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Anti-fungal activity of *Trichoderma atroviride* against *Fusarium oxysporum* f. sp. *lycopersici* causing wilt disease of tomato

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ABSTRACT

***Fusarium oxysporum* f. sp. *lycopersici* causing tropical wilt of tomato is a destructive phytopathogen. To study bio efficacy of fungal bio agents, fifteen isolates of *Trichoderma* species were isolated from rhizosphere soil of healthy tomato plants. Among the isolates, TA12 showed higher antagonistic efficacy against the pathogen. Upon analysis of the rDNA of internal transcribed spacers (ITS) and molecular data, the isolate was identified as *Trichoderma atroviride*. The *in vitro* antagonistic assessment indicated that the *T. atroviride* isolate caused significant inhibition of *F. oxysporum* f. sp. *lycopersici*. *Trichoderma atroviride* (TA12) showed antagonistic activity against *F. oxysporum* with mycelial inhibition of 71.25%. The culture filtrates of *Trichoderma atroviride* exhibited its antifungal activity against *F. oxysporum* with a suppression of 77.77%. Moreover, the ethyl acetate extracts of *T. atroviride* TA12 showed the highest antifungal potency against *F. oxysporum* f. sp. *lycopersici*. The main bioactive constituents of *T. atroviride* were 6-pentyl - 2H-pyran-2-one, quinoline, phenol, 2-(6-hydrazino-3-pyridazinyl) and heptadecane. In conclusion, the isolate TA12 could be exploited to develop an effective biocontrol management practice for combating Fusarium wilt disease of *Fusarium oxysporum* f.sp. *lycopersici* in tomato.**

Keywords: Anti-fungal activity, *Fusarium oxysporum* f. sp. *lycopersici,* tomato and *Trichoderma* and GC-MS.

INTRODUCTION

Tomato (*Lycopersicum esculentum*) belonging to solanaceae family, ranks first and second in processing crops and vegetables respectively in India (Fatima and Anjum, 2017). It is commercially cultivated globally in both indoor and outdoor conditions. It contains lycopene, a rich source of anti-oxidant property (Miller *et al*., 2002). Cultivation of tomato becomes limited due to invasion of wide pests *viz.,* insects, diseases, weeds and nematodes which accounts for major yield loss. *Fusarium oxysporum* f. sp. *lycopersici* (Fol) infecting tomato is a destructive pathogen, causing severe economic losses all over the world. Major symptoms include yellowing of lower leaves, stunted growth, wilting of leaves and finally death of plant (Prihatna *et al*., 2018). Chemical control of plant disease management is commonly employed approach (Hirooka and Ishii, 2013). The efficiency of fungicides chiefly depends on the timing of application, method of application, disease intensity, the efficiency of disease forecasting systems and the rate of emergence of fungicide resistant strains (Skamnioti and Gurr, 2009). Since, plant disease management using fungicides have constraints on environment and paves for evolution of resistant in pathogen, biological control using potential antagonists play a key approach in managing tomato wilt disease (Horinouchi *et al*., 2010, Zhao *et al*., 2011). *Trichoderma* spp. (*Hypocrea*) have found to be the most effective antagonists as they have mechanisms like mycoparasitism, antibiosis, competition and induced systemic resistance in host plants (Rodriguez *et al.,*2020). Numerous *Trichoderma* isolates secreted many volatile and non-volatile substances that one anti-fungal in nature against soil borne pathogens (Nagamani *et al.,* 2017). Besides disease control, *Trichoderma harzianum* also associated with enhancing soil fertility (Liton *et al.,* 2019). This study exploits the anti-fungal efficiency of *Trichoderma atroviride* against wilt disease of tomato.

MATERIALS AND METHODS

Isolation and identification of pathogen

The various isolates of the pathogen tomato wilt were collected from infected tomato plants in different places of Madurai district. The isolate FO (Maa)-5 was found highly virulent. This isolate was identified as *Fusarium oxysporum* f.sp. *lycopersici* based on sequencing of ITS region (Accession number: MZ043720). The pathogen was maintained on PDA slants and used for further studies.

Isolation of *Trichoderma*

Soil samples from rhizosphere region (3 cm) of healthy tomato plants were collected from 15 different locations of Madurai district, Tamil Nadu. The collected samples were dried and subjected to serial dilution (up to 10^{-4}). The biocontrol agent was isolated using the selective medium of *Trichoderma* (TSM) and incubated for 7 days at 25±3^{o}C (Awad *et al.,* 2018). Later the putative colonies were purified by single hyphal tip method. General biochemical tests were done to confirm the biocontrol agent. Later these cultures were preserved in PDA slants for further studies.

