RESEARCH ARTICLE

EFFICACY OF FUNGICIDES, PLANT EXTRACTS AND BIO-AGENTS AGAINST STEM ROT OF CORIANDER INCITED BY *SCLEROTINIA SCLEROTIORUM*

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Received-06.03.2021, Revised-16.03.2021, Accepted-25.03.2021

Abstract: Coriander (*Coriandrum sativum* L.) an important annual herb used extensively all over the world. In India, it is intensively cultivated in the almost all the states of the country but Rajasthan, Madhya Pradesh, Andhra Pradesh, Tamil Nadu, Orissa, Uttar Pradesh and Uttarakhand are the major coriander growing states. Stem rot is an major destructive soil borne disease of coriander incited by *S. sclerotiorum.* The aim of the present study, *S. Sclerotiorum* causal organism of stem rot coriander has been the integrated management strategies through different concentrations of fungicides, plant extracts and bio-agents in *In vitro* and *In vivo* during *Rabi* season 2015-16. The results revealed that, the *Trichoderma viride* showed highest mycelial inhibition zone of the pathogen followed by *T. harzianum.* Seed + soil application of *T. viride* was most effective in reducing disease intensity under field conditions. Garlic clove extract was found most effective in inhibiting mycelial growth followed by safeda leaf extract. Among the plant extracts studied garlic clove extract was found most effective in reducing the disease intensity followed by eucalyptus leaf extract. Among the fungicides carbendazim and carbendazim + mancozeb inhibited mycelial growth completely at all concentrations.Fungicides were used as seed application, foliar application and seed-cum-foliar application against stem rot of coriander. Carbendazim was found most effective in reducing the disease intensity followed by carbendazim + mancozeb.

Keywords: Bio agent, Coriander, Fungicide, Plant extracts, Stem rot

INTRODUCTION

Coriander is an important spice crop belonging to the family Apiaceae. It's all the tender aerial parts, stem, leaf, flower, fruits are used due to aromatic flavour. Since from the ancient period it can be used as an important ingredient of different food. Coriander is popularly used in soups, salads, seasoning and chutney all over the world. In India, coriander is largely cultivated in states of Rajasthan (54%), Madhya Pradesh (17%) contributing about 2/3 of countries total production. Other states include Andhra Pradesh, Gujrat, Assam, Karnataka, Punjab, Orissa, Tamil Nadu, Uttar Pradesh and Uttarakhand. Rajasthan is major coriander growing and producing state with its share of about 60% in the total area and production of the country. Coriander contains 24 g carbohydrates, 1.3 g protein, 19.6 g fat, 5.3 g minerals and 6.3 g moisture in 100 g seed. The seeds have 0.4 per cent essential oil. Linalool is the main component up to 90% and up to 7% thymol. The essential oil has carminative, antiseptic, bactericidal, fungicidal and muscle relaxant. The coriander plant parts and seeds are used by people as short-cut medicines for various body problems (Singh *et al.*, 2007).

This crop is highly affected with soil borne diseases which causes heavy yield loss year by year. These soil borne pathogens are microscopic, hidden and unevenly distributed in the soil or in infected plant material enters through roots and become systemic causing broad range of diseases on various host plants, such as vascular wilts, pre and post emergence blights as well as root and stem rots (Pascale *et al.*, 2002; Schollenberger *et al.*, 2006). Stem rot disease has been reported on coriander by Mehta *et al.*, (1946). The disease appears mainly around flowering under high soil moisture conditions in patches. First water soaked spots are formed on the main stem which turn brown, later the whole stem is girdled. The surface gets covered with white fungus hyphae and looks like a cottony growth. The plant gradually dries. On splitting the diseased stem black sclerotia are observed. The disease is caused by *Scelotinia sclerotiorum* which contains hyaline, branched, septate hyphae. They are inter and intra cellular both. These hyphae form black Sclerotia within the stem. The sclerotia germinate in winter in soil where they remain from previous crop or disseminate with seed as inert matter. Stipes are formed on germination which contain apothecia and produce ascus. Each ascus produces ascospores which disseminate and attack the crop causing the disease. The sclerotia should be removed from seed lot. Deep ploughing is helpful in burying the sclerotia deep in soil. Paddy after coriander helps in the elimination of the inoculum source. So this situation compels to focus on integrated disease management by utilizing bioagents, plant extracts and fungicides in lower concentration. Application of bio agents and plant extracts were sustainable approach apart from being a promising alternative to fungicide application. Thus the present study was aimed to evaluate *in vitro* and *in vivo* of different bioagents,

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plant extracts and fungicides for integrated disease management of stem rot of coriander.

