Int.J.Curr.Microbiol.App.Sci (2020) *9*(8): 2472-2484

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 9 Number 8 (2020)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2020.908.282

Comparative Study on Biocontrol Potential of Local Isolates with Commercial Formulations of *Trichoderma harzianum* for the Management of Collar Rot of Chickpea Caused by *Sclerotium rolfsii* Sacc.

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Keywords

Chickpea, Collar rot, *Sclerotium rolfsii*, *Trichoderma harzianum* and Vermicompost

Article Info

Accepted:
22 July 2020
Available Online:
10 August 2020

A B S T R A C T

Collar rot disease is a major constraint in chickpea production. Comparative efficacy of local isolates and commercial formulations of *Trichoderma harzianum* were evaluated in lab and field against collar rot of chickpea caused by *Sclerotium rolfsii* during 2018-2019. Tested local isolates and commercial formulations significantly inhibited mycelial growth of *S. rolfsii in-vitro* were evaluated in field condition as seed treatment @8gm/kg seed (cfu $1x10^8$/gm) & soil treatment @ 5kg/ha (cfu $1x10^8$/gm) with 100kg vermicompost prior to sowing and recorded the germination percentage, shoot length, root length, nodulation/plant, disease incidence and yield/ha. Maximum seed germination (88%), nodulation (44/plant), pod (306/plant) and highest yield (21.66 q/h) was recorded in soil treated with local *T. harzianum* isolate CRC and minimum seed germination (77.33%), nodulation (20/plant) in seed treated with *T. harzianum* (Bioharz) and lowest yield (15.83q/h) was observed in soil treated with *T. harzianum (Bioharz).* Where as Maximum shoot length (55.33cm) & root length (24.33cm) was observed in seed treated with local *T. hargianum* isolate CRC and minimum shoot length (37.33cm) & root length (13.33cm) seed treated with local *T. harzianum* isolate KVK Hastinapur. Minimum disease incidence (3.57%) was found soil treated with *T. harzianum* isolate CRC multiplied in vermicmpost and maximum disease incidence (11.85%) soil treated with *T. harzianum* commercial formulation (Bioharz). However, local isolates as well as commercial formulation of *T. harzianum*) decreased disease incidence and increased pod yield comparison to control.

Introduction

Chickpea is an important and major pulse crop throughout the world including India. Chickpea is a good source of protein for majority of population and used to feed animals. Chickpea is a good source of nutrition among dry edible grain legumes.

Chickpea seeds contain 17-22% highly digestible protein, 60.8% total carbohydrates, 2.70-6.48% fat (primarily linoleic and oleic acids), 5% crude fibre, 6% soluble sugar and 3% ash (Williams and Singh, 1987). It holds 75 percent production among pulses. There are two types of chickpea cultivated Viz. Desi and Kabuli types. Of them, 85 per cent area occupies Desi types while and remaining area covered by Kabuli types. In India, major chickpea growing states are Madhya Pradesh (MP), Maharashtra, Rajasthan, Uttar Pradesh, Karnataka and Andhra Pradesh and all contributes collectively up to 90 per cent area and 91 per cent production in the country (Singh, 2010). Chickpea covers cultivated area of 105.73 lakh hectares with production of 111.18 lakh tons with productivity level of 1056 kg/ha (Anonymous 2018). In Uttar Pradesh, chickpea is grown in 6.11 Lakh hectares area with a total production of 6.84 Lakh tone (Anonymous 2018), while productivity is 901 kg / ha (anonymous 2017). Chickpea also helps to maintain the soil health and takes 80% of its nitrogen (N) needs from symbiotic microbial association. It also gave considerable amount of residual nitrogen to the successive crops and helps to add organic matter to improve the soil health (Saraf *et al*., 1998).

