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Evaluation of Fungicides, Botanicals and Bio-Control Agents against Sclerotinia Rot of Cauliflower Caused by *Sclerotinia sclerotiorum* (Lib.) de Bary under *in-vitro* Condition

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ABSTRACT

The present investigation entitled "Evaluation of Fungicides, Botanicals and Bio-control agents against Sclerotinia rot of Cauliflower caused by *Sclerotinia sclerotiorum* (Lib.) de Bary under *in-vitro* condition" was carried out at Veer Chandra Singh Garhwali, Uttarakhand University of Horticulture and Forestry, Bharsar, Pauri Garhwal during year 2021– 22. Cauliflower is important vegetable crop rich in Vitamin A, C, ascorbic acid, potassium, sodium, iron, copper and manganese. Sclerotinia rot is very destructive disease in cauliflower which cause serious problem during rainy and humid climate. The result obtained in the present research study maximum mycelium growth of pathogen was observed on potato dextrose agar media (88.75 mm) at 7th day and the maximum mycelium growth of pathogen was observed at 20^{0}C (90.00 mm) at 7th day. The pH 5 shown maximum dry weight of mycelial growth (195.25 mg) at 14th. *In vitro* effect of different botanicals, fungicides and biocontrol agents were tested against the *Sclerotinia sclerotiorum.* Result revealed that Garlic was effectively inhibited 89.24% mycelial growth of the test pathogen at 15% concentration. The fungicides Carbendazim and Hexaconazole inhibited 100% mycelial growth of the test pathogen at 50 ppm and Propiconazole inhibited 100% mycelial growth of the test pathogen at 200 ppm. The biocontrol agent *Trichoderma viride* and *Trichoderma harzianum* were effectively inhibited 72.21 and 63.11% mycelial growth of the test pathogen.

Keywords *Sclerotinia sclerotiorum*, Mycelium, *Trichoderma viride, Trichoderma harzianum*, Carbendazim.

INTRODUCTION

Cauliflower (*Brassica oleracea* var. *botrytis* L.) belonging to family Brassicaceae is one of the important cole crop and has obtained a popular place in vegetable crops because of its delightful taste, flavor and nutritive value. It has been rightly designated as the aristocrat of cole crops.

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Cauliflower is one of the most important winter vegetables grown in India. India is the second largest vegetable producer in the world, next only to China with the area of 472 thousand hectares and production of 9370 thousand metric tonnes. In India major cauliflower growing states are West Bengal, Bihar, Madhya Pradesh, Haryana, Odisha, Assam, Chattisgarh, Uttar Pradesh, Punjab, Jharkhand. In Uttarakhand cauliflower is grown in an area of 3367.06 hectares with the production of 43292.95 metric tons.

Among these Sclerotinia rot of cauliflower caused by *Sclerotinia sclerotiorum* (Lib.) de Bary is one of the major soil borne fungal disease of cauliflower causing losses both in field, especially in the seed crop and in storage. The loss from this disease is as high as 40 – 80 %.

Among the major pathogens *Sclerotinia sclerotiorum* (Lib.) de Bary. The causal organism of Sclerotinia rot is the most ubiquitous, omnivorous, soil – borne and destructive plant pathogen, inciting disease on more than 500 plant species. Sclerotinia rot is more common and severe in temperate and sub – tropical regions of cool and wet seasons (Sharma 2014).

Though, cauliflower is an important crop of Uttarakhand yet the production of cauliflower for seed and vegetable has been threatened with the serious recurrence of Sclerotinia rot, a destructive disease caused by *Sclerotinia sclerotiorum* (Lib.) de Bary. This is major soil born fungal pathogen. In India, the disease was first observed in Saproon valley in the year 1973. Low temperature and high humidity condition favors the disease development to and epidemic form and the crop is badly damage at curd formation and flowering stages. The disease is a serious menace and has been reported to reduce the potential seed yield by 89 -90%. It is feared that it may become a limiting factor in seed production of cauliflower in the country regions. The plants are vulnerable to attack the pathogen at all stages of its growth depending upon the favorable temperature and moisture condition.

