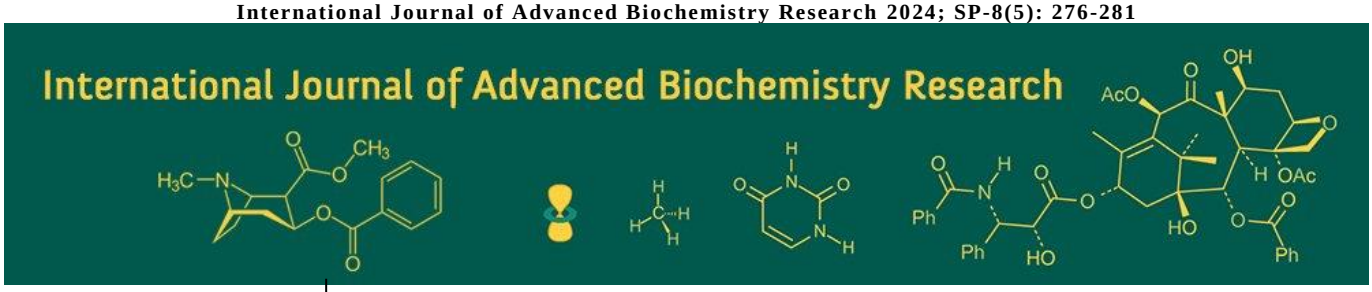

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Studies on disease management of *Alternaria brassicicola* of cabbage

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Abstract
Cabbage is one of the most important cole crops. Now it is grown almost throughout the year for its economic and nutritional value. India occupies 2nd position in cabbage production worldwide. It suffers from a wide array of fungal, bacterial and viral diseases which is causing severe loss in yield. *Alternaria brassicae,* the incitant of leaf blight of cabbage is a fungal pathogen has been reported from all the continents of the world as well as in the state of Uttar Pradesh in wet seasons and in areas with relatively high rainfall. Symptomatology study revealed the seedlings developed spots on leaves leading to damping off. Initially small yellow specks are produced on leaves and stem which gradually darken and enlarge into circular, dark to tan coloured concentric rings giving a target board effect. In pure culture, the fungus produced profuse cottony mycelium, whitish to grey in colour at first and turned dark with age. The conidia are brownish black, obclavate with 4-11 transverse and 0-4 longitudinal septa measuring 28.85-67.28×11.39-13.91 µm with the average of 41.90×12.94 µm. Conidiophores found to be distinctly geniculate arise in fascicles, dark brown unbranched, straight with 1-5 septate. Cultural studies revealed that Potato dextrose agar maximum growth (86.150 mm) of the test fungus at 10 days of inoculation. The optimum pH for the growth of the test fungus was recorded to be 7.0 corresponding to maximum dry weight growth (360 mg). The plate cultures of the fungus put under 16 hours of light along with 8 hours of darkness could facilitate highest radial growth (69.97mm) followed by the plates subjected to 12 hours light along with 12 hours dark (68.92mm), both being statistically at par with each other. From management study it was revealed that among the five phytoextracts evaluated, leaf extract of Datura at 20% concentration was found to be superior in inhibiting the mycelial growth (53.15-57.48%). Five biocontrol agents were tried *in vitro* against *Alternaria brassicae* in terms of the percent inhibition of mycelial growth. *Trichoderma viride* (79.26%) found promising followed by *Trichoderma harzianum* (76.91%), both being statistically at par with each other. Eight agrochemicals were evaluated against *Alternaria brassicae* in terms of the percent inhibition of the mycelial growth. Propiconazole at 0.15% was the best chemical resulting in significantly the maximum growth inhibition of the test fungus (100%) followed by Difenconazole showing 84.16% growth inhibition.

Keywords: Disease management, *Alternaria brassicicola,* cabbage, *Trichoderma viride*

Introduction
Cabbage (*Brassica oleracea* L. *var capitata*) is one of the most important cole crops belonging to family Cruciferae and is grown for the thickened main bud called "Head". The word cabbage is an anglicised form of the french "Cobbache" meaning head and it is mostly used as culinary and dietic articles, salad, pickles, boiled vegetables, cooked in curries, dehydrated vegetables and can also be used for feeding livestock.

