In case of sugarcane, some preliminary studies with limited number of rhizospheric *Trichoderma* isolates have been conducted previously. Gawade *et al*. (2012) evaluated six *Trichoderma* isolates from rhizosphere soil against wilt of sugarcane and reported that all six strains showed antagonistic activity against *F. moniliforme*. The study also revealed that different isolates within same species showed different degree of inhibition. Similarly, Sabalpara *et al.* (2009) also assessed *in vitro* biocontrol potential of seven *Trichoderma* isolates against *F. moniliforme*, associated with sugarcane wilt and identified promising isolates. Similarly, Jena and Panigrahi (2017) also tested *Trichoderma* isolates against wilt in sugarcane. They identified different species of *Trichoderma* like *T. viride, T. harzianum* and *T. pseudockei* which showed antagonistic activity against *F. moniliforme* with *T. viride* exhibiting complete inhibition of *F. moniliforme.* However, in all these studies very limited number of *Trichoderma* isolates (six to seven only) have been evaluated for their antagonistic potential and the assessed isolates are restricted to those isolated from rhizospheric soils only. Moreover, in these studies *Trichoderma* isolates have been evaluated against *F. moniliforme*, as opposed to *F. sacchari* which has been recently established as the causal agent of sugarcane wilt based on molecular studies (Viswanathan *et al.,* 2011).

In recent times, there has been major thrust on exploring various endophytic microbes including *Trichoderma* spp. for management of various plant diseases (Moghaddam and Soltani, 2013; Mulaw *et al.,* 2013; Bailey *et al.,* 2008). In the case of sugarcane wilt in particular, endophytic *Trichoderma* isolates can provide added advantage compared to rhizospheric isolates for disease management, since wilt is primarily sett borne in nature and as such, endophytic *Trichoderma* strains, which will be already adapted to the ecological niche where they have to function to suppress the plant pathogen i.e. within plant tissue, may be more successful as biocontrol agents as compared to rhizospheric isolates for wilt management. Also, evaluating a large number of isolates can increase chances of identifying highly potent antagonistic strains for further use.

Keeping these aspects in mind, the present study was undertaken to evaluate a substantive number of both rhizospheric and endophytic *Trichoderma* isolates against sugarcane wilt pathogen *F. sacchari* by different screening techniques *in vitro* and to identify potent isolates for further studies.

MATERIALS AND METHODS

Isolation and identification of Fusarium sacchari

For isolating *Fusarium sacchari*, the stalks of sugarcane variety Co 7717 showing symptoms of wilt were collected during October 2018 from the research farm of ICAR-Indian Institute of Sugarcane Research, Lucknow, India (GPS coordinates of the location: 26°56¹ N, 80°52¹ E and 111 m above mean sea level).

The collected stalks were washed with running tap water to remove adhering mud and soil particles and the infected canes were split open longitudinally with a sharp knife. The internal pith portion of internodes showing typical wilt symptoms was cut into small segments. The segments were surface disinfected with 95% ethanol for 30-45 seconds followed by washing thrice with sterile distilled water. The disinfected tissue was dried in a laminar flow on sterile filter paper and then plated on Petri dishes previously poured with Potato Dextrose Agar (PDA) (HiMedia) with three pieces per plate. The Petri dishes were incubated at 28±1°C for 7-8 days and monitored on routine basis for growth of the hyphae from the tissue margins. Growing hyphal tips were picked from typical colonies of *Fusarium*, and transferred to fresh PDA plates and maintained on PDA. These isolates produced abundant white mycelia on

the PDA medium for three days. The colors of the colonies on both sides of the petri dishes were determined according to Summerell *et al.* (2003). Morphological characteristics of the fungus largely matched the descriptions in The *Fusarium* Laboratory Manual (Leslie and Summerell, 2006) Further molecular confirmation of the isolate was performed as per O'Donnell *et al.* (1998). Briefly, DNA was isolated from the 50 mg grown mycelium and Translation elongation factor 1-alpha (TEF1-α) gene was amplified using primer pairs of EF1/EF2 (O'Donnell *et al*., 1998). *F. sacchari*, colonies were purified on PDA medium and preserved at 4°C for further antagonistic studies.

