Plant Archives Vol. 24, Special Issue (GABELS), 2024 pp.306-314 e-ISSN:2581-6063 (online), ISSN:0972-5210

Plant Archives

Journal homepage: http://www.plantarchives.org
DOI Url : https://doi.org/10.51470/PLANTARCHIVES.2024.v24.SP-GABELS.044

ANTIFUNGAL ACTIVITY OF *TRICHODERMA ASPERELLUM* TRND14 AGAINST *FUSARIUM OXYSPORUM* F. SP. *CICERI*

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ABSTRACT

Chickpea (*Cicer arietinum* L.) is one of the most important rabi season legume crop belonging to family Fabaceae. The present study investigated the antifungal potential of *T. asperellum* (TrND14) against *F. oxysporum* f. sp. *ciceri* under in vitro and in vivo conditions. The antagonistic potential of *T. asperellum* TrND14 was tested in vitro by dual culture assay and found it effective in arresting the growth of *F. oxysporum* f.sp. *ciceri*. The root colonization of Trichoderma at 30 DAS was recorded. The colonization of Trichoderma with the roots of chickpea seedlings were observed in all the treatments except T7 (Tebuconazole (54% w/w FS) @4ml/10kg) and T8 (Control). Maximum root colonization of Trichoderma was observed in T6 (*T. asperellum* TrND14 @ 20g/kg). Also, the effect of *Trichoderma asperellum* TrND14 on wilt incidence of chickpea caused by *Fusarium oxysporum* f. sp. *ciceri* was tested using susceptible JG-62 under field condition in randomised block design. Chickpea seeds treated with Tebuconazole (54% w/w FS) @4ml/10 kg (T7) was found most effective to reduce incidence of chickpea wilt at 30, 60 and 90 DAS, respectively. Thus *T. asperellum* TrND14 can be used as a potential bio control agent for controlling the chickpea wilt pathogen.

Keywords: *Cicer arietinum, T. asperellum, F. oxysporum* f.sp. *ciceri,* Tebuconazole.

Introduction

Chickpea (*Cicer arietinum* L.) is an important pulse crop in India and ranks first in production and consumption in the world. Although, chickpea is predominantly consumed as a pulse, dry chickpea is also used in preparing a variety of foods, processed foods, sweets and condiments and green fresh chickpeas are commonly consumed as a vegetable (Wallace *et al*., 2016). In India, productivity of chickpea is very low as compared to other countries due to various biotic and abiotic factors. More than fifty soil and seed borne pathogens have been reported on this crop which causes significant crop losses. In Maharashtra during 2020-2021, the area of chickpea crop was 25943.4 ha while production was 28658.8 tonnes. Likewise in India the area of chickpea crop in India was 8422.2 ha while production was 10472.6 tonnes.

Chickpea wilt caused by *Fusarium oxysporum* f. sp. *ciceri* (Foc) is the most destructive disease in India. It is seed-borne as well as soil-borne pathogen (Pande, 2007). The wilt syndrome results in a rapid flaccidity and desiccation of the leaves and stems by 20 days after inoculation, whereas yellowing syndromes results in a progressive foliar yellowing followed by necrosis 30-40 days after introduction. The disease can affect the crop at any stage of growth. Characteristic symptoms are sudden drooping of leaves and petiole, no external rotting of roots and black internal discoloration involving xylem and pith (Dubey, 2001). The fungus survives in soil for at least 6 years (Haware *et al*., 1986). It is more prevalent in lower latitudes where growing season is relatively dryer and warmer than in the higher latitudes. It causes complete loss in grain yield if the disease occurs in the vegetative and reproductive stages of the crop (Haware and Nene, 1980; Haware *et al*., 1990). The disease manifests as mortality of young seedlings (within 25 to 30 days after sowing) to wilting or death of adult plants. The fungus Foc is a primarily soilborne pathogen, however, few reports indicated that it can be transmitted through seeds (Haware *et al*., 1978).

