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Response of fungicides on selective cultivar of chickpea against collar rot in chickpea

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Abstract

Five Fungicides- Propiconazole, Hexaconazole, Bavistin, Topsin M and Vitavax at 100, 250 and 500 ppm concentrations were evaluated for their efficacy against *S. rolfisii* *in vitro*. Propiconazole, Hexaconazole and Vitavax completely inhibited the growth of *S. rolfisii* *in vitro* while Bavistin and Topsin M showed 79.52 and 71.78% growth inhibition respectively at 500 ppm. Investigation on host resistance against collar rot of chickpea *viz.*, DKG 964, BG 372, BG 3051, PUSA 256, BAUG 15, C 235, GAG 1107, JG 315, JG 62 and BG 3043 revealed that out of 10 cultivars tested in pot soil artificially infested with *S. rolfisii*, none showed resistant reaction against the disease. Seed treatment with fungicides significantly reduced the seedling mortality of chickpea when compared with control. Seed treatment with Vitavax @ 2 g/kg of seed proved that best and showed 66.70% disease control followed by Propiconazole @ 2 g/kg.

Keywords: *Sclerotium rolfisii*, *Trichoderma harzianum*, fungicides, chickpea genotypes.

Introduction

Chickpea (*Cicer arietinum* L.), family Fabaceae, is one of the most important leguminous crops grown all around the world (Knights *et al.*, 2007) [13]. Firstly, it was cultivated in south eastern areas of the world but now it is also cultivated in semi-arid regions (Agarwal *et al.*, 2012) [3]. It is not only a major source of dietary protein for human consumption but it also plays an important role in the management of soil fertility because of having the ability of nitrogen fixation in its root nodules (Hossain *et al.*, 2010) [10]. There is a growing demand of chickpea due to its nutritional value. It is the better source of carbohydrates and proteins as compared to other important pulses (Chibbar *et al.*, 2010) [5]. It is free of cholesterol and provides several vitamins and minerals (Wood and Grusak, 2007) [32]. Globally total chickpea cultivated area is 12.0 million ha, with 10.9 million MT production and an average yield of 913 kg ha⁻¹ (Sheehy and Sharma, 2012) [28]. In India, its total production quantity was 7.1 mt with average yield of 885 kg/ha and total cultivated area is 8 m ha (Ghosh *et al.*, 2013) [6]. Under field conditions, *S. rolfisii* has been reported to cause 22 to 50 per cent reduction in yield of chickpea. Ghosh *et al.* (2013) [6] surveyed four chickpea growing states of India i.e. Andhra Pradesh, Karnataka, Madhya Pradesh and Chhattisgarh and reported that losses from collar rot disease ranged from 7.1 to 10.5%.

S. rolfisii control has met with very limited success. This may be due to the prolific growth, extensive host range of the pathogen and having the ability to produce large number of sclerotia that may persist in the soil for several years (Sennoi *et al.*, 2013) [18]. This pathogen causes many diseases like collar rot, sclerotium wilt, stem rot, charcoal rot, seeding blight, damping off, foot rot, stem blight, and root rot in many economically valuable crops (Gopalakrishnan *et al.*, 2005) [7].

Mycelial growth of *S. rolfisii* or the sclerotia germination can be restricted by the use of several fungicides (Zamora *et al.*, 2008) [34]. Some fungicides like vitavax-thiram and vitavax-captan

