First report of Leaf spot of Aswagandha (*Withania somnifera* Dunal) caused by *Colletotrichum gloeosporioides* from West Bengal, India

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First report of Leaf spot of Aswagandha (*Withania somnifera* Dunal) caused by *Colletotrichum gloeosporioides* from West Bengal, India

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Aswagandha (*Withania somnifera* Dunal) suffers from many fungal diseases which include damping off and seedling blight caused by *Alternaria alternata;* leaf blight and die back caused by *Alternaria alternata*; leaf spot caused by *Myrothecium roridum;* wet rot caused by *Choanephora cucurbitarum.* During fixed plot survey a new disease was observed and after studying morphometric characters of the pathogen and identification of the pathogen upto species level the pathogen was identified as *Colletotrichum gloeosporioides* by National Center of Fungal Taxonomy, Inderpuri, near Pusa Institute, New Delhi 110012. This is the first report of *Colletotrichum gloeosporioides* on *Withania somnifera* Dunal.

Key words: Aswagandha, *Colletotrichum gloeosporioides*, leaf spot

INTRODUCTION

Aswagandha (*Withania somnifera* Dunal) belongs to the family Solanaceae. It is a xerophytic plant, found in the drier parts of India, Sri Lanka, Afghanistan, Baluchistan and Sind and is distributed in the Mediterranean regions, the Canaries and Cape of Good Hope. The plant is used as an aphrodisiac, liver tonic, anti-inflammatory agent, astringent, and to treat bronchitis, asthma, ulcers, emaciation, insomnia and senile dementia. It has also been used for strengthening the body to prevent disease. Aswagandha suffers from many fungal diseases which include damping off and seedling blight caused by *Alternaria alternata* (Janardanan, 2002); leaf blight and die back caused by *Alternaria alternata* (Pandey and Nigam, 1985); leaf spot caused by *Myrothecium roridum* (Mahrishi, 1986); wet rot caused by *Choanephora cucurbitarum* (Saroj *et al*.2012). During fixed plot survey a new disease was observed and later diseased samples were collected from field and isolated the pathogen. The pathogen was confirmed by pathogenicity test and the symptoms of the disease and morphomatric characters of the pathogen were studied carefully.

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Therefore, the main objective of this study was to identify the actual cause of leaf spot of *Withania somnifera* for further study.

MATERIALS AND METHODS

Study of the disease symptoms

Disease conditions in the plants were recognized according to the symptoms produced by the pathogens. The maladies observed on the plants were recorded. The plants were carefully studied for fixed and targeted spots on the leaves of *Withania somnifera*.

Isolation of the fungi causing foliar diseases on the Withania somnifera

The leaves showed characteristic symptoms were collected from the field and brought to the laboratory for isolation of the pathogen. Isolation was carried out on a sterilized zone of the laminar air flow. The diseased specimens were washed with running tap water. The washed leaves were taken into laminar air flow and cut into small pieces by a sterilized scissor which contained the half diseased portion and half healthy portion. The pieces were dipped in $HgCl_2$ solution for 1 minute and later

rinsed several times with sterile distilled water. With the help of a sterilized forcep, each piece was placed on the solidified PDA / Water agar media on the sterilized plates. About 3-4 such pieces were placed on each plate maintaining some distance from each other. All the plates were kept into BOD incubator at 28± 1^0C for 4 days. Then growing hyphal tips were transferred into PDA slant. The cultures were maintained in PDA slants and kept in refrigerator at 5^0C. Sub culturing of the isolated pathogens were made at 15 days interval.

Fig. 1 and 2 : Leaf spot of *Withania somnifera* caused by *Colletotrichum gloeosporioides*

Fig. 3: Culture of *Colletotrichum gloeosporioides* on PDA media

Preparation of slides of the fungal cultures to be observed under the phase contrast microscope

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, conidiophores, spores etc. for easy identification of the fungal species. The prepared slides were observed under phase-contrast microscope.

Pathogenicity test

Artificial inoculation of sporulated mycelium mat on the plants

For artificial inoculation, a spore suspension (5 x 10^5 spores/ml) was prepared from 8 days old culture grown on PDA media and was sprayed on plants, under field condition. Control plants also maintained by spraying only sterile distilled water and then the whole plants was covered by the polypropylene packet to maintain the humidity and favourable condition for disease development.

Confirmation of pathogens

15-20 days after inoculation on the test plants, disease symptoms were developed. 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.

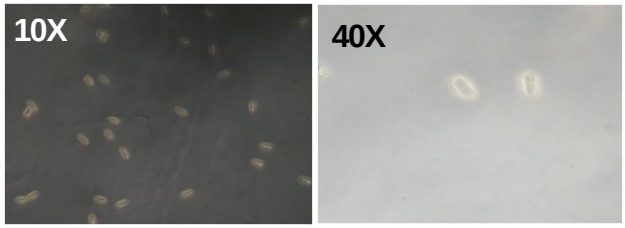

Fig. 4 and 5: Spores of *Colletotrichum gloeosporioides*

Study of the morphometric characters of the pathogens and identification

To identify the pathogens and to know their taxonomic position, morphometric studies were carried out. To study the morphometric characters of fungi, size of the spores were measured under microscope with the help of stage and ocular micrometer and the length and breadth measurements of different fungal spores were recorded. Later fungal cultures were sent to National Centre of Fungal Taxonomy, Inderpuri, Near Pusa Institute, New Delhi 110012 for confirmation up to species level.

Symptom was studied very carefully on the leaves. At first small and irregular yellowish to brown spots were appeared on the leaves and gradually spots become enlarge. Concentric ring was appeared on the spot. Spots coalesced with each other and appeared as a large spot. Some times in severe case whole leaf become blighted (Fig. 1 and 2).

Pathogen was isolated in PDA media and confirmed by Koch's postulates.

The pathogen was grown on PDA medium from infected leaf and identified as *Colletotrichum* sp. on the basis of cultural and morphometric characters. On PDA media the colony of the pathogen was whitish thin thread like mycelial growth appears from the centre and gradually it turns to greenish colour. At last greenish black colour growth are formed on the media and whitish cottony over growth of the pathogen were also found (Fig. 3). Conidia of the fungus were hyaline and cylindrical in shape and spore size ranging from 16.45-20.53 × 6.04-7.17 µm (Fig. 4 and 5). The confirmation of the pathogen was done from National Centre of Fungal Taxonomy, New Delhi (Identification No. 6646.15). The records showed that this disease has not yet been reported from any other else. Therefore, this is the first report of *Colletotrichum gloeosporioides* on *Withania somnifera* Dunal from West Bengal.

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