The Pharma Innovation Journal 2022; 11(5): 2202-2206

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; 11(5): 2202-2206

www.thepharmajournal.com
Received: 05-02-2022
Accepted: 15-04-2022

Rashmi Nigam
Department of Plant Pathology, Janta Vedic College, Baraut, Baghpat, Uttar Pradesh, India

Pradeep Saxena
ICAR-Indian Grassland and Fodder Research Institute, Jhansi, Uttar Pradesh, India

Joginder Singh
Department of Horticulture, Janta Vedic College, Baraut, Baghpat, Uttar Pradesh, India

Rajendra Singh
Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut, Uttar Pradesh, India

Corresponding Author:
Rashmi Nigam
Department of Plant Pathology, Janta Vedic College, Baraut, Baghpat, Uttar Pradesh, India

Efficacy of biocontrol agents on dry root weight of cowpea infected with *Rhizoctonia solani* and *Fusarium oxysporum*

Rashmi Nigam, Pradeep Saxena, Joginder Singh and Rajendra Singh

Abstract
The efficacy of biological control agents on dry weight of cowpea (*Vigna unguiculata* (L.) Walp) was studied at Bundelkhand region. Study reveals that maximum increase in the dry weight of root was observed due to *Trichoderma harzianum* followed by *Trichoderma viride* at various doses. Both the biological agents, *Trichoderma harzianum* and *Trichoderma viride* acted more effectively at 10 to15 g dose. The study also reveals that other two *Trichoderma* species namely *Trichoderma pseudokoningii* and *Trichoderma koningii* did not visualize any significant difference in their efficacies when compared from each other. The least effective biological agents were *Aspergillus flavus* and *Aspergillus niger* when compared with other bioagents. The data also indicate that the efficacy of all treatments significantly varied at different concentration. However, a gradual increase in dry root weight of cowpea plant was recorded with increase of dose. The most effective dose was recorded as 15g/kg seed dose but a reverse trend was observed at lower dose 2g/kg seed dose.

Keywords: Cowpea, root rot, *Rhizoctonia solani, trichoderma, aspergillus*

Introduction
Cowpea (*Vigna unguiculata* L. Walp) is warm weather drought tolerant, annual leguminous fodder crops and has the useful ability to fix atmospheric nitrogen through its root nodules. It is highly rich in protein and form excellent mixture with sorghum, maize, pearl millet and tenosite for increasing the production in milk. Its fresh or dried seed, pods and leaves are commonly used as food for human and livestock. The tender green pods used as vegetables are rich in proteins, minerals, vitamins and dietary fibres (Ibrahim *et al.*, 2010) [8]. The crops is grown in tropics, sub tropics and warm temperature regions. In India, It is commonly grown in Northern and Central India, besides this it is also grown in some parts of Rajasthan, Gujarat, Maharashtra, Karnataka and Tamilnadu. It is now rapidly spreading to the rest of the country due to evolution of better varieties suited to different agro-climatic zones/region. Cowpea is also attacked by wide variety of pest and diseases which hamper crops establishment, decrease forage production, reduce green fodder and seed yield. Besides causing direct yield losses they also suppress nodulation and consequently negating the maximum nitrogen fixation (Saxena and Bhaskar, 2015) [14]. It is proved vulnerable to root rot disease caused by *Rhizoctonia solani* and *Fusarium oxysporum* which attack the roots. The fungi *Rhizoctonia solani* and *Fusarium oxysporum* is the most widespread and destructive plant pathogen causing root rot. These pathogens are restricted to infect the crop either alone or as a complex there by resulting in rots before and after emergence of seedling and wilting of plants (Sumner, 1985; Singh and Gurha, 1996; Bhatnagar and Bansal, 2003; Gokulapalan *et al*., 2006) [17, 16, 2, 6]. These diseases causes substantial losses to this crops. The losses in green fodder and seed yield were estimated to be about 28.8 and 39.7 per cent, respectively (Ram and Gupta, 1988) [13]. Reducing such losses has long been a high priority objective for forage production. Management of soil borne pathogens is of course very difficult and costly too. Now a days, many microorganism have been used as biological control agent of plant disease. *Trichoderma* is known as world-wide fungus with high antagonistic properties against soil borne pathogens. In addition to disease management, *Trichoderma* also promoted growth of the crops that resulted into significantly higher crop production. Seed treatments with biological agents are gaining importance in management of many soil borne pathogens as it of low cost technology and environment friendly. Hence the study was conducted to study the effect of biological agents on dry root weight of cowpea infested with root rot pathogens (*Rhizoctonia solani* and *Fusarium oxysporum*).

