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Studies on Leaf spot and Tip/Foliar blight of Bach (*Acorus calamas*) caused by *Nigrospora oryzae* (Berk. et Br.) Petch

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Bach (*Acorus calamas*) is one of the most important medicinal plants in West Bengal and suffers from foliar disease caused by *Nigrospora oryzae.* In this paper, fixed plot survey of leaf spot of bach, isolation of pathogen, pathogenicity test of isolated pathogen for confirmation of disease, colony characters and growth of pathogen in different semi solid and liquid media were made. The results revealed that the severity of the leaf spot disease of *Acorus calamus* caused by *Nigrospora oryzae* were more during the period from November to February and maximum disease incidence was recorded in the month of November, thereafter the incidence decreased slowly and reached minimum during July - August. From September onward the incidence gradually increased and reached to the peak during November–February. Symptoms of leaf spot of Bach showed that minute brown to black spots appeared at the leaf tip, later it spread from leaf tip to lower part of the leaves. Spots coalesced with each other and appeared as a large spot. Blight symptom was shown from tip and drying of the leaves occurred from leaf margin. In severe infection whole leaf was blighted. The pathogen was established through pathogenicity test. Visual observations of the colony characters were made after re-isolation from the inoculated plants with the pathogens revealed that the colony of the pathogen was deep black in colour with white cottony mass present on the growth. The highest growth of *Nigrospora oryzae* was recorded in Oat meal agar and Richards media and lowest in PDA medium where as in liquid media highest dry weight was recorded in Richards medium (1.54 g) and lowest in Oat meal agar medium (0.14 g). Micrometric measurement of the pathogen were made after growing in PDA medium and observed under the high power microscope. *Nigrospora oryzae* produced smaller size spores (6.12 - 13.60 µm x 5.06 - 12.14 µm). The colony character of the pathogen in different media revealed that in different media the colony character of the pathogen were different.

Key words: *Acorus calamus*, *Nigrospora oryzae*, Richards medium, Oat meal medium

INTRODUCTION

Plants have been used since ancient time mostly to heal and cure human and animal diseases. Recently considerable attention has been paid to utilize eco-friendly and bio-friendly plant based products for the prevention and cure of different human diseases. Considering the adverse effect of synthetic drugs, the western world is looking for natural remedies which are effective as well as safe. It is documented that 80% of world population has faith in traditional medicine, particularly plant drugs for their primary health care (WHO). Cultivation of medicinal plants in West Bengal is yet to take a noble shape. Government of West Bengal has recommended some medicinal plants like Aswagandha, Sarpagandha, Senna, Bach, Tulsi etc for commercial cultivation in different zones. However, several biotic and abiotic factors limited the production of these crops. Several biotic factors like fungi, viruses, bacteria, phytoplasmas, nematodes and abiotic factors like deficiencies in soil, lack of proper irrigation, etc. are responsible for the maladies of medicinal plants. *Acorus calamus* Linn. commonly known as

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Sweet Flag, belongs to the family Araceae (Adoraceae) suffers from bacterial blight disease caused by *Xanthomonas campestris* pv. *oryzae*, leaf spot caused by *Uromyces acori* (Nirmalkar and Lakpale, 2007) and leaf spot and tip/foliar blight caused by *Nigrospora oryzae* (Paul and Dasgupta, 2014). Other than the work conducted under A.I.C.R.P on Medicinal and Aromatic plants, very little work has been made on diseases of Bach (*Acorus calamas*). In this present investigation, attempts have been made to study the leaf spot and tip/foliar blight of Bach caused by *Nigrospora oryzae*.

MATERIALS AND METHODS

Fixed plot survey

Observations of the plots were done at monthly intervals starting from the month of December, 2011 to November, 2012 where the incidence and severity of the disease which appeared were recorded. For percent disease incidence total no. of leaves infected in a plot were recorded and for percent disease index, no. of leaves infected per 10 plants in each plot were rated on a 0-4 scale, where 0= healthy leaves; 1= 1 - 6% leaf area infected; 2= 7 - 12% leaf area infected; 3= 13 - 25% leaf area infected; 4= above 25% leaf area infected (Momin, 2009). Percent disease incidence and percent disease index were calculated according to Paul (2013)

Study of the disease symptom

Disease conditions in the plants were recognized according to the symptoms produced by the pathogen. The maladies observed on the plants were recorded. The plants were carefully studied for any kinds of symptoms on the leaves and the rest of the above ground portions. Detailed escriptions of the symptoms are required for the establishment and diagnosis of the diseases later on.

