Journal of AgriSearch, 7(3):172-176
An Open Access International Peer Reviewed Quarterly
ISSN : 2348-8808 (Print), 2348-8867 (Online)
https://doi.org/10.21921/jas.v7i03.18694
Short Note

Management of collar rot disease in chickpea by Trichoderma species

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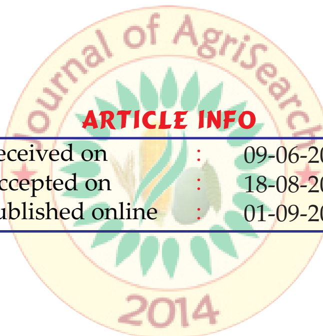
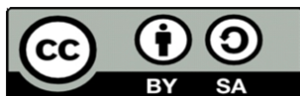


ARTICLE INFO

Received on	:	09-06-2020
Accepted on	:	18-08-2020
Published online	:	01-09-2020



ABSTRACT

Nine isolates of *Trichoderma spp.* were isolated from different agro- ecological regions of Nepal *viz*; Jumla, Palpa, Chitwan, Tarahara, Banke, Illam and Salyan and screened against *Sclerotium rolfsii Sacc*. Adreded soil borne phytopathogen causing collar rot of chickpea in chickpea; *In-vitro* efficacy of nine fungal antagonist (*Trichoderma spp.*) against *Sclerotium rolfsii* were screened. Pot experiment was done to find out the effective management of S. rolfsi through Tricoderma using different methods i.e. Seed treatment, soil drenching and soil application. All the tested isolates of *Trichoderma spp*. were found effective on mycelial growth inhibition and sclerotial parasitization of *S. rolfsii*. *Trichoderma* isolated from Palpa district showed maximum growth inhibition (%) of pathogen periodically after 48(93.78%), 72(96.00%), 96(97.96%) and 120(100.00%) hours of inoculation. Parasitized sclerotium showed minimum sclerotial germination on agar plates. Moreover, *Trichoderma* species isolated from Palpa districts showed second best percent mycelial growth inhibition periodically at 72(25.00%), 120(29.16%), 168(29.16%) and 216(29.16%).In pot experiment at 40 days after sowing, Seedling height was maximum in soil drenching with 30g per 100ml of water (22.27cm) and Mortality percentage of seedlings was least or highest disease control was observed in seed treated with 10^9cfu/ml (0.000%).

KEYWORDS

Chickpea, Collar rot, Disease management, Trichoderma *spp*., Food poisoned technique

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is an important legume food and excellent source of vegetable protein (Singh *et al*., 2015). Chickpea crop can be attacked by several diseases (Singh *et al*., 2015). Collar rot of chickpea caused by *Sclerotium rolfsi* Sacc. is prevalent in areas with high soil moisture and warm temperature with the mortality ranging from 10-100 percent (Kumari and Ghatak, 2018 and Waware *et al*., 2017). Mortality of seedling due to this pathogen is reported to vary from 54.7 to 95 percent and yield reduction in the field condition is reported from 22 to 50 percent (Ahsan *et al*., 2018). It causes stem, crown and root rots and wilt in chickpea (Agrios, 2005). Under field conditions, the pathogen has been reported to the yield reduction from 615 kg/ha to 597 kg/ha from 1995 to 2005 (Amber *et. al*., 2012). Under conducive conditions it can causes 10-100% mortality of the crop at seedling stage (Rajput *et al*., 2010).

Since use of chemicals are hazardous to soil and users, use of biological control agents are the best alternative to the toxic chemicals. Among the bio control agents, *Glio caladium virens* and *Trichoderma viride* were found to be the most effective against the *S. rolfsii*. *Trichoderma* spp. has been found as an effective BCA against many soil borne pathogens (Eziashi *et al*., 2006). *Trichoderma* isolates exhibited inhibition to the mycelial growth of all pathogens. This could be due to the production of diffusible components, such as lytic enzymes or water-soluble metabolites (Anees *et al*., 2010).

