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Biological Control of Soilborne Pathogens (*Fusarium oxysporum* F. Sp. Cucumerinum) of Cucumber (*Cucumis sativus*) by *Trichoderma* sp.

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Abstract: In this study, the efficacy of the native isolates of Trichoderma species to promote the growth and yield parameters of cucumber and to manage Fusarium wilt disease under greenhouse conditions were investigated. Ninety native Trichoderma antagonists were isolated from Grassland and forest soil in different geographical regions of Inner Mongolia, China. Applications of *T. cf. harzianum* T1, T2, T3, T4, T5, T6, T7, T8, T10, T11 and *T. atroviride* (T9) exhibited the least disease incidence (by 0%) also the same strains shows 100% of relative control effect. Cucumber seedling treated with *T. cf. harzianum* (T2 and T1) isolates showed a significant stimulatory effect on plant height (by 13 and 14 cm respectively) and the highest shoot and root fresh weight were recorded by *T. asperelloides* (T27) and *T. gamsii* (T17) by 1.62 gm and 0.97 gm respectively, in comparison to untreated control and treated control (10 cm and 4 cm, 1.27 gm and 0.22 gm and 0.82 gm and 0.10 gm). Therefore, the antagonist (T1, T2, T3, T4, T5, T6, T7, T8, T9, T10, and T11) is chosen to be the most promising bio-control agent for *Fusarium oxysporum* f. sp. Cucumerinum and further study have to be exploited for sustainable disease management program.

Key words: Cucumber, biocontrol, *Fusarium*, *T. cf. harzianum*, *Trichoderma* spp.

1. Introduction

Cucumber (*Cucumis sativus* L.) is one of the most important economic crops, which belongs to family cucurbitaceae and is as important as a tomato in vegetable production in China. Cucumber is grown widely throughout the country, especially in North China, where it is the number one summer vegetable. China produces 70% of all the cucumbers in the world. According to data from the FAOSTAT, the statistical agency of the UN's FAO (Food and Agriculture Organization), with a total area of 1,150,000 ha and a total production of 48,000,000,000 kg. Cucumber is grown either in the open field or under protected houses. The purpose of growing crops under protected house conditions is to extend their cropping season and to protect them from adverse conditions as well as diseases and pests [1].

Fusarium species are worldwide pathogenic fungi of many crop plants. Cucumber Fusarium wilt disease may occur at all growth periods of the cucumber plant [2]. The pathogens can survive as durable spores for many years with or without plant debris in soil, and it retains the ability to infect cucumber plants causing pre- or post-emergence damping-off, vascular discoloration of roots and stems, and eventually the entire plant wilts or dies. In China, an epidemic cucumber Fusarium wilt disease often occurred and led to a major yield loss in cucumber production. Generally, it caused cucumber yield losses of ~10% to 30% and poor quality products resulting in severe economic losses [3]. In China, cucumber Fusarium wilt was caused by several Fusarium species, including *F. oxysporum*, *F. equiseti*, *F. solani*, *F. moniliforme* and *F. proliferatum*. Among them, *F.*

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oxysporum is the major pathogen [4-6] and it is widespread in North China and is especially serious in glasshouse cropping systems [7]. *Fusarium oxysporum* f. sp. cucumerinum is an important soil-borne plant pathogen affecting cucumber plants (*Cucumis sativus* L.). Infected plantlets may develop root rot or stem canker, often leading to wilting and to plant death in severe cases [8].

Current cucumber disease management is based mainly on cultural practices and the use of fungicides, although chemical control is not always efficient or economically feasible for control of the disease. Biological control of the pathogen using mutualistic microorganisms has been suggested as a potential alternative to chemical control [9]. The biological control is the best alternative, especially against soil-borne pathogens. Biological control of pathogens, i.e., the total or partial destruction of pathogen populations by other organisms, occurs routinely in nature [10]. Among the various antagonists used for the management of plant diseases, *Trichoderma* spp. plays a vital role. These filamentous fungi are very common in nature, with high population densities in soil and plant litters [10]. Many studies have reported that *Trichoderma* spp. has the ability to reduce several plant diseases by inhibiting plant pathogens mainly found in the soil roots, through antagonistic and mycoparasitism potential [11]. For instance, studies conducted by Ref. [12] in Egypt and Ref. [13] in Vietnam, indicated that *Trichoderma* spp. had the ability to suppress the growth of fungal pathogens and enhance plant growth. However, no research has been conducted to evaluate the bio-efficacy of *T. cf. harzianum* in plant growth promotion and *Fusarium oxysporum* f. sp. cucumerinum disease management especially in cucumber seedling. Therefore, the objectives of this study were to evaluate the bio-efficacy of *Trichoderma* spp. with antagonistic activities against *Fusarium oxysporum* f. sp. cucumerinum and plant growth-promoting properties in cucumber seedling.