Isolation of the pathogen
Collection of diseased specimen

The leaves which showed some spots or lesions were collected from the field for isolation of the fungi causing disease on them and brought to the laboratory.

Method of isolation

Isolation was carried out in a sterilized zone of the laminar air flow. The diseased specimens already washed with tap water were taken and with the help of a sterilized scissor, leaf was cut into small pieces which contained the diseased portion as well as the healthy tissue. The pieces were dipped in 0.1% $HgCl_2$ solution for 1 min. and were later rinsed three times with sterile distilled water. With the help of a sterilized forceps, each piece was placed aseptically on the solidified PDA / Water agar media on the sterilized plates depending upon the diseased specimen. About 3-4 such pieces were placed on each plate maintaining some distance from each other and the Petriplates were incubated at 28 ±1°C. After 5 days, the growing fungus was examined under microscope for sporangial production. The isolates were maintained on potato dextrose agar medium. All the isolates were preserved at 5°C. Sub cultures were made at 15 days intervals. One set of isolates was preserved in liquid paraffin at 5°C.

Pathogenicity test

Pathogenicity of isolated fungi were tested on potted plants by inoculating the leaves after removing all diseased leaves. The test was conducted with 3 replications and 5 plants per replication. Suitable control was maintained by spraying water. A spore suspension (5 x 10^5 spores/ml) was prepared from 8 days old culture grown on potato dextrose agar medium was sprayed on leaves, with an all glass automizer and the whole set up was placed in the humid chamber. The lesion appeared after 2-3 days of inoculation were observed.

Confirmation of pathogens

After the appearance of disease symptoms, the diseased leaves were collected and again re-isolated the pathogens to compare with the previous isolated pathogens and to get confirm about the disease causing pathogens.

Morphometric character of the pathogen

The slides of the selected fungal cultures or colony were prepared in order to study the fungal morphology such as the characteristics of the hyphae and spores, etc. for easy identification of the fungal species infecting a particular specimen. The prepared slides were observed under Phase Contrast microscope using ocular and stage micrometer.

Colony characters and radial growth of Colletotrichum gloeosporiodes in different semisolid media

Nigrospora oryzae was grown in PDA, Czapek dox, maize meal and Oat meal media. Molten medium was poured into each sterile Petriplate and allowed to solidify. Small discs (6 mm) of the fungus mycelium was cut with a sterile disc cutter from margin of 7 days old culture grown in PDA and was transferred asceptically to the plates and incubated at 28 ± 1°C. Different changes of fungal colony in different media were recorded every day up to 10 days. Colony diameters were measured up to 10 days from 2nd day of inoculation.

Growth of Colletotrichum gloeosporiodes in different liquid media

Colletotrichum gloeosporiodes was grown in PDA, Czapek dox , Maize meal and Oat meal broth i.e. liquid media (50 ml in 250 ml Erlenmeyer flasks). All the flasks were inoculated with 6 mm mycelia disc grown on Potato dextrose agar medium and incubated at 28 ± 1°C for 8 days. After 8 days dry weight of mycelial mats were recorded.

Dry weight determination

Coherent mycelium was removed from liquid medium washed thoroughly and dried on a pre weighted filter paper at 65-70°C for 48 hrs. The dried mycelial mat with filter paper was kept in a desiccator over anhydrous P_2O_5 and then weighed. Weighing was repeated till constant weight was obtained.

RESULTS AND DISCUSSION

Fixed plot survey

The results (Table 1) revealed that the highest per cent disease incidence and per cent disease index of the leaf spot disease of *Acorus calamus* caused by *Nigrospora oryzae* were recorded during the period from November to February and maximum per cent disease incidence (49.50%) and per cent disease index (38.67%) were recorded in the month of December, thereafter both the disease incidence and index decreased slowly and reached minimum (23.33%) during July and 19.32% during August respectively. From November onwards the incidence and index were gradually increased and reached to the peak during December month.