The present study was undertaken in an attempt to isolate *Trichoderma* spp. from the various agro ecological regions of Nepal and also to study the morphological characteristics of isolates of *Trichoderma* spp. in order to evaluate the most potential *Trichoderma* isolates to control *S. rolfsii*. Apart from that it was hypothesized that most of *Trichoderma* isolates can inhibit the growth of *S. rolfsii* according to their parallel evolution in the same location. The experiments includes following activities:

- Management of *Sclerotium rolfsii* through different methods of Trichoderma applications viz: soil application, seed treatment and Soil drenching.
- Management of *Sclerotium* using different concentrations of *Trichoderma* isolate.

Sample collection

The pathogen, *S. rolfsii* was isolated from the stems of infected tomato grown in Chitwan, Nepal by tissue segment method on PDA. Small pieces of tissue of about 1 cm from infected collar region with some healthy tissue were cut with sterile blade, surface sterilized with 1% sodium hypochlorite solution for 1 minute followed by three subsequent wash with sterilized distilled water, kept onto PDA medium in Petri dishes and were incubated at 25± 2°C for 2 day. The pathogen was identified as *S. rolfsii* based on its mycelial and sclerotial characteristics reference.

Isolation of *Trichoderma* spp.

Trichoderma spp. Was isolated from Chitwan district and some of the isolates were used from the lab of Plant Pathology, Agriculture and Forestry University (AFU), Rampur, Chitwan, Nepal. For the isolation of *Trichoderma* spp. *Trichoderma* selective media (TSM) was used as a reference. Each soil samples were weighed to one gram

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and were dissolved in 10ml of distilled water in a test tube. One ml of this suspension was then poured into 9ml of distilled water in second test tube and similarly 3rd, 4th and 5th dilutions were prepared. The suspension of the diluted soil was then poured in solidified TSM media using spread plate technique. All the *Trichoderma* spp. growing on TSM was then isolated into pure culture (Fig. 1 and 2).

Fig. 1: *TSM for Trichoderma* Isolation

Fig. 2: *Trichoderma* in soil growing on TSM

Efficacy of *Trichoderma* isolates against *S. rolfsii*

Potential ability of each *Trichoderma* isolate in controlling *S.rolfsii* was studied using dual culture technique by using the following formula (Dubey *et al.*, 2007):

$$P_i = \frac{(C - T)}{C} \times 100\%$$

Where, P_i = Percent growth reduction of test pathogen
C = Radial growth of test pathogen in control (mm)
T = Radial growth of test pathogen in treatment (mm)

Antagonistic properties of the isolated *Trichoderma* were evaluated on the basis of sclerotial parasitization of the pathogen and inhibition of sclerotial germination (Fig. 3).

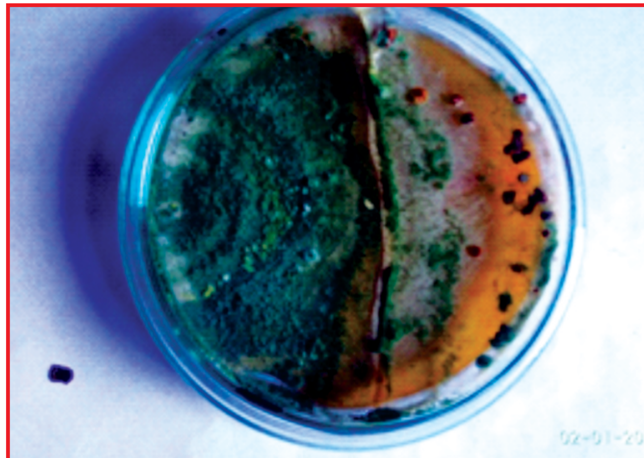

Fig. 3: *Trichoderma* parasitizing *Sclerotia* of Pathogen

For the identification of the effective strain of *Trichoderma* spp. Dual culture of the different isolates against *Sclerotiumrolfsii* was done. Different isolates taken as a treatments were I (Palpa isolate), II (Rampur rice field isolate), III (Rampur vegetable field isolate, IV (Illam isolate), V (Jumla isolate), VI (Nepalgunj, Banke isolate), VII (kapurkot, salyan isolate, VIII (Tarahara, sunsari isolate) and IX (Control (sclerotia without antagonist).