Antifungal assay using *Trichoderma* spp. against pathogen

Antifungal assay was carried out to evaluate the antimicrobial efficacy of the potential isolates of *Trichoderma* spp. against the pathogen.

(i) Dual culture assay

The dual culture described by Yassin *et al.* (2021) was followed to test the antagonistic ability of *Trichoderma* species against the pathogen. Small block (5 mm disc) of *Fusarium* cut from the periphery was placed at one cm away from the periphery of the Petri dish previously poured with PDA. Similarly, the *Trichoderma* isolate was placed one cm away from the edge of the same Petri plate aseptically on the opposite end and plates were incubated at room temperature for 5 days. The experiment was replicated thrice and per cent growth inhibition was calculated by using the following formula,

$$I = \frac{(A\text{-}B)}{A} \times 100$$

Where A is mycelial growth of pathogen in control plate, B is mycelial growth of pathogen in treatment plate and I is the percent inhibition of mycelial growth.

(ii) Effect of culture filtrates on inhibition of pathogen

Mycelial plugs were taken from the freshly grown *Trichoderma* cultures and inoculated into conical flask containing fresh 100 ml potato dextrose broth and incubated for 7 days at 150 rpm at 28^{o}C (You *et al.*, 2016). Supernatant of the cultures were collected and centrifuged at 9000 rpm for 10 min. Then the cell free filtrates were sterilized through a 0.22 µm millipore filters and mixed with unsolidified PDA medium at 10% (v/v) concentration. Uninoculated PDB was added to PDA with same ratio for control. Mycelial disc of the pathogen was placed in all PDA plates and kept for incubation at 28^{o}C for 5 days. Reduction in mycelial growth of the pathogen was measured and per cent inhibiton over control was arrived by the formula of Sreedevi *et al.* (2011),

$$I = \frac{(C\text{-}R)}{C} \times 100$$

Where,

C - Mean linear growth of pathogen in control ,

R - Mean linear growth of pathogen in treatments

Extraction of *Trichoderma* DNA and PCR amplification

The potent cultures were inoculated in conical flask containing 100 ml of potato dextrose broth and incubated in shaker 150 rpm for 7 days. The mycelial mat was sieved and pierced into powder using liquid nitrogen (Liu *et al*., 2020). DNA extraction of virulent isolates was done by using the procedure of Zhang *et al.* (2010). Genomic DNA was isolated by using CTAB method. PCR amplification was carried out using universal primers – internal transcribed spacer ITS1 (5'TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') (White *et al*., 1990). Polymerase chain reaction was performed in a reaction mixture of 50 µl with 35 cycles including 63 ng of genomic DNA, 50 pmol of each primer, 500 µM concentrations of dNTPs and 1.25 units of *Taq* DNA polymerase in an eppendorf thermal Cycler. The PCR programme was performed with initial denaturation (95°C for 2 mins), followed by the repeated cycles of denaturation (94°C 1min), annealing (56°C for 30 sec) and extension (72°C for 1 min), and final extension of 72°C for 10 min. Amplicons were detected by 2% (w/v) agarose gel electrophoresis. Sequencing of purified PCR product was done at Eurofins Genomics India Pvt. Ltd. Bangalore.

Identification of the *Trichoderma* sp. and phylogenetic relationships

ITS region of potential isolate was sequenced and BLAST searched with sequences in the NCBI, GenBank. Phylogenetic dendrogram was constructed by the neighbor-joining method in MEGA 10.0 software depending over multiple sequence alignment with an evolutionary distance of 0.05. The tree topologies were evaluated by performing analysis of 1000 data sets. The sequence was submitted to GenBank for obtaining accession number.

Preparation of crude extracts

Mycelial disc from an actively growing colony of *Trichoderma* isolate was inoculated into fresh potato dextrose broth and incubated for seven days. The culture filtrates were collected by filtering using Whatmann no.1 filter paper followed by centrifugation at 9000 rpm for 15 min and finally the metabolites were extracted using ethyl acetate as solvent (Jantarach and Thanaboripat, 2010). Further concentration of extracts and elimination of solvents were done using rotary evaporator (Sharma *et al.,* 2016).

