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Evaluation of antifungal activities of some plant extracts against *Alternaria brassicae* causing spot disease of mustard

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Abstract
Spot disease is a serious but underestimated pathogenic disease in India. In this study an extensive field survey was conducted to record the disease incidence and severity in Indian mustard caused by fungal pathogen *Alternaria brassicae*. In the month of January 2022, 100% disease incidence was recorded while, disease severity ranged between 11 to 22 % with average 15.4 % (SD = 3.1 %). Typical symptoms of spot disease on the leaves caused by *A. brassicae* were identified. The pathogen was macroscopically as well as microscopically characterized and cultured in the laboratory on potato dextrose agar medium. In order to explore the botanical fungicide aqueous extract of 14 different plants *viz.*, Lemon, Bael, Curry-leaf, Neem, Duranta, Nepata, Zinger, Garlic, Tecoma, *Parthenium*, Cinnamon, Asparagus, *Zamia* and *Pteris* were tested against pathogen in the laboratory. Out of the 14 plants, only aqueous extract of garlic exhibited the antifungal property and inhibited 100 % conidial germination within 5 hours of incubation in cavity slides under germination box. Minimum Inhibitory Concentration (MIC) of the garlic extract against pathogen was recorded as 0.75 %. This garlic extract after successful field-trials could be used to control the spot disease. As the garlic extract is a botanical fungicide, this can be applied without causing any significant hazards to the environment.

Keywords: Spot disease, *Alternaria*, disease severity, garlic extract, conidial germination, MIC

Introduction

Indian mustard [*Brassica juncea* (L.) Czern and Coss.] is a herb of the Brassicaceae (Cruciferae) family and is the third most important oilseed crop in the world in terms of production. It is the source of edible oil which cannot be replaced with other substitutes. World leader countries in rapeseed and mustard production are India, Canada, China, Pakistan, Bangladesh, Germany, France, Sweden, and Poland. India's share in the world area is 25.6%, but it contributes only 14.7% of world's production (Mahapatra and Das 2017) [13].

In India its cultivation is mainly confined to Uttar Pradesh, Madhya Pradesh, Rajasthan, Haryana, Assam, Gujarat, and West Bengal. In Uttar Pradesh it is grown in 7.23 lakh ha area with the production of 8.7 lakh metric tons and productivity of 1326 kg/ha and ranks third in area after Madhya Pradesh and Rajasthan and second in production after Rajasthan.

Mustard is prone to attack by many pathogenic diseases. Among, spot disease (Blight of Mustard) caused by a necrotrophic fungal pathogen *Alternaria brassiceae* and *A. brassicicola* cause significant loss in the yield (Downey and Rimmer 1993) [6]. The pathogen is highly influenced by weather conditions with the highest disease incidence reported in wet season and in areas of relatively high rainfall. The fungus is capable of infecting the aerial plant parts at all the stages of growth. The disease symptoms are manifested as black/brown necrotic lesions on the leaves, leaf petiole, stem (especially lower and older leaves), inflorescence, siliquae and seeds. The characteristic feature of these lesions is the presence of concentric rings which gives it a 'bull's eye' or 'target-board' appearance and are seldom surrounded by a chlorotic halo (Conn *et al*. 1990; Sharma *et al*. 2002) [5, 18]. Leaf spots vary in size from pinpoints up to 5 cm in diameter. Pod lesions may extend into the pod interior and attack the seeds, causing them to shrivel.

As most of the times the symptoms are confined to the leaves only, the disease remains neglected. However, it can reduce the photosynthetic area of the leaves significantly and therefore can cause yield loss up to 70 % (Gupta *et al*. 2020) [8]. As the pathogen is a necrotroph, it produces lytic enzymes as well as mycotoxins (Bhat *et al*. 2017) [4]. Hence, disease can also change the quality of oil in case of severe infection (Saharan *et al*. 2016) [17].

Based on various researches a number of fungicides are available to protect and control the *Alternaria* blight disease of mustard. Seed treatment with Apron @ 6g/kg and three sprays of Trifloxystrobin 25% + Tebuconazol 50% were suggested by Singh *et al*. (2021) [22]. While using chemical fungicides in the fields on the edible crops, their hazardous effects on human as well environment cannot be ignored. Development of resistant varieties is another eco-friendly and sustainable approach to reduce the losses caused by blight disease. Recently, Singh *et al*. (2021) [22] investigated disease resistant in 15 genotypes of mustards. Among 15 genotypes only 4 genotypes including GSL-5, Pusa Aditya and GSL-2 exhibited moderate levels of resistance. Other 11 genotypes including RH-749, Pusa gold, Pusa mustard-27 and Jhumka, were susceptible. The achievement in the field of transfer of disease resistance in mustard is yet not up to the mark. Two strains of *Pseudomonas fluorescens* were recently reported to carry antagonistic activity against the *Alternaria brassicae*, however, only 38.6 % inhibition could be achieved (Gupta *et al*. 2020) [8].

In view of the above facts, there is an urgent need to focus on the losses in mustard caused by blight disease. Also, some cost-effective approach should be developed so that it

can be used at a large scale with least damage to the environment. Therefore, this research study has been planned: i) to assess the spot disease incidence and severity in some Indian mustard at some fields of Sultanpur, and ii) to test some botanical fungicides in the form of herbal extract, which could be recommended to farmers for managing the spot disease of Indian mustard.

