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Integrated Approach for the Management of Collar Rot of Chickpea

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A B S T R A C T

Five Fungicides- Propiconazole, Hexaconazole, Bavistin, Topsin M and Vitavax at 100, 250 and 500 ppm concentrations were evaluated for their efficacy against *S. rolfsii in vitro*. Propiconazole, Hexaconazole and Vitavax completely inhibited the growth of *S. rolfsii in vitro* while Bavistin and Topsin M showed 79.52 and 71.78% growth inhibition respectively at 500 ppm. The integration of soil application of maize grain based culture of *T. 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 59.90% disease control which was higher than Vitavax 0.2% seed treatment alone but did not differ significantly with each other. 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 73.32% disease control followed by Propiconazole @ 2 g/kg.

Introduction

Chickpea (*Cicer arietinum* L.) commonly known as gram, is an important grain legume in Asia including India and the world. In India, chickpea is one of the major pulses grown in *rabi* season. India accounts for approximately 75 per cent of world's chickpea production. Chickpea contributes about 71% to *rabi* pulse production and 46% of the total pulse production in India. It occupies an area of 8 m ha and its production is 7.1 mt with an average productivity of 885 kg/ha (Ghosh *et al.*, 2013).

Chickpeas are also a good source of minerals, such as Ca, P, Mg, Fe, and K. The contents of these compounds decrease the treatment of chickpea grain thermal processes. Chickpea has a higher content of manganese, zinc, and phosphorous than other legumes (Wang *et al.*, 2010). Chickpea is valued for its nutritive seeds with high protein content 12.6-30.5% (Singh *et al.*, 1997). Although chickpea is a rich source of protein, its protein quality is limited by sulphur containing amino acids, methionine and cystine. Chickpea generally meets adult human requirement for all essential amino acids with trace amount of methionine and cystine and rich in fiber, minerals (phosphorus, calcium, magnesium, iron and zinc) and β-carotene. Chickpea seeds are eaten fresh as green vegetables, parched, fried, roasted and boiled; as snack food, sweet

and condiments; seeds are ground and the flour can be used as a soup, dhal and to make bread; prepared with pepper, salt and lemon and served as a side dish. Dhal is the splited grain without its seed coat, dried and cooked into a thick soup or ground into flour for snacks and sweetmeats. Sprouted seeds are eaten as a vegetable or added to salads. Young plants and green pods are eaten like spinach.

Animal feed is another use of chickpea in many developing countries. An adhesive may also be prepared; although not water resistant, it is suitable for plywood. Gram husks, and green or dried stems and leaves are used for livestock feed; whole seeds may be milled directly for feed. Leaves are said to yield an indigo like dye. Chickpeas yield 21% starch suitable for textile sizing, giving a light finish to silk, wool, and cotton cloth.

Despite the high total production and more nutritive value, yields of chickpea are low due to many biotic and abiotic constraints. Among the biotic constraints more than 50 diseases have so far been reported on chickpea. Among them soil borne diseases such as Fusarium wilt (*Fusarium oxysporum* f. sp. *ciceri*), dry root rot (*Rhizoctonia bataticola*) and collar rot (*Sclerotium rolfsii*) are the major limiting factors in chickpea production. 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).

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). Under field conditions, *S. rolfsii* has been reported to cause 22 to 50 per cent reduction in yield of chickpea. Ghosh *et al.,* (2013) 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%.

The disease appears in the early stages of the crop growth i.e. up to six weeks from sowing. Drying plants scattered in the field whose foliage turns slightly yellow before death is an indication of the disease. After infection, seedlings become chlorotic. The joint portion of stem and root turns soft, slightly contracts and begins to decay. Whitish fungal growth with white to dark brown mustard grain like sclerotia are seen on the white infected parts of the plant.

The fungus can overwinter as mycelium in infected tissues or plant debris or as sclerotia near soil surface or buried in soil which serve as a major source of primary infection by germinating in response to alcohols and other volatile compounds released from decomposing plant material (Punja, 1985). Sclerotia disseminate by cultural practices with infected soil and contaminated tools, infected seedlings, water, wind and possibly as concomitant contaminants along with seeds. The pathogen being soil-borne, polyphagous in nature and longer persistence in soil, due to which its control with chemicals alone seems to be ineffective and uneconomical. The combination of bio-control agent (*Trichoderma harzianum*) with fungicides as seed treatment could be very effective against chickpea collar rot, as this pathogen make the plant vulnerable throughout its life starting from rotting of seeds to the death of mature plants. Therefore, present study was conducted during *rabi* 2015 at the Department of Plant Pathology, Dr. rpcau., Pusa. Samastipur, for eco-friendly and economical management of chickpea collar rot disease by integrating fungicides with seed and soil treatment of *Trichoderma harzianum*

Materials and Methods

In vitro evaluation of fungicides against *Sclerotium rolfsii* and *T. harzianum*

Effect of fungicides on radial growth of *S. rolfsii* 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, Propiconazle 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 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 rolfsii* taken from 4 days old culture and incubated at 28 ± 1ºC. in a B.O.D. incubator. PDA plates inoculated centrally with *S. rolfsii* 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:

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 $\sqrt{}$ percentage transformation and then analyzed statistically using completely randomized design.