MATERIALS AND METHODS

Efficacy of bioagents on mycelial growth inhibition of the *S. sclerotiorum* (*In vitro*): *In vitro,* screening of bio agents was done by dual culture technique (Dennis and Webster, 1971). The following bio agents were used for study viz., *Bacillus subtilis*, *Trichoderma harzianum* and *T. viride*. All the bioagents were obtained from Department of Plant Pathology, RARI- Durgapura (Jaipur, India). Single colonies of the isolate were sub-cultured in PDA and stored in refrigerator to maintain their genetic purity. Fifteen ml of PDA medium was poured into sterile Petri plate and allowed for solidification. 5 mm dia. discs from actively growing colony of pathogen was cut with a sterile cork borer and placed near the periphery of PDA plate. Similarly, bio agents were placed on the other side *i.e.*, at an angle of 180˚. Plates with no antagonists served as control for the pathogen. The plates were incubated at 25 ± 1˚ C for seven days. In each treatment five replications were maintained. The extent antagonistic activity by bioagents was recorded after incubation period of 7 days by measuring the growth of the test pathogen in dual culture and in control plates. The per cent mycelial inhibition zone of pathogen was calculated using formula as suggested by Bliss, 1934.

$$I = \frac{C - T}{C} X\ 100$$

Where,

I= Per cent inhibition

C= Colony diameter in control

T= Colony diameter in treatment

Fungitoxicity of plant extracts against *S. Sclerotiorum* (*In vitro*): Incubation study was carried out to find out the fungi-toxicity of six plant extracts (Aak, Dhatura, Garlic, Marigold, Sadabahar and Eucalyptus) of different concentrations (5%, 10% and 15%) against the pathogen. One hundred gram leaves/cloves from each was collected and washed 2-3 times with water and before extraction leaves/cloves of each plant (100 g) were crushed separately with 100 ml sterilized water. The extract was filtered through muslin cloth and centrifuged at 5000 rpm for 30 min. and the extract was sterilized. The extract of each plant species was diluted in order to achieve three concentrations viz., 5, 10 and 15 per cent. Petri plates containing PDA supplemented with different plant extracts, each with three concentrations and replicated three times were inoculated with seven days old culture (5 mm dia. disc). A suitable check (without plant extract) was also maintained. Fungal colony was measured after 7 days of inoculation at 25 ± 1 ˚C. The linear growth of test fungus was recorded and per cent mycelial growth inhibition was calculated by Bliss (1934) formula referred above methods.

Efficacy of fungicides against *S. Sclerotiorum* (*In vitro*): Five fungicides (Propineb 70WP, Carbendazim 50WP, Carbendazim 12%+ Mancozeb 63%WP, Mancozeb 75%WP and Captan 70% + Hexaconazole5 % WP) were evaluated with three (50,100 and 150 ppm) concentrations against the *S. Sclerotiorum* under laboratory conditions to find out per cent inhibition on growth of the pathogen in culture by poisoned food technique. Requisite quantity of each fungicide was incorporated in sterilized two per cent PDA medium, thoroughly mixed by shaking prior to pouring in sterilized Petri plates and were allowed to solidify. These Petri plates were inoculated with 5 mm dia. disc of seven day old culture of the pathogen in the centre of the plate and incubated at 25±1˚C. Each treatment was replicated thrice with suitable control. The efficacy of fungicides in each treatment and average of three replications were calculated. Per cent mycelial growth inhibition was calculated by following formula given in above methods.

Efficacy of *Trichoderma viride* against *S. sclerotiorum* (*In vivo*): Field studies were conducted at Department of Plant Pathology (SKNCOA, Jobner) to manage the stem rot through *T. Viride* during *Rabi* season of the year 2015-16 under artificial soil inoculation conditions. The field study consists four different treatments of *T. Viride* and control, with five replications using coriander variety RCr-41 in RBD design with a plot size of 1.0 sq m. *T. viride* was added @ 6 g/plot. The talc based formulation of *T. Viride* formulation ($2x10^8$cfu) procured from market was used as seed application, soil application and seed + soil application. The pathogen multiplied on sorghum grains at 25±1˚C for one week was used as soil inoculum. Sowing was done using *T. viride* as seed treatment @ 10 g/kg seed and soil application @ 2.5 kg/ha pre-incubated in 50 kg well decomposed farm yard manure for fifteen days. The row spacing (30 cm) and plant spacing (10 cm) were maintained 10 days after sowing. To protect the crop from aphids, oxy-demeton methyl (0.1%) was sprayed. The observations for per cent disease intensity of sclerotinia rot on stem lesions (0-4 scale) were recorded 20 days before the maturity of crop (Yadav *et al.*, 2012 and Meena *et al.*, 2013).

