Indian J. Applied & Pure Bio. Vol. 38(1), 363-374 (2023).

ISSN: 0970-2091
A web of Science Journal

Morphological and molecular characterisation of *Trichoderma asperellum* (*T. viride*) and evaluating bio-control efficacy against *Fusarium* wilt of chilli caused by *Fusarium incarnatum*

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Abstract

Chilli fusarium wilt of one of the destructive diseases of chilli production that affects yield and quality. There are various species of fusarium associated with chilli fusarium wilt in India. Use of synthetic chemicals are commercially employed from years will affect the soil health, environment and food chain. By concerning environmental safety and biodegradability bio control agents are seems to be best alternative cost-effective management practise which helps to recover the soil health and environment. Biocontrol agents like *Trichoderma* has been reported to be effective against many soil borne pathogens, it not only protect the plants from invading pathogens but also helps to activate plant defense mechanism. A total of 10 *Trichoderma* spp. were isolated and tested their antagonistic activity against fusarium wilt of chilli pathogen, *Fusarium incarnatum*. Among the isolates tested T9 found to be effective and recorded the maximum percent inhibition over control (75.6) followed by T6 (71.0) and least was recorded in T2 (48.9) in dual culture technique, Ten *Trichoderma* isolates are further tested in poison food technique at various concentrations 5%, 10%, 15 % and 20% and the reading were recorded. ITS region of rDNA amplification with ITS1 and ITS4 universal primers produced approximately 570 bp product shows the virulent samples were confirmed as *T. asperellum*. The sequence was deposited in GenBank with the accession number, ON909758).

Key words : Chilli, fusarium wilt, *Fusarium*, Biocontrol, *Trichoderma.*

Chilli (*Capsicum annuum* L.) is one of the most significant vegetable and spice crop[12] across India and it alone contributes 25% of the total chilli production in the world[26].

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Chilli occupies an important place in Indian diet[46]. Chilli is today one of India's major export attractions. It is the major spice contributing 40-42 per cent by volume, 22 per cent by value of total spices exported from India[18]. Chilli is an annual plant comes in a wide variety of shapes, sizes, colour and in different degrees of pungency. It is thus several called Capsicum, Paprika, Pimento, Sweet pepper, Red pepper, Cayenne pepper and Bird's eye chilli depending on the type of chilli and the manner in which it is prepared and used[46]. Chilli is rich in vitamins like, vitamin[35] A, C, E, K, B_6 and minerals like calcium, magnesium, folic acid, potassium, Thiamine, copper, iron[7], antioxidants (Capsaicin, capsaicinoids and carotenoids) and flavonoids (β-carotene, α-carotene, lutein, zea-xanthin, and cryptoxanthin). Hot chillies are rich in digestive stimulant capsaicin[4].

There are various factors which affects the production of chilli. Chilli crop is vulnerable to more than 100 different types of pathogens during its various growth stages[19]. Among the various diseases that affect chilli production, fusarium wilt of chilli is one of the most important considerable disease in major chilli growing areas and causes serious damage of up to 40 % of total chilli production[44]. Soil borne diseases can be major limitation in crop production, particularly for vegetables[6]. *Fusarium* wilt disease is commonly caused by many species of fusarium, *F. oxysporum* [10], *F. solani* [10,29], *Fusarium incarnatum* [10], *F. proliferatum* [28], *F. pallidoroseum* [24], *F. equiseti* [10,12], *F. chlamydosporum*[10].

By concerning the environmental safety and biodegradability, biocontrol agents are pretended to be the alternative practises to the chemicals for the management of soil borne pathogens[39]. The major goal of implementing biological control methods is to decrease the density and activity of the pathogen[59]. Several *Trichoderma* species to form mutualistic endophytic relationships with several plant species[13]. *Trichoderma* are potential biocontrol agents for several soil born phytopathogens[15]. Different volatile and non-volatile secondary metabolites have been identified and characterized from *Trichoderma* spp. by antibiotic assay-guided isolation and the production of antibiotic molecules by certain strains is often well correlated with their biocontrol ability[17]. *Trichoderma* have great versatility of mechanisms of action, acting against other fungi through antibiosis and production of volatile metabolites, mycopara-sitism, production of cell wall degrading enzymes and competition for nutrients and substrate[13,56,59]. The aim of the current investigation was to examine the antagonistic potential of different *Trichoderma* species for bio control of *F. incarnatum*.

