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Management of Wilt and Root Rot of Chickpea caused by *Fusarium oxysporum* f. sp. *ciceri* and *Macrophomina phaseolina* through Seed Biopriming and Soil Application of Bio-Agents

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A B S T R A C T

Wilt and root rot diseases of chickpea caused by *Fusarium oxysporum* f. sp. *ciceri* and *Macrophomina phaseolina* are serious biotic constraints for chickpea (*Cicer arietinum* L.) production. These are most important and widespread soil- and seed-borne diseases of chickpea grown where the climate is relatively dry and warm. To find out the effective management of the diseases through seed biopriming and soil application of biocontrol agents, the field studies were conducted during Rabi season of 2013-14 and 2014-15. Seed biopriming checked the incidence of wilt and root rot in the range of 45%-60% and increased the yield of chickpea by 10%-20%. However, combined applications of seed biopriming as well as soil application significantly checked the disease incidence in the range of 46%-78% and increased the grain yield by 13%-27%. The disease control and yield enhancement were highest with *T. viride* followed by *T. harzianum*. The pooled result of two years revealed that soil application of *Trichoderma viride* or *T. harzianum* ($2x10^8$ cfu/g) enriched FYM (10 kg bioagent/ ton FYM) in furrow @ 1 ton/ ha, followed by seed biopriming at the time of sowing i.e. soaking of chickpea seeds for 10 hrs in suspension of talc based formulation 1% WP ($2x10^8$ cfu/g) of *T. viride* or *T. harzianum*, respectively @ 50 g product/ 250 ml of water/ kg seed and shade dried for the effective management of wilt and root rot complex.

Introduction

Wilt and root rot are the common and frequently occurring diseases of chickpea and causes considerable yield loss (Haware *et al.*, 1996; Kaur and Mukhopadhyay, 1992). *Fusarium oxysporum* f. sp. *ciceri* (Padwick) Synd. and Hans. is considered to be the primary cause of wilt disease in chickpea (Chattopadhyay and Sen Gupta, 1967), whereas, *Rhizoctonia solani* Kuhn is concomitantly associated with the disease (Bhatti *et al.*, 1987; Jalali and Chand, 1992). *R. solani* alone is capable of causing wet root rot (Singh, 2005), but its occurrence with *F. oxysporum* f. sp. *ciceri* has been observed quite frequently (Andrabi *et al.*, 2011). India is a major chickpea growing country producing around 75% of the world's supply (Tomar *et al.*, 2010). Chickpea wilt and root rot are soil- and seed-borne; facultative saprophyte and survive in soil for two to three years (Haware *et al.*, 1978). These cause complete losses in grain yield, if the diseases

occur in the vegetative and reproductive stages of the crop (Haware and Nene, 1980). Researches have shown that commonly grown cultivars of chickpea in India may suffer from 9-41% seed yield loss due to wilt, depending on the cultivar and disease severity (Khan *et al.,* 2004).

Biological control is one of the best low-cost and ecologically sustainable methods for managing plant diseases caused by soil-borne pathogens like *Fusarium, Macrophomina, Rhizoctonia*, etc. Among various biocontrol agents (BCAs) evaluated against the plant pathogenic fungi, *Trichoderma* spp. have been found to possess biocontrol ability (Abd El-Khair *et al.,* 2010; Mohiddin *et al.,* 2010), these fungi mycoparasitize the pathogenic fungi via hyphal coiling and enabling enzymatic lysis through 1, 3-glucanase, cellulase, chitinase, and proteinase (Jefries and Young, 1994). *Trichoderma* species can also combat plant pathogens by exerting antagonism in the form of antibiosis; the production of antifungal metabolites such as trichodermin, gliotoxin, or viridin (Bruckner and Przybylski, 1984; Lorito *et al.,* 1993). Research shows that seed and soil applications of different strains of *T. harzianum* and *T. viride* successfully control root rot and wilt disease caused by *R. solani* and *F. oxysporum* f. sp. *ciceri* under pot conditions (Rudresh *et al.,* 2005; Kumar *et al.,* 2008) and field conditions (Prasad *et al.,* 2002; Dubey *et al.,* 2012). However, these studies have tested two or three strains of *Trichoderma* spp. against monopathogenic diseases caused by *Fusarium* or *Macrophomina* or *Rhizoctonia* spp. Information on the relative effectiveness of important species of *Trichoderma* under multipathogenic conditions is largely lacking. The present study was undertaken to examine the performance of three important species of *Trichoderma* (*T. viride, T. harzianum* and *T. virens*), as well as *Pseudomonas fluorescens* through seed biopriming and soil application with the objective to evaluate the bioefficacy of bioagents as well as to assess the effect of the bioagents in growth and yield parameters of the chickpea.