Gas chromatography mass spectrometry (GCMS)

The extract possessing high antimicrobial property has been subjected to GCMS analysis. The antibiotics, volatiles and secondary metabolites present in the sample were detected by injecting one microlitre of sample in Capillary Standard Non – Polar Column of GC - MS in which Helium was used as carrier gas. The analytical conditions were adjusted by following the procedures given by Yassin *et al.,* (2020). The m/z peaks representing mass to charge ratio, characteristic of the antimicrobial fractions were compared with those of the corresponding organic compounds in the NIST library (Manigundan *et al.,* 2020).

Thin layer chromatography (TLC)

Thin Layer Chromatography was performed to identify the presence of antifungal compounds in crude extract of *Trichoderma* isolate. TLC tank was filled with acetone and chloroform solvents in the ratio of 3:1 and sealed the tank immediately (Vivek *et al.,*2013). Desired size of TLC plate (60 F254, Merck, India) was taken and marked 0.5 cm above the bottom corner of plate. Samples were spotted at 1 cm distance and labelled. Spotted TLC plate was allowed to run in TLC tank. Then the plate was removed and visualized in laminar under UV fluorescence light (254 nm) and marked the dark purple fluorescence with pencil. The R_f value was calculated based on the distance covered (Fried and Sherma, 1982),

R_f= Distance travelled by substance / Distance travelled by solvent

Statistical analysis

Statistical analysis were performed using analysis of variance (ANOVA) by SPSS software version 16 (SPSS.Chicago). The data were tabulated as mean of triplicates ± standard error and will be considered significant when the $P < 0.05$ and the means were compared by Duncan's Multiple Range Test (DMRT).

RESULTS

Antifungal assay

The results of antifungal assay revealed that all the *Trichoderma* isolates possessed certain amount of antifungal activity both in dual and culture filtrate assays.

(a) Dual culture assay

A total of fifteen isolates of *Trichoderma* spp. were isolated from the rhizosphere soil of healthy tomato plants. Among the isolates, *Trichoderma* isolate TA 12 was found superior against *Fusarium oxysporum* with 71% mycelial inhibition over control (Fig 1). The next best isolate was TA 2 with mycelial inhibition of 68.75%. Isolate TA 5 recorded minimum inhibition percentage of 46.22 (Table 1).

(b) *Trichoderma* culture filtrate assay against Fol

The experimental results revealed that all the isolates inhibited the mycelial growth of pathogen at significant level.. Among the isolates tested, TA12 showed the maximum mycelial inhibition of 77.77% (Fig 2).This was followed by the isolate TA 2 (75.65%). The least

Table 1. Antifungal assay of *Trichoderma* isolates against *Fusarium oxysporum* f.sp. *lycopersici* in dual culture assay

S.No.	Treatments	Mycelial growth (cm)*	Per cent mycelial inhibition
1.	TA – 1	4.2^{ij}	47.50(43.57)
2.	TA – 2	2.5^{b}	68.75(56.01)
3.	TA – 3	3.9^{h}	51.23(45.70)
4.	TA – 4	3.3^{e}	58.75(50.04)
5.	TA – 5	4.3^{j}	46.22(42.83)
6.	TA – 6	3.5^{f}	56.23(48.58)
7.	TA – 7	2.7^{c}	66.25(54.48)
8.	TA – 8	3.2^{e}	60.00(50.77)
9.	TA – 9	4.1^{i}	48.77(44.30)
10.	TA – 10	3.5^{f}	56.21(48.57)
11.	TA – 11	3.0^{d}	62.50(52.24)
12.	TA – 12	2.3^{a}	71.25(57.58)
13.	TA – 13	4.1^{i}	48.74(44.28)
14.	TA – 14	2.9^{d}	63.76(52.99)
15.	TA – 15	3.7^{g}	53.73(47.14)
	Control	8.0	0.00(0.00)
	CD (P=0.05)	1.37	

*Mean of three replications
Values with different superscripts are significantly differ from each other at $p<0.05$
Values in the parenthesis are arc sine transformed values

Fig. 1. ***In vitro*** **dual confrontation assay of antagonistic *Trichoderma* isolates against *Fusarium oxysporum* f.sp. *lycopersici***

Fig. 2. Anti-mycotic potency of culture filtrate of *Trichoderma* spp (TA12) against *Fusarium oxysporum* f.sp. *lycopersici*

mycelial growth was observed in TA 5 at the rate of 52.20% (Table 2).

Molecular confirmation of potential *Trichoderma* isolate TA12

PCR of *Trichoderma* isolate with ITS-1 and ITS-4 primer pairs resulted in amplification of a fragment of size 636 bp (Fig 3).

