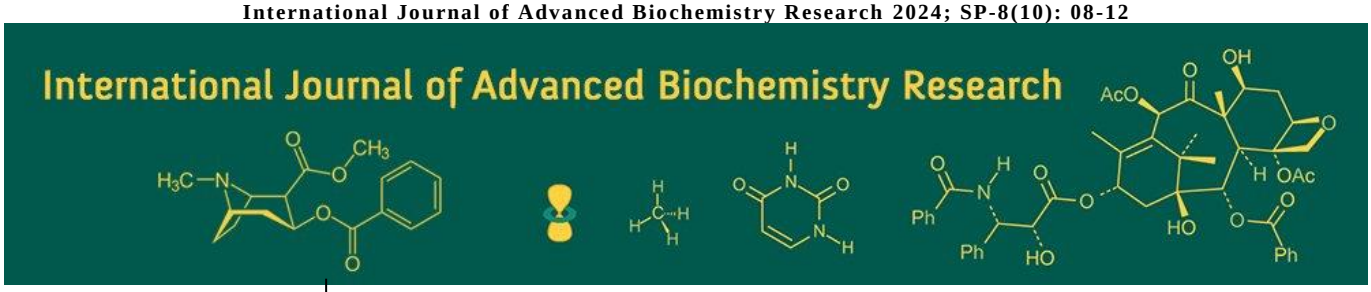

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Effect of phytoextracts on stem rot caused by *Sclerotinia sclerotiorum* (Lib.) de Bary on mustard (*Brassica juncea* L.)

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Abstract
Mustard (*Brassica. juncea* L.) is the most important oil seed crop among the major *Rabi* growing oil seeds of India. Stem rot disease of mustard is caused by cosmopolitan soil-borne hemibiotrophic fungus, is one of the utmost important disease of mustard, causing considerable losses in seed yield and deteriorates the oil quality.The general inadequacy of chemical fungicides to tackle stem rot in mustard has led to the search for ecofriendly management to this disease. Therefore, present study "Effect of phytoextracts on stem rot (*Sclerotinia sclerotiorum* Lib.) de Bary on mustard (*Brassica. juncea* L.)" was carried out during *Rabi* season in the year 2023-24 at Central Research field, Department of Plant Pathology, SHUATS, Prayagraj. Among the various phytoextracts tested against the stem rot of mustard under field conditions, revealed that disease incidence (%) at 60, 75 and 90 DAS was significantly decreased in treatment T_3– garlic bulb extract (*allium sativum*) (16.66%), (22.20%) and (25.92%), followed by neem leaf extract (19.40%), (24.06%) and (29.62%). The number of sclerotia per stem at harvest was significantly decreased in T_3– garlic bulb extract (*allium sativum*) (3.60), followed by neem leaf extract (5.60). The yield (q/ha) was significantly increased in T_3 - garlic bulb extract (*allium sativum*) (13.04 q/ha), followed by neem leaf extract (12.42 q/ha) respectively as compared to control T_0.

Keywords: Mustard, phytoextracts, *Sclerotinia sclerotiorum*, hemibiotroph and soil-borne fungus

Introduction
Mustard (*Brassica juncea* L.) is one of most important oil seed crops in all over India. Mustard is third important oil seed crop in the world after Soya and Palm and India is third largest producer with contribution globally of 28.3 per cent acerage and 19.8 per cent production [7]. Its belonging to family of Cruciferae (Brassicaceae).White rust, Alternaria blight, Downey mildew, Powdery mildew and Sclerotinia stem rot are important diseases of mustard crop [8]. Sclerotinia rot caused by *Sclerotinia sclerotiorum* (Lib.) de Bary is a soil-borne hemibiotrophic fungus causing one of the most devastating, notorious disease worldwide of *Brassica* sp., has switched from being of minor significance to major significance since the last decade due to changes in climatic conditions and presently, one of the most devastating diseases of mustard at the global level causing losses up to 90-100% in Indian mustard [15]. *S. sclerotiorum* exhibits dual infection mode in its host as its resting bodies (sclerotia) and can germinate either myceliogenically (soil-borne infection) to cause disease in the basal stem or can germinate carpogenically (air-borne infection) to cause disease in leaves and siliquae.

