International Journal of Agriculture Sciences
ISSN: 0975-3710&E-ISSN: 0975-9107, Volume 10, Issue 8, 2018, pp.-5880-5884.
Available online at https://www.bioinfopublication.org/jouarchive.php?opt=&jouid=BPJ0000217

Research Article

BIOCONTROL EFFICACY OF *Trichoderma* ISOLATES AGAINST *Sclerotium rolfsii* CAUSING COLLAR ROT DISEASE IN CHICKPEA

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Received: April 20, 2018; Revised: April 26, 2018; Accepted: April 27, 2018; Published: April 30, 2018

Abstract- Chickpea is an important cool season food crop grown mainly in dry land. The crop suffers from serious diseases which affect it in all growth stages. The pathogens that affect chickpea include fungi, bacteria, viruses and mycoplasma. Among them collar rot caused by *Sclerotium rolfsii* is an important fungal pathogen in chickpea root disease complex causing serious losses. To find the biocontrol potential of *Trichoderma* isolates applied through seed and soil application methods, pot culture experiment was conducted. In pot culture, isolate Th4 (64.4%), T22 (60.2%), Tckp (60.2%), T14 (56.0%), T15 (56.0%), Th2 (52.3%), Th3 (52.3%) and Trice (51.4%) gave more than 50% control of chickpea collar rot when applied to soil. Isolate T12 (63.4%), Th4 (60.2%), Tv3 (52.3 %), T2 (51.4%) and Trice (51.4%) gave more than 50% disease control when applied as seed treatment.

Keywords- Chickpea, *Sclerotium rolfsii*, biocontrol potential, *Trichoderma*

Citation: Swathi B., *et al.,* (2018) Biocontrol Efficacy of *Trichoderma* Isolates Against *Sclerotium rolfsii* Causing Collar Rot Disease in Chickpea. International Journal of Agriculture Sciences, ISSN: 0975-3710 & E-ISSN: 0975-9107, Volume 10, Issue 8, pp.-5880-5884.

Academic Editor / Reviewer: Dr B P Bhaskar

Introduction

The Chickpea (*Cicer arietinum* L.) is one of the major pulse crops grown throughout the world and is an important legume crop of dry lands of South East and Western Asia, Northern and Eastern Africa and, Central and Southern America. It is one of the important pulse crop of India contributing about 30% to total pulse acreage and about 40% to total pulse production. It is mostly cultivated under rainfed condition in a variety of soils varying in residual moisture. In Andhra Pradesh, chickpea is cropped in an area of 3.92 Lakh ha producing 4.34 Lakh tones [1]. Among various factors attributed to the low productivity of chickpea, susceptibility to diseases is the most important. A serious threat to chickpea production potential is due to cause of diseases especially, soil borne plant pathogenic fungi *viz*., *Macrophomina phaseolina, Sclerotium rolfsii, Fusarium solani, Rhizoctonia solani* and *Pythium spp*. Among them, special focus is kept back on collar rot caused by *S. rolfsii* which causes considerable loss to plant stand when soil moisture is high and temperature are warmer (30^0C) at sowing time. *S. rolfsii* is an economically important pathogen with extensive host range of at least 500 species in 100 families. The most common hosts are legumes, crucifers and cucurbits, and commonly occur in the tropics, subtropics, and other warm temperate regions [2]. Chemical means of managing the diseases caused by this soil borne plant pathogenic fungi are not practicable owing to high cost of chemical inputs besides causing environmental pollution and resistance development in pathogenic fungi [3]. Biological control of plant pathogens by the addition of antagonistic microorganisms to the soil or seed has become a powerful tool because of fast developing resistance in fungal pathogens against site specific fungicides and advancement in fermentation and genetic manipulation technologies [4]. Among the successful bioagents, *Trichoderma spp*. is being extensively used to manage several soils borne plant pathogens. Though several species of *Trichoderma* are found to be effective in managing plant diseases, isolate variation existed in their antagonistic efficacy [5]. Rhizosphere competence is important because a biocontrol agent cannot compete for space and nutrients if it is unable to grow in the rhizosphere. When applied either to seed or soil, *T. harzianum* remain in soil, multiplies and offers protection to the plant roots and multiplies along with the developing root system by improving the biological soil suppressive. Further, seed treatment or soil application of the antagonist may offer a scope to mitigate losses due to *S. rolfsii.*

Material and Methods

Collar rot susceptible chickpea variety, Annegiri was used as test variety. Test pathogen *Sclerotium rolfsii* was isolated from collar rot affected chickpea plants in College Farm, Agricultural College, Bapatla and twenty-five test antagonistic isolates of *Trichoderma* spp. available in the Department of Plant Pathology, Agricultural College, Bapatla were used in the present investigation.