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can successfully retard the *in vitro* growth of *S. rolfisii*. Other commonly used fungicides to control *S. rolfisii* in several crops are thiram, quitozene, captan, carbendazin, benomyl, oxycarboxin and triadimenol. Mixture of carboxin plus thiram and quitozene has been found most impressive in prohibiting sclerotial formation and mycelial growth (Yaqub and Shahzad, 2006, Singh *et al.* 2017a; Singh *et al.* 2017b; Singh *et al.* 2017c; Singh *et al.* 2018; Tiwari *et al.* 2018; Tiwari *et al.* 2019a; Tiwari *et al.* 2019b; Kour *et al.* 2019; Singh *et al.* 2019) [33, 19, 20, 21, 22, 23, 24, 25, 26]. Virulence of pathogen and serious threat to chickpea that may cause 55-95% mortality of the crop at seedling stage under favourable environmental conditions (Gurha and Dubey, 1982) [9] and susceptibility of chickpea genotypes. Of the various methods used to control plant diseases, the use of chemical Fungicides is very common. However, in view of the complexities arising from the use of chemical pesticides, such as harmful effect on environment and non-target organisms including man, domestic animals, beneficial insects, wild life, the use of highly resistant and less susceptible host as a cultivar has provided a very promising alternative and more effective method for plant disease control. As the genetic resistance is regarded, the only practicable and cost-effective control for such a devastating soil-borne pathogen is selection of cultivars. Therefore, the present study was carried under two headings- *in vitro* screening of fungicides and chickpea genotypes against *S. rolfisii* in pots for resistance in available germplasm accessions.

Materials and Methods

In vitro evaluation of fungicides against *Sclerotium rolfisii* and *Trichoderma harzianum*

Effect of fungicides on radial growth of *S. rolfisii* was studied by poison food technique on PDA. One hundred ml stock solution of 5000 ug/ml a.i. strength of Carbendazim 50 WP, Carboxin, Topsin-M 70 WP, Propiconazole 25 EC and Hexaconazole 5 EC were prepared in sterilized distilled water in 250 ml Erlenmeyer flasks. To obtain the desired concentrations of fungicide in the medium, amount of stock solution to be added in PDA was calculated by using the following formula:

$$C_1V_1 = C_2V_2$$

Where,

C1 = Concentration of the stock solution (ug/ml).

V1 = Volume (ml) of the stock solution to be added to the measured volume of PDA.

C2 = Concentration of desired fungicide (ug/ml).

V2 = Measured volume (ml) of PDA in which fungicide is to be amended.

Required amount of stock solution was poured into 150 ml Erlenmeyer flask containing 60 ml of sterilized melted PDA so as to get final concentrations of 100, 250, and 500 ug/ml PDA for *Sclerotium rolfisii* and concentrations of 25, 50 and 100 ug/ml PDA for *T. harzianum* poisoned with different concentrations of different fungicides was poured into sterilized Petri plates @ 20 ml per plate.

After solidification, each plate was centrally inoculated with 6 mm disc of *Sclerotium rolfisii* taken from 4 days old culture and incubated at $28 \pm 1^\circ\text{C}$. In a B.O.D. incubator. PDA plates inoculated centrally with *S. rolfisii* but not amended with fungicide served as check. Three replicates were maintained for each treatment. Observation on linear growth of the

fungus was recorded after 96 hours of incubation. The data were then converted to per cent inhibition of growth by using the following formula:

$$\text{Per cent growth inhibition (I)} = \frac{C-T}{C} \times 100$$

Where,

C = Colony diameter in check (mm)

T = Colony diameter in the treatment (mm) i.e. in fungicide amended medium.

The per cent inhibition data were then transformed in arc sin $\sqrt{\text{percentage}}$ transformation and then analyzed statistically using completely randomized design.

Screening of chickpea varieties against collar rot

Ten cultivars of chickpea viz., DKG 964, BG 372, BG 3051, PUSA 256, BAUG 15, C 235, GAG 1107, JG 315, JG 62 and BG 3043 were used for screening against collar rot chickpea in pots. The pot soil was infested with mars cultivars of *S. rolfisii* @ 10 g per pot at 10cm depth. Six seeds were sown in each pot at depth of 5cm. Three replications were maintained. Observation of germination was recorded in pot soils infested with *S. rolfisii* as well as in natural pathogen non infested soil. Final observation on post emergence mortality was recorded at 75 days after sowing.

