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Antagonistic Potential of Different Isolates of *Trichoderma* against *Fusarium oxysporum*, *Rhizoctonia solani*, and *Botrytis cinerea*

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Abstract

Trichoderma spp. are widely used as bio-fungicides in agriculture. Induction of plant defense and mycoparasitism (killing of one fungus by another) are considered to be the most important mechanisms of *Trichoderma*-mediated biological control. In the present study, 380 isolates of 21 *Trichoderma* species were isolated from Grassland forest soil of Iner Mongolia, China and screened for their antagonistic and antibiosis efficacy against *Fusarium oxysporum*, *Rhizoctonia solani* and *Botrytis cinerea* by dual culture. The result indicated that the antagonistic potential of 380 isolates of *Trichoderma* strains against *Fusarium oxysporum*, *Rhizoctonia solani* and *Botrytis cinerea* were varied which inhibited *Fusarium oxysporum* ranges 10.12-70.70%, *Botrytis cinerea* (44.18-82.98%) and *Rhizoctonia solani* (35.07-88.07). Among isolates of *Trichoderma*, 195 isolates showed strong antagonistic potential which inhibited >50% mycelial growth of *F. oxysporum*, 319 and 377 *Trichoderma* isolates inhibited >50% mycelial growth of *R. solani* and *B. cinerea* respectively. Furthermore, 47 *Trichoderma* strains have inhibited >50% mycelial growth and have >30 Mycoparasitism for the three tested pathogens. Moreover, one hundred eighty five (185) isolates were also showed inhibitory but their antagonistic potential <50% of the mycelial growth while 50 isolates showed <40% mycelial growth of *F. oxysporum*, 61 isolates showed <50% mycelial growth for R. solani and 3 isolates showed <50% mycelial growth for *B. cinerea*. These potential isolates of *Trichoderma* may be further exploited as a biocontrol agent against *F. oxysporum*, *R. solani* and *B. cinerea* as well as other soilborne phytopathogenic fungi.

Keywords: *Trichoderma spp;* Biological control; Antagonistic potential; Mycelial growth

Introduction

Plant diseases play a direct role in the destruction of natural resources in agriculture. In particular, soil-borne pathogens cause important losses, fungi being the most aggressive. The distribution of several phytopathogenic fungi, such as Botrytis, Rhizoctonia, and Fusarium, has spread during the last few years due to changes introduced in farming, with detrimental effects on crops of economic importance [1].

Soilborne plant pathogens can be a major limitation to yield and quality in vegetable crops. These pathogens are predominantly challenging because they often survive in soil for many years and each vegetable crop may be susceptible to several pathogen species. They are often difficult to control, even with conventional strategies. Different approaches are used to control crop diseases. The use of chemicals in controlling plant diseases has contributed significantly to improvements in crop productivity and quality over the past 100 years [2].

Management of soil born pathogen by chemical fungicides was expensive and tedious. No effective fungicides are available in the market. Moreover, the negative effect of chemical fungicides causes phytotoxicity and environmental pollution [3,4]. Intensified use of fungicides has resulted in the accumulation of toxic compounds potentially hazardous to humans and environment also in the build-up of resistance of pathogens [5]. In order to tackle these national and global problems, alternatives to chemical control are investigated by the use of antagonistic microbes [6]. Therefore, the alternative method of crop disease control is the use of biological control agents. Biological control agents are cost-effective without having any adverse effects on human health or environment, self-sustaining, development of host resistance is unlikely and it is compatible with other crop disease control techniques.

Among the microbes, *Trichoderma* spp. is a common saprophytic filamentous fungus which habitat in soil and rhizospheric soil. It acts as a biocontrol agent against various plant pathogenic causes several diseases in mono and dicotyledonous crop plants [7-9]. Being commercialized as biopesticides, biofertilizers, and soil enhancers. They are non-pathogenic microorganisms that provide protection against fungal diseases caused by *Phytophthora*, *Rhizoctonia*, *Sclerotium*, *Pythium*, and *Fusarium genera* [10]; additionally, they promote high yields in crops [11]. These traits derive from their ability to produce antifungal metabolites, release

hydrolytic enzymes, and their mycoparasitic behavior, as well as the production of other substances that enhance plant growth [12].

The objectives of this study were to evaluate the antagonistic activity of *Trichoderma* isolates originating from Inner Mongolia Grassland and forest soils against *F. oxysporum*, *R. solani* and *B. cinerea in vitro* conditions and to identify isolates with the highest capacity for pathogen inhibition.

