Journal of Applied and Natural Science
13(2): 537 - 543 (2021)
Published online: May 22, 2021
ISSN : 0974-9411 (Print), 2231-5209 (Online)
journals.ansfoundation.org

Research Article

Evaluation of Trichoderma consortia against *Fusarium udum* causing wilt of Pigeonpea

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Article Info
https://doi.org/10.31018/jans.v13i2.2656
Received: April 4, 2021
Revised: May 13, 2021
Accepted: May 18, 2021

How to Cite
Patel, M. and Kumar, S. (2021). Evaluation of Trichoderma consortia against *Fusarium udum* causing wilt of Pigeonpea. *Journal of Applied and Natural Science*, 13(2), 537 - 543. https://doi.org/10.31018/jans.v13i2.2656

Abstract
Pigeonpea is one of the important pulse crop of Madhya Pradesh, a State of India. The plant gets infected by the pathogen *Fusarium udum* causing wilt disease, which is one of the major constraints in the production and productivity of pigeonpea. The present study aimed to carry out *in vitro* condition to assess the possible use of biocontrol consortia in field conditions. Six *Trichoderma* consortia viz., T_1- *T. viride* + *T. harzianum* (JC-I), T_2- *T. viride* + *T. virens* (JC-2), T_3- *T. harzianum* +*T. virens* (JC-3), T_4- *T. hamatum* +*T. viride* (JC-4), T_5- *T. hamatum* + *T. harzianum* (JC-5), T_6- *T. hamatum* + *T. virens* (JC-6) and T_0- Control were evaluated for their antagonistic activity against *F. udum*under *in vitro* conditions. The consortia of T_4- *T. hamatum* +*T. viride* (JC-4) was found most effective(58.82 %) in inhibiting the radial growth of *Fusarium udum*. The volatile compound from consortium of T_5-*T. hamatum* + *T. harzianum* (JC-5) exhibited maximum growth inhibition (81.84%) and sporulation of *Fusarium udum* followed by T_1-*T. viride* + *T. harzianum* (JC-I) (55.49% inhibition) . The culture filtrate of consortia of T_4- *T. hamatum* +*T. viride* (JC-4) showed 100% inhibition of test pathogen followed by T_5-*T. hamatum* + *T. harzianum* (JC-5) (82.89%) at 5 % concentration. It was also observed that with an increase in the concentration of culture filtrates of all the *Trichoderma* species, the radial mycelial growth of the test pathogen was proportionally decreased. The Trichodema consortium *viz*., T_4- *T. hamatum* +*T. viride* (JC-4) may be tried in the field to manage wilt of pigeonpea because they worked synergistically and gave the high impact of their use.

Keywords: Consortia, *Fusarium udum,* Pigeonpea, Trichoderma , Wilt

INTRODUCTION

Pigeon pea is one of the key pulse crops grown in India. Pigeonpea occupies a unique place on Indian agriculture scene as the country accounts for about 71.5% of the global production, covering an area of around 5.40 m ha and production of 4.87 million tonnes. The average productivity of pigeon pea is about 750 kg/ha, which is much lower than their potential yields. (Singh *et al.,* 2020) . Pigeonpea is known to be infected by more than 200 pathogens reported from 23 different countries (Nene *et al.,* 1981). Wilt is predominant in all major pigeon pea growing areas throughout India and causes 30-100% yield loss (Biswas and Ghosh, 2016). The intensive use of fungicides results in environmental pollution, the resistance of pathogens towards fungicides , hazardous to human and animals. This necessitates the need to adopt sustainable management of disease like using antagonistic fungi against the pathogen. (Harman, 2011; Singh *et al*., 2011; Kumar *et al*., 2014).

There are many traditional strategies as well as chemical approaches for managing the wilt disease in pigeon pea. Lesser emphasis on understanding the application of biological approaches for managing *Fusarium udum* in pigeon pea fields. (Sharma *et al*., 2012). Over the years, numerous studies have described the application of microbial consortia for plant disease management throughout the world. Studies revealed that plants treated with anagonistic microbial

consortia showed a significant disease reduction compared to individual isolates. Biocontrol attributes are also more in consortia than using single isolates (Thakkar and Saraf, 2015). Studies on employing indigenous fungal antagonistic consortia are very limited against the wilt of pigeonpea in Madhya Pradesh. Concerning this, the present study was focused on the approach to evaluating Trichoderma consortium against *Fusarium udum* that could help in effective management of wilt of pigeonpea.

