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Inhibitory effects of phytoextracts on mycelial growth and conidial germination of the *Alternaria brassicae* I-6A1y21 isolate causing leaf spot disease in mustard

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ABSTRACT

The study of inhibitory effects of 13 plant extracts such as Lemon, Curry-leaf, Neem, *Duranta*, Ginger, Garlic, *Parthenium*, Cinnamon, *Asparagus*, *Zamia*, *Lantana*, *Eucalyptus* and *Datura* was taken to observe the effect on mycelial growth and conidial germination of *Alternaria brassicae* I-6A1y21 isolate. Among these plant extracts, only five plants extracts (Neem, Garlic, *Lantana*, *Eucalyptus* and *Datura*) were used in radial growth and ten plants extracts (Lemon, Curry-leaf, Neem, *Duranta*, Ginger, Garlic, *Parthenium*, Cinnamon, *Asparagus* and *Zamia* were used against conidial germination. Among the selected plants, the leaf extracts that showed maximum inhibition in mycelial growth was observed with Neem leaf extracts followed by Garlic, *Eucalyptus*, *Datura* and *Lantana*. The chemical Mancozeb fungicide was used at 0.2% level, which caused 100% inhibition in growth of selected isolate I-6A1y21 of *A. brassicae*. Other plant extracts inhibited the growth of test isolate which varied from 28.24% to 94.97% over control. The radial growth percent inhibition of treatments T3, T5, T4, T1, and T2 was 24.28, 35.45, 51.58, 79.29, and 94.97%, respectively, compared to the radial growth percent inhibition (100%) of treatment with 0.2% mancozeb (test treated, T6). Among the aqueous extracts of ten plants, the aqueous extracts of *Azadirachta indica*, *Zingiber officinale*, and *Allium sativum* were the substances that showed an inhibitory effect on the conidial germination of the *A. brassicae* I-6A1y21 isolate.

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KEYWORDS: *Alternaria brassicae* I-6A1y21 isolate, Inhibitory effects, Phytoextracts, Mycelial growth, Conidial germination, Leaf spot diseases, Mustard crop

INTRODUCTION

Mustard is an economically significant genus in the Brassicaceae family, which contains oil seed crops such as mustard (*Brassica juncea*) and rape (*Brassica campestris*). These crops are grown worldwide under various agro-climatic conditions including India. These crops are commercially significant in both national and international trade because they provide edible oil, which is the primary cooking medium in Northern India. In respect to India, estimates for the area, production, and yield of rapeseed mustard in 2018-2019 and 2019-2020 were 6.12 mha, 9.26 mt, and 1511 kg/ha and 6.86 mha, 9.12 mt, and 1331 kg/ha, respectively (DRMR, 2020-21). The fungal diseases, including white rust, downy mildew, powdery mildew, stem rot, clubroot, damping-off, and Alternaria blight, are recognized as major causes of the losses in mustard production. Among several diseases, Alternaria blight, also referred to as Alternaria dark spot or Alternaria leaf spot, is the most devastating disease, which is caused by the *Alternaria brassicae*. The symptom of Alternaria blight, which appears as brown to black circular spots on the upper and lower parts of the mustard leaf. Mustard crop is affected by several diseases, among which blight is also a serious disease with yield losses of up to 35-38% (Kolte *et al.*, 1987). The *A. brassicae* caused losses of up to 47% in Indian mustard (*B. juncea*) (Tamayo *et al.*, 2001; Chattopadhyay, 2008). Alternaria blight that severely damages foliage and inhibits the germination of seeds in mustard (Tewari, 1983; Weiss, 1983; Verma & Saharan, 1994). The disease negatively impacts seed quality by lowering seed size, discoloring

seeds and lowering oil content in addition to directly affecting yield (Prasad *et al.*, 2006). Furthermore, the disease lowers the protein content and the germinability of seeds.

The A. *brassicae* I-6A1y21 isolate, which causes leaf spot disease in mustard, was isolated from the mustard crop of the Barwa agricultural area of Allahabad (Prayagraj) (Kumar *et al.*, 2024). The present study has been undertaken to investigate the inhibitory effects of plant extracts on mycelial growth and conidial germination of A. *brassicae* I-6A1y21 isolate, isolated from the Allahabad (Prayagraj) mustard crop (Figure 1).