Material and methods

Site of study

The area of survey study (Fig. 1) was located at the northern side of Sultanpur city (26.2952128 N, 82.1075212E). The area under study has three crops, *viz.*, Kharif, Rabi and Zayad. Mustard, Pea, Arhar, etc., are chief crops grown during Rabi season. Field study was conducted during January 2022 when the mustard crop was in full bloom. Maximum temperature of the month ranged between 13-20 °C, while minimum temperature ranged between 6-15 °C. During the January month the area faced 31.3 mm precipitation on 22nd January with relative humidity 68 %. All the weather parameters were accessed at the Meteorological office and observatory of Indian Meteorological Department, Government of India, located in the campus of KNIT.

Sample collection

A total of 7.5 ha area was taken into study (Fig. 1). In the area undertaken in survey RH 749 variety of Indian mustard [*Brassica juncea* (L.) Czern and Coss.] was being cultivated by most of the farmers. Systematic sampling method was adopted to assess the disease incidence and disease severity by using following formulae.

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

$$\text{Disease severity (\%)} = \frac{Total\ infected\ leaf\ area\ per\ plant}{Total\ leaf\ area\ of\ infected\ plant} \times 100$$

For assessing disease incidence and disease severity a total 16 samples were collected from the intersect mentioned in Fig. 1. From each intersect, a total five mustard plants were taken into study. Thus, a total of 80 plants were studied. In each plant 5 lowermost (oldest) leaves were selected to assess the disease incidence and disease severity. The leaf area was measured by using "Image J" software on Windows 10.

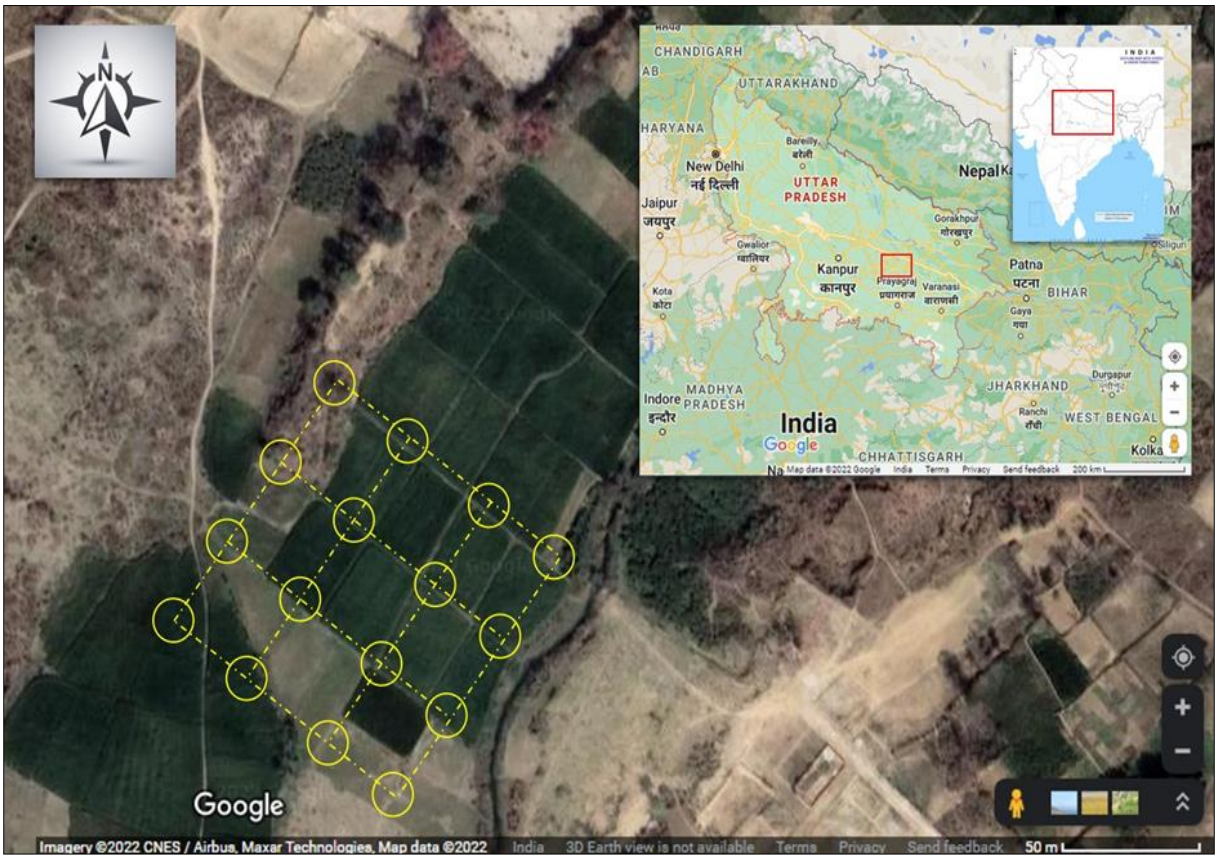

Fig 1: Satellite view of the area of mustard cultivation undertaken to assess the losses caused by spot disease. Area under the yellow square was surveyed and samples were drawn at intersects encircled yellow.