Material and Methods

The present investigations and experiments were carried out for three consecutive crop seasons at the Indian Grassland and Fodder Research Institute, Jhansi on cowpea.

Source of Culture

Isolation of Pathogens: Infected cowpea plant showing the root rot symptoms were collected from the field and the diseased tissue from the root and stem portion were surface sterilized with 0.1% mercuric chloride, aseptically transferred into potato dextrose agar (PDA) medium supplemented with amoxicillin (200mg/l). All the petriplates were incubated for 5 days at 28±ºC. The morphological and cultural characters of the fungus were studied for the identification of isolated pathogens. Isolation of *Rhizoctonia solani* and *Fusarium oxysporum was* done in culture media from diseased plant part collected from field.

Isolation of Bioagents: Antagonistic mycoflora isolated from rhizosphere of cowpea field which was collected from bundelkhand region and further isolation carried out by serial dilution. *Trichoderma* species *viz.*, *Trichoderma. harzianum, Trichoderma koningii, Trichoderma pesudokonigii* and *Trichoderma viride* were isolated on *Trichoderma* specific media (TSM). Cultural characteristics of each isolate of *Trichoderma* and *Aspergillus* spp. were studied in PDA plate. All the Petri plates were incubated for five days at 28 ±1 ºC. Observations were taken from 48 hrs after incubation till 120 hrs.

Effect of bioagents on dry root weight of cowpea

Different bioagents like *Trichoderma harzianum, Trichoderma koningii, Trichoderma pesudokonigii, Trichoderma viride, Aspergillus niger* and *Aspergillus flavus* were used as seed treatment against *Rhizoctonia solani* and *Fusarium oxysporum* in pot condition to determine the plant growth promoting activity in terms of dry weight by seed treatment. Dry powder of the antagonist was prepared by growing the antagonists on soaked and sterilized sorghum grains for 20 days. Healthy sorghum seeds were soaked in sucrose solution (5.0%) for 16 hr. The seeds were strained and placed in 500 ml conical flasks to give 200 cm^3 of sorghum seed/flask. Flasks with sorghum seed were plugged with cotton and sterilized by autoclaving for 30 min at 1 kg/cm^2 pressure. The conical flasks containing sterilized sorghum seeds were inoculated with 1 cm dia PDA discs punched from the peripherally of actively growing 5 day old culture of *Trichoderma* spp. Flasks were placed in a BOD incubator at 27±1^{0}C and the fungus were allowed to grow with periodic shaking of the flasks, so that the surface of all sorghum seeds were colonized and its colony farming units reached above 10^8 cfu/g culture.

Aspergillus spp. were multiplied on sorghum seeds in the same manure as *Trichoderma* spp. After the full growth, grains were taken out and dried in shed and grinded it to make powder. This powder was used as seed treatment Fifteen cm diameter earthen pots were filled with 1 kg autoclaved soil manure mixture of 3:1 and were pre inoculated with the test pathogen separately. Treated seed of cowpea (*Vigna unguiculata* L. walp) were sown with different doses of inoculum *viz*. 2 g, 5 g, 10 g and 15 g. The pots were arranged in complete randomized block design with 3 replicates of each treatment including the treated control as well as untreated control. After 50% flowering of the plant, observations were recorded the plants were uprooted and dry root weight were calculated. The analysis of data was done by the standard statistical methods.