MATERIALS AND METHODS

Preparation of Plant Extracts

The antifungal activity against *Alternaria brassicae* was tested on thirteen different plants: Lemon, Curry-leaf, Neem, *Duranta*, Ginger, Garlic, *Parthenium*, Cinnamon, *Asparagus*, *Zamia*, *Lantana*, *Eucalyptus* and *Datura*. Among these plant extracts, only five plant extracts (Neem, Garlic, *Lantana*, *Eucalyptus* and *Datura*) were used in radial growth and ten plant extracts

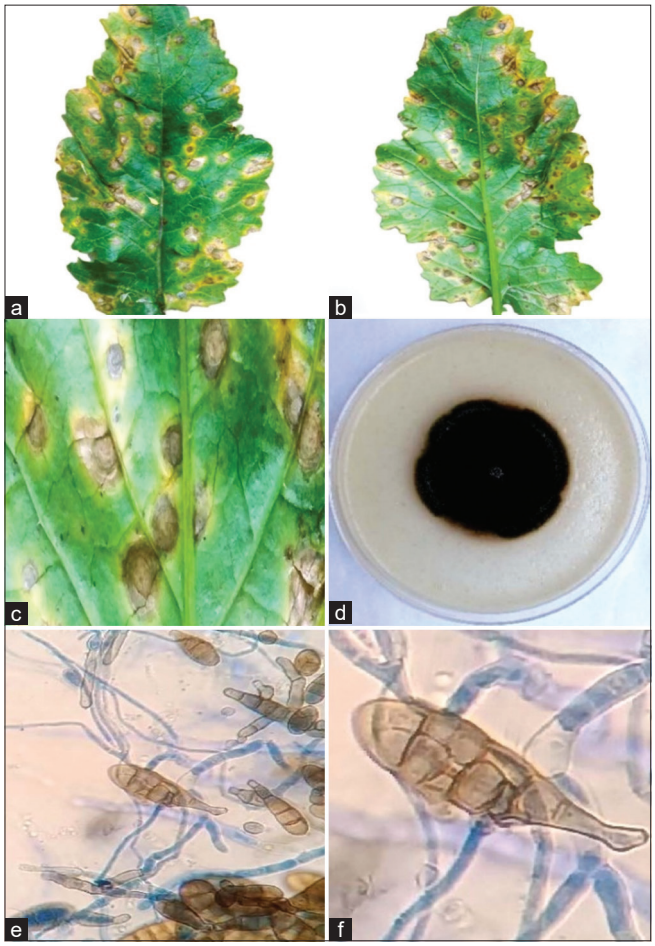

Figure 1: Survey of field, morphological and cultural characteristic of *A. brassicae* I-6A1y21 isolate. a) Dorsal view of an infected leaf with pathogen, b) ventral view of an infected leaf, c) Leaf with concentric ring, d) Growth on culture medium, e) Fungal hyphae with spore and f) A single spore of *A. brassicae* I-6A1y21 isolate

(Lemon, Curry-leaf, Neem, *Duranta*, Ginger, Garlic, *Parthenium*, Cinnamon, *Asparagus* and *Zamia* were used against conidial germination. The bulbs of garlic and the rhizome of ginger were processed into crude extracts. Crude extracts were made from a new set of leaves for each of the remaining plants. Using a mortar and pestle, 1.0 g of each plant material was crushed in 10 mL of distilled water before being placed in a centrifuge tube.

After that, it was centrifuged at 10,000 rpm. The antifungal activity was screened using clear supernatant.

Evaluation of Inhibitory Effects of Phytoextracts on Mycelial Growth of *A. brassicae* I-6A1y21 Isolate

The poison food technique was used to measure the antifungal properties of plant extracts. The plant extract (Neem leaf extract 15%, Garlic bulb extract 15%, *Eucalyptus* leaf extract 15%, *Datura* leaf extract 15%, *Lantana* leaf extract 15%) used for the treatment. The plant extract was filtered through the double layer of muslin cloth and finally through a sterilized Whatman No.1 filter paper. This provides a standard plant extract solution of 100%. Furthermore, sterile water was used to dilute it to the necessary concentration. To obtain a 15% concentration, the standard leaf extract solution (100%) and Potato Dextrose Agar (PDA) medium were mixed in the amount that was needed. The plant leaf extract of the necessary concentration dissolved in PDA was placed in the center of each petri dish, with a five mm diameter culture disc of A. *brassicae*. For Inhibitory tests on mycelial growth, three replicates were maintained. During the seven days when the plates were incubated at 27±2 °C and the colony diameter was determined as well. The following formula was used to determine the percent inhibition of mycelial growth:

$$\text{Percent inhibition (I)} = \frac{\text{Growth in check (C)} - \text{growth in treatment (T)}}{\text{Growth in check (C)}} \times 100$$