Isolation and identification of pathogenic culture responsible for Fusarium wilt of chilli :

Diseased chilli samples collected from various chilli growing areas of Andhra Pradesh and Tamil Nadu we used for isolation. The pathogen, *Fusarium incarnatum* was isolated by tissue segment method[3]. The infected stem portion is cut into small pieces along with healthy portions by using sterile scalpel. The bits were cut into small pieces of 3-5 mm slices and surface sterilized with 1% sodium hypochlorite (Na_2OCl) for 30sec followed by washing with three variants of sterile water

for 20-30 sec, and dried with filter paper. 2-4 bits were placed in a Petri Plate containing solidified agar medium supplemented with antibiotic streptomycin sulphate to prevent bacterial contamination. The culture is incubated at 28±2°C for 3-5 days. The axenic cultures of the pathogen were obtained by single hyphal tip method[37] and were maintained in PDA slants for further studies. The purified isolates are identified on the basis of cultural and morphological features like white- and purple-coloured mycelia and presence of microconidia on short conidiophore[51]. Based on the molecular identification the pathogen was identified as *Fusarium incarnatum* and deposited in NCBI (Accession number - OK598964).

Isolation of fungal antagonist (Trichoderma asperellum) :

Ten soil samples are collected for isolation of *Trichoderma* spp. from healthy rhizosphere zone of chilli plants. *Trichoderma* was isolated by serial dilution technique[20] using *Trichoderma* specific medium (TSM)[11]. One ml of required final dilution (10^{-3} & 10^{-4}) of soil suspension was poured on to sterilized petri plates containing sterilized solidified PDA agar medium. Plates were rotated gently to get uniform distribution of soil suspension in the medium. The plates were incubated at 28±2°C for 3-5 days[35] and observed at frequent intervals for the development of colonies. *Trichoderma* spp. were purified by hypal tip culture technique[43] and preserved in refrigerator at 4°C for further use[33]. *Trichoderma* sp. isolates were identified based on mycological keys[5] and molecular sequence data reveals, the virulent isolate is identified as *Trichoderma asperellum* (GenBank accession number – ON909758) and used for further studies.

Isolation of genomic DNA of Trichoderma sp.:

A small mycelial disc (5 mm) of *Trichoderma* sp. (T9) was inoculated into a conical flask containing 100 ml of potato dextrose broth, incubate in orbital shaker @ 120-150 rpm for 7-14 days at 28±2°C. After incubation the fungal mycelium was filtered by using sterile Whatman No. 1 filter paper (HIMEDIA Labs, India). 100 mg dried mycelial mat was collected and taken to pestle and mortar make it into fine powder by adding liquid nitrogen. Total fungal DNA was extracted by CTAB method with minor modifications[1]. The purified DNA was dissolved in 50µl TE buffer (Tris 10mM + EDTA 1mM pH 8.0). Integrity of genomic DNA was checked in 1.5 per cent agarose gel (MEDAUXIN). The quality and quantity of DNA was assessed by using NanoDrop spectrophotometer (Thermo Fisher Scientific NanoDrop 2000c, USA). The concentration of DNA was adjusted to 50 ng/µl and stored at 4°C for further use[1].

PCR amplification :

The PCR-amplification reactions were performed in a 50 µl mixture containing 50 mM KCl, 20 mM Tris HCl (pH 8.4), 2.0 mM $MgCl_2$, 200 µM of each of the four deoxynucleotide triphosphates (dNTPs), 0.2µmM of each primer, 40 ng/µl of template and 2.5 U of Taq polymerase. The cycle parameters included an initial denaturation at 94°C for 5 min, followed by 40 cycles of denaturation at 94°C for 1 min, primer

annealing at 55°C for 2 min and primer extension at 72°C for 3 min and a final extension for 10 min at 72°C. Amplified products were separated on 1.2% agarose gel in TAE buffer, prestained with ethidium bromide (1µg/ml) and electrophoresis was carried out at 60 volts for 3 hours in TAE buffer.

DNA sequencing of the 18S rDNA fragment:

The 18S rDNA amplified PCR product (100 ng concentration) was used for the sequencing with ITS-2 using Primers ITS1- F (5' TCC GTA GGT GAA CCT TGC GG 3') and ITS4- R (5' TCC TCC GCT TAT TGA TAT GC 3') primers.

Sequence analysis :

A number of *Trichoderma* sequences were selected on the basis of a similarity score of over 90% with database sequences. Multiple sequence alignment of these selected homologous sequences and 18S rRNA gene sequence of test strain was performed using Clustal W. Subsequently, an evolutionary distance matrix was then generated from these nucleotide sequences in the dataset. A phylogenetic tree was constructed using the Neighbour Joining method[38]. Phylogenetic and molecular evolutionary analyses were conducted using MEGA (Molecular Evolutionary Genetics analysis) version X [47].

