Effect of phytoextracts on Alternaria blight disease (*Alternaria brassicae* (Berk.) Sacc.) of mustard (*Brassica juncea* L.)

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ABSTRACT

Mustard is the one of the most important oil seed crops among the major *Rabi* oil seed of India. Alternaria blight disease which is caused by *Alternaria brassicae* (Berk.) Sacc. is considered to be the most devastating disease of this crop. The general inadequacy of chemical fungicides to tackle alternaria blight in mustard has led to the search for ecofriendly management to these diseases. Therefore, present study entitled "Effect of phytoextracts on Alternaria blight disease (*Alternaria brassicae* (Berk.) Sacc.) of mustard (*Brassica juncea* L.)" was carried out in *Rabi* season in the year 2023-24 *in - vivo* and in situ. Effects of treatments were evaluated on the radial growth (mm) and disease intensity (%) *in - vitro* and in situ. Among the plant extracts, garlic bulb extract (T_2) showed the significant inhibitory effect, reducing the mean colony diameter to 23.12 mm and achieving a redial growth per cent inhibition of 62.54% respectively as compared to control T_0. The disease intensity (%) at 45, 60, 75 and 90 DAS was significantly decreased in treatment T_2 - garlic bulb extract (16.62), (23.48), (31.72) and (35.63) respectively as compared to control T_0.

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Key words: *Alternaria brassicae,* mustard and phytoextracts

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1.1 INTRODUCTION

Rapeseed-mustard is the third most important oilseed commodity in the world after Soybean *(Glycine max)* and Palm *(Elaeis guineensis Jacq)* in world agriculture and India is the third largest producer with global contribution of 28.3 per cent acreage and 19.8 per cent production. (Rakesh *et al*., 2016). The oil contains usually 38-57% of erucic acid, 4.7-30% of linoleic acid and 20% of oleic acid which are the high nutritive value required to human health. Mustard has a wide range of industrial applications and the oil cake from them may be used as manure (Bora *et al.,* 2021).

Indian mustard, *Brassica juncea* (Linn.) Czernj and Cosson is ravaged by several diseases, *viz.,* Alternaria blight, Sclerotinia stem rot, White rust and nutrient deficiency causing substantial yield loss and among there alternaria blight is a major concern (Gupta *et al.,* 2016). Alternaria blight is the global headache of crop scientists, it can reduce yield by up to 70%. Currently, 50% of rapeseed yield is lost due to alternaria blight around the globe. This disease is mainly caused by two fungal organisms- *Alternaria brassicae* and *Alternaria brassicicola* (Panday *et al.,* 2023).

Currently, due to the increasing use of chemicals in the management of alternaria blight of mustard, public health and environmental hazards have become a major problem. Therefore, the current emphasis is on use of local sources for managing the plant disease which is cheaper and does not harmful humans or nature. Thus, the paper aims for management of alternaria blight *in-vitro* and in situ through phytoextracts.

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2.1 MATERIALS AND METHODS

2.1.1 Experimental place: The experiment was analysed in randomized block design (RBD) with three replications in a plot size 2x1 m^2.

2.1.2 Symptoms: Alternaria blight attacks all the green aerial parts of the plant reducing its photosynthetic area and vigor. On older leaves, the spots turn into circular, dark-brown, sunken necrotic lesions surrounded by light yellow halo and bear conidiophores and conidia in concentric rings, at the grayish-white center, giving them a target board effect (Dahiya *et al.,* 1988; Dahiya and Tiwari,1991; Saharan and Mehta, 2002).

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Plate 1. Symptoms of alternaria blight of mustard

2.1.3 Isolation: Potato dextrose agar (PDA) medium 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 leaf 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 leaf bits were surface sterilized with 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 (Tuite, 1969). 2-3 leaf bits were transferred on PDA medium contained in petri plates aseptically with the help of sterilized forceps. These petri plates were incubated at 27±2°C. After 3 days mycelia growth was observed around leaf bits from this colony growth, a portion from the periphery, that is, a single hyphal tip was separated and transferred.

2.1.4 Morphological characters of test fungus: The species of alternaria affecting mustard were differentiated on the basis of morphological characters of mycelium, conidia and conidiophores. The mycelium of *Alternaria brassicae* is septate and it becomes brown to brownish grey in color. The conidiophores are dark septate, measuring 14-74×4-8um. The structure of conidia is brownish black, singly borne or sparingly in chains with 2-4, muriform along with beak (Kolte, 1985).

