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EVALUATION OF NATIVE *Trichoderma* ISOLATES AGAINST FUSARIAL WILT OF CHILLI (*Capsicum annum* L.)

D. R. PATEL[1], J. R. PANDYA[1], S. H. JOSHI[2] and R. P. PANDYA[1]

[1]Department of Plant Pathology, College of Agriculture, Navsari Agricultural University, Campus Bharuch 392012 Gujarat, India.
[2]Department of Plant Pathology, N.M. College of Agriculture, Navsari Agricultural University, Navsari 396450 Gujarat, India.

Corresponding Email *jrpandya@nau.in*

Abstract

Chilli (*Capsicum annum* L.) is one of the most important vegetable and spices crop grown across the world for the value for its valuable fruits and is popularly known as "red pepper" which belongs to night shade family Solanaceae. Isolate of *Fusarium* spp. were collected from the different location of villages of Bharuch districts. Six native *Trichoderma* spp. isolates *viz*., BVVT-1, BVDT-1, BJGT-2, BJGT-1, BNBT-2 and BJST-1 were isolated from the rihzospheric soil of chilli wilt infected fields. The maximum number of colony 4×10^{-4} was observed in BVVT-1 and minimum number observed as 1×10^{-4} in BJGT -1 and BNBT-2 isolate. The anti-fungal efficacy of native *Trichoderma* spp. studied using dual culture method revealed strong antagonism of isolate BVVT-1 against most virulent *Fusarium spp.* whereas remaining five isolates *i.e*., BVDT-1, BJGT-2, BJGT-1, BNBT-2 and BJST-1 isolates also appeared as potential antagonists. The six native isolates were also tested in pot condition against susceptible Eagle C4 chilli variety. The T_2 treatment (BVVT-1 isolate) recorded significantly highest 17.60 cm shoot length and higher root length of 7.20 cm which remained at par with T_1 treatment (BVDT-1 isolate) i.e., 6.83 cm root length. While the lowest root length 4.67 cm and shoot length 13.20 cm was recorded in BNBT-1 isolate and it also recorded the highest 36.67 per cent disease incidence with lowest 63.33 per cent disease control, so it was considered as least effective among all the isolates. The results concluded that the BVVT-1 isolate was the most effective and found to be best as it recorded the highest 83.33 per cent disease control with lowest 16.67 per cent disease incidence.

Key words: *Trichoderma*, *Fusarium*, Chilli, dual culture

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Introduction

Chilli is considered as one of the most important vegetable and spices crop grown in world and is popularly known as "red pepper". The Scientific name of Chilli is *Capsicum annum* L. which belongs to nightshade family Solanaceae. Capsicum is a Greek word derived from "Kapisino" meaning "to bite" (Anon., 2009). It is commonly known as mirchi, hot pepper, garden pepper, salad pepper, paprika, bell pepper, red pepper, capsicum, pod pepper, *etc*. It is believed to be originated around 7500 BC in Mexico. In India, it was introduced by Portuguese during end of 15th century in Goa. It is

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an often cross-pollinated annual herb having diploid chromosome number 2X = 24. The five cultivated species of capsicum are *C. annum, C. frutescens, C. chinense, C. baccatum* and *C. pubescens*. In India, *C. annum* and *C. frutescens* is the most commercially cultivated species of capsicum (Bose *et al*.2002).

India is the world's largest producer, consumer and exporter of chilli followed by China, Ethopia, Thailand and Pakistan. During the year of 2019-20, the area occupied by Indian chilli was 387 thousand ha and production was 4119 thousand MT (Anon., 2021). The major chilli growing areas in India are Andhra Pradesh (805.03 thousand ton), Telangana (436.38 thousand ton), Madhya Pradesh (208.63 thousand ton), Karnataka (129.24 thousand ton) and Orissa (69.28 thousand ton) accounting for 43.87, 23.78, 11.37, 7.04 and 3.78 per cent share in chilli production of India, respectively (Anonymous2020).

Fusarium being soil-borne disease is difficult to control. Different methods *viz*., cultural, mechanical, chemical and biological methods have been adopted to control the disease. The most widely used method is chemical method as it is very effective. But the use of excessive chemical is leading to environmental pollution and is also affecting the soil health. It is not economically feasible as it increases the cost of cultivation. In recent year, the trend of using biological method for control is on as this method is most effective, economically feasible and most importantly eco-friendly (Panth *et al*.2020).