As things stand now, the rising application of chemicals to control the stem rot of mustard is turning into a menace public health and environmental concerns. Therefore, the present focus is on utilization of alternative method to manage this disease in eco-friendly manner by using indigenous materials *i.e.* neem leaf, Eucalyptus leaf, garlic bulb, onion bulb and ginger rhizome for management of the plant disease which is less expensive and least disadvantageous to the health of people and the environment. Thus, the paper seeks to achieve *in-situ* management of stem rot using phytoextracts.

Materials and Methods

Experimental place: The present investigation was carried out in the *Rabi* season (2023-24). Central Research Farm (CRF), Department of Plant Pathology, Sam Higginbottom University of Agriculture, Technology And Sciences (SHUATS), Prayagraj.The experiment was analysed in randomized block design (RBD) with three replications in a plot size 2x1 m^2.

Symptoms: *Sclerotinia sclerotiorum* causes more or less similar symptoms on leaves, stem and siliquae as fluffy white mycelia and sclerotia are produced after mycelial growth when the nutrition is not sufficient or other conditions are favourable for sclerotia development [5]. Sclerotinia stem rot in rapeseed mustard starts as elongated, water-soaked lesions on stem especially at base or at internodes and later white mycelial growth covers these lesions and affected plants look whitish from distance. The disease becomes air borne and spread through infected flower petals which fall and become lodged between the main stem and side branches. Large oval to round shaped holes are also formed on leaves due to air borne infection. Under severe infection, defoliation, shredding of stem, wilting and drying of plants occurs. Infected plants was ripe earlier and stand out among green plants [12].

Plate 1: Symptoms of stem rot of mustard

Isolation and maintainance of pure culture: 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. Pathogen was isolated both from infected tissue and the sclerotia obtained from the diseased stem. Infected tissue was cut into small pieces and these small pieces/sclerotia were surface sterilized by dipping them in 0.1 per cent Sodium hypochlorite (NaOCl) solution for one minute and washed three times with sterile distilled water to remove any traces of Sodium hypochlorite adhered with leaf bits [16]. 2-3 leaf bits was 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 leaf bits from this colony growth, a portion from the periphery that is, single hyphal tip was separated and transferred to other petri plates to obtain pure culture and maintain it for further study.

Plate 2: Pure culture of *Sclerotinia sclerotiorum* on petri plate

Morphological characters of test fungus: *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. Apothecia are formed on a slender stalk of 20-80 mm in length called stipe [8]. Asci are arranged on periphery of ascocarp, measuring 119- 162.4x6.4-10.9µ in size, and are inoperculate, cylindrical, narrow, rounded at the apex with eight ascospores per ascus. Ascospores are uniform, hyaline, ellipsoid with smooth walls, measuring 10.2-14.0µ x 6.4-7.7µ in size [14].

Mass multiplication of *Sclerotium sclerotiorum* on sorghum grain medium:

The fungus was mass cultured on sorghum seeds. The seeds were soaked overnight in 5% sucrose solution. The soaked seeds were transferred to 500 ml conical flasks and autoclaved twice at 15 Kg/cm^3, 121 °C, for 15-20 minutes. Thereafter, the flasks were inoculated with the pure culture of *Sclerotinia sclerotiorum* and incubated at 25+1 °C for 8-10 days in an incubator [13]. For soil inoculation, fungus colonized seeds (532g) were ground in a mixer-grinder and suspended in 10 liter tap water. The suspension was spread uniformly on micro plots of 2x1 m^2 to achieve an inoculum level of 1.5 g colonized seeds/kg soil. Soil inoculation was done two days before seed was sown [1].

