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# Antagonistic potential and growth promoting activities of native *Trichoderma* isolates against *Fusarium oxysporum* f. sp. *ciceri*

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ABSTRACT

Chickpea wilt caused by *Fusarium oxysporum* f. sp. *ciceri* (Foc) has been considered as a devastating one which appears every year and causes heavy losses in yield. *Trichoderma* is a potential biocontrol agent against many diseases. Present investigation was carried out during winter (*rabi*) season 2021 to assess the antagonistic potential and growth promoting activities of native *Trichoderma* isolates against Foc. Thirteen *Trichoderma* isolates from chickpea rhizosphere were isolated and evaluated under *in vitro* conditions for their potentiality to antagonise Foc. The highest and lowest per cent of mycelial growth inhibition observed among these isolates was 88.1% (HST-1) and 62.2% (HCdT), respectively. Seed treatment with native *T. asperellum* plays an important role by enhancing plant growth parameters. Experimental results indicated that seed treatment with native *T. asperellum* exhibited significant increase in germination (94.29%), vigour index (2883.60), vigour index mass (117.16), plant height (25.64 cm), root length (4.96 cm), fresh plant weight (10.92 g), dry plant weight (1.24 g) and number of primary and secondary branches as compared to control. Additionally, *T. asperellum* caused plants to accumulate more lignin and showed an inhibitory effect on the occurrence of chickpea Fusarium wilt disease. Seed treatment with *T. asperellum* reduced disease incidence to 36% as compared to 96% in control (chickpea+Foc).

**Keywords**: Bio-control, Chickpea wilt, *Cicer arietinum, F. oxysporum* f. sp. *ciceri, Trichoderma*

Chickpea (*Cicer arietinum* L.) is third most important self-pollinated legume crop and is believed to have originated from south-eastern Turkey and the adjoining part of Syria (Singh 1997). Global area of chickpea is about 13.72 million hectares with production of 14.25 million metric tonnes, in which, India contributes 70% of global production with producing 112.29 lakh tonnes chickpea with an area of 105.61 lakh hectare (FAOSTAT 2021). It has high nutritional value and contributes to sustainable production by fixing atmospheric nitrogen thereby reducing the need for nitrogenous fertilization. Along with these benefits, it is highly prone to many diseases. Among them, Fusarium wilt caused by *F. oxysporum* f. sp. *ciceris* is the most prevalent disease in almost all chickpea growing areas. Under favourable conditions, chlamydospores cause infection and lead to yellowing and drying of leaves from base to upward, drooping of petioles and rachis, improper branching, withering of plants, browning of vascular bundles and finally wilting of plants (Argikar 1970). The yield losses caused by this disease under favourable conditions are up to 60–100% (Singh *et al.* 2007). Its incidence varies from 14–32% in the different states of India (Dubey *et al.* 2010). Owing to the destructive nature of disease, it is of major concern in agriculture for farmers and researchers. As this disease is both seed and soil borne, therefore, crop protection strategies used to manage this disease are chemical control along with the use of resistant varieties. Chemical control may result in environmental pollution and emergence of resistant strains of the pathogen. In recent times, utilisation of eco-friendly practices is becoming a promising tool for protecting the crops from this devastating disease. *Trichoderma* has drawn attention as a threat against most of the soil borne pathogens because of their multifaceted actions such as production of antibiotics, cell wall degrading enzymes, competition for key nutrients and space synthesis of antifungal metabolites, parasitism, rhizosphere competent and so on (Larkin and Fravel 1998, Harman *et al.* 2004). The current study aims to investigate antagonistic potential and growth promoting activities of native *Trichoderma* isolates against Foc.

## MATERIALS AND METHODS

The present study was carried out in the Biological Control Laboratory of Department of Plant Pathology,

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Chaudhary Charan Singh Haryana Agricultural University, Hisar, Haryana during *rabi* 2021. Different *Trichoderma* isolates were isolated from the soil samples collected from chickpea growing regions of Haryana, viz. Bawal (Rewari), Gurugram, Sirsa, Dadri, Rohtak, Faridabad, Fatehabad, Jhajjar, Bhiwani and Mahendragarh district and one isolate from Indian Agricultural Research Institute, New Delhi. All the isolates were assigned with specific code (Supplementary Table 1). Isolation was done by using Serial dilution method (Johnson 1957) which was diluted up to 18 dilution and then 1 ml of the suspension from eighth dilution was spread on petri plates containing Potato dextrose agar (PDA) media. These plates were incubated in BOD incubator at 26±1°C for 5–7 days. Preliminary screening for *Trichoderma* species was carried out by observing both macroscopic and microscopic features of the fungal colonies such as colony colour, mycelia growth, shape, size and colour of conidia. After screening, pure culture of *Trichoderma* was obtained by hyphal tip method (Rangaswami 1972) and was stored at 4±1°C for further studies.

