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Anamika Manhas
Division of Plant Pathology, Faculty of Agriculture, Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu, Jammu and Kashmir, India

Richa Sharma
Division of Plant Pathology, Faculty of Agriculture, Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu, Jammu and Kashmir, India

Stanzin Dorjey
KVK Leh SKUAST-Kashmir, Jammu and Kashmir, India

Corresponding Author
Richa Sharma
Division of Plant Pathology, Faculty of Agriculture, Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu, Jammu and Kashmir, India

Evaluation of fungicide and bio-control agents against Sclerotinia stem rot of chickpea (*Sclerotinia sclerotiorum*)

Anamika Manhas, Richa Sharma and Stanzin Dorjey

Abstract
Present studies on stem rot of chickpea caused by *Sclerotinia sclerotiorum* were carried out with an aim to determine the prevalence of Sclerotinia stem rot, development of symptoms, role of weather factors on disease development and morphological and cultural characters of the pathogen. Morphological and cultural studies revealed that fungus produced fluffy, white colour, gelatinous mycelium on potato dextrose agar media. Sclerotia were formed on potao dextrose agar. Epidemiological studies revealed that that the stem rot commenced from 2nd January (2nd standard week of 2013) reaching its highest peak 32.55 per cent in C-235 and 27.88 per cent in L-550. The disease development was maximum during 2nd to 6th standard week when maximum temperature ranged from 17.80 to 20.50 ^{0}C and relative humidity 91.30 to 96.10 per cent. Newer group of fungicide evaluated against sclerotinia stem rot causing pathogen, best results were obtained with tebuconazole which recorded 62.38 per cent inhibition followed by hexaconazole at 100 ppm. In case of traditional group of fungicides best results were obtained with carbendazim which recorded 73.83 per cent inhibition followed by SAAF (carbendazim 12% + mancozeb 63 WP) at recommended concentration. The antagonistic activity of two fungal and two bacterial biocontrol agents namely *Trichoderma harzianum*, *Trichoderma viride*, *Pseudomonas fluorescence* and *Bacillus subtillis* was studied by dual culture. *Trichoderma harzianum* yielded better results by inhibiting 63.41 per cent growth of *Sclerotinia sclerotiorum*. In case of bacterial biocontrol agents *Bacillus subtillis* yielded better results by inhibiting 57.77 per cent growth of the pathogen.
Compatibility of fungal bioagents were tested with newer and traditional group of fungicides and the best compatibility was found between tebuconazole and *Trichoderma harzianum* and *Trichoderma harzianum* and copper oxychloride at 50 ppm. In case of bacterial bioagent maximum compatibility was observed between hexaconazole and *Pseudomonas fluorescence* at 50 ppm under newer group and under traditional group *Pseudomonas fluorescence* showed maximum compatibility with thiophanate methyl followed by carbendazim.

Keywords: chickpea, pathogenicity, bio-agents

Introduction
Chickpea (*Cicer arietinum* L.) is an important grain legume in world, and being a rich and cheap source of protein it improves the nutritional quality of mankind. It ranks second in area and third in production among the pulses worldwide. The status of the chickpea in the world is 11.9 M ha area with the production of 10.89 MT, respectively. India is the largest producer of chickpea in the world sharing 65.25 and 65.49 per cent of the total area and production. In India the total area sown under chickpea is around 9.01 M ha, with a production of 7.58 MT and with a productivity of 841 kg/ha. In India Madhya Pradesh, Uttar Pradesh, Rajasthan, Maharashtra, Gujarat, Andhra Pradesh and Karnataka are the major chickpea producing states sharing over 95 per cent area (Singh, 2012).
More than 70 pathogens have been reported to infect chickpea in India (Nene *et al.*, 1984), but only a few cause serious economic losses. Among them *Sclerotinia* spp. cause crown and stem rot, a disease of worldwide economic importance. There are three economically important species of the genera Sclerotinia *viz*; *Sclerotinia minor* Jagger, *S. sclerotiorum* (Lib.) de Bary and *S. trifoliorum* Erikss causing diseases on a wide range of crops. Among the three species, *Sclerotinia sclerotiorum* is the most cosmopolitan and versatile. It infects more than 400 species of plants, including field crops, vegetables and weeds (Boland and Hall, 1994). The disease is also known as white mold disease of chickpea. Fungicide application is the only option used to manage the disease so far. Use of fungal and bacterial biocontrol agents against the pathogen could be a better option for the management of disease.