Materials and Methods

The study was conducted at College Agronomy Farm, B. A. College of Agriculture, Anand Agricultural University, Anand during two consecutive years in the Rabi: 2013-14 and 2014-15 in Randomized Block Design with ten treatments along with three replications using cultivar Gujarat Gram 2. The crop was sown with 30×10 cm spacing having a gross plot size of 5.0 x 3.0 m and net plot size of 4.8 x 2.4 m. The seed rate was used at 50 kg/ha. The bioagents were used in the present investigations were *T. viride, T. harzianum, T. virens* and *P. fluorescens*.

The seeds of chickpea were treated with suspension of talc-based formulation of *Trichoderma* spp. and *P. fluorescens* multiplied by liquid fermentation individually (2x108 cfu/g) @ 50 g product/ 250 ml of water/kg of seed for 10 hrs. The bioprimed seeds were shade dried. An untreated control was also maintained. Similarly, the soil application of bioagents ($2x10^8$ cfu/g) enriched FYM (10 kg bioagent/ ton FYM) in furrow @ 1 ton/ ha was done as per treatments. The observations were recorded on seed germination (%), growth parameters i.e. root and shoot length (cm), vigour index, wilt and root rot (complex) incidence (%) and grain yield (kg/ha).

The percent disease incidence (PDI) was calculated by using the following formula:

Disease incidence (%) = Total No. of diseased plants/ Total No. of Plants x 100

The seedling vigour index was calculated using the formula as given by Abdul Baki and Anderson (1973).

Vigour index = (Mean root length + Mean shoot length) x Per cent germination

Results and Discussion

The pooled data of the year: 2013-14 and 2014-15 for the management of wilt - root rot complex of chickpea through seed biopriming and soil application of bioagents (Table 1) revealed significantly lowest incidence of wilt and root rot (8.59%) and highest seed germination (96.69%), vigour index (2734) and grain yield (1535 kg/ha) in the treatment T_5 i.e. seed biopriming for 10 hrs with suspension of talc based formulation (2x108 cfu/g) of *T. viride* @ 50 g in 250 ml of water/ kg of seed + soil application of *T. viride* enriched FYM (10 kg bioagent/ ton FYM) in furrow @ 1 ton/ ha, which was on par with the treatment T6 i.e. seed biopriming for 10 hrs with suspension of talc based formulation (2x108 cfu/g) of *T. harzianum* @ 50 g in 250 ml of water/ kg of seed + soil application of *T. harzianum* enriched FYM (10 kg bioagent/ ton FYM) in furrow @ 1 ton/ ha having low incidence of wilt and root rot (9.78%) and higher seed germination (94.34%), vigour index (2552) and grain yield (1466 kg/ha) as compared to untreated check, which recorded highest incidence of wilt - root rot complex (38.53%) and lowest grain yield of 1117 kg/ha.

Considering the efficacy, additional income and ICBR of the treatments (Table 2), treatment T_5 and T_6 i.e. seed biopriming for 10 hrs with suspension of talc based formulation ($2x10^8$ cfu/g) of *T. viride* (T_5) or *T. harzianum* (T_6) @ 50 g in 250 ml of water/ kg of seed + soil application of *T. viride* (T_5) or *T. harzianum* (T_6) enriched FYM (10 kg bioagent/ ton FYM) in furrow @ 1 ton/ ha was found significantly effective for disease management (8.59% and 9.78% disease incidence, respectively) and economical (ICBR 1: 10.09 and ICBR 1: 8.42, respectively) in reducing the wilt and root rot incidence of chickpea.

Khan *et al.,* (2014) studied the effects of *T. harzianum, T. hamatum, T. viride, T. polysporum,* and *T. koningii* on the wilt disease complex of chickpea caused by *Fusarium oxysporum* f. sp. *ciceri* and *R. solani*. Soil application of biocontrol agents checked the severity of wilt by 25%–56% and 39%–67% and increased the yield of chickpea by 12%–28% and 8%–24% in the two years i.e. 2004-2006, respectively. The disease control and yield enhancement were highest with *T. harzianum*, followed by T. hamatum and *T. viride*.

Manjunatha *et al.,* (2013) reported minimum root rot incidence of chickpea (2.67%) with higher seed germination (97.60%) and seed yield (1274 kg/ha) achieved through seed treatment of *T. viride* + soil application of FYM at 4 kg/plot.