Table 2. Antifungal assay of culture filtreates of *Trichoderma* isolates against *Fusarium oxysporum* f.sp. *lycopersici*

S.No.	Treatments	Mycelial growth (cm)*	Per cent mycelial inhibition
1.	TA – 1	4.2^{j}	53.33(46.91)
2.	TA – 2	2.2^{b}	75.65(60.43)
3.	TA – 3	3.7^{hi}	58.88(50.11)
4.	TA – 4	3.0^{de}	66.67(54.74)
5.	TA – 5	4.3^{j}	52.20(46.26)
6.	TA – 6	3.1^{ef}	65.55(54.06)
7.	TA – 7	2.3^{bc}	74.45(59.64)
8.	TA – 8	2.8^{d}	68.88(56.09)
9.	TA – 9	4.0^{ij}	55.54(48.18)
10.	TA – 10	3.3^{fg}	63.33(52.73)
11.	TA – 11	2.5^{c}	72.22(58.19)
12.	TA – 12	2.0^{a}	77.77(61.87)
13.	TA – 13	3.8^{i}	57.76(49.46)
14.	TA – 14	2.5^{c}	72.22(58.19)
15.	TA – 15	3.4^{gh}	62.20(52.06)
	Control	9.0	0.00(00.00)
	CD (P=0.05)	1.97	

*Mean of three replications

Values with different superscripts are significantly differ from each other at $p<0.05$

Values in the parenthesis are arc sine transformed values

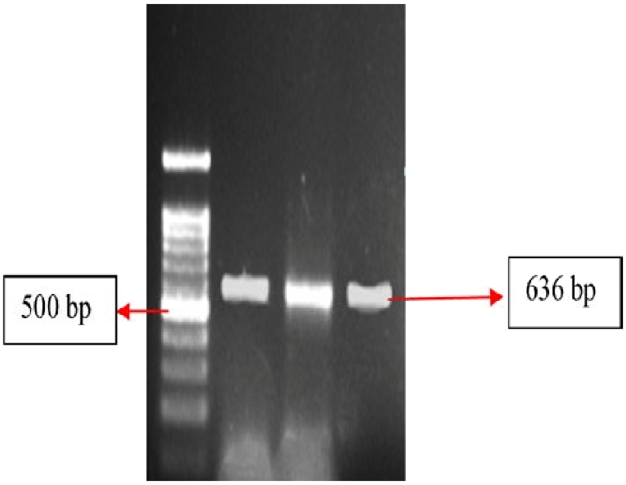


Fig. 3. The results of ITS amplification in potent *Trichoderma* isolates

Phylogenetic analysis of the sequence (TA 12) with existing sequences in the NCBI database showed 99% sequence similarity with *Trichoderma atroviride* (Fig 4). The sequence was deposited in Genbank and obtained accession number (MW984524; Fig 5).

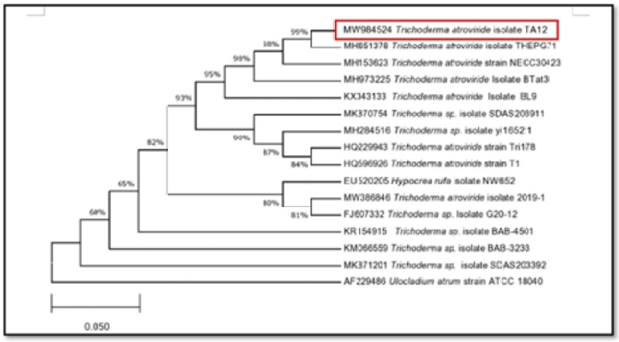


Fig. 4. Phylogenetic analysis of *Trichoderma atroviride* using neighbor joining method. The numbers over branches indicates bootstrap coefficient.

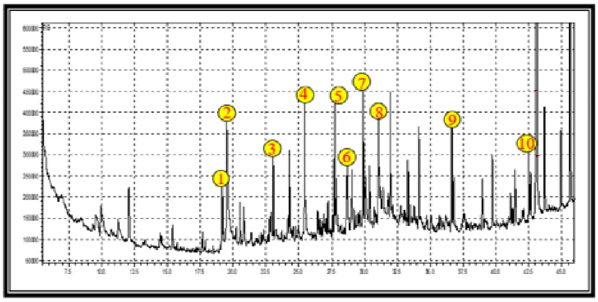


Fig. 5. GCMS chromatogram for detection of secondary metabolites in *Trichoderma atroviride* using ethyl acetate as solvent.