Material and Methods

Isolation and identification of pathogen associated with the sclerotinia stem rot of chickpea

Isolation of pathogen and Purification of Cultures

Isolation of fungal pathogens was done from infected plant parts of chickpea. Sterilized bits were aseptically transferred onto petriplates having pre-poured PDA + streptomycin sulphate and then incubated at 25±2 ^{0}C in BOD incubator. The plates were regularly observed for fungal growth. The growing fungal colonies were transferred to PDA slants by hyphal tip culture technique. After proper growth, pure cultures were maintained in a refrigerator at 4±1 ºC. Stock cultures were maintained by continuous sub-culturing at an interval of 45 days in winter and 30 days in summer for further investigations.

Studies of Morphological Characteristic and Identification

The morphological characters of the isolated fungi were studied on host as well as in culture on potato dextrose agar. The infected Plants and their parts were taken to laboratory for studies, the symptoms on plants parts were recorded and the fungal growth on the diseases spots was examined under microscope. Pure culture of fungal isolates was grown on PDA and incubated at 25±2 ºC.

Microscopic studies of the morphological features of the causal pathogen were carried out in the laboratory for identification of the fungal pathogen on water agar plate method. One sterilized slide was kept in the sterilized Petri-plate and the water agar media was poured in it so that the thin layer of media should come on the slide. The culture was introduced in the centre of the slide and covered with the cover slip. The whole plate was incubated in the incubator at 25 ± 2 ^{0}C for four days. After four days the slides was taken out from the petridish and observed under the microscope for microscopic studies. Colony colour, mycelial width mycelial growth, shape and size of sclerotia and other characteristic features were studied and recorded. Identifications were made after comparing the morphological features of the pathogenic fungi with the available standard literature.

Measurement of fungal mycelia

The ocular scale was first calibrated at 40 X magnification of the microscope with the help of stage micrometer following the standard method of calibration of the microscope. The water agar plate of the culture was placed under the microscope. The size of fungal mycelia was measured by using calibrated ocular scale by placing the organism to be measured under focus and positioned under the scale.

Measurement of sclerotia

The sclerotia were measured with the help of scale. In case of circular sclerotia, the measurement was taken from one side and another at right angle and the average of these two is presented as the diameter of the sclerotia.

Pathogenicity Test

In order to carry out the Pathogenicity test, healthy chickpea seeds were used and sown in earthen pots (30) filled with sterilized soil. Ten chickpea plants were grown in each pot. It was kept for 48 hours. Earthen pots were sterilized using cotton swab dipped in 5 per cent formalin solution. 10 pots were left uninoculated, 10 pots were inoculated with the pathogen after 15 days of sowing. Again 10 pots were inoculated after 30 days of sowing by introducing the mass multiplied mycelia and sclerotia of the pathogen around the collar region. Inoculated sites were covered with moist absorbent cotton. Proper humidity and moisture was maintained by covering with polythene bags and regular watering for disease development. Uninoculated plants were kept as control. The plants were regularly observed for the development of disease symptoms and the observations were recorded. After the development of the symptoms the pathogen was re-isolated from the diseases pots and the morphological characteristics were compared with the isolated fungus for confirmation of Koch's postulates.

In vitro management of sclerotinia stem rot of chickpea

Evaluation of newer fungicides against the pathogen

In order to find out the efficacy of fungicides against *Sclerotinia sclerotiorum* different fungicides were tested *in vitro* by using poisoned food technique (Dhingra and Sinclair, 1995) [8].

Evaluation of newer fungicides viz; hexaconazole, propiconazole and tebuconazole was done at three concentration i.e. 50, 75, and 100 ppm. PDA without fungicides served as control. Each Petri plate containing PDA poisoned with fungicide was inoculated separately with 5 mm discs in inverted position. The discs of the pathogen were taken from the actively growing 7 day old fungal cultures of *Sclerotinia sclerotiorum*. Then the plates were incubated at 25±1 ºC in incubator. The experiment was conducted under completely randomized design (CRD). Each treatment was replicated four times.

Evaluation of traditional group of fungicides against the pathogen

Evaluation of older fungicides viz., Bavistin (carbendazim-50 WP), Saaf (carbendazim 12% + mancozeb 63% WP), Topsin-M (thiophanate methyl 50 WP), Ridomil-MZ (Metalaxyl-M 41%+ Mancozeb 64%), Blue copper (Copper oxychloride) was done at two concentrations of fungicides (recommended dose and half of the recommended dose). PDA without fungicides served as control treatment. This disc was taken from the actively growing 7 day old fungal cultures of *Sclerotinia sclerotiorum.* Then the plates were incubated at 25±1 ºC in incubator. The experiment was conducted under completely randomized design (CRD). Each treatment was replicated three times.