Fungicidal control of collar rot of chickpea

Non-autoclaved soil collected from chickpea plot was used for pot experiments. The texture, pH and electrical conductivity of the soil were, silty loam, 7.5 and 0.16 mhos respectively. Earthen pots of 24 cm diameter (4 kg soil capacity) were filled with soil and then inoculated with culture of *S. rolfisii* grown on sorghum grains at the rate of 10 g per pot. Chickpea seeds of variety Pusa 256 were treated with fungicides viz., Propiconazole, Hexaconazole, Bavistin, Vitavax and Roko @ 2gm per Kg, seed. Six seeds of chickpea were sown in each pot. Each treatment was replicated three times. The pots sown with untreated chickpea seeds in soil infested with culture of *S. rolfisii* @ 10g/plot served as control. Observation on total stand and affected plants were recorded. Final observation on affected plants was recorded 45 days after sowing. Per cent disease control was calculated by applying the following formula:

$$\text{Per cent disease control} = \frac{C-T}{C} \times 100$$

Where,

C = Per cent mortality in check inoculated with *S. rolfisii*.

T = per cent mortality in treatment.

Results and Discussion

In vitro evaluation of different fungicides against *Sclerotium rolfisii* and *Trichoderma harzianum*

Five fungicides viz., Propiconazole, Hexaconazole, Bavistin, Topsin M and Vitavax at 100, 250, and 500 $\mu\text{g/ml}$ concentrations were evaluated against *Sclerotium rolfisii*. The results indicated that all the fungicides at each concentration significantly inhibited the growth of *Sclerotium rolfisii* when compared with control. Three fungicides namely Propiconazole, Hexaconazole and Vitavax proved highly effective and showed complete inhibition of radial growth of *Sclerotium rolfisii* at all concentration i.e. 100, 250, and 500 ppm. Bavistin showed 32.44, 62.07 and 79.52 per cent

inhibition of growth of *Sclerotium rolfsii* at 100, 250, and 500 µg/l respectively. Topsin M was least effective in order of efficacy and showed 5.50, 49.41 and 71.78 per cent inhibition of growth of *S. rolfsii* (Table 1). Of the five fungicides (Bavistin, Topsin M, Hexaconazole, Propiconazole and Vitavax) tested. Propiconazole, Hexaconazole and Vitavax at all three concentration i.e. 100, 200 and 500 ppm completely inhibited the growth of *S. rolfsii* *in vitro*. Bavistin and Topsin M were less effective and cause 79.52 and 71.78 percent inhibition of growth of *S. rolfsii* respectively in present study. Six fungicides *viz.*, Benomyl, Sancozeb, Thiovit, Dithane M-45, Carbendazim and Topsin M were tested against *Sclerotium rolfsii* by food poison method. At low concentration, no fungicide inhibited the growth of *S. rolfsii*. However, at high concentration Dithane M-45 and Sancozeb significantly reduced the growth (Yaqub *et al.*, 2006)^[33].

In the present study, Bavistin, Hexaconazole and Propiconazole were inhibitory to *T. harzianum* even at lower concentration of 25 ppm. Vitavax and Topsin M at lower concentrations were partially inhibitory to the radial growth of

T. harzianum and slowed down the growth. However, on further incubation of such plates *T. harzianum* attained good growth on PDA. Vitavax had fungi static effect on *T. harzianum* since it inhibited the growth temporarily. Therefore, it may also be integrated. Bavistin, Hexaconazole and Propiconazole may not be integrated with *T. harzianum* because it is toxic to *T. harzianum* *in vitro*. The fungi static effect of Vitavax and Thiram against *T. harzianum* has been reported by Mukherjee (1987)^[15] and Kaur (1989)^[12]. The insensitivity of *T. harzianum* to Vitavax-200, Metalaxyl at considerably high concentrations has been reported by Nagpal, 1987 and Kaur, (1989)^[12].

Topsin M was not inhibitory to the radial growth of *T. harzianum* *in vitro* in the present investigation. Since it is not very effective inhibiting the growth of *S. rolfsii* *in vitro*, its integration may not be beneficial from disease control point of view. Vitavax showed fungistatic effect and accordingly, the growth was very slow. Abd-El moity *et al.* (1982)^[1] developed new biotypes of *T. harzianum* tolerant to chlorothalonil (Kavach).