Materials and Methods

The present experiments were conducted in Chinese Academy of Agriculture, Institute of plant protection, *Trichoderma* research Team laboratory, Beijing, China.

Collection of soil simple

The soil sample was collected from different localities of Grassland and forest regions of Inner Mongolia, China. A five-point sampling method was used. We collected soil core from each of the four corners and a center of the given field (10 m × 10 m) by using a hand probe, these soil core samples were combined and mixed evenly and took the central part combined sample into bags. Each sample contained about 200 g of soil from a depth of approximately 5-7 cm. Samples were placed in sterile polyethylene bags, transported to the laboratory, and stored at 4°C until isolation.

Isolation of *Trichoderma* from the soil

Isolation of different isolates of *Trichoderma* was made by the collected soil serial dilution technique of the soil sample. One (1) ml of 10^{-3} dilution was poured onto *Trichoderma* selective Medium ($MgSO_4$: 0.20 g, KH_2PO_4: 0.90 g, NH_4NO_3: 1.0 g, KCl: 0.15 g, Glucose: 3.0 g, Rose Bengal: 0.15 g, Chloramphenicol: 0.25 g, streptomycin: 0.05 g, Agar: 15 g, Pentachloronitrobenzene (PCNB): 0.3 g, Distilled water: 1 L) for selective isolation of *Trichoderma* and after the appearance of the colonies of *Trichoderma* on Petri dishes purified by hyphal tip isolation techniques. *Trichoderma spp*. was identified, picked on the basis of their morphological, microscopic characteristics and Molecular Methods using ITS5 (5'-GGAAGTAAAAGTCGTAACAAGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') according to [13] with modifications. The purified and identified cultures of *Trichoderma spp.* were maintained on Potato Dextrose Agar (PDA) medium and stored at 4°C for further experimentation. Pure cultures were kept in 20% (w/v) glycerol at -20°C.

Collection of pathogens

Three pathogenic isolates namely *Fusarium oxysporum*, *Rhizoctonia solani* and *Botrytis cinerea* initially isolated from diseased tomato and cucumber seedling were obtained from China Academy of Agricultural, Institute of plant protection Research Laboratory (Beijing). The Isolate was maintained on PDA medium and placed until use at 4°C.

Identification of *Trichoderma* species

Fungal hyphae of *Trichoderma* species are septet, hyaline and smooth-walled which produces numerous conidiophores are highly branched. Normally, the branches will form at or near 90° with respect to the main branch. The typical conidiophore terminates with one or a few phialides that usually arising directly from the axis near the tip. Phialides, also known as conidiogenous cells, are typically enlarged in the middle like a flask-shape and may be cylindrical or nearly sub-globose. Conidia are one-celled, and either ellipsoidal (3-5 × 2-4 µm, L/W ≥ 1.3) or globose (L/W <1.3). They are typically light to dark green, or sometimes colorless, greyish or brownish which typically smooth surface.

Some strains are also produced chlamydospores which play important role in survival. They are normally found as thick-walled, enlarged vegetative cells with condensed cytoplasm which are unicellular, globose to subglobose chlamydospores are either formed within hyphae or at the hyphal tips [14,15].

Typically, they are colorless, pale yellowish or greenish. Morphological characters were compared with morphologically and molecularly identified strains.

The nucleotide sequences of 5.8S-ITS region were aligned using Molecular Evolutionary Genetics Analysis (MEGA) version 6.06. The forward and reverse sequences were checked and edited manually when needed. Then, a consensus sequence was generated from each alignment made. The sequences were then compared with the sequences deposited in GenBank database using Basic Local Alignment Search Tool (BLAST), where a nucleotide blast program was chosen. Besides, the 5.8S-ITS sequences were compared to a specific database for *Trichoderma* using TrichOKEY 2 program, which available online from the International Subcommission on *Trichoderma* and Hypocrea Taxonomy (ISTH, www.isth.info) [16].

