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POTENTIALITY OF BIOAGENTS ON SEED QUALITY ENHANCEMENT IN CHICKPEA

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A B S T R A C T

Planting healthy seeds and other inputs have an impact on seed quality, fungal invasion deteriorates the seed quality in soil and storage. Studies on chickpea carried out at Seed Research and Technology Center, Rajendranagar, Hyderabad during spring 2010-11 & 2011-12 revealed that benomyl was found effective followed by *Pseudomonas fluorescens* in the inhibition of mycelial growth of *Fusarium solani*. Maximum germination percentage was recorded with Benomyl (Benomyl 500 WP) @ 2 g/kg seed (treated check) and Tebuconazole @ 1 ml/ kg seed (95 per cent) followed by treatment with *P. fluorescens* @ 10 g/kg seed along with soil application of *P. fluorescens* @ 3 kg/acre (94 per cent). Maximization of growth parameters like root length, shoot length and total seedling length were observed with Benomyl @ 2 g/ kg seed as 17.0 cm, 10.3 cm and 27.3 cm, respectively. Considering seedling vigour index as an important seed quality character, *P. fluorescens* and Benomyl @ 2 g/kg seed recorded high seedling vigor index. The per cent recovery of infested seeds was found to be low with treated seeds when compared to the control.

Keywords: Chickpea, Seed quality, *P. fluorescens*, Bio-agents.

INTRODUCTION

Chickpea is the most nutritive pulse among different food legumes being extensively used as protein adjunct to starchy diet. Of the various diseases, fungal diseases especially, the wilt caused by species of *Fusarium* remains to be a challenging task in terms of management since it is soil-borne in nature (Agrios, 2000; Singh *et al*., 1986).

Planting high quality seeds, one of the cheapest input in crop production, is the key to agricultural progress. Response of other inputs in crop production depends on seed material used. Seed quality of chickpea was affected by incidence of seed borne diseases like wilt and root rot. Earlier workers reported influence of cultural, regulatory, physical, chemical and biological methods on the control of the seed and soil borne pathogens. In modern agriculture, agrochemicals are unavoidable, but bio-agents are important components in integrated pest management programme and sustainable agriculture (Chaudhary *et al.,* 2007). The combined use of bio-control agents and chemical pesticides has attracted much attention as a way to obtain synergistic or additive effects in the control of soil borne pathogens (Maurya *et al*., 2008). Among these, chemical methods offer a good choice to farmer to combat the diseases (Wise *et al*., 2009). But owing to so many problems in view of ecosystem and environment, a rapid shift has been made from synthetic products to bio products, which are eco friendly and beneficial. Keeping these aspects in view, the present study was carried out based on preliminary studies to find out the effect of various bio-agents on seed quality by minimizing the incidence of seed borne diseases and also increasing soil micro flora.

MATERIAL AND METHODS

Preliminary studies on seed mycoflora of chickpea were carried out under laboratory conditions and their inhibition was tested with four fungicides viz., Azoxystrobin, Tebuconazole, Carbendazim and benomyl and three bio-agents ie., *Trichoderma viride*, *Bacillus subtilis* and *Pseudomonas fluorescence* by rolled towel and seed inoculation technique. Fungicides (0.2 and 0.3%) and bio-agents (0.8 and 1%) were tested in two concentrations against *Fusarium oxysporium* seed pathogenic (root rot and wilt) fungi. Per cent inhibition of mycelia growth was carried out by the seed inoculation and seed rolled technique (Pankaj Sharma, 2010).

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Seed Inoculation Technique: Healthy surface sterilized seeds (1:1000 mercuric chloride) of cultivar Annegiri were artificially inoculated with 7 days old culture of fungal species separately. Inoculated seeds were treated with fungicides. The treated seeds were placed on the moist blotter paper in plates @ 5 seeds/plate. Inoculated untreated seeds served as control. Observations were recorded after 10 days of incubation.