*Assessment of antagonistic activity of* Trichoderma i*solates against* F. oxysporum *f. sp.* ciceris *under* in vitro *conditions*: In the Biological control laboratory of Department of Plant Pathology, 13 native isolates of *Trichoderma* along with two previously identified isolates (KBN-29 and RKTV) and two commercial formulations (*T. harzianum* and *T. viride*) were tested against *F. oxysporum* f. sp *ciceri* using dual culture technique (Johnson and Curl 1972). Plates were incubated at 26±1°C in a BOD incubator and the radial growth of Foc was measured when the control plate was completely filled, i.e. after five days of inoculation. The per cent growth inhibition was calculated as per formula given by Vincent (1947).

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

Where, I, Per cent mycelial growth inhibition; C, Colony diameter of *F.oxysporum* f. sp. *ciceris* in control; T, Colony diameter of *F. oxysporum* f. sp. *ciceris* in treatment. Characterization and identification of most promising native HST-1 isolate which exhibited maximum mycelial growth inhibition among all isolates was carried out by Division of Plant Pathology, Indian Agricultural Research Institute, New Delhi and was identified as *Trichoderma asperellum* (ITCC No. 9071) which was further used for *in vivo* studies.

*Evaluation of* Trichoderma asperellum *against* F. oxysporum *f. sp.* ciceris: Most promising isolate of *Trichoderma* (HST-1) was evaluated for its growth promoting activity and antagonistic potential during *rabi* 2021 using completely randomised design with four treatments and each treatment carried five replications in screen house of Department of Plant Pathology, Chaudhary Charan Singh Haryana Agricultural University, Hisar by using standard procedures.

*Preparation of liquid formulations*: The *Trichoderma* isolates were grown in 250 ml flasks containing 150 ml potato dextrose broth (PDB), inoculated with 5 mm mycelial discs of seven days old culture growing on PDA and incubated at 26±1°C for 10 days. The fungal growth was harvested, homogenised for about 10 s in a blender and suspension was prepared by filtering through double layer of muslin cloth and then appropriately diluted by adding calculated, required quantity of sterile distilled water (SDW), containing two to three drops of 0.05% Tween 80 per litre to provide a spore count of $5 \times 10^6$ conidia per ml as counted on haemocytometer.

*Seed treatment with liquid formulation of* T. asperellum: Seed of chickpea cultivar JG-62 were treated with liquid suspension of *T. asperellum.* For seed germination assay, seeds of variety JG-62 without any visual irregularity were selected followed by surface sterilisation with mercuric chloride (0.1%) or sodium hypochlorite (1%) for 30 s and then washed thrice with double-distilled water and dried aseptically. 100 g seeds were soaked in liquid suspension of *Trichoderma* isolate ($5 \times 10^6$ CFU per ml) for 6 hours. After soaking of seeds in formulations for 6 hours, seeds were placed in moist chambers and incubated for 48 hours into BOD incubator at 26±1°C. Radicals emerged out from seeds and then sowing of the germinated seed was done in pots containing mixture of autoclaved soil (at 121°C and 15 lbs) and vermicompost (3:1) at the rate of seven seeds per pot accordingly. Soil of pots was inoculated with *F. oxysporum* f. sp. *ciceris* spore suspension having $1 \times 10^7$ cfu at time of sowing. Surface sterilised seeds without any coating were considered as control. Treatments consisted of $T_1$, Control (Chickpea plant); $T_2$, Control + *F. oxysporum* f. sp. *ciceris*; $T_3$, Control + *T. asperellum*; $T_4$, Control + *F. oxysporum* f. sp. *ciceris* + *T. asperellum.*