Mass Culture of Test Fungi: For mass multiplication of the test pathogenic fungus *S. rolfsii*, 50g of sorghum grains were kept in 250 ml conical flask, soaked overnight and later 2% dextrose solution was added and sterilized consecutively for two days at 15 psi pressure for 15 min and 121.6^0C in an autoclave. These autoclaved sorghum grain flasks were allowed to cool, inoculated with the test pathogen under aseptic conditions and incubated at 29 ± 1^0C for 10 days. The flasks were shaken on alternate days for uniform colonization on sorghum grains by the inoculated fungus. The sorghum grains completely covered with fungal growth were used as inoculum [Plate-1]. Seed treatment and soil application were two different application methods used to assess the biocontrol potential in decreasing the collar rot of chickpea caused by *S. rolfsii*.

Plate-1 Mass cultured *Sclerotium rolfsii* on sterilized sorghum grains

Twenty-day old sorghum grain culture of *S. rolfsii* was inoculated @6 g/pot of the 6 kg soil pot in the top two inches soil and watered sufficiently.

Seed treatment of *Trichoderma*: Spores of individual *Trichoderma* isolate were harvested from 7day old culture plate. The spore concentration was estimated using haemocytometer and adjusted to 108 spores/ml using serial dilution method. Seeds were treated individually with these spore suspensions using carboxy methyl cellulose (CMC) as base. Such treated seeds were sown in pots @ 10 seeds/pot 24 h after pathogen inoculation.

Soil application of *Trichoderma*: Individual *Trichoderma* isolate mass multiplied on sorghum grain was mixed in the soil 24 h after pathogen application @ 10 g per pot. Seeds were sown in pots @10 seeds/pot. Pathogen inoculated and uninoculated pots were maintained as checks. Observations were recorded on the number of seeds germinated, plant stand and disease incidence. The following formulae were used to assess treatment effects in pot culture.

$$\text{Plant stand (\%)} = \frac{\text{Number of plants in treatment}}{\text{Plant stand in absolute check}} \times 100$$

$$\text{Mortality (\%)} = \frac{\text{Number of plants in absolute check} - \text{Number of plants in treatment}}{\text{Number of plants in absolute check}} \times 100$$

$$\text{Disease Control (\%)} = \frac{\text{Mortality \% in pathogen inoculated check} - \text{Mortality \% in treatment}}{\text{Mortality \% in pathogen inoculated check}} \times 100$$

Result and Discussion

To find the biocontrol potential of *Trichoderma* isolates applied through seed and soil application methods, pot culture experiment was conducted. Pot pre-inoculated with sorghum grain culture of *S. rolfsii* @6 g/6 kg soil pot and *Trichoderma* applied through seed (108 spores/ ml) or soil (10 g/pot).

