



Study of fungal diseases on *Swertia chirayita* from cultivated fields of Dolakha district, central Nepal

Rabindra Thapa and Sanjay Kumar Jha*

Central Department of Botany, Tribhuvan University, Kirtipur, Kathmandu, Nepal

*E-mail: sanjay.jha@cdb.tu.edu.np

Abstract

Swertia chirayita is a medicinally and economically important herb of the family Gentinaceae. It is one of the highly traded, traditionally important medicinal plants. Fungal Pathogens corrupt the quality and amount of *Swertia chirayita* development, and production and cause terrible well-being of plants as well as monetary problem to the traders. This study was carried out to identify some diseases associated with *S. chirayita* cultivated fields in Bigu, Khartal, and Boch regions of Dolakha district. The samples were collected from selected sites and cultured on Potato Dextrose Agar media for fungal pathogens. During the investigation, five species of fungal pathogens were identified. *Alternaria alternata*, *Colletotrichum gleosporioides* and *Curvularia* sp. were isolated from leaf. They caused *Alternaria* leaf spot, *Colletotrichum* leaf blight, and *Curvularia* leaf spot. Similarly, stem possesses canker disease and root had root canker, *Fusarium oxysporum* causing *Fusarium* wilt, *Rhizoctonia* sp. causing *Rhizoctonia* root rot. The presence of illnesses in *S. chirayita* shows the need for proper plant care.

Keywords: *Alternaria* leaf spot, fungal pathogens, Gentinaceae, poison food technique, traders

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Introduction

Swertia chirayita is a Himalayan herb. It is a widely used medicinal plant of the family Gentinaceae; has ethnomedicinal, historical, environmental, ethnomedicinal, and phytochemical properties (Barakoti, 2004). It is an annual/ biennial herb of 0.6-1.5m tall; generally flowering from August to October having greenish-yellow flowers, leafy panicles, and numerous seeds (Chandra *et al.*, 2012). It has an ideal elevation of 600-5600m asl. The plant is reported to contain potent bitter compounds like chiratin and ophelic acid (Sultana *et al.*, 2011) and plenty of 'amelogenin' (the bitterest compound recorded to the date (Joshi and Dhawan, 2005). *S. chirayita* plays bioactive functions like hepatoprotective, digestive, astringent, laxative, anti-inflammatory, and anti-malarial with its bioactive like Xanthenes (Tabassum *et al.*, 2012).

The greater parts of the rural population are reliant upon *Swertia* sp. for collection and trade. They also use it for therapeutic purposes. Among 100 species of *Swertia* around the world, Nepal exports about 31 sps. with 5 varieties having wide distribution in mountains and terrains of Eastern, Western, and Central Nepal (Joshi, 2008). The Trade of *S. chirayita* has a very long history in different places in China, India, and Nepal. 61 districts out of 77 districts in Nepal are involved in the trade of medicinal plants with an account of 3% of trade being occupied by the trade of *S. chirayita* (Phoboo and Jha, 2010). In recent days, due to biological and physical causes, quality and quantity losses are prevalent in *S. chirayita* dropping production from 711 metric tons per year to 503.25 Mt. / yr. during 2013 to 2014 AD while the price dropped from Nrs. 750 per Kg to 250 per Kg (Cunningham *et al.*, 2018).

Alternaria leaf spot was reported multiple times on *S. chirayita* leaves (Baskey *et al.*, 2016b; Chandel *et al.*, 2014; Yadav and Negi, 2016). Similarly blight by *Phyllosticata* sp., *Colletotrichum* sp. was reported by Chandel *et al.* (2014). In India, *Cladosporium tenuissium* Cooke caused pale greenish-yellow spots on the upper surface of *S. chirayita* leaves (Baskey *et al.*, 2016a) along with other foliar pathogens like *Septoria* sp., *Cercospora* sp., and *Curvularia* sp. Such illnesses cause quality and quantity losses of plants.