*Observations recorded*

Morphological observations: Root development and architecture was observed by uprooting the diseased and healthy plants and biomass (root and shoot) was observed by taking the fresh and dry weight of the plants 45 days after sowing. Germination per cent, vigour index per cent and vigour index mass data was also recorded. Calculation of vigour index per cent and vigour index mass was done by the formula given by Abdul Baki and Anderson (1973).

$$\text{Germination per cent} = \frac{\text{Number of germinated seedlings}}{\text{Total number of seeds sown}} \times 100$$

$$\text{Disease incidence (\%)} = \frac{\text{Number of disease plants}}{\text{Total number of plants}} \times 100$$

$$\text{Vigour index (\%)} = \text{Mean germination (\%)} \times \text{Mean seedling length (cm)}$$

$$\text{Vigour index mass} = \text{Mean germination (\%)} \times \text{Mean seedling dry weight (g)}$$

Microscopic observation: Pathogen ingress was determined through histopathological study of root sectioning and staining of lignin deposited in vascular bundles of the roots of the plant. For this, Phloroglucinol 1.0%, prepared in 95% ethanol was used for mounting

the hand transverse section taken from the first node of chickpea stem. Then, the transverse section was placed on glass slides and covered with the coverslip. To verify the lignin accumulation, concentrated hydrochloric acid was augmented near the coverslip border, so that it could reach the transverse section (Guo *et al.* 2001) and leading to the development of pink colour indicating lignin deposition.

## RESULTS AND DISCUSSION

*Antagonistic activity of different* Trichoderma *isolates against* F. oxysporum *f. sp.* ciceris *under* in vitro *conditions*: Data presented in Supplementary Fig 1, Supplementary Table 2 and Fig 1 clearly indicates that different *Trichoderma* isolates showed wide range of antagonistic activity against *F. oxysporum* f. sp. *ciceris* that ranged from 88.1–62.22%. The highest mycelial growth inhibition of 88.1% was reported by the use of isolate HST-1 followed by HBhT (83%), HJT (81.5%) and HMT-2 (80%) while, the least mycelial growth inhibition was recorded in HCdT (62.2%). On the other hand, isolate 1 (RKTV) showed mycelial growth inhibition of 71.10% and isolate 2 (KBN-29) of 66.67%. Commercial formulations *T. viride* and *T. harzianum* exhibited 69.99% and 67.74% mycelial growth inhibition, respectively. Therefore, it has been recorded that native isolates were found more effective when compared to commercial formulations under *in vitro* conditions. Our results are similar to the findings of Singh *et al.* (2018) who determined the potential of twenty native *Trichoderma* isolates against *F. oxysporum* f.sp. *ciceri* and recorded that isolate T3 exhibited maximum per cent mycelial growth inhibition of 86.4%, followed by T15 (84.59%) under *in vitro* conditions. Yusnawan *et al.* (2019) evaluated biocontrol activity of native *Trichoderma* antagonistic fungi against *Fusarium* sp. and reported that isolate T 20A showed the highest inhibition of 90.8% followed by isolates of T 15C and T 16A (77.1%). Nagamani *et al.* (2020) evaluated antagonism of *Trichoderma* sp. against soil borne disease of chickpea and observed that among all twenty native *Trichoderma* isolates, ATPU 1 showed 84.1% mycelial growth inhibition of *F. oxysporum* f. sp. *ciceri*. Mishra *et al.* (2021) isolated twelve *Trichoderma* isolates and recorded

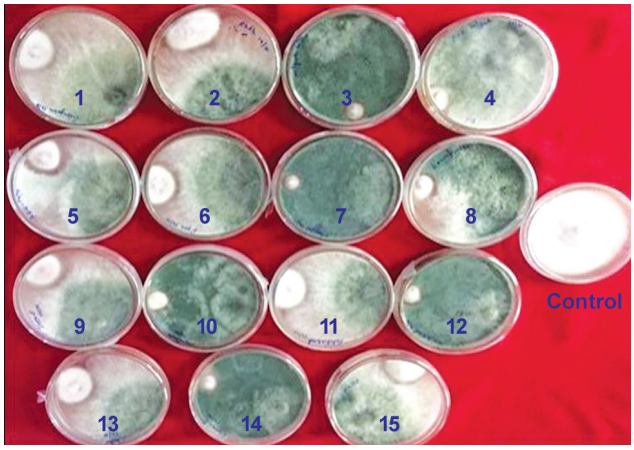


Fig 1 Mycelial growth inhibition of *F. oxysporum* f. sp. *ciceris* by various *Trichoderma* isolates.

that isolates IIPRTas-1 and IIPRTas-2 were most promising and showed maximum mycelial inhibition of *F. oxysporum* f. sp. *ciceris* (76.58 and 73.29%, respectively). However, the lowest per cent inhibition of 44.66% was observed in IIPRTas-8 isolate.

