JOURNAL OF PURE AND APPLIED MICROBIOLOGY, March 2017. Vol. **11**(1), p. 401-405

# Bioefficacy of Native *Trichoderma* spp against Pathogenic *Fuusrium* Sp Causing Wilt Diseases

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http://dx.doi.org/10.22207/JPAM.11.1.52

(Received: 09 August 2016; accepted: 03 October 2016)

**Effective native isolates of *Trichoderma* spp. collected from the rhizosphere of different plant growing in the field. Twenty seven isolates of *Trichoderma* spp 13, *T. viride* (Pers.), 9, *T. harzianum* (Rifai), 2, *T. longibrachiatum* (Rifai), 1, *T. koningii* (Oudem) and 2, *Gliocladium virens* were identified. The antagonistic potential of the isolates as evaluated through dual culture plate using three soil borne pathogens of wilt of Safflower, Chickpea and Pigeonpea respectively, *T. harzianum* Jalna isolate was found highly antagonistic compared to *T. viride* isolates as it inhibited mycelia growth of *Fusarium oxysporum* f.sp. *carthami*, *F. oxysporum* f.sp. *ciceri* and *F. oxysporum* f.sp. *udum* by 78.06 %, 89.99 % and 82.49 per cent respectively after six days of *in vitro* incubation.**

**Keyword:** *Trichoderma* spp., Rhizosphere, Biocontrol, *Fusarium sp*., Safflower, Pigeonpea and Chickpea.

*Trichoderma* species are used as biocontrol agent in agriculture. *Trichoderma*, a genus of asexually reproducing saprophytic fungi, frequently present in nearly all temperate and tropical soils, decaying plant tissues and root ecosystems. The strains of *Trichoderma* spp. are strong opportunistic invaders, fast growing, prolific producers of spores and powerful antibiotic producers. The antagonism of *Trichoderma* involves several mechanisms, such as competition for nutrient antibiosis and production of fungal cell wall degrading enzymes. The mycoparasitic ability of *Trichoderma* species against plant pathogenic filamentous fungi allows for development of biocontrol strategies (Benitez *et al.*, 2004).

The *Fusarium* spp. is one of the most important soil borne fungi which causes wilt diseases in several field crops like chickpea, pigeonpea and safflower. Yield losses in different field crops due to *Fusarium* are upto tune of 90 per cent (Singh and Dahiya 1973).

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## MATERIALS AND METHODS

### Sample Collection

A total of 35 samples were randomly collected from different sites in Jalna and Parbhani district from August to Sept. 2014. The soil samples were taken from a depth of 05 to 15 cm around the rhizosphere area of fruit plants, cereal, vegetable, pulses and oilseed crops.

### Isolation of *Trichoderma* spp. from rhizospheric soils

Serial dilution technique was used to isolate the *Trichoderma* from the samples collected. The collected samples were air dried in shade. These samples were finely ground before serial dilutions. *Trichoderma* Selectve Medium (TSM) was used for isolation. Isolation was carried out

under aseptic conditions of laminar air flow in aseptic condition. Isolation were carried out by serial dilution and plating method. The culture of bioagents *Trichoderma* spp isolated from different region was identified on the basis of morphological and cultural characteristics.

***In vitro* evaluation for antagonistic potential**

Twenty seven *Trichoderma* spp isolates, thirteen *T. viride* (Pers.), nine *T. harzianum* (Rifai), two *T. longibrachiatum* (Rifai), one *T. koningii (*Oudem) and two *G. virens* were evaluated *in vitro* against *Fusarium oxysporum* f.sp. *carthami*, *F. oxysporum* f.sp. *ciceri* and *F. oxysporum* f.sp. *udum* by employing dual culture technique by Dennis and Webster (1971). Seven days old cultures of bioagents and test fungus grown on agar media were used for the study. Disc (5 mm diameter) of PDA along with culture grown of the test fungus and bioagents were cut out with sterilized cork borer. Then two cultures disc, one each of the test fungus and bioagents were placed at equidistance and exactly opposite with each other on solidified PDA medium in plates under aseptic conditions and plates were incubated at 28±2° C. plates inoculated with culture disc of test fungus were maintained and untreated control. Observations on linear mycelia of 24 hours and continued till untreated control plate was fully covered with