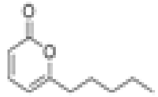
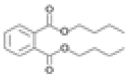
GC- MS analysis of extracts of *Trichoderma*

The extracts of *T. atroviride* were analyzed to determine its active chemical constituents. Active constituents of *T. atroviride* extract were demonstrated in Fig 5 and Table 3. The results showed that a numerous compounds produced by *Trichoderma atroviride,* possessing high antimycotic property.

TLC of *Trichoderma* spp.

The TLC plate with the sample was observed under UV laminar fluoroscence. The spot was resolved without any smear or streak pattern in TLC plate. In case of chitinase (developed in acetone:chloroform (3:1)) distinct spots were visualized under UV light (254 nm) with R_f value of 0.84 The distance travelled by the substance was 5.1 cm.

Table 3. Identification of secondary metabolites from *Trichoderma atroviride* through GCMS analysis

Peak	Retention Time	Compound Name	Structure	Molecular Formula	Molecular Weight (g/mol)	Peak area %
1	19.162	Quinoline		C_9H_7N	129.16	1.92
2	19.518	2H-Pyran-2-one, 6-pentyl		$C_{10}H_{14}O_2$	166.22	6.25
3	23.056	Nonadecane		$C_{19}H_{40}$	268.5	1.81
4	25.450	Heptadecane		$C_{17}H_{36}$	240.5	3.20
5	27.725	Heneicosane		$C_{21}H_{44}$	296.57	3.11
6	27.866	Eicosane		$C_{20}H_{42}$	282.54	1.68
7	28.690	Phenol		C_6H_5OH	94.11	1.77
8	31.062	Dibutyl phthalate		$C_{16}H_{22}O_4$	278.34	2.79
9	36.633	Hexadecane,1-iodo		$C_{16}H_{33}I$	352.34	3.54
10	42.653	Benzene propanoic acid		$C_9H_{10}O_4$	182.17	1.78

DISCUSSION

Trichoderma species have a global range of distribution and live in a variety of ecological niches, including decaying bark and wood, other fungus, soil, and healthy plant roots, stems, and leaves (Du Plessis *et al*., 2018; Mukherjee *et al*., 2013). The number of *Trichoderma* species used in biocontrol has drastically increased in modern era. Up to date, more than 290 *Trichoderma* species have been discovered (Bissett *et al*., 2015; Du Plessis *et al*., 2018; Zhu *et al*., 2017).

In this study, a survey was conducted and obtained fifteen isolates of *Trichoderma* after isolation. Among the isolates, *Trichoderma* isolate TA12 showed greater inhibition against the *Fusarium* strain than the other *Trichoderma* isolates. TA12 suppressed the mycelial growth of the pathogen (*Fusarium oxysporum* f. sp. *lycopersici*) by 71%. The results were in accordance with Schoffen *et al.* (2020), who reported that *T. atroviride* strain suppressed the mycelial growth of *F. oxysporum* in the range of 52.37% – 70.56%. *Trichoderma virens* exhibited a mycelial inhibition percentage of 80 against Fusarium wilt (Banerjee *et al.,* 2020). Sallam *et al.* (2019) confirmed the antagonistic potency of *T. atroviride* strain against *Fusarium* wilt of tomato with a mycelial inhibition rate of 66.80%.

The metabolites produced by *Trichoderma* spp. inhibited *Fusarium* isolates. Among the 15 isolates tested, 12 were able to inhibit the growth of *Fusarium oxysporum* (>50%) within which six isolates showed relatively strong inhibitory effect (>60%). Further in vitro assay of *Trichoderma* culture filtrates against Fol confirmed the similar trend as the TA 12 isolate recorded the highest inhibition of the pathogen (77%). Our findings are consistent with those of Rudresh *et al.* (2005), who reported the antimicrobial efficiency of culture filtrates of *T. harzianum* against *F. oxysporum* strain, recording mycelial inhibition rates of 78.5%. Alvarez-Garcia *et al.* (2020) also reported the suppression of mycelial growth of *Fusarium* spp. by the culture filtrates of *T.harzianum* and recorded the inhibition rate of 76.27%. Findings of Tomah *et al.* (2020) proved that *Trichoderma citrinoviride* retarded the growth of fungal pathogen at 77.8%.

The potent antagonist (TA 12) was subjected to sequencing of ITS regions and phylogenetic analysis. The phylogenetic analyses indicated that the isolate shown 99% similarity with other *T. atroviride* isolates thus TA12 confirmed as *T. atroviride*.

