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Management of Wilt Complex of Chickpea with Seed Biopriming and Soil Application of *Trichoderma* spp.

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ABSTRACT

Background: Chickpea wilt complex caused by several soil-borne pathogens is a serious biotic constraint for chickpea production. **Methods:** To find out the effective management of the disease through seed biopriming and soil application of biocontrol agents under *in vivo* and *in vitro* conditions experiments were carried out during *rabi* 2018-19 and 2019-20 at Anand Agricultural University, Anand, Gujarat. **Result:** Seed biopriming showed a positive impact producing vigorous plant shoot and root system, besides disease control during *in vitro* conditions. While under *in vivo* conditions, the pooled results of two years revealed that seed biopriming for 10 h with the suspension of talc-based formulation (2×10^8 CFU/g) of *Trichoderma viride* or *T. asperellum* @ 50 g in 250 ml of water/kg of seed followed by soil application of *T. viride* or *T. asperellum* enriched FYM (10g/kg FYM) @ 100 g/m^2 of soil found significant for the disease management as well as higher yield. The seed biopriming alone control the disease in the range of 23-34% and increased the yield of chickpea by 23-29%. However, combined applications of seed biopriming as well as soil application significantly control the disease in the range of 51-70% and increased the grain yield by 41-51% over untreated control.

Key words: Chickpea wilt complex, *Fusarium oxysporum* f. sp. *ciceri*, *Fusarium solani*, *Macrophomina phaseolina,* Seed biopriming, *Trichoderma* spp.

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is the largest produced food legume of South Asia and the third-largest produced food legume globally (Suhasini *et al*., 2009). Nearly 172 pathogens (67 fungi, 3 bacteria, 22 viruses and 80 nematodes) have been reported infecting chickpea world-wide (Nene *et al*., 1996), but only a few of them have the potential to devastate the crop. Among them, the chickpea wilt complex is considered the most vital, devastating and challenging disease. The problem is widespread in several countries of the world like India, Iran, Pakistan, Nepal, Burma, Spain, Mexico, Peru, Syria and the USA (Nene *et al*., 1989; Jalali and Chand, 1992).

Wilt complex is caused by several soil-borne pathogens, among them, Fusarium wilt [*Fusarium oxysporum* Schlechtend.: Fr. f. sp. *ciceri* (Padwick) T. Matuo and K. Satõ], black root rot [*Fusarium solani* (Mart.) Sacc.], dry root rot [*Macrophomina phaseolina* (Tassi) Goidanich], wet root rot [*Rhizoctonia solani* Kühn] and collar rot [*Sclerotium rolfsii* Sacc.] are of considerable importance (Nene *et al*., 1981). Management of wilt complex of chickpea is difficult to achieve as the pathogens are soil-borne, surviving through resistant structure *i.e.* chlamydospores and sclerotia in the soil for years even in the absence of host and the crop remains susceptible throughout all the growth stages (Haware *et al*., 1986; Kaiser *et al*., 1994).

The use of chemical fungicides for the effective management of these pathogens is not possible because of the physical heterogeneity of the soil, which might prevent effective concentrations of the chemical from reaching the target pathogen (Tewari and Mukhopadhyay, 2001). To overcome such issues, biological control is one of the best, low-cost and ecologically sustainable methods. Also, the adverse effects of chemicals used in agriculture over decades have changed the mindset of farmers and consumers who are now producing and buying organic foods for their health (Vyas *et al*., 2019). Among various biocontrol agents (BCAs) examined against the plant pathogenic fungi, *Trichoderma* spp. has been widely studied for its biocontrol ability (Sharma *et al*., 2014).

A seed is the preferred technique of agriculture scientists for disseminating any technology as it is an easy means of adaptation. Seed biopriming is a comprehensive approach to agricultural sustainability. It allows rapid seed colonization by beneficial organisms and more uniform coverage of seed surface compared to the other techniques and also very effective technology in suppressing many diseases caused by seed and soil-borne pathogens (Sabalpara, 2015).

Because of the complex nature of the disease and based on the above facts, the present experiment was

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conducted to examine the performance of three important species of *Trichoderma* (*T. viride, T. harzianum* and *T. asperellum*) through seed biopriming alone as well as soil application of bioagents with enrichment of FYM for the disease management and yield parameters of chickpea in pot and field conditions during *Rabi* 2018-19 and 2019-20.

MATERIALS AND METHODS

Isolation of the pathogens and collection of *Trichoderma* spp.