2. Materials and Methods

2.1 Isolation of Trichoderma spp.

Trichoderma spp. were isolated from the Grassland and forest soil of Inner Mongolia, China. Isolation of different *Trichoderma* spp. was made by the serial dilution technique. The soil solution was shaking for 30 min. And 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 based on morphological and molecular methods using ITS5 (5'-GGAAGTAAAAGTCGTAACAAGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') according to Ref. [14] with modifications. The purified and identified cultures of *Trichoderma* spp. were maintained on PDA (Potato Dextrose Agar) medium and stored at 4 ℃ for further experimentation. Pure cultures were kept in 20% (w/v) glycerol at -20 ℃.

2.2 Collection of Pathogens

Fusarium oxysporum f. sp. cucumerinum was obtained from China Academy of agricultural institute plant protection Research Laboratory (Beijing). The Isolate was maintained on PDA medium and placed until use at 4 ℃.

2.3 Seed Treatment and Germination Testing

All tested cucumber seeds were disinfected using 10% sodium hypochlorite and soaked for 5 min then washed with distilled water for 4-5 times. After disinfection, the seeds are soaked in 3% (w/v) sodium carboxymethyl cellulose which serves as adhesive and stirring to improve the better stickiness of *Trichoderma*

spp., on the surface of the seed. The seed is coated with tested *Trichoderma* spp. and transferred to 90 mm petri dish with wet filter paper and each Petri plate will have 20 seeds then incubate at 28 ℃ for 5 days. Germinated seeds were recorded at the 5th day after incubation. Germination rate was calculated as the formula below:

$$\text{Seed germination rate} = \frac{\text{No. of germinated seed}}{\text{Total No. of seeds}} \times 100$$

The experiment was conducted twice with five replicates per treatment. The germination rates were analyzed by Friedman test, LSD (Least Significant Different) [15].

2.4 Bio-Efficacy Evaluation of the Selected Trichoderma spp.

The bio-efficacy of the selected Trichoderma isolates was conducted under Glasshouse condition using a test tube. After the treated seed germinated 5 cucumber seedlings were transferred to 5 tubes containing MS (Murashige and Skoog with vitamins) at a rate of 4.43 g/lt and kept for one day. The next day we added 2 mL of *Fusarium oxysporum* f. sp. cucumerinum and kept for 3 weeks. We use two different controls Ck1 the same treatment but without Trichoderma and without pathogen and Ck2 is with only *Fusarium oxysporum* f. sp. cucumerinum.

The disease severity, incidence and growth parameters of the cucumber seedlings in the test tube were evaluated at the vegetative stage: 3 weeks after transplanting (MacLean et al., 2002). The disease incidence was calculated by using the formula as described by Ref. [16]:

$$Disease\ incidence(\%) = \frac{\text{Total No. of infected plants per tube}}{\text{Total No. of plants per tube}} \times 100$$

The disease symptoms were evaluated based on to the disease scales from 0-5 (Table 1). The DSI (Disease Severity Index) was calculated following the calculation described by Ref. [17] using the following equation:

$$DSI(\%) = \frac{\Sigma(Number\ on\ scale\ X\ Number\ of\ seedlings\ in\ that\ scale)}{\Sigma(Number\ of\ treated\ seedlings)(highest\ rating\ value)} \times 100$$

$$Reduction\ (\%) = \frac{Control - Treatment}{Control} \times 100$$

Table 1 Cucumber seedling disease symptoms scoring scale.

Disease scale	Disease symptoms
0	Healthy and uninfected plants (no external symptoms)
1	Initial signs of wilting (yellowing)
2	Up to 25% of the leaves and root with symptoms
3	Up to 50% of the leaves and root with symptoms
4	Up to 75% of the leaves and root with symptoms
5	Plants dead

2.5 Statistical Analysis

All experiments were performed in five tubes per treatment and arranged in a randomized complete block design. Disease incidences (%) and disease severity index were analyzed using an ANOVA (analysis of variance) and grouped by Duncan test.

3. Result

3.1 Isolation of the Potential Trichoderma spp.

Total of 90 Trichoderma strains were isolated under 8 species from 76 soil samples collected from seven different regions of Xilingol, Hulunbuir, Tongliao, Chifeng, Ulanqab, Xing'an and Baotou within three Grassland and Forest Ecosystem in Inner Mongolia, Northern China. All isolated were confirmed as *Trichoderma* spp. based on morphological and Molecular results.

The isolated *Trichoderma* spp. is *T. cf. harzianum* (68), *T. longibranchiatum* (8), *T. asperelloides* (6), *T. afroharzianum* (2), *T. rossicum* (2), *T. hamatum* (1), *T. atroviride* (1) and *T. gamsii* (2).