Table 1: Evaluation of different fungicides against *S. rolfsii* *in vitro*

Fungicides.	Conc. µg/ml	Colony diameter (mm)	Growth inhibition (%)
		96 hrs	96 hrs
Propiconazole	100	0.00	100.00* (89.96)**
	250	0.00	100.00 (89.96)
	500	0.00	100.00 (89.96)
Hexaconazole	100	0.00	100.00 (89.96)
	250	0.00	100.00 (89.96)
	500	0.00	100.00 (89.96)
Bavistin	100	60.80* (37.43)**	32.44 (18.92)
	250	34.13 (19.95)	62.07 (38.35)
	500	18.43 (10.62)	79.52 (52.65)
Topsin-M	100	85.00 (58.19)	5.50(3.18)
	250	45.53 (27.08)	49.41 (29.60)
	500	25.40 (14.71)	71.78 (45.85)
Vitavax	100	0.00	100.00 (89.96)
	250	0.00	100.00 (89.96)
	500	0.00	100.00(89.96)
Control(<i>S. rolfsii</i>)	-	90.00 (64.13)	0.00
SME		0.14	0.23
C.D at 5%		0.41	0.67
C.V		2.12	0.61

*Mean of 03 replications.

**Values given in parentheses are Arcsin $\sqrt{\quad}$ transformaion

Screening of chickpea varieties against collar rot in pot

Ten chickpea varieties were screened against collar rot under pot conditions at Tirhut College of Agriculture, Dholi, Muzaffarpur, during rabi season of 2014-2015. Six seeds of each genotype were sown per pot, and 3 replications were maintained. Final observation on collar rot incidence was recorded at 75 days after sowing. Percent incidence of collar rot was calculated as per formula given in materials and methods.

Healthy seeds of selected ten genotypes *viz.*, BAUG 15, BG 3043, BG 3051, BG 372, C 235, DKG 964, GAG 1107, JG 315, JG 62, PUSA 256 were sown in infested pots. It was found that all genotypes were highly susceptible to *S. rolfsii*. Genotypes BAUG 15, BG 3043, BG 3051, BG 372, DKG 964, JG 315 and PUSA 256 showed 100 per cent germination where as C 235, GAG 1107 and JG 62 recorded 94.4 per cent germination in uninoculated soil. On the other hand per cent germination in inoculated soil was maximum in DKG 964

(83.3%) followed by BG 3043 (77.8%) where as the germination percentage in remaining varieties reduced. Pre emergence mortality was maximum in BAUG 15 (83.3%) followed by GAG 1107(77.8%). On the other hand the pre emergence mortality is minimum in DKG 964 (16.7%) followed by BG 3043 (22.2%). Post emergence mortality was maximum i.e. 33.30 per cent in DKG 964, JG 62 and Pusa 256. Total mortality per cent was maximum in BAUG 15 (100%) followed by GAG 1107 (94.5%) and BG 372 (84.3%).

The utilization of resistant varieties is a classical approach to prevent losses caused due to diseases. Keeping this in view, 10 cultivars of chickpea were screened against *S. rolfsii* in artificially inoculated pot soil. The disease incidence in different varieties ranged between 50.0 to 100.0 percent. All the genotypes recorded highly susceptible reaction against collar rot of chickpea (Table 2).

Table 2: Evaluation of Chickpea genotypes for resistance against *S. rolfisii* in pots

Genotypes	Germination test (%)	Germination in <i>S. rolfisii</i> infested pot soil (%)	Pre emergence mortality (%)	Post emergence mortality (%)	Total mortality (%)	Disease reaction
BAUG 15	100*	16.7*	83.3*	16.7*	100.0*	HS
BG 3043	100	77.8	22.2	27.8	50.0	HS
BG 3051	100	66.7	33.3	27.8	61.1	HS
BG 372	100	33.3	66.7	16.7	84.3	HS
C 235	94.4	38.9	61.1	16.7	77.8	HS
DKG 964	100	83.3	16.7	33.3	50	HS
GAG 1107	94.4	22.2	77.8	16.7	94.5	HS
JG 315	100	44.4	55.6	22.2	77.8	HS
JG 62	94.4	61.1	38.9	33.3	72.2	HS
PUSA 256	100	55.6	44.4	33.3	77.7	HS

*Mean of 03 replications.