Trichoderma is used as a bio-agent and have been reported to be quite effective, inexpensive, and eco-friendly. It can be used as seed treatment, applied direct to the soil before planting and added to organic fertilizers. *Trichoderma* controls the pathogenic organism by competition, mycoparasitism and antagonism. It excretes enzymes like *viridin* and *gliotoxin* thereby enhancing the root growth. Hence, it has got significant importance in eco-friendly disease management programs (Komla *et al*., 2019). Hence the present study is mainly focused on the ecofriendly management of chickpea wilt pathogen using *Trichoderma asperellum* (TrND14) isolate both in vitro and under field conditions.

Materials and Methods

1. Collection and isolation of pure culture

The pure culture of *Trichoderma asperellum* isolate (TrND14) and *Fusarium oxysporum* f.sp. *ciceri*was procured from Department of Plant Pathology, Dr. PDKV, Akola.

2. Antagonistic activity of *Trichoderma asperellum* isolate (TrND14) against *Fusarium oxysporum* f.sp. *ciceri*

The antagonistic activity of *Trichoderma asperellum* isolate (TrND14) against *Fusarium oxysporum* f.sp. *ciceri* was done by dual culture technique. About 20 ml of potato dextrose media was poured into petridishes and allowed to cool down. The fungal mycelial disc (5 mm) was transferred to one end of the plate and fungal antagonist culture disc placed opposite to it leaving 5-6 mm distance from the periphery of the plates. Each treatment was replicated thrice. The inoculated plates were incubated at room temperature. After five days the observation was taken. The efficacy of *Trichoderma* isolates was expressed as percentage inhibition of mycelia growth over control. The per cent inhibition over control was calculated according to formula given by Vincent (1947) as follows :

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

Where,

I= Per cent inhibition

C= Radial growth (mm) in control

T= Radial growth (mm) in treatment.

3. Mass multiplication of *T. asperellum*

The grains of sorghum were taken for mass multiplication of *Trichoderma asperellum*. 200 g of sorghum grains were transferred in to 500 ml capacity conical flask and 250 ml distilled water was added. Sorghum grains will be soaked overnight. Then the flasks were autoclaved at 15 lbs for 30 min. After cooling the flask were inoculated with 7 days old fresh culture of the *T. asperellum* TrND14. The flasks were incubated at 28°C for 15 days. Then the grains along with the growth of *T. asperellum* was dried in shed and crushed into fine powder.

4. Preparation of talc-based formulation

The crushed growth of *Trichoderma* (TrND14) was added into talc powder to prepare talc-based formulation @ 2% WP. Also, CMC was added @ 0.5 g/kg and formulation was stored for further use. (Krishna and Kumar, 2013)

5. Mass multiplication of *Fusarium oxysporum* f. sp. *ciceri*

The pure culture of *Fusarium oxysporum* f. sp. *ciceri* was grown on sterilized sand maize medium in the ratio 9:1 (sand: broken maize). The flasks were incubated in the room temperature at 27±1^{o} C for 15 days. (Vijayashanthi, 2020).

6. Disease severity and root colonization study

Fusarium oxysporum f.sp. *ciceri* was used to tested against *Trichoderma*. The target pathogen was mass multiplied on sand maize medium. Once the culture had grown well, the sand maize medium was mixed along with the fungal growth in the plastic cups in the proportion recommended by Srivastav *et al*. (2002). The plastic cups (5-6 diameter) filled with soil and FYM (3:1) was used. In each cup the filling was done up to 3/4th level. The pathogen inoculum was mixed with sand was applied up to 2 cm depth in the plastic cups. The seeds of chickpea JG-62 were treated as per treatment details and was used for sowing in this plastic cups.

The key for grading efficacy was used as mentioned by Srivastav *et al*., 2002.

Table 1: Disease grading key

Sr. No.	Disease Incidence (%)	Rating of Bioefficacy of Bioagent
1	0	Highly efficient
2	1-15	Efficient
3	16-30	Moderately efficient
4	31-45	Moderately inefficient
5	46-60	Inefficient
6	Above 60	Highly inefficient

Root colonization assay

For root colonization assay, the plants were uprooted, and root bits were cut into 1 cm bits and randomly 25 bits were selected for each treatment. The

bits were plated on TSM, and percentage of bits root colonized was recorded**.**

7. Bioefficacy of *T. asperellum* TrND14 against wilt of Chickpea caused by *Fusarium oxysporum* f.sp. *ciceri.*

The study was performed under field condition. The chickpea wilt susceptible variety JG-62 was sown in rabi 2021 season and followed recommended standard agronomic practices. The experiment was conducted in randomized block design (RBD).