Screening of antagonistic potential of *Trichoderma* strains with *Fusarium oxysporum, Rhizoctonia solani, and Botrytis cinerea*

Dual culture technique was used to conduct the antagonistic test. The *Trichoderma* isolates and a pathogen species to be tested were cultured separately on PDA for 7 days. After 7 days, 5 mm mycelial plugs (taken from the edge of fungal colonies) of each species to be tested were transferred to PDA plates using cork borer. The mycelial plug of *Trichoderma* species and pathogens was placed opposite side to each other on a PDA surface. PDA plates inoculated with the pathogens spp. were included as negative controls. The antagonistic tests were conducted in duplicate. The controls consisted of pure *Fusarium oxysporum*; *Rhizoctonia solani* and *Botrytis cinerea* cultures all culture plates were incubated at 28°C and observations were made after 5 days of and the hyper parasitization were recorded after 10 days of incubations. The percent inhibition of mycelia growth over control was calculated by following equation [17].

$$I\% = (C-T)/C \times 10$$

Where, I=Percent inhibition of mycelial growth, C=Growth of mycelium in control. T=Growth of mycelium in treatment in the pathogens.

Statistical analysis

Data were analyzed using statistical package SPSS version 20.

Result and Discussion

Twenty-one Trichoderma species were identified from 380 strains isolated from 711 soil sample collected from Grassland and forest soil of Inner Mongolia and individual culture of every isolate was maintained for further experimentation. The isolates were studied for species identification and the same revealed that they belong to *Trichoderma Isolates*. The isolates of *Trichoderma* were a dual culture in Petri plates with *Fusarium oxysporum*, *Rhizoctonia solani*, and *Botrytis cinerea*. Further, the average percent inhibition by all 21 *Trichoderma* species isolates against *Fusarium oxysporum*, *Rhizoctonia solani* and *Botrytis cinerea* have been evaluated (**Table 1**). The average Growth inhibition of *Trichoderma spp.* on *B. Cinerea* was higher by the rate of 63.07 ± 2.73, followed by *R. solani* with an inhibition rate of 57.42 ± 2.16 and the inhibition of *F. oxysporum* was 46.09 ± 2.77. The Mycoparasitism rate of *Trichoderma spp.* and the Growth rate of the pathogens was also varied, the highest rate of Mycoparasitism was recorded by *R. Solian* with 36.49 ± 20.79 followed by *F. oxysporum* with the rate of 23.60 ± 10.88 and *B. cinerea* (20.60 ± 10.88). Highest mycelial growth was recorded with the rank of *R. solani* (31.08 ± 1.86), *B. cinerea* (22.24 ± 1.80) and *F. oxysporum* (15.80 ± 0.69).

Table 1. The average dual culture assays of the 21 *Trichoderma* species against *F. oxysporum*, *B. cinerea*, *and R. solani* (in mm).