To establish a phylogenetic tree, we first calculated haplotypes from 90 ITS5 and 4 sequences. Finally, 14

Table 2 Haplotypes (14) of Trichoderma identified in this study.

Haplotype	Species name	Number of strains	Representative strain	accession number
Hap1	*T.cf. harzianum*	12	T1	MF108890
Hap2	*T.cf. harzianum*	51	T5	MF108865
Hap3	*T. atroviride*	1	T9	-
Hap4	*T. gamsii*	2	T17	MF150096
Hap5	*T. asperelloides*	2	T63	MF114227
Hap6	*T. hamatum*	1	T34	MF144562
Hap7	*T. rossicum*	2	T23	MF144556
Hap8	*T. asperelloides*	4	T29	MF114228
Hap9	*T. longibranchiatum*	7	T64	MF102151
Hap10	*T.cf. harzianum*	1	T39	MF108907
Hap11	*T. afroharzianum*	2	T81	MF116243
Hap12	*T.cf. harzianum*	3	T57	MF108905
Hap13	*T. longibranchiatum*	1	T46	MF102164
Hap14	*T.cf. harzianum*	1	T50	MF109010

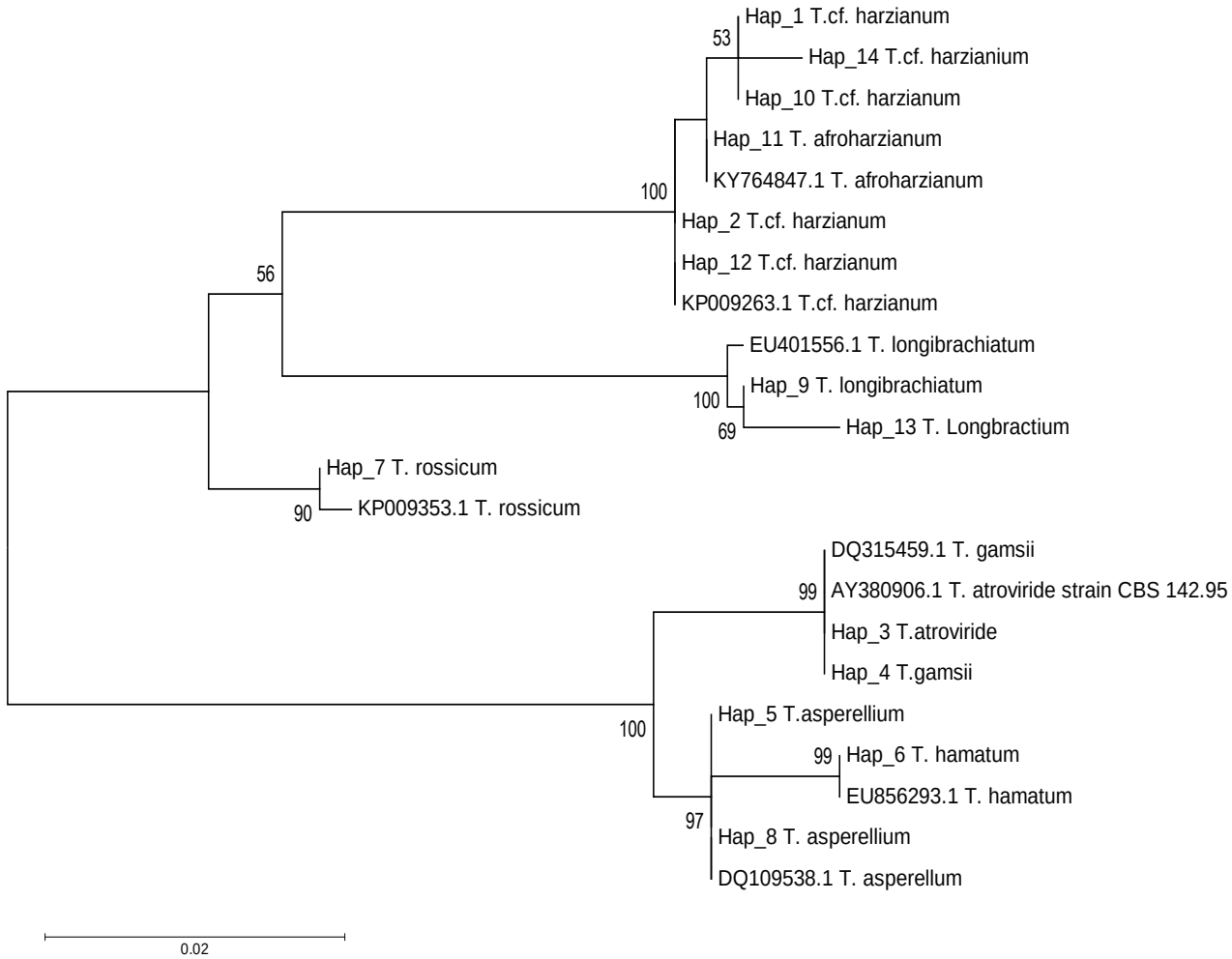

Fig. 1 Phylogeny reconstruction based on the ITS1-5.8S-ITS2 sequences from the rDNA (MEGA 6.0).

haplotypes (Table 2) were subjected to parsimony and Bayesian analysis. The result of this phylogenetic analysis is shown in Fig. 1. The 14 haplotypes belonging to nine Trichoderma species were positioned into seven clusters with strong bootstrap supports.