Samples of chickpea root and stem showing characteristic symptoms were collected from infected chickpea fields of Gujarat. A portion of each diseased root/stem/bark tissue of the sample was surface-sterilized with 1% sodium hypochlorite for 30 sec. and incubated on Potato Dextrose Agar (PDA). Among the different isolates three fungi *viz., Fusarium oxysporum* f. sp. *ciceri, F. solani* and *Macrophomina phaseolina* were isolated.

Different *Trichoderma* spp. for the experiment were obtained from the Department of Plant Pathology, BACA, Anand Agricultural University, Anand, the Department of Plant Pathology, CoA, Junagadh Agricultural University, Junagadh and the Department of Plant Pathology, NMCA, Navsari Agricultural University, Navsari.

Pot trial (*In vitro*)

The experiment was conducted at the Department of Plant Pathology, B. A. College of Agriculture, AAU, Anand during *rabi* 2019-20 in completely randomized design with nine treatments along with three replications using variety Gujarat gram 2. Sterile soil was filled in 30 cm earthen pots. The chickpea seeds were treated with a suspension of talc-based formulation of respective *Trichoderma* spp. (2×10^8 CFU/g) @ 50 g product/ 250 ml of water/kg of seed. The bioprimed seeds were shade dried. Ten bioprimed seeds of chickpea were sown in each pot. For pathogen treated check sick pot technique developed by Nene *et al.* (1981) was followed. In which the fungi were grown on a 9:1 sand maize meal medium and added at 100 g/2 kg of soil.

The observations on seed germination (%), growth parameters *i.e.* shoot length (cm) and weight (g), root length (cm) and weight (g), vigour index at 15 DAS and seedling mortality (%) were recorded.

$$\text{Germination (\%)} = \frac{\text{Number of seed germinated}}{\text{Total no. of seed sown}} \times 100$$

Vigour index (Abdul Baki and Anderson, 1973) was calculated as follows.

$$\text{Vigour index} = (\text{Mean root length} + \text{Mean shoot length}) \times \text{Germination (\%)}$$

Seedling mortality was calculated (Pande *et al.,* 2012) as follows.

$$\text{Seedling mortality (\%)} = \frac{\text{Number of infected seedings}}{\text{Total no. of seedlings}} \times 100$$

Field trials (*In vivo*)

The field trials were conducted at Agronomy Farm, BACA, AAU, Anand (22°35′N, 72°-55′E) during *rabi* 2018-19 and 2019-20 in randomized block design with eight treatments along with three replications using variety Gujarat gram 2. The crop was sown with 30 × 10 cm spacing having a gross plot size of 5.0 × 3.0 m and a net plot size of 4.2 × 2.4 m. The seed rate was used at 60 kg/ha. Chickpea seeds were bioprimed similarly as mentioned in pot trial and soil application of respective *Trichoderma* spp. enriched well-decomposed FYM (10 g/kg FYM) @ 100 g/m^2 of soil was done as per the treatments. A pathogen-treated check and an untreated control were also maintained. The observations on seed germination (%), disease incidence (%), seed yield (kg/ha), disease control (%) and yield increase over control (%) were recorded.

$$\text{Disease incidence (\%)} = \frac{\text{Number of disease plants}}{\text{Total no. of plants}} \times 100$$

The per cent disease control was calculated by the following formula given by Wheeler (1969).

$$\text{Disease control (\%)} = \frac{\text{PDI in control- PDI treatment}}{\text{PDI in control}} \times 100$$

The per cent yield increase over control was calculated as,

$$\text{Yield increase over control (\%)} = \frac{\text{Yield in treated plot - Yield in control plot}}{\text{Yield in treated plot}} \times 100$$

The data on all the parameters were analyzed by DNMRT in both of the trials and the economics of the treatments was calculated.

RESULTS AND DISCUSSION

Pot trial (*In vitro*)

The data presented in Table 1 revealed that maximum root length (13.90 cm), root weight (0.97 g), shoot length (24.90 cm), shoot weight (1.95 g), vigour index (3362) and minimum seedling mortality (15.23%) was recorded in treatment T_7 *i.e.* seed biopriming for 10 hrs. with the suspension of talc-based formulation (2×10^8 CFU/g) of *T. asperellum* (AAU isolate) @ 50 g in 250 ml of water/kg of seed followed by T_6 *i.e.* seed biopriming for 10 hrs. with the suspension of talc-based formulation (2×10^8 CFU/g) of *T. viride* (NAU isolate) @ 50 g in 250 ml of water/kg of seed which also recorded better root length (12.50 cm), root weight (0.81 g), shoot length (22.00 cm), shoot weight (1.86 g), vigour index (2875) and seedling mortality (19.88%). While data revealed a non-significant result in the germination per cent was in the range of 83.33 to 86.67%.