Where
I = Colony growth inhibition percent
C = Growth diameter of fungal colony in control
T = Growth diameter of fungal colony after treatment

Evaluation of Inhibitory Effects of Phytoextracts on Conidial Germination

The conidial germination method was used to measure the inhibitory effect. In a germination box, conidia were allowed to germinate. For this purpose, a transparent plastic box sized 25.5×16.5×6.5 cm and inside the box, layers of blotting paper were taken. The blotting paper was made by pouring sterilized distilled water into the box and the extra water was poured out. In order to initiate the bioassay, a five-day-old culture of A. *brassicae* was utilized. In a watch glass filled with sterile distilled water, conidia were scraped from the culture plate using a glass spreader and the hemocytometer was used to produce a homogeneous suspension. Using a calibrated micropipette, 200 µL of conidial suspension was put into the well on a cavity slide. The slide was maintained at 24 to 25 °C for five hours in

the germination box. A tungsten lamp was used to maintain the temperature.

Calculation of Minimal Inhibitory Concentration (MIC)

To determine the minimum inhibitory concentration (MIC), an aqueous extract of *Allium sativum L.* (Garlic) was produced at graded concentrations (1%, 0.75%, 0.5%, 0.25%, and 0.10%). A 1% aqueous extract of Garlic was made. Using distilled water to dilute the original solution, several concentrations were prepared from this solution. The conidial germination experiment was carried out in the various graded aqueous extract concentrations (1%, 0.75%, 0.5%, 0.25%, and 0.10%) of garlic.

RESULTS

Inhibitory Effect of Plant Extracts on Mycelial Growth of *A. brassicae* I-6A1y21 Isolate

The effectiveness of five plant extracts such as Neem leaf extract, Garlic bulb extract, *Eucalyptus* leaf extract, *Datura* leaf extract, and *Lantana* leaf extract was used against the radial growth of A. *brassicae* (Table 1 and Figures 2-4). The food poison method is used to measure the effect of different treatments on the radial growth and percent of inhibition of A. *brassicae*. The Garlic bulb extract (T2) was the most effective plant extract, exhibiting a strong inhibitory effect that resulted in the mean radial growth and percent of inhibition being 4.49 mm and 94.97%, respectively. In the control (T0), the pathogen showed the highest radial growth (89.34 mm), while in plant extracts containing Neem leaf extract (T1), *Eucalyptus* leaf extract (T4), *Datura* leaf extract (T5), and *Lantana* leaf extract (T3), significant inhibitory effects were likewise exhibited with respective measurements of 18.50, 43, 57.67, and 67.67 mm. In comparison to the radial growth percent inhibition (100%) of treatment with 0.2% mancozeb (test treated, T6), the radial growth percent inhibition of treatments T3, T5, T4, T1 and T2 was 24.28, 35.45, 51.58, 79.29, and 94.97%, respectively.

Inhibitory Effect of Plant Extracts on Conidial Germination of *A. brassicae* I-6A1y21 Isolate

After five hours of incubation, cavity slides were examined for germination inhibition using a compound microscope (Carl Zeiss, Germany) at a 10X magnification (Table 2).

The inhibitory effect of ten distinct plants from one gymnosperm family and nine angiosperm families against A. *brassicae* was examined. The aqueous extract of *Azadirachta indica*, *Zingiber officinale* and *Allium sativum* was the material that showed antifungal activity in the form of conidial germination inhibition. All conidia germinated within 5 hours of incubation in the germination box, and no antifungal activity was detected in any other plants examined. After 4 hours of incubation all of the conidia germinated in distilled water. The Department's Botanical Garden has the plants used in the present study easily available.

Table 1: Effects of phytoextracts on radial growth and percent inhibition

No. of treatment	Treatment	Radial growth (mm)	Inhibition (%)
T0	Control	89.34	-
T1	Neem leaf extract	18.5	79.29
T2	Garlic bulb extract	4.49	94.97
T3	*Lantana* leaf extract	67.67	24.28
T4	*Eucalyptus* leaf extract	43	51.85
T5	*Datura* leaf extract	57.67	35.45
T6	Mancozeb (0.2%)	0	100
SD =		29.82	28.95