The Food and Agriculture Organization of the United Nations (FAO) reports that world production of cabbage for 2019 was almost 71 million metric tons. Almost half of these crops were grown in China, where Chinese cabbage is the most popular *Brassica* vegetable. China has the 1st position in cabbage production worldwide and India is on the 2nd position. The Food and Agriculture Organization of the United Nations (FAO) reporting that total world production of all Brassicas for 2019 was 70,104,972 metric tons. The nations with the largest production were China which produced 47% of the world total and India which produced 12%. China and India used a surface area of 980,000 hectares (2,400,000 acres) and 375,000 hectares (930,000 acres) respectively.

In India largest producing state West Bengal Production 1490.39 ha Area with production 29.55 million ton, however Uttar Pradesh the production near 3.1 million tonnes

Materials and Methods

This chapter deals with the materials and methods which were used during the course of investigations and experiments. The diseased specimens of cabbage grown in Central Farm, Orissa University of Agriculture & Technology, Bhubaneswar were collected, used for study. The symptoms were minutely observed and the disease was suspected to be *Alternaria* leaf spot.

Cleaning of glasswares

Borosil glasswares were used in laboratory experiments. They were cleaned by using standard procedures (Riker and Riker, 1936). All the glasswares were dipped overnight in solution containing 60 ml of potassium dichromate, 60 ml of conc. sulphuric acid per litre of water. Then they were washed with detergent followed by repeated rinsing with tap water. The glasswares were again rinsed with distilled water before use.

Sterilization

a. **Sterilization of glasswares:** All glasswares were sterilized in at 160-180 ℃ temperature in hot air oven for a period of three hours. Before sterilization, the glasswares were wrapped with brown paper/news paper.
b. **Sterilization of media:** After the media were prepared, approximately 7-8ml of the medium was taken in each culture tube for the preparation of slant. The culture tubes and conical flasks containing melted agar medium were properly plugged with dry non absorbent cotton and were sterilized at 15p.s.i (121.6 ^{0}C) for 20 minutes in an autoclave Sterilization of water
   For the preparation of sterilized water, desired quantity of distilled water was taken in suitable container like conical flask, plugged with non-absorbent cotton and sterilized at 15p.s.i for 20 minutes in an autoclave.
c. **Sterilization of blotting paper:** It was sterilized in hot air oven at 160 ºC for two hours by placing it inside petridish. Before keeping petridish inside oven, it was wrapped with brown paper/news paper.
d. **Sterilization over flame:** The inoculation needle, forecep and cork borer were sterilized by dipping them in 70% ethanol followed by flaming over spirit lamp. The slide and cover slip were placed over flame just before use.
e. **Sterilization of plant material:** Surface sterilization of plant material was done by cutting it into small bits (2-3mm) and then dipping these bits in (0.1%) mercuric chloride solution for 30 seconds or 0.5% sodium hypochlorite solution for 2 minutes followed by repeated rinsing with distilled water 3 to 4 times to remove the trace of chemical from the plant sample.

Maintenance of aseptic condition

To avoid contamination, all the operations were carried out inside an inoculation chamber under the laminar air flow in aseptic condition.

Collection of the diseases sample

Disease samples were collected from nearby farms and from central farm of Orissa University of Agriculture and Technology Bhubaneswar. Each sample was brought to the laboratory separately in polythene bags and being washed with distilled water. Affected portions of the leaves were teased and examined under microscope which revealed the presence of characteristic mycelia, conidiophores and conidia of the fungus, *A. Brassicae*. Isolation of the pathogen

Purification and identification of fungi

Each isolate of fungus maintained on PDA slants, were transferred to agar plates. Hyphae from the periphery of young colonies were carefully examined 'and transferred to PDA slants. This process was repeated for 2-3 times till the concerned fungus was found to be free from other fungi and bacteria. Pure culture of fungus was obtained through 'single spore' and 'hyphal tip' methods. The characteristics of the fungal colony on PDA and the details of their morphology were recorded for each isolate. Each isolate was taxonomically identified with the help of available cultures and literature.