Materials and Methods

Study Area

The study area was Bigu, Khartal, and Boch in Dolakha district. The cultivated lands of *Swertia chirayita* were visited for the study. The total area of this district is 2.191 km² and is located between 27.7784° and 86.1752°.

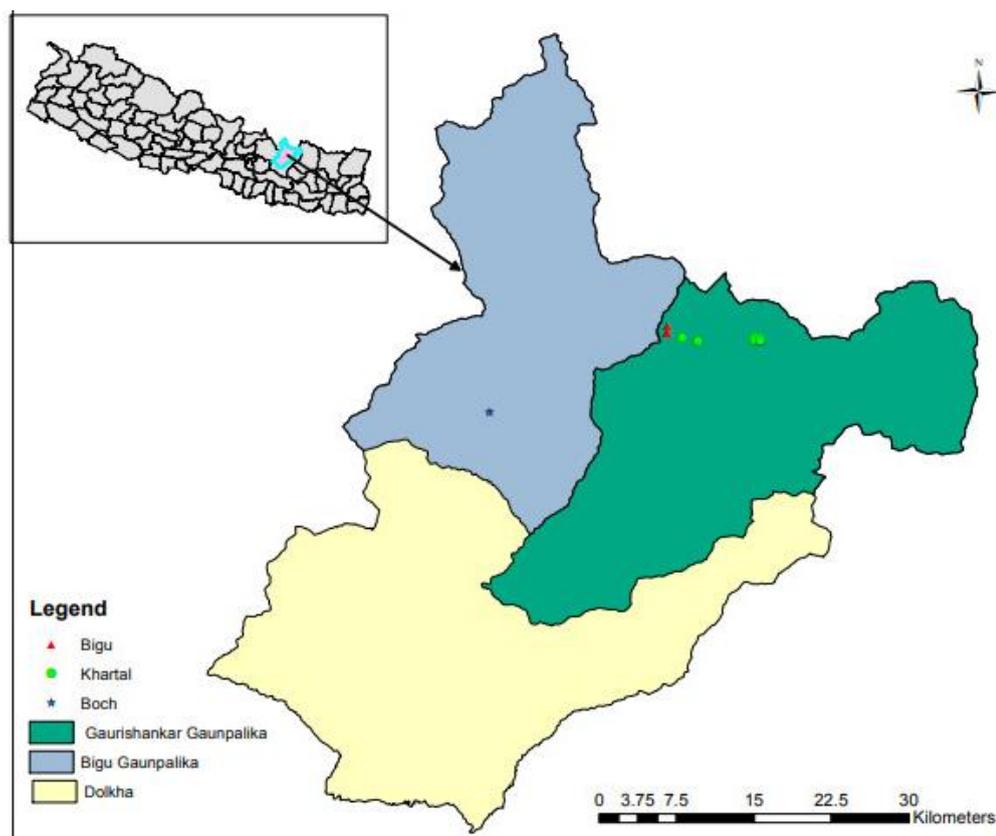


Figure 1. Map of the study area.

Collection of samples

The field visit was done in the month of March/April in the year 2019. Plants parts were collected by purposive sampling technique using surface-sterilized forceps (using 70% ethanol). Samples collection was done after observing symptoms, tagged, and kept in zip bags. The wilted plants and the plants with symptoms of root diseases were uprooted with soil. Laboratory analysis for root disease pathogen identification. Samples were collected from the field. The photographs were taken on the spot (Figure 4) and brought to the Central Department of Botany, Plant Pathology Unit Laboratory, Tribhuvan University, Nepal.

Laboratory Analysis

Culturing

The sterilized Petri plates were poured with sterilized PDA media and kept in an incubator for 7 days after placing 2-3 mm of surface-sterilized (using 70%) ethanol plant pieces. The plates were sealed with paraffin tapes and kept upside-down position.