Results and Discussion

Infected seedling as well as adult plants of cowpea showing the symptoms of root rot, frequently yielded the colonies of *Rhizoctonia solani* and *Fusarium oxysporum* on potato dextrose agar medium (Plate 1). Repeated isolations from infected plant also exhibited the frequency of these pathogens. Identification of these causal pathogens was confirmed on the basis of their cultural and morphological characteristics recorded in pure cultures as well as in temporary mount, respectively and testimonised with the literature. The fungi *Rhizoctonia solani* and *Fusarium oxysporum* are the most widespread and destructive plant pathogens causing root rot/dry root rot in cowpea. These pathogens are restricted to infect the crop either alone or as a complex there by resulting in rots before and after emergence of seedling and wilting of plants (Sumner, 1985; Bhatnagar and Bansal, 2003; Gokulapalan *et al*., 2006, Nigam 2009, Saxena and Bhaskar, 2015) [17, 2, 6, 12, 14]. These diseases causes substantial losses to this crops. Therefore there is need to develop effective management strategy.

The rhizospheric soil of cowpea collected from different localities/areas of Bundelkhand region, more frequently yielded fungal antagonists. During the study period six fungal antagonists were isolated and evaluated. These fungal antagonists includes *Trichoderma harzianum, Trichoderma viride, Trichoderma pseudokoningii, Trichoderma koningii, Aspergillus niger* and *Aspergillus flavus*. These fungal species were identified and confirmed on the basis of their cultural and morphological characteristics as documented in the literature (Windham *et al*., 1986; Cook, 1993; Mukhopadhyay, 1994; Sen, 2000 and Nagmani *et al*., 2002) [18, 3, 9, 15, 11]. Results of the effect of biological control agents on the dry root weight of cowpea is depicted in Table 1, Table 2 and Figure 1. It is clear from Table 1, that all treatments exhibited a significant effect on dry root weight of cowpea plants under *Rhizoctonia solani* infestation at varying level of doses. The pooled data of three year experimentation (Table 1) revealed that all the treatments were significantly superior in increasing the dry root weights of the plant as compared to untreated control. However, maximum increase in dry root weight of cowpea plant was recorded at 15g/kg seed dose and minimum at 2g/kg seed dose. In present study, *Trichoderma harzianum* was also found effective in increasing the dry root weight by 172.1 per cent followed by *Trichoderma viride* (156.4%), *T. pseudokoningii* (141.4%) and *T. koningii* (126.4%), respectively over untreated control. The minimum increase in dry root weight (93.6%) of cowpea plant was recorded by *A. flavus*. Bavistin was also showed a significant effect in increasing the dry root weight (167.1%) and it was also statistically *at par* in its efficacy with *Trichoderma* spp.

The data presented in Table 2 clearly indicate that all the treatments showed significant effect in increasing the dry root weight of cowpea plant infested with *Fusarium oxysporum*. Table 1, that all treatments exhibited a significant effect on dry root weight of cowpea plants under *Fusarium oxyspor*um infestation at varying level of doses. Among the treatments, *T. harzianum* yielded maximum increase (160.1%) in dry root weight followed by *T. viride* (142.6%), *T. pseudokoningii*

(126.4%) and *T. koningii* (114.3%), respectively over untreated control. Among the *Aspergillus* spp., *A. niger* was most effective and *A. flavus* was the least effective as they exhibited 102.0 and 88.5 per cent increase in dry root weights, respectively over untreated control.

Several scientist (De *et al*. 1990, 1996, Aggarwal, *et al*. 2001, Zareen *et al*. 2001 and Harman *et al*. 2004) [4, 5, 1, 20, 7] also reported the similar results as reported in the present study. The possible reason, for increase in dry weight, is accumulation of nutrients in roots. Number of nodules was higher when the treatments were given with *Trichoderma* species. *Trichoderma harzianum* recorded the best results in increasing the dry weight against *Rhizoctonia solani* and *Fusarium oxysporum* infested plants (Fig 1). Mukhopadhyay (1996) and Zaidi *et al.* (2004) [19] also showed positive results in increased growth and weight of crop plants with *Trichoderma harzianum* and *Trichoderma virens*. These responses may be due to suppression of deleterious root micro-flora including those not causing obvious disease, production of growth stimulating factors, increased nutrient uptake through solublization and sequestration of nutrients and Enhanced root growth.