MATERIALS AND METHODS

The present investigations entitle "Evaluation of Fungicides, Botanicals and Bio-control agents against Sclerotinia rot of Cauliflower caused by *Sclerotinia sclerotiorum* (Lib.) de Bary under *in-vitro* condition" was conducted in the Department of Plant Pathology Laboratory, Vegetable Research and Demonstration Block, Department of Vegetable Science, College of Horticulture, VCSG UUHF, Bharsar (Pauri Garhwal) Uttarakhand during March 2022. The details of experimental material used and procedure are described here under.

Sterilization

Isolation and cultural studies were conducted under aseptic condition in the laminar air flow cabinet using sprit and flame for sterilizing inoculating needles and forceps tips. The working surface of laminar air flow was sterilized by swabbing with 70% ethanol. Sterilization of media was done in vertical autoclave. In this method, moist heat (steam) was provided for the sterilization at 121^{0}C with a pressure of 15 lbs psi for 20 minutes. All the glassware was sterilization in an electronic oven at 180^{0}C for 3 hrs. Any glassware coming from outside was flamed before and after use. The forceps, inoculation loop were sterilized by heating in the flames and hands were sterilized with ethanol.

Preparation of medium

Potato dextrose agar (PDA) medium was prepared by peeled potato and agar-agar dissolving in 1000 ml of water. The solution was heated to completely dissolve the solid components of the medium and then sterilized by autoclaving.

Collection, isolation, identification of the pathogen

Collection of diseased material

Diseased samples were collected from Vegetable research and Demonstration Block, Bharsar and used for isolation of the pathogen for further examinations.

Isolation and purification of pathogen

All the glassware's were cleaned with Potassium dichromate sulphuric acid solution, washed with

sterilized water, sterilized in hot air oven at 1800^{0}C for two hrs. Media (PDA) was sterilized by autoclaving at 1.045 kg/cm^{2} pressure for 20 minutes.

Diseased stem portions were washed with sterilized water and cut into small pieces of 1-2 mm size with the help of sterilized blade. Each piece was surface sterilized with 1% sodium hypochlorite solution for one minute followed by three consecutive washing with sterilized water and dried on sterilized blotter paper. One bit was placed aseptically in 2% PDA (Potato Dextrose Agar) slant culture tubes then incubated for 4 days at 20 ± 1^{0}C

Similarly, isolation was also made from black sclerotia present inside the diseased stem tissues. Sclerotia after surface sterilization were cut into small pieces with the help of sterilized blade. Each piece was surface sterilized with 1% sodium hypochlorite solution for one minute followed by three consecutive washing with sterilized water and dried on sterilized blotter paper. One bit was placed aseptically in 2% PDA slant culture tubes then incubated for 4 days at 20 ± 10^{0}C.The fungus was purified by hyphal tip method. The culture was maintained in refrigerator at 10^{0}C and renewed after every fifteen days.

Pathogenicity test

Pathogenicity test was conducted on healthy cauliflower plants in glass house, Vegetable Research and demonstration Block College of Horticulture, VCSG UUHF Bharsar. Different inoculation methods were applied on potted plants grown in sterilized soil to prove the pathogenicity of isolated pathogen. Un-inoculated healthy cauliflower plants were kept as control. Observation was made regularly for the appearance and development of symptoms. Within 7-10 days of inoculation plant showed typical Sclerotinia rot symptoms. After appearance of disease symptoms, re-isolation was made from the diseased tissues of artificially infected plants using PDA plate technique. The isolate obtained was compared with the original culture for confirmation of the same pathogenic isolates, which were inoculated.

Botanical preparation: The botanicals were prepared by crushing (plant part) mixing in distilled water (1:1 w/v). The mixture was passed through muslin cloth. Desire concentration was made by mixing calculated volume of plant extract into calculated volume of water (Upadhyay 2019).

Effect of physical parameters on the growth and sclerotia formation of the pathogen

All the glassware's were thoroughly cleaned and rinsed with distilled water. Chemicals of annular grade were used. Different synthetic and semi-synthetic media were prepared by weighing the different constituents of each medium and then adding the distilled water to make up the volume 1000 ml and autoclaved at 1.045 kg/cm^{2} for 20 minutes. Inoculation was done with 5 mm disc of mycelial mat taken from 7 days old fungal culture and incubated at 20±10^{0}C (except temperature study) for 10 days. Each experiment of physical parameter studies was arranged in Completely Randomized Design (CRD) with four replications.