Table 1 : Fixed plot survey of Leaf spot and Tip/Foliar blight of Bach

Months	%disease incidence	%disease index
December,11	49.50	38.67
January,12	46.48	35.49
February,12	43.49	32.56
March,12	28.52	22.48
April,12	26.53	21.64
May,12	25.46	22.78
June,12	24.34	20.53
July,12	23.33	19.57
August,12	23.89	19.32
September,12	28.70	26.54
October,12	30.98	28.39
November,12	40.82	30.65

Table 2: Colony character and the length and breadth of the spores

Colony character of the pathogen	Length (µm)	Breadth (µm)
Deep black coloured mycelial growth and white cotony mass present on the growth	6.12 - 13.60	5.06 - 12.14

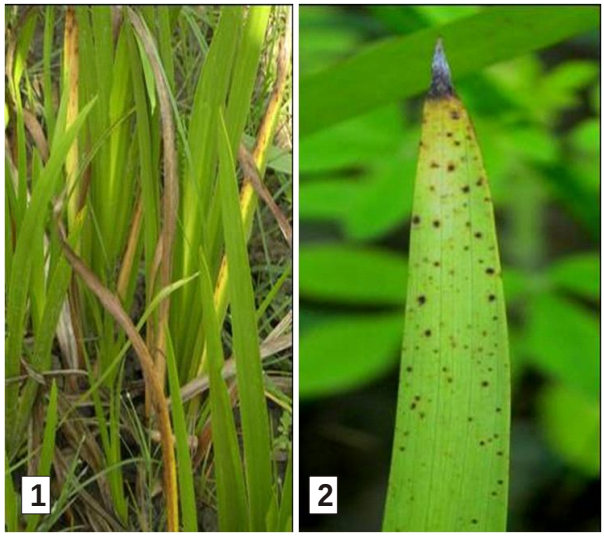

Fig. 1 and **2 :** Leaf spot and Tip blight of *Acorus calamus*

Symptom of leaf spot and tip/foliar blight of Bach

Symptom was studied very carefully on the leaves. First minute brown to black spots were appeared at the leaf tip, later it spread from leaf tip to lower part of the leaves. Spots coalesced with each other and appeared as a large spot. Blight symptom was shown from tip and drying of the leaves occurred from leaf margin. In severe infection whole leaf is blighted (Fig. 1 and 2).

Table 3: Cultural Characteristics and radial growth of the pathogen in different media

Growth of Pathogen in different media	Potato Dextrose Agar medium	Czepek Dox Agar medium	Richards Agar medium	Oat Meal Agar medium	Maize Meal Agar medium
	Whitish mycelial growth started on media. After 3 days centre became blackish. Full plate is covered with blackish white mycelia growth. Colour of mycelium became more deep blackish.	Transparent mycelial growth from the centre. It was not so dense. The colour of mycelium became light green and turned to black in colour. Whitish cottony growth started from the centre.	Transparent not so dense mycelia growth is occurred and finally it became black in colour. White cottony growth on the centre.	First off -white mycelium growth are occurred. It became dark black in colour. White cottony growth present on it but it not so dense.	Same as oat media but white cottony growth are not there.
Radial growth in different media	90mm	90mm	90mm	90mm	90mm
Dry wt. (gm) in different media	1.16	1.18	1.54	0.50	0.14

Colony characters of the pathogens

Visual observations of the colony characters were made after re-isolation from the inoculated plants (Table 2).

Micrometric measurements

Micrometric measurement of the pathogen were made after growing in PDA medium and observed under the high power microscope (Table 2 and Fig. 3).

Cultural characteristics of pathogens in different media

Characteristics of *Nigrospora oryzae* was studied in five different media such as Potato Dextrose Agar medium (PDA), Czepek dox medium (CZA), Richards medium (RM), Oat meal agar medium(OMA) and Maize meal agar media (MMA). The general cultural fea

Radial growth of Alternaria sp*. in different semi solid media*

The fungus was allowed to grow in five different media such as Potato Dextrose Agar medium (PDA), Czepek dox medium (CZA), Richards medium (RM), Oat meal agar media (OMA) and Maize meal agar media (MMA). The results (Table 3) showed that the highest growth of *Nigrospora oryzae* was recorded in Richards Agar medium.

The results thus obtained gives a primary knowledge of the disease for future study by the research workers.

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