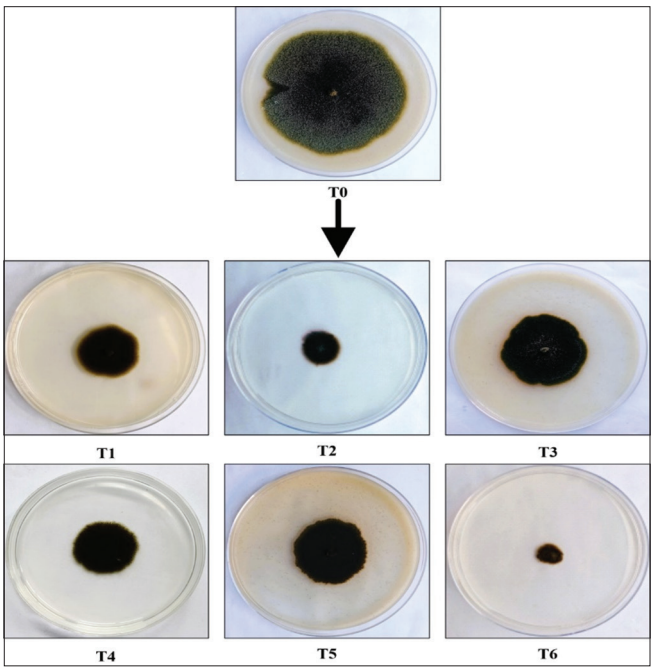

Figure 2: Effects of Phytoextracts on Radial growth of *Alternaria brassicae* I-6A1y21 isolate T0) Radial growth on PDA medium (pH 6.5) without treatment (control), Treatment with T1) Neem leaf extract @ 15% conc., T2) Garlic bulb extract @ 15% conc., T3) Lantana leaf extract @ 15% conc., T4) Eucalyptus leaf extrac @ 15% conc., T5) Datura leaf extract @ 15% conc. and T6) Mancozeb Fungicide @ 0.2% conc

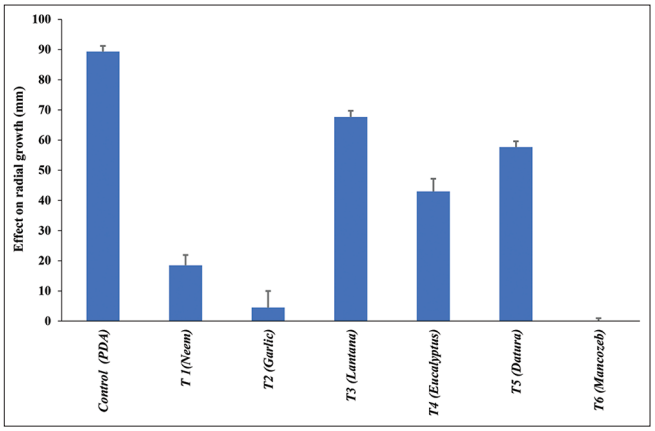

Figure 3: Effect of plant's leaf extract on radial growth of *Alternaria brassicae* I-6A1y21 isolate

Table 2: The obsevation of *Alternaria brassicae* isolate (I-6A1y21) conidial germination inhibition in aqueous extracts of different plant parts

Plant name	Family	Parts of plant used for extract	Antifungal activity	Conidia germination
Citrus limon (L.) Osbeck	Rutaceae	Leaf	-	+
Azadirachta indica A. Juss.	Meliaceae	Leaf	+	-
Murraya koenigii (L.) Sprengel	Rutaceae	Leaf	-	+
Duranta erecta L.	Verbenaceae	Leaf	-	+
Zingiber officinale Roscoe	Zingiberaceae	Rhizome	+	-
Allium sativum L.	Amaryllidaceae	Bulb	+	-
Parthenium hysterophorus L.	Asteraceae	Leaf	-	+
Cinnamomum zeylanicum Blume	Lauraceae	Bark	-	+
Asparagus racemosus Willd.	Asparagaceae	Rhizome	-	+
Zamia furfuracea L. f.	Zamiaceae	Leaf	-	+

*: '+' sign showing antifungal activity and conidial germination was observed, while the '-' sign showing no antifungal activity and conidia germination was not observed. No conidial germination inhibition was observed in doubled distilled water and double distilled water was used as negative control.