Mass culture of *S. rolfsii*

S. rolfsii was mass cultured in 250 ml Erlenmayer flask on sorghum grains pre-soaked in 2% sucrose solution for overnight and sterilized at 15 lb P.S.I for 20 minutes in an autoclave. Each flask was inoculated with 11mm mycelial disc of *S. rolfsii* obtained from margin of 4 day old colony and incubated at 28^0C for 3- weeks for mycelium and sclerotial production. Three flasks of inoculum were sufficient to inoculate 12 kg of soil and 10 g per pot.

Mass culture of *T. harzianum*

Conical flasks of 250 ml capacity were used for mass culture of *T. harzianum*. Food grain *i.e* maize was soaked in distilled water for 72 hrs to make them soft, excess water was drained off. The mouth of the flasks was closed using cotton plugs. All conical flasks were autoclaved 121ºC (15 psi) for 20

minutes. All flasks were inoculated with 5 mm mycelial discs of 4 days old of *T. harzianum* and incubated for 15 days at room temperature. After 15 days, biomass of *T. harzianum* produced and 10 g mass culture of *T. harzianum* was used per pot for soil application.

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.

Surface sterilized (0.1% $HgCl_2$) healthy seeds of chickpea were sown (10 seeds/pot) in the earthen pots containing *S. rolfsii* sick soil/potting mixture and maintained as untreated control. All these pots (treated and untreated) were watered regularly and maintained in the screen house for further observations. Observations on seed germination and pre-emergence seed rot (PESR) were recorded at seven days after sowing and that of post emergence seedling mortality (PESM) at 30 days after sowing. The percentage seed germination, pre-emergence seed rot and postemergence seedling mortality were calculated by the formulae.

$$\text{Germination (\%)} = \frac{\text{Number of seeds germinated}}{\text{Total number of seeds sown}} \times 100$$

$$\text{PESR (\%)} = \frac{\text{Number of seeds ungerminated}}{\text{Total number of seeds sown}} \times 100$$

$$\text{PESM (\%)} = \frac{\text{Number of seedlings died}}{\text{Total number of seedlings}} \times 100$$

$$\text{Reduction (\%) in PESR \& PESM} = \frac{C-T}{C} \times 100$$

Where,

C= Per cent rot/mortality in treatment pots and

T = Per cent rot/mortality in untreated control pots

Results and Discussion

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

Five fungicides viz., Propiconazol, Hexaconazol, Bavistin, Topsin M and Vitavax at 100, 250, and 500 µg/ml concentrations were evaluated against *Sclerotium rolfsii.*

The results indicated that all the fungicides at each concentration significantly inhibited the growth of *Sclerotium rolfsii* when compared with control. Three fungicides namely Propiconazle, Hexaconazole and Vitavax proved highly effective and showed complete inhibition of radial growth of *Sclerotium rolfsii* 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 ug/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).

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 *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) developed new biotypes of *T. harzianum* tolerant to chlorothalonil (Kavach).

Tolerance of fungicides by *T. harzianum* isolate 4 *in vitro*

All the five fungicides viz. Propiconazol, Hexaconazol, Bavistin, Topsin M, and Vitavax which were tested against *S. rolfsii*, also evaluated at lower concentrations like 25, 50 and 100 ppm against *T. harzianum* isolates 4 *in vitro* to study their sensitivity to fungicides. Observations on radial growth were recorded at 96 hours of incubation.

The data given in table 2 clearly indicate that Bavistin, Propiconazole and Hexaconazole at all three concentrate i.e. 25, 50 and 100ug/ml were absolutely inhibitory to *T. harzianum.* Per cent growth inhibition of *T. harzianum* in Topsin M at 25, 50 and 100 ug/ml were 35.17, 49.63, and 60.80% while in Vitavax the inhibition were 48.90, 65.53, and 78.55% respectively. It is obvious from the result that Topsin M (Roko) and Vitavax were comparatively less inhibitory to *T. harzianum.* Hence, these fungicides at lower concentrations may be used in integration with *T. harzianum* isolate 4.

