

Effect of Biopriming with Biocontrol Agents *Trichoderma harzianum* (Th. Azad) and *Trichoderma viride* (01pp) on Chickpea Genotype (Radhey)

Vipul Kumar, Mohammad Shahid, Anuradha Singh, Mukesh Srivastava*, Abhishek Mishra, Y.K. Srivastava, Sonika Pandey and Antima Sharma

Department of Plant Pathology, CSA University of Agriculture & Technology, Kanpur-208002, Uttar Pradesh, India

Abstract

Chickpea is an important pulse crop of India. Its productivity is quite low due to several biotic and abiotic stresses. Among the biotic stress disease are the major constraints. Wilt caused by *Fusarium oxysporum* f. sp. *ciceri* has been considered as devastating one to cause up to 10 per cent loss in yield every year. For eco-friendly and sustainable management of the disease, two species of antagonists (*Trichoderma viride* 01PP and *Trichoderma harzianum* Th azad) and chemical fungicide (Bavistin @ 0.2%) were evaluated against the pathogen. Two bio-agents *Trichoderma harzianum* Th azad and *Trichoderma viride* 01PP were evaluated for their efficacy on colony growth by dual culture plate method. The results showed that the two bio-agents suppressed the colony growth of *Fusarium oxysporum* f. sp. *ciceri*, which ranged between 53.38-57.99 per cent the suppression of the growth of the pathogen was significantly higher with *Trichoderma harzianum* Th azad. Seed treatment *in vivo* showed that out of six treatments including control, T₁ (5%) followed by T₂ (10%), T₃ (20%), T₄ (2% @ 2.5 kg h⁻¹), T₅ (Bavistin @ 0.2%) and T₆ (Control) were found superior seed treatments in enhancing quality seed parameters (germination, plant death on different dates, plant survival and yield), which can be finally converted in superior yield even in adverse conditions. T₁ treatment (5%) was found to be significantly superior and effective in increasing 79% and 71.67% respectively (in both strains) more germination from control followed by bavistin (T₅). Results of the study show that bio-agents significantly reduced the wilt incidence, and increased seed germination and plant growth parameters as compared to chemical fungicides.

Keywords: Chickpea; Bavistin; Inhibition; *Fusarium*

Introduction

Among the pulses, the major contribution to the total pulse production comes from chickpea. It holds an important position as for as area and consumption are concerned. Chickpea belongs to sub-family *Papilionaceae* of family *leguminosae* and is said to be one of the oldest pulses known to be cultivated from ancient time both in Asia and the Europe. It spread to different countries including India and it is now grown as pulse crop throughout tropical and sub-tropical Asia, Northern Africa, Southern Europe, Central and Southern America [1]. Chickpea (*Cicer arietinum*) is also known as gram, Bengal gram or Spanish pea and is considered to be one of the important pulse crops of the world. In India, it is an important source of protein in human diet. It plays a significant role in sustaining production of the subsistence forming system. Major production of chickpea comes from central and northern India. The chickpea is consumed in different forms. Dry chickpea is used as split chickpea (dal) and 'Besan' for various salty and sweets preparations. Both husks and bits of dal are valuable cattle feed. Fresh green leaves are used as vegetable (sag). Straw of chickpea is an excellent fodder for cattle. The grains are also used as vegetable (chhole). It is a leading vegetable among frozen foods. Chickpea is considered to be having great medicinal value and it is used for blood purification.

Chickpea is affected by the diseases caused by fungi, bacteria, viruses and nematodes. More than 30 pathogens have been reported on this crop from different parts of the world [1]. However, during the surveys conducted at different locations in U.P., the wilt caused by

Fusarium oxysporium f. sp. *ciceri* [2-5] was observed in moderate to severe form. It is potentially a serious disease in India, Iran, Pakistan, Myanmar, Spain, Tunisia, Bangladesh, Ethiopia, Mexico, Peru, Syria, and USA. In India, it is the major biotic constraint in successful cultivation of chickpea in all the chickpea growing states. According

to estimates 10 per cent losses in yield due to wilt is considered as a regular feature [6]. Losses amounting to \$1 m have been reported from Pakistan [7]. Early wilting is known to cause more losses than that of late wilting [8]. In India, however, it was reported by Butler in 1910, but more work is still required on this disease of chickpea. Therefore, in view of the seriousness of disease and the importance of crop, the main aim of this work was to conduct a study for the management of wilt disease of the chickpea through seed treatment with pesticides in combination with bio agents.

Materials and Methods

Collection of diseased material

Healthy as well as diseased chickpea plant showing characteristic wilt symptoms were collected from Nawab ganj Research Farm, C. S. Azad university of Agriculture & Technology, Kanpur during the Rabi season of 2013-2014 and brought to the laboratory for examination.