Trichoderma isolates

One hundred and three *Trichoderma* isolates were used in the present study. Forty three of the selected isolates had been previously established from sugarcane rhizosphere soils collected from various geographical regions and farming systems (conventional/ organic) of sub-tropical India (Joshi and Misra, 2013; Joshi *et al*., 2019). In addition, 60 isolates of endophytic *Trichoderma*, established from root, stalk and leaf tissue of 10 different sugarcane varieties were also selected for the present study (Joshi *et al.,* 2018). For further reference the rhizospheric isolates are designated as STr-1 to STr-126 while endophytic isolates are designated as SER1 to SER44 for root endophytes, SEL1 to SEL7 for leaf endophytes and SES1 to SES 13 for stalk endophytes. The source of the selected *Trichoderma* isolates is given in table 1.

Evaluation of antagonistic activity of *Trichoderma* isolates

Screening by Dual Culture Method

Antagonistic effect of *Trichoderma* isolates against *F. sacchari* was evaluated using the dual culture technique (Dennis and Webster, 1971a). Briefly, 6 mm discs of the pathogen were cut from the edge of a 6-7 days old culture and placed at the edge of Petri dishes, previously poured with PDA.

Another disc cut from the edge of a 3-4 days old colony of test *Trichoderma* isolate was inoculated in the opposite direction of pathogen disc. The plates were incubated at 28±1 ºC for 72 hours with three replications of each *Trichoderma* isolate.

Table1. Source of the 103 *Trichoderma* isolates selected in the study.

Sources*	Isolates Number
	Rhizospheric Isolates
IISR Farm Lucknow (U.P)-C	STr-1, 3, 8, 10, 12.15, 21, 23, 26, 28, 29, 30, 35, 44, 74, 117, 118, 119, 120, 122, 123, 125, 126
IISR Farm Lucknow (U.P)-O	STr-80, 81, 83, 85, 88, 90, 91, 93, 94, 96, 108, 114, 116, 121, 124
Motipur (Bihar)-C	STr-52
Gajraula (U.P)-C	STr-64
Palia (U.P)-C	STr-72
Deoband (U.P)-C	STr-47, 79
	Endophytic isolates
Root Tissue (40 Isolates)	SER1, SER2, SER3, SER4, SER5, SER6, SER7, SER8, SER9, SER10, SER11, SER13, SER15, SER16, SER17, SER18, SER19, SER20, SER21, SER22, SER23,SER24, SER25, SER28, SER29, SER30, SER31, SER32, SER33, SER34, SER35, SER36, SER37, SER38, SER39, SER40, SER41, SER42, SER43, SER44
Leaf Tissue (7 Isolates)	SEL1, SEL2, SEL3, SEL4, SEL5,SEL6, SEL7
Stalk Tissue (13 isolates)	SES1, SES2, SES3, SES4, SES5, SES6, SES7, SES8, SES9. SES10, SES11, SES12, SES13

**C- Conventional Farming, O- Organic Farming*

Inoculated Petri plates with pathogen alone was maintained as a control. Radial growth of the pathogen was recorded after 72 h for both dual culture and control plates. The radial growth inhibition of pathogen by different isolates of *Trichoderma* was calculated as Inhibition % (I) by the formula: Inhibition % (I) =((R1-R2)/R1) × 100, where R1: Radial growth of pathogen in control and R2: Radial growth of pathogen in treatment.

Screening by Poison Food Technique

Based on results of Dual culture technique, superior isolates of endophytic *Trichoderma* were further evaluated for production of soluble inhibitory metabolites against *Fusarium sacchari* by using the poison food technique (Dennis and Webster, 1971b). Minimal salts broth was used as culture media for production of soluble inhibitory metabolites by *Trichoderma* (Srinivasan *et al*., 1992). Conical flasks (150 ml) with 100 ml minimal salts broth were inoculated with 1 ml of a spore suspension (10^6 cfu ml^{-1}) of *Trichoderma* isolate prepared in sterile distilled water. These flasks were then incubated at 28 ± 1 °C.