Sub-Culture

The seven days old fungal culture was taken out of the incubator and transferred a piece (using cork borer) for subculture into three fresh PDA plates. The plates were sealed and again placed

in an incubator at 25±2°C for 5-6 days. The subculture plates were photographed and the culture was used to identify the pathogens and pathogenicity test.

Identification of Pathogen and pathogenicity test

The slides of sub-cultured pathogens were prepared by cellophane technique using lactophenol cotton blue. It was observed under a digital compound microscope and identified

concerning physical and morphological characteristics. Various literature (Barnett and Hunter, 1972), expertise, online databases, etc. were used to identify the pathogens. The pathogen's conidial size or mycelial size was measured and photographed. The stem and root canker disease were just recorded as per observation and no borer insect was present on the infected part during collection time. A pathogenicity test was done on potted plants following Koch's Postulates.

Results

Five fungal pathogens namely *Alternaria alternata*, *Colletotrichum gleosporioides*, *Fusarium oxysporum*, *Curvularia* sp. *Rhizoctonia* sp. was identified from the collected samples. The former four were found to be pathogenic to the leaf of *S. chirayita* while the latter one was a root pathogen. The symptoms were somehow similar in the case of leaf pathogens while observing but the infection part, rate of infections, and key major signs and symptoms on close observation were different (Table 1 and 2). Four species of pathogens (*Alternaria alternata*, *Colletotrichum*

gleosporioides, *Fusarium oxysporum*, and *Curvularia* sp.) were obtained in all three sites and while *Rhizoctonia* sp. was collected from the Boch area only. The entire cultivated field possessed spread diseases in every part of the plants. Each fungal pathogen varied with morphological and cultural characteristics (Figures 2 and 3). The insect pathogen on the stem was recorded from all three areas and root canker was reported from the storehouse of Chirayita plants in the Boch region only. Boch region had more diseases than Khartal and Bigu areas (Table 3).

Table1. Symptoms of Diseases caused by listed pathogens.

S.N.	Diseases	Disease symptoms
1.	<i>Alternaria</i> leaf spot	<i>Alternaria</i> leaf spot was characterized by dark brown spherical leaf spots spread over the infected leaves. The lesion finally collapses the entire leaf surface.
2.	<i>Colletotrichum</i> blight	Small chlorotic spots appear on leaf tips. Spots enlarge rapidly and coalesced, turning into brown patches. Thus, finally, it covers the entire leaf surface.
3.	<i>Fusarium</i> wilt	The symptoms were clear on older plants with the falling of an older leaf. Mid- age plant was stunted and the plant finally dies due to wilting. The stem on cutting shows a blockade of the vascular part. The plant finally dies.
4.	<i>Rhizoctonia</i> root rot	In the affected plant withering and dropping off can be observed. The main root of the plant underneath becomes fragmented into threads structure. The plant wilts and the upper parts dry. Specifically, seedlings dry and die.
5.	<i>Curvularia</i> leaf spot	Small tan lesions appear on leaves. The spots are surrounded by a yellow halo on the upper leaves' surface. It gradually increases with time; firstly scatters on the leaf surface and later covers with a yellowish surface of the leaf.

Table 2. List of pathogens and their characteristics.