Wilt incidence was recorded after 30, 60 and 90 DAS (days after sowing). Based on infected and total number of plants, per cent disease incidence was calculated according to the following formula (Nirmalkar, 2017).

$$\text{Wilt incidence}\,(\%) = \frac{\text{Total no. of wilted plants}}{\text{Total no. of plants observed}} \times 100$$

8. Statistical analysis

Statistical analysis was done for the interpretation of the results obtained. Completely randomized design (CRD) (for *in vitro* experiments) and randomized block design (RBD) (for *in vivo* experiments) were employed to analyses the data. 'F' test of significance was used to know whether observed treatment effects were real or not from the data in which the treatment effects were significant. The standard error (SE) and critical difference (CD) at 5% level of probability were calculated. Values of critical difference were used to interpret the results. The data have been illustrated graphically at appropriate place in the text.

Result and Discussion

1. Collection and isolation of pure culture

The pure culture of *Trichoderma asperellum* TrND14 and *F. oxysporum* f.sp. *ciceri* were procured from Department of Plant Pathology, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola. (Plate: 1 and 2)

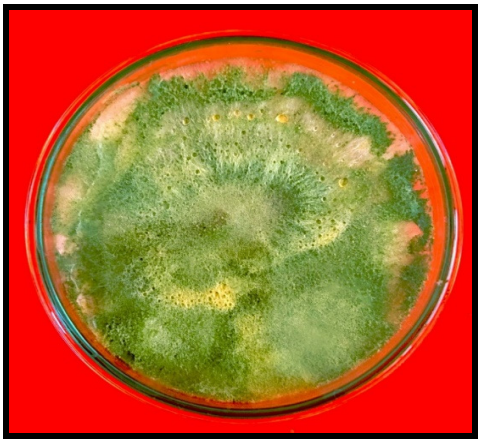


Plate 1: Pure culture of *Trichoderma asperellum* TrND14

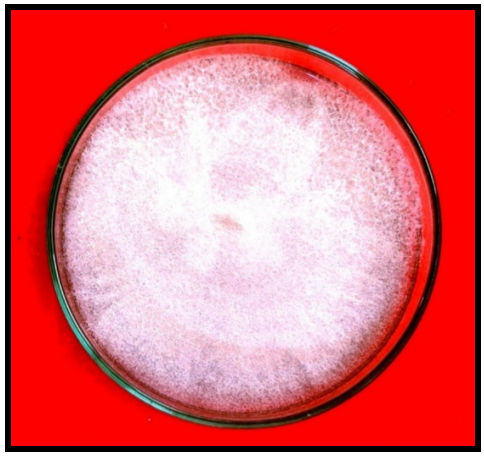
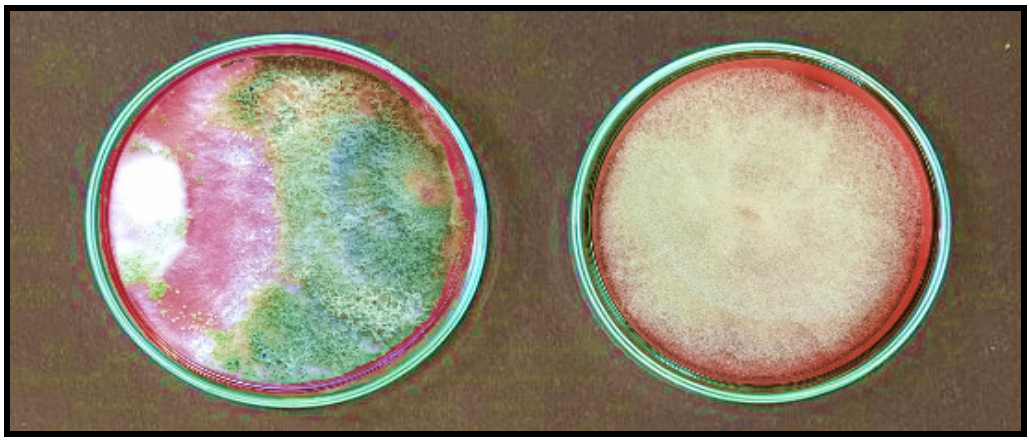