Evaluation of biocontrol agents against pathogen

Antagonistic activity of *Trichoderma harzianum, Trichoderma viride, Pseudomonas fluorescence* and *Bacillus subtillis*, were tested by dual culture technique (Dennis and Webster, 1971) [7]. Five mm culture discs of each fungal antagonists and *Sclerotinia sclerotiorum* were used. The disc of *Sclerotinia sclerotiorum* placed at the same place in separate Petri-plates alone served as check. The inoculated plates were incubated in BOD incubator 25±2 ^{0}C. Experiment was replicated four times. Observations on growth of *Sclerotinia sclerotiorum* were recorded at 4th and 8th day.

Compatibility of biocontrol agents with fungicides

Compatibility of newer fungicides with potential biocontrol agents was evaluated using food poison technique in case of fungal biocontrol agents and using seeded plate technique for bacterial biocontrol (Skinner, 1955) [33].

Compatibility of fungal biocontrol agents with newer groups of fungicides

Evaluation of three newer fungicides i.e., hexaconazole, propiconazole and tebuconazole was done at three concentration i.e. 50, 75 and 100 ppm. The concentrations of fungicides were obtained by adding appropriate amount of stock solution to the 80 ml PDA was poured into four sterilized Petri-plates in equal volume. PDA without fungicides served as control treatment. A 5 mm mycelial disc of seven days old culture was inoculated in inverted position at the centre of the Petri-plates after solidifying of the medium and incubated at 28±2 °C for eight days. Experiment was conducted in four replications. Per cent reduction in radial growth over control was calculated by using the following formula as per Vincent (1947) [34].

Compatibility of bacterial biocontrol agents with newer groups of fungicides

Evaluation of newer fungicides including hexaconazole, propiconazole and tebuconazole was done at three concentration i.e. 50, 75, and 100 ppm. Bits of sterilized blotting paper dipped in three different concentrations of three chemicals viz; Propiconazole, hexaconazole and tebuconazole was kept for 30 minutes and dried in shade and then was inoculated at the centre of Petri-plates. A control was maintained by placing bit treated with Sterilized distilled water in the centre. Experiment was conducted in CRD and was replicated four times. Readings regarding diameter of the inhibition zone were taken on 4th day and 8th day.

Compatibility of fungal biocontrol agents with traditional group of fungicides

Evaluation of traditional group of fungicides viz: Bavistin (carbendazim-50 WP), Saaf (carbendazim 12% + mancozeb 63% WP), Topsin-M (thiophanate methyl 50 WP), Ridomil-MZ (metalaxyl-M 41% + mancozeb 64%), Blue copper (copper oxychloride) was done at two concentration i.e. at recommended and half of the recommended concentration using food poison technique. The PDA plates without fungicides served as control. Experimentation was conducted in three replications. Per cent reduction in radial growth over control was calculated by using the following formula as per Vincent (1947) [34].

Compatibility of bacterial biocontrol agents with traditional group of fungicides

Evaluation of traditional group of fungicides including Bavistin (carbendazim-50 WP), Saaf (carbendazim 12% + mancozeb 63% WP), Topsin-M (thiophanate methyl 50 WP), Ridomil-MZ (Metalaxyl-M 41%+ Mancozeb 64%), Blue copper (Copper oxychloride) was done at two concentration i.e. at recommended and half of the recommended concentration. Bits of sterilized blotting paper dipped in two different concentrations of five chemicals mentioned above for 30 minutes and dried in shade and then placed at the centre of Petri-plates. A control was made without placing chemical immersed bit in the centre. Readings were taken by measuring the inhibition zone.

Evaluation of soil amendments, biocontrol agents and chemicals against pathogen under field conditions.

Soil amendments viz ; FYM, Mustard oil cake, Spent compost of Dhingri (*Pleurotus* sp). and white button (*Agaricus bisporus*), biocontrol agents viz; *Trichoderma harzianum*, *Bacillus subtillis* and chemicals viz; Carbendazim and Tebuconazole alone and in possible combinations were evaluated under field conditions. FYM and spent compost of dhingri and white button were added @ 5 kg/ m^2 area in the plot of 2.25 m^2 whereas, mustard oil cake was added @ 400gm/ m^2 area at the time of field preparation. In case of biocontrol agents one loop of bacterial biocontrol agents was used. The susceptible variety C-235 was grown in the management trial. In case of tebuconazole and carbendazim the seeds were soaked in the solution of recommended dose of carbendazim and tebuconazole for 20 minutes and dried in shade before sowing. Two foliar sprays of recommended dose of the chemicals carbendazim, tebuconazole, topsin-M and blue copper was given after 45 days and 60 days done after the appearance of symptoms.