S.N.	Name of Pathogen	Morphological characteristics
1.	<i>Alternaria alternata</i>	Dark ash colored at the center and the fluffy margin appeared, the conidia were pale brown, obclavate to obpyriform or ellipsoid, short conical beak at the tip or beakless, surface smooth to verruculose. The hyphae septed, branched arise singly or in the group. The conidia size was 28.82-49.82 $\mu\text{m} \times 8.12$ -15.23 μm . It is cosmopolitan in nature found in food, soil, and water.
2.	<i>Colletotrichum gleosporioides</i>	The colony color was pale orange color towards the center and cottony white on the edge that becomes colorless at mature with hyaline cylindrical conidia rounded on both ends. <i>C. gleosporioides</i> produces colonies and is formed typically cylindrical conidia with rounded tips on PDA media. The colony was sparse and cottony with conidia ranging 9.55-18.65 $\mu\text{m} \times 3.24$ -6.65 μm in size with cylindrical broadly rounded ends. The pathogen is found in crops and mostly perennial plants of tropical areas.
3.	<i>Fusarium oxysporum</i>	The coloration is initially white later becomes purple/pink with discrete orange sporodochia (mass of hyphae) present in some strains. The aerial mycelium in sparse to dense and floccose appears as cottony white colonies with enormous conidia production. The conidiophores are short and bear micro and macroconidia. The microconidia are curved, oval, ellipsoid cylindrical, aseptate, or singly septate and measure 6-13 $\mu\text{m} \times 2.5$ -5.1 μm . The macroconidia were born on more elaborate branched conidiophores which are thin-walled, 3-septated, and sickle-shaped and pointed at both ends measuring 27.45-49.5 $\mu\text{m} \times 3.35$ -5.65 μm . The pathogen is ubiquitous soil inhabiting and also exists as a saprophyte.
4.	<i>Rhizoctonia</i> sp.	On PDA, <i>Rhizoctonia</i> colony varies with color appearance as per the stage of development. It appeared as a whitish colony firstly and on maturation turns brownish-black colored cottony colony and submerged over the media. Under the microscope, it is observed with numerous branching is characterized by the right-angled branching of hyphal branches, Thread-like hyphae are highly interlocked with each other with numerous black scleroital masses having spherical and other various irregular shapes. The hyphal thread measured 90.51 μm -161.13 $\mu\text{m} \times 4.13$ μm -5.12 μm in length. The pathogen is saprophytic soil fungi with a cosmopolitan nature of the distribution.
5.	<i>Curvularia</i> sp.	The fungi have velvety black and fluffy colony growth on PDA media. The colony previously is white and later changes color with fluffy, cottony raised on media surface having entire margin. Conidia are black, septed, and have an intermediate broad appearance between both ends. They are curved slightly distinct or indistinct; much is transversely septed. Conidia measures 11.34 μm -15.55 $\mu\text{m} \times 7.5$ μm -13.1 μm in size. It is dark-colored facultative fungi prevalent in plants and cereals around varied environments.

On stems of *S. chirayita* long incisions like damages were noted stems of wilted and dead

plants. Stem borer characteristics were noted in the plants. Similarly, stored and dried plants

had deep holes and other n roots too. The symptoms and signs indicate it to be the insect disease but no insects were collected from infected

parts. Both diseases were clearly visible with canker-like hollow plant parts (Figures 4F and G).

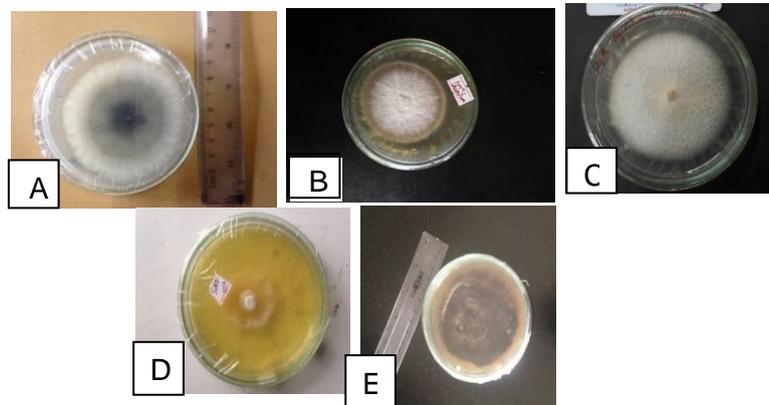


Figure 2. Pure culture of fungal pathogens; (A) *Alternaria alternata*; (B) *Fusarium oxysporum*; (C) *Colletotrichum gloeosporioides*; (D) *Rhizoctonia* sp.; (E) *Curvularia* sp