From in vitro evaluation of antagonist property of *Trichoderma* isolates against pathogen the most effective *Trihchoderma* isolate was identified and that isolate was mass multiplied in saw dust and wheat bran in 2:1 ratio by their weight. In order to inoculate soil with pathogen the *Sclerotiumrolfsii* was mass multiplied in wheat grain (Fig. 4).

Fig. 4: Mass culturing of *Sclerotium rolfsii in wheat grain*

Pot experiment:
The most effective *trichoderma* isolate evaluated from the lab work was used to see its effect of controlling collar rot disease of chickpea (*Sclerotium rolfsii* Sacc.) in pot condition (Fig. 5). Variety; Koseli released from National grain legumes research program, Khajura Banke was used for the study. Trial was repeated twice for evaluating the most effective delivery methods and concentration of antagonist for the management of disease. Each pot was filled and inoculated with 20g of fully grown pathogen wheat grain preparation in each pot. The grains were thoroughly mixed with upper 3 inches soil layer of the pot or plant root region. Pots were watered and left for three days for better colonization of the pathogen in pots.

Fig. 5: Experimental setup for the Pot culture of chickpea

Management of *Sclerotium* collar rot through seed treatment:
Conidial suspension of *Trichoderma* isolates was prepared for seed treatment. 10 ml of distilled water was added with *Trichoderma* isolates to fully grown PDA plates and shaken vigorously to dissolve the colony. A thick suspension was formed which was sieved through double layer of muslin cloth and the suspension was used as first concentration of *Trichoderma* for seed treatment. One ml of first suspension was taken through sterilized pipette and poured into 9ml of distilled water in second test tube and shaken vigorously as before and was used as second concentration for seed treatment. Similarly, third concentration of *Trichoderma* was also prepared as third concentration. The three concentrations of suspensions were kept in three different sterilized petri plates and 10 gm of seeds were kept on them and shaken vigorously. Control was maintained by soaking the seeds in distilled water. Nine treated seeds were sown in a pot at the depth of 5cm and watered daily. Watering was done daily early in the morning or late in the afternoon.

Management of *Sclerotium* collar rot through soil application:
Preparation of pots and its inoculation with pathogen and sowing is same as that for seed treatment. Three days after inoculation with pathogen *Trichoderma* mass culture preparation was applied and mixed thoroughly in upper layer of the soil. Three different concentration of mass culture of *Trichoderma*; includes three different amount of mass culture were applied in soil. 10gm, 20gm and 30gm of Trichodermal mass culture were mixed in three different pots and control was maintained as pathogen inoculated pot without antagonist mass culture. The method of seed sowing was same as above. Watering was done early in the morning or late in afternoon every day.

Management of Sclerotium collar rot through soil drenching:
Preparation of pots and its inoculation with pathogen and sowing was done as mentioned above two methods. Three days after pathogen inoculation in pots, soil was drenched with *Trichoderma* suspension of three different concentrations. 10gm of mass culture of *Trichoderma* was weighted and mixed with 100ml of distilled water and shaken vigorously, the mixture was then filtered through muslin cloth and the suspension thus obtained was drenched in pathogen inoculated soil as first concentration. Similarly, 20gm and 30gm of *Trichoderma* mass culture were also mixed and filtered as above and drenched in soil. Control was maintained as soil drenched with distilled water. The method of seed sowing was same as above. Watering was done early in the morning or late in afternoon every day.

Observation on number of plants germinated, number of healthy plants on respective days after sowing till 40 days of sowing, number of infected/wilted plants till 40 days after sowing, height of plants at every 10 days after sowing were recorded.