Trade, common and chemical name, doses, active ingredient and source of fungicides used

Trade / common name of fungicide	Chemical names	Active ingredient	Dose (%)	Source
Derosal (Carbendazim)	2-Methoxy (carbomoyl)- benzimidazole	50WP	0.10	Bayer Crop Science Ltd.
Blue Copper	Copper oxychloride	50 WP	0.25	Sygenta Crop Protection.
ROKO (Thiophanate methyl)	Dimethyl [(1,2-phenylene) bis-(iminocarbonothioyl)] bis [carbamate] (56)	70WP	0.10	Nippon Soda Co. Ltd., Japan
Ridomil MZ	Metalaxyl-M 41%+ Mancozeb 64%	68WP	0.20	Sygenta Crop Protection.
Saaf	Carbendazim 12%+ Mancozeb 63%	WP	0.20	Sygenta Crop Protection
Tilt	Propiconazole	25 EC	0.10	Sygenta Crop Protection.
Folicur	Tebuconazole	25.9 EC	0.10	Bayer Crop Science Ltd.
Jahaan	Hexaconazole	5 EC	0.10	Aries agro limited.

Result and Discussion

Morphological and cultural characters of the pathogen

Studies on morphological and cultural characteristics of *Sclerotinia sclerotiorum* revealed that the fungus produced fluffy white to dull colour, gelatinous mycelium on the potato dextrose agar (Table. 1). Under microscopic examination, the mycelium appeared hyaline, profusely branched, closely septate, thin walled and filled with dense protoplasm. This further confirmed the findings that for the induction of sclerotia by *Sclerotinia sclerotiorum*, factors namely contact with mechanical barrier, competition of substrate and deprivation of nutrients plays an important role. Our findings are confirmatory with Khalequzzaman and Hossain (2012) [17], Willet. (1972) [36], Christias and Lockwood (1973) [6]. Chet and Henis (1975) [5] suggest that sudden changes in some of factors results in sclerotial production. It was established fact that Potato Dextrose Agar medium is routinely used for laboratory studies (Dingra and Sinclair, 1995) [8], for growing fungi in culture. So *Sclerotinia sclerotiorum* showed rich mycelial growth and sclerotial

production on Potato dextrose Agar.

Table 1: Morphological and cultural characters of *Sclerotinia sclerotiorum*

Morphological characters		Characteristic developed on Potato dextrose agar (PDA)
Colony	• Colour • Shape • Growth	White Regular Fluffy
Mycelium	• Colour • Septation • Branching	Hyaline Septate Dichotomos
Sclerotia	• Colour • Shape • Size	Brown to Black Roughly rounded 2-6

Pathogenicity

The pathogenicity test of Sclerotinia stem rot causing fungus, *Sclerotinia sclerotiorum* was conducted on chickpea plants and symptoms of stem rot were appeared on the plants after one week of inoculation (Table. 2). The causal pathogen was re-isolated from the infected plant parts to confirm Koch's postulates. Patel *et al.* (2011) [26] confirmed *Sclerotinia sclerotiorum* causing stem rot and *Rizoctonia bataticola* causing root rot chickpea on healthy chickpea plants grown on sterilized soil in pots. *Sclerotinia sclerotiorum* causing stem rot of chickpea were confirmed on healthy chickpea plants grown on sterilized soil in pots by Goel *et al* (2002) [11]. The plants were inoculated with mycelial mats and the pathogen was re-isolated from artificially infected plants (Singh and Gill 1979) [30].