Isolation of pathogen

Infected leaves of Indian mustard plants were collected in sterilized polybags and brought to the laboratory for further analyses. The pathogen was isolated on the same day. For the isolation pathogen potato dextrose agar (PDA) medium. Infected leaf of 1 cm^2 area was inoculated on PDA medium (containing 30 mg/l streptomycin) in the BOD incubator at 26 ±2 ºC in darkness.

On 4th day after incubation all the colonies were subcultured on PDA medium. Colonies similar to the *Alternaria* in appearance were transferred to the fresh PDA medium for identification and further experimentation.

Identification of pathogen

The pathogen was identified based on colony morphology and microscopic characteristics. In morphological characteristics, appearance, and color of the colonies from

the upper as well as lower surfaces were taken into consideration. In microscopic characteristic shape, size, color of the mycelium as well as conidia was taken into consideration. Slides were prepared in lectophenol-cotton blue. For microscopic observation Magnüs CH20i compound microscope was used. All the photomicrographs were captured by using FMAO5 accessed on a computer in the MagVision 3.0 software. For identification and naming purposes, characteristics of pathogen were matched with the characteristics described by Simmons (1967) [20].

Preparation of extract

A total of 14 plants *viz.*, Lemon, Bael, Curry-leaf, Neem, Duranta, Nepata, Zinger, Garlic, Tecoma, *Parthenium*, Cinnamon, Asparagus, *Zamia* and *Pteris* were selected to test the antifungal activity against *Alternaria brassicae.* Crude extracts from rhizome of ginger and bulb of garlic were prepared. For the rest of the plants, crude extracts were prepared from a fresh second pair of leaves. From each plant material 1.0 g was crushed in 10 ml of distilled water in a mortar pestle and taken into a centrifuge tube. It was then centrifuged at 10000 rpm. Clear supernatant was used for screening of antifungal activity.

Antifungal bioassay

Antifungal activity was determined by conidial germination method. Conidia were germinated in a "germination box". A transparent plastic box of 25.5 ×16.5 × 6.5 cm dimension was taken for this purpose. Blotting paper (10 layers) was kept inside the box. Sterilized distilled water was poured inside the box to make the blotting paper. Excess water was decanted.

Four days old culture of *Alternaria brassicae* was used for germination for bioassay. Conidia from the culture plate was taken through a glass spreader and suspended in sterilized distilled water in a watch glass. A homogenous suspension was prepared by using a hemocytometer. On a cavity slide 200 µl of conidial suspension was loaded into the well with the help of calibrated micropipette. Slide was kept in the germination box for 5 hours at 26±2 ºC. Temperature was maintained by a tungsten lamp.

Determination of Minimum Inhibitory Concentration (MIC)

For determination of MIC graded concentration (1%, 0.75%, 0.5%, 0.25%, 0.10%) of aqueous extract of *Allium sativum* L. (Garlic) was prepared. One percent aqueous extract of Garlic was prepared by the method as described earlier. From this solution different concentrations were Prepared by diluting the original solution with distilled water. In the different graded aqueous extract of Garlic conidial germination assay was conducted as described in earlier section.

Statistical analysis

The data Table 1 were analysed by single-way analysis of variance (ANOVA). Means were separated by Duncan's multiple range test of homogeneity at P=0.05 level. Microsoft 365 Excel and SPSS 16.0 on Windows 10 were used for conducting the statistical analyses.

Results and discussion

Disease incidence and disease severity

At the time of study all the plants were infected with spot disease of mustard and therefore, 100 % disease incidence was reported. Disease severity in all the samples drawn is presented in (Table 1). The sum of five lower leaves of a single plant ranged between 420-913 cm^2 with a mean area of 608.6 cm^2 (Table 1). Per plant total infected area was ranged between 51-131 cm^2 with infected area of 91.5 cm^2 (Table 1). The disease severity ranged between 11-22 % with mean of 15.4 % (Table 1). The leaf area of plants sampled at different intersects were significantly different at P≥0.05 (Table 1). However, the infected area of leaves among the different samples were homogeneous (Table 1). This suggests that once disease is established, growth of the pathogen is dependent on the external weather conditions rather than on age and of leaves and plants. Thus, progress of the symptoms is somewhat equal in all the mustard plants of an area. The mild rain on 22 Jan 2022 (Supplementary Material 1) might have favored the dispersal of the conidia and their germination, thus secondary infection. Thus, 100 % disease incidence could be the result of this rain. Spot disease is a well-established disease of mustard in the parts of South-Asian countries. Shrestha *et al*. (2005) [19] recorded 32 to 57% yield loss in two localities of Nepal. Infection in the leaves reduces the photosynthetic area and hence oil content is reduced by 14.58 to 35.97% (Ansari *et al*. 1988) [2]. Percentage infection and disease severity also depends upon the variety of mustard. Ansari *et al*. (1988) [2] reported Varuna variety as most susceptible whereas Kranti to be least susceptible. In the field environmental conditions play a significant role in the development of disease. Maximum temperature ranging between 18-25 ºC and minimum between 10-14 ºC with related humidity more than 80% promote the disease incidence (Sinha *et al*. 1992; Shrestha *et al*. 2005) [23, 19]. These findings were in accordance with the environmental conditions recorded in this research.