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Plate 2. Conidium of *Alternaria brassicae*

2.1.5 Preparation of plant extracts: The botanical part used for the treatment were neem leaf extract 15%, garlic bulb extract 15%, eucalyptus leaf extract 15%, datura leaf extract 15%, lantana leaf extract 15%. The fresh leaf extracts were gently washed under running tap water and finally in sterile distilled water. The sample was separately ground using sterile water at the rate 1 mlg^{-1} of plant material in pestle and mortar. It was 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 2 gm of leaves in 100 ml of sterile water @2% of leaf extract was obtained (Kamlesh and Gurjar, 2002; Prasad and Barnwal, 1994).

2.1.6 Treatments used: Foliar sprays with the botanicals used for the experiment were neem leaf extract 15%, garlic bulb extract 15%, eucalyptus leaf extract 15%, datura leaf extract 15%, lantana leaf extract 15%. The standard leaf extract solution (100%) and Potato Dextrose Agar (PDA) medium were mixed at required quantities to get 15% concentration.

2.1.7 Inhibitory tests on mycelial growth: Five mm diameter of culture disc of *Alternaria brassicae* was kept at the centre of each petri plate containing the botanicals of required concentration dissolved in PDA. Five replications were maintained. The plates were incubated at 27±2˚C for seven days and colony diameter was also recorded (Vincent, 1947). Per cent inhibition of mycelial growth was calculated by using the following formula:

$$\text{Per cent inhibition} = \frac{\text{Growth in check-growth in treatment}}{\text{Growth in check}} \times 100$$

2.1.8 Disease intensity (%): Assessment of disease was done with the grading method following a grade chart of 0-9. Disease intensity (%) was calculated using the formula given by Wheeler (1969).

$$\text{Disease intensity} = \frac{\text{Sum of all the disease rating}}{\text{Total number of ratings} \times \text{maximum disease grade}} \times 100$$

Its disease intensity (%) was recorded at 45, 60 and 75 days after sowing.

3. RESULTS AND DISCUSSION

3.1 Effect of phytoextracts on the radial growth (mm) of pathogen:

3.1.1 *In-Vitro* evaluation of botanicals: Five plant extracts *viz.,* neem leaf extract, garlic bulb extract, eucalyptus leaf extract, datura leaf extract, lantana leaf extract) were evaluated using poisoned food technique to check the efficacy of botanicals against *Alternaria brassicae.* The effects of various treatments on the radial growth of the mycelium of *Alternaria brassicae*, measured by the mean colony diameter in millimeters (mm) and the corresponding percent inhibition are presented in Table 1.

Among the plant extracts, garlic bulb extract (T_2) showed the significant inhibitory effect, reducing the mean colony diameter to 23.12 mm and achieving a percent inhibition of 62.54%. Other plant extracts, including neem leaf extract (T_1), eucalyptus leaf extract (T_4), datura leaf extract (T_5) and lantana leaf extract (T_3) also demonstrated substantial inhibitory effects of 26.33, 27.33, 28.16 and 28.42 mm. T_1, T_4, T_5 and T_3 showed percent inhibition of 58.46, 57.46, 56.58 and 56.31%, respectively.

The control (T_0)- untreated check exhibited the highest mean colony diameter of 90.00 mm. Whereas, treated check mancozeb (T_6) demonstrated the reduction in radial growth with a mean colony diameter of 16.52 mm and a percent inhibition of 70.63%.

Comparing the treatments for radial growth with the CD value 0.42, all the treatments were found to be significant over untreated check (T_0). Among the treatments (T_1, T_2 and T_4) were found statistically significant over other treatments, However, T_3 and T_5 were not statistically significant to each other. Similarly at CD value 0.53 for inhibition (%), all the treatments were found to be significant over untreated check (T_0). Among the treatments (T_1, T_2 and T_4) were found statistically significant over other treatments, However, T_5 and T_3 were not statistically significant to each other.

