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RESEARCH ARTICLE

FIRST REPORT OF LEAF SPOT (*ALTERNARIA ALTERNATA*) ON SWERTIA CHIRAYITA – A CRITICALLY ENDANGERED MEDICINAL PLANT OF SUB-HIMALAYAN ZONE

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ABSTRACT

Swertia chirayita (Roxb.ex Flem.) Kart (Family: Gentianaceae), is a high value endangered medicinal plant used in traditional therapies. The species is distributed in temperate Himalayas at an altitude of 1200-1500 m amsl. The dried herbage is the source of raw drug. The species is valued for its bitter principles as hepatic stimulant, blood purifier, tonic etc. The bitterness, antihelmintic, hypoglycemic and antipyretic properties are attributed to amarogentin, swerchirin, swertiamarin and other active principles of the herb (Chassot, 2000). The species is on the verge of extinction due to massive unscientific exploitation from its wild natural areas. Considering the demand and importance of genuine chirayita, work on exploration, domestication and development of cultural practices and chemical profiling of *S. chirayita* has been initiated. *S. chirayita* suffers from many diseases like seedling blight, Leaf blight, Leaf spot etc. Among them, leaf spot is an important disease. It becomes severe in major chirata growing area of Darjeeling district. Efforts were made to identify and isolate the harmful pathogen. Hence, laboratory experiments were conducted to detect and identify the causative pathogen on leaves of *Swertia chirayita* collected from naturally contaminated commercial plants. Identification of the pathogen was done on the basis of morphological parameters. Koch's postulates confirmed the presence of *Alternaria alternata*.

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INTRODUCTION

Chirata (*Swertia chirayita*) belongs to the family gentiniaceae, a potential medicinal plant used as bitter tonic in treatment of fever and for curing various skin diseases, is a perennial herb and distributed throughout the eastern Himalayan region of India. The plant is a native of temperate Himalayas, found at an altitude of 1200–3000 m (4000 to 10,000 ft), from Kashmir to Bhutan and in the Khasi hills at 1200–1500 m (4000 to 5000 ft). It can be grown in sub-temperate regions between 1500 and 2100 m altitudes. The bitterness, antihelmintic, hypoglycemic and antipyretic properties are attributed to amarogentin (most bitter compound isolated till date) 1, swerchirin, swertiamarin and other active principles of the herb [2]. Chirata plants have been so exploited that many of the species are now extinct and a very many are endemic [4, 5]. Due to the high demand, this plant is being cultivated in many places of Darjeeling district both for domestic use and for exporting purpose. But leaf spot disease becomes a major problem in cultivation of *Swertia chirayita*.

The quality of plant is deteriorated due to the occurrences of this disease. *S. chirayita* suffers from many diseases like seedling blight, Leaf blight etc. Among them, leaf spot is an important disease. It is severe in all the chirata growing area of Darjeeling district. Hence, efforts were made to identify and isolate the harmful pathogen. Finally, the pathogen was identified *Alternaria alternata*. Hence, the present investigation deals with the detection and identification of the pathogen *Alternaria alternata* from the infected *Swertia chirayita* plant.

Sample Collection

The infected leaves showing the typical symptoms of leaf spot were collected during 2011-13 from farmer's field of major chirata growing areas of Darjeeling district in West Bengal. The collected samples were transported to plant health clinic (PHC) in fresh condition in plastic bags and stored at 28^oC for 24 h for further analysis. All the leaves showed typical external symptoms like yellowish brown spot, decolouration, cankers lesions and irregular yellow and brown patches (Fig.1).

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Isolation of *Alternaria alternata* from infected *Swertia chirayita* leaves

Leaves of chirata showing typical symptoms of leaf spot caused by *Alternaria alternata* were collected during 2011-13 from major chirata growing areas of Darjeeling district. The fungus was isolated by taking small pieces of infected leaves were cut aseptically from the edge of typical spots with a little portion of healthy tissue. The infected leaf bits were surface sterilized in 70% alcohol or 1% sodium hypochloride and washed in three series of sterile water to remove traces of alcohol. The infected leaf bits, after slightly air drying, were poured onto the sterilized petriplates containing PDA media. The inoculated plates were incubated at 30°C for 72 hours. Observations were made for development of mycelia growth on PDA media.