After 20 days mycelium was removed by filtration using filter paper (Whatman No. 1) and the obtained culture filtrate was passed through a 0.22 µm membrane disposable filter unit (Whatman) for removal of spores. Culture filtrate obtained after filtration was added in potato dextrose agar (PDA) medium just before pouring in Petri plates to get a final concentration of 20% culture filtrate. After solidification a disc (5 mm) was cut from 5 days old culture of *F. sacchari* and inoculated in the center of the PDA poured plates. Control plates were amended with similar amount of un-inoculated minimal salts broth. For each isolate, four replications were maintained at 28 ± 1°C and *Fusarium* sp. isolate colony diameter after 7 days was recorded in all plates. Percent inhibition in growth was calculated as: Inhibition % (I) =((R1-R2)/R1) × 100, where R1: Radial growth of pathogen in control and R2: Radial growth of pathogen in treatment.

RESULTS AND DISCUSSION

With regard to the macroscopic characteristics of the fungus, the color of the colony on PDA was initially orange-yellow and became purple-white when aged. On PDA, the growth was rapid with abundance of mycelia and the colony exhibited cottony hyphal growth (Figure 1 a, b). Colonies reached 2.3-3.7 cm in diameter at 25 °C and 2.1-3.4 cm at 30 °C after 3 days on PDA. Morphological characteristics of the isolated fungus were consistent with the description of *Fusarium sacchari*, which is a member of the *Gibberella fujikuroi* species complex (Leslie and Summerell, 2006). Abundant microconidia were observed in the aerial mycelium and were oval to ellipsoidal in shape and aseptate. Macroconidia, were slender or slightly falcate and three to five septate. The identification of *F. sacchari* was done based on the taxonomic guidelines by Leslie and Summerell (2006). For molecular identification of the putative *Fusarium* isolate, TEF1-α gene specific primer was used which resulted in an amplicon of 656 base pair size (O'Donnell *et al*., 1998).

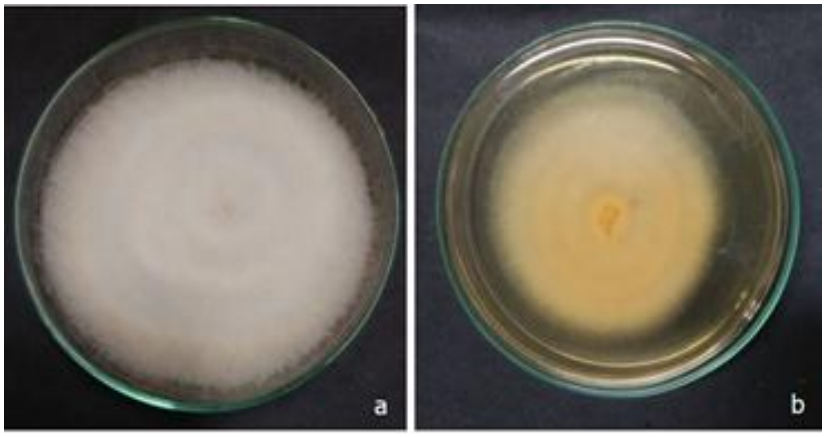

Figure 1. *Fusarium sacchari* on PDA. (a) Pure culture (b) Pigmentation.

Evaluation of antagonistic potential of *Trichoderma* isolates against *F. sacchari*

The two evaluation techniques (dual culture and poison food technique) applied to assess the inhibitory potential of *Trichoderma* isolates on the growth of *F. sacchari*. shown results (Table 2 and Table 3). In dual culture technique, considerable variability was observed in the inhibition of *F. sacchari* growth by the 103 *Trichoderma* isolates which ranged from a highest inhibition of 27.2% by isolate SER42 to lowest inhibition of 1.4% observed in isolate SER11 (Table 2, Figure 2).

On examining the inhibitory activity of the rhizospheric and endophytic *Trichoderma* isolates separately, it was observed that among the 43 rhizospheric isolates, the inhibitory percentage ranged between 1.6% (STr-64) to 26.1% (STr-29 & STr-80). Thirteen rhizospheric isolates exhibited inhibition in *F. sacchari* growth between the range of 20 to 30% while in 26 isolates inhibition was recorded in range of 10 to 20% and the remaining four isolates showed very poor inhibitory activity of < 10%.

