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**RESEARCH ARTICLE**

**FIRST REPORT OF LEAF BLIGHT (CLADOSPORIUM TENUISSIMUM COOKE) ON SWERTIA CHIRAYITA –A CRITICALLY ENDANGERED MEDICINAL PLANT OF SUB-HIMALAYAN ZONE**

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**ABSTRACT**

A new disease in Chirata (*Swertia chirayita*) characterized by circular or irregular reddish brown leaf spots that become tan to brown with age, pale greenish-yellow spots on the upper leaf surface that correspond with the spots on the lower leaf surface, and coalesced lesions that form larger necrotic areas. Based on cultural characters the pathogen was identified as *Cladosporium tenuissimum*. Upon conducting pathogenicity tests, the fungus was reisolated from the inoculated leaves. This is thought to be the first report of the leaf blight disease affecting both cultivated and non-cultivated chirata in India.

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**INTRODUCTION**

*Swertia chirayita* (Roxb.ex Flem.) Kart (Family: Gentianaceae), commonly known as *chirayita* or *kirata tikta* or *bhunimba* 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 from Kashmir to Bhutan and in the Khasi hills in Meghalaya. 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 (most bitter compound isolated till date) 1, 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. The situation is so alarming that it

has been classified as critically endangered plant species and has been put in negative export list of Ministry of Commerce, GOI. This is one of the 32 species short listed by NMPB for extensive cultivation and research. Most of the domestic demand is met from imports from Nepal and other areas. Due to overexploitation of this species and Low germination percentage, viability of seeds, long gestation periods and delicate field-handling are some of the factors which discourage commercial cultivation and created lot of pressure on its natural resources which has led to considerable depletion of it stock. Due to lack of genuine raw material of this species and great demand from industries, substitute species like *S. angustifolia*, *S. paniculata*, etc. and even *Andrographis paniculata* are traded in the name of *S. chirayita*. 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

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initiated at AICRP on MAP&B Kalimpong on 2006 under the aegis of Uttarbanga Krishi Viswa Vidyalyaya. Commercial cultivation of Chirata is being initiated in many places of Darjeeling district both for domestic and for exporting purpose. But leaf blight disease becomes a major in cultivation of *Swertia chirayita*. The quality of plant is affected due to the occurrences of this disease as whole plants part being used as medicinal purpose. *S.chirayita* suffers from many diseases like seedling blight, Leaf blight etc. Among them, leaf blight 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. Finally, the pathogen was identified *Cladosporium tenuissimum* Cooke.

Hence, the present investigation deals with the detection and identification of the pathogen *Cladosporium tenuissimum* from the infected *Swertia chirayita* plant.

## MATERIALS AND METHODS

### Sample Collection

The infected leaves showing the typical symptoms of leaf blight were collected. The collected samples were transported to plant health clinic (PHC) in fresh condition in plastic bags and stored at 22°C for 24 h for further analysis. All the leaves showed typical external symptoms like yellowish brown spot, blighting from the tip of the leaf and irregular yellow and brown patches(Fig.1).



Fig.1 Leaf Blight disease of *Swertia*

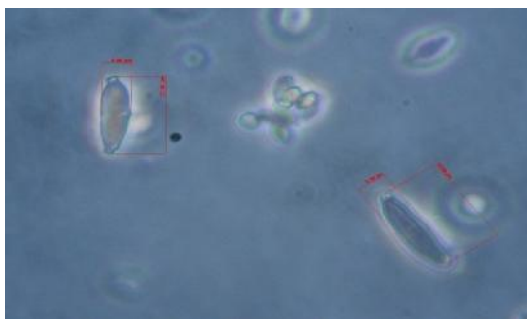


Fig.2 Conidia of *Cladosporium tenuissimum*

### Isolation of *Cladosporium tenuissimum* from infected *Swertia chirayita* leaves

Leaves of chirata showing typical symptoms of leaf blight caused by *Cladosporium tenuissimum* were collected during 2012-13 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 slighty air drying, were poured onto the sterilized petriplates containing PDA media. The inoculated plates were incubated at 22°C for 72hours. 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 22°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 colony appearance, texture and morphology, growth/temperature relationships and its micro-morphology (type of conidia and conidiogenesis) were studied.

### 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 blight 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 10<sup>6</sup> 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 *Cladosporium tenuissimum* by studying colony morphology, colour and characters.

## RESULTS AND DISCUSSION

### Isolation of *Cladosporium tenuissimum* from infected *Swertia chirayita* leaves

The pathogen was isolated on potato dextrose agar (PDA) media. On PDA, the fungus grew fast with colonies reaching approximately 70 to 80 mm in diameter in 7 days when incubated at 22°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. it was very pale and greyish. The pathogen produced tall, dark, and upright conidiophores that may branch near the top. Conidiophores were very long, often up to 800  $\mu$  or more, straight or slightly curved, mid to dark brown, smooth, 3-6  $\mu$  thick, not swollen or only slightly and unilaterally swollen at the apex and without the swollen nodes characteristic of the closely related *C. oxysporum*. Conidia were catenate, cylindrical, ellipsoidal, limoniform or subspherical, very much variable in size as well as shape with well-defined scars, 0- to 2-septate, pale or very olivaceous brown, smooth or occasionally minutely verruculose, 3-25micron x 3-6 micron. Colour of the conidia was Pale or very Olivaceous brown (Fig.2). The pathogen was identified as *Cladosporium tenuissimum* Cooke on the basis of morphological characters.

### Pathogenicity on *Swertia chirayita*

The conidia of *Cladosporium tenuissimum* artificially inoculated to 6-month chirata plants. 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 re-isolated from inoculated plants. It started producing small, light brown spots, gradually becoming irregular, dark brown, with a diffuse margin, in the advance stage of infection on the leaves become wither and shrivel. The lesion become necrotic leading to blighting of infected leaves.

The dematiaceous hyphomycete *Cladosporium tenuissimum* has long been known as a polyphagous saprophyte occurring in the air, on the soil and on plant surfaces (Ellis, 1976). It is a frequent colonizer of senescent or dead plant material and is common on the phyllosphere and rhizosphere of plant species, but it is also reported as an endophyte (Fisher and Petrini, 1992) and a facultative plant pathogen, causing blights, leaf spots, and seed, fruit and blossom-end rots (Pandey and Gupta, 1983; Xiang *et al.*, 1989; Dohroo and Sharma, 1992; Sharma and Majumdar, 1993; Fujii *et al.*, 1995; Dhal *et al.*, 1997; Moricca *et al.*, 1999).

It is observed that the chirata plant is severely affected by fungal pathogen. Review of literature on diseases of chirata yielded only one disease reported till date, i.e. Seedling blight disease of *Swertia Chirayita* (Roxb. ex Flem.) Karst. incited by *Rhizoctonia Solani* Kuhn by T.K.Bag (2005) from Shilong, Meghalaya, India. This means that information on the diseases of Chirata in particular is scanty. The study of diseases in medicinal plants is an important aspect of assessing plant health and excels the production technology for greater benefit of farming community. However, a critical problem in the study of fungal pathogen is the correct identification of the infectious agent. 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 *Cladosporium tenuissimum* as causative pathogen for leaf blight disease in the infected leaves of *Swertia chirayita*. This result also indicate that the chirata plants grown in Darjeeling district of west Bengal is highly infected by the fungal pathogen.

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