MATERIALS AND METHODS

Isolation and identification of test pathogen

Pigeonpea plants showing typical symptoms of Fusarium wilt were collected from the experimental field of Jawaharlal Nehru Krishi Vishwa VidyalayaJabalpur for isolation and identification. The infected plant parts were cut into small pieces and surface sterilized with 0.1 per cent Sodium hypochloride solution and washed thoroughly 3 to 4 times with sterilized water to remove the traces of sodium hypochlorite. The pieces were transferred in petri dishes containing potato dextrose agar medium and incubated at 27 ± 1ºC for 7 days. The pure culture was isolated from inoculated petriplates separately in aseptic condition.(Chaudhary *et al.,* 2017).

The following six Trichoderma consortium were evaluated to test the antagonism against *Fusarium udum* in the Department of Plant Pathology Jawaharlal Nehru Krishi Vishwavidyalaya, Jabalpur (M.P.) during 2020-21.

T_1-*Trichoderma viride* + *Trichoderma harzianum* (JC-I)
T_2-*Trichoderma viride* + *Trichoderma virens*(JC-2)
$T_{3:}$-*Trichoderma harzianum* +*Trichoderma virens*(JC-3)
T_4-*Trichoderma hamatum* +*Trichoderma viride* (JC-4)
T_5-*Trichoderma hamatum* + *Trichoderma harzianum* (JC-5)
T_6-*Trichoderma hamatum* + *Trichoderma virens*(JC-6)
T_{0-} Control

The Trichoderma used in the consortium were isolated and identified in the laboratory as per the method given by Gams and Bisset (1998) and will be submitted to Indian type culture collection, Division of Plant Pathology, Indian Institute of Agriculture Research (IARI), New Delhi for accession numbers.

Effect of Trichoderma consortia on the radial growth of *F. udum* in dual culture

A dual culture technique developed by Morton and Straube (1955) and Patole et al (2017) was adopted to study the effect of Trichoderma consortia against *F. udum*,. Twenty ml sterilized melted PDA media was poured into sterilized petriplates @ 20 ml/plate aseptically, allowed to solidify. For the Trichoderma consortium, 8 mm disc of Trichoderma was placed at equidistance on potato dextrose agar media in a petriplate. Immediately after inoculation , the plates were sealed with plastic film and incubated at 27 ± 1ºC for 1 week period. Observations were recorded after 3, 5,7 days of inoculation on the growth of individual Trichoderma in the presence of its co-inoculant. Pairs of consortia were considered compatible if they grow without any inhibition zone in the culture plate. Then, 8 mm disc of test pathogen and the consortia cut with the help of sterilized cork borer were placed on PDA approximately 4 cm apart from each other and incubated in BOD incubator at 27± 1ºC for 144 hours. Three replications were maintained for each treatment. Observations on colony diameter of individual antagonist and the pathogen were recorded after 144 hours of incubation. Inhibition of radial growth of *F. udum* over control was calculated by the formula given by Vincent (1947).

Effect of volatile and non volatile compounds from Trichoderma consortia on the radial growth of *Fusarium udum*

The effect of volatile compounds from Trichoderma consortia *viz*., T_1-*T. viride* + *T. harzianum* (JC-I), T_2- *T. viride* + *T. virens* (JC-2), $T_{3:}$-*T. harzianum* +*T. virens* (JC-3), T_4- *T. hamatum* +*T. viride* (JC-4), T_5-*T. hamatum* + *T. harzianum* (JC-5) and T_6-*T. hamatum* + *T. virens* (JC-6) on radial growth of test pathogen was performed as per the method given by Dennis and Webster (1971a and1971b). Two bottom portion of petridplates containing potato dextrose agar were inoculated with 8 mm disc of test pathogen and Trichoderma consortia, respectively and both inoculated bottom plates were placed facing each other and sealed with cellophane adhesive tape. The petriplate containing PDA without antagonist served as control. The observations on the radial growth of the test pathogen were recorded after 144 h of incubation at 27 ± 1ºC.

To study the effect of non volatile compounds, the Trichoderma consortia were grown on potato dextrose broth at 27±1ºC with intermittent shaking at 150 rpm. Themetabolites were collected after 15 days and filtered. The sterilized filtrate was amended in potato dextrose agar to make 2 and 5 % concentration in petriplates. The solidified agar plates were inoculated at the centre with 5 mm mycelial disc of pathogen and incubated at 27±1ºC for 96 hours. The plates without filtrate served as control. Observations on radial growth of individual Trichoderma consortia and the test pathogen were recorded after 96 hours of incubation. Inhibition of radial growth of test pathogen over control was calculated by formula given by Vincent (1947).