Chickpea crop affects by different diseases *viz.*, Dry root rot, *Fusarium* wilt Collar rot, *Verticillium* wilt, Ascochyta blight, Black root rot, *Phytophthora* root rot, Grey mould and seed rot. Of them, collar rot (*Sclerotium rolfsii* Sacc.) is a very damaging to chickpea. Under all favorable conditions, collar rot disease may be a serious threat, which causes very high mortality (55-95%) at seedling stage of this crop (Gurha and Dubey, 1982). Collar rot is causes high losses in yield and production if persists longer. It is well known fact that collar rot is a soil-borne pathogen and produced symptom on the collar region of the plant that is why named collar rot. It also affects many other plant species of families Leguminosae and Compositae, while Graminae family is less susceptible to collar rot disease (Mahen *et al*., 1995). *Trichoderma* and its various species are widely used as a potent biological control agent of soil borne plant pathogens and is a key area of research in the present days in all over the world (Mukhopadyay, 1987). Many research groups confirmed that *Trichoderma* has potential capacity to control different soil borne plant pathogens (Papavizas, *et al.*, 1984). *Trichoderma* spp. is among them and recognized as a broad range biological control agent that shows good activity for their growth in soil. In present day agricultural systems, the usage of fungicides has become vital. Seed treatment with combination of fungicides and bio-agents is a common method used in different crops. It alters the microbial symmetry in soil which helps to reduce disease incidence in a particular area. *Trichoderma* spp., is well proven to establishes symbiotic rather than parasitic relationships among the plant and crop species through increasing plant growth and yield that helps to overcome stress and stimulates nutrient absorption (Harman *et al.*, 2004).

Materials and Methods

Sample collection, isolation of *Trichoderma* spp.

Soil samples from different locations collected for the present investigation. Locations which were used are CRC, HRC, KVK Hastinapur, all comes under jurisdiction of SVPUA&T, Meerut. Samples were collected randomly with the help of an open soil borer (approx. 20 cm depth, 2.5 cm diameter). Collected samples were air-dried at an optimum temperature for 8-10 days and passed through a 0.8 mm fine mesh sieve. After that, samples were stored in a

polyethylene bags for further use in the experiment.

Preparation of *Trichoderma* selective medium

Di-potassium Hydrogen Phosphate (DHP) (0.9g,), Magnesium Sulphate (0.2g), Ammonium Nitrate (1.0g), Potassium Chloride (0.15g), Glucose (3g), Metalaxyl (0.3g), Penta Chloro Nitro Benzene (PCNB) (0.2g), Chloromenicol (0.25g), Rose Bengal (0.15g), Agar –Agar (15g) and the required amount of double distilled water (1000 ml) used in the present study..

Prepared medium through mixing of all these ingredients and sterilized them at 121^0C and 1.1 Kg/cm² pressure for around 15 minutes with an autoclave. Then cool the medium up to 45-47^0C. After that poured the sterilized medium in a pre sterilized 90 mm petri plates under laminar air hood and keep them to solidify.

Potato dextrose agar medium recipe

Take small piece of potato (200 gms) and peeled them, dextrose (20 gms), agar powder (20 gms), and double distilled sterilized water (1000 ml) in a container. Potatoes were cleaned, washed, peeled and chopped into slices. After that 200 gm of these slices were heat boiled in 500 ml of double distilled water and the extract was carefully sieved through clean and intact muslin cloth. Next step is to take dextrose (20 gm), and of agar powder (20 gm) and dissolved in a 500 ml deionized water. Heat slowly and stirred with the help of a glass rod. The potato extract and agar solution mixed and make the final volume 1000 ml by adding deionized distilled water. The conical flasks containing PDA medium were properly sterilized at recommended temperature (121^0C) and 1.1 kg/cm^2 pressure for at least 15 minutes in a autoclave.

Collection of diseased specimens

The infected chickpea plants produced the typical symptoms of collar rot were collected from 'Crop Research Centre' (CRC) field of University during *Rabi* season of 2017-18 for isolation of pathogens of *Sclerotim rolfsii*. The specimens were then brought to the laboratory and examined carefully for symptoms of the disease.

Isolation, purification and identification of the pathogens

Isolation of the pathogen was done with the help of standard tissue isolation technique. Infected plant parts were thoroughly washed in sterilized water for removing the dust and other surface contaminants. A small portion of diseased parts (only collar region) were cut into very small pieces with the help of a sterilized scalpel. Thereafter, complete surface sterilization was done with 70 percent ethyl alcohol. Then pieces washed thoroughly with sterilized distilled water thrice. A small piece of infected part was transferred in petriplates containing appropriate amount of PDA. Theses plates were incubated carefully at 27 ± 1^0 C for 72 hours. The fungal growth, which arose through the infected tissue in the petri plates, was transferred aseptically to a PDA slants and in a petri-plates. The pathogen was identified with various morphological characters.

