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# Management of chickpea collar rot by integration of biological and chemical seed treatment

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#### Abstract

Biologically and chemically treated chickpea seeds observed after 45 DAS showed that the integration of soil application of maize grain based culture of *Trichoderma harzianum* (10 g per pot) with Vitavax seed treatment @ 2 g/kg seed proved best combination and gave maximum disease control over check (79.95%). Integration of seed treatment with *T. harzianum* and Vitavax showed 66.70% disease control which was higher than Vitavax 0.2% seed treatment alone but did not differ significantly with each other.

Keywords: Sclerotium rolfsii, Trichoderma harzianum, fungicides, chickpea genotypes

#### Introduction

Collar rot of chickpea caused by Sclerotium rolfsii is an important soil borne and fast spreading fungal pathogen, which causes considerable damage to the plant stand. Seedling mortality in chickpea due to S. rolfsii has been reported to vary from 54.7 to 95.00 per cent (Shrivastava et al., 1984) [15]. Under field conditions, S. rolfsii has been reported to cause 22 to 50 per cent reduction in yield of chickpea. Ghosh et al. (2013) [9] 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%. Chickpea diseases may cause yield losses of up to 100% depending on time of infection. Dry root rot and collar rot are emerging as a major threat to chickpea production due to drastic climate change (Pande et al., 2010, 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) [13, 17, 18, 19, 20, <sup>21, 22, 23, 24, 19]</sup>. The combination of bio-control agents with fungicides as seed treatment could be very effective against chickpea collar rot, as these pathogens make the plant vulnerable throughout its life starting from rotting of seeds to the death of mature plants. The present study was conducted for eco-friendly and economical management of chickpea collar rot disease by integrating fungicides with T. harzianu.

#### Evaluation of fungicides against Sclerotium rolfsii

Bhuiyan *et al.* (2012) <sup>[2]</sup> tested six fungicides namely Provax-200, Bavistin, Ridomil, Dithane M-45, Rovral 50 WP and Tilt at 100, 200 and 400 ppm concentration for their efficacy against the radial growth of *S. rolfsii*. They observed complete inhibition with Provax-200 at all the selected concentrations. Complete inhibition also obtained at the highest concentration of Tilt. The highest concentration of Rovral 50WP inhibited 93.88% radial growth and significantly superior to Dithane M-45 at the highest concentration. Bavistin and Ridomil were found to be significantly lower when used against the test pathogen.

Thakur *et al.*, (2002) <sup>[26]</sup> evaluated six fungicides, Bavistin (carbendazim), Thiram, Benomyl, Captan, Prochloraz, and Mancozeb for their efficacy against the collar rot (*Sclerotium rolfsii*) of chickpea in pot cultures. Bavistin, Benomyl and Captan were significantly superior to the other fungicides and reduced colony diameter to 1.09, 2.14 and 2.77 cm respectively

Corresponding Author: Md. Shahzaman Ahsan Department of Plant Pathology, Dr. RPCAU, Pusa. Samastipur, Bihar, India (compared with 8.60 cm in the control). These respective fungicides also recorded the lowest collar rot infection (11.0, 22.0 and 27.6%), the highest chickpea seed germination (91.6, 83.3 and 75.0%), and high values for shoot and root lengths, fresh and dry weights, and nodules per plant.

Six fungicides, Benomyl, Sancozeb, Thiovit, Dithane, Carbandazim 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 and Sancozeb significantly reduced the growth (Fouzia and Saleem, 2006) [8].

### Management of S. rolfsii through introduction of Trichoderma in soil

Wells *et al.* (1972) [30] were the first to report conclusively field control of *S. rolfsii* on blue lupines, tomatoes and peanuts by infestation of soil surface with *T. harzianum* grown on an autoclaved mixture of rye grass seed and soil. Backman and Rodriguez-Kabana (1975) [1] used a diatomaceous earth granule impregnated with 10 per cent molasses solution as food base for growing *T. harzianum* and to facilitate dispersal in field. They observed a significant reduction in *S. rolfsii* damage and increase in yield over a 3 year test period in peanuts.

#### **Materials and Methods**

The antagonist *T. harzianum* isolate-4 was selected for the present investigation.

#### Seed treatment Seed coating

100 ml spore suspension (10<sup>8</sup> spores<sup>-ml</sup>) collected from 6 days old culture of *T. harzianum* grown on potato dextrose agar (PDA) plates, was used to coat one kg seed.

#### Seed dressing

*T. harzianum* was multiplied on sterilized pre-boiled maize grains, incubated at  $27 \sim 1$  °C for 20 days. Colonized grains were air dried and powdered ( $10^9$  spores<sup>-g</sup>) and used as seed treatment @ 3 g<sup>-kg</sup>seed. The formulation of antagonist was prepared by thoroughly mixing of carboxymethyl cellulose with *T. harzianum* and used as seed treatment @  $4g^{-kg}$  seed.

#### **Integrated seed treatment**

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. rolfsii* @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. rolfsii* 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 at Tirhut College of Agriculture, Dholi, Muzaffarpur, during rabi season. 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. rolfsii* 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. rolfsii* @ 10g/plot served as control. Standard agronomical practices were followed as per recommendations. Observations on seedlings emergence and final plant stand were made at 15 DAS and at crop maturity stage, respectively 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:

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

Where.