In case of the 60 endophytic *Trichoderma* isolates, highest inhibition of 27.2% was recorded in isolate SER42 (a root endophyte). Thirty endophytic *Trichoderma* isolates (16 from roots, five from leaf and nine from stalk) showed inhibition ranging between 20 to 30% against *F. sacchari* while 19 isolates (13 from root, two from leaf and four from stalk) showed inhibition between 10 to 20%. The remaining 11 isolates (all isolated from root tissue) showed <10% inhibition in *F. sacchari* growth.

Overall, it was observed that endophytic isolates were more effective in suppressing *F. sacchari* growth than rhizospheric isolates as indicated by the fact that almost 50% (30 out of 60) of the endophytic isolates showed >20% inhibition in *F. sacchari* growth as compared to only 30% (13 out of 43 isolates) of the rhizospheric isolates showing >20% inhibition.

Since in dual culture studies endophytic isolates were observed to show higher inhibitory activity against *F. sacchari* than rhizospheric isolates, 12 endophytic isolates (five from roots, two isolates from leave and five isolates from stalk) were selected for further studies and examined for production of soluble inhibitory metabolite against *F. sacchari*.

All the selected 12 isolates had exhibited >20% inhibition in *F. sacchari* growth in dual culture studies (Table 3, Figure 3). The results of the study revealed significant variability across the 12 isolates. The inhibition percentage varied from 1.4% (SER 43) to 44.2% (SER10). Eight out of the 12 isolates showed inhibition percentage less than 11% while three isolates (SER15, SER42, SES12) exhibited inhibition between 20% to 29.9% and one isolate (SER10) showed highest inhibition of 44.2%. Overall, four of the 12 isolates showed >20% inhibition in *F. sacchari* growth in poison food technique (Table 3, Figure 3).

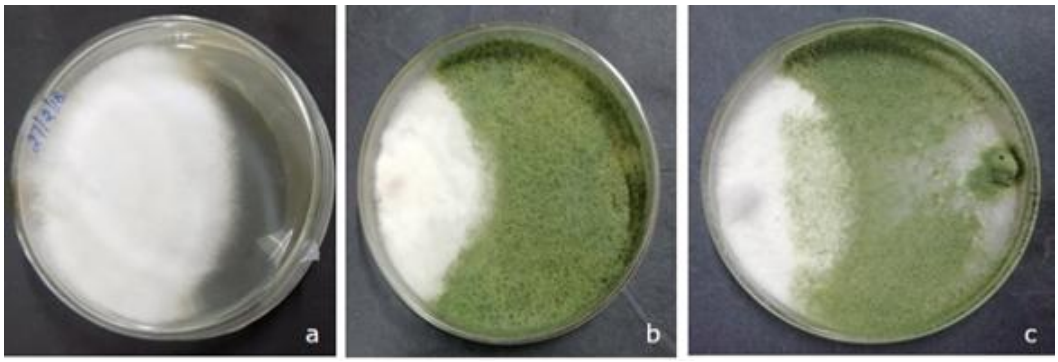

Figure 2. Antagonistic activity of endophytic *Trichoderma* against *Fusarim sacchari* in dual culture studies, (a) Control Plate, (b) Trichoderma SER 32 isolate showing antagonistic activity, (c) Overgrowth of *Trichoderma* over *Fusarium.*

Table2. Antagonistic activity of rhizospheric and endophytic *Trichoderma* isolates against *Fusarium sacchari* in dual culture assay.