Table 3: Observation of the minimum inhibitory concentration bioassay of aqueous extract of some plant against an *Alternaria brassicae* isolate (I-6A1y21)

Concentration of Aqueous neem extract	Concentration of Aqueous garlic extract	concentration of Aqueous ginger extract	Antifungal activity	Conidial germination
1%	1%	1%	+	-
0.75%	0.75%	0.75%	+	-
0.50%	0.50%	0.50%	-	+
0.25%	0.25%	0.25%	-	+
0.10%	0.10%	0.10%	-	+
Distilled water	Distilled water	Distilled water	-	+

*: '+' sign showing antifungal activity and conidial germination was observed, while the '-' sign showing no antifungal activity and conidia germination was observed. No conidial germination inhibition was observed in doubled distilled water, was used as negative control

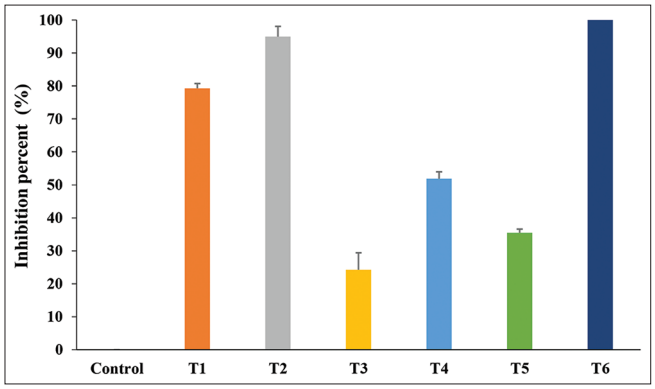

Figure 4: Effect of phytoextracts on radial growth of *Alternaria brassicae* isolate I-6A1y21 (Percent Inhibition)

Minimum Inhibitory Concentration (MIC) Bioassay

No conidial germination was observed in Garlic bulbs, Neem extract and Ginger extract at a 0.5% and lower aqueous concentration (Table 3). In the 0.75% or higher aqueous extract, conidial germination was inhibited. All conidia were fully germinated in distilled water. Extract of Neem, Ginger and Garlic bulb was shown high antifungal activity at 1% concentration against *Alternaria brassiace* isolate (I-6A1y21).

DISCUSSION

In vitro application of botanicals extract such as Neem, *Eucalyptus*, *Datura*, Pudina, Tulsi, and *Lantana* were tested against A. *brassicae* in both crude and boiling forms. *Eucalyptus* and Neem were also assessed in oil form. The fungal growth was strongly suppressed by the botanicals that were examined in oil and crude form (Sasode *et al.*, 2012). Plant extracts, like Garlic bulb extracts, *Datura*, and *Mentha* leaf extracts, significantly inhibited the mycelial growth of A. *brassicae* at different tested concentrations (Ganie *et al.*, 2013; Khalse *et al.*, 2017). According to a previous study by Hayat *et al.* (2016), the active ingredient in aqueous Garlic extract is known as "allicin, a phytoalexin that inhibited the growth of phytopathoge nic fungus. The freshly prepared aqueous extract of Garlic was tested against Alternaria spot of mustard, and its aqueous extract affected the mycelial growth of *Alternaria brassicae* (Yadav *et al.*, 2023). The present study revealed that among the five plant extracts, such as Neem leaf extract, Garlic bulb extract, *Eucalyptus* leaf extract, *Datura* leaf extract and *Lantana* leaf extract, the garlic bulb extract (T2) was the most effective, exhibiting a strong inhibitory effect on mean radial growth and a percent inhibition rate of 4.49 mm and 94.97%, respectively. The present study's findings regarding the inhibition of spore germination confirm previous research conducted by Yadav *et al.* (2023), which reported 100% inhibition of conidial germination *in vitro* with a 10% aqueous extract of Garlic. The results of the Neem and Adrakh, *Allium* extracts with our findings as all of the conidia not germinated and the extracts exhibited antifungal action against A. *brassicae*. The reason for these results could be that neem acts as a good insecticide and fungicide. The age, strain, and quantity of subculturing performed all affect a fungal pathogen's inoculum potential. According to Wani *et al.* (2022), Garlic clove is an effective fungicide against *Phoma exigua*, which causes Ascochyta blight

in common beans. Additionally, the researcher observed that the conidial germination of *Alternaria spp*. was inhibited by the extracts of two additional plants, namely *Ziziphus spina-christi* and *Rumex vesicarius* (Alotibi *et al*., 2020). The solvent used in the extraction process has an impact on the plant's antifungal activity as well. Methanolic extract of *Agave americana* leaves showed high antifungal activity against *A. brassicae*, according to Guleria and Kumar (2009). The freshly made aqueous extract of garlic was tested in the field against Alternaria spot of mustard by Yadav *et al*. (2023), however, the recommended dose was greater (2.0%, w/v) than the MIC recorded (0.75%) in this experiment.

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