Effect of Soil Application of *Trichoderma* Isolates on Collar Rot of Chickpea

In pathogen inoculated check, germination percent was 40.0% whereas plant stand at 30 DAS was only 16.7% equivalent to 83.3% disease incidence [Table-1]. In comparison with pathogen inoculated check, all the treatments showed significantly higher germination percent and plant stand at 30 DAS. However, all the treatments involving *Trichoderma* soil application showed significantly superior germination % and superior plant stand (except T3, T4, T8 and Tv3) compared to pathogen inoculated check. Soil application with *Trichoderma* isolate T14 resulted in significantly increased germination percent (80.0%) compared to other isolates in the presence of *S. rolfsii* and was significantly higher compared to pathogen check (40.0%). However, soil application with Th4 (76.7%), T2 (73.3%), T12 (73.3%), T22 (73.3%), Tckp (73.3%), T4 (70.0%), T15 (70.0%) and Th3 (70.0%) isolates did not differ significantly with each other when germination percent was assessed besides being on par with T14. In pathogen inoculated check, plant stand was only 16.7% compared to pathogen uninoculated check (100%) at 30 DAS. The mortality percent in pathogen inoculated check was 83.3%. Soil application of *Trichoderma* isolates resulted in increased plant stand when compared to pathogen inoculated check (16.7%). When percent plant stand was observed, highest plant stand was observed in isolate Th4 (70.0%) in the presence of pathogen and was on par with T22 (66.7%), Tckp (66.7%), T14 (63.3%), T15 (63.3%), Th2 (60.0%), Th3 (60.0%), Trice (60.0%), T12 (53.3%), Tl2 (53.3%) and Th5 (53.3%) isolates. The lowest percent plant stand was noticed in isolates T3, T4, T8 and Tv3 (30.0%) which was on par with pathogen inoculated check (16.7%). In other words, percent mortality of Th4 isolate was only 30.0% which has significant difference compared to pathogen uninoculated check (83.3%). When percent disease control was estimated, the isolate Th4 was found to offer better disease control (64.4%) against *S. rolfsii*. Isolate Th4 (64.4%), T22 (60.2%), Tckp (60.2%), T14 (56.0%), T15 (56.0%), Th2 (52.3%), Th3 (52.3%) and Trice (51.4%) gave more than 50% disease control when applied as soil application [Fig-1]. Significant control of *S. rolfsii* in groundnut by the application of *T. harzianum* granules to the soil was reported by Elad, *et al*., (1980) [6]. Soil application of bioagents can also quickly manage the disease as these being natural soil inhabitants, establish and multiply more quickly in soil. Reduction in *S. rolfsii* growth when *T. harzianum* applied both as soil and bulb treatment was observed by Chet, *et al*., (1982) [7]. The efficacy of *T. harzianum* Rifai as soil application against sclerotial wilt of groundnut was evaluated by Patibanda, et al., (2002). Disease reduction was maximum (92.6%) when *T. harzianum* was applied @10 g/kg soil. Efficacy of *Trichoderma* in decreasing *S. rolfsii* through soil application method was reported by Dutta and Das, (2002) and Jegathambigai, *et al*., (2010) [8,9]. Application of *Trichoderma* strains successfully decreases the stem rot incidence and also increases the growth of the chickpea plants was observed by Nagamma and Nagaraja, (2015) [10]. Application of *Trichoderma* in every cropping was effective in lowering the population of the pathogen's propagules, the sclerotia, and its effect was long-lasting [11]. Under greenhouse conditions, the application of *Trichoderma harzianum* (ANR-1) exhibited the least wilt incidence in tomato [12].

Effect of Seed Treatment of *Trichoderma* Isolates on Collar Rot of Chickpea

In pathogen inoculated check, germination percent was 40.0% whereas plant stand at 30 DAS was only 16.7% equivalent to 83.3% disease incidence [Table-2]. In comparison with pathogen inoculated check, all the treatments showed significantly higher germination percent and plant stand at 30 DAS. However, all the treatments involving *Trichoderma* seed treatment showed significantly superior germination %, except Tps1 (50.0%) and superior plant stand (except T18, Tps1, T3, Tmaruteru and Tmachli) compared to pathogen inoculated check. Seed treatment with *Trichoderma* isolate T12 resulted in significantly increased germination percent (76.7%) compared to other isolates in the presence of *S. rolfsii* which was significantly higher compared to pathogen check (40.0%). However, seed treatment with T2 (73.3%), Th4 (73.3%), T8 (70.0%) and Tckp (70.0%) isolates did not differ significantly with each other when germination percent was assessed and were on par with T12. The lowest germination percent was observed with Tps1 (50.0%) isolate. Thirty days after sowing in pathogen inoculated check, plant stand was only 16.7% compared to pathogen uninoculated check (100%). In other words, the mortality percent in pathogen inoculated check was 83.3%. When percent plant stand was observed, 70.0% plant stand was noticed in isolate T12 in the presence of pathogen, but significantly differed from other isolates but it was on par with Th4 (66.7%), T2 (60.0%), Tv3 (60.0%), Trice (60.0%), Tckp (56.7%), T8 (53.3%), Th3 (53.3%) and Tpudivada (53.3%) isolates. The lowest percent plant stand was noticed in isolates T18 (20.0%), Tps1 (20.0%), Tmaruteru (30.0%) and Tmachli (30.0%) which were on par with pathogen inoculated check (16.7%).