*Evaluation of* Trichoderma asperellum *against* F. oxysporum *f. sp. ciceris*

Morphological observations of diseased plants: Data of Table 1 and Fig 2 clearly revealed that out of four treatments, seed treatment with native *T. asperellum* was found to be the most effective treatment with maximum plant height (25.64 cm) and root length (4.96 cm), fresh weight (12.1 g) and dry weight (2.25 g), number of primary (16.8) and secondary branches (9.6) which were statistically significant with other treatments followed by uninoculated chickpea plants. Whereas, chickpea plants inoculated with *F. oxysporum* f. sp. *ciceris* was found to be the least effective with minimum plant height (19.16 cm), root length (2.60 cm), fresh weight (6.94 g) and dry weight (1.69 g). Similar findings had been reported by Mishra *et al.* (2021) who evaluated the effect of 12 native *Trichoderma* isolates on growth promoting activities of chickpea plant and recorded that IIPRTas-1 isolates had maximum root and shoot lengths

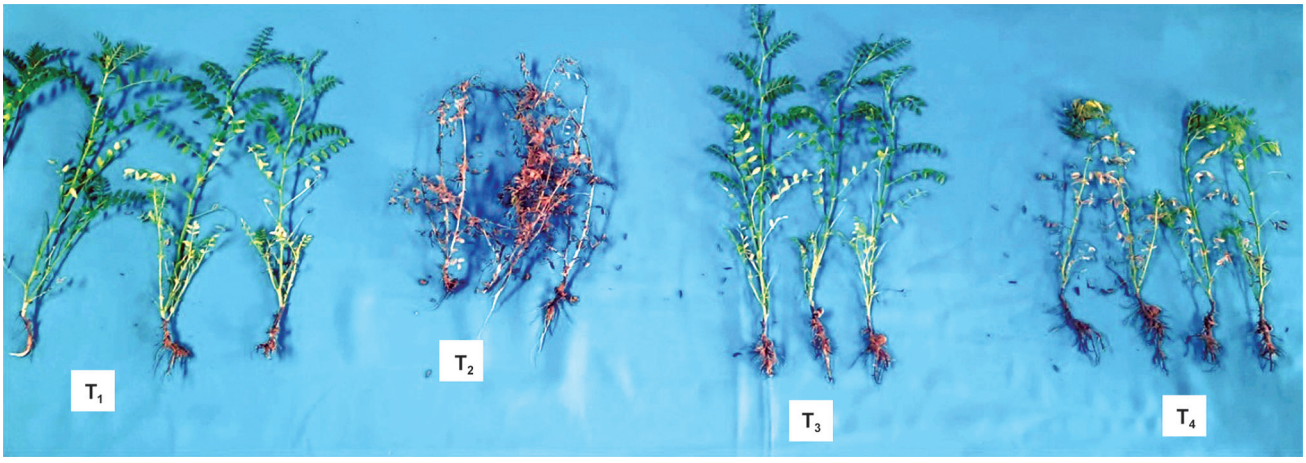


Fig 2 Effect of different *T. asperellum* treatments against Foc on plant growth parameter.

Table 1 Effect of different *T. asperellum* treatments against *F. oxysporum* f. sp. *ciceris* on plant growth parameters