Table 2: Pathogenicity test of isolated *Sclerotinia sclerotiorum*

Treatments	Infected plants /10	Mortalitiy percent
Sterilized soil	00.00	00.00
Sterilized soil + Pathogen after 15 days of sowing	04.00	40.00
Sterlized soil + Pathogen after 30 days of sowing	03.00	30.00
C. D. (P=0.05)	00.80	
SEm ±	00.25	

***In vitro* evaluation of newer group of fungicides against *Sclerotinia sclerotiorum* causing stem rot of chickpea**

The results of the experiment in table 3 shows that all the three fungicides significantly inhibited the growth of the pathogen over the control at three concentrations i.e. 50, 75 and 100ppm. Maximum inhibition was recorded in tebuconazole in which less growth of pathogen was obtained at all the three concentrations (50, 75, 100 ppm) followed by hexaconazole. Nene and Thapliyal, (1973) reported the effectiveness of triazoles, which inhibit sterol biosynthetic pathway in fungi. McMillan 1973 [21] reported that *in vitro* screening of three chemicals, Azoxystrobin, Flubioxonil and Tebuconazole has shown activity in reducing the growth of *Sclerotinia sclerotiorum.* Mueller *et al.* (1999) reported that tebuconazole at 10 µg a.i./ml of agar inhibited mycelial growth of *Sclerotinia sclerotiorum*.

Table 3: *In vitro* evaluation of newer fungicides against *Sclerotinia sclerotiorum* causing stem rot of chickpea

Treatment	50 ppm				75 ppm				100 ppm			
	4th day		8th day		4th day		8th day		4th day		8th day	
	Radial growth	Inhibition	Radial growth	Inhibition	Radial growth	Inhibition	Radial growth	Inhibition	Radial growth	Inhibition	Radial growth	Inhibition
	(mm)	%	(mm)	%	(mm)	%	(mm)	%	(mm)	%	(mm)	%
Hexaconazole	15.50	60.75	27.13	50.22	14.50	63.29	26.50	51.37	14.50	63.29	25.50	53.20
Propiconazole	16.25	58.86	28.75	47.24	15.50	60.75	27.00	50.45	14.75	62.26	26.75	50.09
Tebuconazole	13.25	66.45	23.75	56.42	12.50	68.35	22.88	58.01	11.13	71.82	20.50	62.38
Control	39.50		54.50		39.50		54.50		39.50		54.50	
CD (P=0.05)	01.09		01.76		01.64		02.36		00.98		02.26	
SEm ±	03.39		05.47		05.12		07.36		03.06		07.03	

***In vitro* evaluation of traditional fungicides against *Sclerotinia sclerotiorum* causing stem rot of chickpea**

The results of the experiment in Table 4 show that all the five fungicides significantly inhibited the growth of the pathogen over the control at recommended and half of the recommended dose. Maximum inhibition was recorded in Derosal (Carbendazim) and Saaf (Carbendazim 12% + Mancozeb 63%) and in which very less growth of the pathogen was obtained at recommended dose and half of recommended dose, of followed by Topsin –M (Thiophanate methyl). Carbendazim belonging to benzimidazole group of fungicides interferes with energy production and cell wall synthesis of fungi (Nene and Thapliyal, 1973). Similar results were found by Kumawat and Jain (2003) [19] who found that under *in vitro* condition benlate and bavistin were found most effective in checking growth of *Sclerotinia sclerotiorum* followed by topsin-M, subeej, Captan under *in vitro* conditions. Handoro *et al.* (2001) [12] reported that carbendazim and Bayleton at 0.1% and Captan at 0.25% gave significant disease control and better seed yield.

Table 4: *In vitro* evaluation of traditional fungicides against *Sclerotinia sclerotiorum* causing stem rot of chickpea

Treatment	Recommended dose				Half of the recommended dose			
	4th day		8th day		4th day		8th day	
	Radial growth (mm)	Inhibition %	Radial growth (mm)	Inhibition %	Radial growth (mm)	Inhibition %	Radial growth (mm)	Inhibition %
Carbendazim	08.33	76.64	11.00	80.81	11.00	69.16	15.00	73.83
Mancozeb+Metalaxyl	11.67	67.28	17.00	70.34	14.00	60.75	20.25	64.67
Mencozeb+Carbendazim	10.67	70.08	12.67	77.89	12.50	64.95	18.00	68.60
Thiophanate methyl	11.33	68.23	15.00	73.83	13.15	63.13	19.00	66.85
Copper oxychloride	14.75	58.64	20.75	63.80	16.67	53.26	23.67	58.57
Control	35.67		57.33		35.67		57.33	
CD (P=0.05)	01.27		02.64		01.05		00.91	
SEm ±	03.97		08.23		03.26		02.82	

Effect of biocontrol agents on mycelial growth of *Sclerotinia sclerotiorum* in dual culture.