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

……..Eq.1

Where,
C = Radial growth in check plate (mm)
T = Radial growth in the treated plate (mm)

RESULTS AND DISCUSSION

Compatibility among Trichoderma

All the six Trichoderma consortium (JC-1, JC-2, JC-3, JC-4, JC-5 & JC-6) were found compatible with each other as no isolates inhibited the growth of one another. They were growing simultaneously on the PDA without inhibiting the growth of other or formation of inhibition zone in their combinations. All the six Trichoderma consortium were found compatible with each other as no isolates inhibited the growth of one another. They were growing simultaneously on the PDA without inhibiting the growth of other or formation of inhibition zone in their combinations.

Evaluation of antagonistic efficacy of consortium of Trichoderma against *F. udum*

The consortium of T_4- *T. hamatum* +*T. viride* (JC-4) was found most effective(58.82 %) in inhibiting the radial growth of *Fusarium udum* (Table-1, and Plate-1)The percent growth inhibiton recorded in T_2- *T. viride* + *T. virens* (JC-2), $T_{3:}$-*T. harzianum* +*T. virens* (JC-3) and *T_5*-*T. hamatum* + *T. harzianum* (JC-5) were, respectively, 57.20, 56.79 , 56.38 % and were statistically at par with each other. The percent inhibition recorded in T_1-*T. viride* + *T. harzianum* (JC-I) *and T_6*-*T. hamatum* + *T. virens* (JC-6) *were* 44.21 and 19.83 *%,*respectively.

Evaluation of antagonistic efficacy of volatile and non volatile compounds from consortium of Trichoderma against *Fusarium udum*

The volatile compound from consortium of *T_5*-*T. hamatum* + *T. harzianum* (JC-5) exhibited maximum growth inhibition (81.84%) and sporulation of *Fusarium udum* followed by T_1-*T. viride* + *T. harzianum* (JC-I) (55.49%) and T_4- *T. hamatum* +*T. viride* (JC-4) *(*43.23 *%)* (Table-2, and Plate-2). *T_6*-*T. hamatum* + *T. virens* (JC-6) and T_2- *T. viride* + *T. virens* (JC-2) inhibited 35.13 % and 34.74% growth of *F. udum*. Minimum growth inhibition 29.54 % recorded in $T_{3:}$-*T. harzianum* +*T. virens* (JC-3) after 144 hours of incubation.

The culture filtrate of consortia of T_4- *T. hamatum* +*T. viride* (JC-4) showed cent per cent inhibition of test pathogen followed by *T_5*-*T. hamatum* + *T. harzianum* (JC-5) (82.89%) at 5 % concentration (Table-3, and Plate-3) . T_2- *T. viride* + *T. virens* (JC-2) and *T_6*-*T. hamatum* + *T. virens* (JC-6) exhibited 78.52 % and 77.63 % mycelial inhibition of test pathogen and statistically at par with each other.The consortia of $T_{3:}$-*T. harzianum* +*T. virens* (JC-3), and T_1-*T. viride* + *T. harzianum* (JC-I) exhibited respectively 67.10 and 61.84 % growth inhibition of *F. udum* . The test pathogen exhibited 38.00 mm growth after 96 hour of incubation.It was also observed that with an increase in concentration of culture filtrates of all the Trichoderma consortia, the radial mycelial growth of test pathogen was proportionally decreased.

Over the years, numerous studies have described the application of microbial consortia for plant disease management throughout the world. Studies revealed that plants treated with anagonistic microbial consortia showed a significant disease reduction when compared to using individual isolates (Nikam *et al.,* 2007, Sharma *et al*., 2012,Patole*et al.,* 2017). The principal biocontrol mechanisms involved include mycoparasitism, antibiosis, competition, and induced resistance (Kumar *et al.* 2009, Kumar. 2013, Chaudhary *et al.,* 2017**,** Kushwaha *et al*., 2018). Application of bioagents in a consortium may improve the efficacy, reliability and consistency of the bioagents even under diverse soil and environmental conditions (Sharma *et al*. 2012; Amirthalingam *et al.*

Table 1. Evaluation of antagonistic efficacy of consortium of Trichoderma against *F. udum.*