The length of lesion on infected stem was considered for recording the disease intensity (Sharma, 1987). The infected area was calculated from 15 randomly selected plants in each plot and then average for each treatment was worked out. The per cent disease intensity was calculated using the formula of Wheeler (1969):

Per cent Disease Intensity =

$$\frac{\text{Sum of individual ratings}}{\text{No. of plants observed x Maximum disease rating}} \times 100$$

The per cent disease control was calculated by using the following formula:

Per cent disease control =

$$\frac{\text{Disease in control} - \text{Disease in treatment}}{\text{Disease in control}} \times 100$$

Efficacy of plant extracts against *S. sclerotiorum* (*In vivo*): The experiment was carried out in earthen pots (30 cm dia.) with host cultivar RCr-41. The pathogen multiplied on sorghum grains at 25 ± 1 ˚C for one week was used as soil inoculum. Prior to sowing, pots were filled with sterilized soil. The soil was sterilized at 1.045 kg/cm^2 for one hour for three consecutive days. RCr-41 variety of Coriander was sown in these pots as susceptible check with four replications. Six plant extracts (Aak, Dhatura, Garlic, Marigold, Sadabahar and Eucalyptus) at 5% concentration were tested by applying as seed application (for 15 min), foliar application (30 DAS) and seed–cum–foliar application. Seed were dipped in freshly prepared, aqueous leaf and clove (garlic) extract (5 % v/v) for 15 min., the seeds were drained of water and then air dried before sowing. The pots were inoculated with fungal inoculum multiplied on sorghum grains before sowing. For inoculation, the upper 5 cm layer of soil of each pot was thoroughly mixed with inoculum @ 20 g/pot. 10 seeds were maintained per pot and kept in cage house. At 30 DAS, each extract of the 5 per cent concentration was used as single foliar spray (Meena *et al.*, 2013). The disease intensity and per cent disease control were calculated 60 DAS by formula given in above methods.

Efficacy of fungicides against *S. sclerotiorum* (*In vivo*):The experiment was carried out in earthen pots (30 cm dia.) with cultivar RCr-41. The pathogen multiplied on sorghum grains at 25 ± 1˚C for one week was used as the soil inoculum. Prior to sowing, pots were surface sterilized with copper sulphate solution and filled with sterilized soil. The soil was sterilized at 1.045 kg/cm^2 for one hour for three consecutive days. Five fungicides (Propineb 70 WP, Carbendazim 50WP, Carbendazim 12% + Mancozeb 63% WP, Mancozeb 75% WP and Captan 70% + Hexaconazole 5 % WP) different concentrations were tested by applying as seed application (as per table), foliar application (30 DAS) and seed–cum–foliar application in four replications. These pots were inoculated with fungal inoculum multiplied on sorghum grains before sowing. For inoculation the upper 5 cm layer of soil of each pot was thoroughly mixed with inoculum @ 20 g/pot. 10 seeds were maintained per pot and kept in cage house. At 30 DAS, 0.1% of carbendazim and 0.2% of rest of the fungicides were used as single foliar spray. The disease intensity and per cent disease control were calculated 60 DAS by formula given in above methods.

Table 1. Efficacy of *Trichoderma viride* against stem rot of coriander *(in vivo)*

Treatments	**Disease intensity (%)**	**Disease control (%)**
Seed application @ 10 g/kg seed	45.17(42.23)	34.95
Soil application @ 2.5 kg/ha	36.72(37.30)	47.11
Seed @ 10 g/kg seed + soil application @ 2.5 kg/ha	25.00(30.00)	63.99
Control	69.44(56.44)	0.00
SEm±	0.79	-
CD (p=0.05)	2.73	-

Table 2. Efficacy of plant extracts against Stem rot of coriander (*In vivo*)