It can be therefore concluded that besides disease control, biopriming has a positive impact on germination and producing vigorous plant shoot and root system, which is key for better production.

Table 1: Effect of seed biopriming of bioagents on growth parameters and wilt complex incidence of chickpea in pot condition.

Tr. no.	Treatments	Germination (%)	Root length (cm)	Root weight (g)	Shoot length (cm)	Shoot weight (g)	Vigour index	Seedling mortality (%)
1	Seed biopriming for 10 hrs. with the suspension of talc-based formulation (2×10^8 CFU/g) of *T. harzianum* (AAU isolate) @ 50 g in 250 ml of water/kg of seed	83.33	10.57^{c}	0.59^{e}	18.33^{cde}	0.82^{e}	2408^{f}	36.91^{cd} (36.06)
2	Seed biopriming for 10 hrs. with the suspension of talc-based formulation (2×10^8 CFU/g) of *T. harzianum* (JAU isolate) @ 50 g in 250 ml of water/kg of seed	86.67	12.00^{bc}	0.69^{cd}	19.00^{cd}	0.91^{d}	2687^{d}	32.57^{bc} (28.98)
3	Seed biopriming for 10 hrs. with the suspension of talc-based formulation (2×10^8 CFU/g) of *T. harzianum* (NAU isolate) @ 50 g in 250 ml of water/kg of seed	83.33	11.23^{bc}	0.60^{e}	18.40^{cde}	0.82^{e}	2468^{f}	36.74^{cd} (35.78)
4	Seed biopriming for 10 hrs. with the suspension of talc-based formulation (2×10^8 CFU/g) of *T. viride* (AAU isolate) @ 50 g in 250 ml of water/kg of seed	86.67	12.50^{ab}	0.75^{bc}	19.50^{c}	1.05^{c}	2773^{c}	28.74^{abc} (23.12)
5	Seed biopriming for 10 hrs. with the suspension of talc-based formulation (2×10^8 CFU/g) of *T. viride* (JAU isolate) @ 50 g in 250 ml of water/kg of seed	86.67	11.47^{bc}	0.63^{de}	18.63^{cde}	0.88^{d}	2608^{e}	33.70^{bc} (30.79)
6	Seed biopriming for 10 hrs. with the suspension of talc-based formulation (2×10^8 CFU/g) of *T. viride* (NAU isolate) @ 50 g in 250 ml of water/kg of seed	83.33	12.50^{ab}	0.81^{b}	22.00^{b}	1.86^{b}	2875^{b}	26.48^{ab} (19.88)
7	Seed biopriming for 10 hrs. with the suspension of talc-based formulation (2×10^8 CFU/g) of *T. asperellum* (AAU isolate) @ 50 g in 250 ml of water/kg of seed	86.67	13.90^{a}	0.97^{a}	24.90^{a}	1.95^{a}	3362^{a}	2.97^{a} (15.23)
8	Treated check with *Fusarium* spp. + *M. phaseolina*	73.33	7.80^{d}	0.38^{g}	16.50^{e}	0.57^{f}	1782^{h}	50.12^{e} (58.89)
9	Control (Untreated check)	83.33	8.63^{d}	0.50^{f}	17.00^{de}	0.79^{e}	2136^{g}	43.92^{de} (48.12)
S.Em.±		3.33	0.47	0.02	0.68	0.02	22.03	2.63
CD at 5%		NS	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.
CV %		6.89	7.36	5.90	6.12	2.84	1.49	13.13

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 retransformed values of arcsine transformation.

Table 2: Effect of seed biopriming and soil application of bioagents in management of wilt complex of chickpea under field conditions (pooled: *Rabi* 2018-19 and 2019-20).