Table 1: Showing total of leaves per plant and total infected area of leaves of a single plant along with disease severity.

No. of sample	Total leaf area of single plant (cm^2)	Total infected leaf area of single plant (cm^2)	Disease severity (%)
1	527 ± 26^{c}	75 ± 8.9^{bcd}	14 ± 1.3^{abc}
2	477 ± 27^{bc}	51 ± 7.7^{a}	11 ± 1.4^{a}
3	475 ± 22^{bc}	65 ± 8.0^{ab}	14 ± 2.3^{abc}
4	914 ± 50^{g}	122 ± 15.8^{f}	13 ± 1.6^{abc}
5	866 ± 82^{f}	128 ± 11.7^{f}	15 ± 1.8^{abc}
6	421 ± 24^{a}	91 ± 11.3^{cde}	22 ± 3.5^{e}
7	439 ± 36^{ab}	84 ± 8.4^{bcde}	19 ± 3.2^{de}
8	612 ± 25^{d}	96 ± 14.4^{de}	16 ± 2.4^{cd}
9	719 ± 35^{d}	99 ± 5.2^{e}	14 ± 1.3^{abc}
10	774 ± 35^{e}	132 ± 23.6^{f}	17 ± 3.0^{cd}
11	444 ± 32^{ab}	93 ± 16.0^{cde}	21 ± 3.8^{e}
12	508 ± 35^{c}	78 ± 18.5^{bcd}	15 ± 2.1^{abc}
13	432 ± 27^{ab}	71 ± 10.6^{bc}	17 ± 3.1^{cd}

14	717 ± 33^{d}	97 ± 26.0^{e}	14 ± 3.8^{abc}
15	694 ± 33^{d}	101 ± 16.5^{e}	15 ± 2.1^{bc}
16	720 ± 28^{d}	80 ± 11.1^{bcde}	11 ± 1.9^{ab}
Average	608.57	91.5	15.4
Minimum	420	51	11
Maximum	913	131	22
SD	164.1	22.4	3.1

Data in the column are the sum of the area of five lowermost leaves of a single plant identified by systematic sampling method.
Data in each column were analyzed for homogeneity test by Duncan's multiple range test in SPSS 16.0 software on Window 10 platform.
Means in each column with same superscripts are not significantly different at P ≥ 0.05 (n = 5).

Symptoms of the disease and morphology of the pathogen

All the mustard plants taken into study were found to be infected with spot disease (Fig. 2a and b). Typical symptoms of spot disease of mustard were observed in all the leaves studied (Fig. 2 b). At the time of study spots were seen on the leaves only. These spots were circular in shape ranging from tiny dots of 1 mm up to 9 mm in diameter with alternating gray and black concentric rings (Fig. 2 c). Sometime more than two spots were found to be coalesced but with distinct concentric rings (Fig. 2 c). The symptoms of spot disease in this study were in corroboration with the observation by Singh *et al*. (2007) [21], where they have recorded the same symptoms of 3-11.5 mm diameter. On host leaves mature conidia were observed (Fig. 2 d). These conidia were dark brown to black colored, found in the black parts of concentric rings, in chains of 2-3 conidia or solitary (Fig. 2 d). Sub-cylindrical to oblong with blunt tapered beak. Measuring 117-284 µm in total length (Fig. 2 d). Body straight to slightly curved, maximum broad at 4-6th cells ranging between 22-28 µm with up to 19 transverse and 11 longitudinal septa (Fig. 2 d). Basal cell ranging between 8-19 µm, sometimes with longitudinal septum dividing into two. Beak straight, unbranched, non-filamentous, 4-7 µm broad (Fig. 2 d). In the field conditions the morphology of the conidia varies greatly with the variations in host variety and weather condition of the season (Saharan and Kadian 1983; Singh *et al*. 2007) [16, 21].

