Original Research Article

Evaluation of selected plant extracts and carbendazim against stem rot of mustard (*Brassicajuncea* L.) caused by *Sclerotiniasclerotiorum* (Lib.) de Bary

ABSTRACT

Sclerotinia stem rot disease caused by *Sclerotinia sclerotiorum* (Lib.) de Bary is a serious problem of mustard. The Effect of five plant extracts using of neem, garlic, eucalyptus, datura, and calotropis were tested against *S. sclerotiorum*. Maximum inhibition (%) of mycelial growth of *Sclerotinia sclerotiorum* was recorded in garlic bulb extract at 10% followed by calotropis extract after 96 hours of inoculation. In field experiment during rabi season 2023-24, seed treatment with carbendazim 2g/kg and foliar sprays of garlic bulb extract at 10% was most effective and recorded lowest disease incidence (20.08%) of sclerotinia stem rot of mustard (Brassica juncea L.), maximum production (13.26 q/ha) and maximum cost benefit ratio (1:1.87).

Keywords: Botanicals, carbendazim, mycelial growth, Sclerotinia sclerotiorum, stem rot,

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1. INTRODUCTION

Mustard (*Brassica juncea* L.) is most important oil seed crop in all over India. Its belonging to family of Cruciferae. It is grown in certain tropical and subtropical regions as a cold-season crop. Mustard is the key source of income mainly for the marginal and small farmers cultivated mainly in the rain-fed and resource vulnerable regions of the country [11]. Mustard is second in oilseed production, with soybean leading at 12.4 million metric tons, while mustard production is 11.8 million metric tons. The third most important source of edible oil next to soybean and palm oil [4].

Sclerotinia stem rot was first reported by Shaw and Ajreker (1915) from Pusa (Bihar). Later on, the occurrence of this disease has also been observed in other parts of the country [8]. Stem rot now become a serious problem in mustard crop in northern India. It's now economically important yield reducing disease that has been widely reported in the last few years in India. This disease has now attained next position to alternaria blight in terms of its economic.

Botanical pesticides or plant extracts as vital components of an integrated pest management programme due to their environmentally friendly approach that possesses low persistence, biodegradability and low mammalian toxicity. Hence, use of botanicals for management of plant diseases is needed for research due to their easy availability, ecofriendly nature, cost effective and safe for human as well as animal health [13].

2. MATERIAL AND METHODS

2.1.1 Symptoms of stem rot of mustard

Symptoms of stem rot of mustard caused by *Sclerotinia sclerotiorum* were observed on lower leaves turning yellow and wilting, while a white, fluffy mold appeared around the base of the plant or on the stem collar [5]. As the disease advance, the stems become soft and can be peeled away easily. Water-soaked spots then form and grow into larger, sunken lesions that might completely encircle the stem, leading to plant collapse [3]. Black, irregularly shaped sclerotia, ranging from 2 to 12 mm, develop on the infected areas [2]. Wet conditions make this disease worse, and it tends to affect older plants more than young ones. Flowers and pods can also be affected, causing stunted growth and lower yields [10].

2.1.2 Morphological characters of *Sclerotinia sclerotiorum*

Sclerotinia sclerotiorum hyphae are hyaline, septate, multinucleate, thin walled 9-18 µm in width and branching is never at right angles. Mycelia may appear white to tan in culture. Individual sclerotia are embedded in white mycelial net and are round, semi spherical to irregular in shape, measuring 2-10 x 3-15 mm in size. Sexually produced apothecia are cup shaped with concave disc, light yellowish brown, and vary in size from 2-11 mm (average 4-5mm) in diameter [9].

2.1.3 Potato Dextrose Agar (PDA) medium:

The culture media used in experiment was prepared according to standard formula. For isolating and culturing of pathogen (*Sclerotinia sclerotiorum*) Potato Dextrose Agar (PDA) medium was used.

Procedure:

The potato was peeled and cut in to small pieces and boiled in 500 ml of distilled water till they become soft. The extract obtained was filtered through muslin cloth and all the liquid was squeezed in beaker. Twenty gram of dextrose was added bit by bit to the remaining 500 ml of hot water. Then 20 g of agar was added to solidify. Volume of broth were made up to 1000 ml by adding more distilled water. Then in each conical flask 200 ml of this solution was dispensed and sterilized at 121°C at 15 lbs. pressure/square inch for 15 minutes in an autoclave.