Trt. no.	Treatments	Germination (%)	Disease incidence (%)	Disease control (%)	Seed yield (kg/ha)	Yield increase over control (%)
T_1	SB for 10 hrs. with talc-based formulation (2× 10^8 CFU/g) of *T. viride* @ 50 g in 250 ml of water/kg of seed	86.84^{bc}	32.01^{c}	33.67	1222^{d}	28.40
T_2	SB for 10 hrs. with talc-based formulation (2 × 10^8 CFU/g) of *T. harzianum* @ 50 g in 250 ml of water/kg of seed	84.25^{cd}	37.12^{c}	23.08	1137^{d}	23.04
T_3	SB for 10 hrs. with talc-based formulation (2 × 10^8 CFU/g) of *T. asperellum* @ 50 g in 250 ml of water/kg of seed	85.88^{bc}	33.65^{c}	30.27	1197^{d}	26.90
T_4	T_1 + SA of *T. viride* enriched FYM (10 g/kg FYM) @ 100 g/m^2 of soil	93.84^{a}	14.72^{a}	69.50	1775^{a}	50.70
T_5	T_2 + SA of *T. harzianum* enriched FYM (10 g/kg FYM) @ 100 g/m^2 of soil	90.34^{bc}	23.48^{b}	51.35	1494^{c}	41.43
T_6	T_3 + SA of *T. asperellum* enriched FYM (10 g/kg FYM) @ 100 g/m^2 of soil	92.03^{b}	16.99^{a}	64.79	1727^{b}	49.33
T_7	Treated check with *Fusarium* spp. + *M. phaseolina*	68.49^{e}	57.10^{e}	-	722^{e}	-
T_8	Control (Untreated check)	78.58^{d}	48.26^{d}	-	875^{e}	-
	S.Em± T	2.10	1.71		57.01	
	Y	1.16	0.87		31.44	
	T x Y	3.29	2.48		88.92	
	C.V. %	6.70	13.06		12.14	

Note: SB -Seed biopriming; SA- Soil application.

Treatment means with the letter/letters in common are not significant by Duncan's New Multiple Range Test at 5% level of significance.

Table 3: Economics of the treatments.

Trt.no.	Quantities of bioagent required (kg/ha)	Cost of bioagent (` /ha)	Quantities of FYM required (kg/ha)	Cost of FYM (` /ha)	Labour charges (` /ha)	Total cost of treatments (` /ha)	Seed yield (kg/ha)	Net gain over control (kg/ha)	Realization (` /ha)	Net profit (` /ha)	ICBR
T_1	3.00	360.00	-	-	318.40	678.40	1222	347	17350	16671.60	1:24.57
T_2	3.00	360.00	-	-	318.40	678.40	1137	262	13100	12421.60	1:18.31
T_3	3.00	360.00	-	-	318.40	678.40	1197	322	16100	15421.60	1:22.73
T_4	13.00	1560.00	1000	1000.00	674.40	3234.40	1775	900	45000	41765.60	1:12.91
T_5	13.00	1560.00	1000	1000.00	674.40	3234.40	1494	619	30950	27715.60	1:8.57
T_6	13.00	1560.00	1000	1000.00	674.40	3234.40	1727	852	42600	39365.60	1:12.17
T_7	-	-	-	-	-	-	722	-	-	-	-
T_8	-	-	-	-	-	-	875	-	-	-	-

Note: Labour charges @ 318.40 ` /labour per day; 178.00 `/unskilled labour per day.

The selling price of chickpea seeds: 50 ` /kg.

Price: Bioagents: 120 ` /kg; FYM: 01` /kg.

