



# Effect of Phytoextracts on Alternaria Blight Disease (*Alternaria brassicae* (Berk.) Sacc.) of Mustard (*Brassica juncea* L.)

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## Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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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

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alternaria blight in mustard has led to the search for ecofriendly management to this disease. Therefore, present study entitled "Effect of phytoextracts on Alternaria blight disease (*Alternaria brassicae* (Berk.) Sacc.) of mustard (*Brassica juncea* L.)" was carried out during 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<sub>2</sub>) showed the significant inhibitory effect, reducing the mean colony diameter to 23.12 mm and achieving a radial growth per cent inhibition of 62.54 respectively as compared to control T<sub>0</sub>. The disease intensity (%) at 45, 60, 75 and 90 DAS was significantly decreased in treatment T<sub>2</sub> - garlic bulb extract (16.62), (23.48), (31.72) and (35.63) respectively as compared to control T<sub>0</sub>.

**Keywords:** *Alternaria brassicae*; mustard and phytoextracts.

## 1. INTRODUCTION

"Rapeseed-mustard is the third most important oilseed commodity in the world after Soybean (*Glycine max*) and Palm (*Elaeis guineensis* Jacq) India is the third largest producer of Rapeseed with global contribution of 28.3 per cent acreage and 19.8 per cent production" [1]. "The oil contains usually 38-57% of erucic acid, 4.7-30% of linoleic acid and 20% of oleic acid which are of high nutritive value that are essentially required to maintain good human health. Mustard has a wide range of industrial applications and the oil cake obtained out of it is used as manure" [2].

"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 them alternaria blight is of major concern" (Gupta *et al.*, 2016). "Alternaria blight has been viewed as a potential biotic stress to rapeseed globally by plant protection scientists, as it can reduce yields up to 70%. Currently, 50% of rapeseed yield is globally lost due to alternaria blight. This disease is mainly caused by two fungal organisms- *Alternaria brassicae* and *Alternaria brassicicola*" [3].

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 eco-friendly and non harmful to humans. Thus, the paper aims for management of alternaria blight *in-vitro* and *in situ* through phytoextracts.

## 2. MATERIALS AND METHODS

**Experimental place:** The experiment was laid out in a randomized block design (RBD) with three replications in a plot size of 2x1 m<sup>2</sup>.

**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 [4-6].



**Plate 1. Symptoms of alternaria blight of mustard**

**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 [7]. Two or three 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.

**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 14-74x4-8 µm. The structure of conidia is brownish black, singly borne or sparingly in chains with 2-4, muriform along with beak [8].



Plate 2. Conidium of *Alternaria brassicae*

**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<sup>-1</sup> 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 [9,10].

**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.

**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 [11]. Per cent inhibition of mycelial growth was calculated by using the following formula:

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

**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 [12].

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

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

**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<sub>2</sub>) showed significant inhibitory effect, reducing the mean colony diameter to 23.12 mm and achieving a per cent inhibition of 62.54. Other plant extracts, including neem leaf extract (T<sub>1</sub>), eucalyptus leaf extract (T<sub>4</sub>), datura leaf extract (T<sub>5</sub>) and lantana leaf extract (T<sub>3</sub>) also demonstrated substantial inhibitory effects of

26.33, 27.33, 28.16 and 28.42 mm respectively. The treatments T<sub>1</sub>, T<sub>4</sub>, T<sub>5</sub> and T<sub>3</sub> showed per cent inhibition of 58.46, 57.46, 56.58 and 56.31%, respectively.

Untreated check(T<sub>0</sub>) exhibited the highest mean colony diameter of 90.00 mm. Whereas, treated check mancozeb (T<sub>6</sub>) demonstrated the reduction in radial growth with a mean colony diameter of 16.52 mm and a per cent 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<sub>0</sub>). Among the treatments (T<sub>1</sub>, T<sub>2</sub> and T<sub>4</sub>) were found statistically significant over other treatments, However, T<sub>3</sub> and T<sub>5</sub> 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<sub>0</sub>). Among the treatments (T<sub>1</sub>, T<sub>2</sub> and T<sub>4</sub>) were found statistically significant over other

treatments, However, T<sub>5</sub> and T<sub>3</sub> were not statistically significant to each other.

Among the plant extracts tested, garlic clove extract showed the highest inhibitory effect, reducing the mean colony diameter and achieving highest per cent inhibition of radial growth of *Alternaria brassicae*. Garlic contains sulphur compounds like allicin, which have potential antifungal properties, that effectively reduce fungal growth [13,14]. Similar findings were also reported by Yadav *et al.* [15], Upadhyay *et al.* [16] and Choudhary *et al.* [17]. Meena *et al.* [18] who observed garlic clove extract (10%) was most effective in inhibiting mycelial growth and sporulation of *Alternaria brassicae*, the causal agent of alternaria blight disease of mustard. Garlic extract was followed by neem leaf extract and ginger rhizome extract. Similarly, Bochalya *et al.* [19] reported that garlic bulb extract at 15% concentration was highly effective against *A. alternata* in brinjal, with neem leaf extract also showing significant inhibition.