2.1.4 Isolation of *Sclerotinia sclerotiorum*:

Potato Dextrose Agar (PDA) was prepared and 80 mg of streptomycin, an antibiotic was added to each 500 ml preparation of the PDA to inhibit probable bacterial growth. The infected stem parts were cut into small pieces of two to three mm dimension in a manner so that pieces may have some green portion also. Such stem bits were surface sterilized with 1 per cent sodium hypochlorite (NaOCl) solution for 30 seconds and washed three times with sterile distilled water to remove any traces of sodium hypochlorite adhered with stem bits [12]. Two to three stem bits were transferred on PDA medium contained in Petri plates aseptically with the help of sterilized forceps. These Petri plates were incubated at 25±1°C. After 3 days mycelia growth was observed around stem bits from this colony growth, a portion from the periphery that is, single hyphal tip was separated and transferred to other.

2.1.5 Preparation of plant extracts

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The fresh leaves of selected plants and bulb extract were gently washed under running tap water and finally in sterile distilled water. They were be separately grinded in sterile water at the rate 1 ml/g of plant material in pestle and mortar. Then were filtered through double layer of muslin cloth and finally through sterilized Whatman no.1 filter paper. This forms 100 % standard plant extract solution. Further its dilution was performed of required concentration with sterilized water. By grinding 10 g of leaves in 100 ml of sterile water 10 % of leaf extract can be obtained.

2.1.6 Poisoned food technique:

Five mm diameter of culture disc of *Sclerotinia sclerotiorum* was kept at the center of each Petri plate containing the botanicals of 10 per cent concentration dissolved in PDA. Three replications were maintained. The plates were incubated at 25±1˚C for seven days and colony diameter was recorded [14].

Per cent inhibition of mycelial growth was calculated by using the formula:

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

where,

I = Per cent inhibition,

C=Growth (mm) of test fungus in untreated control plates,

T=Growth (mm) of test fungus in treated plates.

2.1.7 Mass multiplication of pathogen:

The fungus inoculum was multiplied on sterilized sorghum grains. The sorghum grains were soaked in sterilized water overnight. The excess water drained out and 40 grams of grains were taken in each 250 ml conical flask and sterilized in autoclave at 1.045 kg cm^{-2} pressure for 20 minutes. The sorghum grains in flasks were inoculated aseptically with 5 days old mycelial discs (5 mm) of the pathogen and inoculated for 15 days at 20±2 °C. The inoculum was mixed in rows during sowing, using 20 g of inoculum per meter of row [1].

2.1.8 Disease incidence:

The incidence of disease was visually assessed in all the plots at 15 interval from first appearance of disease for each treatment. The data was analyzed statistically. Disease intensity was calculated by following formula:

$$\text{Disease incidence} = \frac{Number\ of\ infected\ plants}{Total\ number\ of\ plants} \times 100$$

2.1.9 Cost benefit ratio:

Cost benefit ratio is the ratio of gross return to cost of cultivation, which can also be expressed as return per rupee invested. This index provides an estimate of the benefit a farmer derives from the expenditure incurs in adopting a particular cropping system. Any value above 2.0 is considered safe as the farmer gets Rs. 2 for every rupee invested. The cost benefit ratio was calculated using the formula [7]:

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C:B ratio= (Gross return)/ (Total Cost of treatment)

3. RESULTS AND DISCUSSION

The *in vitro* screening aimed out to find out the efficacy of selected botanicals against *Sclerotiniasclerotiorum*using poisoned food technique. The experiment was analysed by using CRD (Completely randomized design) with six treatments including control which were T_1- neem leaf extract @ 10 %, T_2 –@ 10 %, T_3 - eucalyptus leaf extract @ 10 %, T_4 - datura leaf extract @ 10 %, T_5 – garlic bulb extract @ 10 %, T_6 –carbendazim @ 0.1% and T_0 – untreated check observations were recorded at 96 hours. Minimum radial growth at 96 hours was recorded in garlic bulb extract @ 10 % (37.50 mm) followed by calotropis leaf extract @ 10 % (44.37 mm), as compared to treated control carbendazim (30.87 mm) and untreated control (90.00 mm). Maximum per cent inhibition of radial growth was recorded with garlic bulb extract @ 10 % (58.33) followed by calotropis leaf extract @ 10 % (50.70%), as compared to treated control carbendazim (65.70%) and untreated control (0.00%).