Purification of Fungal Culture

The suspected fungal colonies were picked up with the help of sterilized inoculating needle and place onto the surface of sterilized petriplate containing PDA media. The inoculated plates were incubated at 30°C for 48 to 72 hours and the observation made for the development of fungal colonies. The purified fungal colonies were placed on PDA slants and store at 5°C in refrigerator for future use.

Identification of Causal organism

The morphological characteristics such as Mycelial growth rate, Shape, Size and colour of Conidia and Conidiophore were studied [3].

Pathogenicity on *Swertia chirayita*

Pathogenicity test was carried out to find out whether the isolated fungal culture was capable of producing typical symptoms of leaf spot under artificial inoculation condition on chirata plants or not. Pathogenicity tests were carried out three times on 6-month-old plants (n = 10). Plants were sprayed with a conidial suspension of 10⁶ conidia/ml; control plants were sprayed with sterilized water. Plants were covered with polyethylene bags for 10 days. Observations were made for the development of symptoms of leaf spot. The isolated and re-isolated fungal culture was compared with the original culture of *Alternaria alternata* by studying colony morphology, colour and characters.

RESULTS

Isolation of *Alternaria alternata* from infected *Swertia chirayita* leaves

The pathogen was isolated on potato dextrose agar (PDA) media. On PDA, the fungus grew slowly with colonies reaching approximately 35 to 40 mm in diameter in 7 days when incubated at 30°C.

Purification of Fungal Culture

Well separated out colonies isolated from the infected leaves were purified by re-culturing on the surface of PDA media.

The culture was stored on PDA slants at 5°C. These were kept as the stock cultures for further studies.

Identification of Causal organism

The pathogen was isolated on potato dextrose agar (PDA) media. On PDA, the fungus grew slowly with colonies reaching approximately 35 to 40 mm in diameter in 7 days when incubated at 30°C. Conidiophores arose singly or in groups, straight or flexuous, cylindrical, septate, pale to olivaceous brown, as much as 155 µm long, 4 to 5.5 µm thick; conidia were straight, obclavate, pale olivaceous brown, smooth, with up to 15 transverse and rarely 1 or 2 longitudinal or oblique septa and measured 50 to 115 × 5 to 10 µm (Fig.2.).



Fig. 1. Leaf Spot of *Swertia chirayita*



Fig. 2. Conidia of *Alternaria alternata*

Pathogenicity on *Swertia chirayita*

The conidia of *Alternaria alternata* artificially inoculated to 6-month chirata plants. It started producing small, light brown spots, gradually becoming irregular, dark brown, concentrically zonate with a diffuse margin, frequently surrounded by light yellow haloes, conspicuous brownish concentric rings in the advance stage of infection on the

leaves. The lesion becomes necrotic leading to blighting of infected leaves. The first symptoms of the disease observed 15 days after inoculation. Symptoms on the leaves were similar to those of a naturally occurring diseased plant. The fungal pathogen was consistently reisolated from inoculated plants. The pathogen was identified as *Alternaria alternata* on the basis of morphological characters

DISCUSSION

The chirata plant is severely affected by fungal pathogen. It has become more serious in chirata growing areas of Darjeeling district as these medicinal plants cultivation is getting popular among the hill farmers. A new seedling blight disease of *Swertia Chirayita* (Roxb. ex Flem.) Karst. incited by *Rhizoctonia Solani* Kuhn was reported from Shilong, Meghalaya [1]. A critical problem in the study of fungal pathogen is the correct identification of the infectious agent. The study of diseases in medicinal plants is an important aspect of assessing plant health. Hence, in the present investigation the identification and isolation of pathogen from infected plants of *Swertia chirayita* has been demonstrated for the first time in West Bengal as well as India.

Conclusion

The experiments confirmed the presence of *Alternaria alternata* as causative pathogen for leaf spot disease in the infected leaves of *Swertia chirayita*. This result also indicates that the chirata plants grown in Darjeeling district of west Bengal is highly infected by the fungus.

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