Among the various biocontrol agents, 90 per cent of fungal biocontrol agents belong to different strains of *Trichoderma* and on evaluation has also shown promising antagonistic effect against the Fusarium wilt pathogen. They are common saprophytic filamentous fungus, widely distributed and ubiquitous in almost all type of soils (Olabiyi and Ruocco, 2013). The various characteristics of *Trichoderma i.e*., ability to survive under adverse conditions, high reproducibility, strong aggressiveness against various pathogenic fungi, act as plant growth regulator and defense mechanism has made it as one of the most successful biocontrol agents among the other fungal agents (Sinha *et al*. 2018). The concept of evaluation on native Trichoderma spp. isolates was also adopted by Pandya and Sabalpara, 2018 and found efficient native Trichoderma isolate. With this view the present investigation was carried out to explore the potentiality of native Trichoderma isolates against Fusarium.

Materials and Methods

Isolation and Purification of *Fusarium* spp.

The collected infected samples were subjected through microscopy and positive (+) samples were subjected to isolation using tissue isolation technique. The pathogen *Fusarium* spp. was isolated from the freshly infected collar portion of the plant. After the development of fungal colony, a small bit of single mycelium was transferred on another petri-plate containing PDA medium to obtain pure culture. This pure culture was maintained in PDA slants for further studies.

Isolation of native *Trichoderma* spp. isolates from chilli wilt infected soils

An extensive field survey was conducted in major chilli growing areas of Bharuch district at flowering and fruiting stage of the chilli crop. For the isolation of native *Trichoderma* spp. from the rhizosphere soils, samples were collected from the infected fields of chilli. Soil samples weighing approximately 250-300 g were collected separately from each field in a polythene bag and were

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tagged with information related to host, locality, soil type, *etc*. The soil sample was collected from the area around root zone (rhizosphere soil) and brought to the laboratory for further studies. These samples were subjected to standard serial dilution plate technique (Johnson 1957) and *Trichoderma* selective medium (TSM) or PDA medium was used for isolation. The fungal colonies developed in the plates were sub cultured and purified on TSM or PDA slants for the further studies (Wani *et al.*, 2014). Number of colonies developed was recorded and *Trichoderma* population was calculated among the other colonies if developed.

Anti-fungal efficacy of native *Trichoderma* spp. isolates against most virulent *Fusarium* spp.

The isolates of *Trichoderma* spp. were evaluated against *Fusarium* spp. in the laboratory by using the standard dual culture technique (Morton and Stroube, 1955). Based on the growth in a control treatment the per cent growth inhibition was calculated by formula:

$$PGI = \frac{C - T}{C} \times 100$$

Here,

PGI= Per cent growth inhibition

C= colony diameter (mm) in control plate

T = colony diameter (mm) in treated plate

Table 1: List of native *Trichoderma* spp. isolates tested under *in vitro* and pot condition

Treatment	Name of Isolate
T1	BVDT-1
T2	BVVT-1
T3	BJGT-2
T4	BJGT-1
T5	BJST-2
T6	BNBT-1
T7	Control

Evaluation of native *Trichoderma* spp. isolates against chilli fusarial wilt in pot condition

The seed of chilli variety Eagle C4 which is susceptible to Fusarial wilt disease was obtained from a local market. The seeds were surface sterilized by 1 per cent NaOCl for 60 seconds, followed by washing them thrice with distilled sterile water to remove the traces of NaOCl. Sterilized soil was filled in small pots and placed in greenhouse. Before one month, the seeds were grown in the plug

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tray in which coco-peat and vermicompost mixture was used under the greenhouse condition. The four - weeks old seedlings were transplanted in pots. The ten chilli seedlings were transplanted in the one pot *i.e.*, 10 seedlings per pot. Later, the conidial suspension of *Fusarium* spp. adjusted to 1×10^6 conidia ml^{-1} was applied near the root zone of the plants in each pot. After 10 days of inoculum, the conidial suspension of *Trichoderma* isolates adjusted to 1×10^8 conidia ml^{-1} was applied near the roots of chilli plants in their respective pots as per the treatment. The plants were placed in greenhouse at 28-30°C temperature for one month and the disease severity was calculated (Anjum *et al*.2020). The phonological parameters of the crops were also observed and recorded and transformation applied if necessary.

The per cent disease incidence was calculated using the formula:

$$\text{Per cent diseases incidence} = \frac{\text{No of infected plants}}{\text{Total no. of plants observed}} \times 100$$

Results and Discussion

Isolation and Purification of *Fusarium* spp.