3.2 Effect of Treating Cucumber Seeds with Trichoderma Isolates on the Severity of Fusarium Wilt Disease

A total of ninety *Trichoderma* spp. strains were tested for their control efficiency against *Fusarium oxysporum* f. sp. cucumerinum on cucumber (*Cucumis sativus* L.). Data present in the Table 3 show that all tested isolates of *Trichoderma* spp. were antagonistic to *F. oxysporum* f. sp. cucumerinum. The disease incidence shows that the isolates have wide ranges from the lowest of 0 to the highest of 43. The relative control effect between tested strain was highly different compared to the control from the total tested strains. Eleven of them have reducing disease severity by 100% which consist two species which are ten strains of *T. cf. harzianum* and One strain of *T. atroviride* and the lowest was 53% which has been recorded by six strains of *Trichoderma* spp. under three species which are four strains of *T. cf. harzianum*, one strain of *T. rossicum* and one strain of *T. asperelloides*. In terms of growth parameters, significant differences were noticed between the tested isolates. The results on relative seed germination index on cucumber (*Cucumis sativus* L.) treated with Trichoderma isolates are presented in Table 3. The results show that the germination percentage ranges from the lowest of 85 to 96% indicating that the seeds were highly viable and the lower germination may be due to pre-emergence germination failure.

3.3 Screening the Relative Controlling Effect of the Trichoderma spp.

Eight *Trichoderma* spp., biological control agents i.e. *T. cf. harzianum, T. longibrachiatum, T. asperelloides, T. rossicum, T. afroharzianum, T. gamsii, T. atroviride* and *T. hamatum* were used to study their effect against *Fusarium oxysporum* f. sp. cucumerinum i.e. (the most virulent isolates), the causal organism of cucumber wilt under greenhouse conditions. Data in Table 4 indicate that all tested biological control agents minimized the disease symptoms parameters in comparing with the infected control treatments. DI (disease incidence) was at lowest value by *T. atroviride* (0), followed by *T. hamatum* (7%) which both has one strain each while the highest DI value was recorded by *T. afroharzianum* (37%), *T. longibranchiatum* (28%) and *T. rossicum* (25%).

The highest number of strain isolated species was *T. cf. harzianum* (68) with average DI of 13%. The highest reducing disease severity was recorded by *T. atroviride* (100%) and *T. hamatum* (93%), followed by *T. cf. harzianum* (86%), while the lowest value was recorded by *T. afroharzianum* (60%), followed by *T. longibranchiatum* (70%). Table 4 indicates that all growth parameters of cucumber plants were improved by application of biological control agents against *Fusarium oxysporum* f. sp. cucumerinum compared with infested control treatments (Fig. 2). The highest shoot fresh weight was recorded by *T. asperelloides* (2 gm), followed by *T. hamatum* (1.30 gm) and *T. gamsii* (1.26 gm), while the lowest shoot fresh weight was recorded by *T. rossicum* (1.04 gm). Also, root fresh weight was at the highest value by *T. hamatum* (0.92 gm), followed by *T. gamsii* (0.87 gm), whereas the lowest root fresh weight was recorded by *T. rossicum* (0.56 gm). The biological control agents improved vegetative growth characters in treated cucumber plants. The highest vigor index was recorded by *T. atroviride* (936) followed by *T. hamatum* (902.5), while the lowest vigor index was recorded by *T. rossicum* (666.4).

3.4 Screening of the Relative Controlling Effect of Trichoderma Isolates Based on Their Isolation Regions and Ecosystem

The highest isolates were recorded in Typical Grassland Ecosystem with the rates of 71 strains

Table 3 Effect of Antagonistic fungi on disease incidence and growth parameters of FOC (F. O. f. sp. *cucumerinum*).