C = Per cent mortality in check inoculated with S. rolfsii.

T = per cent mortality in treatment.

#### Biological control collar rot of chickpea

Non-autoclaved soil filled in earthen pots of 24 cm diameter (4 kg soil capacity) was used for the experiment. Soil was inoculated with mass culture of *S. rolfsii* grown on sorghum grains @ 10 g/pot as described earlier. Thereafter, maize grain based preparation of *T. harzianum* was mixed in upper 5 centimeter soil @ 5 g, 10g, 15 g, 20 g per pot. These pots were then left for 5 days to allow the antagonist to grow and interact with the test fungus in the soil. Appropriate moisture was maintained in pot soil by watering as and when required. Six seeds of chickpea variety Pusa 256 was sown in each pot inoculated with *S. rolfsii* five days after *T. harzianum* application. Observation on pre and post emergence mortality was recorded. Final observation on the disease was recorded 45 days after sowing. Percent disease control was calculated by using the following formula:

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

Where.

C = Per cent mortality in check inoculated with *Sclerotium* rolfsii

T = per cent mortality in treatment.

#### Integrated management of collar rot

Chickpea seeds of variety Pusa 256 were used for integrated management of collar rot. Seeds were treated with *T. harzianum* preparation @ 0.6%. Fungicides Vitavax and Topsin M were also used for seed treatment @ 0.2%. In integrated treatment full dose of *T. harzianum* (0.6%) and half dose of fungicide (0.1%) of Vitavax or Topsin M was used. In integration with soil application of *T. harzianum* (10 g/pot), seed was treated with full dose of fungicide i.e. 0.2%. Three replications were maintained for each treatment. Untreated seeds sown in *S. rolfsii* in infested soil (10 g/pot) served as control. The experiment was conducted in pots and data was analyzed through RBD.

#### Results and Discussion 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. rolfsii* @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 (Table1).

Table 1: 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	

<sup>\*</sup>Mean of 03 replications.

Variety-Pusa 256

Date of sowing- 27/11/2015

Results of the pot experiment presented in table 1 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 66.70% disease control followed by Propiconazole @ 2 g/kg of seed treatment but this did not differ significantly with Vitavax 2 g. 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. rolfsiihas been reported by Shukla et al. (1981) [16] also. Chaube et al. (1984) [3] 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 germinability, 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) [12] reported lowest percentage of seedling mortality of chickpea caused by *F. oxysporum* f. sp. *Cicero* 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) [27].

### Integration of fungicide and *T. harzianum* isolate 4 for management of collar rot of chickpea

Fungicides Vitavax and Topsin M @ 0.1 % were integrated with T. harzianum @ 0.6% per kg seed as seed treatment. Besides, the above fungicides @ 0.2% i.e. 2g/kg seed were integrated with soil application of T. harzianum isolate 4 @ 10g/pot. Results are presented in table 2 and revealed that integration of seed treatment with fungicide and soil application of T. harzianum was most effective in reducing

the mortality of chickpea seedlings. All the treatments proved significantly superior in controlling the disease when compared with check. Maximum disease control of 79.95% was achieved in pots when soil application of *T. harzianum* @ 10 g/pot was integrated with Vitavax @ 0.2 % per kg seed followed by integration of *T. harzianum* @ 6 g with Vitavax @ 0.2 % per kg seed. As seed treatment which gave 59.90 per cent disease control over check, which was at par from seed treatment with Vitavax @ 0.2% alone (53.26%). Seed treatment with Topsin M @ 0.1% + *T. harzianum* @ 0.6% was at par with seed treatment with *T. harzianum* @ 0.6% only which showed 20.01% and 19.92% disease control over check respectively.

It was observed that the post emergence mortality was minimum (0.0%) when Vitavax @ 0.2 % per kg seed was integrated with *T. harzianum* @ 10 g/pot followed by combination of seed treatment with Vitavax @ 0.1% and *T. harzianum* 0.6% per kg seed. Thus result leads to conclusion that maximum management of collar rot of chickpea (79.95%) can be obtained when Vitavax was applied at full dose of seed treatment i.e. 0.2% or 2g/kg seed and integrated with soil application of *T. harzianum* @ 10g/pot. The next best treatment was integration of seed treatment with Vitavax @ 0.1% and seed treatment with *T. harzianum* @ 6g/kg seed. Management of collar rot of chickpea was not satisfactory when Topsin M was integrated with *T. harzianum* either as seed treatment or soil application.