The antagonistic activity of fungal biocontrol agents (Table 5), namely *Trichderma viride*, *Trichoderma harzianum*, *Pseudomonas fluorescence*, *Bacillus subtillis* in dual culture showed that *Trichoderma harzianum* established better results by inhibiting 63.41 per cent in dual culture followed by *Trichoderma viride* and among bacterial bioagents *Bacillus subtillis* showed better antagonistic activity as compared to *Pseudomonas fluorescence*. Yuen *et al.*, 1991 [37] observed that the filtrate from cells of *Bacillus subtillis* inhibits twinning of ascospores depending on the concentration. The studies on antagonism of *Trichoderma harzianum*, *Trichoderma viride*, *Pseudomonas fluorescence*, *Bacillus subtillis* in different *Sclerotinia sclerotiorum* host combinations have been studied under *in vitro* conditions by number of workers (Fernando *et al.*, 2007; Singh *et al.*, 2003; Sharma *et al.*, 1999; Sharma and Basandrai 1997 and Dos and Dhingra, 1982) [10, 32, 28, 27, 8]. The results on the use of different biocontrol agents corroborate with the findings of Sharma 1994 who reported that *Trichoderma harzianum* caused maximum inhibition (75%) followed by Trichoderma viride (65.93%) over control.

Whipps (1993) [35] also evaluated the inhibitory effect of the isolated fungal and bacterial antagonistic agents namely *Trichoderma harzianum*, *T. viride*, *T. hamatum*, *Bacillus subtilis* and *Pseudomonas fluorescens* against the linear growth of *Sclerotinia sclerotiorum* and *S. minor* using the modified dual culture technique. Fernando *et al.*, 2007 [10] concluded that *Bacillus subtillis* has an antagonistic effect on all stages of the cycle of *Sclerotinia sclerotiorum.* Zhang and Xue (2010) achieved reduction in mycelia growth upto 75% by cultivating *Bacillus subtillis* for 10 days which attest the great potential on biological control to *Sclerotinia sclerotiorum*.

Table 5: Effect of biocontrol agents on mycelial growth of *Sclerotinia sclerotiorum* in dual culture.

Treatment	4th day		8thday	
	Mycelial growth (mm)	Inhibition (%)	Mycelial growth (mm)	Inhibition (%)
Trichoderma harzianum	14.00	60.00	20.58	63.41
Trichoderma viride	15.50	55.71	22.00	60.88
Pseudomonas fluorescence	18.25	47.85	25.00	55.55
Bacillus subtillis	16.50	52.85	23.75	57.77
Control	35.00		56.25	
CD (P=0.05)	01.07		00.78	
SEm ±	03.26		02.37	

Compatibility of fungal amd bacterial bioagent viz; *Trichoderma harzianum, Trichoderma viride, Pseudomonas flourecence and Bacillus subtillis* were evaluated with fungicides.

Compatibility of fungal bioagents with newer groups of fungicides

Among fungal biocontrol agents *Trichoderma harzianum* showed compatibility with tebuconazole in case of newer fungicides as compared to hexaconazole which was least compatible (Table. 6). Similar results were found by Hausvater and Trnkova (1993) [13]. He reported that the toxicity of hexaconazole is more than tebuconazole against all the four species of *Trichoderma*.

Compatibility of fungal bio control agents with traditional group of fungicides at recommended concentration.

In case of traditional fungicides copper oxychloride showed maximum compatibility with *Trichoderma harzianum* (Table. 7). These finding are in conformity to the observation made by Bagwan (2010) [2] who reported that copper oxychloride (0.2%) is compatible with *Trichoderma harzianum*. Trichoderma has the capability of degrading xenobiotic compounds and can survive in environment with remnants of fungicides molecules (Chapparro *et al.*, 2011) [4].

Table 6: Compatibility of fungal bioagents with newer groups of fungicides

Treatments	*Trichoderma viride*				*Trichoderma harzianum*			
	4th day		8th day		4th day		8th day	
	Radial growth (mm)	Inhibition %	Radial growth (mm)	Inhibition %	Radial growth (mm)	Inhibition %	Radial growth (mm)	Inhibition %
At 50 ppm								
Hexaconazole	00.00	100.00	03.25	94.09	05.50	84.28	08.00	85.71
Propiconazole	05.00	86.48	06.75	87.72	06.25	82.14	08.75	84.37
Tebuconazole	06.00	83.78	08.50	84.54	08.50	75.71	09.00	83.92
Control	37.00		55.00		35.00		56.00	
CD (P=0.05)	02.25		02.92		02.81		02.54	
SEm ±	07.02		09.11		08.75		07.93	
At 75 ppm								
Hexaconazole	00.00	100.00	02.50	95.45	05.00	85.71	05.25	90.62
Propiconazole	02.75	92.56	05.75	89.54	05.50	84.28	06.00	89.28
Tebuconazole	05.75	84.45	07.75	85.90	08.00	77.14	08.50	84.82
Control	37.00		55.00		35.00		56.00	
CD (p=0.05)	02.16		02.45		02.55		02.72	
SEm ±	06.72		07.64		07.93		08.48	
At 100 ppm								
Hexaconazole	00.00	100.00	00.00	100.00	00.00	100.00	00.00	100.00
Propiconazole	00.00	100.00	00.00	100.00	02.50	92.85	05.00	91.07
Tebuconazole	05.00	86.48	05.50	90.00	05.50	84.28	06.50	88.39
Control	37.00		55.00		35.00		56.00	
CD (p=0.05)	01.40		01.60		02.03		02.38	
SEm ±	04.50		04.99		06.33		07.48	