The infected samples collected from the surveyed field were subjected to isolation using tissue isolation technique. Total twenty isolates were obtained from the twenty different villages surveyed. These isolates were further identified as *Fusarium* spp. isolates on the basis of their morphological and cultural characteristics. These isolates were then grown on PDA media and were further sub-cultured for purification and maintained on PDA slant at 4°C. Then the further investigation was carried out using this pure culture.

Isolation of native *Trichoderma* spp. isolates from chilli wilt infected soils

The native Trichoderma spp. isolates obtained from the rihzospheric soils of chilli wilt infected plants collected from the different villages of the Bharuch district. Total of six native *Trichoderma* spp. isolates *viz.*, BVVT-1, BVDT-1, BJGT-2, BJGT-1, BNBT-2 and BJST-1 were isolated and further tested. The various characters of native *Trichoderma* isolates *i.e.*, number of Trichoderma colonies, other colony and population recorded in terms of cfu (%) and cfu g^{-1} studied during experiment are furnished in Table 2. The Number of colonies of *Trichoderma* spp. isolates ranged from 1×10^{-4} to 4×10^{-4} cfu g-1. The maximum numbers of Trichoderma colonies were observed in the BVVT-1 isolate obtained from the soil sample collected from Vadfaliya village while the BJGT -1 isolate of Govali village and BNBT-2 isolate of Baldava village recorded the minimum number of *Trichoderma* colonies *i.e.*, 1×10^{-4} cfu g^{-1}. The colonies of other fungi rather than *Trichoderma* were also found which ranged from 5-7.

The result revealed that there was abundant amount of Trichoderma population present in the rhizospheric soil samples collected from chilli wilt infected regions of different villages. The highest 75 per cent of cfu was noted in BVDT -1 isolate obtained from the soil sample of Deli village followed by BJST -1, BVVT -1, BJGT -1, BJGT -2 and BNBT -2 isolates with 70.5, 70, 69.56, 69.11 and 66.66 per cent of cfu of the native *Trichoderma* spp. isolates obtained from the chilli wilt infected rihzospheric soils, respectively (Table-2). The colony forming units of native *Trichoderma* spp. isolates obtained in 1 g of rihzospheric soils collected from the region of chilli wilt infected

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plants ranged from the 1×10^5 to 1.8×10^5 cfu g^{-1}. The highest 1.8×10^5 cfu g^{-1} was recorded for the BVDT -1 isolate of the Deli village of Bharuch district. While the BNBT -2 isolate of Baldava village of Bharuch district recorded the lowest 1×10^5 cfu g^{-1}.

The result found in this experiment is in conformity with the results of Sundaramoorthy and Balabaskar (2013). Both of them isolated fifteen native *Trichoderma* antagonists from healthy tomato rhizosphere soil collected from different geographical regions noted colony of dark green in colour.

Table 2: Colony characteristics of native *Trichoderma* spp. isolates from chilli wilt infected rihzospheric soils

Sr. No.	Villages	Trichoderma Isolates	Number of Trichoderma Colonies (10^{-4})	Other Colony	cfu (%)	Cfu g^{-1} (10^5)
1.	Vadfaliya	BVVT-1	4	6	70.00	1.4
2.	Deli	BVDT-1	3	6	75.00	1.8
3.	Govali	BJGT-2	2	6	69.11	1.1
4.	Govali	BJGT-1	1	7	69.56	1.6
5.	Baldava	BNBT-2	1	5	66.66	1.0
6.	Sultanpura	BJST-1	2	5	70.5	1.2

Anti-fungal efficacy of native *Trichoderma* spp. isolates against most virulent *Fusarium* spp.

The six native isolates of *Trichoderma* spp. were tested against the isolates of *Fusarium* spp. causing Fusarial wilt disease in chilli using the dual culture technique. The details regarding the result obtained have been indicated in Table 3.