Efficacy of antagonistic Trichoderma sp. against Fusarium incarnatum :

In vitro antagonistic potential of *Trichoderma* sp. were evaluated against the virulent isolate of *Fusarium incarnatum* isolate through dual culture technique in *in vitro* condition[23]. 5 mm of the growing edges of the antagonist (*Trichoderma* sp.) and pathogenic culture were placed on opposite direction of PDA plates at the edges and incubated at 27±2 °C in incubator for 5 days[41]. The pathogen alone inoculated was served as a control. Observations of colony growth were recorded. The growth inhibition was measured using Vernier callipers and percentage of the inhibition rate was calculated using the formula described by Dennis and Webster[9]; Rahman *et al.*,[36]; Sarvanakumar *et al.*,[41].

$$\text{Percent inhibition (I)} = \frac{C - T}{C} \times 100$$

Where,

I = Per cent inhibition in mycelial growth
C = Growth of pathogen in control plates (mm)
T = Growth of pathogen in dual culture plates (mm)

Preparation of culture filtrate of Trichoderma spp. *:*

The effective *Trichoderma* isolates were selected and grown in conical flasks containing 50 ml of sterilized potato dextrose broth (PDB) and incubated in orbital shaker (150 rpm) for 5 days at room temperature (28±2°C)[58]. Then the liquid culture filtrate was filtered through Whatman filter paper No. 1 to remove mycelial mat and Seitz filter was used to separate the spores[2].

Effect of antagonistic culture filtrates on the mycelial growth of Fusarium incarnatum (Poisoned food technique) :

The antagonist of the culture filtrate was evaluated by Poisoned food technique[30]

to evaluate the effect of non-volatile compounds[53]. The culture filtrates of the antagonists were separately incorporated into sterile PDA melted medium at 5, 10, 15 and 20 per cent concentration by means of a sterile pipette. The amended media were transferred to sterile Petri dishes separately @ 15 ml and allowed to solidify. The PDA medium without the culture filtrate served as control. Each plate was inoculated at the centre with a five days old PDA culture disc of *Fusarium incarnatum*. Three replications were maintained for each treatment. The diameter of the mycelial growth (mm) of *F. incarnatum* was measured after 5 days of incubation. The per cent inhibition of the test fungi was calculated by using the formula of Wonglom *et al*.[57].

$$\text{Percent inhibition (I)} = \frac{C - T}{C} \times 100$$

Where,

I = Per cent inhibition of mycelial growth

C = Growth of pathogen in control plates (mm)

T = Growth of pathogen in poisoned plates (mm)

A survey was carried out in major chilli growing areas of Andhra Pradesh and Tamil Nadu and collected 20 fusarium wilt infected samples. Pathogenicity was conducted to identify the virulent isolates of the pathogen and recorded F1 with the highest disease incidence. Based on morphological and molecular characters, F1 isolates collected from Peesapadu village of Guntur district of Andhra Pradesh identified as *Fusarium incarnatum*. Mishra *et al*.[27] was conducted an extensive survey on 36 locations of Pratapgarh, Amethi, Sulatanpur, Kanpur, Etawah, Allahabad, Faizabad, Jaunpur, and Mirzapur districts of Uttar Pradesh and observed the maximum incidence of anthracnose followed by fusarium wilt are the predominant pathogens in all locations surveyed. Likewise, Priya and Mesta[34] surveyed for fusarium wilt incidence in Belagavi, Gadag and Haveri districts of Karnataka and recorded the percent incidence of the disease, which was ranges from 5.45 to 95 percent.

Cultural characters of Trichoderma spp. isolates :

Ten different *Trichoderma* isolates were isolated from different locations are grown in PDA plates for studying colony growth, colony colour, conidiophore, conidial colour, conidial shape, conidial length and breadth were measured. Among the different isolates, T9 were shown fully occupied with the mycelial growth in five days, the colonies were observed with dull green, light green, dark green, pale green, whitish to greyish green in colour with floccose to arachnoid in mycelial form (Table-1). Conidial characters like conidial shape, colour, length and breadth were recorded (table-2). Conidial length and breadth were measured and ranges from 2.10-3.92 µm and 2.15-5.18 µm respectively. *Trichoderma* is a fast-growing fungus, it reached the pathogen within 3-4 days. Similar findings of the interaction of *Trichoderma* spp. against *Fusarium* spp. were recorded previously [25,52]. Morphological characters of three different isolates (CSR-T2, CSR-T3, CSR-T4), Green to dark dirty green colony colour with smooth edge, floccose to arachnoids mycelial form, flat to ring like conidiation, green conidial colour with regular to densely branched conidiophores. Supported by Praveen *et al*.[33]

reported that cultural and morphological character of 10 *Trichoderma* isolates (Tr1-Tr10) exhibited whitish green to pale dull green colony colour and high to moderately conidiophore branching, conidial shape of globose, obovoid to ellipsoidal and conidial colour of green to dull green. Sankara Reddy *et al.*[39], observed cultural characters of native isolates of *Trichoderma viride* isolated from rhizosphere soil and assed the colony morphology of ten isolates of *T. viride*, the cultural characters were recorded, green to dark green mycelial with dull to deep green sporulation.