Species name	Number of strain	IR F	MR-F	GoF	IR B	MR-B	GoB	IR R	MR-R	GoR
T.cf. harzianum	148	50.73 ± 0.74	25.51 ± 1.45	15.38 ± 0.16	66.61 ± 0.44	28.63 ± 1.07	20.13 ± 0.26	57.08 ± 0.81	38.46 ± 0.85	31.53 ± 0.58
T. longibrachiatum	120	50.68 ± 0.59	42.62 ± 0.74	14.20 ± 0.14	66.71 ± 0.39	4.31 ± 0.81	20.13 ± 0.24	68.12 ± 0.93	39.72 ± 0.78	23.84 ± 0.69
T. afroharzianum	30	45.10 ± 1.54	25.58 ± 2.01	16.28 ± 0.43	66.82 ± 0.97	40.5 ± 2.50	20.43 ± 0.65	59.25 ± 1.29	43.17 ± 1.52	30.23 ± 1.01
T. koningiop	23	47.49 ± 1.60	28.70 ± 3.42	15.60 ± 0.31	65.40 ± 1.40	31.09 ± 3.04	20.94 ± 0.74	52.99 ± 1.67	37.61 ± 1.56	34.62 ± 1.22
T harzianum	9	39.47 ± 3.30	12.22 ± 5.97	16.79 ± 0.55	63.91 ± 1.80	29.16 ± 5.36	21.55 ± 1.06	58.31 ± 4.46	43.33 ± 1.66	31.74 ± 3.33
T. hamatum	9	48.71 ± 3.44	30.38 ± 5.57	16.22 ± 0.9	62.90 ± 1.26	26.12 ± 5.09	23.52 ± 0.95	52.34 ± 1.85	37.85 ± 3.63	34.88 ± 1.26
T. citrinovi	7	49.61 ± 2.13	22.85 ± 5.18	14.88 ± 0.63	62.46 ± 2.71	13.92 ± 6.42	22.82 ± 0.67	70.03 ± 2.98	38.21 ± 5.30	22.64 ± 2.10
T. rossicum	7	47.85 ± 5.95	33.30 ± 6.04	15.97 ± 1.18	62.73 ± 2.95	19.26 ± 6.74	23.29 ± 1.24	55.00 ± 3.00	38.61 ± 4.03	32.26 ± 1.50
T. gamsii	5	49.40 ± 3.82	45	14.17 ± 0.56	70.70 ± 2.59	45	17.86 ± 1.24	58.58 ± 1.41	45	31.30 ± 1.11
T. guizhoue	3	42.74 ± 0.53	20 ± 13.22	14.79 ± 0.53	66.86 ± 1.15	30.83 ± 11.76	20.50 ± 1.49	59.55 ± 4.27	45	30.31 ± 3.42
H. alni	2	28.9 ± 5.28	3.75 ± 3.75	19.65 ± 1.85	61.37 ± 2.72	3.75 ± 3.75	24.45 ± 4.17	53.18 ± 2.72	18.75 ± 26.25	24.45 ± 4.17
H. atrovirides	2	47.39 ± 2.11	29.34 ± 12.28	14.34 ± 0.27	69.41 ± 1.47	18.98 ± 13.24	19.3 ± 2.88	60.10 ± 2.51	29.34 ± 12.28	29.43 ± 1.70
T. saturnisporum	2	29.72 ± 3.47	0	20.33 ± 2.29	48.56 ± 3.72	7.5 ± 7.5	30.09 ± 3.93	48.57 ± 0.63	3.75 ± 11.25	39.97 ± 1.61
T. asperelloides	2	39.33 ± 6.07	37.5 ± 7.5	17.44 ± 0.57	62.54 ± 8.62	45	23.36 ± 1.90	58.33 ± 1.29	45	31.35 ± 0.29
T. crassum	2	47.24 ± 0.65	15 ± 15	15.63 ± 0.47	63.39 ± 4.37	11.25 ± 3.75	21.70 ± 2.28	55.60 ± 6.30	45	33.93 ± 4.77
T. koningii	2	52.90 ± 6.31	22.5 ± 22.5	15.23 ± 0.07	55.97 ± 3.04	7.5	24.90 ± 5.48	51.12 ± 1.82	37.5 ± 7.5	36.29 ± 2.41
T. konilangbra	2	41.19 ± 2.66	22.5 ± 7.5	15.55 ± 0.4	61.49 ± 3.33	22.5 ± 7.5	21.45 ± 2.16	54.23 ± 2.65	45	33.99 ± 2.23

T. velutinum	2	53.3 ± 8.01	26.25 ± 18.75	14.73 ± 0.11	66.60 ± 3.33	26.25 ± 18.75	19.77 ± 0.36	59.57 ± 4.70	45	29.25 ± 2.94
T. polysporum	1	68.76	45	13.51 ± 1.79	63.73	7.5	26.90 3.48	52.08	30	33.42 ± 0.76
T. virens	1	41.95	7.5	15.66 ± 0.50	68.37	15	21.81 ± 0.09	68.6	45	22.10 ± 0.94
T. sinosum	1	45.49	0	15.40 ± 0.68	54.69	0	22.13 2.71	53.15	15	35.21 ± 1.00
	380	46.09 ± 2.77	23.60 ± 10.88	15.80 ± 0.69	63.07 ± 2.73	20.60 ± 10.88	22.24 ± 1.80	57.42 ± 2.16	36.49 ± 20.79	31.08 ± 1.86

IRF=% Inhibition rate of *Fusarium oxysporum*
IRR=% inhibition rate of *Rhizoctonia solani*
IRB=% inhibition rate of *Botrytis cinerea*
MR-F=Mycoparasitism rate of *Trichoderma* on *Fusarium oxysporum*
MR-R=Mycoparasitism rate of *Trichoderma* on *Rhizoctonia solani*
MR-B=Mycoparasitism rate of *Trichoderma* on *Botrytis cinerea*
GoF=Radial growth of *Fusarium oxysporum*
GoR=Radial growth of Rhizoctonia solani; GoB=Radial growth of *Botrytis cinerea*

