IDENTIFICATION OF FUNGAL DISEASES OF *Swertia chirayita* OF SANKHUWASABHA DISTRICT

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ABSTRACT

Swertia chirayita is one of the important medicinal and highly traded plant for commercial purpose. However, its production has significantly declined in recent days due to incidence of various diseases. Unfortunately, the knowledge on disease and its causative agent is insufficient. Therefore, the present study focuses on incidence and severity of disease and identification of causative agents. The disease incidence of plant of study cultivated field of Sankhuwasabha district was 53.20% and the disease severity was 23.87%. Leaf spot disease caused by *Alternaria alternata, Botrytis cinera* and *Bipolaris sorokiniana,* leaf blight caused by *Colletotrichum gloeosporioides* and *Fusarium* wilt by *Fusarium oxysporum* and rot disease caused by *Trichoderma harzianium* were identified in *Swertia chirayita*.

Keywords: Disease incidence, disease severity, medicinal plant, pathogens

INTRODUCTION

Swertia chirayita (Chiraito) is acritically endangered medicinal herb that grows at high altitudes between 1200 and 3600m altitudes (Negi *et al*., 2011) on the slopes of moist shady places considered to be superior in its medicinal properties in comparison to other species of *Swertia* (Rijal, 2010). *S. chirayita* is an annual/biennial herb 0.6–1.5m tall (Kumar & Staden, 2016). The leaves are lanceolate, root is simple, tapering, stout and short and flowers are tiny, stalked, green-yellow, and purple-tinged (Chandra *et al.,* 2012). It prefers to grow on moist shady places and forms a colony with other plants and grows on acidic soil condition with pH 4.7-5.5 (Bhattarai & Shrestha, 1996). In Nepal, it is widely distributed from tropical to alpine habitat of eastern, central and western regions (Joshi & Joshi, 2008).

The bitter taste of this ethno-medicinal herb is due to the presence of many chemical elements such as amarogentin, swerchirin, swertiamarin, and other bioactive substances that are directly linked to human health (Joshi & Dhawan, 2005). It is a traditional Ayurvedic herb, is used for medical purposes by numerous indigenous ethnic groups. Locals regularly use the entire plant tocure hepatitis, inflammation, and digestive issues (Bhatt *et al*., 2006). Chronic fever, malaria,anemia, bronchial asthma, hepatotoxic disorders, liver disorders, hepatitis, gastritis, constipation, dyspepsia, skin diseases, worms, epilepsy, ulcers, scanty urine, hypertension, melancholia, and certain types of mental disorders are among the many medicinal uses, as are bile secretion, blood purification, and diabetes (Shivaji *et al.,* 2000; Saha *et al.,* 2004; Chen *et al.,* 2011). Anti-hepatitis B virus (anti-HBV) activities have recently been discovered in its extracts (Zhou *et al*., 2015). Ayush-64, Diabecon, Mensturyl syrup, and Melicon V ointment are examples of herbalproducts (Edwin & Chungath, 1988; Mitra *et al.,* 1996) that use varied concentrations of its extract for antipyretic, hypoglycaemic, antifungal, and antibacterial effects. This herb's medicinal value has also been reported in ancient Ayurvedic medicine and other traditional medical systems. In the world market, Nepal supplies 45 percent of the dried Chiraito. In the fiscal year 2073/74, the government of Nepal received NRs 2,31,640 in revenue by providing collection permissions to various Distict Forest Officers (DFOs). The Government of Nepal (GON) collected roughly US$ 61,000 in Chiraito royalties from all districts' DFOs (DOF, 2016).

Plant disease is the most significant limitation to agricultural crop output. Fungal and bacterial illnesses are the most common in Nepal. The pest detection survey on *S. chirayita* conducted by DPR in 2015 at Ilam district found 11 fungal species *Fusarium solani, F. oxysporum, F. moniliformae, Alternaria alternata, Bipolaris sorokiana, Verticillium arbo-artum, Colletotrichum gloeosporioides, Trichoderma harzianum, Geotrichum candidum* and *Verticillium* sp. cause different diseases in plant. Bacteria such as *Agrobacterium tumefaciens* and several type of stem borers caused by insects. There is critical problem due to seedling blight in *S. chirayita* from Shilong, Meghalaya, India which was caused by *Rhizoctonia solani* Kuhn (Bag, 2005 cited in Baskey *et al*., 2016). New disease in *S. 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 was found. The pathogen was identified as *Cladosporium tenuissimum* (Baskey *et al*., 2016). Further, *S. chiraita* suffers from many diseases like seedling blight, leaf spot, leaf blight, etc. due to the presence of *Alternaria alternata* as causative pathogen for leaf spot disease in the infected leaves of *S. chirayita* (Baskey *et al.,* 2018). Medicinal plants are not exception and are vulnerable to the attack of several pathogens resulting crop loss in terms of both quantity and quality. Hence, the present study was conducted to identify the fungal diseases of Swertia chirayita in Sankhuwasabha district, eastern Nepal.