Mycelial growth and sclerotia formation of *Sclerotinia sclerotiorum* on different solid media

The variation in culture characters of *Sclerotinia sclerotiorum* studied on the following solid media for the fungus growth. The growth characters of the fungus were studied different solid media namely Potato dextrose agar (PDA), Potato carrot agar, Oatmeal agar, Malt extract agar, Corn meal agar, Richard's agar media. All the media were sterilized at 121^{0}C at 15 psi for 15 minutes. 20 ml of the each of the medium poured in to 90 mm diameter petri plates. Such plates were inoculated with 5 mm disc of fungal growth and incubated as 25±1^{0}C. Each treatment was replicated four times. The fungal colony was measured after 5th and 7th days.

Effect of temperature on mycelial growth and sclerotia formation of *Sclerotinia sclerotiorum*

Effect of temperature on mycelial growth of *Sclerotinia sclerotiorum* was studied *in vitro*. Twenty ml of sterilized PDA medium was poured in each sterilized petri plate. Inoculation was made with 5 mm disc of 7 days old culture of *Sclerotinia sclerotiorum* with the help of sterilized cork borer and incubated at 5 differ-

ent levels of temperature viz., 10, 15, 20, 25 and 30 ^{0}C for 7 days. Observations on mycelial growth and sclerotia formation was recorded after 5th and 7th days of incubation, respectively.

Effect of pH on mycelial growth of *Sclerotinia sclerotiorum*

To study the effect of different levels of pH on mycelial growth, the pH of medium (broth) was adjusted at 4.0, 5.0, 6.0, 7.0 and 8.0 using citrate phosphate buffer before sterilization with the help of pH meter. Flasks having liquid medium of each pH level were inoculated with 5 mm disc of seven days old fungus culture. Flasks were incubated at 25±1^{0}C and the observation on mycelial growth (on dry weight basis) was recorded after 14 days of inoculation.

Efficacy of fungicides against *Sclerotinia sclerotiorum (in vitro)*

Six different fungicides namely Mancozeb, Captan, Carbendazim, Propiconazole, Hexaconazole and Copper oxychloride with a control were tested on different concentrations like 50, 100, 150 and 200 ppm by use of poison food technique and mycelial growth of the test fungus will be observed after 7th day after inoculation.

Poisoned food technique

The poisoned food technique was adopted for *in vitro* testing of botanicals. The calculated quantities of fungicides were aseptically added to 1000 ml of molten PDA in conical flasks, so as to get the desired concentration of active ingredient of each fungicide separately. The flask was shaken gently to ensure the proper mixing of chemicals in PDA, 20 ml of molten and cooled PDA was poured in each petri plate. After solidification of media, mycelial disc (5mm) was cut from the edges of three weeks old cultured of test pathogen with the help of sterilized cork borer and placed in the middle of Petri plates. Then it was incubated at 25±1^{0}C. Suitable control was kept in which the culture disc was grown under same conditions on PDA without fungicides. The radial colony growth was measured after 7th day of incubation. The efficacy of fungicides was expressed as per cent inhibition of mycelial growth over control, calculated by using following formula suggested by (Vincent 1947).

Efficacy of botanials against *Sclerotinia sclerotiorum* (*in vitro*)

Six different botanials namely Garlic, pine, kapoor, lemongrass, tulsi and onion with a control were tested on different concentrations like 5, 10 and 15 % by use of poison food technique and mycelial growth of the test fungus will be observed after 7th day after inoculation.

Efficacy of bio-control agents against *Sclerotinia sclerotiorum* (*in vitro*)

In vitro, screening of bio agents was done by dual culture technique. The following six biocontrol agents were used for study viz., *Trichoderma harzianum, T. asperellum, T. viride* (IARI isolate), *Pseudomonas fluorescence, Cladosporium herbarum* and *Aspergillus niger.* All the biocontrol agents were obtained from Department of Plant Pathology, College of Horticulture, VCSG UUHF Bharsar, India. Single colonies of the isolate were sub-cultured in PDA and stored in refrigerator to maintain their genetic purity.