Isolation and purification of the pathogen

Isolation of the fungus was done from the part of the plant showing

*Corresponding author: Dr. Mukesh Srivastava, Biocontrol Laboratory, Department of Plant Pathology, CSA University of Agriculture & Technology, Kanpur-208002, Uttar Pradesh, India, Tel: 9454646550; E-mail: vipulpathology@gmail.com

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initial wilt symptoms [9-11]. The affected roots were first washed in tap water to removed dust particles. The affected pieces of root surface were sterilized in 0.1 percent aqueous solution of mercuric chloride for one minute and subsequently washed thoroughly 3-4 times with distilled water. Excess water was removed by putting the pieces in between the folds of sterilized blotting papers. These pieces were inoculated on PDA plates kept in incubator at $25 \pm C$ for 24 hours.

Single spore isolation technique was done for the purification of the culture and the culture thus obtained was maintained on potato dextrose agar medium slants for further studies.

Identification of pathogen

Identification of the pathogen was made by comparing the cultural and morphological characters of the fungus with that of described by Booth [12] for *Fusarium oxysporum* f. sp. *ciceri* following growth habit, cultural and morphological characters.

Laboratory screening of antagonists against the test pathogen

Two bioagents viz. *Trichoderma viride* and *Trichoderma harzianum* were assessed for comparative efficacy against *Fusarium oxysporum* f. sp. *ciceri* by using dual culture plate technique [13]. Five mm disc of test fungus and the antagonistic fungi, cut from the edge of five days old culture were used for inoculation. Test fungus was inoculated before 72 hour of bioagent inoculation, on potato dextrose agar medium petriplates. The test fungus and bioagents were inoculated opposite to each other at a distance of 5 mm from the periphery of the petriplate. Control without bioagent was prepared for each treatment. Three replicates of each treatment were made. All treatments were incubated at $25 \pm 1^{\circ}C$; the data were recorded after 96 hours of bioagents inoculation. When the inhibition zone was formed, it was expressed as percent inhibition.

In vivo effect of seed treatment with different concentrations of biopesticides

The seeds were soaked for overnight in a suspension of different concentrations of bioformulation of the bioagent separately [14,15]. The seeds were sown on the next day early morning in a well pulverized plot of 28 x 46 m. The distance between two rows kept as 30 cm. Three rows were sown for each treatment in a block. Regular observations were taken at 10 days interval from 30 days after sowing; the sowing was done on 20 October 2013. Number of plant were counted row wise and fixed for further studies. Number of plants dead was also counted at 20 days interval. After five regular observations, number of plants survived was counted and observations were made for calculating the effectiveness of bioagent in checking the attack of *Fusarium oxysporum* f. sp. *ciceri* on chickpea

The field experiment was conducted during the Rabi season 2013-2014 in a design consisting of seed treatment with different concentrations of bioformulations (*Trichoderma viride* and *Trichoderma harzianum*) and Bavistin [16,17]. Recommended dose of different concentration of bio-formulations viz., 5%, 10%, 20%, 2% WP @ 2.5 kg h⁻¹ in Furrow, Bavistin @ 0.2% and control) applied to infected seeds in three replication with five treatments and one without seed treatment served as control. Different pre-sowing seed treatments showed different responses against all the seed quality attributes (germination, plant death on different dates, plant survival and yield/plant).

Seeds sown without any treatment were considered as check (control). The soil was inoculated with inoculum of *Fusarium*

oxysporum f.sp. *cicero* @ 5.0 g/m². The inoculum was mixed with the seeds before the time of sowing of seeds. The soil in the field was sandy loam in texture with pH-7.2, organic matter: 0.4 per cent with medium fertility status and medium water holding capacity. Seeds of susceptible variety (Radhey) were sown. Wilting of plant was carefully monitored right after emergence of seedlings to crop maturity. Wilt incidence, per cent wilt control and per cent increase in yield over check were also observed. Chickpea seeds of the susceptible variety (Radhey) treated with antagonists *Trichoderma viride* (01PP) and *Trichoderma harzianum* (*Th azad*) was sown in 28 x 46 m size wilt sick plot at the Nawabganj farm, C. S. Azad University of Agriculture & Technology, Kanpur to test the efficiency of the bioagent during the Rabi season of 2013-2014. The bio-agent was also procured from Biocontrol Lab, Department of Plant Pathology, CSAUA&T, and Kanpur. The experiment was sown in RBD. The untreated seeds served as control.

Results and Discussion

Laboratory screening of the antagonist against the test pathogens by dual culture method

Trichoderma harzianum and *Trichoderma viride* were evaluated *in vitro* against *Fusarium oxysporum* f. sp. *ciceri* by adopting dual culture technique. The results are interpreted in terms of per cent inhibition over the fungal growth of control and presented in Table 2.