Study area

The experimental site for this study is located in cultivated sites of Madi Gaupalika (Chowki, Mangalbareand Mawadin) of Sankhuwasabha district which is shown in Fig. 1 and tabbulated in Table 1.

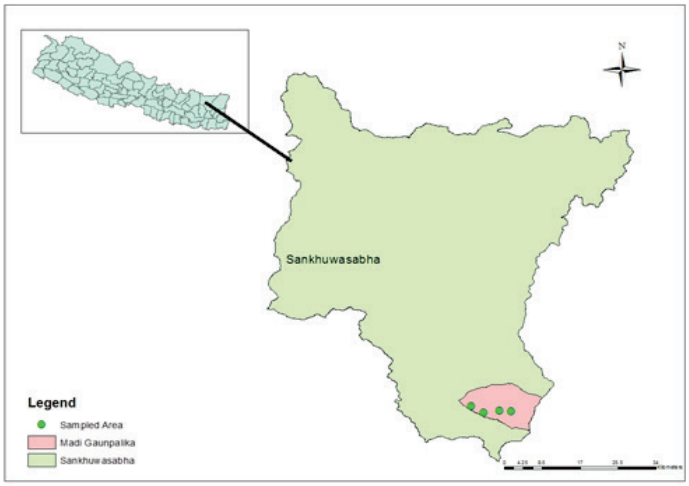

Fig. 1. Map of study area.

Table 1. Description of study area.

Site	Places	Latitude	Longitude	Altitude	Areaof cultivation (Km^2)
I	Mangalbare	$27^0$13.8690'N	$87^0$27.9610'E	2512 m	0.9(1.4 x 0.75)
II	Chowki	$27^0$13.8690'N	$87^0$27.9610'E	2560 m	0.84(1.2 x 0.7)
III	Mawadin	$27^0$16.7463'N	$87^0$25.8907'E	2600 m	1.17(1.3 x 0.9)
IV	Mawadin	$27^0$16.8220'N	$87^0$25.7433'E	2590 m	0.48(0.8 x0.6)

MATERIALS AND METHODS

Collection of samples

Diseased plants were collected and stored under 2-5˚C as soon as possible in ice box. Ventilated bags with dry tissues were used to keep sample to absorb excess moisture.

Isolation

The collected samples were brought to the Central Department of Botany, Tribhuvan University laboratory. Infected plant samples (root, stem, leaves) were cut into 3-5 mm pieces, washed well in sterilized water, surface sterilized using 70% ethanol for 1-2 minutes and then again washed by sterilized water. Sample pieces were blotted to remove excess water. Small portion of the infected plant parts from the center was cut off and inoculated insterilize PDA media on aseptic condition.

Culture

The petri plates containing surface sterilized materiel on sterilized medium were sealed with paraffin tape and incubated in inverted position at 25˚C for 72 hours for growth (Pathak, 1984).

Sub-culture

After 72 hours, the petri plates were taken out from the incubator. Then, they were brought tosub-culture process in which three inoculums dishes were prepared with the help of borer from 3days old culture and transferred to three new petri plates filled with new PDA media separately. The petri plates were sealed with paraffin tapes and kept for incubation at 25˚C for 10 days. Thereafter, pure culture was photographed.

Identification

The fungi were carefully transferred on the cellotape and mounted on the slide containing mixture of lactophenol and cotton blue in orderto stain the material. Thus, prepared slides were examined under digital microscope (Olympus microscope Model No. CX22 Japan). The photographs were taken under immersion oil. The morphological characters of the fungi were studied under high power (10X x 40X). The pathogen were identified with the help of diagnostic morphological characteristics seen under microscope as well as concerning standard literatures (Barnett,1960; Ainsworth *et al*., 1972) and web surfing on online database.

RESULTS AND DISCUSSION

The incidence of disease along with severity and symptoms with identified pathogerns are tabulated in Table 2 and Table 3 respectively. The incidence of disease of *S. chirayita* which was 48.81%, 67.90%, 50.26% and55.83% in the site I, II, III, IV of cultivated field and the disease severity was 19.20%, 22.20%, 26.40% and 25.68% respectively. The mean incidence of disease of that area was 55.70 % and mean severity was 23.37% (Table2).

Table 2. Mean severity and mean disease incidence of different sites in study area.