Plant extracts	**Conc. used (%)**	**Seed application***		**Foliar application***		**Seed-cum-foliar* application**	
		Disease intensity (%)*	**% disease control**	**Disease intensity (%)***	**% disease control**	**Disease intensity (%)***	**% disease control**
Aak	5.0	73.48 (59.00)	6.39	77.75 (61.86)	5.47	72.25 (58.21)	8.83
Datura	5.0	66.87 (54.86)	14.81	72.55 (58.40)	11.79	65.37 (53.95)	18.14
Garlic	5.0	53.77 (47.16)	31.50	59.55 (50.51)	27.59	48.75 (44.28)	38.48
Marigold	5.0	70.25 (56.95)	10.50	79.12 (62.81)	3.80	69.15 (56.26)	13.74
Sadabahar	5.0	74.33 (59.56)	5.31	80.17 (63.56)	2.52	73.25 (58.86)	8.15
Eucalyptus	5.0	61.50 (51.65)	21.65	68.75 (56.01)	16.41	57.25 (49.17)	27.76
Control	-	78.50	0.00	82.25	0.00	79.75	0.00

		(62.38)		(65.08)		(63.26)	
SEm±		1.31	-	0.93	-	1.35	-
CD (p=0.05)		3.90	-	2.75	-	4.01	-

Table 3. Efficacy of fungicides against stem rot of coriander (*in vivo*)

Fungicide	**Dose (%)**	**Seed application***		**Foliar application***		**Seed-cum-foliar application***	
		Disease intensity (%)*	**% disease control**	**Disease intensity (%)***	**% disease control**	**Disease intensity (%)***	**% disease control**
Propineb	0.2	65.87 (54.25)	15.27	70.61 (57.17)	14.04	61.33 (51.55)	21.94
Carbendazim	0.1	31.62 (34.22)	59.33	39.75 (39.09)	51.61	21.25 (27.45)	72.92
Carbendazim + Mancozb	0.2	36.62 (37.24)	52.90	42.15 (40.48)	48.69	24.50 (29.67)	68.78
Mancozeb	0.2	52.70 (46.55)	32.21	59.12 (50.25)	28.03	47.50 (43.57)	39.49
Captan + Hexaconazole	0.2	37.25 (37.61)	52.09	45.17 (42.23)	45.01	29.75 (33.05)	62.10
Control	-	77.75 61.86)	0.00	82.15 (65.01)	0.00	78.50 (62.38)	0.00
SEm±		0.82	-	0.70	-	0.65	-
CD (p=0.05)		2.46	-	2.12	-	1.96	-

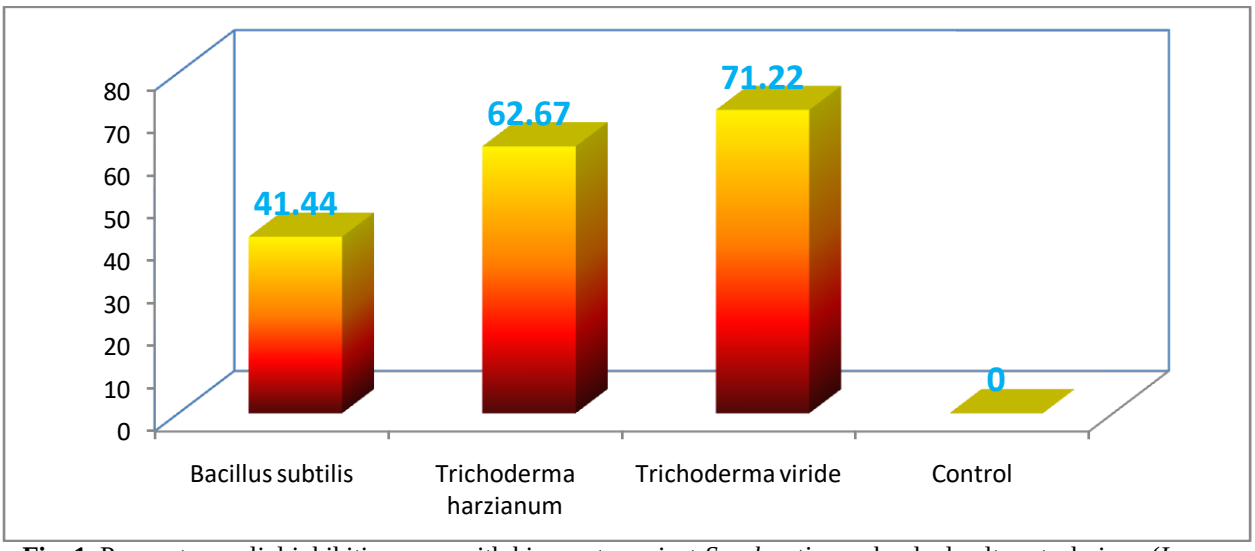

Fig. 1. Per cent mycelial inhibition zone with bioagents against *S. sclerotiorum* by dual culture technique (*In vitro*)