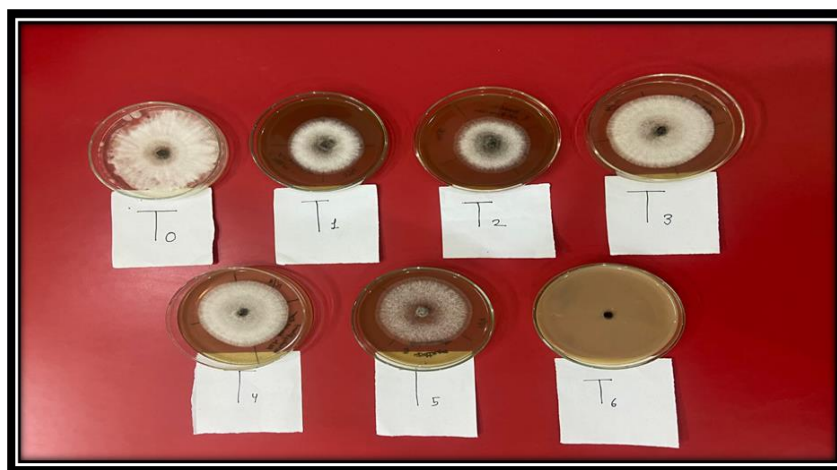


Plate 3. Effect of various plant extracts on the radial growth (mm) of pathogen

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

Tr. No.	Treatment	Mean colony diameter (mm)	Inhibition (%)
T <sub>0</sub>	Control (untreated check)	90.00	0.00
T <sub>1</sub>	Neem leaf extract @15 %	26.33	58.46
T <sub>2</sub>	Garlic bulb extract @15%	23.12	62.54
T <sub>3</sub>	Lantana leaf extract @15%	28.42 <sup>a</sup>	56.31 <sup>a</sup>
T <sub>4</sub>	Eucalyptus leaf extract @15%	27.33	57.46
T <sub>5</sub>	Datura leaf extract @15%	28.16 <sup>a</sup>	56.58 <sup>a</sup>
T <sub>6</sub>	Mancozeb 75% (0.2%)	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

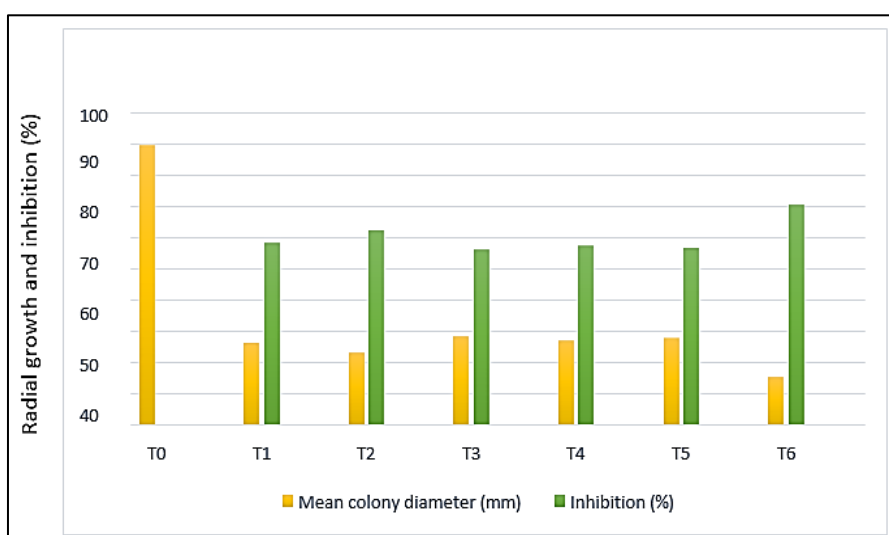


Fig. 1. Effect of plant extracts on radial growth of *Alternaria brassicae* by poison food technique