Effect of seed treatment with biocontrol agents in the field

Treated seeds were subjected to assess the germination, plant death on different dates, plant survival and yield per plant during Rabi season (2013-14). The observation on the number of plants dead and the average yield was recorded at 10 days interval starting after the 30 days of the sowing. The experimental results of different seed treatments in chickpea (Radhey) revealed significant different responses against all the four seed quality attributes (Tables 1 and 2). T1 was found to be superior and effective in increasing germination over control followed by T2, T3, T4 and T5. Similarly, the impact of seed treatment was also recorded for plant survival where, T1 excelled over other treatments followed T2, T3, T4 and T5. The average yield was also obtained maximum in case of T1. Percent plant survival and yield per plant are important attributes, which determine the quality of seed. From the data presented in Table 2, it is inferred that T1 has achieved the highest germination as well as yield per plant. It is concluded from the above observation, that out of 5 treatments including control, T1 followed by T2, T3, T4 and T5 were found superior seed treatment in enhancing quality seed parameters, which can be finally converted in superior yield. The observations recorded are summarized in Table 2.

Different pre-sowing seed treatments showed different responses against all the seed quality attributes (germination, plant death on different dates, plant survival and yield/plant).

From the data presented in Tables 2a and 2b, it is inferred that T₁ Treatment (5%) achieved the highest germination as well as yield per plant. It is concluded from the above observations, that out of

Treatment	Radial growth (mm) (F.o.c.)	Percent inhibition
Foc + Th	26.5	57.99
Foc + Tv	28.3	53.38
Control	60.7	
C.D. at 5 per cent		5.55

Table 1: Percent inhibition of F.o.c. over control in presence of bioagents *in vitro*, Where, F.o.c. – *Fusarium oxysporum* f. sp. *ciceri*, Th – *Trichoderma harzianum*, Tv – *Trichoderma viride*.

a: Average effect of seed treatment with *T. harzianum* (*Th. Azad*) on chickpea during 2013-14.

Sl. No.	Treatments	Germination (%)	No of Plant dead on different dates					Plant survival (%)	Yield per treatment (kg)
			02.01.2014	22.01.2014	22.02.2014	22.03.2014	22.04.2014		
1.	T1 (5%)	79.00	1.00	0.67	2.00	0.33	0.33	73.67	4.64
2.	T2 (10%)	72.33	0.67	0.67	1.33	0.67	1.00	71.67	3.48
3.	T3 (20%)	67.67	1.33	1.00	2.00	0.67	0.33	62.67	3.44
4.	T4 (2% @ 2.5kg h ⁻¹) Furrow (Bavistin @ 0.2%)	69.00	1.67	0.67	2.67	0.67	0.67	63.33	3.31
5.	T5 (Bavistin @ 0.2%)	63.00	1.00	1.00	2.67	0.67	0.33	62.00	2.77
6.	Control	56.33	2.33	1.33	3.33	1.67	1.00	50.67	2.51
	CD at 5%	9.35						6.14	1.080

b: Average effect of seed treatment with *T. viride* (01PP) on chickpea (*Radhey*) during 2013-14.

Sl. No.	Treatments	Germination (%)	No of Plant dead on different dates					Plant survival (%)	Yield per treatment(kg)
			02.01.2014	22.01.2014	22.02.2014	22.03.2014	22.04.2014		
1.	T1 (5%)	71.67	1.67	1.00	1.33	0.33	1.00	67.33	3.69
2.	T2 (10%)	65.67	1.00	1.00	1.00	1.00	1.00	61.67	3.43
3.	T3 (20%)	62.00	1.33	1.33	1.67	1.00	1.00	58.00	3.29
4.	T4 (2% @ 2.5kg h ⁻¹) Furrow	59.00	2.33	0.67	2.00	0.67	0.67	57.67	3.20
5.	T5 (Bavistin @ 0.2%)	56.00	2.00	1.00	2.00	0.67	1.33	54.33	2.99
6.	Control	51.67	3.00	1.33	3.00	1.33	1.67	47.67	2.78
	CD at 5%	10.86						5.06	NS

Table 2a,2b: Effect of seed treatment in chickpea during Rabi season 2013-14 through bioagent *T. harzianum* (*Th. Azad*) and *T. viride* (01PP).

six treatments including control, T₁ (5%) followed by T₂ (10%), T₃ (20%) T₄ (2% @ 2.5 kg h⁻¹ furrow application), T₅ (*bavistin* @ 0.2%) and T₆ (control) were found superior seed treatments in enhancing quality seed parameters, which can be finally converted in superior yield even in adverse conditions. Scientists also reported that bioagent *Trichoderma* enhance the seed treatment on germination and vigour in chick pea and lentil crop [18-22]. Srivastava (2004) [23,24] reported that root colonization by *Trichoderma* strains frequently enhances root growth and development. The strains of *Trichoderma* increased root development in several crops, under both green-house and field conditions [22].

Conclusion

In vivo results showed that *T. viride* 01 PP and *T. harzianum* *Th. azad* significantly inhibited the mycelial growth of the pathogen. Treatments T₁ followed by T₂, T₃, T₄ and T₅ were found the best seed treatment in enhancing the quality of the seed including reducing wilt incidence. Thus, instead of treating seed in a row with different concentration of bio-formulation, fungicide and insecticide, seed treatment with only bio-formulation @ 5% (*Trichoderma* @ 5 g/kg) is now recommended.

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