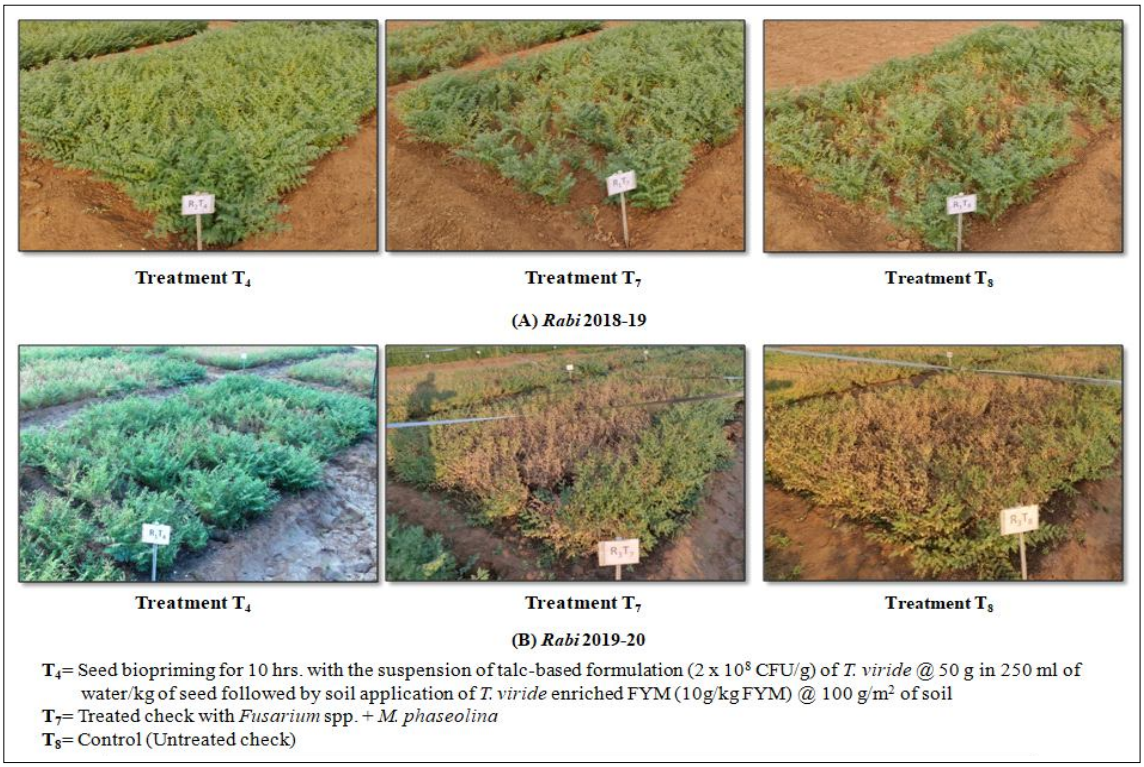


Fig 1: Managment of wilt complex of chickpea under field condition.

Field trials (*In vivo*)

The pooled data of the year 2018-19 and 2019-20 presented in Table 2 and Fig 1 revealed that treatment T_4 *i.e.* seed biopriming for 10 hrs. with the suspension of talc-based formulation (2×10^8 CFU/g) of *T. viride* @ 50 g in 250 ml of water/kg of seed followed by soil application of *T. viride* enriched FYM (10 g/kg FYM) @ 100 g/m^2 of soil recorded highest germination (93.84%), lowest disease incidence (14.72%), highest disease control (69.50%) with the highest yield (1775 kg/ha) and maximum yield increase over control of (50.70%) followed by treatment T_6 *i.e.* seed biopriming for 10 hrs. with the suspension of talc-based formulation (2×10^8 CFU/g) of *T. asperellum* @ 50 g in 250 ml of water/kg of seed followed by soil application of *T. asperellum* enriched FYM (10 g/kg FYM) @ 100 g/m^2 of soil which recorded better germination (92.03%), lower disease incidence (16.99%), disease control (64.79%) and yield (1727 kg/ha) as compared to the treated and untreated check.

Economics of various treatments (Table 3) indicated that the maximum net realization was obtained in treatment T4 (45000 ` /ha) followed by T6 (42600 ` /ha). Looking to the ICBR, the highest return was obtained in treatment T1 (1:24.57) followed by T3 (1:22.73), however, the efficacy of these treatments was moderate against wilt complex.

It is evident from the data that seed biopriming followed by soil application of *Trichoderma* spp. found significant for control of the disease as well as gaining higher yield as compared to their single-use.

Similar results were also found by earlier workers. Prasad *et al.* (2002) and Mehta and Gangopadhyay (2017) found seed treatment + soil application of bioagents provided better control as compared to their use as either seed or soil treatment alone in reducing wilt (*F. oxysporum* f. sp. *ciceri*), wet root rot (*R. solani*) of chickpea and dry root rot (*M. phaseolina*) of mungbean under artificial soil inoculation condition. Shamarao *et al.* (2002) also recommended *T. viride* as an eco-friendly approach for the management of chickpea wilt as seed treatment with *T. viride* (Co) + soil application recorded 12.1 per cent wilt incidence compared to control (42.7%).

Animisha *et al.* (2012), Manjunatha *et al.* (2013), Sayyad *et al.* (2015), Lakhran and Ahir (2018) and Jat and Kumawat (2020) also found *T. viride* more effective compared to other different bioagents. In the present experiment, *T. asperellum* was found at par with *T. viride* in many parameters which were supported by Nagamani *et al.* (2018) as they found seed treatment and soil application of *T. asperellum* recorded the lowest dry root rot disease incidence (12.93%) and highest yield (1751 kg/ha) of chickpea.

CONCLUSION

Chickpea wilt complex was effectively and economically managed under *in vivo* and *in vitro* conditions through seed biopriming of *Trichoderma* spp. Under *in vitro* conditions, *T. asperellum* (AAU isolate) recorded maximum root length and weight, shoot length and weight, vigour index and minimum seedling mortality. Whereas, under *in vivo* conditions, the pooled results of two years revealed that seed biopriming of *T. viride* or *T. asperellum* followed by soil application of *T. viride* or *T. asperellum* enriched FYM found significant for

the disease management as well as higher yield. Here, the combined application of seed biopriming followed by soil application was found significant as compared to only seed biopriming.

Conflict of interest

All authors declared that there is no conflict of interest.

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