Dual culture technique

Twenty-five ml of PDA medium was poured into sterile Petri plate and allowed for solidification. Five mm diameter discs from actively growing colony of pathogen was cut with a sterile cork borer and placed near the periphery of PDA plate. Similarly, biocontrol 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^{0}C for seven days. In each treatment three replications were maintained. The extent antagonistic activity by biocontrol agent was recorded after incubation period of 7th days by measuring the growth of the test pathogen in dual culture and in control plates. The efficacy of Bio-control agents was expressed as per cent inhibition of mycelial growth over control, calculated by using following formula suggested by (Vincent 1947).

Per cent mycelium inhibition

Percent inhibition of in growth was calculated in relation to growth in control using the following formula.

$$PGI = \frac{C\text{-}T}{C} \times 100$$

RESULTS AND DISCUSSION

Isolation, identification, morphological characters and pathogenicity test

In (Table 1) isolation was made from infected portion of plant. The culture of *S. sclerotiorum* was purified by hyphal tip method. Cultural characters of isolated disease material did not show variation i.e., amount of color of the aerial mycelium on PDA. The pathogen produced typical symptoms in 25- 30 days after inoculation. Gill *et al.* (2014) also isolated and proved pathogenicity of *S. sclerotiorum* on *Brassica* sp.

Growth study of pathogen on different culture media

In (Table 2) the fungus was capable of growing on different media, but variation in growth was observed on six media. Result showed that *S. sclerotiorum* grows best on potato dextrose agar medium (88.00 mm) followed by Richard's medium (86.00 mm) while minimum mycelial growth was observed in potato carrot agar (61.92 mm) on 8th day of incubation. Similar results were found by Bharti *et al.* (2015) that *S. sclerotiorum* have maximum growth on PDA (82.66 mm) after 07 day of inoculation.

Table 1. Effect of different inoculation method on symptom development of *S. sclerotiorum.*

Treatment details	Number of days ± SE (m)	Types of symptoms development
T_1 (Control)	0.00±0.00	No symptoms
T_2 (Soil inoculation)	57.88*±0.52	Water-soaked brown lesion
T_3 (Foliar spray inoculation)	15.72*±0.39	Water-soaked lesion
T_4 (Syringe inoculation)	19.05*±0.41	Water-soaked lesion
T_5 (Wound inoculation)	22.31*±0.27	Water-soaked lesion
SE (d)	0.51	
CD (0.05)	1.11	

*Significant at 5 % level of significance as compared with control.

Table 2. Effect of different media on mycelium growth (mm) of the pathogen.

Media	05 days ± SE (m)	07 days± SE (m)
T_1 (Potato dextrose agar)	68.68±0.69	88.75±0.31
T_2 (Czapeck's Dox agar)	30.16±0.83	43.05±0.47
T_3 (Oat meal agar)	56.51±0.71	77.98±0.61
T_4 (Malt extract agar)	45.97±0.63	62.93±0.82
T_5 (Corn meal agar)	48.12±0.12	65.39±0.34
T_6 (Richard's medium)	43.14±0.36	59.66±0.47
SE (d)	0.90	0.75
CD (0.05)	1.90	1.60

Growth study of pathogen at different temperature

In (Table 3) among the physical parameter's temperature is an important factor which play important role in growth, reproductive structures and survival of fungi and for each fungus there is a definite range of temperature for growth having three fundamental points viz., minimum, maximum and optimum Panchal *et al.* (2012). The fungus was grown on wide range of temperature i.e., from 5^{0}C to 25^{0}C. Results showed that maximum mycelial growth and sclerotia formation of the fungus were found at 20^{0}C.

Growth study of pathogen at different pH

In (Table 4) studies on *Sclerotinia sclerotiorum* have indicated that the pathogen can tolerate a wide range of pH i.e., 4.0 to 8.0 for growth. Better growth was recorded between pH 4.0 to 6.0. The results are in closeness with those obtained by Panchal *et al.* (2012).