**Table 1.** Bioefficacy of *Trichoderma* isolates against *Fusarium oxysporum* f. sp. *carthami*

| Treatment | Isolates | Avg. colony dia. of *F. oxysporum* f. sp. *carthami**(mm) | Per cent inhibition of mycelial growth |
|---|---|---|---|
| $T_1$ | *T. viride*(BDN-1) | 30.50 | 66.10 (54.39) |
| $T_2$ | *T. harzianum*(BDN-2) | 21.00 | 76.66 (61.11) |
| $T_3$ | *T. harzianum*(BDN-3) | 24.00 | 73.33 (58.90) |
| $T_4$ | *T. viride*(BDN-4) | 23.50 | 73.88 (59.26) |
| $T_5$ | *T. harzianum*(BDN-5) | 32.50 | 63.88 (53.05) |
| $T_6$ | *T. longibrachiatum*(BDN-6) | 43.75 | 51.38 (45.79) |
| $T_7$ | *T. harzianum*(BDN-7) | 19.75 | 78.05 (62.06) |
| $T_8$ | *T. koningii*(JN-1) | 40.75 | 54.71 (47.70) |
| $T_9$ | *G. virens*(JN-2) | 50.75 | 43.61 (41.32) |
| $T_{10}$ | *T. harzianum*(JN-3 ) | 22.25 | 75.27 (60.17) |
| $T_{11}$ | *T. viride*(JN-4) | 27.50 | 69.44 (56.43) |
| $T_{12}$ | *T. harzianum*(JN-5) | 35.75 | 60.27 (50.92) |
| $T_{13}$ | *T. longibrachiatum*(MNT-1) | 44.75 | 50.27 (45.15) |
| $T_{14}$ | *T. viride*(MNT-3) | 28.75 | 68.05 (55.58) |
| $T_{15}$ | *G. virens*(MNT-4) | 59.50 | 33.88 (35.59) |
| $T_{16}$ | *T. viride*(PTR-1) | 26.25 | 70.82 (57.30) |
| $T_{17}$ | *T. harzianum*(PTR-2) | 20.25 | 77.49 (61.67) |
| $T_{18}$ | *T. viride*(PTR-3) | 26.75 | 70.27 (56.95) |
| $T_{19}$ | *T. viride*( PRT-1) | 28.50 | 68.33 (55.75) |
| $T_{20}$ | *T. viride*(SLU-1) | 33.00 | 63.33 (52.73) |
| $T_{21}$ | *T. viride*(SLU-2) | 34.50 | 61.66 (51.74) |
| $T_{22}$ | *T. viride*(SLU-3) | 35.75 | 60.27 (50.92) |
| $T_{23}$ | *T. harzianum*(PBN-2) | 22.25 | 75.27 (60.17) |
| $T_{24}$ | *T. viride*(PBN-3) | 31.75 | 64.71 (53.55) |
| $T_{25}$ | *T. harzianum*(PBN-4) | 31.25 | 65.27 (53.89) |
| $T_{26}$ | *T. viride*( PBN-5) | 25.25 | 71.94 (58.01) |
| $T_{27}$ | *T. viride*(PBN-6) | 23.00 | 66.38 (54.56) |
| $T_{28}$ | control | 90.00 | 90.00 (71.56) |
| SE± | | 1.38 | 1.22 |
| CD at 1% | | 3.60 | 3.52 |

*-Mean of two replications, Dia.: Diameter
Figure in Parentheses are angular transformed values.

mycelia growth of the test fungus. Per cent inhibition of the test fungus over untreated control was calculated by applying the formula given by Arora and Upadhyay (1978).

$$\text{Per cent growth Inhibition} = \frac{\text{Colony Growth Control plate} - \text{Colony Growth in intersecting plate}}{\text{Colony Growth in Control plate}} \times 100$$

After incubation radial growth was calculated by applying the formula (Vincent, 1927)

Per cent inhibition (I) = C-T/C x 100

Where,

C = Growth of test fungus in control in mm.

T = Growth of test fungus in treatment in mm.

## RESULT AND DISCUSSION

### *In vitro* evaluation of bioagents

### *In vitro* bioefficacy of different *Trichoderma* isolates against *F. oxysporum* f. sp. *carthami.*

The results (Table1) obtained on mycelial growth and inhibition of *Fusarium oxysporum* f. sp. *carthami* (Klisiewicz and Houston) with