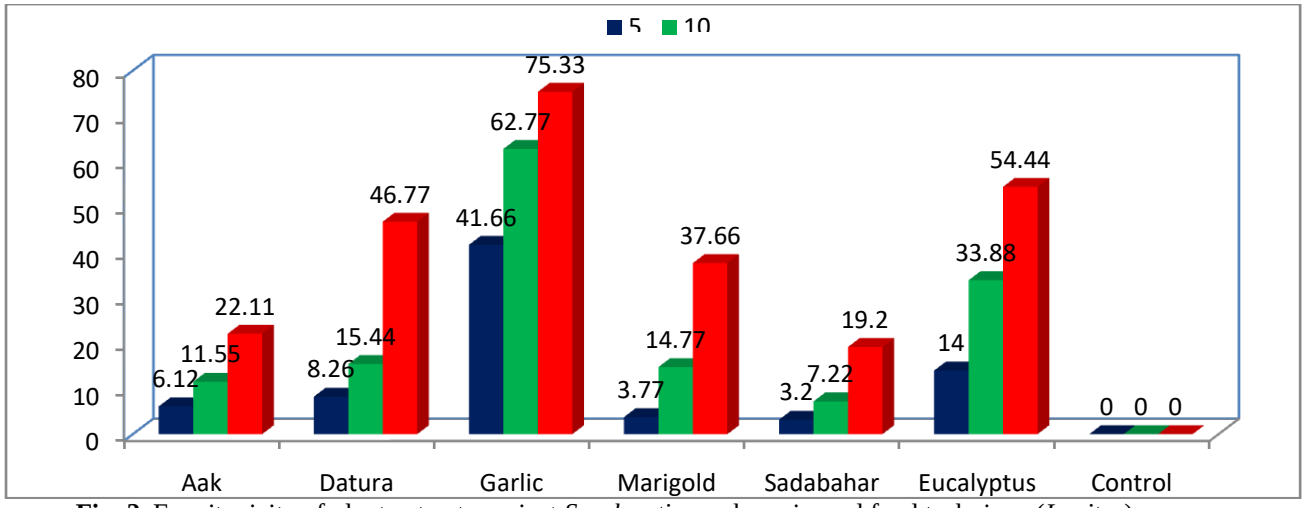

Fig. 2. Fungitoxicity of plant extracts against *S. sclerotiorum* by poisoned food technique (*In vitro*)

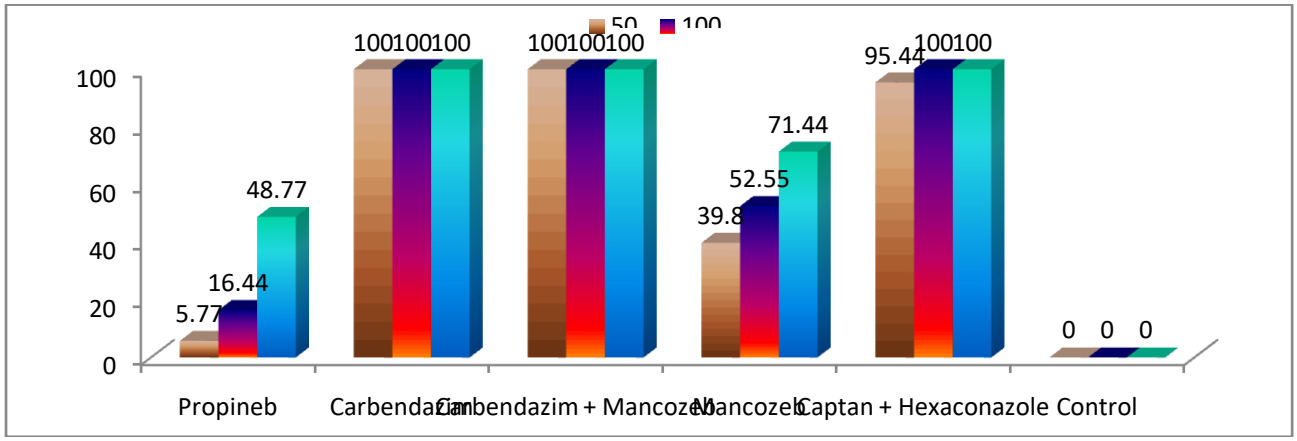

Fig. 3. Efficacy of fungicides against *S. sclerotiorum* by poisoned food technique (*In vitro*)