Table 7: Compatibility of fungal bio control agents with traditional group of fungicides at recommended concentration.

Treatments	*Trichoderma viride*				*Trichoderma harzianum*			
	4th day		8th day		4th day		8th day	
	Radial growth (mm)	Inhibition %	Radial growth (mm)	Inhibition %	Radial growth (mm)	Inhibition %	Radial growth (mm)	Inhibition %
Carbendazim	00.00	100.00	00.00	100.00	03.33	91.00	06.67	88.50
Mancozeb + Metalaxyl	00.00	100.00	03.33	94.25	06.67	81.97	07.33	87.36
Mancozeb + Carbendazim	04.00	88.57	04.33	92.53	03.33	91.00	07.00	87.93
Thiophanate methyl	21.33	39.05	38.00	34.48	32.33	12.62	40.33	30.46
Copper oxychloride	28.33	20.00	54.33	06.32	35.00	05.40	55.67	04.01
Control	35.00		58.00		37.00		58.00	
CD (p=0.05)	01.82		02.29		02.42		02.72	
SEm ±	05.66		07.12		07.54		08.48	

Compatibility of bacterial bio-agents with newer group of fungicides

Among the bacteria, *Pseudomonas fluorescens* was found to be more compatible with fungicides than *Bacillus subtilis* and the *Pseudomonas flourescence* was found to be more compatible with hexaconazole among newer group and thiophanate methyl followed by carbendazim in traditional group of fungicides (Table 8). Incorporation of thiophanate methyl and carbendazim in growth medium of *Pseudomonas fluorescens* did not affect its growth instead fungicides favoured the growth of antagonistic strains at a lower concentration of 100 ppm. By increasing the fungicidal concentration to 500 ppm also, the antagonistic bacteria were able to tolerate and multiply as in control without fungicide (Mohiddin and Khan, 2013) [22].

Table 8: Compatibility of bacterial bio-agents with newer group of fungicides

Treatment	Inhibition zone in mm					
	Bacillus subtillis			*Pseudomonas fluorescence*		
	50 ppm	75 ppm	100 ppm	50 ppm	75 ppm	100 ppm
Propiconazole	00.00	05.00	08.00	07.75	09.25	10.75
Tebuconazole	15.75	18.25	20.25	12.50	13.75	15.00
Hexaconazole	08.75	09.50	11.50	00.00	00.00	02.50
Control	90.00	90.00	90.00	90.00	90.00	90.00
CD (p=0.05)	04.71	06.76	04.84	04.16	05.00	03.97
SEm ±	01.51	02.17	01.55	01.34	01.60	01.27

Table 9: Compatibility of bacterial bio-agents with older group of fungicides

Treatment	Inhibition zone in mm			
	Bacillus subtillis		*Pseudomonas fluorescence*	
	Half Conc.	Rec. conc.	Half Conc.	Rec. conc.
Carbendazim	07.00	10.33	03.00	06.00
Mancozeb + Carbendazim	22.33	26.33	13.33	15.00
Mancozeb + Metalaxyl	28.00	30.33	10.33	13.67
Thiophanatemethyl	05.00	08.00	00.00	00.00
Copper oxychloride	15.00	18.00	05.00	09.66
Control	90.00	90.00	90.00	90.00
CD (p=0.05)	01.85	01.75	01.20	01.34
SEm ±	00.59	00.56	00.38	00.43

Compatibility of bacterial bio-agents with older group of fungicides

The biocontrol bacteria viz., *Pseudomonas fluorescens and Bacillus subtilis* were found more tolerant to fungicides than fungi (Table 9). This may be due to the reason that, some bacteria can use pesticides as nutrients and hence can tolerate higher concentrations of chemicals (Kishore and Jacob, 1987 and Aislabie and Jones, 1995) [18, 1].