The antifungal ability of *T. atroviride* was confirmed by performing GCMS. Previous studies indicated that these compounds inhibited the mycelial growth of different pathogenic fungal strains (Keszler *et al.,* 2000**,** Jelen *et al.,* 2014, Mallaiah *et al.,* 2016). The main constituents alone do not attribute to the antifungal activity but also the presence of other bioactive substances attributed to antifungal potency. The anti-fusarial potency of *T. atroviride* extract may be attributable to the presence of many bioactive compounds such as 6-pentyl-2H-pyran-2-one, quinoline, phenol, 2-(6-hydrazino-3pyridazinyl), heptadecane, 17-methoxy-4-methyl-d-homo-18-norandrosta, nonadecane, heneicosane, eicosane, dibutyl phthalate, hexadecane and benzene propianoic acid. The antifungal efficacy of the extract may also be referred to the synergistic effect among the bioactive components (Khan *et al*., 2020).

Thin layer chromatography was done to separate and identify antifungal compound of *T. atroviride.* The R_f value calculated was similar to the values obtained with separation of enzymes from *Trichoderma* isolates (Rabinal and Bhat, 2017). Vinale *et al.* (2008) also revealed the same range of values when isolated from *Trichoderma.* The R_f value of 0.86 was identified in TLC separation of *T. harzianum* isolates (Kiss *et al.,* 2000).

Many studies indicated that *Trichoderma* spp. possess the multiple mechanisms, including mycoparasitism, extracellular enzymes such as cellulase, amylase, pectinase, protease and chitinase, antagonistic compounds and induced resistance, to inhibit pathogens and reduce diseases (Cherkupally *et al.,* 2017). Thus, the *Trichoderma atroviride* TA12 possibly uses multiple mode of action to inhibit pathogen, while antifungal compounds secreted by it could have played a major role in inhibiting pathogen and controlling fusarium wilt incidence of tomato.

The potent antagonist, *Trichoderma atroviride* isolate exhibited excellent antimycotic activity against Fusarial phytopathogen of tomato. Hence its antimicrobial potency of culture filtrates and organic solvent extracts against fusarial pathogen of tomato highlights the ability to employ novel and safe biofungicide in order to neglect the hazards of chemical fungicides on the human health and environment.

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REFERENCES

Alvarez-Garcia, S., Mayo-Prieto, S., Gutierrez, S and Casquero, P. A. 2020. Self-inhibitory activity of Trichoderma soluble metabolites and their antifungal effects on Fusarium oxysporum. *Journal of Fungi*, **6(3)**: 176.

Awad, N.E., Kassem, H.A., Hamed, M.A., El-Feky, A.M., Elnaggar, M.A.A., Mahmoud, K and Ali, M.A. 2018. Isolation and characterization of the bioactive metabolites from the soil derived fungus *Trichoderma viride. Mycology*. **9 (1):** 70-80.

Banerjee, S., Singh, S., Pandey, S., Bhandari, M. S., Pandey, A and Giri, K. 2020. Biocontrol potential of *Pseudomonas azotoformans, Serratia marcescens* and *Trichoderma virens* against Fusarium wilt of *Dalbergia sissoo*. *Forest Pathology*, **50(2):** e12581.

Bissett, J., Gams, W., Jaklitsch, W and Samuels, G.J. 2015. Accepted *Trichoderma* names in the year 2015. *IMA fungus* .**6**: 263–295.

Cherkupally, R., Amballa, H and Reddy Bhoomi, N. 2017. In vitro screening for enzymatic activity of *Trichoderma* species for biocontrol potential. *Ann. Plan. Sci.* **6**: 1784–1789.

Du Plessis, I.L., Druzhinina, I.S., Atanasova, L., Yarden, O and Jacobs, K. 2018. The diversity of *Trichoderma* species from soil in south Africa, with five new additions. *Mycologia*. **110**: 559–583.

Fatima, S and Anjum, T. 2017. Identification of a potential ISR determinant from *Pseudomons aeruginosa* PM12 against Fusarium wilt in tomato. *Front. Plant Sci.* **8**: 848.

Fried, B. and Sherma, J. 1982. Thin layer chromatography: techniques and applications. Marcel Dekker, P.308.

Hilje-Rodriguez, I., Albertazzi, F.J., Rivera-Coto, G and Molena-Bravo, R. 2020. A multiplex qPCR TaqMan-assay to detect fungal antagonism between *Trichoderma atroviride* (Hypocreaceae) and *Botrytis cinerea* (Sclerotiniaceae) in blackberry fruits using a de novo tef1-α- and an IGS-sequence based probes. *Biotechnology reports.* 27: e00447

Hirooka, T and Ishii, H. 2013. Chemical control of plant diseases. Journal of General *Plant Pathology*, **79 (6):**390-401

Horinouchi, H., Muslim, A and Hyakumachi, M. 2010. Biocontrol of Fusarium wilt of spinach by the plant growth promoting fungus fusarium equiseti Gf183. Journal of *Plant Pathology,* **1**: 249-254.