The effect of native *Trichoderma* on radial growth of *Fusarium* was noted on 3rd, 4th, 5th and 7th day. It was observed that there was decrease in radial growth of *Fusarium* spp. due to inoculation of different isolates of the native *Trichoderma* spp. From the six different isolates used, the BVVT-1 isolate recorded the best result as it showed minimum radial growth of *Fusarium i.e.*, 16.33 mm, 19.67 mm, 23.33 mm and 15.33 mm on 3rd, 4th, 5th and 7th day, respectively. While, BNBT-1 isolate showed poor result because in comparison to other isolates maximum radial growth of *Fusarium* spp. was noted in it i.e., 24.67 mm, 31.00 mm, 34.33 mm and 25.33 mm on 3rd, 4th, 5th and 7th day, respectively. The highest radial growth of *Fusarium* spp. was observed in control where no inoculation of *Trichoderma* spp. was made *i.e.*, 31.00 mm, 51.00mm, 81.00 mm and 90.00 mm on 3rd, 4th, 5th and 7th day, respectively. The obtained results have been furnished in Table 3.

Looking to the pooled analysis data, the radial growth on 3rd was highly suppressed due to BVVT-1 isolate (16.33 mm) followed by BVDT-1 (18.67 mm) isolate, BJGT-2 (20.33 mm) isolate, BJGT-1

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(22.00 mm) isolate and BJST-2 (22.67 mm) isolate. While the BNBT-1 (24.67 mm) isolate was least effective in inhibiting the radial growth of *Fusarium* spp.

The BVVT -1 isolate was also highly effective on 4th day as it recorded minimum radial growth followed by the BVDT-1, BJGT-2 and BJGT-1 isolates. While the BNBT-1 isolate was least effective in inhibiting the radial growth and remained at par with BJST -2 isolate. The mean data of radial growth recorded on 5th day concluded that the BVVT-1 isolate recoded 23.33 mm was highly effective and at par with BVDT-1 isolate recorded 25.67 mm radial growth. The least effective BNBT-1 isolate recorded 34.33 mm radial growth on 5th day.

On 7th day, the BVVT-1 isolate was most effective as it recorded radial growth of only 15.33 mm followed by BJGT-2 isolate with 18.33 mm radial growth. While the BNBT-1 isolate was considered as the least effective as it recorded maximum radial growth of 25.33 mm and it remained at par with isolate BVDT-1 (23.67 mm).

The per cent growth inhibition recorded on the 3rd, 4th, 5th and 7th day was found to be highest for the BVVT-1 *i.e.*, 47.31, 61.17, 71.19 and 82.96 per cent, respectively followed by BJGT-2 *i.e.*, 34.41, 48.36 and 65.84 per cent on 3rd, 4th and 5th days, respectively also showed good result leading to 79.63 per cent growth inhibition on the 7th day. The lowest PGI was observed for BNBT-1 isolate i.e., 20.43, 39.21, 57.61 and 71.85 per cent growth inhibition on 3rd, 4th, 5th and 7th day, respectively. The result of PGI has been depicted in Table 3.

The results obtained are in accordance with the Choudhary *et al*. (2007). They assess the antagonist effect of native *Trichoderma* isolates against *F. oxysporum* pathogen in chilli and observed that the Th 011 isolate recorded lowest mycelial growth 28 mm with highest 69 per cent growth inhibition over control of *F. oxysporum* followed by Th 008 and Th 005 isolates.

Evaluation of native *Trichoderma* spp. isolates against chilli fusarial wilt in pot condition

The results observed during the examination *i.e.*, root length and shoot length of chilli crop, per cent disease incidence and disease control have been tabulated in the Table 4.

The results recorded for the root length stated that the significantly higher root length was (7.20 cm) obtained by the T_2 treatment where BVVT-1 isolate was inoculated and it was found to be at par with T_1 treatment (BVDT-1 isolate) recording 6.83 cm root length. The control where no inoculation of native *Trichoderma* isolates was done resulted in lowest root length of 3.23 cm. In comparison of the six isolates, the treatment T_6 showed poor result with only 4.67 cm root length where BNBT -1 isolate was used.

Viewing to the results of shoot length, the significantly highest 17.60 cm shoot length was found in the T_2 treatment where BVVT-1 isolate was used followed by 16.37 cm in T_1 treatment where BVDT-1 isolate and 16.07 cm in T_3 treatment (BJGT-2). The T_6 treatment where BNBT-1 isolate was used recorded the lowest shoot length of 13.20 cm among the six treatments where *Trichoderma* isolates inoculum was made.

The lowest disease incidence 16.67 per cent was recorded in T_2 treatment where BVVT-1 isolate was inoculated whiles the highest 36.67 per cent disease incidence was recorded in the T_6 were treatment BNBT-1 native *Trichoderma* isolate was inoculated.