Management of disease through different treatments under field conditions

Under field conditions most effective disease control was observed in the plots treated with carbendazim (8.33%) (Table. 10). These findings are in conformity with that of Singh and Tripathi (1997) [31] who reported that among the various fungicides, carbendazim (0.2%) was the most effective. Tebuconazole (11.33%) followed the carbendazim in the field trials. Similar results were found by McMillan (1973) [21] who found that Flubioxonil and Tebuconazole restricted growth of *Sclerotinia sclerotiorum* through 96 hrs in the field. Among organic soil amendments FYM was most effective in reducing the disease incidence followed by mustard oil cake. Huang *et al.* (2002) reported that organic soil amendments with agricultural residues can be an effective method for controlling carpogenic germination of sclerotia of *Sclerotinia sclerotiorum* and therefore reducing the production of ascospores, the primary source of inoculation for Sclerotinia diseases. The mode of action for suppression of carpogenic germination of sclerotia of *Sclerotinia sclerotiorum* in these amendments may be related to the production of ammonia related compounds (Huang *et al* 1993) [14]. The findings are in conformity with Kapoor *et al.*, 2006 who report that FYM alone and in combination with *Trichoderma harzianum* showed reduction of disease incidence (64.85%) and increase in grain yield up to 38.17 per cent.

In case of integration *Pseudomonas flourescent* + Topsin-M showed 14.33 per cent disease incidence. These findings are in confirmation with Malathi *et al.* (2002) [20] who reported that the treatment receiving thiophanate methyl and *Pseudomonas fluorescens* recorded the maximum plant survival of 81.67%, while other combined treatments had comparable effect. *Trichoderma harzianum* + copper oxychloride showed 16.66 per cent disease incidence. The findings are in confirmation with Bagwan. (2010) [2] who evaluated the compatibility of *Trichoderma sp*. with fungicides and reported that seed treatments and soil applications with *Trichoderma sp*. would be compatible with blue copper fungicide.

Table 10: Management of disease through different treatments under field conditions

Treatment	60 DAS		75 DAS		90 DAS	
	PDI (%)	Inhibition over Control	PDI (%)	Inhibition over Control	PDI (%)	Inhibition over Control
T_1 (FYM)	33.66	20.48	36.66	17.92	40.33	10.37
T_2 (Mustard oil cake)	35.00	17.31	39.00	12.67	41.33	08.15
T_3 (Spent compost of dhingri)	40.33	04.72	42.66	04.48	45.33	03.71
T_4 (Spent compost of white button mushroom)	38.33	09.44	40.66	08.96	41.66	07.55
T_5 (*T. harzianum*)	31.00	26.76	33.33	25.37	36.33	19.26
T_6 (*Bacillus subtillis*)	32.66	24.39	36.00	19.39	38.33	14.82
T_7 (Tebuconazole)	09.33	77.94	10.33	76.89	11.33	74.81
T_8 (Carbendazim)	06.66	84.25	07.66	82.88	08.33	81.48
T_9 (FYM + *T. harzianum*)	12.66	70.08	17.66	60.48	17.00	62.21
T_{10} (FYM+ *Bacillus subtillis*)	16.00	62.19	18.33	58.17	21.66	51.86
T_{11} (Mustard oil cake + *T. harzianum*)	21.33	49.60	23.00	48.51	24.33	45.92
T_{12} (Mustard oil cake + *Bacillus subtillis*)	24.00	43.29	25.33	43.29	28.00	73.77
T_{13} (Dhingri spent compost + *T. harzianum)*	25.00	40.93	30.66	31.36	31.66	29.64
T_{14} (Dhingri spent compost + *Bacillus subtillis*)	28.00	33.81	32.33	27.61	35.33	21.48
T_{15} (White button spent compost + *T. harzianum*)	18.33	56.68	19.33	56.73	22.66	49.63
T_{16} (White button spent compost + *B. subtillis*)	20.33	51.96	22.33	50.01	23.33	48.15
T_{17} (*P. fluorescence* + topsin-M)	11.33	73.22	12.66	71.68	14.33	68.14
T_{18} (*T. harzianum* + blue copper)	12.00	71.63	15.33	65.69	16.66	62.97
T_{19} (Control)	42.33	0.00	44.66	0.00	45.00	0.00
CD (p=0.05)	01.05		02.04		01.61	
SEm ±	00.57		00.71		0.563	

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