Inhibition Percentage	Endophytic Isolates	Number	Rhizospheric Isolates	Number
20%-29.9%	SER4, SER5, SER10, SER15, SER16, SER17, SER19, SER21, SER23, SER32, SER33, SER36, SER38, SER41, SER42, SER 43, SEL 1, SEL 2, SEL 3, SEL 6, SEL 7, SES1, SES2, SES3, SES4, SES5, SES8, SES10, SES11, SES 12	16 (Root) 5 (Leaf) 9 (Stalk)	STr-12, STr-21, STr-23, STr-29, STr-30, STr-80, STr-81, STr-85 STr-93, STr-114, STr-119, STr-120, STr-125	13
10%-19.9%	SER2, SER3, SER13, SER22, SER24,SER28,SER29,SER30, SER31, SER34, SER37, SER40, SER 44, SEL4, SEL5, SES6, SES7, SES9, SES13	13 (Root) 2 (Leaf) 4 (Stalk)	STr-3, STr-8, STr-10, STr-15, STr-26, STr-28, STr-35, STr-44, STr-47, STr-52, STr-74, STr-79, STr-83, STr-88, STr-90, STr-91, STr-94, STr-96, STr-108, STr-116, STr-117, STr-118, STr-121, STr-123, STr-124, STr-126	26
1%-9.9%	SER1, SER6, SER7, SER8, SER9, SER11, SER18, SER20, SER25, SER35, SER 39	11 (Root)	STr-1, STr-64, STr-72, STr-122	4
Total		60		43

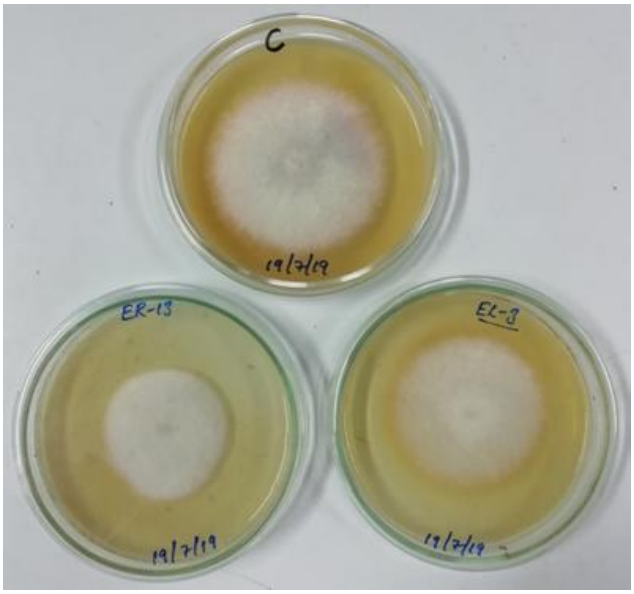

Figure 3. Inhibition of *Fusarium sacchari* by soluble inhibitory metabolites of endophytic *Trichoderma* isolates ER13 and EI-3. C-control

Overall, the results of the dual culture and poison food assay of the *Trichoderma* isolates revealed considerable variability in the inhibitory potential of the isolates in both studies. Several previous workers have also reported varying levels of inhibition of *Fusarium* spp. by both endophytic and rhizospheric strains of *Trichoderma*. Both, Sabalpara *et al.* (2009) and Gawade *et al.* (2012) had *in vitro* evaluated *Trichoderma* isolates against *F. moniliforme* and observed variability in antagonistic activity among

Table 3. Inhibition of *Fusarium sacchari* by diffusible secondary metabolite produced by endophytic *Trichoderma* isolates by Poison Food Technique.

Trichoderma Isolate	Growth inhibition (%)
SER10	44.2
SER15	21.8
SER41	4.50
SER42	21.8
SER43	1.40
SEL3	11.4
SEL6	7.27
SES1	8.14
SES2	7.31
SES4	10.0
SES10	9.70
SES12	21.8

Trichoderma isolates. Dolatabadi *et al.* (2012) reported variability in suppression of *Fusarium* spp. causing wilt of lentil by root endophytic strains of *Trichoderma*. Similarly, Taribuka *et al*. (2017) reported variability in inhibitory activity of endophytic *Trichoderma* isolates against *Fusarium* causing banana wilt.