Plate 2: Pure culture of *Fusarium oxysporum* f.sp. *ciceri*

2. Antagonistic activity of *T. asperellum* TrND14 against *F. oxysporum* f. sp. *ciceri*.

The antagonistic activity of *Trichoderma asperellum* TrND14 against *Fusarium oxysporum* f. sp. *ciceri* was tested by dual culture technique. The experiment was conducted as per method suggested by Denis and Webster (1971) and the observations were recorded and it revealed that the *T. asperellum* TrND14 showed growth inhibition of pathogen up to 65.11% against *F. oxysporum* f.sp. *ciceri* as compared to control. **(Plate 3)**

The observations were in line with the findings of Nikam *et al*. (2007), who reported the combined effect of three *Trichoderma* spp. (*T. viride* + *T. harzianum* + 15 *T. hamatum*) was found to be most effective against *F. oxysporum* f. sp. *ciceri* thereby causing maximum inhibition (87. 77%) followed by individual isolates i.e. *T. viride* (83.33%), *T. harzianum* (76.66%) and *T. hamatum* (67. 77%). Also, Shrivastava and Agrawal (2010) reported that the antagonistic activity of *Fusarium oxysporum* f.sp. *ciceri* was inhibited by *Trichoderma harzianum* and *Trichoderma viride* under *in vitro* conditions and concluded that *Trichoderma viride* was most effective than *Trichoderma harzianum* in controlling the chickpea disease. Similarly, Korde (2011) studied the antagonistic potential of different biocontrol agent against *Fusarium oxysporum* f. sp. *lentis* and *Fusarium oxysporum* f. sp. *ciceri* and reported the highest inhibition by *Trichoderma viride* (58.82%), while Ranjitha and Mane (2014) reported inhibition of *Fusarium oxysporum* f. sp. *ciceri* by two fungal bioagents *T. viride* and *T. harzianum,* showing maximum per cent growth of inhibition by *T. harzianum* (76.66%) followed by *T. viride* (72.77%), respectively.



A - F. oxysporum f.sp. ciceri B - T. asperellum TrND14

Plate 3 : Antagonistic activity of *T. asperellum* TrND14 against *F. oxysporum* f.sp. *ciceri* by dual culture technique

3. Mass multiplication and preparation of talc-based formulation of *Trichoderma asperellum*.

The mass multiplication of *T. asperellum* TrND14 was carried out on sorghum grains in which 200 g of sorghum grains were transferred in to 500 ml capacity conical flask and 300 ml distilled water was added in it. Then the sorghum grains were soaked overnight, and excess water was drained out. The flasks were autoclaved at 15 lbs for 30 min and later were inoculated with seven days old culture of the *T. asperellum* TrND14. The flasks were incubated at 28 °C for 15 days and later the grains along with the growth of *T. asperellum* were dried in shed and crushed into fine powder.

The two grams of crushed growth of *Trichoderma* (TrND14) was added into 100 g talc powder to prepare 2% WP talc-based formulation. Also, CMC was added @ 0.5 g/100 g and formulation was stored for further use.

Mass multiplication of *F. oxysporum* f. sp. *ciceri*

Mass multiplication of *F. oxysporum* f. sp. *ciceri* was carried out on sand maize media, which was used to prepared wilt sick soil in earthen pots.

4. Root colonization of *T. asperellum*

The efficacy test of *Trichoderma asperellum* TrND14 against *Fusarium oxysporum* f. sp. *ciceri* was conducted under greenhouse condition**.** The seeds of chickpea susceptible variety (JG-62) were treated as per the seed treatment details and sown in pots containing sick soil (*Fusarium oxysporum* f. sp. *ciceri* infested soil). (Plate 3 and 4)

Table 2: Root colonization of *Trichoderma* with Chickpea.