In the present studies maximum per cent inhibition at 96 hours was recorded with garlic bulb extract followed by calotropis extract which were found effective over other treatments. The probable reasons may be the fungicidal properties of *Allium sativum* due to the presence of allicin and di-allyl sulphide. Allicin is the most important biologically active substance of garlic, it is formed from its precursor, allin, by the action alliinase enzyme. Antifungal activates of allicin can be attributed to its interaction with the thiol group of proteins and amino acids and that, especially with the latter, allicin forms S-allyl derivates. These results were similar to the findings of Shivpuri and Gupta (2001), Chattopadhyay *et al*. (2007), Sharma *et al.* (2016) and Kewate *et al.* (2020). Maximum per cent inhibition was recorded in garlic bulb extract against *Sclerotinia sclerotiorum*.

Table 1. Mean colony diameter and per cent inhibition

Treatment Number	Treatments	Concentration (%)	Meancolony diameter(mm)	Per cent inhibition (%)
T_0	untreated control	-	90	0
T_1	Neem leaf extract	10%	61.96	31.15
T_2	Calotropis leaf extract	10%	44.37	50.70
T_3	Eucalyptus leaf extract	10%	85.65[a]	5.94[a]
T_4	Datura leaf extract	10%	83.90[a]	6.77[a]
T_5	Garlic bulb extract	10%	37.50	58.33
T_6	Carbendazim	0.1%	30.87	65.70
			C.D. (5%)	0.825
			SEM (±)	0.26

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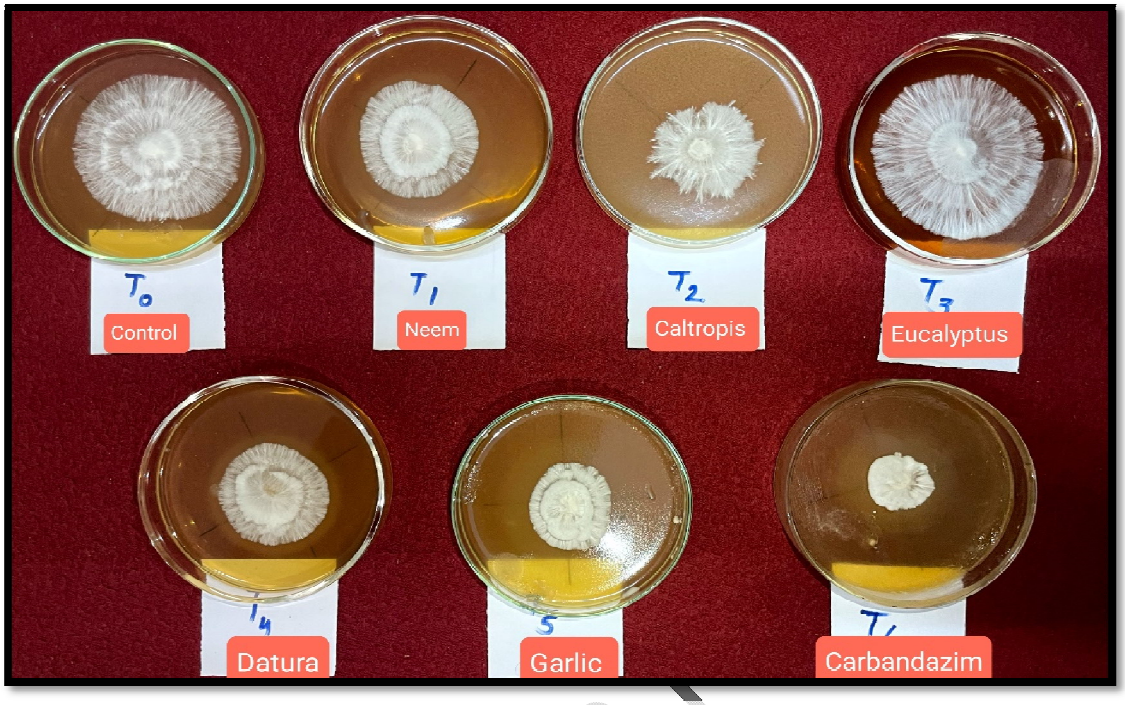