Gupta and Anita Babbar (2006)^[8] reported that the genotypes H 99-264, PG 9425-5 and PG 9425-9 (desi) and HK 00297 and PG 97-313 (Kabuli) exhibited resistance against collar rot of chickpea. Abida Akram *et al.*, (2008)^[2] and Vannia Rajan *et al.*, (2011)^[31] also reported differential varietal reaction from highly resistant to tolerant response among the genotypes tested. Similarly Saifulla *et al.*, (2011)^[17] reported 67 chickpea genotypes as resistant to collar rot disease.

Fungicidal control of collar rot

Five fungicides *viz.*, Propiconazole, Hexaconazole, Bavistin, Topsin-M, and Vitavax were evaluated as seed treatment @ 2.0 g/kg seed for control collar rot of chickpea in pots. The pot soil was infested with mass culture of *S. rolfisii* @10g mass culture/pot.

All the treatment proved significantly superior in controlling the disease when compared with untreated control. Vitavax @ 2.0 g/kg of seed treatment proved to be the best and showed maximum disease control (66.70%). The disease control obtained in Bavistin, Propiconazole and Hexaconazole at 2 g/kg seed showed 46.69, 53.42 and 60.02 respectively and did not differ significantly among each other *i.e.* indicating equally good performance. Minimum disease control of 34.99 percent was recorded in Topsin M seed treatment (Table 3).

Table 3: Fungicidal control of collar rot of chickpea in pot

Fungicides	Seed treatment (g/kg)	Mortality (%)	Disease control (%)
Propiconazole	2.0	35.23* (33.30)**	60.02* (50.76)**
Hexaconazole	2.0	38.80 (38.49)	53.42 (46.91)
Bavistin	2.0	44.44 (41.73)	46.69 (43.09)
Topsin M	2.0	55.5 (48.23)	34.99 (33.37)
Vitavax	2.0	27.81 (22.22)	66.70 (59.20)
Control	-	83.33 (65.86)	0
SME		3.337	4.15
C.D at 5%		9.789	12.24
C.V		13.198	18.41

*Mean of 03 replications.

**Values given in parentheses are Arcsin $\sqrt{\quad}$ transformation

Variety- Pusa 256

Date of sowing- 27/11/2015

Results of the pot experiment presented in table 9 clearly indicated that treatment with fungicides proved significantly superior in controlling collar rot of chickpea when compared with untreated control. Seed treatment with Vitavax @ 2 g/kg of seed proved the best and showed 73.32% disease control followed by Propiconazole @ 2 g/kg of seed treatment but this did not differ significantly with Vitavax 2g. Topsin-M was least effective in controlling the collar rot of chickpea in

pots. Superiority of Vitavax in controlling the collar rot of chickpea caused by *S. rolfisii* has been reported by Shukla *et al.* (1981)^[29] also. Chaube *et al.* (1984)^[4] studied the efficacy of ten fungicides against chickpea wilt complex under field condition and reported that Bavistin 1.0 g or mixture of Bavistin + Thiram (1:4) @ 2.5 g/kg seed used as seed dressers improved germ inability, plant stand and yield as compared to check. Other effective fungicides were Panorm 2.5 g, Bayleton 1.0 g and mixture of Brassicol + Thiram (1:1) 2.5 g/kg seed.

Mahmood (1981)^[14] reported lowest percentage of seedling mortality of chickpea caused by *F. oxysporum* f. sp. *ciceri* and *R. solani* in plots treated with Difolatan closely followed by Thiram + Bavistin and Thiram. Difolatan and Bavistin proved effective in increasing the number of nodules when combined with *Rhizobium*.

In a related pot experiment, the effect of 0.2% Captan, Benomyl, Prochloraz, Mancozeb, Bavistin and Thiram on chickpea growth under artificial inoculation conditions was studied. Bavistin gave the highest seed germination percentage, shoot length and fresh weight, while it also gave the lowest infection percentage. Captan, Benomyl and Mancozeb gave the highest root length, dry weight and number of nodules reported by Thakur *et al.* (2004)^[30].

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