| Treatment | Plant height (cm) | Root length (cm) | No. of primary branches | No. of secondary branches | Fresh weight (g) | Dry weight (g) |
|---|---|---|---|---|---|---|
| $T_1$ | 24.36* (29.56±0.44)** | 3.84 (11.28±0.33) | 15.40 (23.09±0.40) | 7.00 (15.26±0.80) | 10.26 (18.67±0.28) | 1.202 (6.29±0.03) |
| $T_2$ | 19.16 (25.95±0.25) | 2.60 (9.27±0.21) | 12.20 (20.43±0.33) | 4.20 (11.77±0.55) | 6.4 (14.63±0.43) | 0.692 (4.77±0.11) |
| $T_3$ | 25.64 (30.41±0.27) | 4.96 (12.83±0.16) | 16.80 (24.18±0.29) | 9.60 (18.02±0.50) | 10.92 (19.27±0.47) | 1.24 (6.40±0.03) |
| $T_4$ | 24.04 (29.35±0.21) | 3.64 (10.97±0.36) | 15.00 (22.77±0.36) | 6.00 (14.08±0.86) | 10.20 (18.61±0.28) | 1.196 (6.28±0.04) |
| CD (P=0.05) | (0.92) | (0.84) | (1.05) | (2.10) | (1.13) | (0.18) |
| CV | (2.37) | (5.57) | (3.43) | (10.53) | (4.69) | (2.28) |

Treatment details are given under Materials and Methods.

(9.25 cm and 25.9 cm, respectively) as compared to control (6.1 cm and 21.89 cm, respectively). Patel and Saraf (2017) evaluated the efficacy of *T. asperellum* MSST for the growth and yield parameters of tomato and observed significant increase in root length, shoot length and plant fresh and dry weight on 60 days after sowing. Suresh *et al.* (2011) tested the efficacy of native isolates of *Trichoderma* against four isolates of Fusarium wilt of chickpea and recorded that seeds treated with native isolate of *T. viride* exhibited highest shoot length (41.13 cm) and root length (35.26 cm) as compared to control (31.28% and 25.01%, respectively) .

In addition, seeds treated with *T. asperellum* had the highest germination per cent (94.29), vigour index (2883.6%) and vigour index mass (117.16), respectively followed by treatments in which seeds were treated with *T. asperellum* which was later inoculated with *F. oxysporum* f. sp. *ciceris* while, the lowest germination percentage (68.60), vigour index (1483.07) and vigour index mass (47) was recorded in plants inoculated with only *F. oxysporum* f. sp. *ciceris* (Table 2). The present findings are in agreement with the results reported by Meher *et al.* (2018) who evaluated the growth promoting activity of eight native isolates of *Trichoderma* spp. and recorded that seed treatment with isolate Tr-7 exhibited maximum vigour index (3383.3), vigour index mass (16.40) with 100% germination per cent as compared to pathogen treated control (1782, 8.24 and 66.67%, respectively) and untreated control (2324.6, 10.14 and 80%, respectively).

Results presented in Table 3 and Supplementary Fig 2 indicated that *Trichoderma* has a significant effect in reducing wilt disease in chickpea plants. Mean of three time intervals (25, 35 and 45 DAS) showed that seed treatment with native isolate *T. asperellum* resulted in minimum disease incidence (25.33%) as compared to control (65.33%). Thus, results indicated that *F. oxysporum* f. sp. *ciceris* inhibited plant growth and reduced biomass of plant whereas *Trichoderma* acts as a plant growth promoter which reduced the effect of *F. oxysporum* f. sp. *ciceris* by reducing disease incidence and thereby enhancing plant growth and biomass. The results of the investigation were similar to the findings of Suresh *et al.* (2011) who tested the antagonistic efficacy of native isolates of *Trichoderma* sp. against four isolates of Fusarium wilt of chickpea and recorded that

Table 2 Effect of different *T. asperellum* treatments against *F. oxysporum* f. sp. *ciceris* on chickpea germination and vigour index

| Treatment | Germination (%) | Vigour index (%) | Vigour index mass |
|---|---|---|---|
| $T_1$ | 88.57 (72.21±4.45) | 2505.38 | 106.36 |
| $T_2$ | 68.60 (55.97±1.70) | 1483.07 | 47.00 |
| $T_3$ | 94.29 (81.11±5.45) | 2883.60 | 117.16 |
| $T_4$ | 91.43 (76.66±5.45) | 2527.64 | 109.23 |
| CD (P=0.05) | (13.69) | - | - |
| CV | (14.16) | | |

Treatment details are given under Materials and Methods.