Plate 1.Effect of selected botanicals onradial growth of *Sclerotinia sclerotiorum*

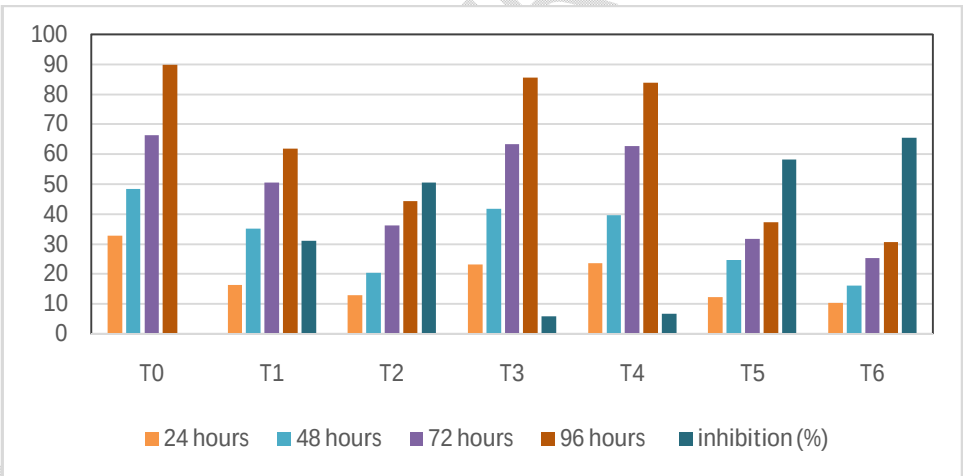

Figure1. Effect of selected botanicals on per cent inhibition and radial growth of *Sclerotinia sclerotiorum*

The experiment was analysed by using RBD (randomized block design) with three replications having plot size 2 x 1 m^2 with six treatments including control which were T_1- neem leaf extract @ 10 % (Foliar Spray) + carbendazim @ 2g/kg (Seed Treatment), T_2 – calotropis leaf extract @ 10 % (Foliar Spray) + carbendazim @ 2g (Seed Treatment), T_3 - eucalyptus leaf extract @ 10 % (Foliar Spray) + carbendazim @ 2g (Seed Treatment), T_4 - datura leaf extract @ 10 % (Foliar Spray) + carbendazim @ 2g (Seed Treatment), T_5 – garlic bulb extract @ 10 % (Foliar Spray) + carbendazim @ 2g (Seed Treatment), T_6 –carbendazim @ 2g (Seed Treatment) and T_0 – untreated control observations were recorded at 75 and 90 DAS.

Minimum per cent disease incidence (%) at 75 DAS was recorded in garlic bulb extract + carbendazim (14.28%) followed by neem leaf extract + carbendazim (16.66%) as compared to treated check carbendazim (20.27%) and untreated control (34.28%).

Minimum per cent disease incidence (%) at 90 DAS was recorded with garlic bulb extract + carbendazim (20.08%) followed by neem leaf extract + carbendazim (20.87%) as compared to treated check carbendazim (27.61%) and untreated control (42.85%).

Minimum number of sclerotia per stem at harvest was recorded with garlic bulb extract + carbendazim (2.13) followed by neem leaf extract + carbendazim (3.73), as compared to treated check carbendazim (13.26) and untreated check (16.13).

Maximum yield (q/ha) was recorded with garlic bulb extract + carbendazim (13.26q/ha) followed by neem leaf extract + carbendazim (12.81q/ha) as compared to treated check carbendazim (9.80q/ha) and untreated check (9.24q/ha).

Maximum cost benefit ratio was recorded with garlic bulb extract + carbendazim (1:1.87) followed by neem leaf extract + carbendazim (1:1.81) as compared to treated check carbendazim (1:1.47) and untreated check (1:1.44).