The inhibitory ability of different *Trichoderma* species to three pathogens was different. Among the *Trichoderma* species, 6 species showed strong antagonistic potential which inhibited >50% mycelial growth of *Fusarium oxysporum*, viz., *T. polysporum* (63.99), *T. velutinum* (53.3), *T. koningii* (52.90), *T. gamsii* (52.45), T.*cf. harzianum* (50.74) and *T. longibranchiatum* (50.68), the highest and lowest Growth and Mycoparasitism of *F. oxysporum* was recorded under *T. saturnisporum* (20.33 ± 2.29), *T. polysporum* (13.51 ± 1.79) and *T. gamsii* (45), *T. polysporum* (45) and *T. sinosum* (0) respectively. Moreover, fifteen species were also showed inhibitory but their antagonistic potential was <50% of the mycelial growth. Among the 21 *Trichoderma spp*. Tested 20 of them has >50% mycelial growth inhibition and only one species has <50%. The two most resistant species of *Botrytis cinerea* were *T. gamsii* (70.70 ± 2.59) and *T. atrovirides* (69.41 ± 1.47) and the lowest antagonistic *Trichoderma* species were *T. saturnisporum* (48.56 ± 3.72). The highest Mycoparasitism capacity of *Trichoderma spp.* Against B. cinerea was recorded by *T. gamsii* and *T. asperelloides* (45) each and the lowest was by *T. sinosum* (0). 20 species out of 21 has shown >50% of growth inhibition of *R. solani* mycelia. The highest inhibition was recorded by *T. citrinoviride* (70.03 ± 2.98) and the lowest was by *T. saturnisporum* (48.57 ± 0.63). The highest Mycoparasitism was recorded by 7 *Trichoderma spp* with the rate of (45). And lowest was by *T. saturnisporum* (3.75 ± 11.25) (**Table 1**).

Further, the percent inhibition by all the 380 *Trichoderma* isolates against *Fusarium oxysporum*, *Rhizoctonia solani* and *Botrytis cinerea* has been evaluated (Appendix 1). In the case of *Fusarium oxysporum* among isolates of *Trichoderma*, 195 isolates showed strong antagonistic which inhibited >50% mycelial growth and from this 35 isolate showed >60% inhabitation and the top five are, CTCCSJ-F-KY40053 (70.70%), CTCCSJ-G-JK41059 (69.86%), CTCCSJ-G-QT41050 (68.76%), CTCCSJ-G-LK41051 (68.76%) and CTCCSJ-G-HB41054 (65.32%). Moreover, 185 isolates were also showed inhibitory but their antagonistic potential <50% of the mycelial growth while 50 isolates showed <40% mycelial growth. From 195 isolated strains, 120 of them have Mycoparasitism of 45.

For *Botrytis cinerea* from 380 *Trichoderma* isolates 377 isolates showed strong antagonistic which inhibited >50% mycelial growth and 81 isolates showed >70% inhabitation and the top five are, CTCCSJ-G-HB41039 (82.98%), CTCCSJ-F-KY40053 (81.09%), CTCCSJ-G-QT40904 (78.36%), CTCCSJ-G-QT40113 (78.31%) and CTCCSJ-G-HB40110 (77.85%).Three isolates were showed antagonistic potential <50% of mycelial growth. From 377 *Trichoderma* isolates 98 of them has Mycoparasitism of 45 and 99 of the isolates has zero (0) Mycoparasitism.

The percent inhibition against *Rhizoctonia solani* by all 380 *Trichoderma* isolated was also recorded 319 isolates has showed antagonistic which inhibited >50% mycelial growth and 80 isolates has shown antagonistic which inhibited >70% mycelial growth and the top five are, CTCCSJ-G-HB40057 (88.07%), CTCCSJ-G-HB40040 (85.86%), CTCCSJ-G-QT40154 (85.40%), CTCCSJ-G-QT40223 (84.02%) and CTCCSJ-G-QT40168 (84.52%) while 61 isolates showed <50% mycelial growth. From 319 *Trichoderma* isolates 219 of them has Mycoparasitism of 45, 74 isolates have Mycoparasitism of 30 and one strain has rate -7.5.

Analysis of the antagonistic effect of *T. cf. harzianum* on three pathogens

The results showed that the antagonistic effect of *Trichoderma spp*., on the three pathogens, was different and the antagonistic effect of the same strain of *Trichoderma spp*. On the same pathogen was different *and T.cf.harzianum* was used as an example.