Molecular characterisation :

Molecular characterisation plays an important role in identification of plant pathogens and biocontrol agents at species level. PCR based analysis by using universal primers ITS 1 and ITS 4 are employed in identification of *Trichoderma asperellum*. Sequence analysis of *Trichoderma asperellum* eluted the DNA at 570 base pairs. The results are in order with Shahid *et al.*[45], who molecularly characterised thirty isolates of *Trichoderma viride* by using ITS 1 and ITS 4 universal primers produced 600 bp products in all isolates. ISSR profiles and dendrogram analysis showed genetic diversity and similarity co-eficient among the isolates *T. viride.* Similarly Singh *et al*. (2014) extracted the genomic DNA of *Trichoderma harzianum* (Th Azad) by using universal fungal primers ITS 1 and ITS 4 by using 18S rRNA gene fragment, a total of 546 bp of the 18S rRNA gene was sequenced and used for the identification of Th Azad strain.

Similarly, Hermosa *et al.*,[16] characterised 16 biocontrol strains of previously identified species of *T. harzianum* and *T. viride*. Sequencing was carried out by using universal primers ITS 1 and 2 reveals three different lengths and four different sequence types. Phylogenetic analysis cluster based on ITS 1 divided into four different groups: *T. harzianum-T. inhamatum* complex, *T. longibrachiatum*, *T. asperellum*, and *T. atroviride-T. koningii* complex. ITS2 sequences were also useful for locating the biocontrol strains in *T. atroviride* within the complex *T. atroviride-T. koningii*. Pandian *et al.*,[31] characterised *Trichoderma asperellum* strain Ta13 based on molecular analysis with five different genes (ITS, cal, act, tef1α and rpb2).

In vitro efficacy of Trichoderma spp. *against Fusarium incarnatum by dual culture technique :*

Out of Ten *Trichoderma* isolates, five isolates are from highest disease incidence and another five isolates from lowest disease incidence areas of Andhra Pradesh and Tamil Nadu, which were screened against *Fusarium incarnatum* by dual culture technique (Table 3). Among the ten isolates, T9 shows the maximum percent inhibition 75.6% followed by T6 (71.0%) and T8 (69.0 %) and least inhibition was observed in T2 (48.9 %). It is observed that among the ten isolates of *Trichoderma*, five isolates from lowest disease incidence shows maximum per cent inhibition against *Fusarium incarnatum* compared to isolates from highest disease incidence areas may reveals the diseases incidence is reduced due to the high rhizosphere population of *Trichoderma* sp. and other microbiota. *Trichoderma* as potential antagonist

Table-1. Morphological and cultural characteristics of different *Trichoderma asperellum*

Isolates	Locality	Mycelial characters			
		Mycelial growth 5th day (mm)	Colony characters	Colony colour	Mycelial form
T1	Peesapadu	88.60	Whitish green sporulation	Dull green	Floccose
T2	Karambilipatti	87.85	Green to light green sporulation	Light green	Cottony
T3	Abburu	89.50	Dark green sporulation	Dark green	Floccose
T4	Ponnampalle	89.86	Green to bright green sporulation	Dark green	Arachnoid
T5	Andukuru	89.95	Complete dark green sporulation	Pale green cottony	Compact
T6	Athicombai	90.00	Green to bright green sporulation	Whitish green to arachnoid	Floccose
T7	Kalanjipatty	89.90	Dark green to dull green sporulation	Dark green	Floccose
T8	Puducharam	90.00	Dark green sporulation	Greyish green	Arachnoid
T9	Aamayapuram	90.00	Green to bright green sporulation	Green	Floccose
T10	Naganampatty	89.92	Dark green sporulation	Dull green	Compact cottony

Table-2. Morphological and cultural characteristics of different *Trichoderma asperellum*