One of the most desirable characteristics of an antagonist is its insensitivity to the fungicides which are effective against the test pathogen. Before going to integration of biological and chemical control methods one must ensure that the fungicide to be integrated should not be toxic to the antagonist. With this view the insensitivity of Bavistin, Topsin M, Hexaconazole, Propiconazole and Vitavax were tested against *T. harzianum* isolates. 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.

Insensitivity of *T. harzianum* to Topsin M at the concentrations inhibitory to *S. rolfsii* clearly indicated that the fungicide can be successfully used in conjunction with biocontrol agent *T. harzianum* for the control of *S. rolfsii*. *In vivo,* integration of *T. harzianum* with other control systems would improve the prospects for single or multiple disease control. 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.*

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 (*S. rolfsii*)	-	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 √¯transformaion

Table.2 Tolerance of fungicides by *T. harzianum* isolate 4 *in vitro*

Fungicides	Conc. µg/ml	Colony diameter (mm)	Growth inhibition (%)
		96 hrs	96 hrs
Propiconazole	25	0.00	100.00* (89.96)**
	50	0.00	100.00 (89.96)
	100	0.00	100.00 (89.96)
Hexaconazole	25	0.00	100.00 (89.96)
	50	0.00	100.00 (89.96)
	100	0.00	100.00 (89.96)
Bavistin	25	0.00	100.00 (89.96)
	50	0.00	100.00 (89.96)
	100	0.00	100.00 (89.96)
Topsin M	25	58.97* (36.12)**	35.17 (20.58)
	50	45.97 (27.35)	49.63 (29.75)
	100	30.33 (17.65)	60.80 (37.43)
Vitavax	25	46.00 (27.38)	48.90 (29.26)
	50	30.33 (17.65)	65.53 (40.93)
	100	19.63 (11.32)	78.55 (1.70)
Control (*T. harzianum* only)	-	89.97 (64.09)	0.0
SEM		0.20	1.15
C.D at 5%		0.58	3.33
C.V		2.78	3.13

*Mean of 03 replications.
**Values given in parentheses are Arcsin √¯transformation

Table.3 Integrated management of collar rot of chickpea in pots

Treatment	Doses	Seed Germination in inoculated soil in pots (%)*	No. of infected plants.	Pre emergence mortality (%)*	Post emergence mortality (%)*	Total 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

*****Mean of 03 replications.

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

The fungi static effect of Vitavax and Thiram against *T. harzianum* has been reported by Mukherjee (1987) and Kaur (1989). The insensitivity of *T. harzianum* to Vitavax-200, Metalaxyl at considerably high concentrations has been reported by Nagpal (1987) and Kaur (1989).

Pandey and Upadhyay (1998) reported that *T. viride* and *T. harzianum* were highly sensitive to Bavistin and should not be used in integration as it completely inhibited the growth at 100 ppm. *T. harzianum* can be integrated with thiram only upto 50ppm.

Malathi *et al.*, (2002) evaluated effect of two fungicides Carbendazim and Thiophanate methyl at 1 ppm and 5 ppm on mycelial growth of *Trichoderma* isolates. They observed that *Trichoderma* isolates were not able to grow even at 1 ppm of Carbendazim. However, 1 ppm of thiophanate methyl did not have any

effect on growth of all strains of *Trichoderma* spp.

New biotypes of *T. harzianum* tolerant to fungicides were such as Chlorothalonil, Procymidone, Iprodione and Vinclozolin have been developed by exposing conidia to increasing concentrations of the fungicides in culture media and then selecting surviving colonies for exposure to even higher concentrations (Abd-El Moity *et al.,* 1982). One of the new benomyl-resistant biotype *T. viride* (T-1-R-9) was also effective against Fusarium wilt of chrysanthemum (Locke *et al.,* 1984) and Rhizoctonia scurf of potato (Beagle-Ristaino and Papavizas, 1984). Howell (1982) has also produced mutants of *G. virens* that differ from wild strains in their ability to produce the antibiotic gliovirin. Davet *et al.,* (1981) found that benomyl inhibited *Trichoderma* growth while thiram enhanced it. Papavizas *et al.,* (1982) found mutants resistant to Benomyl and Captan. This further indicates the possibility of combining some appropriate chemicals with biocontrol agents.

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 *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) developed new biotypes of *T. harzianum* tolerant to chlorothalonil (Kavach).

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 3 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.

Integrated management of collar rot of chickpea

Integrating biological and chemical control seems a very promising way of controlling pathogen with minimum interference with the biological equilibrium (Baker and Cook, 1974). 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). 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 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 Vitvax 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 (Upadhyay and Mukhopadhyay, 1986 and Mukherjee, 1987).

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 m^2), final plant stand (480.4/20 m^2) 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). Chet *et al.*, (1979) 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.

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