It can be seen from (**Figure 1**) that the antagonistic effect of *T.cf. harzianum* on three pathogens is significantly different. From the inhibition rate, *B. cinerea* was the highest value of 66.61% followed by *R. solani* with an inhibition rate of 57.08% but the inhibition rate of *F. oxysporum* was 50.73%. *T. cf. harzianum* can significantly inhibit the growth of *B. cinerea* and *R. solani* but *F. oxysporum* itself grows slowly and inhabitation effect is not higher as others. In terms of Mycoparasitism ability, the highest was *R. solani* (38.46), followed by *B. cinerea* (28.63) and the lowest was *F. oxysporum* (25.51). After 10 days of incubation the Mycoparasitic ability of *T.cf. harzianum* was

higher in the dual culture of *R. solani* and *B. cinerea* but low Mycoparasitism was recorded in *F. oxysporum* compared to others (**Figure 2**).

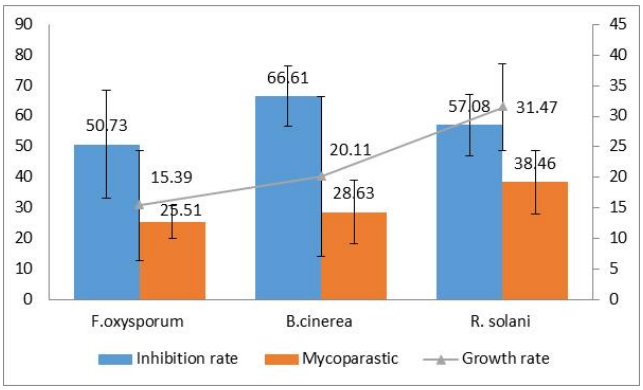

Figure 1. The summary figure of dual culture assays of T.cf. harzianum against three pathogens.

Screening of the antagonistic *Trichoderma* strains

In addition we have found 174 common isolates of *Trichoderma* which inhibited >50% mycelial growth against *Fusarium oxysporum*, *Rhizoctonia solani* and *Botrytis cinerea*, in additions from 174 strains 47 common isolates has higher Mycoparasitism which is greater than or equal to 30 and above, which belongs to 10 *Trichoderma spp*., *T.cf.harzianum* (28), *T. koningiopsis* (4), *T. afroharzianum* (3), *T. gamsii* (3), *T. citrinoviride* (2), *T. longibranchiatum* (2), *T. rossicum* (2), *T.velutinum* (1), *T. harzianum* (1), *T. hamatum* (1) (**Table 2**).

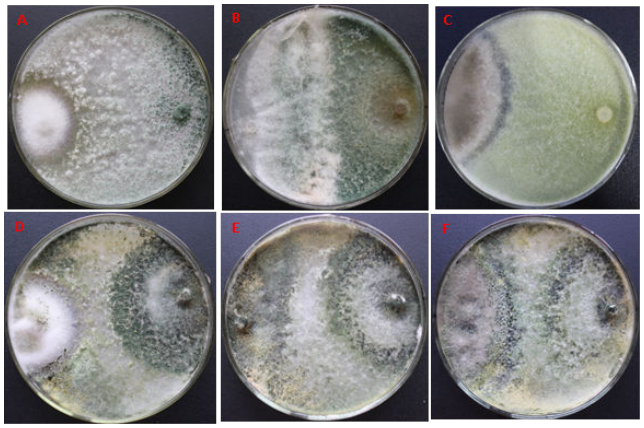

Figure 2. Some *T. cf. harzianum* strains antagonism effect to three pathogens.

Table 2. Primary screened 47 antagonistic Trichoderma strains (in mm).