Pathogenicity test

The pathogenicity tests were carried out to prove the Koch's postulate (1876). During the experiment inoculums of mycelium bits was mixed into pots filled with the sterilized soil before sowing the seeds and the placement of inoculums near plant after sowing the seeds in pots filled with sterilized soil. Soil (sterilized) was used to fill in 30cm diameter earthen pots. Fifteen days old culture were used to

grow on PDA medium and mixed thoroughly in the upper soil layer at 1 per cent weight basis. Then healthy seeds (six seeds) were used to grow in each pot. Control s was used without adding inoculums in pots. Plants were incubated for 30 days to appear the collar rot symptoms. Infected plants were taken out and washed thoroughly in double distilled water. Re-isolations were done from a artificially infected plants and then isolated culture compared with original culture.

Isolation, identification and purification of local *Trichoderma harzianum* isolates

Soil samples were collected from different places and bring to the laboratory. Stock solution was equipped by dissolving 10 g of soil sample into 90 mL of distilled water in test tube. Next, serially diluted the samples as 10^{-1}, 10^{-2} to 10−5. 1ml of each of the diluted sample was spread on petri dish containing *Trichoderma* selective medium (Papavizas and Lumsden, 1982). Then Petri plates were incubated in BOD incubator at 28 ± 1°C for 7 days for growth of *Trichoderma* spp. Purification was made through single spore isolation method (Bisett, 1984) and put at 4^{o}C for further use.

Mass multiplication of local *Trichoderma harzianum* isolates

Wheat grains were used for mass culture of *Trichoderma* isolates. Wheat grains were taken carefully and then rinsed with double distilled water to remove dirt and impurities. Then the grains were soaked in water containing sucrose (2%) for 6 hrs. Drained excess water and then dried under proper shade for reducing the moisture up to 60-70%. 250 gm of wheat grain were filled up in 500 ml capacity conical flasks. Flasks with wheat grains were plugged and wrapped with silver foil and sterilized in autoclave at 121^{0}C temperature (15 lbs pressure/inch^{2}) for 15 minutes. Sterilized wheat grains inoculated vigorously growing 5 days old culture of *Trichoderma* isolates. All inoculated conical flasks were incubated at 26±2 °C temperature in a BOD incubator. *Trichoderma* isolates were allowed to grow after 5 to 6 days shaking of the flasks, the surface of all wheat seeds colonized with a good growth of *Trichoderma* isolates.

Trichoderma harzianum isolates and commercial formulation

Trichoderma harzianum (cfu 1x10 ^{8}/gm) isolate CRC, *Trichoderma harzianum* (cfu 1x10^{8}/gm) isolate HRC, *Trichoderma harzianum* (cfu 1x10 ^{8}/gm) isolate KVK Hastinapur, *Trichoderma harzianum* commercial formulation (cfu 1x10 ^{8}/gm) SVPUAT BCA lab and *Trichoderma harzianum* (cfu 1x10 ^{8}/gm) commercial formulation (Bioharz) of market were used for further studies nin lab and field conditio.

In-vitro evaluation of local *Trichoderma* isolates and commercial *Trichoderma* formulations against pathogen

Dual culture technique was used to in vitro evaluation of local *Trichoderma* isolates and also for commercial *Trichoderma* formulations against pathogen The antagonistic activity of three local *Trichoderma harzianum* isolates and two commercial *Trichoderma harzianum* formulation were tested *in-vitro* in the present study for their ability to inhibit the mycelial growth of *Sclerotium rolfsii*. A mycelial disc (5 mm.), cut from the actively growing of 5-7 day old culture of pathogen on PDA, was positioned on fresh PDA plate (3 cm from centre) then a 5 mm mycelial disc, which was obtained from a actively growing 5-7 day old culture of fungal bio agents. That were placed 3 cm away from the mycelial disk of the pathogen. Three replication of each treatment were maintained with one set of control and without inoculating the bio inoculants and

plates were incubated at 26±1^{0}C. The radial growth of pathogen was measured after 48, 96, 144, and 196 hours well after incubation.