Rudresh *et al.,* (2005) reported significant control of wet root rot and *Fusarium* wilt of chickpea by soil application of *T. harzianum* (PDBCTH) and *T. virens* (PDBCTV12), respectively. However, in another study, Kumar *et al.,* (2008) found *T. virens* was more effective than *T. harzianum* against *R. solani*. Malathi and Sabitha (2004) studied the effect of seed priming with *Trichoderma* spp. i.e. *T. viride, T. harzianum, T. hamatum, T. longibrachiatum, T. koningii* and *T. pseudokoningii* on seed-borne infection of *M. phaseolina* in groundnut and found that seed pelleting with *Trichoderma* spp. protected seeds from *M. phaseolina* infection and maximum reduction of infection (79.6%) was shown by *T. harzianum* and also it improved seedling vigour, dry matter production and prevented loss of oil content up to six months of storage.

Table.1 Effect of seed biopriming and soil application of bioagents on growth parameters, wilt-root rot incidence and yield of chickpea

Tr No	Treatment	Germi-nation (%)	Shoot Length (cm)	Root Length (cm)	Vigour Index (VI)	Wilt and root rot incidence (%)	% disease control over check	Grain yield (kg/ha)	% Increase of yield over check
T_1	Seed biopriming for 10 hrs with suspension of talc based formulation ($2x10^8$ cfu/g) of *Trichoderma viride* @ 50 g in 250 ml of water/kg of seed	74.19 abc (92.58)	23.84 abc	8.21 bc	2379 bc	23.36 c (15.72)	59.20	1392 abc	19.76
T_2	Seed biopriming for 10 hrs with suspension of talc based formulation ($2x10^8$ cfu/g) of *Trichoderma harzianum* @ 50 g in 250 ml of water/kg of seed	73.05 bcd (91.50)	23.15 bc	8.10 bcd	2279 cd	24.41 bc (17.08)	55.67	1330 bcd	16.02
T_3	Seed biopriming for 10 hrs with suspension of talc based formulation ($2x10^8$ cfu/g) of *Trichoderma virens* @ 50 g in 250 ml of water/kg of seed	69.44 cde (87.67)	22.48 cd	7.93 bcde	2114 de	25.70 bc (18.81)	51.18	1313 cd	14.93
T_4	Seed biopriming for 10 hrs with suspension of talc based formulation ($2x10^8$ cfu/g)of *Pseudomonas fluorescens* @ 50 g in 250 ml of water/kg of seed	67.58 de (85.45)	21.03 d	7.31 de	1914 e	27.54 b (21.38)	44.51	1230 de	9.19
T_5	T_1+ Soil application of *T. viride* enriched FYM (10g/ kg FYM) @ 100 g/ m^2 of soil	79.51 a (96.69)	25.22 a	9.12 a	2734 a	17.04 e (8.59)	77.72	1535 a	27.23
T_6	T_2+ soil application of *T. harzianum* enriched FYM (10g/ kg FYM) @ 100 g/ m^2 soil	76.24 ab (94.34)	24.79 ab	8.71 ab	2552 ab	18.22 de (9.78)	74.62	1466 ab	23.81
T_7	T_3+ soil application of *T. virens* enriched FYM (10g/ kg FYM) @ 100 g/ m^2 soil	74.29 abc (92.67)	24.43 abc	8.28 abc	2426 bc	20.14 d (11.86)	69.22	1457 ab	23.33
T_8	T_4+ soil application of *P. fluorescens* enriched FYM (10g/ kg FYM) @ 100 g/ m^2 soil	68.29 de (86.32)	20.65 d	7.57 cde	1929 e	27.14 b (20.81)	45.99	1285 cd	13.07
T_9	Hydropriming of seed *i.e.* soaking of chickpea seed @ 250 ml of water/kg seed for 10 hrs.	65.32 e (82.57)	18.12 e	7.22 e	1660 f	35.52 a (33.75)	12.41	1220 de	8.44
T_{10}	Untreated check	59.52 f (74.27)	15.21 f	6.42 f	1286 g	38.37 a (38.53)	--	1117 e	--
	S.Em.±	--	--	--	--	--	--	--	--
	Year	**0.84**	**0.29**	**0.12**	**31.46**	**0.44**	--	**21.05**	--
	Treatment	**1.75**	**0.60**	**0.26**	**66.95**	**1.02**	--	**42.84**	--
	YxT	**2.67**	**0.91**	**0.38**	**99.49**	**1.38**	--	**66.56**	--
	CD at 5%	--	--	--	--	--	--	--	--
	Treatment	**4.99**	**1.71**	**0.73**	**191.35**	**2.90**	--	**122.10**	--
	YxT	**NS**	**NS**	**NS**	**NS**	**NS**	--	**NS**	--
	CV %	**6.54**	**7.20**	**8.42**	**8.10**	**9.30**	--	**8.64**	--

Note: Treatment means with the letter/ letters in common are not significant by Duncan's New Multiple Range Test at 5% level of significance.
Figures in parentheses are original values, while those outside are arcsine transformed values.