Voucher Sample	Strains	IR-F	MR-F	IR-B	MR-B	IR-R	MR-R
CTCCSJ-G-HB40038	*T. citrinoviride*	55.7	45	68.25	30	76.74	45
CTCCSJ-G-HB40041	*T.cf.harzianum*	55.81	30	73.16	45	65.49	45
CTCCSJ-G-HB40045	*T.cf.harzianum*	62.35	45	72.86	45	72.02	45
CTCCSJ-G-HB40046	*T. citrinoviride*	55.66	30	67.12	45	74.19	45
CTCCSJ-F-KY40053	*T. afroharzianum*	70.7	30	81.09	45	67.44	45
CTCCSJ-G-HB40057	*T. longibrachiatum*	54.41	30	77.49	45	88.07	45
CTCCSJ-G-QT40063	*T. afroharzianum*	54.41	30	76	45	67.19	45
CTCCSJ-G-QT40069	*T.cf.harzianum*	55.22	30	73.05	45	64.39	45
CTCCSJ-G-HB40091	*T.cf.harzianum*	55.12	30	76.21	45	65.66	45
CTCCSJ-G-HB40110	*T.cf.harzianum*	58.22	30	77.85	45	68.93	45
CTCCSJ-G-HB40294	*T. afroharzianum*	64.28	30	65.27	45	54.95	45
CTCCSJ-G-QT40314	*T. koningiopsis*	50.5	30	70.73	45	56.77	30
CTCCSJ-G-QT40328	*T. gamsii*	53.25	45	68.48	45	55.5	45
CTCCSJ-G-QT40330	*T. gamsii*	54.76	45	76.86	45	63.11	45
CTCCSJ-G-HB40468	*T.cf.harzianum*	50.3	30	61.64	30	54.24	45
CTCCSJ-G-HB40473	*T.cf.harzianum*	52.74	30	66.19	45	56.48	45
CTCCSJ-G-HB40481	*T. harzianum*	57.69	45	65.3	30	68.49	45
CTCCSJ-G-HB40484	*T. rossicum*	55.5	30	71.4	30	66.06	45
CTCCSJ-F-ZY40511	*T. gamsii*	57.83	45	76.97	45	59.62	45

CTCCSJ-F-ZY40535	*T. koningiopsis*	56.76	45	72.78	45	56.4	45
CTCCSJ-G-QT40905	*T.cf.harzianum*	62.17	45	71.81	30	66.89	45
CTCCSJ-G-QT40907	*T. longibrachiatum*	50.17	30	68.15	30	51.74	30
CTCCSJ-G-QT40908	*T.cf.harzianum*	52.8	45	72	30	65.79	45
CTCCSJ-G-HB40920	*T.cf.harzianum*	53.86	45	60.9	45	57.64	45
CTCCSJ-G-QT40936	*T.cf.harzianum*	50.26	30	61.96	45	63.04	45
CTCCSJ-G-HB40938	*T.cf.harzianum*	54.28	45	60.47	45	50.45	30
CTCCSJ-G-JK40972	*T.cf.harzianum*	55.35	30	69.53	30	64.08	45
CTCCSJ-G-JK40977	*T.cf.harzianum*	63.42	45	62.08	45	53.03	45
CTCCSJ-G-HB41021	*T.cf.harzianum*	55.59	45	65.16	30	51.04	30
CTCCSJ-G-HB41024	*T.cf.harzianum*	57.11	45	67.69	30	56.46	45
CTCCSJ-G-HB41026	*T.cf.harzianum*	58.5	30	61.96	45	56.33	45
CTCCSJ-G-QT41029	*T. hamatum*	55.8	30	69.45	30	56.13	45
CTCCSJ-G-QT41030	*T. koningiopsis*	54.29	45	64.39	30	55.86	45
CTCCSJ-G-QT41031	*T. koningiopsis*	52.95	45	70.18	45	55.45	45
CTCCSJ-G-QT41032	*T.cf.harzianum*	64.41	45	73.12	45	55.43	45
CTCCSJ-G-QT41035	*T.cf.harzianum*	60.73	45	71.35	45	55.13	45
CTCCSJ-G-HB41036	*T.cf.harzianum*	59.79	45	69.89	45	55.03	45
CTCCSJ-G-QT41037	*T. velutinum*	61.05	45	69.94	45	54.86	45
CTCCSJ-G-HB41038	*T.cf.harzianum*	61.32	45	71.57	30	53.94	30
CTCCSJ-G-QT41041	*T.cf.harzianum*	54.99	30	59.09	30	53.81	30
CTCCSJ-G-HB41044	*T.cf.harzianum*	61.85	45	65.11	30	53.25	45
CTCCSJ-G-HB41045	*T.cf.harzianum*	61.71	45	68.25	45	53.23	45
CTCCSJ-F-KY41047	*T.cf.harzianum*	62.81	45	62.2	30	52.88	30
CTCCSJ-G-QT41050	*T. rossicum*	68.76	45	68	45	52.16	30
CTCCSJ-G-HB41054	*T.cf.harzianum*	65.32	45	63.49	30	51.78	30
CTCCSJ-G-JK41059	*T.cf.harzianum*	69.86	45	63.43	45	51.66	30
CTCCSJ-G-HB41061	*T.cf.harzianum*	56.7	45	67.61	30	50.05	30
		57.81 ± 0.7	38.94 ± 1.8	68.88 ± 0.87	39.26 ± 1.1	59.55 ± 1.2	41.49 ± 0.9