Single spore isolation

A spore suspension was prepared in sterile water and spore contents were ascertained by examining a drop of suspension under the microscope. Culture tubes containing 10 m1 of sterile water agar were melted over water bath and temperature was allowed to come down to 40 °C

Hyphal tip culture

The fungus was grown in a sterilised petridish containing potato dextrose agar medium. As isolated, hyphal tip was located under the microscope and marked with the help of a sharp glass marking pencil. The tip was carefully lifted up and transferred by sterilized inoculating needle to a potato dextrose agar slant at room temperature. After 2-3 days, the growth of the fungus was observed in the culture tube and thus a pure culture of the fungus was obtained. After getting the fungus in pure culture, it was maintained in potato dextrose agar medium and sub cultured in 2 weeks intervals.

Identification of the culture

The culture obtained was compared with the original description of the fungus for morphological characters.
A bit of fungal hypha was taken from 15 days old culture on a slide and teased gently by the help of two sterilized pricking needle. Then it was stained with lactophenol blue, covered with glass cover slips and examined under microscopes to study the morphological characters like its mycelia growth, colony characteristics like its colour, texture, lustre and growth habit etc., size, shape, colour, septations etc. of the conidia with the help of microscope. Microphotograph of the mycelium and conidia was taken and measurement of the conidia was done with the help the computer generated micrometer.

Maintenance of the culture

The fungus was sub cultured on the PDA slants and allowed to grow at room temperature for 15 days. Then the slants were preserved in refrigerators at 4°C and sub-cultured once in 30 days.

Pathogenicity test

Pathogenicity was proved on local variety of cabbage plant. The healthy plants were raised in earthen pots. Sixty days old plants were sprayed with distilled.

Morphological studies
A mycelial tip was taken from the culture. It was teased upon a glass slide and a drop of cotton blue lactophenol was added upon that and was covered by a cover slip. The slide was observed under compound microscope.

Spore germination studies
Spore suspension was made in sterilized water. A drop of spore suspension was taken upon a clean slide. Then the slide was placed in petridish lined with wetted blotting paper to provide high humidity. It was kept for 24 hours. Then the type of germination was studied under microscope.
The growth characters of *Alternaria brassicae* were studied on 9 different solid media *viz*. Potato dextrose agar, Host extract agar, Carrot root extract agar, Oat
meal agar, Malt extract agar, Czapek‟s (Dox) agar, Richard‟s agar, Sabouraud dextrose agar and Asthana & Hawker‟s agar. All the media were sterilized at 15 psi
for 20 minutes. After sterilization, each medium was poured into 90 mm petridishes. Each treatment was replicated thrice. After pouring they were allowed to cool down. Then 5 mm mycelia disc of culture was inoculated into each plate and incubated at room temperature i.e.28±1°C. Colony diameter was recorded by averaging linear growth of the colony in three directions for each plate at 9 days of inoculation with the help of fine transparent plastic scale in millimetres. The colour of the fungal colony, surface elevation and sporulation were also recorded. The recorded data were analysed statistically. The composition of each medium given below. The preparation of various media was done following the procedure given by Ainsworth (1969) [4].

Potato dextrose agar: Potato Dextrose Agar (PDA) medium was prepared by following standard procedure postulated by Riker and Riker (1936) followed with slight modifications wherever necessary.

Peeled and sliced potato: 200g
Agar-agar: 20g
Dextrose: 20g
Distilled water: 1000 ml

Two hundred gram of peeled and sliced potato was boiled in a container with 500 ml of water for some time until it was soften, but not over cooked. The extract was filtered through a piece of muslin cloth. Simultaneously, agar was melted in 500 ml of water in another container. The potato decoction and the melted agar were mixed together to which dextrose was added and mixed thoroughly in another container with constant stirring and the volume was made up to 1000 ml. Streptomycin sulphate was added to the medium @ 0.75g per 1000 ml before autoclaving for suppression of bacterial contamination. The prepared potato dextrose agar medium was kept in suitable flasks or tubes, plugged and sterilized in autoclave at 15 psi for 20 minutes.