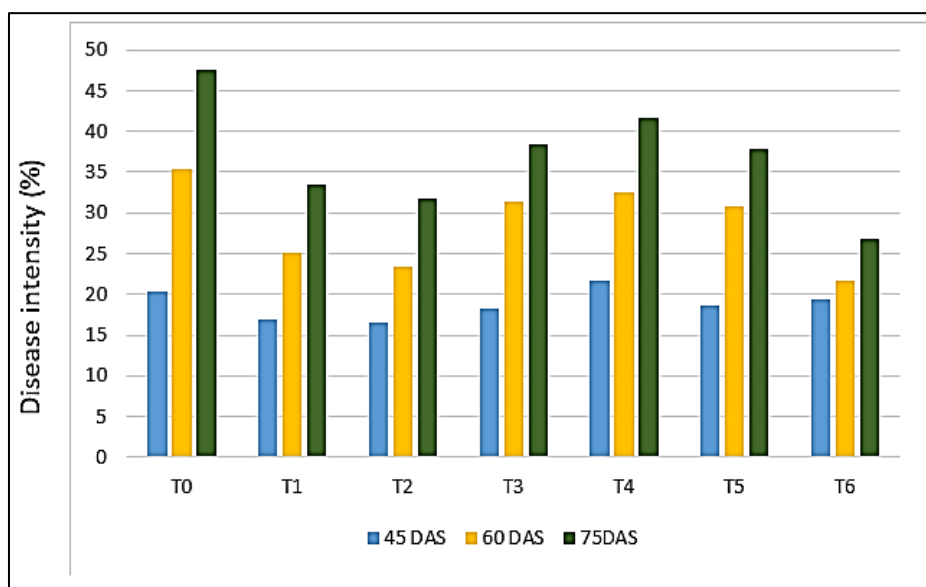


Fig. 2. Effect of various treatments on the disease intensity (%)

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

Tr. No.	Treatment	45 DAS	60 DAS	75 DAS
T <sub>0</sub>	Control (untreated check)	20.28	35.42	47.59
T <sub>1</sub>	Neem leaf extract @15% (F.S.)	16.89 <sup>a</sup>	25.10	33.54
T <sub>2</sub>	Garlic bulb extract @15% (F.S.)	16.62 <sup>a</sup>	23.48	31.72
T <sub>3</sub>	Lantana leaf extract @15% (F.S.)	18.29 <sup>b</sup>	31.34 <sup>a</sup>	38.34 <sup>a</sup>
T <sub>4</sub>	Eucalyptus leaf extract @15% (F.S.)	21.59	32.47	41.73
T <sub>5</sub>	Datura leaf extract @15% (F.S.)	18.58 <sup>b</sup>	30.84 <sup>a</sup>	37.82 <sup>a</sup>
T <sub>6</sub>	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



### 3.2 *In situ* Evaluation of Botanicals

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

**45 DAS (before foliar spray):** Disease intensity (%) of mustard significantly decreased in T<sub>2</sub> (garlic bulb extract @15% (F.S.) (16.62 %) followed by T<sub>1</sub> (neem leaf extract @15 % (F.S.) (16.89 %), T<sub>3</sub> (lantana leaf extract @15% (F.S.) (18.29 %), T<sub>5</sub> (datura leaf extract @15% (F.S.) (18.58 %), T<sub>4</sub> (eucalyptus leaf extract @15% (F.S.), (21.59 %) as compared to T<sub>6</sub> mancozeb 75 % WP (0.2%) (F.S) (19.41%) and T<sub>0</sub> control (20.28 %) Table 2.

**60 DAS:** Disease intensity (%) of mustard significantly decreased in T<sub>2</sub> (garlic bulb extract @15% (F.S.) (23.48 %) followed by T<sub>1</sub> (neem leaf extract @15 % (F.S.) (25.10 %), T<sub>5</sub> (datura leaf extract @15% (F.S.) (30.84%), T<sub>3</sub> (lantana leaf extract @15% (F.S.) (31.34 %), T<sub>4</sub> (eucalyptus leaf extract @15% (F.S.), (32.47 %) as compared to T<sub>6</sub> mancozeb 75 % WP (0.2%) (F.S) (21.6 %) and T<sub>0</sub> control (35.42 %) Table 2.

**75 DAS:** Disease intensity (%) of mustard significantly decreased in T<sub>2</sub> (garlic bulb extract @15% (F.S.) (31.72 %) followed by T<sub>1</sub> (neem leaf extract @15 % (F.S.) (33.54 %), T<sub>5</sub> (datura leaf extract @15% (F.S.) (37.82 %), T<sub>3</sub> (lantana leaf extract @15% (F.S.) (38.34 %), T<sub>4</sub> (eucalyptus leaf extract @15% (F.S.), (41.73 %) as compared to T<sub>6</sub> mancozeb 75 % WP (0.2%) (F.S) (26.76 %) and T<sub>0</sub> control (47.59 %) Table 2.

In the present study minimum disease intensity at 45, 60, 75 and 90 DAS was recorded with garlic bulb extract followed by neem leaf extract and both were found effective over other treatment. All treatments were also significantly reducing 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.* [20], Bugalia *et al.* [21], Kumar *et al.* [22] and Raghuvanshi *et al.* [23]. In the study conducted by Kumar *et al.* [22], minimum disease incidence of 43.10 per cent was recorded with garlic bulb extract through foliar application and was found to be effective over other treatments.

### 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 significantly managed *Alternaria brassicae* in lab tests. The findings also indicate that garlic bulb extract (F.S.) show 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.

### DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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