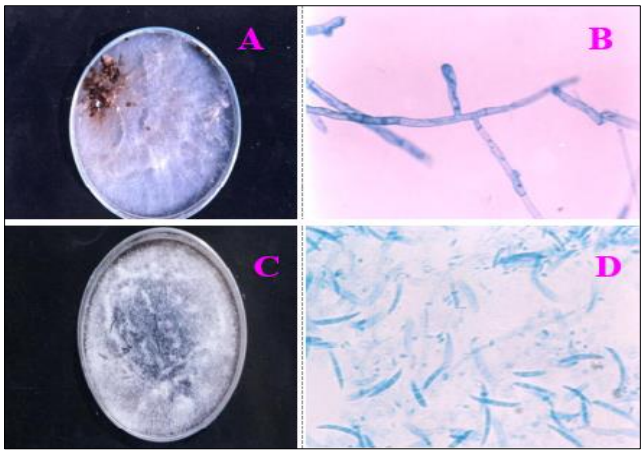

Plate 1: Pathogens (Cultural and Microscopic) of cowpea root rot disease, A & B = *Rhizoctonia solani*, C & D = *Fusarium oxysporum*

Table 1: Efficacy of biological control agents on dry root weight of cowpea infested with *Rhizoctonia solani***

Treatments/Biological agents	Dry root weight* (gm) Doses(g/kg)				Average	Increase over control (%)
	2g	5g	10g	15g		
Aspergillus flavus	0.65	0.67	0.69	0.70	0.68	93.6
Aspergillus niger	0.72	0.74	0.75	0.76	0.74	112.1
Trichoderma koningii	0.77	0.79	0.80	0.81	0.79	126.4
Trichoderma pseudokoningii	0.82	0.84	0.85	0.87	0.85	141.4
Trichoderma viride	0.88	0.89	0.90	0.92	0.90	156.4
Trichoderma harzianum	0.93	0.94	0.96	0.98	0.95	172.1
Bavistin(Treated Control) @ 2g/kg	0.92	0.92	0.92	0.92	0.94	167.1
Control (untreated control)	0.35	0.35	0.35	0.35	0.35	-
CD 5% Treatment 0.01 Doses 0.01 Interaction 0.00						

*Each value is mean of three replicate.
**Pooled data of three year

Table 2: Effect of biological control agents on dry root weight of cowpea infested with *Fusarium oxysporum***

Treatments	Dry root weight* (gm) DOSES(g/kg)				Average	Increase over control (%)
	2g	5g	10g	15g		
Aspergillus flavus	0.67	0.69	0.71	0.72	0.70	88.5
Aspergillus niger	0.73	0.74	0.75	0.77	0.75	102.0
Trichoderma koningii	0.77	0.79	0.8	0.81	0.79	114.2
Trichoderma pseudokoningii	0.82	0.83	0.84	0.86	0.84	126.4
Trichoderma viride	0.87	0.89	0.91	0.92	0.90	142.6
Trichoderma harzianum	0.94	0.96	0.97	0.98	0.96	160.1
Bavistin(Treated Control) @ 2g/kg	0.96	0.96	0.96	0.96	0.96	159.5
Control (untreated control)	0.37	0.37	0.37	0.37	0.37	-
CD 5% Treatment 0.01 Doses 0.01 Interaction 0.00						

*Each value is mean of three replicate.
**Pooled data of three year.