Trichoderma strains	Strain code	Disease incidence (%)	Relative control effect	Germination percent (%)	Growth parameters (gm fresh weight)		Vigor index
					Shoot	Root	
T.cf. harzianum	T1	0	100	96	1.31	0.85	1,324.8
T.cf. harzianum	T2	0	100	93	0.97	0.60	1,199.7
T.cf. harzianum	T3	0	100	93	1.12	0.66	1,106.7
T.cf. harzianum	T4	0	100	95	1.35	0.76	1,045.0
T.cf. harzianum	T5	0	100	96	1.18	0.75	1,048.3
T.cf. harzianum	T6	0	100	96	1.20	0.76	1,038.7
T.cf. harzianum	T7	0	100	95	1.18	0.82	1,018.4
T.cf. harzianum	T8	0	100	92	1.23	0.84	966.0
T. atroviride	T9	0	100	90	1.25	0.85	936.0
T.cf. harzianum	T10	0	100	92	1.45	0.91	947.6
T.cf. harzianum	T11	0	100	92	1.38	0.68	947.6
T. *longibrachiatum*	T12	3	96	92	1.03	0.78	938.4
T.cf. harzianum	T13	3	96	93	1.37	0.74	941.2
T.cf. harzianum	T14	3	96	93	1.28	0.78	937.4
T.cf. harzianum	T15	3	96	93	1.14	0.83	930.0
T.cf. harzianum	T16	3	96	89	1.13	0.75	882.9
T. gamsii	T17	3	96	93	1.43	0.97	920.7
T.cf. harzianum	T18	3	96	93	1.23	0.90	920.7
T.cf. harzianum	T19	3	96	92	1.21	0.69	910.8
T.cf. harzianum	T20	3	96	90	1.31	0.81	882.0
T.cf. harzianum	T21	7	93	93	1.52	0.68	911.4
T.cf. harzianum	T22	7	93	92	1.25	0.91	901.6
T. rossicum	T23	7	93	92	1.27	0.82	899.3
T.cf. harzianum	T24	7	93	90	1.13	0.81	878.4
T.cf. harzianum	T25	7	93	92	1.11	0.75	894.2
T.cf. harzianum	T26	7	93	95	1.16	0.84	921.5
T. asperelloides	T27	7	93	95	1.62	0.69	921.5
T.cf. harzianum	T28	7	93	95	1.21	0.77	919.6
T. asperelloides	T29	7	93	95	0.93	0.64	912.0
T.cf. harzianum	T30	7	93	92	1.33	0.75	877.7
T.cf. harzianum	T31	7	93	95	1.19	0.79	903.3
T.cf. harzianum	T32	7	93	95	1.27	0.77	902.5
T.cf. harzianum	T33	7	93	96	0.86	0.68	912.0
T. hamatum	T34	7	93	92	1.30	0.92	902.5
T.cf. harzianum	T35	7	93	92	1.05	0.87	874.0
T.cf. harzianum	T36	7	93	89	1.19	0.77	845.5
T.cf. harzianum	T37	10	89	92	1.35	0.72	872.2
T.cf. harzianum	T38	10	89	95	1.21	0.76	896.8
T.cf. harzianum	T39	10	89	92	1.32	0.70	866.6
T.cf. harzianum	T40	10	89	90	1.09	0.77	847.8
T.cf. harzianum	T41	10	89	90	0.88	0.59	846.0
T.cf. harzianum	T42	10	89	85	1.16	0.85	797.3
T.cf. harzianum	T43	10	89	95	1.05	0.73	891.1
T.cf. harzianum	T44	10	89	95	1.36	0.71	885.4
T.cf. harzianum	T45	10	89	95	1.03	0.84	883.5
T. *longibrachiatum*	T46	10	89	96	1.12	0.76	890.9