Inhibition percent of the growth in compare to control was calculated with the help of equation given mentioned by Vincent (1927).

$$I = \frac{C - T}{C} X100$$

Where,
I represent percent inhibition
C represents growth (control)
T represents growth (treatment)

Thereafter, evaluation of local *Trichoderma* isolates was done with *Trichoderma harzianum* for commercial formulation to manage collar rot (*Scelorotium rolfsii)* under field conditions.

Field experiment was conducted as Randomized Block Design (RBD) with three replications. One treatment served as control which was without any treatment and plot size was kept 3×4 cm^2 .

Statistical analysis

Data were analyzed statistically and presented in tables 1, 2 and 3. The data on experiments conducted in the laboratory, pots and field were subjected to statistical analysis. The data were transformed whenever required. The critical difference was worked out at 5.0 per cent probability level to find out the difference between treatments (Chandel, 1993).

Results and Discussion

In-vitro evaluation for commercial formulation of *Trichoderma harzianum* against *Sclerotium rolfsii*

Antagonistic activities of three local isolates and two commercial formulations of *Trichoderma harzianum* were used to evaluate against *Sclerotium rolfsii in-vitro.* Data presented in Table 1 showed that there are significant differences in mycelial growth inhibition of *Sclerotium rolfsii* in all the tested bio-agents in the present study. Among them, up to or at 196 hours, maximum inhibition (71.85%) was recorded in commercial formulation of *T. harzianum* (zharioB) followed by (71.11%) in commercial formulation of *Trichoderma harzianum* obtained from biocontrol lab SVPUAT, Meerut. In case of *T. harzianum*, isolate collected from HRC Meerut and isolate from KVK Hastinapur were found same mycelial inhibition (68.88%). Minimum (57.07%) inhibition was observeded in *T. harzianum* isolate of CRC Meerut. All the tested bio-agents showed significant inhibition of *Sclerotium rolfsii* growth over control.

Effect of seed treatment and soil application for commercial formulations of *Trichoderma harzianum* on different traits

Germination percentage

Our findings revealed that percent seed germination varied and were observed from 88 to 65.33 percent. Maximum seed germination (88%) was observed in soil application of *Trichoderma harzianum* followed by seed treatment (86.33%) with *T. harzianum* isolate of CRC, while 85.67% in both soil application & seed treatment with *T. harzianum* isolate of KVK, Hastinapur. While, seed treatment with commercial formulation of *T. harzianum* BCA lab and soil application, of commercial formulation *T. harzianum* Bioharz the percent seed germination was recorded 82.33% and 80.00% respectively (Table 2). The lowest germination percent (77.33%) was observed in seed treatment with commercial formulation *T. harzianum* Bioharz as compared to control65.33%.

Shoot length

Data in table 2 indicated that the shoot length varied from 53.33cm to 33.67cm. The highest shoot length (55.33cm) was recorded in seed treatment with *Trichoderma harzianum* isolate of CRC followed by (51.33cm) soil application of *T. harzianum* isolate KVK, Hastinapur (49.00cm) with proper soil application of *T. harzianum* isolate of HRC and 46.67cm in soil application of *T. harzianum* commercial formulation Bioharz. In case of seed & soil treatment with *T. harzianum* commercial formulation BCA lab shoot length was observed 46.33cm and 45.67cm, respectively. The minimum shoot length was recorded 37.33cm in seed treatment with *T. harzianum* isolate KVK compare to 33.67cm in untreated control.

Root length

In the present study we observed that the root length varied between 26.00cm to 13.00cm and maximum root length (26.00cm) was observed in soil application of *Trichoderma harzianum* commercial formulation Bioharz followed by 24.33cm in seed treatment with *T. harzianum* isolate of CRC. Average 20.66cm root length was recorded in soil application of *T. harzianum* commercial formulation BCA lab and 19.00cm in seed treatment with *T. harzianum* isolate of HRC. 18.00cm and 17.66cm root length was recorded in seed treatment with *T. harzianum* commercial formulation Bioharz and commercial formulation BCA lab, respectively (Table 2). The minimum root length was recorded (13.33cm) in seed treatment with *T. harzianum* isolate of KVK in compare to 13.00cm in untreated control.