For the radial growth of *Alternaria brassicae*, among the plant extracts tested, garlic clove

extract showed the highest inhibitory effect, reducing the mean colony diameter and achieving a percent inhibition. Garlic contains sulfur compounds like allicin, which have potent antifungal properties, effectively reducing fungal growth **(Saniewska *et al.*, 2001; Abdulrahman *et al.*, 2005).** Similar findings were also reported by **Yadav *et al*. (2019), Upadhyay *et al.* (2019)** and **Choudhary *et al.* (2020**). **Meena *et al.* (2022)** observed that garlic clove extract (10%) was the most effective in inhibiting mycelial growth and sporulation of *Alternaria brassicae*, the causative agent of alternaria blight disease of mustard, followed by neem leaf extract and ginger rhizome extract. Similarly, **Bochalya *et al.* (2012)** reported that garlic bulb extract at 15% concentration was highly effective against *A. alternata* in brinjal, with neem leaf extract also showing significant inhibition.

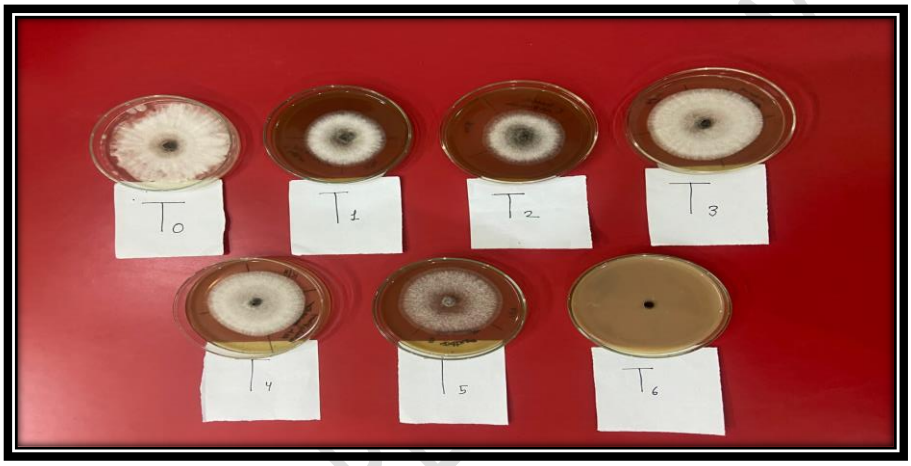

Plate 3. Effect of various plant extracts on the radial growth (mm) of pathogen

Table 1. Effect of various plant extracts on the radial growth (mm) of pathogen

Tr. No.	Treatment	Mean colony diameter (mm)	Inhibition (%)
T_0	Control (untreated check)	90.00	0.00
T_1	Neem leaf extract @15 % (F.S.)	26.33	58.46
T_2	Garlic bulb extract @15% (F.S.)	23.12	62.54
T_3	Lantana leaf extract @15% (F.S.)	28.42[a]	56.31[a]
T_4	Eucalyptus leaf extract @15% (F.S.)	27.33	57.46
T_5	Datura leaf extract @15% (F.S.)	28.16[a]	56.58[a]
T_6	Mancozeb 75% (0.2%) (F.S.)	15.52	70.63
	CD (p=0.05)	0.42	0.53

SEM±	0.14	0.17

*Average of three replications

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

figure 1. Effect of plant extracts on radial growth of *Alternaria brassicae* by poison food technique

3.2 In situ evaluation of botanicals

3.2.1 Effect of phytoextracts on the plant disease intensity (%):

3.2.1.1 45 DAS (before foliar spray): Disease intensity (%) of mustard significantly decreased in T_2 (garlic bulb extract @15% (F.S.) (16.62 %) followed by T_1 (neem leaf extract @15 % (F.S.) (16.89 %), T_3 (lantana leaf extract @15% (F.S.) (18.29 %), T_5 (datura leaf extract @15% (F.S.) (18.58 %), T_4 (eucalyptus leaf extract @15% (F.S.), (21.59 %) as compared to T_6 mancozeb 75 % WP (0.2%) (F.S) (19.41%) and T_0 control (20.28 %) Table 2.

3.2.1.2 60 DAS: Disease intensity (%) of mustard significantly decreased in T_2 (garlic bulb extract @15% (F.S.) (23.48 %) followed by T_1 (neem leaf extract @15 % (F.S.) (25.10 %), T_5 (datura leaf extract @15% (F.S.) (30.84%), T_3 (lantana leaf extract @15% (F.S.) (31.34 %), T_4 (eucalyptus leaf extract @15% (F.S.), (32.47 %) as compared to T_6 mancozeb 75 % WP (0.2%) (F.S) (21.6 %) and T_0 control (35.42 %) Table 2.