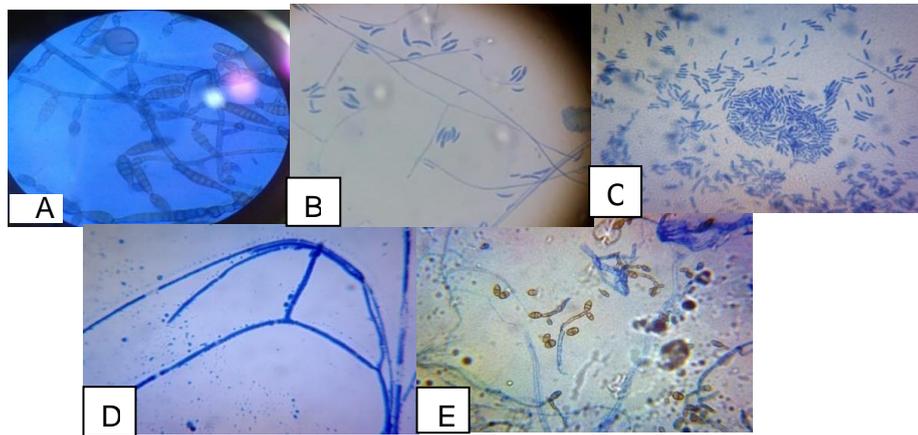


Figure 3. Microscopic view of fungal pathogens; (A) *Alternaria alternata*; (B) *Fusarium oxysporum*; (C) *Colletotrichum gloeosporioides*; (D) *Rhizoctonia* sp.; (E) *Curvularia* sp

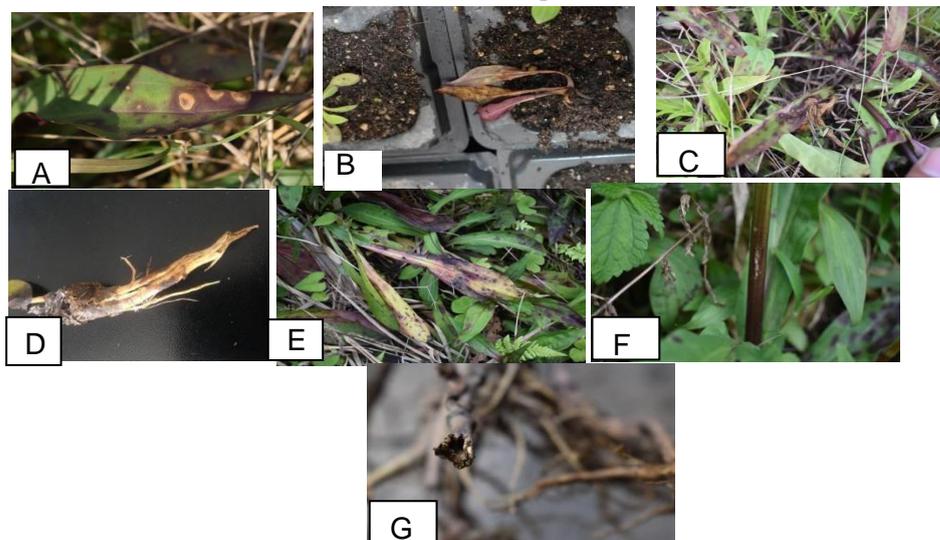


Figure 4. Field photos of Fungal and insects symptoms (A) *Alternaria* leaf spot; (B) *Fusarium*

oxysporum; (C) *Colletotrichum* leaf blight; (D) *Rhizoctonia* Root rot; (E) *Curvularia* leaf spot; (F) Stem borer Disease; (G) Root borer Disease

Table 3. Fungal species, present sites, and corresponding diseases.