Table 3 Effect of *T. asperellum* on incidence of Fusarium wilt in chickpea

| Treatment | Disease incidence (%) | | | Mean |
|---|---|---|---|---|
| | 25 DAS | 35 DAS | 45 DAS | |
| $T_2$ | 32.00 (34.15) | 68.00 (58.46) | 96.00 (83.20) | 65.33 (58.60) |
| $T_4$ | 12.00 (15.93) | 28.00 (31.62) | 36.00 (36.68) | 25.33 (28.08) |
| Mean | 22.00 (25.04) | 47.99 (45.04) | 65.96 (59.94) | |
| | Duration | Treatments | Duration × Treatments | |
| CD (P=0.05) | (8.81) | (10.79) | (15.27) | |
| SEm± | (3.02) | (3.68) | (5.22) | |

Treatment details are given under Materials and Methods.

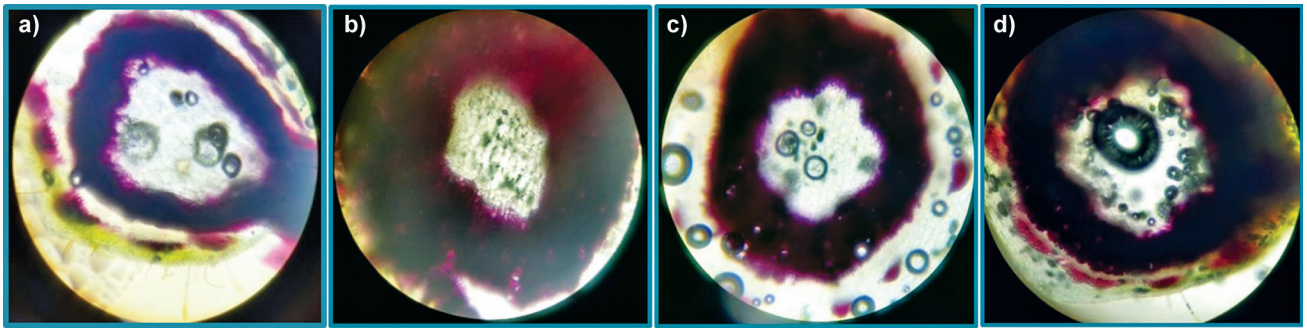


Fig 3 Lignin deposition in a) uninoculated chickpea plant; b) Foc inoculate plant; c) *T. asperellum* inoculated plant and; d) both Foc and *T. asperellum* inoculated plant.

seeds treated with native isolate of *T. viride* exhibited least disease incidence of 23.6% as compared to control (69.1%). Patel and Saraf (2017) evaluated the antagonistic efficacy of *T. asperellum* MSST and recorded 85% reduction in disease incidence of Fusarium wilt of tomato. Younesi *et al.* (2021) tested the Fusarium wilt control efficacy of three *Trichoderma* isolates and recorded that seed treatments with the KT10, KT9 and KT8 showed lower disease incidence (30.0, 33.3 and 35%) than control (98.3%).

Microscopic observations of diseased plants: The amount of lignified tissues were observed in transverse sections of chickpea stems infected with *F. oxysporum* f. sp. *ciceris*, healthy plants and *Trichoderma* treated wilt infected plants as evidenced by the pink hue of the lignified tissues when stained with phloroglucinol-HCl. The intensity of the pink colour intake indicated a larger lignin deposition. The highest quantity of lignin was deposited in plants treated with *T. asperellum* alone, followed by treatment in which seeds were treated with *T. asperellum* along with *F.oxysporum* f. sp. *ciceris* inoculation. However, the lowest amount of lignin was deposited in plants inoculated with only *F. oxysporum* f. sp. *ciceris* (Fig 3). Similar findings were recorded by Singh *et al.* (2016) who observed intense and uniform lignification in vascular tissue of tomato plants grown from seeds bioprimed with *T. asperellum* BHUT8 as compared to control.

Out of 13 native isolates of *Trichoderma* evaluated against *F. oxysporum* f. sp. *ciceri* for their antagonistic and growth promotion potential, HST-1 was identified as the most promising *Trichoderma* isolate which exhibited highest mycelial growth inhibition of test pathogen. Furthermore, it is concluded that isolate HST-1 was identified as the most promising isolate for managing chickpea wilt and its growth promoting activities. The foregoing research findings clearly demonstrated that the identified multi-trait potential strain of *T. asperellum* (HST-1) may be effectively used to create biocontrol formulations as well as consortia for further exploitation in chickpea-based cropping systems.

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