T. No . and Treatment Name	Radial growth of test Pathogen (mm)	Percent growth inhibition	Sporulation
T_1 *T. viride* + *T. harzianum*	45.83	44.21	+++
T_2 *T. viride* + *T. virens*	35.16	57.20	+++
$T_{3:}$ *T. harzianum* +*T. virens*	35.50	56.79	+++
T_4 *T. hamatum* +*T. viride*	33.83	58.82	+
T_5 *T. hamatum* + *T. harzianum*	35.83	56.38	+++
T_6 *T. hamatum* + *T. virens*	65.86	19.83	++++
$T_{0:}$ Control	82.16		++++
SE(m)	0.78		
CD	2.10		

*Average of three replications

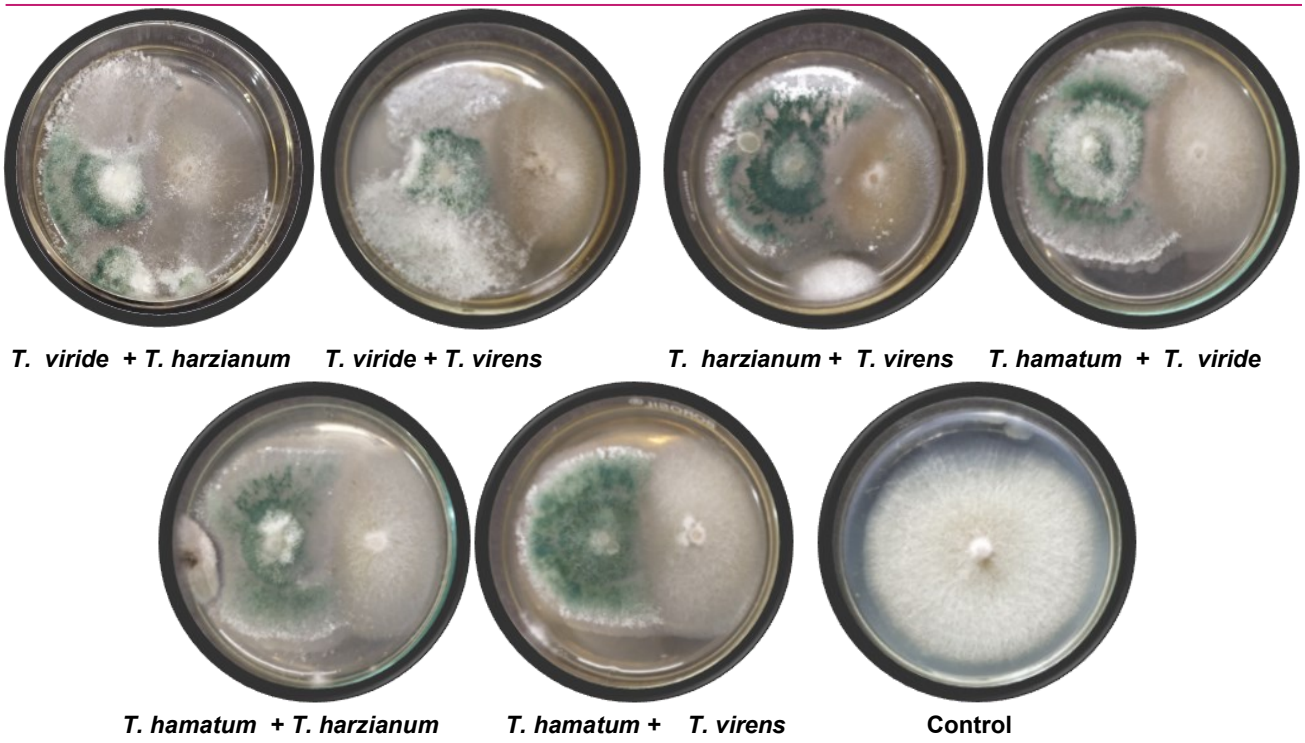

Plate 1: Evaluation of antagonistic efficacy of consortium of Trichoderma against *Fusarium udum.*