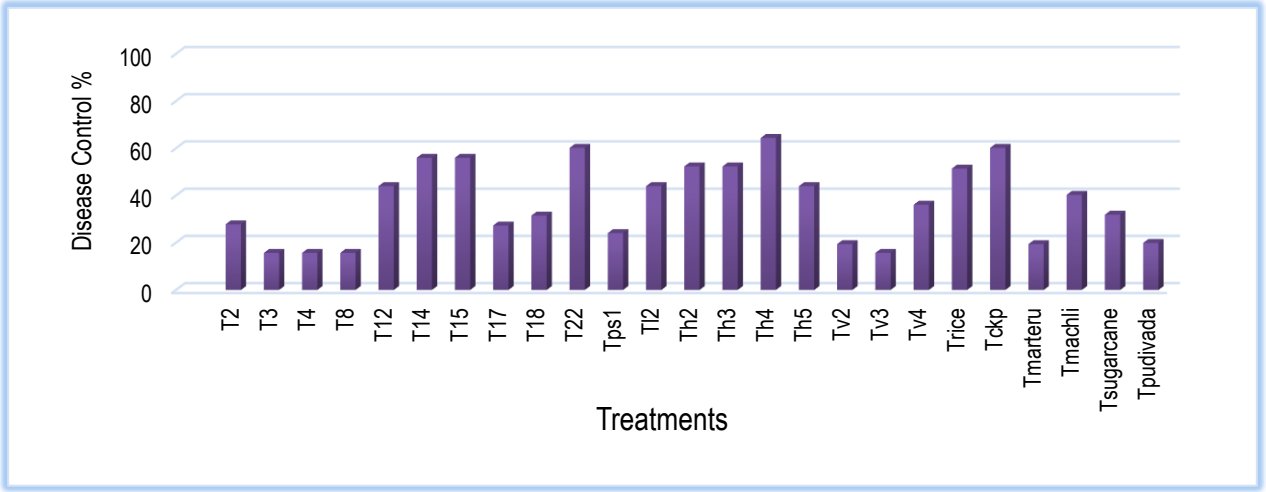

Fig-1 Effect of soil application of *Trichoderma* isolates on chickpea collar rot

Table-1 *Effect of soil application of Trichoderma isolates on the incidence of chickpea collar rot*