Jantarach, J and Thanaboripat, D. 2010. The efficacy of ethyl acetate extract of *Trichoderma* culture broth on growth inhibition and aflatoxin production by *Aspergillus flavus* IMI 242684. *Curr. Appl. Sci. Technol*. **10 (1):** 19–29.

Jelen, H., Blaszczyk, L., Chelkowski, J., Rogowicz, K., and Strakowska, J. 2014. Formation of 6-n-pentyl-2H-pyran-2-one (6-PAP) and other volatiles by different *Trichoderma* species. *Mycological Progress.* **13(3):** 589-600.

Kesler, A., Forgacs, E., Kotai, L., Vizcaino, J.A., Monet, E and Garcia-Acha, I. 2000. Separation and identification of volatile components in the fermentation broth of *Trichoderma atroviride* by solid-phase extraction and gas chromatography–mass spectrometry. *Journal of Chromatographic Science*, **38(10):** 421-424.

Khan, R. A. A., Najeeb, S., Hussain, S., Xie, B and Li, Y. 2020. Bioactive secondary metabolites from *Trichoderma* spp. against phytopathogenic fungi. *Microorganisms*. **8(6):** 817.

Kiss, G.C., Forgacs, E., Cserhati, T and Vizcaino, J.A. 2000. Colour pigments of *Trichoderma harzianum* preliminary investigations with thin-layer chromatography–Fourier transform infrared spectroscopy and high-performance liquid chromatography with diode array and mass spectrometric detection. *Journal of Chromatography A*. **896** : 61-68.

Lee, S., Yap, M., Behringer, G., Hung, R and Bennett, J. W. 2016 . Volatile organic compounds emitted by Trichoderma species mediate plant growth. *Fungal Biology and Biotechnology*, **3(1):** 1-14.

Liton, M. J. A., Bhuiyan, M. K. A., Jannat, R., Ahmed, J. U., Rahman, M. T and Rubayet, M.

T. 2019. Efficacy of *Trichoderma*-fortified compost in controlling soil-borne diseases of bush bean (*Phaseolus vulgaris* L.) and sustainable crop production. *Advances in Agricultural Science.* **7(2):** 123-136.

Liu, B., Ji, S., Zhang, H., Wang, Y and Liu, Z. 2020. Isolation of *Trichoderma* in the rhizosphere soil of *Syringa oblata* from Harbin and their biocontrol and growth promotion function. *Microbiological Research*. **235**: 126445.

Mallaiah, B., Rajinikanth, E and Muthamilan, M. 2016. Isolation and identification of secondary metabolites produced by *Trichoderma viride* inhibiting the growth of *Fusarium* in Carnatum (Desm.)Sacc. incitant of crossandra Wilt. *The Bioscan*. **11(3):** 1525-1529.

Manigundan, K., Joseph, J., Ayswarya, S., Vignesh, A., Vijayalakshmi, G., Soytong, K., Gopikrishnan, V and Radhakrishnan, M. 2020. Identification of biostimulant and microbicide compounds from Streptomyces sp. UC1A-3 for plant growth promotion and disease control. *International Journal of Agricultural Technology* **16 (5):** 1125-1144

Miller, E.C., Hadley, C.W., Schwartz, S.J., Erdman, J.W., Boileau, T.W.M

and Clinton, S.K. 2002. Lycopene, tomato products, and prostate cancer prevention. Have we established causality?. *Pure Appl. Chem*. **74**: 1435–1441.

Mukherjee, P.K., Horwitz, B.A., Singh, U.S., Mukherjee, M and Schmoll, M. 2013.

Trichoderma: Biology and Applications .*CAB*. 344.

Nagamani, P., Bhagat, S., Biswas, M. K and Viswanath, K. 2017. Effect of volatile and non volatile compounds of *Trichoderma* spp. against soil borne diseases of chickpea. *International Journal of Current Microbiology and Applied Sciences*, **6(7):** 1486-1491.

Prihatna, C., Barbetti, M.J and Barker, SJ. 2018. A novel tomato Fusarium wilt *Tolerance Gene. Front.* Microbiol. **9**: 1226.