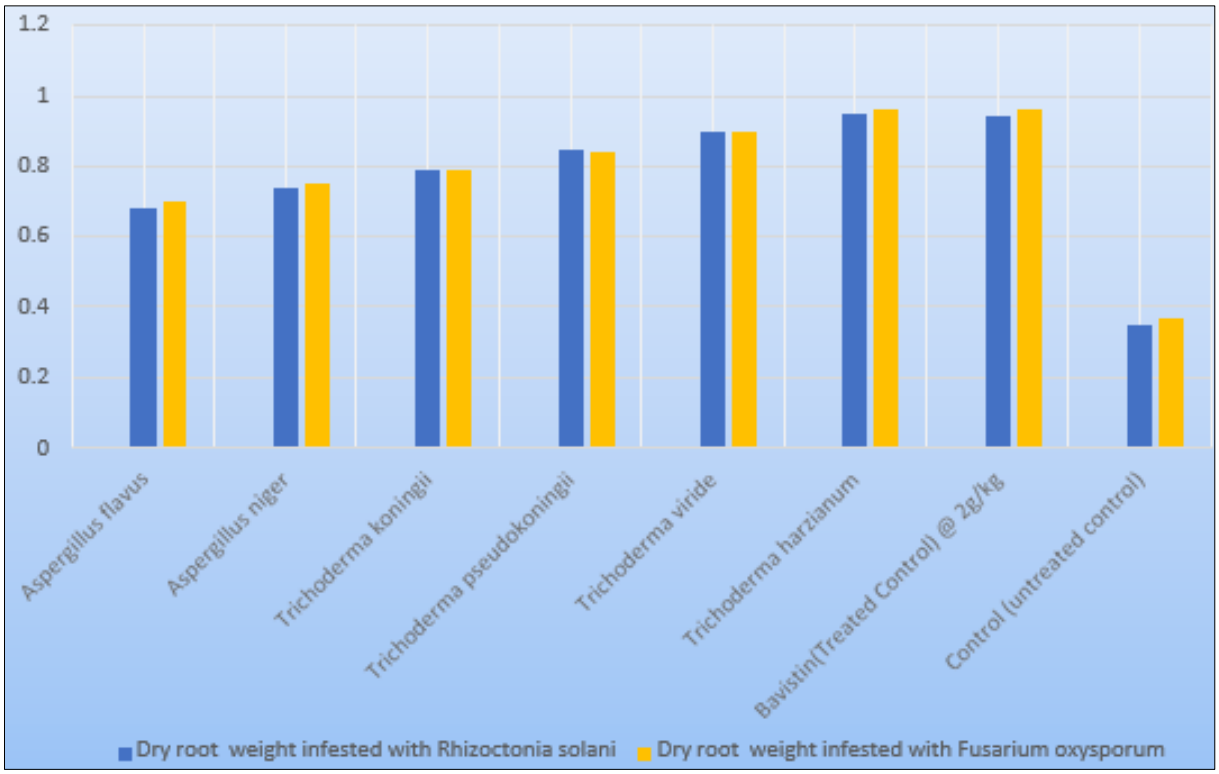

Fig 1: Comparative efficacy of biological control agents on dry root weight of cowpea infested with root rot pathogens

Conclusions

From the results of the experimentation it was concluded that seed treatment with *Trichoderma* spp. (*T. harzianum, T. viride, T. pseudokoningii*) was effective in controlling the root rot disease, enhancing the growth parameters dry root weight of cowpea. *Trichoderma harzianum* at 15 g dose was significantly superior and offered maximum root weights. Hence, use of these bioagents is recommended as environment friendly and cost effective technology for sustainable management of cowpea root rot diseases and can be exploited for enhancing the cowpea production. These bioagents are cheaper and can be applied to control the menace of soil borne pathogens especially *Rhizoctonia solani* and *Fusarium oxysporum.*