Treatments	Actual germination	Germination (%)	Actual Plant stand	Plant stand (%)	Plant mortality (%)	Disease reduction over control (%)
T2	7.3	73.3 (59.0)^{bcd}	4.0	40.0 (39.2)^{fgh}	60.0	27.8 (31.7)^{defgh}
T3	6.7	66.7 (54.8)^{cde}	3.0	30.0 (33.2)^{hi}	70.0	15.7 (23.2)^{gh}
T4	7.0	70.0 (56.8)^{bcde}	3.0	30.0 (33.2)^{hi}	70.0	15.7 (23.2)^{gh}
T8	6.7	66.7 (54.8)^{cde}	3.0	30.0 (33.2)^{hi}	70.0	15.7 (23.2)^{gh}
T12	7.3	73.3 (59.0)^{bcd}	5.3	53.3 (46.9)^{bcdef}	46.7	44.0 (41.5)^{abcdef}
T14	8.0	80.0 (63.4)^{b}	6.3	63.3 (52.8)^{bcd}	36.7	56.0 (48.5)^{abc}
T15	7.0	70.0 (56.8)^{bcde}	6.3	63.3 (52.8)^{bcd}	36.7	56.0 (48.5)^{abc}
T17	6.3	63.3 (52.8)^{de}	4.0	40.0 (39.2)^{fgh}	60.0	27.3 (30.8)^{defgh}
T18	6.3	63.3 (52.8)^{de}	4.3	43.3 (41.1)^{efgh}	56.7	31.5 (33.4)^{cdefgh}
T22	7.3	73.3 (59.0)^{bcd}	6.7	66.7 (55.0)^{bc}	33.3	60.2 (51.0)^{ab}
Tps1	6.3	63.3 (52.8)^{de}	3.7	36.7 (37.2)^{fgh}	63.3	24.1 (29.4)^{efgh}
Tl2	6.3	63.3 (52.8)^{de}	5.3	53.3 (46.9)^{bcdef}	46.7	44.0 (41.5)^{abcdef}
Th2	6.7	66.7 (55.0)^{cde}	6.0	60.0 (50.8)^{bcde}	40.0	52.3 (46.3)^{abcd}
Th3	7.0	70.0 (56.8)^{bcde}	6.0	60.0 (50.8)^{bcd}e	40.0	52.3 (46.3)^{abcd}
Th4	7.7	76.7 (61.2)^{bc}	7.0	70.0 (57.0)^{b}	30.0	64.4 (53.5)^{a}
Th5	6.7	66.7 (55.0)^{cde}	5.3	53.3 (47.0)^{bcdef}	46.7	44.0 (41.3)^{abcdef}
Tv2	6.0	60.0 (50.7)^{e}	3.3	33.3 (35.2)^{gh}	66.7	19.4 (25.5)^{fgh}
Tv3	6.0	60.0 (50.7)^{e}	3.0	30.0 (33.0)^{hi}	70.0	15.7 (19.4)^{h}
Tv4	6.0	60.0 (50.7)^{e}	4.7	46.7 (43.1)^{defgh}	53.3	36.1 (36.7)^{bcdefg}
Trice	6.7	66.7 (55.0)^{cde}	6.0	60.0 (50.8)^{bcde}	40.0	51.4 (45.8)^{abcd}
Tckp	7.3	73.3 (59.0)^{bcd}	6.7	66.7 (55.0)^{bc}	33.3	60.2 (51.0)^{ab}
Tmarteru	6.0	60.0 (50.7)^{e}	3.3	33.3 (35.2)^{gh}	66.7	19.4 (25.5)^{fgh}
Tmachli	6.3	63.3 (52.8)^{de}	5.0	50.0 (45.0)^{cdefg}	50.0	40.3 (39.3)^{abcdef}
Tsugarcane	6.0	60.0 (50.7)^{e}	4.3	43.3 (41.1)^{efgh}	56.7	31.9 (34.3)^{cdefgh}
Tpudivada	6.0	60.0 (50.7)^{e}	3.3	33.3 (35.2)^{gh}	66.7	19.9 (26.3)^{fgh}
Pathogen check	4.0	40.0 (39.2)^{f}	1.7	16.7 (23.8)^{i}	83.3	
Uninoculated Check	10.0	100.0 (90.0)^{a}	10.0	100.0 (90.0)^{a}		
SEm±		2.1		2.8		4.2
CD (P=0.01)		8.0		10.9		16.0
CV (%)		8.3		11.2		19.8

Values in parenthesis are arc sine transformed values, Each pot was sown with 10 chickpea seeds of cv. Annegiri, Germination was recorded 8 DAS, Plant stand was recorded 30 DAS