Isolates	Locality	Conidial characters				
		Conidiophore	Conidial shape	Conidial colour	Conidial length (μ)	Condial breadth (μ)
T1	Peesapadu	Long, infrequently branched, regular	Sub globose to ellipsoidal	Pale Green	2.15-3.05	2.55-3.85
T2	Karambilipatti	Infrequent branching, verticillate	Globose	Light Green	2.10-3.15	2.15-3.10
T3	Abburu	Highly branched	Ellipsoid	Dirty green	2.25-3.15	2.40-3.54
T4	Ponnampalle	High branched, regular	Ellipsoidal to obovoid	Dirty green	2.22-3.20	2.30-4.05
T5	Andukuru	High branched, irregular	Globose	Pale green	2.45-3.45	2.54-4.40
T6	Athicombai	Frequent branched	Globose to ellipsoidal	Dark Green	2.61-3.80	2.51-4.95
T7	Kalanjipatty	Narrow, frequent branching	Ellipsoidal	Dark green	2.85-3.92	2.45-4.10
T8	Puducharam	Broad, frequently, branching	Broadly ellipsoidal	Dark green	2.55-3.71	2.45-4.65
T9	Aamayapuram	Frequent branching, verticillate	Globose	Yellowish Green	2.34-3.22	2.55-5.18
T10	Naganampatty	Branched regularly	Obovoid	Light Green to dark green	2.40-3.41	2.40-4.25

against the phytopathogen like *R. solani, A. alternata, C. lunata* and *F. oxysporum* in dual culture technique was reported by Tapwal *et al*.[54] and Prasad and Kumar[32]. Similarly, Choudhary and Reena[8] screened nineteen isolate of *T. harzianum, T. viride* and *T. koningii* by dual culture technique and liquid culture filtrate assay against *Fusarium oxysporum* f.sp. *lentis*; responsible for wilt of lentil and observed significant growth inhibition of pathogen.

Kucuk[22] tested 18 *Trichoderma* isolates against various *Fusarium* species pathogen, among the isolates tested T16 against *F. solani* (84.4%), T10 against *F. moniliforme* (82.8%), T6 against *F. culmorum* (70 %), T3, T4 against *F. verticilloides* (83.3 %), F1 and F5 against *F. chlamydosporum* (92.6 %) showed maximum inhibition rate in dual culture method.

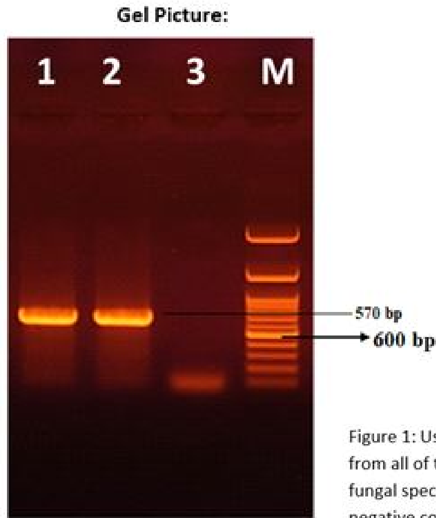

Figure 1: Using the universal fungal primers (ITS1/ITS4), PCR products (570 bp) were obtained from all of the species. Figure 1 showed that the sizes obtained for the full ITS region amplified fungal species. Legends: **1**-TRI sample, **2**-Genomic DNA positive control; **3**-Genomic DNA negative control; **M**-Molecular Weight Marker; 100bp DNA ladder (Genedirex).

Phylogenetic tree

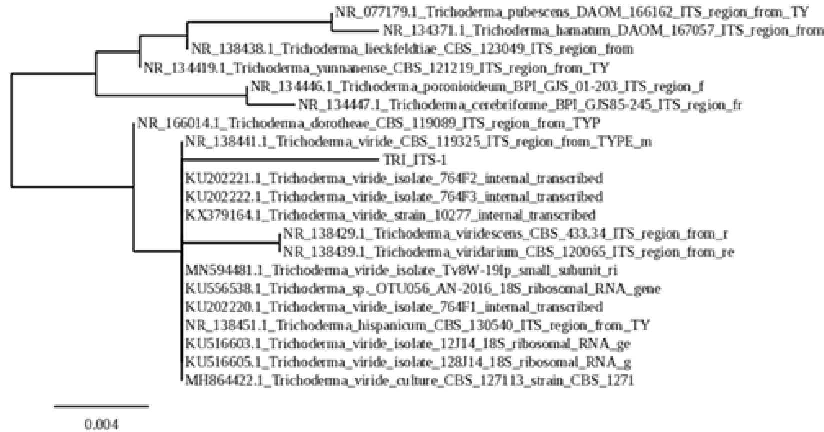

Table-3. *In vitro* efficacy of *Trichoderma* spp. against *Fusarium incarnatum* (F1)