***In-vitro* Evaluation of Fungicides by Seed Rolled Towel Method:** Seeds were treated with fungicides by following wet seed treatment with 0.2 per cent and 0.3 per cent fungicidal solution. Seeds were soaked in the fungicidal solutions for 2 hrs. Then the seeds were dried under shade. Three replications of 50 seeds per treatment were tested in moist paper towel (rolled towel) method. The plates were incubated in BOD incubator at 20±2˚ C under 12 hrs of light and 12 hrs dark. The untreated samples served as control. The per cent germination and per cent infection were recorded. (Pankaj Sharma, 2010).

***In-vitro* Evaluation of Bio-agents by Rolled Paper Towel Method:** The powder formulation of antagonist's viz., *Trichoderma viride, Bacillus subtilis* and *Pseudomonas fluorescence* were used for seed treatment to test their efficacy in overcoming seed borne fungal infection of chickpea under *in-vitro* conditions by rolled towel method. Seeds of moderately infected chickpea were treated with different bio-agents at the rate 10^6 cfu. The seeds were shaken in shaker for 20 min for the coating of bio-agents formulation and then stored in separate boxes for 24 hrs. The treated seeds were tested in 5 replications of 50 seeds per replication. Seeds without treatment served as control. These paper towels were incubated at 20±2˚ C for seven days under 12 hrs light and 12 hrs darkness. After 7 days of incubation, per cent germination, per cent infection and seedling vigor were calculated as germination x seedling length.

Based on the above findings of *in-vitro* conditions, the field study was taken up at Seed Research and Technology Centre, Rajendranagar, Hyderabad during spring, 2010–2012. Treatments comprising of bio-agents, fungicides and their combinations *viz*., *Trichoderma viride* @ 10 g/kg seed, soil application of *T. viride* @ 2 kg/acre, *Bacillus subtilis* @ 5 g/kg seed, Benomyl (Benomyl 500 WP) @ 2 g/kg seed, *Pseudomonas fluorescens* @ 10 g/kg seed, combination of *Trichoderma viride* @ 10 g/kg seed + soil application of *T. viride* @ 2 kg/acre, combination of *B. subtilis* @ 5 g/kg seed + Benomyl @ 2 g/kg seed (treated check), AMISTAR *(*23% w/w a*zoxystrobin)* @ 1 g/kg seed, Tebuconazole (100% Raxil T @ 25 g/l = Tebuconazole 4 g/l + Triflunuron) @ 1 ml/kg seed, *P. fluorescens* @ 10 g/kg seed + foliar spray of *P. fluorescens* @ 6-10 g/l, *P. fluorescens @ 10 g/kg seed* + soil application of *P. fluorescens* @ 3 kg/acre along with untreated check were imposed. The experiment was laid out in a randomized block design in three replications with all the recommended agronomic practices. Observations on various disease parameters such as crop stand, per cent disease incidence of wilt and root rot was assessed. Yield attributing characters *viz.*, plant height, branches per plant, pods per plant, seeds per plot, seed yield per plot (g), seed yield per ha (q) and 100 seed weight (g) were recorded for 10 randomly selected plants from each treatment. Various seed quality parameters like germination percentage, root length (cm), shoot length (cm), total seedling length and seedling vigor index I were studied under laboratory conditions as per the ISTA rules (1985) and expressed in percentage. Ten normal seedlings were selected at random in each replication for recording seedling length in centimeters (cm) and oven dried at 80ºC for 17 h and weighed (g) for seedling dry weight. Seed vigor index I was calculated as product of germination and seedling length (Abdul-Baki and Anderson, 1973). Average values were computed and the data was subjected to statistical analysis (Panse and Sukhatme, 1985).

RESULTS AND DISCUSSION

All the fungicides and bio-agents significantly reduced mycelia growth as compared to control. Minimum per cent mycelia growth was observed in Tebuconazole tested against *Aspergillus niger.* The per cent inhibition in mycelia growth was in the range of 24.9 to 96 per cent in different tested fungicides (Table 1). Among the fungicides tested, benomyl was more effective in maximum germination and minimum seed borne infestation (92 and 16) followed by Pseudomonas (84 and 18). These results are in agreement in the inhibition of mycelial growth of *Fusarium solani*. with those of Singh and Jha (2003).