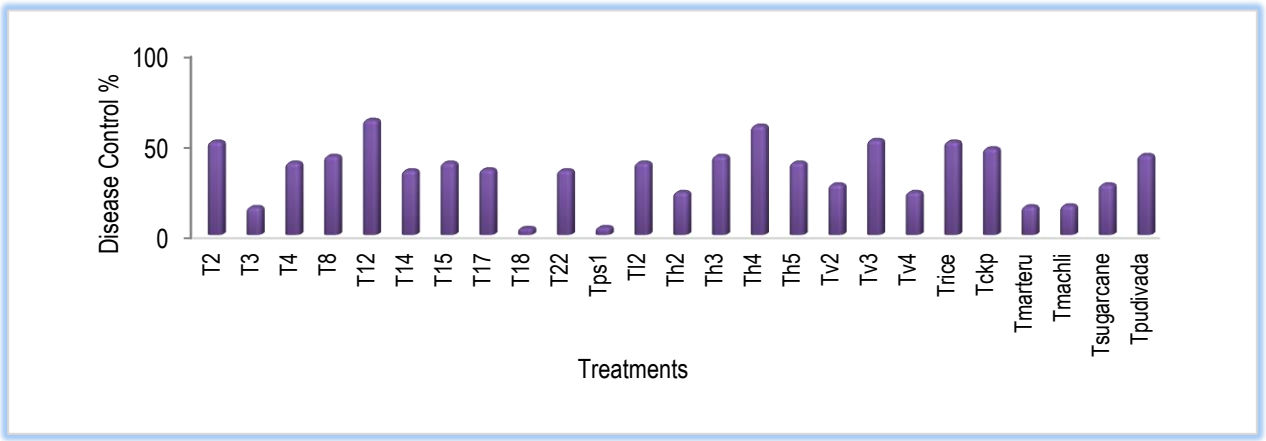

Fig-2 Effect of seed treatment of *Trichoderma* isolates on chickpea collar rot

Table-2 Effect of seed treatment of *Trichoderma* isolates on the incidence of chickpea collar rot

Treatments	Actual germination	Germination (%)	Actual Plant stand	Plant stand (%)	Plant mortality (%)	Disease reduction over control (%)
T2	7.3	73.3 (59.0)^{bc}	6.0	60.0 (50.8)^{bcd}	40.0	51.4 (45.8)^{ab}
T3	6.0	60.0 (50.7)^{efg}	3.0	30.0 (33.0)^{ghi}	70.0	15.3 (18.6)^{de}
T4	6.3	63.3 (52.8)^{def}	5.0	50.0 (45.0)^{cdef}	50.0	39.8 (38.9)^{abc}
T8	7.0	70.0 (57.0)^{bcd}	5.3	53.3 (46.9)^{bcdef}	46.7	43.5 (41.2)^{abc}
T12	7.7	76.7 (61.2)^{b}	7.0	70.0 (57.0)^{b}	30.0	63.4 (53.0)^{a}
T14	6.3	63.3 (52.8)^{def}	4.7	46.7 (43.1)^{defg}	53.3	35.6 (36.5)^{abcd}
T15	6.0	60.0 (50.7)^{efg}	5.0	50.0 (45.0)^{cdef}	50.0	39.8 (39.1)^{abc}
T17	6.0	60.0 (50.7)^{efg}	4.7	46.7 (43.1)^{defg}	53.3	36.1 (36.7)^{abcd}
T18	5.7	56.7 (48.8)^{fg}	2.0	20.0 (26.6)^{hi}	80.0	3.7 (6.5)^{e}
T22	5.7	56.7 (48.8)^{fg}	4.7	46.7 (43.1)^{defg}	53.3	35.6 (36.5)^{abcd}
Tps1	5.0	50.0 (45.0)^{gh}	2.0	20.0 (26.6)^{hi}	80.0	4.2 (6.9)^{e}
Tl2	5.7	56.7 (48.8)^{fg}	5.0	50.0 (45.0)^{cdef}	50.0	39.8 (38.9)^{abc}
Th2	5.7	56.7 (48.8)^{fg}	3.7	36.7 (37.2)^{fgh}	63.3	23.6 (28.6)^{bcd}
Th3	6.3	63.3 (52.8)^{def}	5.3	53.3 (46.9)^{bcdef}	46.7	43.5 (41.2)^{abc}
Th4	7.3	73.3 (59.0)^{bc}	6.7	66.7 (54.8)^{bc}	33.3	60.2 (50.9)^{a}
Th5	6.0	60.0 (50.7)^{efg}	5.0	50.0 (45.0)^{cdef}	50.0	39.8 (38.9)^{abc}
Tv2	6.0	60.0 (50.7)^{efg}	4.0	40.0 (39.1)^{efg}	60.0	27.8 (31.2)^{bcd}
Tv3	6.3	63.3 (52.8)^{def}	6.0	60.0 (50.7)^{bcd}	40.0	52.3 (46.3)^{ab}
Tv4	6.0	60.0 (50.7)^{efg}	3.7	36.7 (37.2)^{fgh}	63.3	23.6 (28.6)^{bcd}
Trice	6.7	66.7 (54.8)^{cde}	6.0	60.0 (50.7)^{bcd}	40.0	51.4 (45.8)^{ab}
Tckp	7.0	70.0 (57.0)^{bcd}	5.7	56.7 (48.8)^{bcde}	43.3	47.7 (43.6)^{ab}
Tmarteru	6.3	63.3 (52.8)^{def}	3.0	30.0 (33.0)^{ghi}	70.0	15.7 (19.4)^{de}
Tmachli	6.3	63.3 (52.8)^{def}	3.0	30.0 (33.0)^{ghi}	70.0	16.2 (23.4)^{cde}
Tsugarcane	6.3	63.3 (52.8)^{def}	4.0	40.0 (39.2)^{efg}	60.0	27.8 (31.7)^{bcd}
Tpudivada	5.3	53.3 (46.9)^{fg}	5.3	53.3 (46.9)^{bcdef}	46.7	44.0 (41.5)^{abc}
Pathogen check	4.0	40.0 (39.2)^{h}	1.7	16.7 (23.8)^{i}	83.3	
Uninoculated Check	10.0	100.0 (90.0)^{a}	10.0	100.0 (90.0)^{a}		
SEm±		1.6		2.8		4.9
CD (P=0.01)		5.9		10.6		18.6
CV (%)		5.1		11.0		24.3