Statistical Analysis :
The data were entered in a excel sheet and were analyzed with R stat. Mean comparison of the treatments were done through Duncan Multiple Range Test (DMRT) and the significance of difference among the means was calculated by LSD (least Significant Difference) test with the use of package agricolae (De Mendiburu, 2014).

Efficacy of *Trichoderma* isolates against *Sclerotium rolfsii* :
All the isolates from different agro ecological regions inhibited the germination of sclerotia of *S. rolfsii* when they

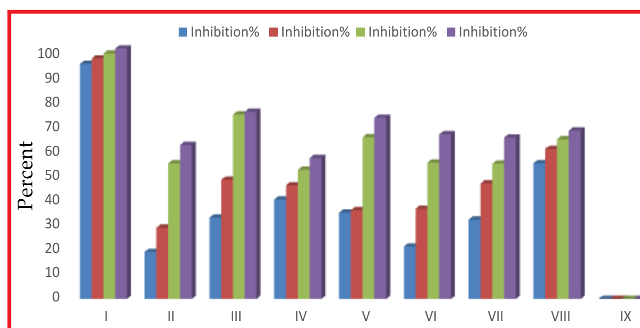

Fig. 6: Percent inhibition of *Sclerotium rolfsii* in dual culture with *Trichoderma* isolates.
I (Palpa isolate), II (Rampur rice field isolate), III (Rampur vegetable field isolate, IV (Illam isolate), V (Jumla isolate), VI (Nepalgunj, Banke isolate), VII (kapurkot, salyan isolate, VIII (Tarahara, sunsari isolate) and IX (Control (sclerotia without antagonist).

were co-cultured on PDA plates as compared to control (without antagonist).*Trichoderma* isolate isolated from Palpa district of Nepal gave the maximum inhibition percent of *S. rolfsii*at 48 (93.78%), 72 (96.00%), 96 (97.96%) and 120(100%) hours after inoculation followed by the isolates from vegetable farm of Rampur and the isolate from Tarahara (Fig. 6). *T. harzianum* caused 80% inhibition of mycelial growth after 72hrs of incubation; and it also caused 35.5% inhibition of *sclerotial* formation after 10 days of incubation.

Among eight sclerotia of *S. rolfsii* inoculated in PDA plates treated with different isolates of *Trichoderma* spp. Least germination percentage of sclerotia was obtained in the plate treated with Palpa isolate at 3(25%), 5(29.16%), 7(29.16%) and 9(29.16%) days after inoculation (Fig. 7). In general, all *Trichoderma* isolates suppressed the germination of *Srolfsii*. Similar results were also observed by Jegathambigai *et al.* (2010), they reported that formation of coils around the hypha of *S.rolfsii* by *Trichoderma*. They also observed sporulation of *Trichoderma* on the *sclerotia*. Jana and Mandal (2017) reported all the five antagonistic isolates suppressed sclerotial germination and completely killed sclerotia within 20 days; only isolate T_{11} required 25 days.

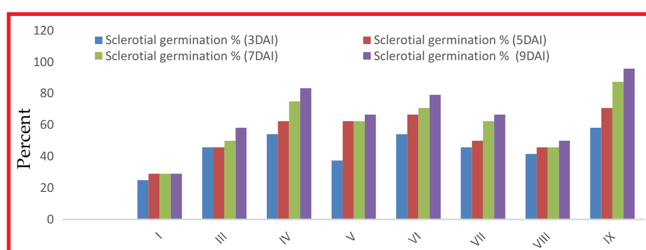

Fig. 7: Effect of *Trichoderma* isolates on sclerotial germination of *Sclerotiumrolfsii*at different Days after inoculation
(Palpa isolate), II (Rampur rice field isolate), III (Rampur vegetable field isolate, IV (Illam isolate), V (Jumla isolate), VI (Nepalgunj, Banke isolate), VII (kapurkot, salyan isolate, VIII (Tarahara, sunsari isolate) and IX (Control (sclerotia without antagonist).