S.No.	Isolate	Mycelial growth (mm)		Percent inhibition over control (%)
		Trichoderma asperellum	*F. incarnatum*	
1	T1	49.88^{e}	37.0^{b}	58.9
2	T2	41.13^{f}	46.0^{a}	48.9
3	T3	50.66^{de}	35.5^{c}	60.6
4	T4	52.78^{bcd}	33.1^{d}	63.2
5	T5	53.42^{bc}	29.0^{ef}	67.8
6	T6	55.29^{b}	26.1^{g}	71.0
7	T7	52.65^{cd}	32.0^{d}	64.4
8	T8	54.02^{bc}	27.9^{f}	69.0
9	T9	59.15^{a}	22.0^{h}	75.6
10	T10	52.89^{bcd}	30.1^{e}	66.6
11	Control	-	90	-

Table-4. Efficacy of Non-Volatile compounds produced by *Trichoderma asperellum* against *Fusarium incarnatum* (F1)

S.No.	Isolate	Concentration of culture filtrate (%)							
		5		10		15		20	
		Mycelial growth of pathogen (mm)	Percent inhibition over control (%)	Mycelial growth of pathogen (mm)	Percent inhibition over control (%)	Mycelial growth of pathogen (mm)	Percent inhibition over control (%)	Mycelial growth of pathogen (mm)	Percent inhibition over control (%)
1	T1	75.58^{b}	16.02	46.34^{ab}	48.51	28.85^{ab}	67.94	8.58^{b}	90.46
2	T2	79.15^{a}	12.05	46.68^{a}	48.13	29.35^{a}	67.38	9.83^{a}	89.07
3	T3	73.73^{b}	18.07	45.12^{bc}	49.86	28.01^{b}	68.87	8.38^{b}	90.68
4	T4	73.24^{bc}	18.62	44.11^{c}	50.98	26.41^{c}	70.65	7.81^{c}	91.32
5	T5	66.51^{ef}	26.10	39.97^{e}	55.58	21.99^{e}	75.56	6.01^{f}	93.32
6	T6	63.50^{fg}	29.44	38.18^{f}	57.57	19.21^{f}	77.79	4.51^{g}	94.98
7	T7	70.11^{cd}	22.10	42.35^{d}	52.94	23.45^{d}	73.94	7.34^{d}	91.84
8	T8	65.54^{ef}	27.17	39.91^{e}	55.65	21.08^{e}	76.57	5.89^{f}	93.45
9	T9	61.81^{g}	31.32	35.41^{g}	60.65	18.53^{f}	79.41	1.82^{h}	97.97
10	T10	68.82^{de}	23.53	40.01^{e}	55.54	23.25^{d}	74.16	6.89^{e}	92.34
11	Control	90							

In vitro efficacy of Trichoderma spp. against Fusarium incarnatum by poison food technique :

Out of Ten *Trichoderma* isolates, half of the isolates are selected from lowest disease incidence areas and another half are from highest disease incidence areas of Andhra Pradesh and Tamil Nadu. All the isolates are screened against *Fusarium incarnatum* by poison food technique and the values are furnished in Table-4. Among the various concentration tested @ 20 % shows maximum percent inhibition over control in all the isolates and the order as follows T9 (97.97), T6 (94.98), T8 (93.45) and least inhibition was recorded in the isolates T2 (89.07). The results are supported by Hasan *et al.*[14], who demonstrated the culture filtrate of *T. asperellum* inhibited the growth of *F. oxysporum* f.sp. *lycopersici*. Among the four concentrations tested (20%, 40%, 60% and 80%), maximum percentage of inhibition value (90.49 mm) was observed at 80% concentration. Likewise, Upadhyay *et al.*[55] tested the antagonistic effect of *Trichoderma* isolates against sugarcane fusarium wilt caused by *F. sacchari* by poison food technique, the growth inhibition ranging between 1.4% (SER 43) to 44.2% (SER10).

In the present study, All the isolates are showed significant inhibition against virulent isolate *Fusarium incarnatum*. Among the isolates ten isolates tested T9 showed highest inhibition against *F. incarnatum* in dual culture assay and in Poison food technique. The species identification of *Trichoderma* was done by using PCR based technique eluted the DNA at 570 bp. *Trichoderma* has been commercial exploited for management of various soil born pathogens since years, the isolates are need to exploit more for further in plant disease management. In future by exploring new species of bioagents we may open new gates to manage plant pathogens effectively.