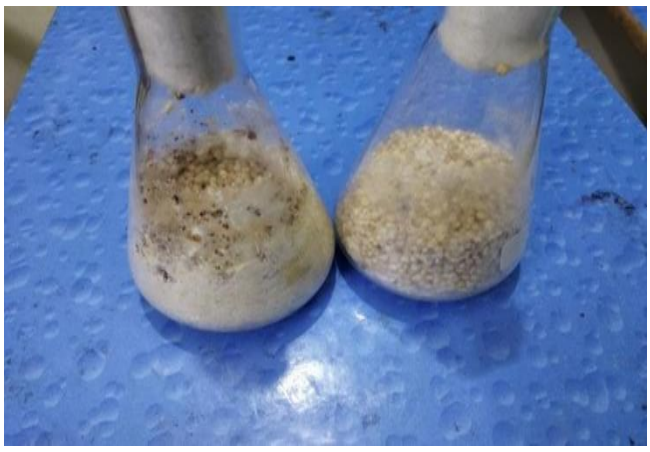

Plate 3: Mass multiplication of pathogen on sorghum grains

Preparation of plant extracts and Application of treatments

Botanical extracts were prepared by using method of standard procedure given by [10]. Matured leaves were collected and sterilized with distilled water, the leaves were homogenized in a pre-chilled pestle and mortar using chilled, sterilized distilled water. Aqueous extract of this botanical (1% w/v) were prepared by mixing 100g fresh leaves of plant with 100 ml of sterile distilled water and crushing in warring blender. The extract was filtered through four layers of moisture muslin cloth. The filtrate thus obtained was considered as 100% plant extract. Further its dilution was performed of required concentration 15% with sterilized water.

All the plant extract were given as foliar spray @ 15 ml/liter of water with the help of sprayer and mancozeb was applied @ 2 ml/litre of water.

Disease incidence (%): The incidence of disease was visually assessed in all the plots at weekly interval from first appearance of disease for each treatment. The data was analysed statistically. Disease incidence was calculated by following formula.

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

Disease incidence (%) was recorded at 60, 75 and 90 days after sowing.

Results and Discussion

***In situ* evaluation of botanicals**

Effect of treatments on disease incidence (%) of mustard

60 DAS: Data recorded in **Table 1** showed that, among all treatments, minimum per cent disease incidence (%) at 60 DAS was recorded with garlic bulb extract (*allium sativum*) (16.66%) followed by neem leaf extract (19.44%), eucalyptus leaf extract (20.83%), ginger rhizome extract (21.29%), onion bulb extract (22.22%) as compared to mancozeb (treated check) and control.

75 DAS: Data recorded in Table 1 showed that, among all treatments, minimum per cent disease incidence (%) at 75 DAS was recorded with garlic bulb extract(*allium sativum*) (22.20%) followed by neem leaf extract (24.06%), eucalyptus leaf extract (25.88%), ginger rhizome extract (26.85%), onion bulb extract (28.70%) as compared to mancozeb (treated check) and control.

90 DAS: Data recorded in Table 1 showed that, among all treatments, minimum per cent disease incidence (%) at 90 DAS was recorded with garlic bulb extract (*allium sativum*) (25.92%) followed by neem leaf extract (29.62%), eucalyptus leaf extract (31.71%), ginger rhizome extract (32.40%), onion bulb extract (34.25%) as compared to mancozeb (treated check) and control.

In the present studies minimum disease incidence at 60, 75 and 90 DAS was recorded with garlic bulb extract followed by neem leaf extract and found effective over other treatments. Garlic possess fungicidal property might be 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 activities 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 Chattopadhyay *et al.* [3], Chattopadhyay *et al.* [4], Yadav [18], Upadhyay *et al.* [17], Meena *et al.* [11] and Jajoriya *et al.* [6] who also reported that garlic bulb extract among other plant extracts emerged superior and inhibited the growth of *sclerotinia sclerotiorum*. In the studies conducted by Upadhyay *et al.* [17] and Jajoriya *et al.* [6], minimum disease incidence (30%) was found in garlic bulb extract (F.S) effective over the other treatments.