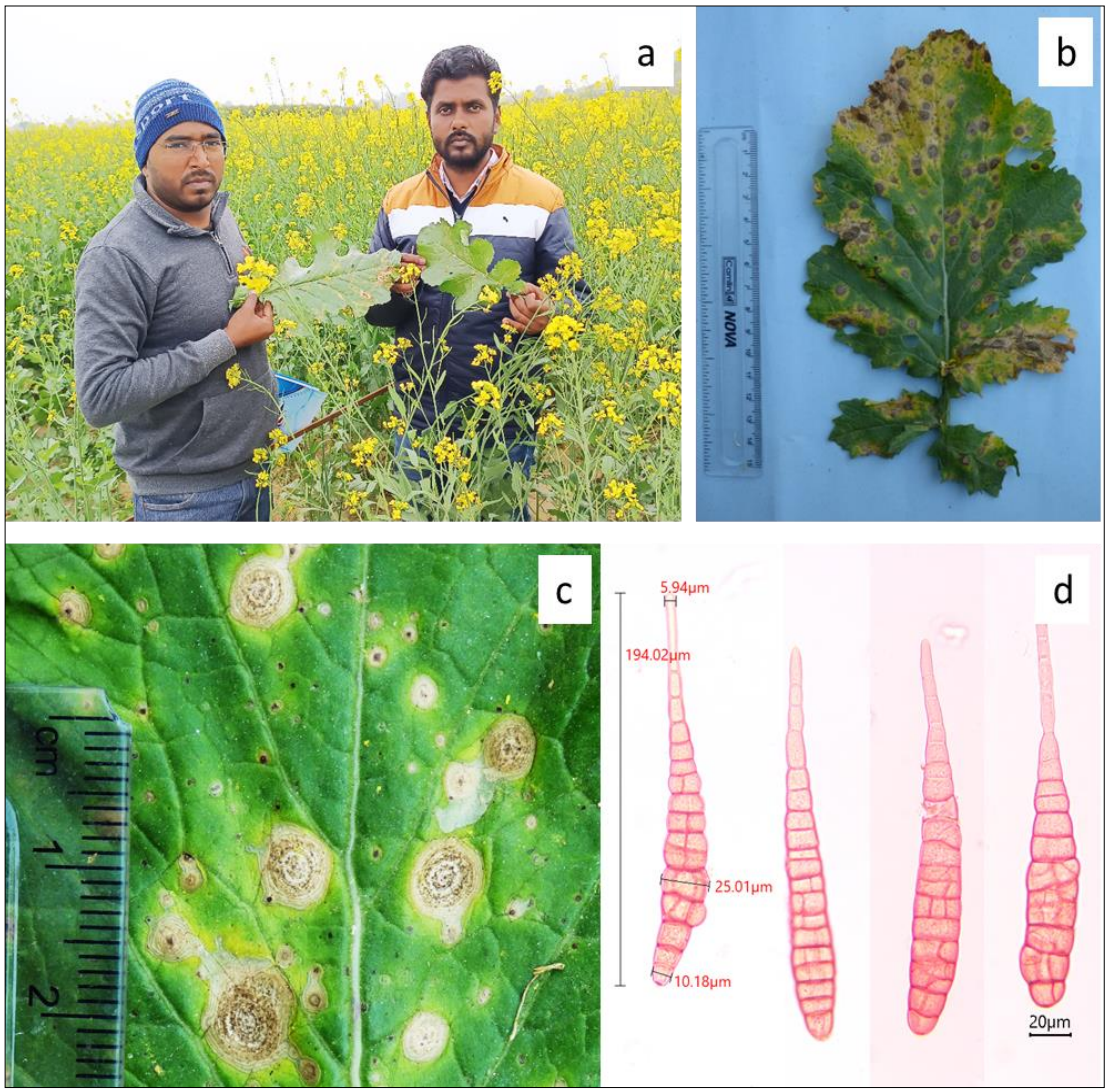

Fig 2a: Showing field and leaf of Indian mustard infected with spot disease, **b:** a heavily infected leaf, **c:** enlarged leaf showing typical symptoms of spot disease in the form of alternating black and grey colored concentric rings, and **d:** microscopic characteristics of conidia of *Alternaria brassicae* (Berk.) Sacc. From and infected leaf of mustard.

Morphological characteristics of pathogen in Potato Dextrose Agar (PDA) medium

The pathogen isolated from the leaf spots by single spore method was morphologically identified as *A. brassicae* (Fig. 3). The colony appeared greyish-black from both the upper as well as lower surface on PDA (Fig. 3a and b). Margin showed loose mycelium growth with a somewhat circular margin (Fig. 3a and b). Mycelial growth was regular and compact with 38 to 45 mm diameter in 7 seven days. Mycelia grown on the surface of culture medium and numerous erect conidiophores produced aerially (Fig. 3 c). Mycelia and conidiophore of same thickness ranging 6 to 9 µm in diameter. Developing mycelia hyaline appeared blue in trypan blue stain, became brown to black at maturity and showed no reactivity in trypan blue (Fig. 3 c). Conidiophores with numerous septations with the number of septa as many as conidia to be produced (Fig. 3 c). Conidia were typical *Alternaria* type, ovoid, obclavate, obpyriform with horizontal and vertical septation (muriform). Conidia range from 16 µm (developing) to 64 µm (mature) with only 6 to 11 horizontal septa and 3 to 5 vertical septa at maturity (Fig. 3 d). Conidia beakless, or with a short conical, narrowly tapered, or cylindrical beak (Fig. 3 d). Outer walls of the conidia were very minutely roughened (Fig. 3 d). Mature conidia germinated from 2 to 4 cells.

The shape and size of conidia of *A. brassicae* observed from culture on PDA medium at 26 ±2 ºC (Fig. 3 d) was significantly different from the characteristics of conidia isolated directly from the mustard leaves (Fig. 2 d). However, when cultured conidia were tested for infectivity (not included in this result) these were again very similar to the conidia described in Fig. 2 d. This phenotypic plasticity may be attributed to the different sources of food the pathogen consumed, temperature and humidity. Also, morphology of the conidia may be determined by the genotypes of isolates from different fields, e.g., Kumar *et al*. (2003) [10] observed average conidial length from 118.62 to 194.52 µm and breadth from 14 to 23 µm along with some variation in beak length and number of septation. Based on the morphology of different isolates of *A. brassiciae*, Mehta *et al*. (2003) [14] categorized isolates in four group *i.e.*, small (100 µm.), medium (101-150 µm.), long (151-200 µm.) and very long (>200 µm.). Wide variability among the *A. brassicae* isolated from different areas was also recorded by Saha *et al*. (2014). The morphology of the conidia grown on PDA medium were very similar to the characteristics described by Simmons (1967) [20].