Nodule formation

Present investigation observations indicate that the number of nodules per plant varied and recorded in the range of 44 to 18. Maximum number of nodules (44/plant) was recorded in soil application of *Trichoderma harzianum* isolate of CRC followed by 41/plant in seed treatment with *T. harzianum* isolate of CRC, while 32 and 31 per plant in seed and soil treatment with *T. harzianum* commercial formulation BCA lab, respectively. Seed treatment with *T. harzianum* isolate HRC nodulation was evaluated 30/plant and 28/plant soil application of *Trichoderma harzianum* isolate of KVK Hastinapur. The minimum nodule formation was recorded (20/plant) in seed treatment with *T. harzianum* commercial formulation Bioharz compare to 18/plant untreated control (Table 2).

Effect of seed treatment and soil application of local isolates and commercial formulations of *Trichoderma harzianum* against collar rot

Disease incidence

Our study revealed that all treatment were significantly reduced the disease incidence in compare to control. Disease incidence was reduces to a minimum level of 3.57% that is recorded in soil application of *Trichoderma harzianum* isolate of CRC followed by 4.05% seed treatment with *T. harzianum* isolate of CRC. Disease incidence recorede at the level of 4.22% and 4.42% with soil application and seed treatment with *T. harzianum* isolate of HRC, respectively. With reference to the case of seed treatment with *T. harzianum* isolate of KVK Hastinapur, 5.77% disease incidence was recorded, on the other hand 7.50% seed treatment with *T. harzianum* for commercial formulation BCA lab was observed. The maximum disease incidence (11.85%) was recorded in soil application of *T. harzianum* commercial formulation Bioharz compared to 41.10% in untreated control at 60 days after sowing (Table 3).

Yield

Yield in the present study is represented in q/ha: Data in table 3 revealed that the yield varied between 14.80q/ha to 21.66q/ha. Highest yield (21.66q/ha) was recorded in soil application of *Trichoderma harzianum* isolate CRC followed by 19.72q/ha seed treatment with *T. harzianum* isolate CRC, 18.89q/ha soil application of *T. harzianum* isolate HRC and 17.78q/ha seed treatment with *T. harzianum* commercial formulation BCA lab. In case of seed treatment with *T. harzianum* isolate HRC and isolate KVK same yield (17.50q/ha) was recorded. The lowest yield (15.83q/ha) was recorded in soil application of *T. harzianum* commercial formulation Bioharz compare to 14.80q/ha untreated control.

Table.1 *In-vitro* evaluation of different of local *Trichoderma harzianum* isolates and commercial formulations of *Trichoderma harzianum* against *Sclerotium rolfsii*

Treatment No.	Treatment Details	Mycelial growth (mm)							
		Mycelium growth (48 hr)	Inhibition percent	Mycelium growth (96 hr)	Inhibition percent	Mycelium growth (144 hr)	Inhibition percent	Mycelium growth (196 hr)	Inhibition percent
T_1	*Trichoderma harzianum* isolate-CRC Meerut (cfu $1x10^8$/gm)	32.33	52.91	38	57.77	31.67	64.82	28.67	57.07
T_2	*Trichoderma harzianum* isolate-HRC Meerut(cfu $1x10^8$/gm)	33.67	50.97	37.33	58.52	32	64.44	28	68.88
T_3	*Trichoderma harzianum* isolate-KVK Hastinapur (cfu $1x10^8$/gm)	30.33	51.45	38.33	57.41	31.33	65.18	28	68.88
T_4	*Trichoderma harzianum* ,formulation BCA lab SVPUAT, Meerut (cfu $1x10^8$/gm)	30.00	56.30	36.67	59.26	29.33	67.41	26	71.11
T_5	*Trichoderma harzianum* formulation of Market Bioharz(cfu $1x10^8$/gm)	31.33	54.36	38	57.77	28.33	68.52	25.33	71.85
T_6	Control	68.67	-	90	-	90	-	90	-
	C.D. at 5%	4.06	-	4.34	-	7.71	-	6.21	-
	S.E.(m) ±	1.30	-	1.39	-	2.47	-	1.99	-