IRF=% Inhibition rate of *Fusarium oxysporum*
IRR=% inhibition rate of *Rhizoctonia solani*
IRB=% inhibition rate of *Botrytis cinerea*
MR-F=Mycoparasitism rate of *Trichoderma* on *Fusarium oxysporum*
MR-R=Mycoparasitism rate of *Trichoderma* on *Rhizoctonia solani*
MR-B=Mycoparasitism rate of *Trichoderma* on *Botrytis cinerea*

The average growth inhibition capacity of the 47 strains in the case of *F. oxysporum* was 57.81 ± 0.74, the highest *Trichoderma* strain recorder are *T. afroharzianum* (70.70%) followed by *T.cf.harzianum* (69.86%) and the average Mycoparasitism was 38.94 ± 1.08 , the highest was 45; the average inhibition capacity of *Trichoderma* strains in the case of B. cinerea was 68.88 ± 0.77 , the highest inhibition was by *T.cf.harzianum* (77.85) followed by *T. longibranchiatum* (77.49) and the average Mycoparasitism was 39.26 ± 1.07, the highest was 45; the average inhibition rate of *R.solani* was 59.55 ± 1.17 and the highest inhibition rate was (88.07) by *T. longibranchiatum* followed by (76.74*) T. citrinoviride* with Mycoparasitism of 45.

Trichoderma species was found to be an effective biological control agent for protecting a number of crop plants from damaged induced by *Fusarium oxysporum*, *Rhizoctonia solani* and *Botrytis cinerea* under both greenhouse and field conditions

in the study conducted by [18,19]. Various agro products are used for biomass production and applied as a biocontrol agent. Several mechanisms may explain the biocontrol activity of these strains [20,21]. Hyperparasitism and volatile metabolites may be involved in the inhibition of Fusarium oxysporum, *Rhizoctonia solani* and *Botrytis cinerea* [22]. Cell wall degrading enzymes (CWDEs) such as chitinase, glucanase, and proteases are thought to be closely related to the mycoparasitism of *Trichoderma* strains [18,23,24]. Inhibitory volatile substances such as alkylpyrons may also contribute to the biocontrol activity of some *Trichoderma* strains [25-27]. Harman et al. [28] reported that the *Trichoderma* have the ability to antagonized soilborne phytopathogens as well as it also induced plant growth promotion and protect plants from biotic and abiotic stresses [29]. Thus, it can be concluded that *Trichoderma* isolates prove to be effective biocontrol agent and native isolates of it may be further explored as a biocontrol agent against *Fusarium oxysporum*, *Rhizoctonia solani*, and *Botrytis cinerea*.

Conclusion

Twenty-one (21) species identified from 380 native strains of *Trichoderma* were isolated from Inner Mongolia Grassland and forest Soil and were characterized on the basis of their morphological features and Molecular techniques, Screening of the antagonistic potential of isolated *Trichoderma* strains against *Fusarium oxysporum*, *Rhizoctonia solani* and *Botrytis cinerea* were conducted and we have found that 195 *Trichoderma* strain showed strong antagonistic potential which inhibited >50% mycelial growth of *Fusarium oxysporum* and 317 and 319 *Trichoderma* strains for *Botrytis cinerea* and *Rhizoctonia solani* respectively. 174 common strain has shown inhibited >50% mycelial growth of *Fusarium oxysporum*, *Rhizoctonia solani* and *Botrytis cinerea*, from the above common strains 47 of them has highest Mycoparasitism >30 while 185 isolates were also showed inhibitory but their antagonistic potential <50% of the mycelial growth while 50 isolates showed <40% mycelial growth of *Fusarium oxysporum*, 61 isolates showed <50% mycelial growth for *Rhizoctonia solani* and 3 isolates showed <50% mycelial growth for *Botrytis cinerea*. Furthermore from 380 Trichoderma strain 259 of them has >30 Mycoparasitism on Fusarium oxysporum, 184 and 354 strain has >30 Mycoparasitism on Botrytis cinerea and Rhizoctonia solani. As per result, 47 potential isolates of Trichoderma may be further exploited as a biocontrol agent against *Fusarium oxysporum*, *Rhizoctonia solani*, and *Botrytis cinerea* as well as other Soilborne phytopathogenic fungi.

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