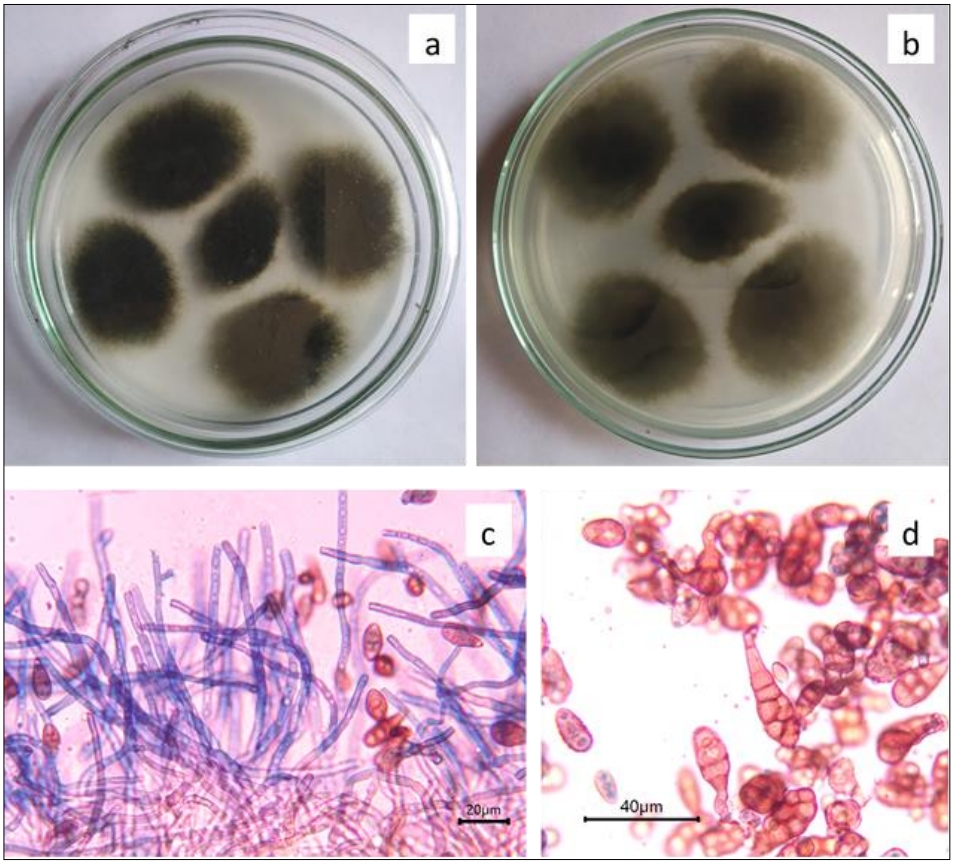

Fig 3: Cultural characteristics of seven days old culture of *Alternaria brassicae* grown on Potato Dextrose Agar (PDA) medium, **a:** upper surface of the culture, **b:** lower surface of the culture, **c:** areal conidiophore stained with trypan blue, **d:** conidia of various stages.

Antifungal assay

After 5 hours incubation cavity slides were observed under the compound microscope (Magnus CH 20i) at 10X magnification for germination inhibition. The result of the germination inhibition is presented in Table 2 photomicrographs are presented in (Fig. 4 a-p). A total of 14 different plants belonging to ten families of angiosperms, one family of gymnosperm and one family of pteridophyte were tested for antifungal activity against *Alternaria brassicae* (Table 2). Antifungal activity in the form of inhibition of conidial germination was observed only in the aqueous extract of bulb of *Allium sativum* (Fig. 4 h). In all the other plants tested no antifungal activity was observed (Table 2) and all the conidia were germinated within 5 hours of incubation in the germination box (Fig. 4). In distilled water all the conidia were germinated within 4 hours of

incubation (Fig. 4 o), whereas, in fungicide carbendazim no conidial germination was observed (Fig. 4 p) even after 5 hours of incubation.

The plants taken into this study were easily available in the Botanical Garden of the Department. The result of spore germination inhibition in this research is in confirmation of the earlier research carried out by Yadav *et al*. (2019) where 10% aqueous extract of garlic showed 100% inhibition of conidial germination *in-vitro*. However, the result of Bael, Neem and Adrakh extracts is contradictory to our findings, where these extracts did not show any antifungal activity against *A. brassicae* and all the conidia were germinated (Table 2; Fig. 4 b, d and g). These findings may be attributed to the fact that, neem is a good insecticide but poor fungicide. Also, inoculum potential of a fungal pathogen depends upon the strain, age and number of subculturing done. Garlic clove is a good fungicide against *Phoma exigua* causing Ascochyta blight in common bean (Wani *et al*. 2022) [24]. The extracts of two other plants *viz.*, *Rumex vesicarius* and *Ziziphus spina-christi* were found to inhibit the conidial germination of *Alternaria* spp. (Alotibi *et al*. 2020) [1]. The antifungal activity of a plant also depends upon the solvent used to make the extract. Guleria and Kumar (2009) [7] found strong antifungal activity of methanolic extract of *Agave americana* leaves against *A. brassicae*.