Integrating biological and chemical control seems a very promising way of controlling pathogen with minimum interference with the biological equilibrium (Baker and Cook, 1983) <sup>[7]</sup>. One of the most attractive ways of reducing the amount of fungicide is the integration of sublethal dose of chemicals with biocontrol agents (Chet, 1987) <sup>[4]</sup>. In the present study, seed treatment with fungicide and soil application of *T. harzianum* was integrated for the management of collar rot of chickpea. Integration of Vitavax either with seed treatment with *T. harzianum* or with soil application of *T. harzianum* proved significantly superior in controlling the disease when compared with control. Maximum disease control was achieved when *T. harzianum* as a soil application integrated with Vitavax as seed treatment @ 10 g and 0.2 % per kg seed respectively followed by

<sup>\*\*</sup>Values given in parentheses are Arcsin \(^\tau\) transformaion

integration of Vitavax with seed treatment with *T. harzianum*. There was no significant difference in integration of *T. harzianum* with Topsin M and *T. harzianum* alone in respect of disease control. This indicates that integration of *T. harzianum* with Topsin M may not prove beneficial for disease control. Topsin M alone was not very effective for for management of collar rot of chickpea. Integration of *T. harzianum* with Vitavax by seed treatment showed 53.265 % disease control which was at par to that obtained by seed treatment with Vitavax alone. The integration of biocontrol agents (*Trichoderma* spp.) with fungicides gave significantly higher disease control in several crops (Sugar beet, Tobacco, cauliflower and chickpea) than that obtained by the biocontrol agent or fungicide alone (Mukhopadhyay *et al.*, 1986; Upadhyay and Mukhopadhyay, 1986 and Kaur *et al.* 1989) [28, 19, 11]

Two fungicides (carboxin and thiram) and two bio-control agents (*Pseudomonas fluorescens* and *Trichoderma harzianum*) were evaluated as seed treatment in different

combinations against *Sclerotium rolfsii*, the causal organism of collar rot of chickpea. Seed treated with *T. harzianum* (4 g/kg seed) + carboxin (0.5 g/kg seed) provided maximum protection to the crop by giving maximum seedling emergence (495.0/20 m²), final plant stand (480.4/20 m²) and grain yield (18.2 q/ha). Other treatment combinations significantly increased seedling emergence, final plant stand and grain yield compared to control (Ravinder *et al.*, 2008) [14]. Chet *et al.* (1979) [5] reported a synergistic effect resulting from the interaction between *T. harzianum* and sublethal doses of PCNB while applied against *S. rolfsii* in peanuts. This synergism is apparently due to partial suppression of soil micro flora, enabling a more effective activity of biocontrol agent.

The results obtained in the present investigation indicates that integration treatments i.e. Vitavax and *T. harzianum* gave additive effect and show higher disease control as compared with *T. harzianum* application alone.

Table 2: Integrated management of collar rot of chickpea in pots

Treatment	Doses	Seed Germination in inoculated soil in pots (%)*		Pre emergence mortality (%)*	Post emergence mortality (%)*	•	Disease control over check (%)*
ST with TH	0.6%	66.7	0.67	16.70* (9.61)**	50.00* (29.99)**	66.70* (41.82)**	19.92* (11.49)**
ST with Topsin M	0.2%	55.6	0.67	33.30 (19.44)	38.87 (22.86)	72.17 (46.17)	13.36 (7.68)
ST with Topsin M and TH	0.1% and 0.6%	61.1	0.33	38.87 (22.86)	27.77 (16.11)	66.63 (41.77)	20.01 (11.54)
ST with Topsin M and soil application of TH	0.2% and 10 g per pot	72.2	0.0	33.30 (19.44)	27.77 (16.11)	61.07 (37.62)	26.68 (15.47
ST with Vitavax	0.2%	83.3	0.0	16.70 (9.61)	22.23 (12.84)	38.93 (22.90)	53.26 (32.17)
ST with TH and Vitavax	0.6% and 0.1%	94.4	0.0	16.70 (9.61)	16.70 (9.61)	33.40 (19.50)	59.90 (36.79)
ST with Vitavax and soil application of TH	0.2% and 10 g per pot	88.9	0.0	16.70 (9.61)	0.00	16.70 (9.61)	79.95 (53.06)
Control (S. rolfsii)	10 g per pot	55.6	2.3	44.40 (26.35)	38.90 (22.88)	83.30 (56.39)	0.00
SME				1.24	2.26	2.67	2.15
C.D at 5%				3.79	6.35	8.20	6.58
C.V				14.31	17.26	14.16	17.40

<sup>\*</sup>Mean of 03 replications.

The integration of soil application of maize grain based culture of *T. harzianum* (10 g/pot) with Vitavax seed treatment proved best combination and gave maximum disease control (79.95%) over check. Integration of seed treatment of *T. harzianum* with Vitavax showed 59.90% disease control which was at par to that obtained by seed treatment with Vitavax 0.2 % alone.

The present investigation leads to the conclusion that isolates of *Trichoderma harzianum* exists coiling around hyphae of *S. rolfsii* and disintegration of protoplasm as well as lysis of host fungus were principal mechanisms of antagonism. None of the chickpea variety found resistant against *S. rolfsii*. Vitavax fungicide was found most effective in management of *S. rolfsii* alone as well as in integration with *T. harzianum*. Soil application of *Trichoderma* in integration with Vitavax fungicide proved most effective for management of collar rot of chickpea. Field trials on management of collar rot of chickpea by integration of chemical and biological control methods are required for validation of results.

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<sup>\*\*</sup>Values given in parentheses are Arcsin \( \structure{-}\text{transformation} \)

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