S.N.	Fungal Pathogens	Site I (Khartal)	Site II (Bigu)	Site III (Boch)	Diseases
1.	<i>Alternaria alternata</i>	+	+	+	<i>Alternaria</i> leaf spot
2.	<i>Colletotrichum gleosporioides</i>	+	+	+	<i>Colletotrichum</i> leaf blight
3.	<i>Fusarium oxysporum</i>	+	+	+	<i>Fusarium</i> wilt
4.	<i>Curvularia</i> sp.	+	+	+	<i>Curvularia</i> leaf spot
5.	<i>Rhizoctonia</i> sp.	-	-	+	<i>Rhizoctonia</i> root rot
6.	<i>Stem borer</i>	+	+	+	Stem bore
7.	<i>Root borer</i>	-	-	+	Root bore
Total number of diseases		5	5	7	
Percentage of Diseases		71.42%	71.42%	100%	

Discussion

Swertia chirayita plant was found to be infected by different fungal pathogens and insects in the field due to unmanaged cultivation and poor sanitation in the field. Pathogen occurrence and insect invasion depend on the environment where the plant grows and the conditions for the pathogens to flourish. The majority of the plant illnesses are favored by a cool climate, light and successive downpours, mist or weighty dews, high dampness, and swarmed or obscure plantings (Pataky, 1998). The cultivated fields in selected areas of Dolakha districts also possess a cool climate and temperature; shades of trees and poorly sanitized fields which were responsible for the occurrence of plant diseases.

Alternaria leaf spot (Figure 4A) has been found to be the most abundant disease of *S. chirayita* and has been reported by various researchers (Baskey *et al.*, 2016b; Chandel *et al.*, 2014; Yadav and Negi, 2016). It is responsible for the degradation of leaf quality thus resulting in leaves with various circular and concentric spots. Similarly, *Colletotrichum gleosporioides* causing leaf blight (Figure 4C) was also supported by Chandel *et al.* (2014). The pathogen is also responsible for leaf disease being patchy and altered colored in the field. *Curvularia* leaf spots appeared as small tan lesions on leaves surrounded by yellow hollow that firstly scatters on the leaf surface and later covers with the yellowish leaf surface. *Rhizoctonia* sp. was found to be causing root rot of *S. chirayita* due to which plant dies too early and cannot live more. Bag (2005) in his paper

mentioned *Rhizoctonia solani* causing a seedling blight on plantlets of *S. chirayita* plants. A report published by the Department of Plant Resources, 2015 has mentioned *Fusarium oxysporum* as a pathogen causing plant wilting of plants (Figure 4B) including *S. chirayita*, and ultimately die. These pathogens have been a major topic of concern that causes economic losses in wide varieties of crops (Okungbowa and Shittu, 2012).

Regarding the insect diseases, the internodal regions on the stem of the plant were straightly bored by an insect (about 3-4 cm long). Insects feed on the inner plant stem making it hollow with few exit holes. The affected parts have remained of insect excretes. Similarly, dry and stored *S. chirayita* plants were found to be having hollow holes on the main roots of the plant (1-2 cm long). No insect pathogens were present on it but the symptoms show the occurrence of diseases to be caused by insects. These might be caused due to short lifecycle of plant-feeding insects.

In spite of all, the study finds out the prevalence of diseases in the cultivated field of *S. chiraiyata* around Dolakha district, Central Nepal. Proper initiatives should be taken in order to manage and control the diseases in order to enhance the quality and quantity grade of the plant which can be helpful to increase the economic as well as medicinal value of the plant.

Conclusion

Most of the fungal pathogens were isolated from the infected plants from *Swertia chirayita* plant around the study areas. The cultivated fields being unmanaged with weeds and other plants are more prevalent with the diseases. Proper manuring and care must be done by the farmers in order to grow the plants properly. This study thus might be helpful to create awareness and buildup a concrete strategy in order to grow more Chiraito plants and export them abroad too. So this study might be a base for upcoming research in this field.

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