3.2.1.3 75 DAS: Disease intensity (%) of mustard significantly decreased in T_2 (garlic bulb extract @15% (F.S.) (31.72 %) followed by T_1 (neem leaf extract @15 % (F.S.) (33.54 %), T_5 (datura leaf extract @15% (F.S.) (37.82 %), T_3 (lantana leaf extract @15% (F.S.) (38.34 %), T_4 (eucalyptus leaf extract @15% (F.S.), (41.73 %) as compared to T_6 mancozeb 75 % WP (0.2%) (F.S) (26.76 %) and T_0 control (47.59 %) Table 2.

In the present studies minimum disease intensity at 45, 60, 75 and 90 DAS was recorded with garlic bulb extract followed by neem leaf extract and found effective over other treatment. All treatments are also significantly decreasing the disease 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. These results were similar to the findings of **Singh *et al.* (2017), Bugalia *et al.* (2013), Kumar *et al.* (2019)** and **Raghuvanshi *et al.* (2021).** In the studies conducted by **Kumar *et al.* (2019),** minimum disease incidence was found with garlic bulb extract through foliar application was 43.10% and effective over other treatments.

Table 2: Effect of various treatments on disease intensity (%) of mustard:

Tr. No.	Treatment	45 DAS	60 DAS	75DAS
T_0	Control (untreated check)	20.28	35.42	47.59
T_1	Neem leaf extract @15 % (F.S.)	16.89[a]	25.10	33.54
T_2	Garlic bulb extract @15% (F.S.)	16.62[a]	23.48	31.72
T_3	Lantana leaf extract @15% (F.S.)	18.29[b]	31.34[a]	38.34[a]
T_4	Eucalyptus leaf extract @15% (F.S.)	21.59	32.47	41.73
T_5	Datura leaf extract @15% (F.S.)	18.58[b]	30.84[a]	37.82[a]
T_6	Mancozeb 75% (0.2%) (F.S.)	19.41	21.60	26.76
	CD (p=0.05)	0.55	0.57	0.52
	SEM±	0.18	0.19	0.17

*Average of three replications

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

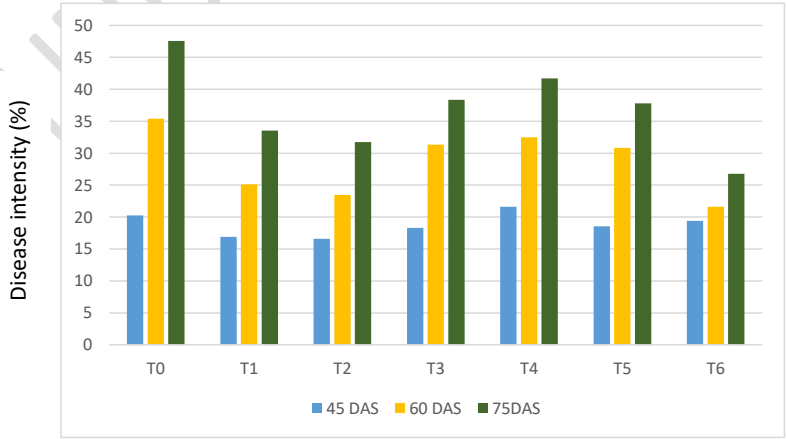

Figure 2: Effect of various treatments on the disease intensity (%)

4. CONCLUSIONS

This study "Effect of phytoextracts on Alternaria blight disease (*Alternaria brassicae* (Berk.) Sacc) of mustard (*Brassica juncea* L.)" found that plant extracts can help manage the disease alternaria blight in mustard plants. The results show that garlic bulb extract worked significant against *Alternaria brassicae* in lab tests. The findings indicates that garlic bulb extract (F.S.) led to the lowest disease intensity (%). The study concludes that plant extracts play a key role to manage alternaria blight in mustard. This experiment proves that farmers can manage alternaria blight in mustard by reducing use of chemicals and using various plant extracts. However, these findings are from just one growing season. To confirm these results, researchers should run more trials in the future.

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