**Table 2.** Bioefficacy of *Trichoderma* isolates against *Fusarium oxysporum* f. sp. *ciceri.*

| Treatment | Isolates | Avg. colony dia. of *F. oxysporum* f. sp. *ciceri.**(mm) | Per cent inhibition of mycelial growth |
|---|---|---|---|
| $T_1$ | *T. viride*(BDN-1) | 25.00 | 72.21 (58.18) |
| $T_2$ | *T. harzianum*(BDN-2) | 13.25 | 85.27 (67.43) |
| $T_3$ | *T. harzianum*(BDN-3) | 9.00 | 89.99 (71.55) |
| $T_4$ | *T. viride*(BDN-4) | 12.25 | 86.38 (68.34) |
| $T_5$ | *T. harzianum*(BDN-5) | 24.25 | 73.05 (58.72) |
| $T_6$ | *T. longibrachiatum*(BDN-6) | 36.75 | 59.16 (50.27) |
| $T_7$ | *T. harzianum*(BDN-7) | 11.75 | 86.94 (68.81) |
| $T_8$ | *T. koningii*(JN-1) | 34.00 | 62.22 (52.07) |
| $T_9$ | *G. virens*(JN-2) | 58.25 | 35.27 (36.43) |
| $T_{10}$ | *T. harzianum*(JN-3 ) | 13.00 | 85.55 (67.65) |
| $T_{11}$ | *T. viride*(JN-4) | 14.00 | 84.44 (66.76) |
| $T_{12}$ | *T. harzianum*(JN-5) | 21.75 | 75.82 (60.54) |
| $T_{13}$ | *T. longibrachiatum*(MNT-1) | 35.75 | 60.27 (50.92) |
| $T_{14}$ | *T. viride*(MNT-3) | 28.75 | 68.05 (55.58) |
| $T_{15}$ | *G. virens*(MNT-4) | 54.50 | 39.71 (39.06) |
| $T_{16}$ | *T. viride*(PTR-1) | 23.25 | 74.16 (59.44) |
| $T_{17}$ | *T. harzianum*(PTR-2) | 12.50 | 86.10 (68.10) |
| $T_{18}$ | *T. viride*(PTR-3) | 28.00 | 68.44 (55.82) |
| $T_{19}$ | *T. viride*( PRT-1) | 23.75 | 73.61 (59.08) |
| $T_{20}$ | *T. viride*(SLU-1) | 22.25 | 75.27 (60.17) |
| $T_{21}$ | *T. viride*(SLU-2) | 23.50 | 73.88 (59.26) |
| $T_{22}$ | *T. viride*(SLU-3) | 24.00 | 73.33 (58.90) |
| $T_{23}$ | *T. harzianum*(PBN-2) | 26.50 | 70.55 (57.13) |
| $T_{24}$ | *T. viride*(PBN-3) | 23.50 | 73.88 (59.26) |
| $T_{25}$ | *T. harzianum*(PBN-4) | 25.25 | 71.33 (57.62) |
| $T_{26}$ | *T. viride*( PBN-5) | 19.00 | 78.88 (62.64) |
| $T_{27}$ | *T. viride*(PBN-6) | 11.50 | 87.21 (69.04) |
| $T_{28}$ | control | 90.00 | 90.00 (71.56) |
| SE± | | 1.24 | 1.28 |
| CD at 1% | | 3.60 | 3.99 |

*-Mean of two replications, Dia.: Diameter

Figure in Parentheses are angular transformed values.

thirteen *T. viride*, nine *T. harzianum*, two *T. longibrachiatum*, one *T. koningii*, two *G. virens* isolates antagonists. It was observed that treatment $T_7$ (*T. harzianum*) isolate obtained from Jalna strain showed highest zone of inhibition i.e. 78.06 per cent followed by $T_{17}$ (*T. harzianum*) Parbhani strain i.e. 77.50 and $T_2$ (*T. harzianum*) Jalna strain i.e. 76.66 per cent.

Results of the present study on effect of bioagents on mycelia growth of *F. oxysporum* f. sp. *carthami* are in consonance with those reported earlier by several workers. Bioagents *viz.*, *Trichoderma viride*, *T. harzianum*, *T. longibrachiatum*, *T. koningii*, and *G. virens* were reported earlier to cause significant inhibition of mycelial growth of *F. oxysporum* f. sp. *carthami* by several workers (Behare *et al.*, 2002; Singh *et al.*, 2003; Waghmare and Datar, 2007 and Waghmare and Datar, 2009).

***In vitro* bioefficacy of different *Trichoderma* isolates against *F. oxysporum* f. sp. *ciceri.***

The results (Table 2) obtained on mycelial growth and inhibition of *Fusarium oxysporum* f. sp. *ciceri* (Padwick) with thirteen *T. viride*, nine *T. harzianum*, two *T. longibrachiatum*, one *T. koningii*, two *G. virens* isolates antagonists. It was observed that treatment $T_3$ (*T. harzianum*) isolate obtained from Jalna strain showed highest zone of