Table.2 Effect of seed treatment and soil application of local *Trichoderma harzianum* isolates and commercial formulations of *Trichoderma harzianum* on plant growth parameter of chickpea

Treatment No.	Treatment Details	Germination (%)	Shoot length (cm)	Root length (cm)	No. of Nodules/plant
T_1	Soil application of *Trichoderma harzianum* isolate CRC Meerut (cfu $1x10^8$/gm) (@ 5kg/ha. with 100kg vermicompost)	88	45.00	15.00	44.00
T_2	Seed treatment with *Trichoderma harzianum* isolate CRC Meerut (cfu $1x10^8$/gm)@8gm/kg seed	86.33	55.33	24.33	41.00
T_3	Soil application of *Trichoderma harzianum* isolate HRC Meerut (cfu $1x10^8$/gm) (@ 5kg/ha. with 100kg vermicompost)	83.67	49.00	16.33	27.00
T_4	Seed treatment with *Trichoderma harzianum* isolate HRC Meerut (cfu $1x10^8$/gm)@8gm/kg seed	81	42.00	19.00	30.00
T_5	Soil application of *Trichoderma harzianum* isolate KVK Hastinapur (cfu $1x10^8$/gm) (@ 5kg/ha. with 100kg vermicompost	85.67	51.33	16.33	28.00
T_6	Seed treatment with *Trichoderma harzianum* isolate KVK Hastinapur (cfu $1x10^8$/gm) @8gm/kg seed	85.67	37.33	13.33	21.00
T_7	Soil application of *Trichoderma harzianum* formulation BCA lab, SVPUAT, Meerut (cfu $1x10^8$/gm) (@ 5kg/ha. with 100kg vermicompost	80.00	45.67	20.66	31.00
T_8	Seed treatment with *Trichoderma harzianum* formulation BCA lab, SVPUAT, Meerut(cfu $1x10^8$/gm) @ 8gm/kg seed	82.33	46.33	17.66	32.00
T_9	Soil application of *Trichoderma harzianum* formulation Bioharz (cfu $1x10^8$/gm) (@ 5kg/ha. with 100kg vermicompost	80.00	46.67	26.00	24.00
T_{10}	Seed treatment with *Trichoderma harzianum* formulation Bioharz (cfu $1x10^8$/gm) @8gm/kg seed	77.33	45.67	18.00	20.00
T_{11}	Control	65.33	33.67	13.00	18.00
	C.D. at 5%	4.66	4.10	3.34	2.11
	S.E.(m) ±	1.56	1.38	1.12	0.71

Table.3 Effect of seed treatment and soil application of local *Trichoderma harzianum* isolates and commercial formulations of *Trichoderma harzianum* on yield and disease incidence of chickpea

Treatment No.	Treatment Details	Disease incidence (%)	Yield (q/ha)	Increase in yield (%)
T_1	Soil application of *Trichoderma harzianum* isolate CRC Meerut (cfu $1x10^8$/gm) (@ 5kg/ha. with 100kg vermicompost	3.57	21.66	46.35
T_2	Seed treatment with *Trichoderma harzianum* isolate CRC Meerut (cfu $1x10^8$/gm) @8gm/kg seed	4.05	19.72	33.24
T_3	Soil application of *Trichoderma harzianum* isolate HRC Meerut (cfu $1x10^8$/gm) (@ 5kg/ha. with 100kg vermicompost	4.22	18.89	27.63
T_4	Seed treatment with *Trichoderma harzianum* isolate HRC Meerut (cfu $1x10^8$/gm) @8gm/kg seed	4.42	17.50	18.24
T_5	Soil application of *Trichoderma harzianum* 1solate KVK Hastinapur (cfu $1x10^8$/gm) (@ 5kg/ha. with 100kg vermicompost	8.16	16.11	8.85
T_6	Seed treatment with *Trichoderma harzianum* isolate KVK Hastinapur (cfu $1x10^8$/gm) @8gm/kg seed	5.77	17.50	18.24
T_7	Soil application of *Trichoderma harzianum* formulation BCA lab SVPUAT(cfu $1x10^8$/gm) (@ 5kg/ha. with 100kg vermicompost	9.24	16.38	10.67
T_8	Seed treatment with *Trichoderma harzianum* formulation BCA lab SVPUAT (cfu $1x10^8$/gm) @8gm/kg seed	7.50	17.78	20.13
T_9	Soil application of *Trichoderma harzianum* formulation Bioharz (cfu $1x10^8$/gm) @ 5kg/ha. with 100kg vermicompost	11.85	15.83	6.95
T_{10}	Seed treatment with *Trichoderma harzianum* formulation Bioharz (cfu $1x10^8$/gm) @8gm/kg seed	8.55	16.25	9.79
T_{11}	Control	40.10	14.80	0.00
	C.D. at 5%	5.44	2.22	
	S.E.(m) ±	1.83	0.74	