Table 1: Effect of various treatments on Disease incidence (%) of mustard:

Tr. No.	Treatment	60 DAS*	75 DAS*	90 DAS*
T_0	Control (untreated check)	28.68	37.03	45.40
T_1	Neem leaf extract @15% (F.S.)	19.40	24.06	29.62
T_2	Garlic bulb extract @15% (F.S.)	16.66	22.20	25.92
T_3	Ginger rhizome extract @15% (F.S.)	21.29 [a]	26.85 [a]	32.40 [a]
T_4	Eucalyptus leaf extract @15% (F.S.)	20.83 [a]	25.87 [a]	31.71 [a]
T_5	Onion bulb extract @15% (F.S.)	22.22	28.70	34.25
T_6	Mancozeb 75% (0.2%) (F.S.)	9.25	13.88	17.59
	CD(p=0.05)	0.702	1.394	0.730
	S. Ed±	0.32	0.57	0.34

*Average of three replications

*Data followed by same alphabets in a column are non-significant to each other at 5% level

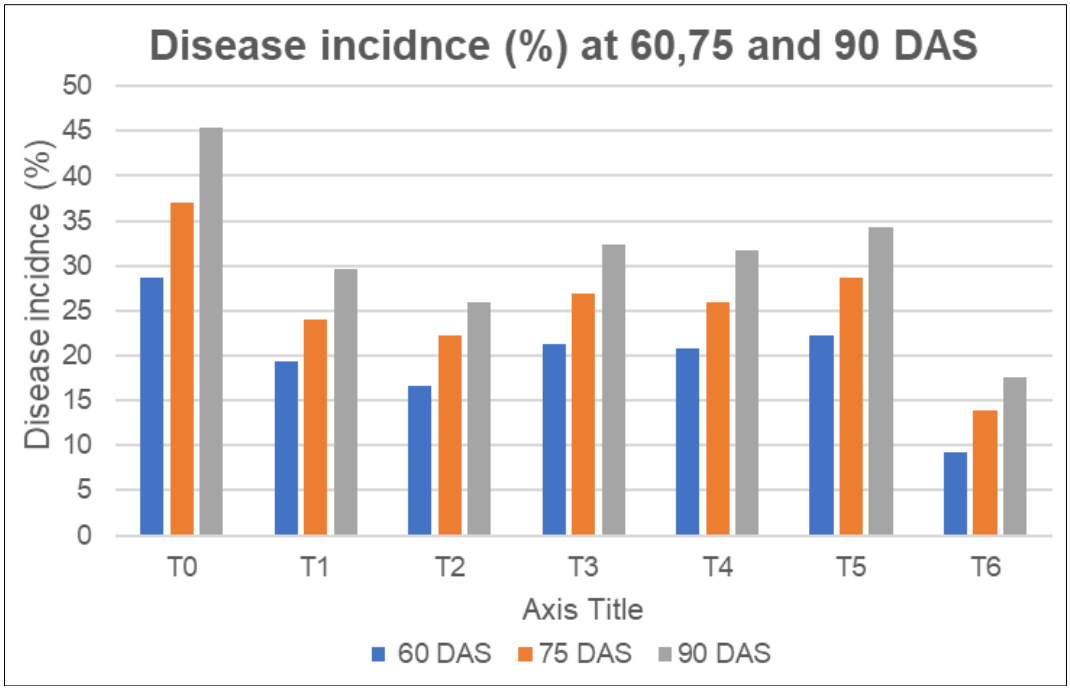

Fig 1: Effect of various treatments on the disease incidence (%)

Effect of treatments on the number of sclerotia per stem of mustard plants

Data recorded in Table 2 showed that, among all treatments, minimum sclerotia per stem was recorded with garlic bulb extract (*allium sativum*) (3.6) followed by neem leaf extract (5.60), eucalyptus leaf extract (7.50), ginger rhizome extract (7.93), onion bulb extract (8.26) as compared to mancozeb (treated check) and control.

In the present studies minimum sclerotia per stem at harvest was recorded in garlic bulb extract (*allium sativum*) followed by neem leaf extract was found to be effective over other treatments. Garlic has been known for its antifungal and antibacterial activities for decades due to the presence of chemical compound such as allicin *i.e.* well known to be effective against bacteria and fungi. This result was similar to the finding of Bharti *et al.* [2] in that the minimum sclerotia per stem at harvest was found in garlic bulb extract and was effective over other treatments.

Effect of treatment on yield (q/ha) of mustard:

Data recorded in Table 2 showed that, among all treatments, maximum yield (q/ha) was recorded with garlic bulb extract (*allium sativum*) (13.04 q/ha) followed by neem leaf extract (12.42 q/ha), eucalyptus leaf extract (11.70 q/ha), ginger rhizome extract (11.33 q/ha), onion bulb extract (10.76 q/ha) as compared to mancozeb (treated check) and control.