On the basis of the results of dual culture and poison food assay, isolate SER10 was identified as the most potent isolate in the study exhibiting high inhibition in *F. sacchari* growth in both the assays. *Trichoderma* species are well documented biological control agents which have been previously explored for management of wilt caused by *Fusarium* spp. in various crops. Sundarmoorthy and Balabaskar (2013) evaluated the efficiency of *Trichoderma* against *F. oxysporum*, causing wilt disease in tomato (*Solanum lycopersicum* L.) and observed it to be effective. Similarly, Adhikary *et al.* (2017) stated that pre inoculation application of *Trichoderma* significantly reduced the severity of the wilt disease in eggplant (*Solanum melongena* L.). Anuragi and Sharma (2016) evaluated *Trichoderma* isolates *in vitro* against *F. oxysporum*, causal agent of wilt disease in chickpea and observed that *T. reesei* showed the strongest antagonistic activity against *Fusarium* in dual cultured followed by *T. viride* and *T. harzianum.*

However, despite there being a number of studies exploring potential of *Trichoderma* in other crops, there have been very limited studies on evaluating *Trichoderma* against sugarcane wilt. A few of these previous studies, the focus has been on evaluating antagonistic potential of six to seven *Trichoderma* isolates against *Fusarium* sp. *in vitro* and potent isolates were identified (Sabalpara *et al.,* 2009; Gawade *et al*., 2012). The major limitation with these studies has been that they have been conducted using a very small number of rhizospheric isolates (six-seven) only and moreover, the isolates were evaluated against *F. moniliforme*, as opposed to *F. sacchari* which is now recognized as the causal agent of sugarcane wilt. Since, sugarcane wilt is primarily a set borne disease, the use of potent endophytic *Trichoderma* strains can give added advantage as compared to rhizospheric strains in managing this disease. The endophytic strains will be better adapted to establish and inhibit target pathogen within plant tissue as compared to rhizospheric strains which may function better in soil and plant rhizosphere. Several endophytic *Trichoderma* spp. has been used against *Fusarium* spp. causing wilt in crops other than sugarcane. For instance, Dolatabadi *et al*. (2012), reported suppression of *Fusarium* spp. causing wilt of lentil (*Lens culinaris* Medikus) by root endophytic strains of *Trichoderma*. Similarly, Taribuka *et al*.

(2017) evaluated endophytic *Trichoderma* isolates against *Fusarium* spp. causal agent of wilt disease in banana (*Musa* spp.).

However, this is the first study reporting evaluation of the antagonistic effect of substantive number (103) of rhizospheric as well as endophytic *Trichoderma* isolates against *F. sacchari* causing sugarcane wilt and identification of promising isolates. Moreover, we also observed that, in dual culture assays, the endophytic isolates were more effective in suppressing *F. sacchari* growth than rhizospheric isolates as indicated by the fact that almost 50% (30 out of 60) of the endophytic isolates showed >20% inhibition in *F. sacchari* growth as compared to only 30% (13 out of 43 isolates) of the rhizospheric isolates showing >20% inhibition.

As such, the potent endophytic *Trichoderma* isolates established from this study can provide advantage in case of sugarcane diseases management since endophytic strains will be better adapted to establish and inhibit target pathogen within plant tissue as compared to rhizospheric strains which may function better against soil borne pathogens. Based on the results of both the assays, *Trichoderma* isolate SER 10, isolated from root tissue of sugarcane, was found most promising showing 44.2% inhibition of *Fusarium* growth in poison food method and 26.3% inhibition in dual culture studies. However, further studies on field evaluation of this isolate for wilt management are essential to establish their efficacy and their further exploitation.

CONCLUSIONS

The present evaluation of study conducted using 103 *Trichoderma* isolates, representing both rhizospheric as well as endophytic isolates, revealed that in general, endophytic *Trichoderma* strains show higher potency in suppressing *F. sacchari* growth as compared to rhizospheric isolates. A potent endophytic *Trichoderma* SER10 strain show high inhibitory activity against wilt pathogen. The isolate needs to be explored further for wilt management in field.

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Conflict of interest

The authors have no conflict of interest to declare. The work is genuinely undertaken and the data in the paper is never published or in consideration for publication in any other journal.

Author contributions

Conceptualization DJ, Data curation MU, Formal analysis MU, Funding acquisition DJ, Investigation MU, Methodology DJ and MU, Project administration DJ, Resources DJ, Supervision AA and DJ, Validation DJ and MU, Visualization DJ and AA, Writing—original draft MU, AA and DJ, Writing—review & editing AA and DJ.