Sr. No.	Treatment	Per cent root colonization (30 DAS)	PDI (30 DAS)
1	T1 - *T. asperellum* TrND14 (2% WP) @ 2g/kg	50.40	23.66*
2	T2 - *T. asperellum* TrND14 (2% WP) @ 4g/kg	60.80	18.66
3	T3 - *T. asperellum* TrND14 (2% WP) @ 8g/kg	73.40	14.33
4	T4 - *T. asperellum* TrND14 (2% WP) @ 12g/kg	81.60	13.33
5	T5 - *T. asperellum* TrND14 (2% WP) @ 16g/kg	82.80	13.33
6	T6 - *T. asperellum* TrND14 (2% WP) @ 20g/kg	86.60	8.00
7	T7 - Tebuconazole (54% w/w FS) @ 4ml/10 kg	0.00	11.66
8	T8 – Control	-	36.00
	F- test	-	Sig
	S.E (m) ±	-	0.41
	C.D. (P= 0.01)	-	1.25

*=average of 3 replications

The seedlings of the chickpea plants were observed periodically for the wilting symptoms. At 30 DAS these seedlings were uprooted for the root colonization study of *Trichoderma.* The roots of uprooted seedlings of chickpea were washed and surface sterilized with (0.1%) sodium hypochlorite (NaOCl) and were cut down into small bits.

Plate 3 : Bioefficacy of *T. asperellum* TrND14 against *F. oxysporum* f.sp. *ciceri*

Plate 4 : Root colonization assay

Which were further kept above the sterilized *Trichoderma* selective medium (TSM). The colonies of *Trichoderma* were observed after 3-4 days of incubation on TSM. The per cent root colonization by the *Trichoderma* and per cent wilt incidence at 30 DAS was recorded and graded according to the grading scale given by Srivastava *et al.* (2002).

The data presented in Table 2. (Plate 3 and 4) revealed that the colonization of *Trichoderma* with the roots of chickpea seedlings were recorded in all the treatments except T_7 andT_8. However, maximum root colonization of *Trichoderma* i.e. 86.60% was observed in T_6. The next best treatments were T_5 and T_4 that were at par with each other. Treatments T_1 andT_2 recorded 50.40 and 60.80% root colonization of *Trichoderma.*

The symptoms of wilt incidence were initiated on chickpea seedlings at 30 DAS under greenhouse condition. The data in Table 5 revealed that the root colonization by *Trichoderma* reduces the wilt incidence. The minimum disease incidence i.e. 8.00% was observed in T_6. The disease incidence in T_7(11.66%), T_5(13.33%), T_4 (13.33%), T_3(14.33%), T_2(18.66%)and T_1 (23.66%) was at par with each other. Maximum per cent wilt incidence was recorded in T_8 (control i.e. without seed treatment) i.e. 36.00%.

The above results are in conformity with findings of Akrami (2013) who studied the effect of *T. harzianum*, *T. asperellum* and *T. virens* against two pathogens of chickpea roots, *Fusarium solani* and *Fusarium oxysporum* alone or in combination under field and greenhouse conditions and revealed that *T. virens* showed least effect on roots infested with both *Fusarium* spp. Also, Yedidia *et al*, (2000) studied the interaction between the antagonistic fungus *Trichoderma harzianum* strain T-203 and cucumber roots and observed that *Trichoderma*'s association with roots reduce root disease through activation of the plants defence response.

5. Efficacy of *Trichoderma* against Chickpea wilt caused by *F. oxysporum* f.sp. *ciceri* under field conditions.

Efficacy of *Trichoderma asperellum* TrND14 against *Fusarium oxysporum* f. sp. *ciceri* was studied under field condition. The study was performed in rabi (2021) at Research field, Department of Plant Pathology, Dr. PDKV Akola **(Plate 5).** The wilt susceptible variety JG-62 of chickpea was taken for the experiment. The experiment was performed in randomized block design (RBD) with eight treatments and three replications and followed recommended standard agronomic practices. The symptoms of wilt disease incidence were observed early at 25 DAS. Based on infected and total number of plants, wilt disease incidence was recorded at 30, 60 and 90 DAS.