Table 3. Effect of different temperature on mycelium growth (mm) of the pathogen.

Temperature (^{0}C)	05 days ± SE (m)	07 days± SE (m)
T_1 (10)	35.94±0.55	62.77±0.42
T_2 (15)	60.22±0.22	79.44±0.25
T_3 (20)	79.26±0.36	90.00±0.00
T_4 (25)	72.07±0.82	88.68±0.29
T_5 (30)	30.13±0.52	54.60±0.47
SE (d)	0.85	0.49
CD (0.05)	1.89	1.08

Table 4. Effect of pH on dry weight of mycelium growth of *Sclerotinia sclerotiorum* on PDA medium.

pH	Dry weight of mycelial growth (mg)* ± SE (m)
T_1 (4)	162.47± 0.26
T_2 (5)	195.25± 0.67
T_3 (6)	155.49± 0.45
T_4 (7)	75.93± 0.53
T_5 (8)	30.90± 0.51
SE (d)	0.71
CD (0.05)	1.53

Effect of Botanicals

In (Tables 5 - 6) Evaluation of six botanicals under laboratory (*in vitro*) condition at three different concentrations i.e., 5, 10 and 15 % was tasted against pathogen (*Sclerotinia sclerotiorum*) by poison food technique. This experiment showed that Garlic clove extract inhibited 89.24 % mycelium growth followed by Rosemary (74.47 %) at 15 % concentration. Ghasolia and Shivpuri (2008) found that Garlic clove extract (88.00 %) showed maximum per cent mycelium inhibition against *S. sclerotiorum.*

Effect of fungicides

In (Tables 7– 8.) Efficacy of six fungicides was tested *in vitro* under laboratory conditions for the per cent mycelial growth inhibition of *S. sclerotiorum.* The results of the experiment clearly show that carbendazim and hexaconazole completely inhibited mycelial growth up to 100 % at 50 ppm concentration. Propiconazole at 150 ppm concentration completely inhibited up to 100 % as compared to 95.45 % inhibition at 50 ppm concentration. Copper oxychloride was found least effective, as they inhibited 32.22% of fungal mycelial growth even at 200 ppm, respectively. Similar findings have reported by Prasad and Kumar (2007).

Table 6. Effect of botanicals on mycelium inhibition of the pathogen at different concentrations at 5, 10 and 15 %.

Treatments details	5% ± SE (m)	10% ± SE (m)	15%± SE (m)
T_1 (Control)	00.00±00.00 (00.00)	00.00±00.00 (00.00)	00.00±00.00 (00.00)
T_2 (Garlic)	72.25*±0.38 (58.19)	78.03*±0.50 (62.03)	89.24*±0.44 (70.84)
T_3 (Pine)	29.19*±0.65 (32.68)	41.28*±0.51 (39.96)	50.44*±0.68 (45.23)
T_4 (Kapoor)	41.47*±0.62 (40.07)	50.48*±1.14 (45.26)	58.68*±0.86 (49.98)
T_5 (Lemongrass)	53.14*±0.48 (46.78)	62.62*±0.73 (52.29)	69.01*±0.63 (56.15)
T_6 (Rosemary)	62.66*±0.61 (52.31)	71.22*±0.36 (57.53)	74.47*±0.47 (59.62)
T_7 (Onion)	40.76*±0.39 (39.66)	48.40*±0.40 (44.18)	59.99*±0.34 (50.74)
SE (d)	0.70	0.87	0.78
CD (0.05)	1.47	1.82	1.63

*Significant at 5% of significance compared with control.
( ) Value in parenthesis are angular transformed.

Effect of bio control agent

In (Tables 9–10) Biological control is of much significance in view of hazards caused by toxic chemicals or in a situation where pathogens develop resistance to fungi toxicants. Six biocontrol agents namely *Trichoderma harzianum, T. viride, T. asperellum,*

Table 5. Effect of botanicals on mycelium growth (mm) of the pathogen at different concentration at 5, 10 and 15 %.