In present study compare the efficacy of potent isolates and commercial formulation of *Trichoderma harzianum. In vitro* there is significant difference in percent inhibition of mycelial growth of *Sclerotium rolfsii* was recorded by all the tested bio-agents up to 196 hours. Maximum inhibition 71.85% of *Sclerotium rolfsii* was recorded in *Trichoderma harzianum* commercial formulation from market (Bioharz). Similar to our findings Nagamma and Nagaraja (2015) evaluated antagonistic effect of *T. harzianum* against under *in-vitro* conditions and representing the same line of research confirmation. They observed that the maximum inhibition (71.67%) of mycelial growth of *S. rolfsii* along with *T. harzianum* (Bacteriology lab isolate) followed by *T.*

viride (Microbiology lab isolate) 63.33%. Least inhibition was recorded with *T. harzianum* isolate GKVK (31.67%). Gaikwad *et al.,* (2018) also evaluated antagonistic activity of *Trichoderma harzianum* against soil borne pathogens under *in-vitro* conditions. They observed that the maximum mycelial inhibition against *Fusarium roseum (*62.18%) and minimum against *Sclerotium rolfsii* (27.73%) in their findings. Yaqub and Shahzad (2005) evaluated in a different finding that *Trichoderma harzianum* and *T. longibrachiatum* against *S. rolfsii in-vitro* and observed sharp inhibition of the mycelial growth of *S. rolfsii.* The observations of our findings are on the similar track and showed similarity with the findings of many research groups.

In field condition, effective local isolates were evaluated for comparison with commercial formulation. The result was significant increase in the growth parameter i.e. germination, shoot length, root length, number of nodule and number of branch in chickpea plant. The maximum germination percentage 88.00% and maximum number of nodules 44.33 were recorded in Soil application of *Trichoderma harzianum* isolate (CRC Meerut) @ 5kg/ha with 100kg vermicompost. Maximum shoot length 55.33cm was recorded in Seed treatment with *Trichoderma harzianum* isolate (CRC Meerut) 8gm/kg seed. In the similar line of our findings, Pandey and Pandey (2005) evaluated that tomato seeds coated with *T. viride* was very much effective against *S. rolfsii* with 80.8 per cent seed germination. They also observed that *Trichoderma* treated seed resulted higher germination up to 48.62% in compare to that of control. Subash *et al.,* (2014) observed the growth and sporulation of *T. harzianum* was faster in sugercane baggase followed by vemicompost, talcum powder and paddy straw in the similar conditions as ours. They also applied *T. harizanum and* mass multiplied with sugarcane baggase directly to the soil and observed that on 7th week, maximum plant height (25%), maximum root length (12%) and more nodules (10%) were recorded in compare to control. The work of above scientists showed similarity with the present work.