Table.2 Economics of various bioagents used for the management of wilt and root rot of chickpea

Sr. No.	Treatments	Total expenditure with labour charges (Rs./ha)	Grain yield (kg/ha)	Income (Rs./ha)	Additional income over control (Rs./ha)	ICBR
T_1	Seed biopriming for 10 hrs with suspension of talc based formulation ($2x10^8$ cfu/g) of *Trichoderma viride* @ 50 g in 250 ml of water/kg of seed	375	1392	97440	19250	1: 51.33
T_2	Seed biopriming for 10 hrs with suspension of talc based formulation ($2x10^8$ cfu/g) of *Trichoderma harzianum* @ 50 g in 250 ml of water/kg of seed	375	1330	93100	14910	1: 39.76
T_3	Seed biopriming for 10 hrs with suspension of talc based formulation ($2x10^8$ cfu/g) of *Trichoderma virens* @ 50 g in 250 ml of water/kg of seed	375	1313	91910	13720	1: 36.59
T_4	Seed biopriming for 10 hrs with suspension of talc based formulation ($2x10^8$ cfu/g) of *Pseudomonas fluorescens* @ 50 g in 250 ml of water/kg of seed	375	1230	86100	7910	1: 21.09
T_5	T_1+ Soil application of *T. viride* enriched FYM (10g/ kg FYM) @ 100 g/ m^2 of soil	2900	1535	107450	29260	1: 10.09
T_6	T_2+ Soil application of *T. harzianum* enriched FYM (10g/ kg FYM) @ 100 g/ m^2 soil	2900	1466	102620	24430	1: 8.42
T_7	T_3+ Soil application of *T. virens* enriched FYM (10g/ kg FYM) @ 100 g/ m^2 soil	2900	1457	101990	23800	1: 8.21
T_8	T_4+ Soil application of *P. fluorescens* enriched FYM (10g/ kg FYM) @ 100 g/ m^2 soil	2900	1285	89950	11760	1: 4.06
T_9	Hydropriming of seed *i.e.* soaking of chickpea seed @ 250 ml of water/kg seed for 10 hrs.	75	1220	85400	7210	1: 96.13
T_{10}	Untreated check	--	1117	78190	--	--

Cost of inputs

S. No.	Inputs	Price (Rs.)	Sr. No.	Inputs	Price (Rs.)
1	*Trichoderma viride*	120/ kg	5	Farm Yard Manure (FYM)	1.25/kg
2	*Trichoderma harzianum*	120/ kg	6	Selling Price of Chickpea	70/kg
3	*Trichoderma virens*	120/ kg	7	Labour charge per day	150/day
4	*Pseudomonas fluorescens*	120/ kg			

The *Trichoderma* spp. (*T. harzianum*, T. hamatum, and *T. viride*) are well documented for being efficacious mycoparasites of soil-borne fungi such as *Fusarium*, *Pythium*, and *Rhizoctonia* (Papavizas *et al.*, 1984; Mohiddin *et al.*, 2010). Soil application of *T. harzianum*, *T. viride*, and *T. virens* has been found to be effective in controlling root rot (Khan and Gupta, 1998; Ganesan *et al.*, 2007; Kumar *et al.*, 2008) and wilt diseases (Prasad *et al.*, 2002; Dubey *et al.*, 2012). These species multiply rapidly in soil infested with *Fusarium* and *Rhizoctonia* (Khan *et al.*, 2011), evidenced by the significantly greater populations of *Trichoderma* spp. in the pathogen-infested soils. Increase in the CFU count of *Trichoderma* spp. can be attributed to the availability of host pathogens (*F. oxysporum* and *R. solani*) on which these mycoparasites grow and multiply rapidly (Jefries and Young, 1994). The present study has demonstrated that *Trichoderma viride* or *T. harzianum* can be used for controlling wilt and root rot disease complexes of chickpeas in organic farming or in low-input sustainable agriculture. The yield enhancement was also good with the BCAs. In the present study, seed biopriming and soil application of *T. viride* or *T. harzianum* provided better disease control with greater crop yield enhancement. The present research may encourage farmers to integrate bioagents into chickpea agronomy.

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