Czapek's (Dox) agar
Sucrose : 30g
Sodium nitrate: 2g
Potassium dihydrogen phosphate: 1g
Magnesium sulphate: 2.5g
Potassium chloride: 0.5g
Ferrous sulphate: 0.01g
Agar-agar: 20g
Distilled water: 1000 ml

All the ingredients were mixed in 500 ml of distilled water. Agar-agar was melted separately in 500 ml water by boiling. Then two mixtures were mixed and streptomycin sulphate 0.75 g was added for avoidance of bacterial contamination. The medium was sterilized in 15 psi for 20 minutes in autoclave.

Richard's agar
Sucrose: 50g
Potassium nitrate: 10 g
Potassium dihydrogen phosphate: 5g
Magnesium sulphate: 2.5g
Ferric chloride: 0.02g
Agar-agar: 20g
Distilled water: 1000 ml

All the ingredients were mixed in 500 ml of distilled water. Agar-agar was melted separately in 500 ml water by boiling. Then two mixtures were mixed and streptomycin 0.8 g was added for avoidance of bacterial contamination. The medium was sterilized at 15 psi for 20 minutes in autoclave.
All the ingredients were mixed in 500 ml of distilled water. Agar-agar was melted separately in 500 ml water by boiling. Then two mixtures were mixed and streptomycin sulphate 0.75 g was added for avoidance of bacterial contamination. The medium was sterilized in 15 psi for 20 minutes in autoclave.

Experimental finding
Growth and sporulation on different solid media
In order to find out a suitable medium for growth and sporulation of the test fungus, nine different solid media of synthetic, semi-synthetic and natural were tried as per the procedure described under "Materials and Methods".
Observations on radial growth of the fungal colony and sporulation were recorded after ten days of inoculation. The data obtained have been presented in Table 3 and depicted in Figure 1.
Data in the table revealed that Potato dextrose agar medium supported significantly the maximum radial growth (86.15mm) of the test fungus followed by Sabouraud dextrose agar medium (76.11mm) and malt extract agar (71.71mm). The other nutrient media namely Richard‟s agar, Oat meal agar, Carrot root extract agar, Czapek‟s agar, were found inferior in respect of radial growth of fungus with the mean colony diameter of 68.65, 64.45, 58.90, and 57.14 mm respectively.
Similarly maximum sporulation was observed in Potato dextrose agar medium. Least sporulation was recorded in case of Carrot root extract agar, Czapek‟s agar medium.

Table 1: Effect of different solid media on the growth of *Alternaria brassicicola*

Sl. No.	Treatments (Solid media)	Mean colony diameter (mm)*	Sporulation
1	Potato dextrose agar	86.150	++++
2	Carrot root extract agar	58.90	+
3	Oat meal agar	64.450	++
4	Malt extract agar	71.7170	+++
5	Czapek's Dox agar	57.140	+
6	Richard's agar	68.650	+++
7	Sabouraud dextrose agar	76.117	+++
	SE.m±	0.6782	
	C.D.(5%)	2.0574	
	CV%	1.70	

No sporulation; + low; ++ moderate; +++ profuse; ++++ abundant ***** Mean of three replications