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Data presented in Table 3 revealed significant differences in the result of disease control. It was noticed that the T_2 treatment in which BVVT-1 native *Trichoderma* isolate was used recorded the significantly higher diseases control percentage of 83.33 per cent which was found to be at par with the T_1 treatment where BVDT-1 native *Trichoderma* isolate was inoculated which recorded 80.00 per cent disease control. It was found that the least 63.33 per cent disease control was recorded in the T_6 treatment where BNBT-1 native *Trichoderma* isolate was used.

The results of the above experiment indicated are in accordance with Ommati and Zaker (2012). From the 14 *Trichoderma* isolates tested against the *Fusarium oxysporum* fungus, the 8 isolates showed antagonist effect from which (T_2) *F. oxysporum* + *T. asperellum* showed only 2.5 per cent disease incidence which was the lowest PDI while, in control (only *Fusarium* infested) highest 73 per cent disease incidence was observed. A similar result was obtained by pandya (2006). Who observed maximum growth inhibition by *T. viride* against *F. solani*.

Conclusion

The results of the present research work concluded that Fusarial wilt disease of chilli caused by *Fusarium* spp. is becoming major problem that for chilli growing farmers of Bharuch district. The result revealed that native *Trichoderma* spp. isolate BVVT-1 have shown potential antagonist effect against the *Fusarium* spp. causing chilli wilt under pot as well as *in vitro* condition. The *Trichoderma* spp. is effective biocontrol agent that parasitize the pathogen in short period and helps to suppress the growth of *Fusarium* spp. by inhibiting the action of pathogen as it favours the sustainable growth of chilli plant. It is eco-friendly bio-control agent which, avoid environmental risk. It helps to avoid the use of chemicals to manage this soil borne diseases as it works as replacement of the fungicides and can be suggested to the organic farmers. The bottom line of this experiment is that *Trichoderma* acts as one of the most powerful biocontrol agent, to overcome the serious problem of chilli wilt disease.

Table 3: Anti-fungal efficacy of native *Trichoderma* spp. isolates against most virulent *Fusarium* spp.

Sr. No.	Isolate	Radial growth (mm)				PGI (%)			
		3rd Day	4th Day	5th Day	7th Day	3rd Day	4th Day	5th Day	7th Day
1.	BVDT-1	18.67	24.00	25.67	23.67	39.78	52.94	68.31	73.70
2.	BVVT-1	16.33	19.67	23.33	15.33	47.31	61.17	71.19	82.96
3.	BJGT-2	20.33	26.33	27.67	18.33	34.41	48.36	65.84	79.63
4.	BJGT-1	22.00	27.33	28.33	21.67	29.03	47.05	65.02	75.93
5.	BJST-2	22.67	30.33	30.67	21.67	26.88	40.52	62.14	75.93
6.	BNBT-1	24.67	31.00	34.33	25.33	20.43	39.21	57.61	71.85
7.	Control	31.00	51.00	81.00	90.00	0.00	-	-	-
S.Em.±		0.35	0.50	0.87	0.87	-	-	-	-
CD at 5%		1.08	1.53	2.66	2.66	-	-	-	-
CV %		2.77	2.91	4.22	4.92	-	-	-	-

** average of three replication

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Table 4: Evaluation of native *Trichoderma* spp. isolates against chilli Fusarial wilt in pot condition

Table 4: Evaluation of native *Trichoderma* spp. isolates against chilli Fusarial wilt in pot condition

Treatments	Isolate (*Trichoderma* spp.)	Root Length (cm)	Shoot Length (cm)	Percent Disease Incidence (%)	Disease Control (%)
T_1	BVDT-1	6.83	16.37	26.57*(20.00)	63.43* (80.00)
T_2	BVVT-1	7.20	17.60	23.86*(16.67)	66.14* (83.33)
T_3	BJGT-2	6.63	16.07	28.78*(23.33)	61.22* (76.66)
T_4	BJGT-1	4.77	13.33	33.21*(30.00)	56.79* (70.00)
T_5	BJST-2	5.83	14.10	31.00*(26.67)	59.00* (73.33)
T_6	BNBT-1	4.67	13.20	37.22*(36.67)	52.78* (63.33)
T_7	Control	3.23	11.30	90* (100)	-
	S.Em.±	0.13	0.33	1.46	1.46
	CD at 5%	0.40	1.02	4.29	4.29
	CV %	4.05	3.97	9.32	7.02

* Figures in parentheses indicate arcsine transformed values **All values represent means of three replications

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