RESULTS AND DISCUSSION

Per cent mycelial inhibition zone with bioagents against *S. sclerotiorum* (*In vitro*): Efficacy of *Bacillus subtilis*, *Trichoderma harzianum* and *T. viride* was tested against *S. Sclerotiorum* (dual culture technique). After 7 days of incubation at25 ± 1˚C mycelium inhibition zone was recorded. Results (Fig-1) indicated that all the bio-agents viz., *Bacillus subtilis*, *T. harzianum* and *T. viride* were antagonistic to the growth of *S. sclerotiorum*. Maximum 71.22 % mycelia inhibition zone of pathogen was recorded in *T. viride* followed by 62.67 % in *T. harzianum* and minimum mycelial inhibition zone was recorded in *Bacillus subtilis* (41.44%). Earlier worker Saxena *et al.*, (2014) reported antagonistic activities of *Trichoderma harzianum* (PBT 23) against five different plant pathogens viz., *S. Sclerotiorum* (mustard), *R. solani* (maize), *S. Rolfsii* (tomato), *F. Oxysporum* f.sp. *ciceri* (chick-pea) and *B. Cineria* (chick-pea) and *Trichoderma* isolate was completely parasitized (100%) mycelial growth and decaying of sclerotia of *S. sclerotiorum*.

Efficacy of *T. viride* against (*S. sclerotiorum*) caused stem rot of coriander (*In vivo*): Field studies were conducted at Department of Plant Pathology (SKNCOA, Jobner) to manage the Stem rot through *T. viride* during *Rabi* season of the year 2015-16 under artificial soil inoculation conditions. To assess the Stem rot intensity slightly modified disease rating (0-4) scale (Lesovoi *et al.*, 1987 and Sansford, 1995) was used. A perusal of data (Table-1) revealed that minimum disease intensity (25.00 %) was recorded with seed + soil application of *T. viride* @ 10g/kg seed and 2.5 kg/ha, respectively, followed by soil application of *T. viride* @ 2.5 kg/ha (36.72 %) as compared to control (69.44 %). Maximum reduction in disease intensity over control was observed with seed + soil application of *T. viride* (63.99 %) followed by soil application of *T. viride* (47.11 %). Minimum per cent disease control (34.95 %) was observed in seed treatment of *T. viride* alone. Hu *et al.*, (2013) reported, *Aspergillus aculeatus* strain ASP-4 parasitize and destroy the sclerotia of *S. sclerotiorum* causing stem rot of mustard, in both the laboratory and field conditions.

Fungitoxicity of plant extracts against *S. sclerotiorum (In vitro)*: The efficacy of six plant extracts (Fig-2) was tested *In vitro* at three concentrations viz., 5, 10 and 15 per cent against *S. Sclerotiorum* on PDA by poisoned food technique. Among six plant extracts, extract of garlic clove was

found most effective in inhibiting mycelial growth (41.66, 62.77 and 75.33 %) of *S. Sclerotiorum* at 5, 10 and 15 per cent, respectively followed by eucalyptus leaf extract (14.0, 33.88 and 54.44 %) over control. Extract of sadabahar leaves was found least effective in inhibiting mycelial growth of *S. Sclerotiorum* over control (90 mm). All the concentrations (5, 10 and 15 %) of garlic clove extract were found significantly superior over other treatments. At 5 per cent concentration sadabahar and marigold were found at par with 3.20 % and 3.77 % mycelial growth inhibition, respectively, whereas at 10 per cent concentration marigold and datura were at par with 14.77 % and 15.44 % mycelial growth inhibition, respectively. Similarly, Mehta *et al.*, (2011) evaluated 13 plant extracts through poisoned food technique against *S. sclerotiorum* causing white stem rot of rapeseed-mustard. All the tested leaf extracts were effective in checking the mycelial growth except *Eucalyptus globulens* (eucalyptus) and *Tagetes erecta* (marigold).