At higher concentration, all fungicides were found effective in controlling seed borne mycoflora. These results are in agreement with the findings of Pankaj Sharma (2010) where the seed borne mycoflora was effectively controlled by fungicides.

Table 1. Effect of fungicides and bio-agents on % germination, % infection and vigour index of chickpea variety.

Treatment	Percent germination			Percent infection			Vigour index		
	0.8 conc.	1.0 conc.	Mean	0.8 conc.	1.0 conc.	Mean	0.8 conc.	1.0 conc.	Mean
Trichoderma sp.	72.02	72.18	72.10	26.58	25.42	22.00	1581	1608	1594
	(58.09)	(59.16)	(50.15)	(24.12)	(24.09)	(20.23)			
Bacillus subtilis	70.46	72.39	71.42	30.14	25.46	27.80	1321	1448	1385
	(56.20)	(59.65)	(58.09)	(27.70)	(22.12)	(21.16)			
Pseudomonas sp.	84.12	84.87	84.49	20.58	16.25	18.41	1706	1728	1717
	(68.07)	(68.28)	(68.02)	(18.09)	(21.25)	(16.36)			
	2% conc.	3% conc.	Mean	2% conc.	3% conc.	Mean	2% Conc.	3% conc.	Mean
Trifloxystrobin	78.00	84.12	81.06	19.50	19.75	20.62	882	776	829
	(62.05)	(66.18)	(62.42)	(15.87)	(16.10)	(15.95)			
Tebuconazole	83.25	83.56	83.40	24.72	20.66	18.60	1417	1522	1469
	(68.65)	(66.61)	(66.16)	(22.01)	(19.36)	(19.25)			
Carbendazim	73.80	75.26	79.03	20.65	18.93	19.79	1267	1348	1323
	(59.61)	(60.23)	(60.14)	(18.96)	(15.26)	(17.68)			
Benomyl	90.60	93.52	92.03	16.25	16.58	16.41	1576	1668	1622
	(72.17)	(75.26)	(74.83)	(24.02)	(22.60)	(22.03)			
Control	54.26	58.50	55.38	53.80	56.25	55.02	758	758	758
	(48.30)	(49.05)	(42.86)	(47.02)	(49.76)	(48.97)			
S.Em.+	0.46	0.17	0.46	0.31	0.28	0.68	21.32	19.76	30.10
CD	1.26	0.68	2.30	1.2	1.57	1.13	78.20	54.32	112

At higher concentration, all the metabolic activities of all fungi could be arrested which in turn results into complete destroy of the internal parts of the seed (Ibiam *et al.,* 2006).

The bio-agents were tested for their efficacy against seed borne fungal infection of chickpea by rolled paper towel method. Among the three (Table 2) bio-agents tested, *Pseudomonas fluorescence* Migula showed minimum seed infection, maximum per cent germination and vigor index of 18.41, 84.49 and 1717, respectively and which differed significantly from seed treatment with *Trichoderma viride* Rifai and *Bacillus subtilis*. Seed treatment with 1.0 percent concentration of *Pseudomonsa fluorescence* Migula exhibited seed infection of 16.25 per cent, germination of 84.87 per cent and vigour index of 1728, where as it was found similar with benomyl treatment in maximizing vigour index. Seed treatment with *Trichoderma viride* was found ineffective as it resulted in seed infection of 22 per cent, with a germination and vigor index of 72.10 and 1594, respectively. Similar work was carried out by Manoranjitham *et al.* (2003) who reported effective control of coriander wilt by seed treatment with *Pseudomonas*. Seed treatment with strains of *Pseudomonas* increased emergence and yield of pigeon pea planted in *Fusarium* infested soil and were equivalent to fungicidal seed treatment (Gupta *et al.,* 2011). Seed quality is the one of the most important basic input characterized in terms of longer viability and good vigor. Besides quantity of seed realized and their effectiveness in reducing disease incidence, quality of seed also plays an important role in adjudging the performance of bio-agents for chickpea crop.

Table 2. Effect of fungicides and bio-agents on mycelial growth inhibition of seed mycoflora.