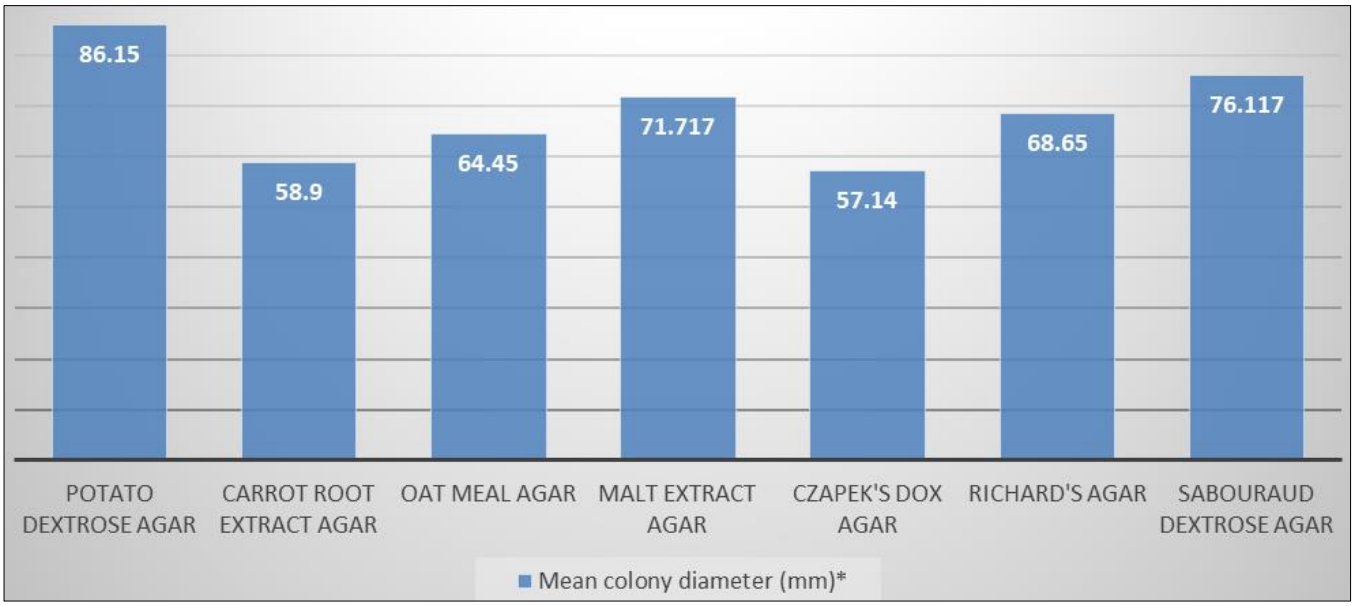
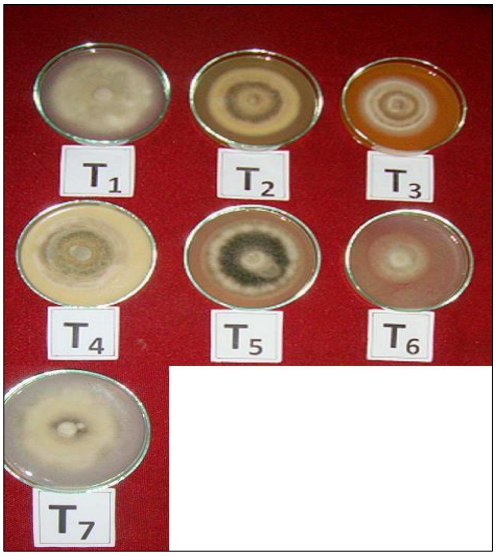


Fig 1: Growth of *Alternaria brassicicola* in different solid media



T_1: Potata dextrose agar
T_2: Carrot root extract agar T_3: Oat meal agar
T_4: Malt extract agar
T_5: Czapek‟s Dox agar
T_6: Richard‟s agar
T_7: Sabouraud dextrose agar

Effect of hydrogen ion concentration on the growth of *Alternaria brassicae*

Hydrogen ion concentration plays a significant role in promoting the vegetative growth as well as sporulation in fungi. In order to ascertain the optimum pH requirement of *Alternaria brassicae*, seven different pH regimes were evaluated *in vitro* in terms of the mean dry mycelial weight of test fungus at ten days of inoculation in Richard‟s medium. The data indicated that pH 7.0 was significantly superior in yielding the maximum mean dry mycelial weight growth of 360 mg. Further, It was observed that both the highly acidic(pH 3.0 and 4.0) and the alkaline (pH 9.0) ranges didn't perform well in promoting the vegetative growth of the test fungus with the mean dry mycelial weight growth of 123.40, 153.42 and 146.21mg respectively. The causal fungus was observed to grow well in the pH range of 5.0-8.0 indicating that it could grow well in neutral to alkaline pH (Table 2 and Figure 2).