Rabinal, C.A and Bhat, S. 2017. Profiling of *Trichoderma Koningii* IABT1252's secondary metabolites by thin layer chromatography and their antifungal activity. *The Bioscan.* **12(1):** 163-168.

Rudresh, D. L., Shivaprakash, M. K and Prasad, R. D. 2005. Potential of *Trichoderma spp.* as biocontrol agents of pathogens involved in wilt complex of chickpea (Cicer arietinum L.). *Journal of Biological Control*, **19(2)**: 157-166.

Sallam, N. M., Eraky, A. M and Sallam, A. 2019. Effect of Trichoderma spp. on Fusarium wilt disease of tomato. *Molecular Biology Reports*, **46(4):** 4463-4470.

Schoffen, R.P., Silva Ribeiro, A., Oliveira-Junior, V.A., Polonio, J.C., Polli, A.D., Orlandelli,, R.C., Santos Ribeiro, M.A., Pamphile, J.A and Azevedo, J.L. 2020. Evaluation of *Trichoderma atroviride* endophytes with growth-promoting activities on tomato plants and antagonistic action on *Fusarium oxysporum*. *Ciencia e Natura*. **42**: 47.

Sharma, D., Pramanik, A and Agarwal, P.K . 2016. Evaluation of bioactive secondary metabolites from endophytic fungus *Pestalotiopsis neglecta* BAB-5510 isolated from leaves of *Cupressus torulosa* D.Don. *3 Biotech*. **6**:210.

Siddiquee, S., Cheong, B. E., Taslima, K., Kausar, H and Hasan, M. M. 2012. Separation and identification of volatile compounds from liquid cultures of Trichoderma harzianum by GC-MS using three different capillary columns. *Journal of Chromatographic Science.* **50(4):** 358-367.

Skamnioti, P and Gurr, S.J. 2009. Against the grain: safeguarding rice from rice blast disease. *Trends in Biotechnology*. **27(3):**141-150.

Sreedevi, B., Charitha Devi, M and Saigopal, D. 2011. Isolation and screening of effective *Trichoderma* spp. against the root rot pathogen *Macrophomina phaseolina. J. Agric. Technol.* **7 (3):** 623–635.

Tomah, A. A., Abd Alamer, I. S., Li, B., and Zhang, J. Z. 2020. A new species of *Trichoderma* and gliotoxin role: A new observation in enhancing biocontrol potential of *T. virens* against

Phytophthora capsici on chili pepper. *Biological Control*. **145:** 104261.

Vinale, F., Sivasithamparamb, K., Ghisalberti, E. L., Marra, R., Barbetti, M. J., Li, H., Woo, S. L. and Lorito, M. 2008. A novel role for *Trichoderma* secondary metabolites in the interactions with plants. *Physiological and Molecular Plant Pathol*, **72**: 80-86.

Vivek, A., Suresh, W., Om, P.S., Jitendra, K., Aditi, K., Jay, S and Paul, Y. S. 2013. Isolation, characterisation of major secondary metabolites of the Himalayan *Trichoderma koningii* and their antifungal activity. *Archives of Phytopathology and Plant Protection,* **47**: 9.

White, T., Bruns, T., Lee, S and Taylor, J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *PCR Protocols*, **18**: 315–322.

Yassin, M.T., Mostafa, A.A., Al-Askar, A.A., Sayed, S.R.M and Rady, A.M. 2021. Antagonistic activity of *Trichoderma harzianum* and *Trichoderma viride* strains against some fusarial pathogens causing stalk rot disease of maize, in vitro. *Journal of King Saud University – Science*, **33(3)**: 101363

You, J., Zhang, J., Wu, M., Yang, L., Chen, W and Li, G. 2016. Multiple criteria-based screening of Trichoderma isolates for biological control of *Botrytis cinerea* on tomato. *Biological control,* **101**: 31-38

Zhang, Y.J., Zhang, S., Liu, X.Z., Wen, H.A and Wang. M. 2010. A simple method of genomic DNA extraction suitable for analysis of bulk fungal strains. *Letters in Applied Microbiology*, **51**: 114-118.

Zhao, Q., Dong, C., Yang, X., Mei, X., Ran, W., Shen, Q and Xu, Y. 2011. Biocontrol of Fusarium wilt disease for *Cucumis melo* melon using bio-organic fertilizer. *Applied Soil Ecology,* **47:** 67-75

Zhu, Z.X., Xu, H.X., Zhuang, W.Y and Li, Y. 2017. Two new green-spored species of *Trichoderma* (sordariomycetes, ascomycota) and their phylogenetic positions. *MycoKeys,* **26**: 61–75

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