Table 3 continued

Trichoderma strains	Strain code	Disease incidence (%)	Relative control effect	Germination percent (%)	Growth parameters (gm fresh weight)		Vigor index
					Shoot	Root	
T.cf. harzianum	T47	10	89	92	1.33	0.70	846.4
T.cf. harzianum	T48	10	89	90	1.25	0.73	819.0
T.cf. harzianum	T49	10	89	95	1.34	0.92	864.5
T.cf. harzianum	T50	10	89	95	1.06	0.73	862.6
T.cf. harzianum	T51	10	89	89	1.32	0.78	805.5
T.cf. harzianum	T52	10	89	92	1.26	0.74	828.0
T.cf. harzianum	T53	10	89	89	0.66	0.84	801.0
T.cf. harzianum	T54	10	89	89	0.94	0.71	799.0
T.cf. harzianum	T55	10	89	92	0.97	0.85	818.8
T.cf. harzianum	T56	13	86	96	1.27	0.69	854.4
T.cf. harzianum	T57	13	86	92	1.25	0.76	816.5
T.cf. harzianum	T58	13	86	95	1.27	0.78	840.8
T.cf. harzianum	T59	13	86	95	1.39	0.79	836.9
T.cf. harzianum	T60	13	86	89	0.91	0.65	783.2
T.cf. harzianum	T61	13	86	92	1.06	0.94	809.6
T.cf. harzianum	T62	13	86	92	1.04	0.66	809.6
T. asperelloides	T63	17	82	90	1.16	0.75	790.2
T. *longibrachiatum*	T64	23	75	96	1.17	0.72	842.9
T. asperelloides	T65	23	75	85	1.21	0.80	744.8
T. *longibrachiatum*	T66	27	71	95	1.25	0.76	822.7
T.cf. harzianum	T67	27	71	95	1.26	0.76	817.0
T. *longibrachiatum*	T68	27	71	96	1.06	0.75	825.6
T. *longibrachiatum*	T69	27	71	95	1.32	0.77	807.5
T. *longibrachiatum*	T70	30	67	95	1.29	0.77	807.5
T. longibrachiatum	T71	30	67	95	1.03	0.77	807.5
T. afroharzianum	T72	33	64	95	1.13	0.75	807.5
T. longibrachiatum	T73	33	64	95	0.84	0.82	798.0
T. longibrachiatum	T74	33	64	95	1.03	0.61	788.5
T.cf. harzianum	T75	33	64	95	0.81	0.77	780.9
T. asperelloides	T76	33	64	95	1.29	0.79	779.0
T.cf. harzianum	T77	33	64	95	1.39	0.81	779.0
T.cf. harzianum	T78	33	64	85	1.23	0.80	688.5
T.cf. harzianum	T79	37	60	85	1.29	0.50	688.5
T. longibrachiatum	T80	37	60	95	1.09	0.73	765.7
T. afroharzianum	T81	40	57	95	1.35	0.74	750.5
T.cf. harzianum	T82	40	57	90	1.13	0.64	703.8
T.cf. harzianum	T83	40	57	93	1.09	0.81	716.1
T.gamsii	T84	40	57	95	1.09	0.77	731.5
T.cf. harzianum	T85	43	53	92	0.45	0.67	690.0
T. asperelloides	T86	43	53	93	0.98	0.59	585.9
T.cf. harzianum	T87	43	53	85	0.43	0.38	520.2
T.cf. harzianum	T88	43	53	85	0.80	0.67	493.0
T.cf. harzianum	T89	43	53	85	0.93	0.61	493.0
T. rossicum	T90	43	53	85	0.81	0.29	433.5
	Ck 1	0	100	96	1.3	0.8	938.4
	Ck 2	83	17	65	0.2	0.1	260.0

Table 4 Evaluation of eight *Trichoderma* spp., for the control of *Fusarium oxysporum* and growth performance on cucumber seedling under greenhouse conditions.

Species	No. of strains	DI (disease incidences)	Reducing disease severity (%)	Germination (G %)	Growth parameters (gm fresh weight)		Vigor index
					Root	Shoot	
T. cf. harzianum	68	13	86	92	0.75	1.15	863.43
T. longibranchiatum	8	28	70	95	0.74	1.10	815.5
T. asperelloides	6	22	76	92	0.78	2.00	788.9
T. rossicum	2	25	73	89	0.56	1.04	666.4
T. afroharzianum	2	37	60	95	0.75	1.24	779
T. gamsii	2	22	76	94	0.87	1.26	826
T. atroviride	1	0	100	90	0.85	1.25	936
T. hamatum	1	7	93	95	0.92	1.30	902.5
Control (Ck 1)		0	100	96	1.3	0.8	938.4
Control (Ck 2)		83	17	65	0.2	0.1	260.0
SEM		7.62	7.68	2.90	0.09	0.15	63.07

within 7 *Trichoderma* spp., i.e. *T. cf. harzianum* (52), *T. longibranchiatum* (8), *T. afroharzianum* (2), *T. asperelloides* (6), *T. hamatum* (1), *T. atroviride* (1), and *T. gamsii* (1) within seven regions of Inner Mongolia which are Chifeng (24), Xilingol (19), Ulanqab (10), Tongliao (7), Xing'an (6), Hulunbuir (3) and Baotou (2). The highest reducing disease severity was recorded by seven *T. cf. harzianum* (100%), five of them were isolated from Chifeng (T4, T5, T6, T7, and T10) and one each from Baotou (T8) and Xilingol (T11) and *T. atroviride* which were isolated from Hulunbuir (T9), while the lowest relative control was 52.9% recorded by 4 strains of *T. cf. harzianum* isolated from Xing'an (T85), Tongliao (T87), Chifeng (T88), Xilingol (T86) and *T. asperelloides* isolated from Tongliao (T86).

Meadow steppe has the second highest isolates rate with seven strains within four species *Trichoderma* spp., i.e. *T. cf. harzianum* (3), *T. rossicum* (2), *T. longibranchiatum* (1) and *T. gamsii* (1) from the regions of Xilingol, Chifeng and Xing'an. The highest reducing disease severity was recorded by *T. cf. harzianum* with rates of 100% with strains of T2 and T3 both are isolated from Xilingol. The lowest control effect was 52.9% with a strain of *T. rossicum* (T90) from Xing'an.