In the present study maximum yield was recorded in garlic bulb extract followed by neem leaf extract was found to be effective over other treatments. All treatments are also significantly increased the yield in comparison to control. Garlic has been known for its antifungal and antibacterial activities for decades due to the presence of chemical compound such as allicin *i.e.* well known to be effective against bacteria and fungi. This result was similar to the finding of Yadav [18] in that the maximum yield was found in garlic bulb extract (13.07 q/ha) and was effective over other treatments.

Table 2: Effect of treatments on the number of sclerotia per stem and yield

Treatment No.	Treatment detail	Sclerotia per Stem	Yield (q/ha)
		Mean*	Mean*
T_0	Control	14.40	9.14
T_1	Neem leaf extract	5.60	12.42
T_2	Garlic bulb extract	3.60	13.04
T_3	Ginger rhizome extract	8.26 [a]	11.33[a]
T_4	Eucalyptus leaf extract	7.50 [a]	11.70 [a]
T_5	Onion bulb extract	7.93	10.76
T_6	Mancozeb 75% WP	1.80	14.00
	CD(p=0.05)	0.919	0.459
	S Ed±	0.87	0.21

*Average of three replications

*Data followed by same alphabets in a column are non-significant to each other at 5% level.

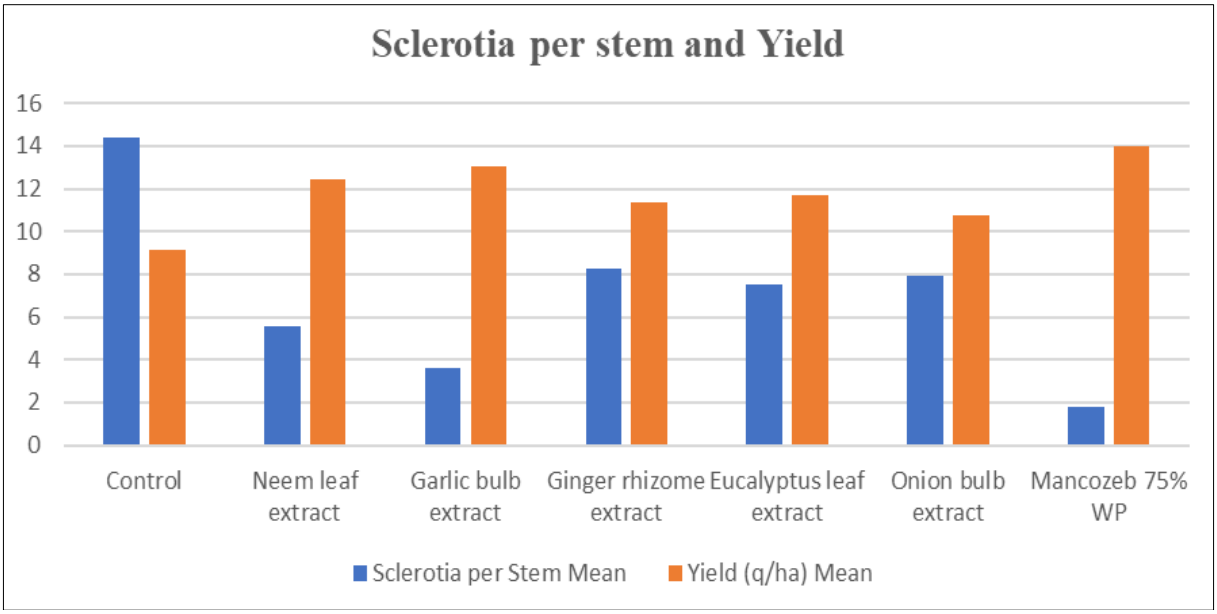

Fig 2: Effect of treatments on the number of sclerotia per stem and yield

Cost Benefit Ratio

Observations regarding the economics of cultivation are shown in the Table 3. Highest total cost of yield (Rs. 58,815) and C:B Ratio (1:1.87) was observed in garlic bulb extract as compared to control (T_0) recorded lowest total cost of yield (Rs. 41,130) and C:B Ratio (1:1.44).