Table 2: Effect of different levels of pH on the growth of *Alternaria brassicicola*

Sl. No.	pH of the medium	Mean dry mycelia weight(mg)
1	3.0	124.15
2	4.0	153.88
3	5.0	217.15
4	6.0	227.89
5	7.0	364.25
6	8.0	311.45
7	9.0	147.52
SE m±		8.752
CD (5%)		26.54
CV %		6.89

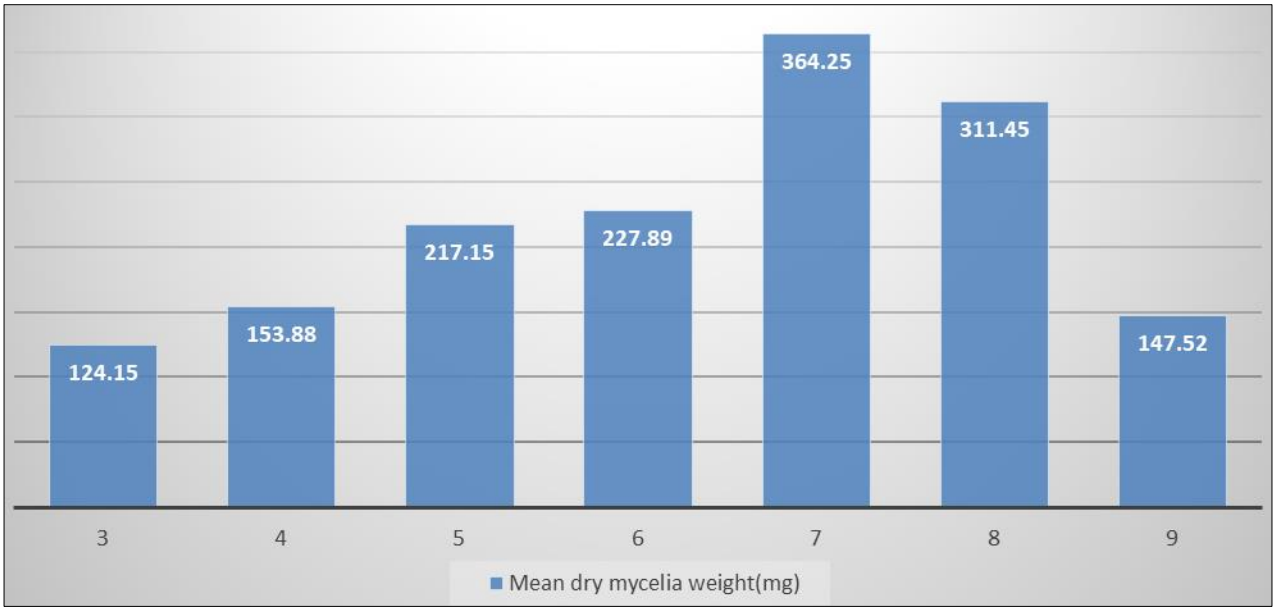


Fig 2: Effect of different levels of pH on the growth of *Alternaria Brassicicola*

Summary and Conclusion

Leaf blight disease of cabbage is an important disease of observed throughout the state of Uttar Pradesh. Samples collected from Central farm, Rama University Mandhana, Kanpur Nagar, were found at all stages of the crop growth. At nursery stages the seedling develop spots on leaves resulting in damping off. The disease spreads to aerial parts of plant as minute yellow specks on leaves and stem, gradually darken and enlarge into circular, tan to black coloured concentric rings giving the appearance of target board surrounding by yellowish halo. In advance stage it changed to tan, brown or black in colour, papery in texture and finally falls off giving the appearance of shot hole which reduced the yield and quality of the crop.

The pathogen was isolated from all the above described symptoms and frequent isolation yielded the same fungus which was isolated and identified as *Alternaria brassicae*. The pathogenicity test was carried out by using the isolated pathogen which developed symptoms those were almost similar to the original symptoms of the sample. Reisolation from the artificially inoculated diseased plants yielded the same fungus which was found to be identical to the originally isolated fungus.

Study on the growth of the fungus in different solid media revealed that Potato dextrose agar medium supported significantly the maximum radial growth (86.15mm) of the test fungus followed by Sabouraud dextrose agar medium (76.17mm) and Malt extract agar (71.71). The other nutrient media namely Richard‟s agar, Oat meal agar, Carrot root extract agar, Czapek‟s agar, were found inferior in respect of radial growth of fungus with the mean colony diameter of 68.65 64.45, 58.90, 57.14 respective

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