Values in parenthesis are arc sine transformed values, Each pot was sown with 10 chickpea seeds of cv.Annegiri, Germination was recorded 8 DAS, Plant stand was recorded 30 DAS

In other words, percent mortality of T12 isolate was 30.0% which is significantly superior compared to pathogen inoculated check (83.3%). When percent disease control was estimated based on percent plant stand, the isolate T12 was found to be best in controlling chickpea collar rot disease (63.4%). Isolate T12 (63.4%), Th4 (60.2%), Tv3 (52.3%), T2 (51.4%) and Trice (51.4%) gave more than 50% disease control when applied as seed treatment [Fig-2]. Biological seed treatment was found to be an efficient method for protecting crops against various diseases [13] and provide enhanced plant growth. Biological seed treatments are more economical as compared to other field application systems and easy to adapt. Biological control of *S. rolfsii* causing collar rot of lentil was evaluated by Agarwal, *et al.*, (1977) [14] reported that, *T. harzianum* was antagonistic to *S. rolfsii* and was more reduction in seedling mortality was observed and effective when applied to seed rather than to the soil. While seed pelleting test was carried out by Prasad, *et al*., (1999) [15] with *T. harzianum* and *T. viride* against *S. rolfsii* which was found to be effective in increasing the germination percent (90-95%) compared to inoculated control (55%). Isolates of *Trichoderma* evaluated in in vivo conditions through seed coating method antagonistic to *S. rolfsii* were reported by several workers [16-18]. While working on Rhizosphere competence, [19] opined that Rhizosphere competence is important because a biocontrol agent cannot compete for space and nutrients if it is unable to grow in the rhizosphere. When applied either to seed or soil, *T. harzianum* remain in soil, multiplies and offers protection to the plant roots. The effectiveness of *Trichoderma* as seed treatment is probably determined not only by their biocontrol qualities but also by their abilities to multiply in the rhizosphere when applied to seed. The success of biocontrol agent depends on its ability to produce inoculum in excess, grow and proliferate well on the plant parts wherever applied. It is widely known that environmental parameters such as abiotic (soil type, soil temperature, soil pH and water potential) and biotic (plant species and variety, microbial activity of the soil) factors as well as method and timing of applications may have influence on the biological control efficacy of *Trichoderma* isolates [20]. *Trichoderma* isolates significantly reduced the amount of diseased bean plants under greenhouse conditions [21].

Application of research: Biological control is an effective, eco-friendly and alternative approach for management of any disease. Research work and application of Biocontrol agents should be carried out continuously for a long period of time in farm fields to achieve best results. The success of biocontrol agent depends on its ability to produce inoculum in excess, grow and proliferate well on the plant parts wherever applied.

Research Category: Biological control, disease management through eco-friendly products.

Acknowledgement / Funding: Author thankful to Acharya N. G. Ranga Agricultural University, Guntur, 522034, Andhra Pradesh

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Research project name or number: PhD Thesis

Author Contributions: All author equally contributed

Author statement: All authors read, reviewed, agree and approved the final manuscript

Conflict of Interest: None declared
Ethical approval: This article does not contain any studies with human participants or animals performed by any of the authors.

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