In Desert steppe all the strains were *T. cf. harzianum* isolated from 3 regions i.e. Chifeng, Ulanqab, and Xilingol. The highest reducing disease severity was recorded by T1 at rates of 100% and the lowest control effect was recorded by four strains of T39, T40, T41, and T42 with the rates of 89%.

In forest, ecosystem 5 strains were isolated within two *Trichoderma* spp., from three regions of Xilingol, Hulunbuir and Xing'an. Three strains of *T. cf. harzianum* (T24, T38, and T47) and *T. longibranchiatum* (T68 and T69). The highest reducing disease severity was recorded by *T. cf. harzianum* (T24) at rates of 92.8%, while the lowest was recorded by two *T. longibranchiatum* (T68 and T69) with the rates of 71%.

3.5 Evaluating the Effectiveness of the Dominant Trichoderma spp., on Fusarium Oxysporum under in Vivo on Cucumber Seedling

The dominant *Trichoderma* spp. has shown significant antagonistic influence on the incidence of *Fusarium oxysporum* f. sp. cucumerinum on the seedling of cucumber. We have tested 68 strains of *T. cf. harzianum* (*=H. 'pseudoharzianum'* sensu Druzhinina et al., 2010a). We have found that from the total tested *T. cf. harzianum* strains 10 of them have 100% relative control effect with the strains of T1, T2, T3, …, T8, T10, T11, which were isolated from three regions Chifeng (6), Xilingol (3) and Baotou (1) under two haplotypes of Hap1 (4 isolates) and Hap2 (6 isolates)

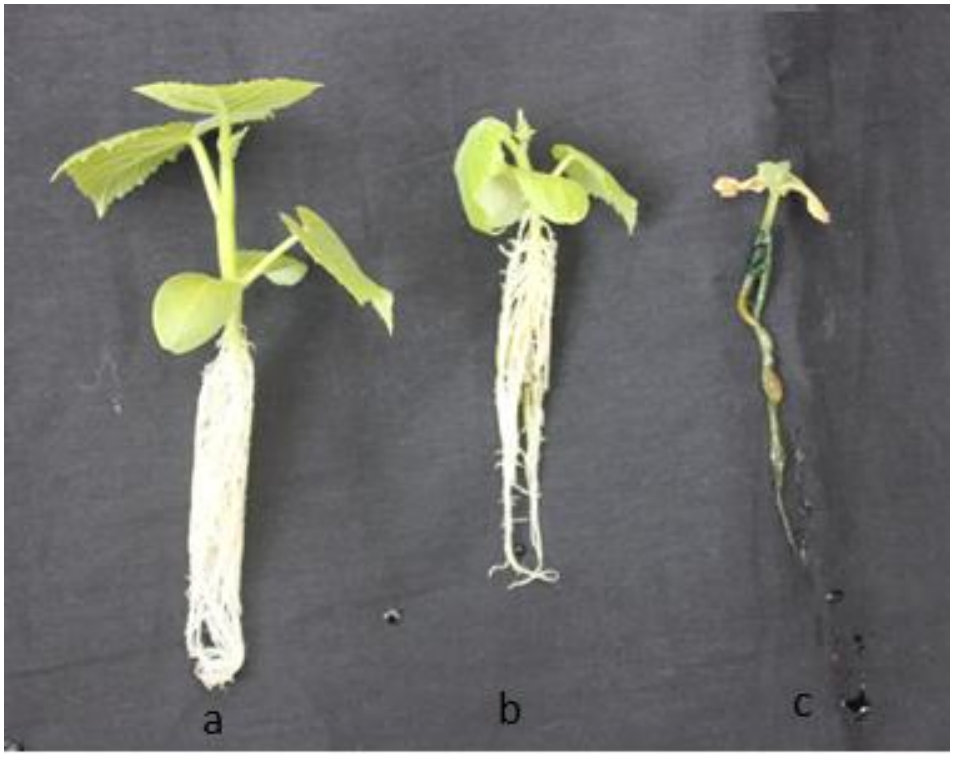

Fig 2 Effect of treating cucumber seeds with cell suspension antagonistic *Trichoderma* spp. isolates on the incidence of Fusarium wilt disease.

N.B. a-*T.cf. harzianum* (T1), b-Control 1 without any treatment and c-Control 2 inoculated with *F. oxysporum* f. sp. cucumerinum.

within three Ecosystem of Typical steppe (7), Meadow steppe (2) and Desert (1) while the lowest relative control effect was 53% recorded by 4 strains isolated from four regions xing'an, Tongliao, Chifeng, Xilingol and from single Ecosystem (Typical steppe) and Haplotype (Hap2).