	Alternaria alternata	*Fusarium solani*	*Aspergillus flavus*	*Colletotrichum spp.*
Trichoderma sp.	80.02 (78.52)	70.62 (67.80)	71.25 (68.45)	83.25 (78.42)
Bacillus subtilis	72.46 (66.20)	82.42 (65.46)	75.03 (72.64)	78.98 (75.86)
Pseudomonas sp.	83.44 (80.82)	86.81 (79.61)	80.64 (78.91)	72.34 (69.75)
Amistar	65.45 (54.61)	76.50 (73.93)	82.78 (79.86)	69.30 (64.74)
Tebuconazole	82.36 (74.90)	84.70 (76.22)	24.96 (21.22)	82.70 (77.62)
Carbendazim	80.22 (73.41)	82.63 (76.10)	83.66 (79.64)	85.84 (81.70)
Benomyl	67.64 (63.61)	96.54 (91.56)	84.05 (80.56)	69.36 (67.12)
Control	86.80 (80.42)	84.63 (79.71)	86.25 (80.06)	75.03 (73.89)
C.V. (%)	1.26	2.86	1.34	1.25
C.D. (0.05)	2.13	4.04	2.07	2.16

Seed treatment with *P. fluorescens* @ 10 g/kg seed recorded minimum germination of 83.0 per cent (Table 3). While, maximum germination was recorded with Binomil seed treatment @ 2 ml/kg seed and Tebuconazole @ 1 ml/ kg seed (95 per cent) followed by treatment with *P. fluorescens* @ 10 g/kg seed along with soil application of *P. fluorescens* @ 3 kg/acre (94 per cent). Similar results of plant growth promotion and reduced wilt incidence by *P. fluorescens* in pigeonpea was reported by Gupta *et al.* (2011). Similarly, root length, shoot length and total seedling length were highest in case of seed treatment with Benomyl @ 2 ml/ kg seed (17.0 cm, 10.3 cm and 27.3 cm respectively).

Considering seedling vigor index as an important seed quality character, Benomyl @ 2g/ kg seed recorded high seedling vigor index (2588) followed by bio-agents. Further, the per cent disease infested seeds were also found to be low with *P. fluorescens* seed treatment. Hence seed quality can be enhanced by *P. fluorescens* instead of chemicals with reduced wilt and root rot incidence.

Table 3. Seed quality characters of chickpea during *rabi*, 2010-12.

Treatments	Germination % First count	Germination % Final count	Root length (cm)	Shoot length (cm)	Total seedling length (cm)	SVII	% disease infested seed
Trichoderma viride@ 10 g/ kg seed	92	82	12.7	7.4	20.1	1849	20
Bacillus subtilis @ 5 g/ kg seed	85	87	14.6	7.1	21.7	1890	21
Benomyl @ 2 ml/kg seed	92	95	17.0	10.3	27.3	2588	12
AMISTAR (*Azoxystrobin)* @ 1 g/kg seed	88	89	14.1	6.6	20.7	1845	23
Tebuconazole @ 1 ml/kg seed	90	95	15.1	7.3	22.4	2115	13
P. fluorescens @ 10 g/ kg seed	90	94	14.8	7.6	22.4	2206	14
Control	78	84	12.9	7.2	20.1	1694	24
Gr. Mean	87.8	90.1	14.1	7.5	21.6	1955	19.33
S. Em	2.92	2.77	0.36	0.30	0.55	105.54	4.81
S. Ed	4.13	3.92	0.51	0.43	0.78	149.23	6.81
C.D. (5%)	8.55	8.10	1.05	0.88	1.62	308.9	14.09
C . V %	5.77	5.32	4.40	6.97	4.42	9.35	43.14

CONCLUSION

Seed treatment with *P. fluorescens* @ 8-10 g/kg results in quality seed. In addition Tebuconazole and Benomyl (treated check) also results in reduced seed rots. Of late use of bio-pesticides for sustainable agricultural production in an eco-friendly manner is an essential component. Hence the bio-agents and fungicide combinations would be incorporated in the integrated management of seed rots and wilt incidence under field conditions in future.

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