Treatments	5 %± SE (m)	10% ± SE (m)	15% ± SE (m)
T_1 (Control)	88.61±0.31	88.74±0.28	88.66±0.29
T_2 (Garlic)	24.58*±0.32	19.40*±0.49	9.52*±0.36
T_3 (Pine)	62.74*±0.44	52.10*±0.49	43.93*±0.50
T_4 (Kapoor)	51.85*±0.38	43.94*±1.13	36.62*±0.70
T_5 (Lemongrass)	41.51*±0.44	33.16*±0.59	27.46*±0.48
T_6 (Rosemary)	33.08*±0.61	25.53*±0.34	22.63*±0.45
T (Onion)	52.48*±0.34	45.59*±0.32	35.47*±0.39
SE (d)	0.59	0.83	0.67
CD (0.05)	1.24	1.74	1.40

*Significant at 5 % level of significance as compared with control.

Table 7. Effect of fungicides on mycelium growth (mm) of the pathogen at different concentration.

Treatment details	50 ppm ± SE (m)	100 ppm ± SE (m)	150 ppm ± SE (m)	200 ppm ± SE (m)
T_1 (Control)	88.81±0.31	88.65±0.20	87.98±0.29	88.48±0.24
T_2 (Mancozeb)	73.70*±0.38	50.01*±0.63	45.49*±0.56	40.09*±0.4
T_3 (Captan)	49.55*±0.31	19.48*±0.52	13.41*±0.57	8.74*±0.40
T_4 (Carbendazim)	0.00*±0.00	0.00*±0.00	0.00*±0.00	0.00*±0.00
T_5 (Propiconazole)	4.03*±0.36	1.68*±0.18	0.00*±0.00	0.00*±0.00
T_6 (Hexaconazole)	0.00*±0.00	0.00*±0.00	0.00*±0.00	0.00*±0.00
T_7 (Copper oxychloride)	80.25*±0.46	72.03*±0.23	66.97*±0.81	59.96*±0.31
SE (d)	0.44	0.48	0.63	0.37
CD (0.05)	0.93	1.00	1.32	0.78

*Significant at 5% of significance compared with control.

Pseudomonas fluorescens, Cladosporum herbarum, Aspergillus niger were tested against *S. sclerotiorum* through dual culture technique. Results showed that maximum growth inhibition of the *S. sclerotiorum* was caused by *T. viride* (72.21%) followed by *T. harzianum* (63.11%) *Pseudomonas fluorescens* (43.81%) and minimum was seen in *Aspergillus niger* (25.51%). They also reported that *T.viride* was found significantly superior in reducing mycelial growth of *S. sclerotiorum.*

Sclerotinia rot appear initially on the collar region and above the ground level. The infected plant dies completely, and white mycelium grows on the plant parts which come in contact to the soil surface. The pith region of attacked stem is filled with large, hard and black sclerotial bodies. Under the microscopic observation the pathogen was identified as *S. sclerotiorum* on the basis of morphological characters, such as hyaline, branched, cottony, closely septate hyphae with dark brown to black sclerotia which were round to irregular in shape. In (Table1) pathogenicity of the isolated pathogen carried out by artificially inoculating methods. Five inoculated methods were used as pathogenicity test and observed the symptoms on the healthy plants. The maximum incubation period was observed for symptoms development with T_2, Soil inoculation (57.88 days) and minimum was recorded in T_3 foliar spray inoculation (15.72 days). In (Table 2) *in vitro,* Potato dextrose agar (88.75 mm) showed

Table 8. Effect of fungicides on mycelium inhibition of the pathogen at different concentrations at 50 ppm, 100 ppm, 150 ppm and 200 ppm.