Table2. Effect of carbendazim and selected botanical extracts on disease incidence (%), number of sclerotia and yield (q/ha) of mustard

Tr. No.	**Treatments**	**Per cent Disease Incidence (%)**			**Number of sclerotia**	**Yield (q/ha)**
		60 DAS	**75 DAS**	**90 DAS**		
T_0	Control (untreated check) Waterspray	28.57	34.28	42.85	16.13	9.24
T_1	Carbendazim (ST) + Neem leaf extract @ 10% (FS)	11.12	16.66	20.87	3.73	12.81
T_2	Carbendazim (ST) + Calotropis leaf extract (aak) @ 10% (FS)	12.14	18.09	22.85	6.40	12.10
T_3	Carbendazim (ST) + Eucalyptus leaf extract @ 10% (FS)	14.28[a]	19.62[a]	25.23[a]	11.00[a]	10.75[a]
T_4	Carbendazim (ST) + Datura leaf extract @ 10% (FS)	13.88[a]	19.44[a]	25.07[a]	10.53[a]	11.07[a]
T_5	Carbendazim (ST) + Garlic bulb extract @ 10% (FS)	8.57	14.28	20.08	2.13	13.26
T_6	Carbendazim (ST) (Treated check)	16.66	20.27	27.61	13.26	9.80
	C.D. (5%)	0.587	0.533	0.527		0.575
	C.V. (%)	1.568	1.469	1.123		1.568

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The probable reasons may be the fungicidal properties of *Allium sativum* due to the presence of allicin and di-allyl sulphide. Allicin is the most important biologically active substance of garlic, it is formed from its precursor, allin, by the action alliinase enzyme. Antifungal activates of allicin can be attributed to its interaction with the thiol group of proteins and amino acids and that, especially with the latter, allicin forms S-allyl derivates. These results were similar to the findings of Shivpuri and Gupta (2001), Chattopadhyay *et al*. (2007),Yadav (2009),Sharma *et al.* (2016) andBharti *et al.* (2021).

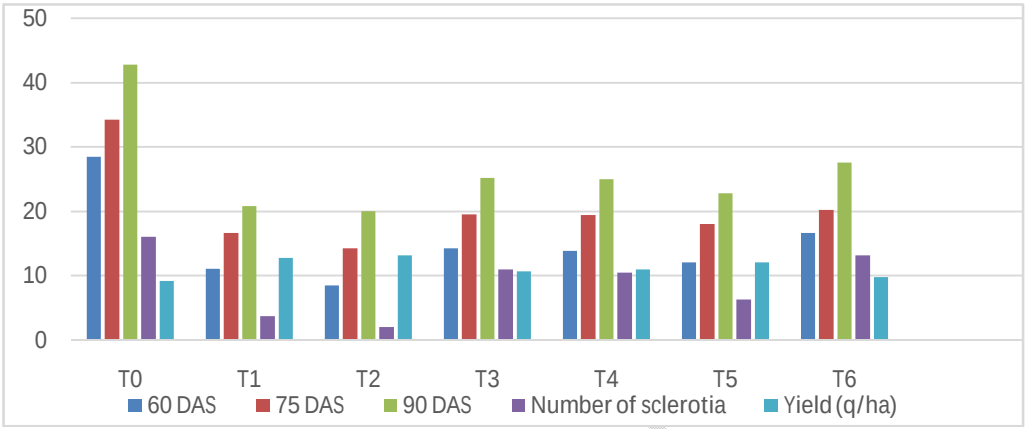

Figure2.Effect of treatments on disease incidence (%), number of sclerotia and yield (q/ha) of mustard

4. CONCLUSION

The present study concludes that the treatment with garlic bulb extract at 10% significantly inhibited the radial growth of *Sclerotinia sclerotiorum* (stem rot of mustard). The minimum disease incidence in stem rot of mustard (*Sclerotinia sclerotiorum*) was observed with garlic bulb extract at 10% (Foliar Spray). The yield and cost-benefit ratio were superiorly recorded in the treatment of garlic bulb extract at 10% (Foliar Spray) + carbendazim (Seed Treatment) when compared to treated check and the untreated check. The present investigation was limited to one crop season (*Rabi*), under the climatic conditions of Prayagraj (U.P.). Therefore, to substantiate the present result, more such trials are required for further recommendation.

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