References :

1. Aboul-Maaty, N.A.F. and H.A.S. Oraby, (2019) *Bulletin of the National Research Centre*, *43*(1): pp. 1-10.
2. Alka P.R., and B. K. Prajapati (2017) *J. Pharmacogn Phytochem 6*(4): 394-398.
3. Al-Taae H.H.W. and A.K. Al-Taae (2019) First molecular identification of *Fusarium oxysporum* causing fusarium wilt of Armenian cucumber in Iraq. In IOP Conference Series: Earth and Environmental Science. IOP Publishing *388*(1): 012005
4. Baloch A.F. (1994) Vegetable crops. In: Malik MK (ed) Horticulture. National Book Foundation, p 498 .
5. Barnett H.L., and B. Hunter (1972) Illustrated genera of imperfect fungi. Burgess publishing company, Minnesota.
6. Baysal-Gurel F., B.M. Gardener, and S.A. Miller (2012) Soil Borne Disease Management in Organic Vegetable Production. Org Agric.
7. Chakrabarty S., A.M. Islam and A.A. Islam, (2017) *Fundamental and Applied Agriculture*, *2*(2): pp. 227-232.
8. Choudhary, S. and M. Reena (2012). *International Journal of Life Science and Pharma Research, 2*(3): 195-202.
9. Dennis C., and J. Webster (1971) *Trans Brit Mycol Soc 57*(1): 25-IN3.
10. dos Anjos I.V., S.S. de Melo, T.A. Gilio, J.P. Kreitlow, SMDS Neves, K.L. Araujo, M.E. Serafim, and L.G. Neves (2019)

J. Agric Sci 11(6): 328.

11. Elad Y. I., Chet, and Y. Henis (1991) *Phytoparasitica 9:* 59-67.
12. Hami A., R.S. Rasool, N.A. Khan, S. Mansoor, M.A. Mir, N. Ahmed, and K.Z. Masoodi (2021) *Sci Rep 11*(1): 1-14.
13. Harman G.E., C.R. Howell, A. Viterbo, I. Chet, and M. Lorito (2004) *Nat Rev Microbiol 2*(1): 43-56.
14. Hasan, Easa Z.A., NAIM Zainudin, A. Aris, M.H. Ibrahim and M.T. Yusof (2021) *Arch Phytopathol Plant Prot 54* (17-18): 1360-1371.
15. Hassan M.M., A. Gaber and E.I. El-Hallous (2014) *Wulfenia Journal 21:* 80-96.
16. Hermosa M.R., I. Grondona, E.T. Iturriaga, J.M. Diaz-Minguez, C. Castro, E. Monte, and Garcia-Acha, IJAEM (2000) *Appl Environ Microbiol 66*(5): 1890-1898.
17. Howell C.R. (1998) The role of antibiosis in biocontrol. In: Harman GE, Kubicek CP (eds.) *Trichoderma* and *Gliocladium*, Vol. 2. Enzymes, biological control and commercial applications. London: Taylor and Francis., pp. 173–184.
18. Jagtap P.P., U.S. Shingane, and K.P. Kulkarni (2014) *J. Spices Aromat crops 23*(1): 32–37.
19. Jayapala N., N.H. Mallikarjunaiah, H. Puttaswamy, H. Gavirangappa, and N.S. Ramachandrappa (2019) *Egypt J. Biol Pest Control 29:* 45.
20. Johnson L.F. and E.A. Curl (1972) Methods for research on the soil borne plant pathogens. Minneapolis U.S. Burgess pub, p 247.
21. Karthika Yadav K., T. Damodaran, N. Kumari, K. Dutt, R. Gopal, and M. Muthukumar (2020) *J. Appl Hortic 22*(1): 38-44.
22. Küçük C. (2017) *J. Biol Environ Sci. 11*(32): 67-74.
23. Kumar R., and I. Honda (2017) *J. Mycol Plant Pathol 37*(2): 259-262.
24. Link K.P., and J. Walker (1933) *J. Biol Chem 100:* 379–383.
25. Lone M.A., M.R. Wani, S.A. Sheikh, S. Sanjay and M.S. Dar (2012) *J. Biol Agric Healthcare 2:* 2224-3208.
26. Manikanta Chowdary G.B.S, and R.S.R. Kumar (2002) *Int J Botany Stud 7* (1): 2188-193.
27. Mishra A., V. Ratan, S. Trivedi, M.R. Dabbas, K. Shankar, A.K. Singh, and Y. Srivastava (2018) *Int J. Pharmacogn Phytochem 7:* 1970-1976.
28. Mushtaq M., and M.H. Hashmi (1997) *Pak J. Bot 29:* 217–222.
29. Naik M. (2006) Wilt of chilli with special reference to cultural, morphological, molecular characterization and pathogenic variability of *Fusarium* isolates of India. In Proceedings of the Midterm Review Meeting of the Project held at Indian Institute of Vegetable Research, Varanasi, India.
30. Nene, Y.L., and P.N. Thapliyal (1993) Fungicides in Plant Disease Control, p. 311–348, Third ed. Oxford and IBH Publishing Co Pvt Ltd, New Delhi India.
31. Pandian R.T.P., M. Raja, A. Kumar, PRATIBHA Sharma (2016) *Indian Phytopathol 69*(3): 297-303.
32. Prasad, N.B. and M.R. Kumar, (2011) *Indian J. Fundamental Appl. Life Sci., 1*(2): 37-42.
33. Praveen A., C. Kannan, V. Jaiganesh and R. Anandan (2021) *Int J Botany Stud 6*(6): 1556-1559.
34. Priya I.N., and R.K. Mesta (2018) *Int J Microbiol Res,* ISSN, pp 0975-5276.
35. Rahman A., M.F. Begum, M. Rahman,