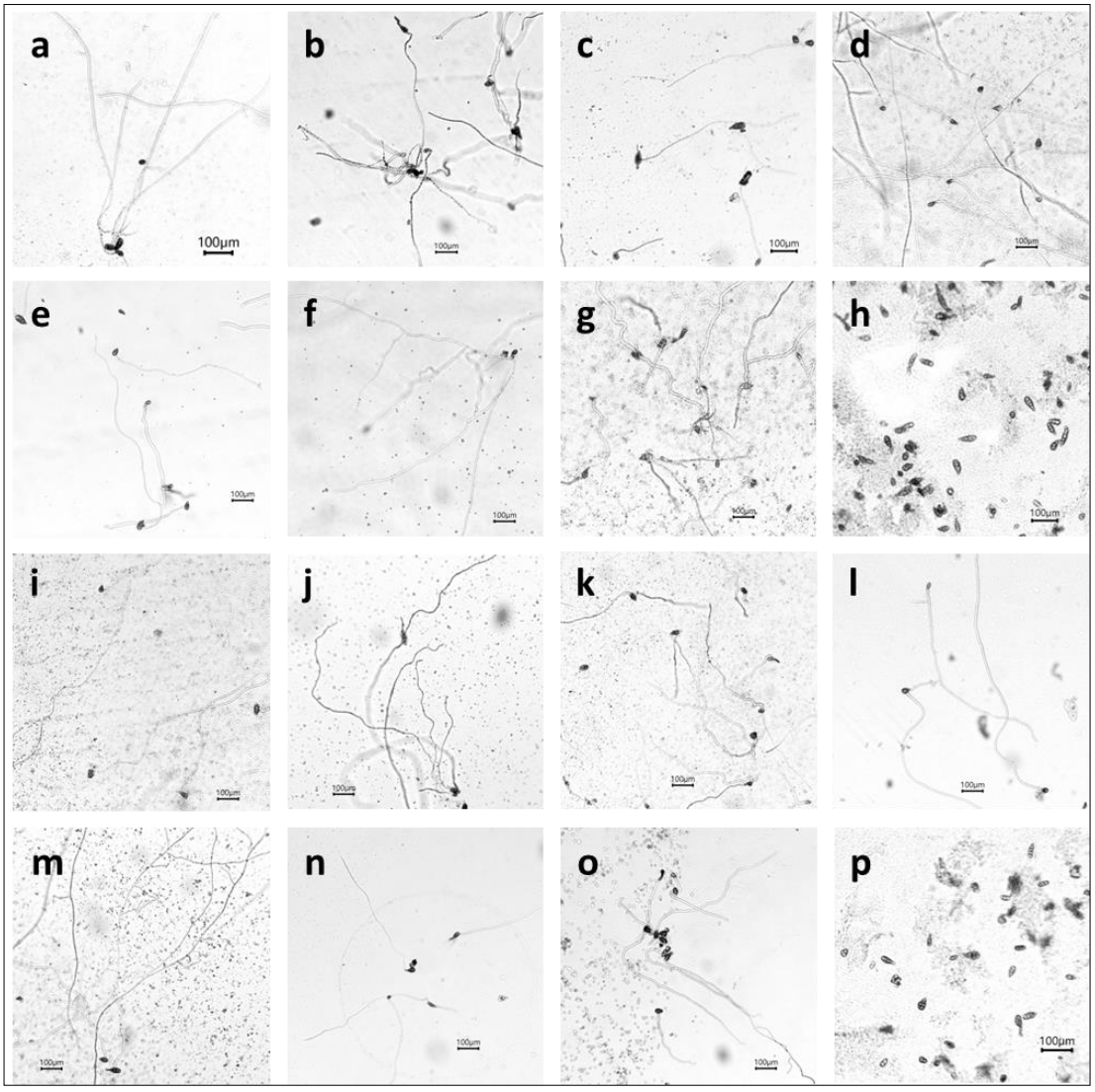

Fig 4: Plate showing photomicrographs of *Alternaria brassicae* conidial germination in aqueous substrates of **a:** *Citrus limon*, **b:** *Aegle marmelos*, **c:** *Murraya koenigii*, **d:** *Azadirachta indica*, **e:** *Duranta erecta*, **f:** *Nepeta hindostana*, **g:** *Zingiber officinale*, **h:** *Allium sativum*, **i:** *Tecoma stans*, **j:** *Parthenium hysterophorus*, **k:** *Cinnamomum zeylanicum*, **l:** *Asparagus racemosus*, **m:** *Zamia furfuracea*, **n:** *Pteris vittata*, **o:** Distilled water, **p:** Carbendazim 20% EC. Conidial germination was inhibited only in the aqueous extract of bulbs of *Allium sativum* (h) and in carbendazim (p). In all the aqueous conidia were germinated within 5 hours, and hyphae are visualized in the rest of the photomicrographs.

Table 2: Result of the conidial germination inhibition of *Alternaria brassicae* in the aqueous extracts of various plant part.