From the Fig. 6 and 7 higher the inhibition percentage of mycelium, lower the *sclerotial* germination percentage of the pathogen. This may be due to the control of pathogen through the various mechanism of disease control by *Trichoderma* isolate.

Pot Experiment:
At 40 days least wilting percentage was observed in seed treatment with 10^{7}, soil drenching with 30g/100ml of water (0.000), soil application with 30g culture (0.000), soil application with 10g culture (0.000) and soil drenching with 10g culture per 100ml of water (0.000) where as in control all the seedlings were wilted before the date of observation. Seedling height was maximum in soil drenching with 30g per 100ml of water (22.27cm) followed by soil application with 10g (22.08cm) at 40 days after sowing. Herman *et al.*, (2004) also reported similar results, they found *Trichoderma* has also a positive impact on plant growth as it produces different kinds of secondary metabolites which are important for plant growth regulation (Fig. 7)

Fig. 8: 40 Days seedling stage with infection

Mortality percentage of seedlings was least or highest disease control was observed in seed treated with 109cfu/ml (0.000%) and soil application with 20g *Trichoderma* culture on 40DAS (0.00%). Similar results were reported by Ahsan *et al.* (2018), they reported highest seedling mortality (61.11%) in pot treated with 5g of culture and least mortality of seedlings in soil application with 20g per pot (53.33%).

Table 1: Effect of *Trichoderm* application on Seedling wilt, seedling height and seedling mortality of chickpea at 40 days after sowing (DAS) in pots inoculated with *Sclerotium rolfsii*

Treatments with Trichoderma	**Seedling wilt 40 DAS**	**Seedling height 40 DAS**	**Seedling mortality 40DAS**
Seed treatment			
(10^{9} cfu/ml)	0.00^{d}(0.000)	16.96^{cd}	0.00^{d}(0.000)
10^{8}cfu/ml	13.25^{c}(21.33)	19.01^{cd}	7.74^{cd} (11.570)
10^{7}cfu/ml	21.75^{a}(27.76)	16.11^{d}	19.58^{bc}(22.95)
Soil drenching			
30g/100ml water/pot	0.00^{d}(0.00)	22.27^{a}	6.69^{cd}(10.72)
20g/100ml water/pot	16.67^{b}(24.06)	17.58^{cd}	5.00^{cd}(6.64)
10g/100ml water/pot	0.00^{d}(0.00)	17.58^{cd}	33.33^{b}(35.05)
Soil application			
30g/pot	0.00^{d}(0.00)	19.74^{abc}	3.57^{cd}(5.55)
20g/pot	22.25^{a}(28.11)	19.25^{bc}	25.00^{b}(29.72)
10g/pot	0.00^{d}(0.00)	22.083^{ab}	18.75^{bc}(18.74)
Control	0.00^{d}(0.00)		100^{a}(89.96)
P- Value	<2e-16***	0.0024**	2.35e-09***t
S.Em±	0.359	1.482	73.08
LSD0.001(P=0.001)	1.229	4.176	17.54
C.V (%)	8.363	10.813	52.357

Values in the parenthesis are arcsine transformed

CONCLUSION

This experiment brings new way to check the efficacy of *Trichoderma* spp. in controlling the pathogen *S. rolfsi.* From the experiment it is found that different isolates isolated from the different ecological region showed antagonistic effect against the pathogen. Among the *Trichoderma* isolates also *Trichoderma* isolates palpa gave the best result with least sclerotial germination and highest inhibition percentage. From this it can be concluded that *Trichoderma* is potential antagonist for the bio-control management of the disease if effective isolates or strains could be obtained as it has shown both the inhibitory effect to the pathogen in laboratory conditions and in field condition. Use of effective *Trichoderma* for the management could be the best option to replace the hazardous chemical application in agriculture.

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Citation:

Pandey P, Sagar GC, Shrestha S, Manandhar H, Yadav RK and Devkota R .2020. Assessment of variability of infiltration characteristics in forage crops. *Journal of AgriSearch* **7**(3):172-176