M.A. Bari, GNM Illias, and M.F. Alam (2011) *Turk J Biol 35*(2): 183-194.

36. Rahman M.A., M.F. Begum, and M.F. Alam (2009) *Mycobiology 37*(4): 277-285.
37. Rangaswami, G. (1972) Diseases of crop plants in India. Prentice Hall of India Pvt. Ltd, New Delhi.
38. Saitou, N. and M. Nei, (1987) *Molecular biology and evolution*, *4*(4): pp.406-425.
39. Sankara Reddy N.H., T. Sivakumar, and P. Balabaskar (2021) *Int J. Curr Microbiol App Sci 10*(11): 407-415.
40. Sankara Reddy N.H., T. Sivakumar, P. Balabaskar, and K. Kuralarasi (2021) *Plant Arch21*(1): 437-443.
41. Saravanakumar K. and M.H. Wang (2020) *Physiol Mol Plant Pathol 109:* 101458.
42. Saravanakumar K., C. Yu, K. Dou, M. Wang, Y. Li, and J. Chen (2016) *Biol Control 94:* 37-46.
43. Sekhar Y.C., S.K. Ahammed, TNVKV Prasad, and RSJ Devi (2017) *Int J. Sci. Environ Technol 6 :* 2056-2063.
44. Shaheen N., U.M. Khan, M.T. Azhar, D.K. Tan, R.M. Atif, M. Israr, S.H. Yang, G. Chung, and I. A. Rana (2021) *A Review. Agronomy 11*(11): 2162.
45. Shahid, M., M. Srivastava, A. Singh, V. Kumar, S. Rastogi, N. Pathak, and A.K. Srivastava (2014) *J. Microbial & Biochemical Technology 6:* 110-115.
46. Shaker BRM, V.J.H. Kumar, P. Chaitanya K. Sriranjitha, R. Kumar, and P.J.M. Rao (2021) *Int J. Curr Microbiol App Sci 10*(02): 893-901.
47. Sharma A., R. Salwan, R. Kaur, R. Sharma, and V. Sharma (2022) *Biocatal Agric Biotechnol,* p 102437.
48. Shomaila I., M. Ashfaq, A.H. Malik, and M.I.U. Haq (2022) *Pak J. Phytopathol 34*(1): 81-91.
49. Singh J.K., M. Kumar, S. Kumar, A. Kumar, and N. Mehta (2017) *J Pharmacogn Phytochem 6:* 2199–2204.
50. Singh, A., M. Shahid, and M. Srivastava, (2014) *International Journal of Advance Research 2*(3): 979-986.
51. Soesanto L., D.S. Utami, and R.F. Rahayuniati (2011) *Can J. Sci Ind Res 2:* 294–306.
52. Subash N., M. Meenakshisundaram, N. Unnamalai, and C. Sasikumar (2013) *Int J. Biol Pharm Appl Sci 2:* 495-500.
53. Tapwal A., G. Thakur, S. Chandra and A. Tyagi (2015) *Int J. Biol Chem Sci 1*(10): 14-19.
54. Tapwal, A., U. Singh, G. Singh, S. Garg and R. Kumar, (2011) *Pest Technology. 5*(1): 59-62.
55. Upadhyay M., A. Awasthi, and D. Joshi (2020) *Biotecnología Vegetal 20*(3): 237-247.
56. Vinale F., K. Sivasithamparam, E.L. Ghisalberti, R. Marra, S.L. Woo, M. Lorito (2008) *Soil Biol Biochem 40:* 1-10.
57. Wonglom P., W. Daengsuwan, S.I. Ito, and A. Sunpapao (2019) *Physiol Mol Plant Pathol 107:* 1-7.
58. Yassin M.T., A.A.F. Mostafa, and A.A. Al-Askar (2021) *Egypt J. Biol Pest Control 31*(1): 1-9.
59. Zhang F., X. Yang, W. Ran, and Q. Shen (2014) *FEMS Microbiology Letters 359:* 116-123 .