References

1. Aggarwal Rashmi, Srivastava KD, Singh DV. Biological control of loose smut of wheat. seed treatment with Trichoderma viride and its influence on plant growth. Annals of Plant Protection Sciences. 2001;9:63-67.
2. Bhatnagar K, Bansal RK. Trichoderma polysporum, a new antagonist against *Macrophomina phaseolina* causing dry root in cowpea. Journal of Mycology and Plant Pathology. 2003;33(2):321-332.
3. Cook RJ. Making greater use of introduced microorganisms for biological control of plant pathogens. Annu. Rev. Phytopathol. 1993;31:53-80.
4. De Mooy CJ, Burke DW. External infection mechanism of hypocotyls and cotyledons of cowpea seedlings by *Macrophomina phaseolina*. Plant Disease. 1990;74:720.
5. De RK, Chaudhary RG, Naimuddin. Comparative efficacy of bio-control agents and fungicides for controlling chickpea wilt caused by *Fusarium oxysporum* f. sp. ciceri. Indian J of Agricultural Sciences. 1996;66(6):370-373.
6. Gokulapalan C, Girija VK, Divya S. *Fusarium pallidoroseum* causes fasciation and yield loss in vegetable cowpea (*Vigna unguiculata* var. *sesquipedalis*), Journal of Mycology and Plant Pathology, 2006, 36(1).
7. Harman GE, Howell CR, Chet AVI, Lorita M. Trichoderma species- opportunistic, avirulent plant symbionts. Nature (Rev. Microbiol.). 2004;2:43-56.
8. Ibrahim U, Auwalu BM, Udom GN. Effect of stage and intensity of defoilation on the performance of vegetable cowpea (*Vigna unguiculata* (L.) Walp). World Journal Agric. 2010;6:460-465.
9. Mukhopadhyay AN. Biocontrol of soil borne fungal plant pathogens current status, future prospect and potential limitation. Indian Phytopathology. 1994;47:119-126.
10. Mukhopadhyay AN. Biological seed treatment with *Gliocladium* and Trichoderma for the control of chickpea wilt complex. Proc. 5th Int. *Trichoderma* and *Gliocladium* workshop Beltsville, U.S.A., 1995 April, 18-20pp.
11. Nagamani A, Manoharachary C, Agarwal SK, Chowdhary PN. Monographic Contribution on Trichoderma. Associated Publishing Co., Post Box No. 2679, Karol Bagh, New Delhi, 2002, 47pp.
12. Nigam Rashmi. Rhizospheric mycflora of cowpea, their antagonistic potential against root rot disease of cowpea. Ph.D Thesis. Bundelkhand University,Jhansi-284003. India, 2009, 164pp.
13. Ram Sri, Gupta MP. Pest Management in Forage Crops. Pastures and Forage Crops Research- A state of knowledge Report. Punjab Singh (Ed.) Range Management Society of India. IGFRI, Jhansi-284003., India, 1988, 261-269pp.
14. Saxena P, Bhaskar RB. Integrated Disease Management of Root rot disease of Cowpea (*Vigna unguiculata* L. Walp). XXII International Grassland Congress Proceeding (Sustainable use of Grassland resources for Forage Production, Biodiversity and Environment

Protection) New Delhi, November 24, 2015, Published by Range Management Society of India, 2015.
15. Sen B. Biological control: A success story. Indian Phytopathology. 2000;53(3):243-249.
16. Singh RA, Gurha SB. Disease of mungbean and Urbean and their management. Disease Senario in Crop Plants. 1996;2:69-93.
17. Sumner DR. Virulence of anastomosis groups of *Rhizoctonia solani* and *Rhizoctonia* like fungi on selected germplasm of snap bean, lima bean and cowpea. Plant disease. 1985;69:25-27.
18. Windham MT, Elad Y, Baker R. A mechanism for increased plant growth induced by *Trichoderma* spp. Phytopathology. 1986;76:518-521.
19. Zaidi NW, Pramila N, Singh, Singh US. Biological control of Plant pathogens: status in India. In: Eco-Agriculture with Bio-augmentation: An Emerging Concept (eds. Singh, S.P. and Singh, S.B.), DASP, Lucknow, 2004, 21-52pp.
20. Zareen A., Zaiki MJ, Khan NG. Effects of fungal filtrates of *Aspergillus* species on the development of root knot nematode and growth of tomato (*Lycopersicon esculentum* Mill.). Pakistan Journal of Biological Sciences. 2001;4(8):995-999.