Treatments details	50 ppm ± SE (m)	100 ppm ± SE (m)	150 ppm ± SE (m)	200 ppm ± SE (m)
T_1 (Control)	0.00±0.00	0.00±0.00	0.00±0.00`	0.00±0.00
T_2 (Mancozeb)	16.99*±0.61	43.57*±0.78	48.28*±0.58	54.68*±0.55
	(24.32)	(41.29)	(44.00)	(47.66)
T_3 (Captan)	44.19*±0.35	78.01*±0.61	84.74*±0.64	92.54*±2.52
	(41.65)	(62.02)	(66.99)	(71.65)
T_4 (Carbendazim)	100.00*±0.00	100.00*±0.00	100.00*±0.00	100.00*±0.00
	(90.00)	(90.00)	(90.00)	(90.00)
T_5 (Propiconazole)	95.45*±0.41	98.10*±0.20	100.00*±0.00	100.00*±0.00
	(77.69)	(82.07)	(90.00)	(90.00)
T_6 (Hexaconazole)	100.00*±0.00	100.00*±0.00	100.00*±0.00	100.00*±0.00
	(90.00)	(90.00)	(90.00)	(90.00)
T_7 (Copper oxychloride)	9.63*±0.61	18.73*±0.11	24.09*±0.83	32.22*±0.47
	(18.04)	(25.63)	(29.37)	(34.57)
SE (d)	0.55	0.54	0.64	0.45
CD (0.05)	1.15	1.14	1.35	0.94

*Significant at 5% of significance compared with control.
( ) Value in parenthesis are angular transformed.

Table 9. Effect of biocontrol agent on mycelium growth (mm) of the pathogen.

Treatment details	Percent mycelium inhibition (%)
T_1 (Control)	0.00 ± 0.00 (0.00)
T_2 (*Trichoderma harzianum*)	63.11*±0.53 (52.58)
T_3 (*Trichoderma viride*)	72.21*±0.40 (58.16)
T_4 (*Trichoderma asperellum*)	42.41*±0.82 (40.61)
T_5 (*Pseudomonas fluorescens*)	43.81*±0.27 (41.42)
T_6 (*Cladosporium herbarum*)	37.83*±0.41 (37.94)
T_7 (*Aspergillus niger*)	25.51*±0.41 (30.32)
SE (d)	0.66
CD (0.05)	1.39

*Significant at 5% of significance compared with control.
( ) Value in parenthesis are angular transformed.

maximum growth of the pathogen while minimum growth was observed in Czapeck's Dox agar (43.05 mm). In (Table 3) studies on the impact of varying temperatures under Maximum mycelial growth was observed *in vitro* condition at 25°C (90.00 mm) and minimum growth was observed at 30°C (54.60 mm). In (Table 4) studies on the impact of varying pH under maximum dry weight of mycelial is observed at pH 5 (195.25 mg) and minimum dry weight is observed at pH 8 *in vitro* condition (30.90mg). In (Table 6) *in vitro,* growth of *S. sclerotiorum* was maximum inhibited by Garlic (89.24%) at 15 % concentration followed by Rosemary (74.47%). In (Table 8) *in vitro*, growth of *S. sclerotiorum* were maximum inhibited by Carbendazim, Propiconazole and Hexaconazole (100%) at concentration 200 ppm. In (Table10) *in vitro*, growth of *S. sclerotiorum* was maximum inhibited by *Trichoderma viride* (72.21%) and followed by *Trichoderma harzianum* (63.11%). After summarization of all the result of experiments conducted *in vitro* conditions *Sclerotinia sclerotiorum* can be managed by botanicals (garlic@15%), fungicides (Carbendazim and Propiconazole @ 200 ppm) and biocontrol agents (*Trichoderma viride)* with perfect integrated can be carried out in the field conditions.

Table 10. Effect of biocontrol agent on mycelium inhibition of the pathogen.

Treatment details	Mycelium growth (mm)
T_1 (Control)	88.25±0.36
T_2 (*Trichoderma harzianum*)	32.55*±0.48
T_3 (*Trichoderma viride*)	24.52*±0.41
T_4 (*Trichoderma asperellum*)	50.81*±0.56
T_5 (*Pseudomonas fluorescens*)	49.58*±0.30
T_6 (*Cladosporium herbarum*)	54.90*±0.41
T_7 (*Aspergillus niger*)	65.73*±0.261
SE (d)	0.58
CD (0.05)	1.21

*Significant at 5% of significance compared with control.

CONCLUSION

The potato dextrose agar medium was found to be the best suitable medium for mycelia growth of *S. sclerotiorum* and the suitable temperature of 20^0C was found to be the best for the growth and suitable pH was 5 and use of botanicals, fungicides and bio control agents can be used to control the disease with perfect integrated can be carried out in the field conditions.

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