Effect of seed treatment and soil application of local *Trichoderma harzianum* isolates and commercial formulations of *Trichoderma harzianum* from market significantly reduced the disease incidence and enhance the yield as compare to control. The minimum disease incidence 3.57% and maximum yield 21.66 q/ha were recorded in soil application of *Trichoderma harzianum* isolate (CRC Meerut) @ 5kg/ha with 100kg vermicompost. The maximum disease incidence 11.85% and minimum yield 15.83 q/ha was recorded in Soil application of *Trichoderma harzianum* formulation commercial formulation from market (Bioharz) @ 5kg/ha with 100kg vermicompost and 41.10% was recorded in case of control. Similarly, Singh *et al.,* (2014) have shown the effects of two isolate of *Trichoderma spp.* against *Sclerotium rolfsii* under field conditions. They also observed that the use of mixture of two compatible *Trichoderma* isolates and proved to be the one of the best crop protection strategies for the management of *Sclerotia rofsii*. Hossain and Hossain (2010) formulated a *Trichoderma* based BAU-bio fungicide that was found effective against tikka disease of groundnut, foot and root rot of pulses and diseases of some vegetable crops. Sultana and Ahsan *et al.,* (2018) observed that maize grain based culture of *T. harzianum* @ 5, 10, 15 and 20 g per pot and showed significant reduction in mortality of chickpea seedlings with the application of *S. rolfsii*. Minimum mortality of collar rot (53.33%) was evident in the treatment with *T. harzianum* applied @ 20 g per pot. Our findings indicates that

application of these micro-organisms successfully reduce the collar rot incidence and consequently increase the growth of chick pea.

Similarly, Sultana and Hossain (1999) evaluated *Trichoderma harzianum* for controlling of foot and root rot (*Sclerotium rolfsii*) of Lentil cv. BARI Masur-1 under field condition. They observed seeds treated with *Trichoderma harzianum* resulted yield up to 1783.33 kg/ha that accounted 81.60% higher yield over control. Mawar *et al.*, (1918) evaluated efficient bio-formulated product of *T. harzianum* and *Bacillus firmus* against dry root rot of guar, and sesame caused by *Macrophomina phaseolina* during rainy season in the year of 2017 at farmer's field. In a study, they also revealed that seed treatment with *T. harzianum* drastically increased yield (14.9-19.0%) with respect to control. However, result of the work carried out by above scientists showed similarity with the present work.

Present study concluded that seed treatment and soil application of *Trihoderma harzianum* is an effective method for management of color rot disease of chickpea. The formulation of *Trichoderma harzianum* (Bioharz) taken from market were also found superior in lab conditions over local *Trichoderma harzianum* isolates. But under field condition local *Trichoderma harzianum* isolates were found more effective in compare to commercial formulations of *Trichoderma harzianum* (Bioharz). Therefore, local isolates of *Trichoderma harzianum* needs to be conserved, formulated, CIB registration under section 9(3B) & 9(3) of the insecticide act 1968, commercially produced by the firms is required to make them available to the end users/famers for the management of soil borne diseases in the field, because local isolates can tolerate adverse climatic conditions prevailing during crop growth.

Acknowledgement

The authors are heartily acknowledged to the Chief Secretary, Government of U.P. and Director of Agriculture, Government of Uttar Pradesh for providing financial assistance to establish Centre of Excellence for Sanitary and Phytosanitary (SPS) under RKVY scheme (Grant No 2235/12-03-11-100(5)/2011 T.C. dated 20-09-2011, Sanction amount Rs. 200 lakh) in the Department of Plant Pathology, Sardar Vallabhbhai Patel University of Agriculture and Technology Meerut, U.P. We all also highly acknowledged to the Uttar Pradesh Council of Agricultural Research (UPCAR), Lucknow for giving resources to conduct the present research.

Conflicts of interest

We solemnly declare that there is no conflict of interest to publish this manuscript.

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How to cite this article:

Anil Kumar, Ramesh Singh Yadav, Kamal Khilari, Prashant Mishra, D. V. Singh, M. K. Yadav and Amit kumar Yadav. 2020. Comparative Study on Biocontrol Potential of Local Isolates with Commercial Formulations of *Trichoderma harzianum* for the Management of Collar Rot of Chickpea Caused by *Sclerotium rolfsii* Sacc.. *Int.J.Curr.Microbiol.App.Sci.* 9(08): 2472-2484. doi: https://doi.org/10.20546/ijcmas.2020.908.282