Efficacy of plant extracts against *S. sclerotiorum* (*In vivo*):

Seed application: A perusal of data (Table-2) revealed that minimum disease intensity was observed with garlic (53.77 %) followed by eucalyptus (61.50 %), as compared to control (78.50 %). Maximum reduction in disease intensity over control was observed with garlic (31.50 %) followed by eucalyptus (21.65 %) over control. Per cent disease intensity of aak (73.48 %) was found at par with marigold (70.25 %). Minimum reduction in disease intensity was observed in aak (6.39 %).

Foliar application: The highest reduction in disease intensity over control (82.25 %) was observed in garlic (27.59 %) followed by eucalyptus (16.41 %) (Table-2). Per cent disease intensity of aak (77.75 %) was found at par with marigold (79.12 %). Minimum reduction in disease intensity was observed in aak (3.02 %). Similar work was reported (Chattopadhyay *et al.*, 2004) that the integrated management of Sclerotinia rot (*S. sclerotiorum*) infecting Indian mustard (cv. Rohini). The treatments comprised seed treatment or foliar spray of *T. Viride*, aqueous blub extracts of *Allium sativum* and carbendazim alone and in combination at 50 and 70 DAS and was observed seed treatment with *A. sativum* blub extract significantly reduced disease incidence by 70.2 % and produced the highest seed yield (34.3 %).

Seed-cum-foliar application: A perusal of data (Table-2) revealed minimum disease intensity in garlic (48.75 %) followed by eucalyptus (57.25 %) over control (79.75 %). Maximum reduction in disease intensity over control was observed in garlic (38.48 %) followed by eucalyptus (27.76 %). Per cent disease intensity of marigold (69.15 %) was found at par with aak (72.25 %) and datura (65.37%). Minimum reduction in disease intensity was observed in aak (8.83 %). Meena *et al.*, (2013) also reported that Sclerotinia rot was reduced in plant that received a combination of seed treatment and foliar spraying with garlic bulb extracts.

Efficacy of fungicides against *S. sclerotiorum* (*In vitro*): The efficacy of fungicides was evaluated against *S. Sclerotiorum* on PDA by poisoned food technique. The data suggested (Fig-3) that increase in concentration of the fungicides caused increased inhibition of mycelial growth of the fungus. Among these, carbendazim and carbendazim + mancozeb inhibited completely the mycelial growth of *S. sclerotiorum* at all concentrations (50,100 and 150 ppm). This was followed by captan + hexaconazole with inhibition of 95.44, 100 and 100 % at 50,100 and 150 ppm respectively. Propineb was found least effective at all concentrations against *S. sclerotiorum*. In chick pea, Pandey *et al.*, (2011) reported *in vitro* efficacy of fungicides against *S. sclerotiorum* causing stem rot and found most effective fungicide including matco, carbendazim, thiophenate methyl, propiconazole, hexaconozle etc.

Efficacy of fungicides against (*S. sclerotiorum*) caused stem rot of coriander (*In vivo*):

Seed application: A perusal of data (Table-3) revealed minimum disease intensity with carbendazim (31.62 %) followed by carbendazim + mancozeb (36.62 %) as compared to control (77.75%). Maximum reduction in per cent disease intensity over control was observed with carbendazim (59.33 %) followed by carbendazim + mancozeb (52.90 %) over control. Per cent disease intensity of captan + hexaconazole (37.25%) was found at par with carbendazim + mancozeb (36.62%). Minimum reduction in disease intensity was observed in mancozeb (32.21 %).

Foliar application: The highest reduction in disease intensity over control was observed in carbendazim (51.61) followed by carbendazim + mancozeb (48.69 %) (Table-6). Minimum reduction in disease intensity was observed in mancozeb (28.03 %).

Seed-cum-foliar application: A perusal of data (Table-3) revealed minimum per cent disease intensity in carbendazim (21.25 %) followed by carbendazim + mancozeb (24.50 %) over control (78.50 %).Maximum reduction in disease intensity over control was observed in carbendazim (72.92 %) followed by carbendazim + mancozeb (68.78 %). Minimum reduction in disease intensity was observed in mancozeb (39.49%). Singh *et al.*, (2014) evaluated *in vitro* and *in vivo* efficacy of fungicides against the *S. sclerotiorum* causing Sclerotinia rot of Mustard.

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