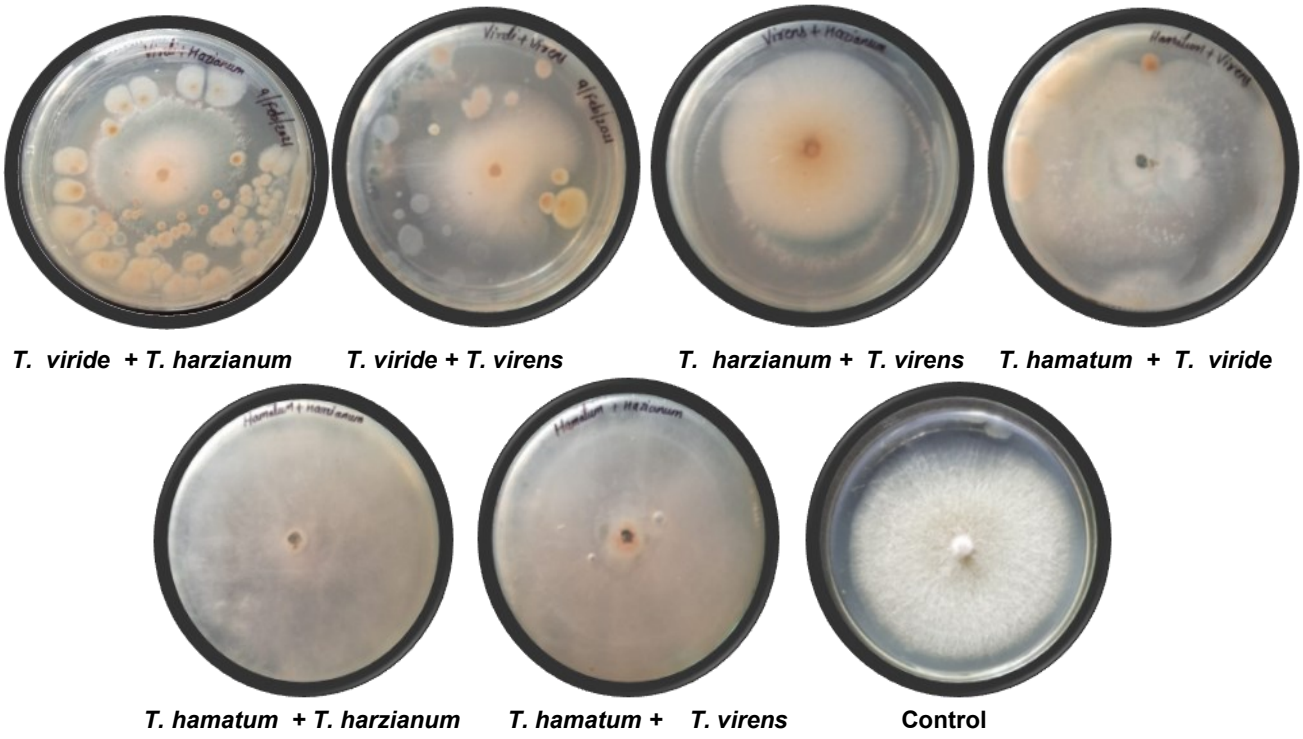

Plate 2. Showing antagonistic efficacy of volatile compound from consortium of Trichoderma against *Fusarium udum.*

2020). A consortium of *T. asperellum* GDFS1009 and *Bacillus amyloliquefaciens* ACCC1111060 was found to be more efficient against infection by *Botrytis cinerea* , causing grey mold disease than the individual strains (Wu *et al.*, 2019). Likewise, when *Trichoderma virens* GI006 was combined with *Bacillus velezensis* Bs006, efficiency against *Fusarium* wilt of cape gooseberry was enhanced (Izquierdo-García *et al*., 2020). Thakkar and Saraf (2015) reported that different biocontrol mechanisms offered by each bioagent in the consortium may help in enhancing pathogen inhibition and may also strengthen the capacity of the partners in an additive or synergistic manner. However, Kumar and