The effect of various strains of *T. cf. harzianum* on the seed germination, vegetative growth, and vigor index of cucumber seedling was studied and the data are furnished in Table 2. Maximum germination percentage of cucumber seeds (96 percent,) was absorbed by six strains of T1, T5, T6, T33, T56, and T68, while the lowest Germination percentage rates were recorded by six strains of T42, T78, T79, T87, T88 and T89 at a rate of 85%. Regarding vegetative growth, the highest shoot and root weight were recorded by T21 (1.52 gm) and T61 (0.94 gm) respectively, while the lowest was recorded by T87 at a rate of the shoot (0.43 gm) and root (0.38 gm). The highest vigor index was shown by T1 (1324.3) and the lowest was recorded by two strains of T88 and T89 at rates of 493.

4. Discussion

Trichoderma spp., bio controlling ability is related to its development of different mechanisms that allow it to compete directly for space and nutrients. In fungi, these mechanisms are associated with the production of antifungal metabolites, the secretion of hydrolytic enzymes, and their mycoparasitic ability, being behaviors that are expressed at different intensities depending on the strain used [18].

This study aimed to evaluate the efficacy of antagonistic Trichoderma isolates in controlling Fusarium wilt disease of cucumber under protected houses. Ninety (90) native strains under nine *Trichoderma* spp., were isolated from seven different regions under four ecosystems of Inner Mongolia soil and were characterized on the basis of their morphological and molecular features, effective in

reducing disease severity and the vegetative parameters were compared with the control and showed strong relative control effects which inhibited >53% infestation of *Fusarium oxysporum* f. sp. cucumerinum and eleven of the isolates showed 100% relative control of *Fusarium oxysporum* f. sp. cucumerinum while six isolates only showed 53% antagonistic potential.

All isolates were effective in reducing disease severity and increasing the average fresh weight of shoot and root. *T. cf. harzianum* (T1, T2, T3, T4, T5, T6, T7, T8, T10 and T11) has reduced disease severity by 100% apart from this *T. cf. harzianum* is a family of *Trichoderma harzianum* complex which was the dominant species isolated from different regions and ecosystem from the study area soil and the effective biological control in our trial. A similar trend was found by Ref. [19] when studying *T. harzianum* species, where it was concluded that each isolate could have a specific mycoparasitic activity. Therefore, it is not possible to generalize and indicate that a specific Trichoderma species can be classified as good antagonistic fungi because that characteristic is specific to each isolate. Sivakumar et al. [20] reported that the effects of *T. harzianum* were due to both antibiosis and mycoparasitism. Mechanisms employed by Trichoderma species to affect biological control of plant diseases are many and complex, and their use varies with the kind of biocontrol agent, pathogen, and host plant involved in the interaction [21]. Many investigators obtained similar results in greenhouse studies for controlling wilt disease on cucumber as well as other diseases [22-29].

Trichoderma atroviride is also well known as a biological control agent which reduces disease severity by (T9) 100%. This species has been confused with the closely related species *T. harzianum* because both species produce smooth, globose to sub-globose conidia. According to Ref. [30], the main difference between these two species is that *T. atroviride* produces a distinctive sweet or coconut odor in culture, whereas *T. cf. harzianum* does not. We confirmed this specific odor from our isolated *T. atroviride* strains and did not detect it from any of the isolated *T. cf. harzianum* strains. On the other hand *T. cf. harzianum* (T85, T87, T88, and T89), *T. asperelloides* (T86) and *T. rossicum* (T90) were the least effective in reducing disease severity by 53%. It was reported that isolates of the *T. harzianum* collected from different soil samples were not equally effective to inhibit the growth of *F. udum* [31, 32]. It was found that one isolate (T1) among the 10 isolates of this antagonist was effective. So, there is need to search a very specific isolate(s) of *Trichoderma* sp. for successful control of *Fusarium oxysporum* f. sp. lycopersici [32].

As per result, ninety (90) potential isolates of Trichoderma may be further exploited as a biocontrol agent against *Fusarium oxysporum* f. sp. cucumerinum, as well as other soil, bore phytopathogenic fungi. In the present study, the better efficacy was observed in treatments including *T. cf. harzianum* (Table 3).

5. Conclusion

By using *Trichoderma* spp. such as *T. cf. harzianum*, *T. atroviride* and *T. gamsii* we could reduce disease incidence percentage of cucumber exposed to Fusarium pathogens such as *Fusarium oxysporum* f. sp. cucumerinum as agents of root and stem rot cucumber under greenhouse conditions. The presented method of bio controlling of pathogens can be considered as an applicable strategy in control measures against pathogens.

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Competing Interests

The authors declare that they have no competing interests.

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