REFERENCES

Abdel-Monaim MF, Abdel-Gaid MA, Zayan SA, Naseef DMT (2014) Enhancement of growth parameters and yield components in eggplant using antagonism of *Trichoderma* spp. against *Fusarium* wilt disease. Int J Phytopathol 03(01): 33-40

Adhikary MC, Begum HA, Meah MB (2017) Possibility of recovering *Fusarium* Wilt affected eggplants by *Trichoderma.* Int J Agril Res Innov & Tech 7(1): 38-42

Agnihotri VP, Rao GP (2002) A century status of sugarcane wilt in India. In: Singh SB, Rao GP, Easwaramoorthy S (eds). Sugarcane Crop Management, pp. 145-160. Sci Tech LLC Publishing, Taxas

Anuragi M, Sharma TK (2016) Biocontrol of chickpea wilt disease by *Fusarium oxysporum* f. sp. *ciceri* with rhizopshere mycoflora. Flora and fauna 22(2): 201-209

Bae H, Roberts DP, Lim HS, Strem MD, Park SC, Ryu CM, Melnick RL, Bailey BA (2011) Endophytic *Trichoderma* isolates from tropical environments delay disease onset and induce resistance against *Phytophthora capsici* in hot pepper using multiple mechanisms. Mol Plant Microbe In 24: 336-351

Bailey BA, Bae H, Strem MD, Crozier J, Thomas SE, Samuels GJ, Vinyard BT, Holmes KA (2008)

Antibiosis, mycoparasitism, and colonization success for endophytic *Trichoderma* isolates with biological control potential in *Theobroma cacao*. Biol Control 46: 24–35

Bhat MN, Mesta R, Yenjerappa ST, Tatagar MH, Sardana HR, Singh D, Vennila S, Sabir N, Ahmad M (2016) Biological control of *Fusarium* wilt of chillies using *Trichoderma* spp. Indian J Hort 73(1): 74-77

Butler EJ, Khan AH (1913) Some new sugarcane diseases, Part I Wilt Mem Dept Agril. India Bot Ser 6: 180-190

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

Dennis C, Webster J (1971b) Antagonistic properties of species groups of *Trichoderma* II, Production of non volatile antibiotics. Trans Br Mycol Soc 57: 41-48

Dolatabadi HK, Goltapeh EM, Mohammadi N, Rabiey M, Rohani N, Varma A (2012) Biocontrol potential of root endophytic fungi and *Trichoderma* species against *Fusarium* wilt of lentils under *in vitro* and green house conditions. J Agr Sci Tech 14: 407-420

Gawade DB, Pawar BH, Gawande SJ, Vasekar VC (2012) Antagonistic effect of *Trichoderma* against *Fusarium moniliforme* the causal of sugarcane wilt. American- Eurasian J Agric & Environ Sci 12(9): 1236-1241

Harman GE, Howell CR, Viterbo A (2004) *Trichoderma* species-opportunistic, avirulent plant symbionts. Natures Review Microbiology 2: 43-56

Jahanshir A, Dzhalilov FS (2010) The Effects of Fungicides on *Fusarium oxysporum* f. sp. *lycopersici* associated with *Fusarium* wilt of tomato. J Plant Prot Res 50(20): 172-178

Jena N, Panigrahi MR (2017) Rhizobacteria and *Trichoderma* spp. The potential biocontrol agents against sugarcane wilt pathogens. Int J Res Engi App Sci 7(10): 1-9

Joshi D, Gupta J, Mishra A, Upadhyay M, Holkar SK, Singh P (2018) Distribution, composition and bioactivity of endophytic *Trichoderma* spp. associated with sugarcane. Proc Natl Acad Sci India Sect B Biol Sci 98(4): 1189-1200

Joshi D, Misra SC (2013) Characterization of *Trichoderma* Isolates from Sugarcane Agroecosystem and their efficacy against *Colletotrichum falcatum* causing red rot of sugarcane. Sugar Tech 15(2): 192-196