Table 3: Economics of cultivation:

Tr. No.	Treatments	Yield q/ha	Cost of Yield (q/RS)	Gross return (RS)	Common cost (RS)	Treatment cost (RS)	Total cost of cultivation (RS)	C:B ratio
T_0	Control	9.14	4,500	41,130	28,550	---	28,550	1:1.44
T_1	Neem leaf extract	12.42	4,500	55,890	28,550	2,450	31,000	1:1.80
T_2	Garlic bulb extract	13.07	4,500	58,815	28,550	2,850	31,400	1:1.87
T_3	Ginger rhizome extract	11.33	4,500	50,985	28,550	2,260	31,150	1:1.63
T_4	Eucalyptus leaf extract	11.70	4,500	52,650	28,550	2,100	30,650	1:1.71
T_5	Onion bulb extract	10.76	4,500	48,420	28,550	2,600	21,150	1:1.55
T_6	Mancozeb 75% WP	14.00	4,500	63,000	28,550	1,590	30,140	1:2.09

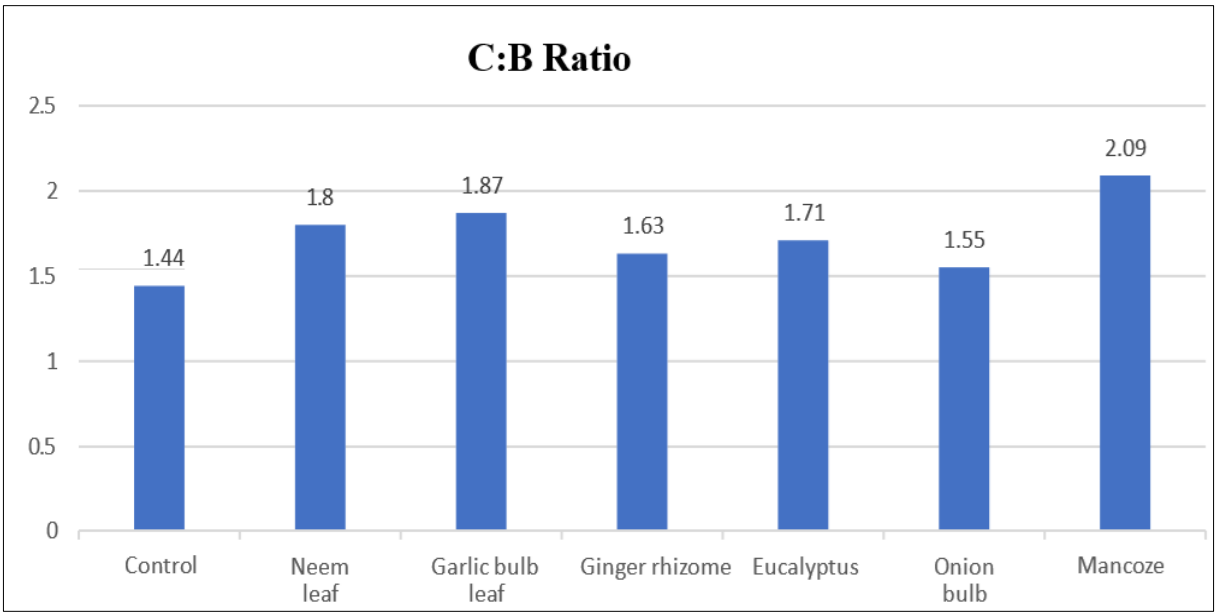

Fig 3: Cost Benefit Ratio

Conclusions

Based on the result obtained from present investigations it was found that phytoextract garlic bulb extract @ 15% foliar application was most effective against *Sclerotinia sclerotiorum* which causes stem rot disease in mustard, under field conditions reducing the Disease incidence significantly and also reducing number of sclerotia per plant. Followed by neem leaf extract and eucalyptus leaf extract, therefore it may be recommended for the better management of stem rot disease of mustard. Results of the

present study were found to be significantly effective under Prayagraj agro-climatic conditions. It may vary with region and climatic conditions, therefore, for validation of the results more such trials should be carried out in future along with *in-vitro* testing of these phytoextracts will be helpful for more effective phytoextracts selection.

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