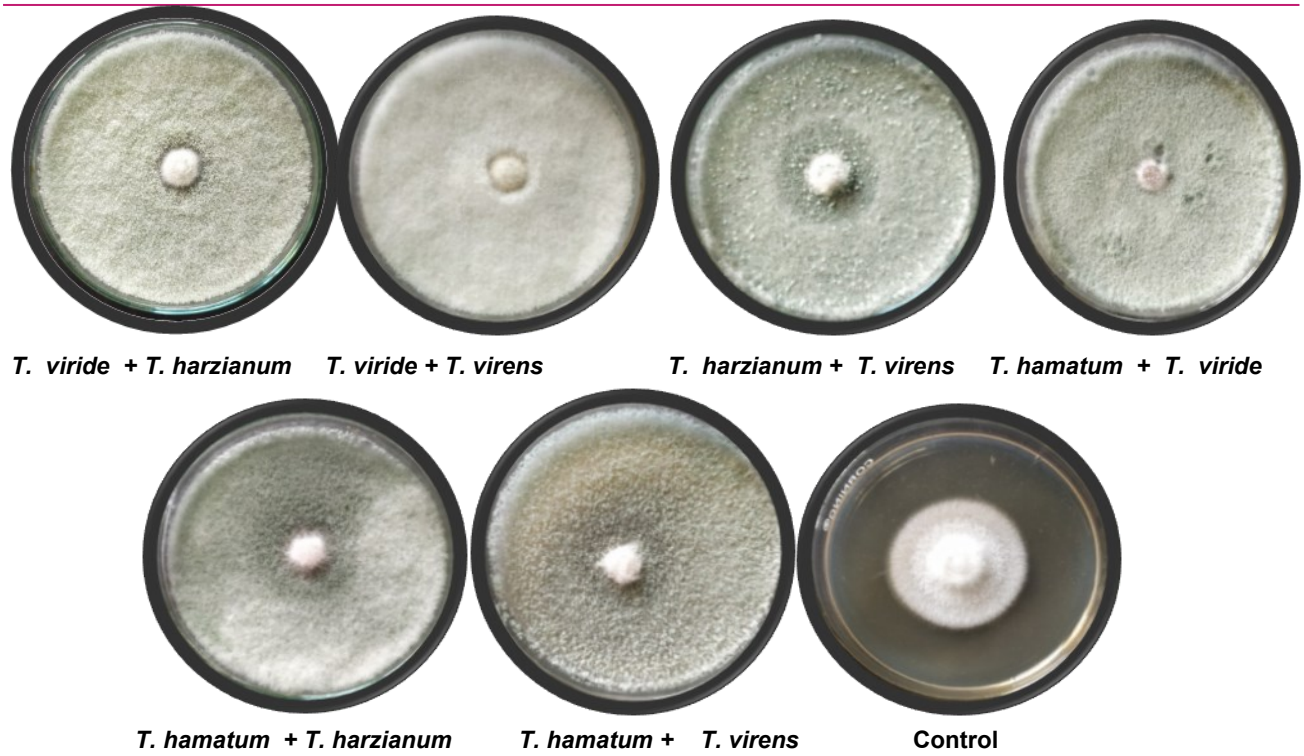

Plate 3 . Showing efficacy of non volatile compound from consortium of Trichoderma against *Fusarium udum* .

Table 2: Evaluation of antagonistic efficacy of volatile compound from consortium of Trichoderma spp. against *F. udum* under *in vitro* condition.

T. No. and Treatment Name	Radial growth of test Pathogen (mm)[#]	Percent growth inhibition	Sporulation
T_1 *T. viride + T. harzianum*	37.16	55.49	++
T_2 *T. viride + T. virens*	54.50	34.74	+++
T_3 *T. harzianum+ T. virens*	58.83	29.54	+++
T_4 *T. hamatum + T. viride*	48.16	43.23	+++
T_5 *T. hamatum + T. harzianum*	15.16	81.84	-
T_6 *T. hamatum + T. virens*	54.16	35.13	+++
T_7 Control	83.50		++++
SE(m)	0.930		
CD	2.84		

*Average of three replications

Jagadeesh (2016) reported that certain microbial consortia were unable to show at least comparable effects on plants with respect to their individual applications, which may be attributed to the incompatibility of the microbes in the mixture with each other and do not have any additive or synergistic effects on disease suppression.

Conclusion

The application of Trichoderma consortia *viz*., *T. hamatum + T. viride* can be more useful than the individual for management of wilt of pigeonpea because of two compatible isolates of different species work synergistically and gave the high impact of their use. Reduction in disease by using Trichoderma consortia may reduce the chemical pesticide loads on the pigeonpea crop, which will be in favour of farmers and consumers as well.

Conflict of interest

The authors declare that they have no conflict of interest.

Table 3. Effect of non-volatile compounds from consortium of *Trichoderma* on growth and sporulation of *F. udum.*

T. No. and Treatment Name	Radial growth of test Pathogen after 96hrs (mm)*		Percent growth inhibition	Sporulation
	2%	5%		
T_1 *T. viride + T. harzianum*	8.66	14.50	61.84	+++
T_2 *T. viride + T. virens*	11.67	8.16	78.52	++
T_3 *T. harzianum+ T.virens*	15.83	12.50	67.10	+++
T_4 *T. hamatum + T. viride*	0.00	0.00	100.00	-
T_5 *T. hamatum + T. harzianum*	8.83	6.50	82.89	+
T_6 *T. hamatum + T. virens*	12.50	8.50	77.63	+
T_7 Control	36.83	38.00		++++
SE(m)	0.40	0.454		
CD	1. 23	1.39		

*Average of three replications

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