Sr. No.	Name of the plant	Family	Part of the plant used for extract	Conidial germination*
1.	*Citrus limon* (L.) Osbeck	Rutaceae	Leaf	-
2.	*Aegle marmelos* (L.) Corrêa	Rutaceae	Leaf	-
3.	*Murraya koenigii* (L.) Sprengel	Rutaceae	Leaf	-
4.	*Azadirachta indica* A.Juss.	Meliaceae	Leaf	-
5.	*Duranta erecta* L.	Verbenaceae	Leaf	-

6.	*Nepeta hindostana* (B.Heyne ex Roth) Haines	Lamiaceae	Leaf	-
7.	*Zingiber officinale* Roscoe	Zingiberaceae	Rhizome	-
8.	*Allium sativum* L.	Amaryllidaceae	Bulb	+
9.	*Tecoma stans* (L.) Juss. ex Kunth	Bignoniaceae	Leaf	-
10.	*Parthenium hysterophorus* L.	Asteraceae	Leaf	-
11.	*Cinnamomum zeylanicum* Blume	Lauraceae	Bark	-
12.	*Asparagus racemosus* Willd.	Asparagaceae	Rhizome	-
13.	*Zamia furfuracea* L.f.	Zamiaceae	Leaf	-
14.	*Pteris vittata* L.	Pteridaceae	Leaf	-
15.	Distilled water			-
16.	Carbendazim 20% EC			+

*: '-' sign showing no antifungal activity and conidial germination was observed, whereas '+' sign showing antifungal activity and conidial germination was not observed.
Double distilled water was used as negative control, where no inhibition in conidial germination was observed.
Carbendazim 20% EC was used as positive control, where conidial germination was inhibited.

Minimum inhibitory concentration (MIC) bioassay

The result of the MIC bioassay was shown in the Table 3. In 0.5% and below aqueous concentration of garlic bulb no conidial germination inhibition activity was recorded. The conidial germination was inhibited only in the 0.75% or above aqueous extract (Table 3). In distilled water also, all the conidia were fully germinated. However, in the fungicide carbendazim prepared in the same distilled water no conidia was germinated and inhibition was recorded (Table 3).

Table 3: Result of the minimum inhibitory concentration bioassay of aqueous garlic extract against *Alternaria brassicae*.

Sr. No.	Concentration of aqueous garlic extract	Conidial germination*
1.	1 %	+
2.	0.75%	+
3.	0.50%	-
4.	0.25%	-
5.	0.10%	-
6.	Distilled water	-
7.	Carbendazim 20% EC	+

*: '-' sign showing no antifungal activity and conidial germination was observed, whereas '+' sign showing antifungal activity and conidial germination was not observed.
Double distilled water was used as negative control, where no inhibition in conidial germination was observed.
Carbendazim 20% EC was used as positive control, where conidial germination was inhibited.

Antifungal activity of garlic is due to the allicin or ajoene (Hayat *et al*. 2016 [9]; Kuatawa *et al*. 2018). These compounds make the garlic extract an excellent antifungal product and it is reported to be active against *Fusarium* spp. and *Rhizopus* spp. even in less than 1.5 mg/l concentration (Kuatawa *et al*. 2018). Li *et al*. (2016) reported as low as 0.35 µg/ml MIC against *Candida albicans*. According to Hayat *et al*. (2016) [9] "allicin" the active principle in aqueous garlic extract is a phytoalexin and not only inhibits the growth of phytopathogenic fungi but also acts as a growth stimulator in cucumbers. Yadav *et al*. (2023) [26] tested the freshly prepared aqueous extract of garlic against *Alternaria* spot of mustard in the field, however the dose they have recommended was higher (2.0 %, w/v) than MIC reported (0.75 %) in this experiment.

Conclusions

From the field survey it can be concluded that, spot of disease of Indian mustard is a serious, but still underestimated problem in the parts of Sultanpur district of Uttar Pradesh. In the field conditions the disease caused up to 22% loss in the photosynthetic area of the leaves. The disease incidence and disease severity are strongly influenced by the weather conditions. Rain and high humidity in the field promoted the secondary infection very rapidly and all the plants were infected within a few days and 100% disease incidence was recorded. As no variety showing complete resistance against *A. brassicae* is available in the region, disease can be checked only by means of fungicides. The hazardous role of fungicides cannot be neglected as the Government of India examines the relevance of available fungicides from time to time. Recently 4 pesticides including fungicide Dinocap were banned (gazette notification vide S.O. 701(E), dated 02.02.2023) in India (Banning of pesticides 2023). Thus, considering the hazardous effects, more than 50 pesticides have been banned till date. Another alternative is the gene manipulation, but ethical clearance is hurdle in general cultivation of such crops. In this scenario farmers should be promoted to shift on other alternatives which should be environment friendly to combat the pathogenic diseases of crops. With this initiative the finding of present study is a milestone to control the spot disease of mustard by using garlic extract, which is household and easily available in every farmer's home. The results of in-vitro conidial germination inhibition by garlic extract in this study is fascinating as 0.75% aqueous solution effectively inhibited the conidial germination in the cavity slide. Based on findings of this research, field trials will be conducted first at microplot level and then in the field conditions in near future to standardize the formulation. Thus, findings of this research study open vistas to formulate botanical fungicide for the safe use to control the fungal diseases of crops. As, allicin, the active principle of aqueous garlic extract also acts as growth stimulator, the possibility of beneficial effects on mustard plant should also be validated in future experimentation.

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