**Table 3.** Bioefficacy of *Trichoderma* isolates against *Fusarium oxysporum* f. sp. *udum.*

| Treatment | Isolates | Avg. colony dia. of *F. oxysporum* f. sp. *udum.**(mm) | Per cent inhibition of mycelial growth |
|---|---|---|---|
| $T_1$ | *T. viride*(BDN-1) | 20.75 | 76.94 (61.30) |
| $T_2$ | *T. harzianum*(BDN-2) | 20.25 | 77.49 (61.67) |
| $T_3$ | *T. harzianum*(BDN-3) | 22.25 | 75.27 (60.17) |
| $T_4$ | *T. viride*(BDN-4) | 22.50 | 74.99 (59.99) |
| $T_5$ | *T. harzianum*(BDN-5) | 17.00 | 81.10 (64.23) |
| $T_6$ | *T. longibrachiatum*(BDN-6) | 39.25 | 56.38 (48.66) |
| $T_7$ | *T. harzianum*(BDN-7) | 22.50 | 74.99 (59.99) |
| $T_8$ | *T. koningii*(JN-1) | 35.75 | 60.27 (50.92) |
| $T_9$ | *G. virens*(JN-2) | 39.50 | 56.05 (48.47) |
| $T_{10}$ | *T. harzianum*(JN-3 ) | 17.00 | 81.10 (64.23) |
| $T_{11}$ | *T. viride*(JN-4) | 23.25 | 74.16 (59.44) |
| $T_{12}$ | *T. harzianum*(JN-5) | 15.75 | 82.49 (65.26) |
| $T_{13}$ | *T. longibrachiatum*(MNT-1) | 40.00 | 55.55 (48.18) |
| $T_{14}$ | *T. viride*(MNT-3) | 31.50 | 64.99 (53.72) |
| $T_{15}$ | *G. virens*(MNT-4) | 47.00 | 47.77 (43.72) |
| $T_{16}$ | *T. viride*(PTR-1) | 24.50 | 72.88 (58.61) |
| $T_{17}$ | *T. harzianum*(PTR-2) | 25.75 | 71.38 (57.65) |
| $T_{18}$ | *T. viride*(PTR-3) | 23.00 | 74.44 (59.63) |
| $T_{19}$ | *T. viride*( PRT-1) | 24.50 | 72.77 (58.54) |
| $T_{20}$ | *T. viride*(SLU-1) | 27.75 | 69.16 (56.26) |
| $T_{21}$ | *T. viride*(SLU-2) | 23.00 | 74.44 (59.63) |
| $T_{22}$ | *T. viride*(SLU-3) | 22.25 | 72.99 (58.68) |
| $T_{23}$ | *T. harzianum*(PBN-2) | 22.75 | 73.21 (58.82) |
| $T_{24}$ | *T. viride*(PBN-3) | 17.25 | 80.83 (64.03) |
| $T_{25}$ | *T. harzianum*(PBN-4) | 23.50 | 74.16 (59.44) |
| $T_{26}$ | *T. viride*( PBN-5) | 24.75 | 72.49 (58.36) |
| $T_{27}$ | *T. viride*(PBN-6) | 18.00 | 79.99 (63.42) |
| $T_{28}$ | control | 90.00 | 90.00 (71.56) |
| SE± | | 1.07 | 1.17 |
| CD at 1% | | 3.11 | 3.38 |

*-Mean of two replications, Dia.: Diameter

inhibition i.e. 89.99 per cent followed by $T_{27}$ (*T. viride*) Parbhani strain i.e. 87.21 and $T_7$ (*T. harzianum*) Jalna strain i.e. 86.94 per cent.

Results of the present study on effect of bioagents on mycelia growth of *F.oxysporum* f. sp. *ciceri* are in consonance with those reported earlier by several workers. Bioagents *viz., Trichoderma viride, T. harzianum, T. longibrachiatum, T. koningii,* and *G. virens* were reported earlier to cause significant inhibition of mycelial growth of *F.oxysporum* f. sp. *ciceri* by several workers ( **Srivastava and Mall,** 2008; Patil *et al*., 2010; Rajput *et.al.,* 2010 and Yadav and Anadani , 2013).

**In *vitro* bioefficacy of different *Trichoderma* isolates against *F. oxysporum* f. sp. *udum*.**

The results (Table 3) obtained on mycelia growth and inhibition of *Fusarium oxysporum* f. sp. *udum* (Butler) with thirteen *T. viride*, nine *T. harzianum*, two *T. longibrachiatum*, one *T. koningii*, two *G. virens* isolates antagonists. It was observed that treatment $T_{12}$ (*T. harzianum*) isolate obtained from Jalna strain showed highest zone of inhibition i.e. 82.49 per cent followed by $T_5$ and $T_{10}$ (*T. harzianum*) Jalna strain i.e. 81.10 and $T_{24}$ (*T. viride*) Parbhani strain i.e. 80.83 per cent.

Results of the present study on effect of bioagents on mycelial growth of *F. oxysporum* f. sp. *udum* are in consonance with those reported earlier by several workers. Bioagents *viz., Trichoderma viride, T. harzianum, T. longibrachiatum, T. koningii,* and *G. virens* were reported earlier to cause significant inhibition of mycelial growth of *F. oxysporum* f. sp. *udum* by several workers (Pandey and Upadhay, 2000; Patel *et al.,* 2011; Ranjana and Chhetry, 2012).

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