Joshi D, Singh P, Holkar SK, Kumar S (2019) *Trichoderma* mediated suppression of red rot of sugarcane under field conditions in subtropical India. Sugar Tech 21: 496-504

Joshi D, Singh P, Singh AK, Lal RJ, Tripathi N (2016) Antifungal potential of metabolites from *Trichoderma* sp. against *Colletotrichum falcatum* Went causing red rot of sugarcane. Sugar Tech 18(5): 529-536

Leslie JF, Summerell BA (2006) The *Fusarium* laboratory manual 1st ed. Blackwell Publishing Ltd Oxford, London

Mirajkar SJ, Devarumath RM, Nikam AA, Sushir KV, Babu H, Suprasanna P (2019) Sugarcane (*Saccharum* spp.): Breeding and Genomics. In: Al-Khayri J, Jain S, Johnson D (eds). Advances in Plant Breeding Strategies: industrial and food crops, pp. 363-406 Springer, Cham; doi: 10.1007/978-3-030-23265-8_11

Moghaddam MSH, Soltani J (2013) Bioactivity of endophytic *Trichoderma* fungal species from the plant family Cupressaceae. Ann Microbiol 64: 753–761

Mulaw TB, Druzhinina IS, Kubicek CP, Atanasova L (2013) Novel endophytic *Trichoderma* spp. isolated from healthy *Coffea arabica* roots are capable of controlling coffee tracheomycosis. Diversity 5: 750-766

O'Donnell K, Kistler HC, Cigelnik E, Ploetz RC (1998) Multiple evolutionary origins of the fungus causing Panama disease of banana: concordant evidence from nuclear and mitochondrial gene genealogies. P Natl A Sci 95: 2044-2049

Rao GP, Viswanathan R, Singh SB (2002) Current situation of sugarcane diseases in India. In: Singh SB, Rao GP, Easwaramoorthy S (Eds). Sugarcane Crop Management, pp.1-9. Sci Tech LLC Publishing, Houstan

Sabalpara AN, Priya J, Waghunde RR and Pandya JR (2009) Antagonism of *Trichoderma* against sugarcane wilt pathogen *Fusarium moniliforme*. Am-Eurasian J Sustain Agric 3(4): 637-638

Sharma MN (1976) Wilt disease of sugarcane. Sugarcane Pathol Newsl 15/16:30-33

Sharma P, Sharma M, Raja M, Shanmugan V (2014) Status of *Trichoderma* research in India: A Review. Indian Phytopath 67(1): 1-19

Srinivasan U, Staines HJ, Bruce A (1992) Influence of media type on antagonistic modes of *Trichoderma* spp. against wood decay basidiomycetes. Material und Organismen 27(4): 301-321

Summerell B, Salleh B, Laslie JF (2003) A Utilitarian Approach to *Fusarium* Identification. Plant Disease 87(2): 117-128

Sundarmoorthy S, Balabaskar P (2013) Biocontrol efficacy of *Trichoderma* spp. against wilt of tomato caused by *Fusarium oxysporum* f. sp. *lycopersici*. J App Biol Biotech 1(3): 36-40

Taribuka J, Wibowo A, Widyastuti S, Sumardiyon C (2017) Potency of six isolates of biocontrol agents endophytic *Trichoderma* against *Fusarium* wilt on banana. J Degrade Min Land Manage 4(2): 723-731

Verma LK, Solanki A (2020) Cost and Returns Analysis of Sugarcane Production in Baghpat district of Western Uttar Pradesh, India. Int J Curr Microbiol App Sci 9(1): 733-739

Viswanathan R, Poongothai M, Malathi P (2011) Pathogenic and molecular confirmation of *Fusarium sacchari* causing wilt in sugarcane. Sugar Tech 13: 68-76

Viswanathan R, Rao GP (2011) Disease scenario and management of major sugarcane diseases in India. Sugar Tech 13: 336-353

Viswanathan R, Poongothai M, Malathi P, Ramesh A (2012) Sugarcane wilt: New insights into the pathogen identity, variability and pathogenicity. In: Viswanathan R, Sundar AR (eds). Functional plant science and biotechnology, pp. 30-39. Global Science Books, Ikenobe

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