Result presented in **Table 3 and Fig 1,** indicates that at 30 DAS, all treatments except T_1 (*T. asperellum* TrND14 (2% WP) @ 2g/kg) were found significantly superior over control for reducing the incidence of chickpea wilt. Among treatments T_7 i.e. Tebuconazole (54% w/w FS) @4ml/10 kg was found most effective (8.67%) and which was found at par with T_6 (*T. asperellum* TrND14 @ 20g/kg) it recorded (9.33%). Other treatments i.e. T_5, T_4, T_3 and T_2 were also effective and at par with each other.

At 60 DAS, chickpea seeds treated with Tebuconazole (54% w/w FS) @4ml/10 kg (T_7) was found most effective to reduce incidence of wilt on chickpea i.e. 9.67% which was at par with T_6 (*T. asperellum* TrND14 @ 20g/kg) and T_5(*T. asperellum* TrND14 @ 16g/kg) it recorded wilt incidence 10.67% and 11.33% respectively. The wilt incidence observed in T_4 (11.67%), T_3 (13.00%), T_2 (13.67% and T_1 (15.33%) were found at par with each other. Maximum wilt incidence was recorded by T_8 (control) i.e. 19.00%.

At 90 DAS, treatment T_7 (Tebuconazole @4ml/10 kg) recorded the minimum wilt incidence which was at par with T_6 (*T. asperellum* TrND14 @ 20g/kg) and T_5 (*T. asperellum* TrND14 @ 16g/kg). Wilt incidence observed in T_4 (14.00%), and T_3(14.00%) were at par with each other.

Plate 5 : General view of experimental field.

(13.00%). T_1 recorded 18.33% wilt incidence which was at par with T_8 (control) which showed maximum incidence of wilt.

Table 3 : Effect of *T. asperellum* TrND14 on Chickpea wilt caused by *F. oxysporum* f. sp. *ciceri.*

Sr. No.	Treatment	PDI		
		30 DAS	60 DAS	90 DAS
1	T1 - *T. asperellum* TrND14 (2% WP) @ 2g/kg	14.33*	15.33*	18.33*
2	T2 - *T. asperellum* TrND14 (2% WP) @ 4g/kg	13.00	13.67	15.33
3	T3 - *T. asperellum* TrND14 (2% WP) @ 8g/kg	12.67	13.00	14.00
4	T4 –*T.asperellum* TrND14 (2% WP) @ 12g/kg	11.33	11.67	14.00
5	T5 - *T. asperellum* TrND14 (2% WP) @ 16g/ kg	10.67	11.33	12.67
6	T6 - *T. asperellum* TrND14 (2% WP) @ 20g/kg	9.33	10.67	12.00
7	T7 - Tebuconazole (54% w/w FS) @ 4ml/10 kg	8.67	9.67	10.67
8	T8 – Control	17.00	19.00	21.67
	F test	Sig.	Sig.	Sig.
	S.E (m) ±	1.03	1.65	1.32
	C.D. (P= 0.05)	3.11	5.01	4.01

*=average of 3 replications

Among the treatments containing different doses of *T. asperellum* TrND14, T_6 (*T. asperellum* TrND14 (2% WP) @ 20g/kg) was found most effective for controlling chickpea wilt caused by *Fusarium oxysporum* f. sp. *ciceri* at30, 60 and 90 DAS.

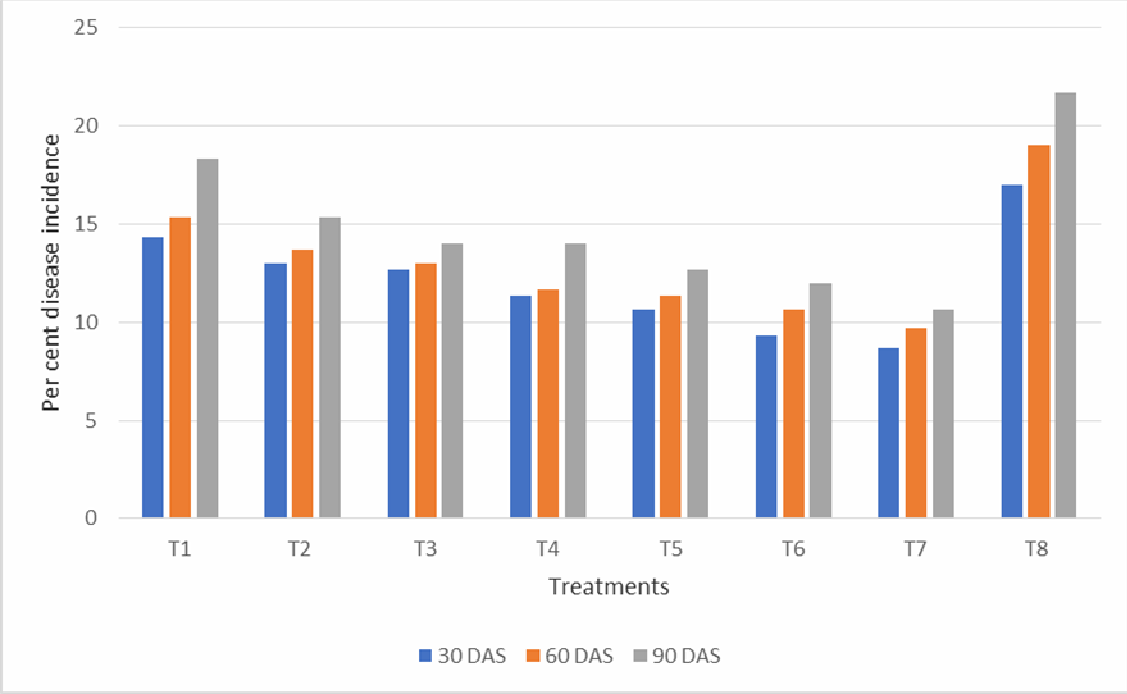


Fig. 1 : Wilt incidence of Chickpea at 30, 60 and 90 DAS

The above results are in lined with the findings of Zote *et al*. (2007) who tested the *Trichoderma viride* in combination with three fungicides (thiram, carbendazim and captan), four oil cakes and observed that soil /seed application of *T. viride* was the most effective recording the lowest wilt incidence (19.04 to 33.33%) with highest wilt reduction (66.67 to 80.86%) over the untreated control. Similarly, Simon and Anamika (2011) found *Trichodermaviride* as most effective against *Fusarium oxysporum* f.sp. *ciceri* and reduced the wilt disease intensity up to 70% in soil application followed by 66% in seed treatment and 49% in foliar application. Also, Kamdi *et al.* (2012) reported lowest disease incidence when chickpea seeds were treated with carbendazim (10.40%) followed by

Trichoderma viride (13.15%) over the control (28.20%).

Khan *et al*. (2014) reported the effects of *T. harzianum, T. hamatum, T. viride, T. polysporum* and *T. koningii* on the wilt disease complex of chickpea caused by *Fusarium oxysporum* f. sp. *ciceri.* Soil application of biocontrol agents (BCAs) checked the severity of wilt by 25%–56% and increased the yield of chickpea by 12%–28%. Also, Nirmalkar (2017) showed that seed treated with *Trichoderma harzianum* + *Rhizobium* @10g/kgalong with soil application of *T. harzianum* enriched FYM @250kg/ha was found most effective to reduce the incidence of wilt complex of chickpea i.e. 7.25%.

Conclusion

The above concluded that**,** *T. asperellum* TrND14 found antagonistic against the *F. oxysporum* f.sp. *ciceri* under in vitro. The maximum root colonization of Trichoderma with minimum disease incidence was observed when seed treated with *T. asperellum* TrND14 @ 20g/kg (86.66%). Maximum seed germination (82.72%) and seedling vigour index (2373.23) were also recorded in T6 (*T. asperellum* TrND14 @ 20g/kg) which was at par with T5 (*T. asperellum* TrND14 @ 16g/kg).The chickpea seeds treated with Tebuconazole (54% w/w FS) @4ml/10 kg (T7) was found most effective for reducing the wilt incidence at 30, 60 and 90 DAS. Among the seed treatments of *T. asperellum* TrND14, T6 (20g/kg) and T6 (16g/kg) were found effective for reducing wilt incidence caused by *Fusarium oxysporum* f. sp. *ciceri* and recorded maximum seed yield of chickpea.

Acknowledgement

We are thankful to all staff of Department of Plant Pathology, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola for their valuable support to do my research work completely. Also special thanks to my research guide and all PhD seniors who guided me in writing this manuscript.

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