S.N.	Sites	Mean Incidence (%)	Mean Severity (%)
1	Site I (Mangalbare)	48.81%	19.20%
2	Site II (Chowki)	57.90%	22.20%
3	Site III (Mawadin)	50. 26%	26.40%
4	Site IV (Mawadin)	55.83%	25.68%
Mean		53.2%	23.37%

The survey revealed that the disease incidence and severity were relatively similar in all cultivated field due similar geographical and environmental conditions prevailing in the area. The incidence and severity of the disease in the area may be due to the presence of shade of tree, shrubs and unwanted associated weeds in the fields. These conditions reduce light intensity and increase in humidity that favors the distribution of the pathogen (Gashaw *et al*., 2014). Most common associate reported species of *Swertia chirayita* are: *Fragaria indica*, *Oxalis corniculata*, *Cynodon dactylon*, *Digitaria adecendens*, *Desmodium oxyphyllum*, *Elsholtzia strobilifera*, *Artemesia vulgaris*, *Anemone obtusiloba*, *Bidens* sp, *Eupatorium adenophorum*, *Rhododendron arboretum* and *Acer* sp. (Phoboo & Jha, 2010; Bhatt *et al*., 2007; Sharma *et al*., 2011).These associated species favour the growth of pathogens providing shade to the cultivated plant of the area. Fungi are known as emerging destructive pathogens of agriculturally important crops, causing the loss of billions of euros every year (Gomes *et al*., 2021). The symptoms, affected plant parts, their pathogens and diseases are shown in Table 3.

Sample collection and identification.

Table 3. Symptoms of collected plant sample which cause several disease in *Swertia chirayita* with identified pathogens.

S.N.	Symptoms and affected plant parts	Pathogens	Diseases
1.	Leafspot: Yellowish brown spot, decolouration, and irregular yellow and brown patches.	*Alternaria alternata*	Leafspot
2.	Dark brown irregular lesions, round or irregulargray spots surrounded by dark brown margins.	*Colletotrichum gloeosporioides*	Leafblight
3.	Wilting, decayed of the base of the plant and root and ultimate death	*Fusarium oxysporum*	Root rot
4.	Brown softspot	*Botrytis cinera*	Leafspot
5.	Reddish to purplish spot	*Bipolaris sorokiniana*	Leafspot
6.	Wilted and decayed plant	*Trichoderma harzianum*	*Trichoderma* rot

Identified species

Colletotrichum gleosporioides

Cultural and morphological characteristics:

Colony is flat, filamentous with entire margin. The colony consists of mass of conidia. Colony is whitish, greyish, or creamish colour and cottony, velvet ymycelium on the top side and the colony is pale yellow and at reverse view. The mycelial had uniform radial growth characterised by circulating-like patterns (Fig. 2).

The mycelium of the fungus is hyaline and septate where conidiophores are highly branched. Thecultures produced spores which were straight with rounded end, ranging within 3.0–5.0 μm in width and 10.3–18.2 μm in length.


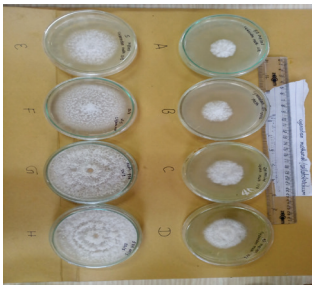
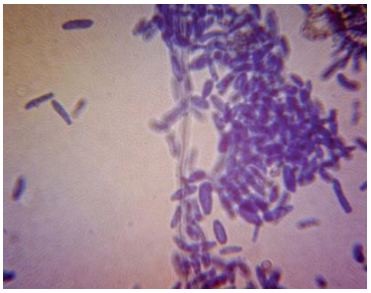

Fig. 2. **(a) Plant infected with leaf blight** **(b) Colony of *Colletotrichum gloeosporioides*** **(c) Conidia of *C. gloeosporioides***

*Alternata alternata*Cultural and morphological characteristics:

Colony appears circular in form, entire margin with raised elevation, powdery to felty. The colony is grey to olive green with white margin in obverse and appears dark brown black with light brown periphery (Fig. 3).

The mycelium is sparse, grayish brown, fluffy, septate hyphae. The conidia is variable in shape often ellipsoidal or ovoid, pale to dark brown, 3-7 transverse septa, 1-several longitudinal septa. The size of conidia ranges from 28-58x10-25 µm. The conidia are polymorphous formed in branching chains.


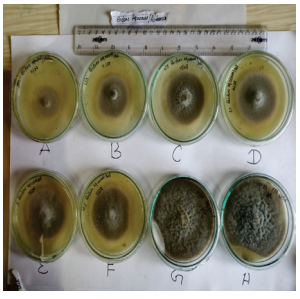
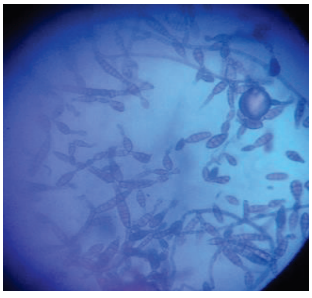

Fig. 3. **(a) Plant infected with leaf spot** **(b) Colony of *Alternaria alternata*** **(c) Conidia of *A. alternata***

Fusarium oxysporum

Cultural and morphological characteristics:

Colony is circular, floccose, crateriform in elevation with entire margin. The obverse view is slightly pink and reverse view of fungus is pinkish white to purple (Fig. 4).

The fungus produces aerial, septate and branched mycelium. The fungus micro conidia is oval toellipsoidal which size ranges 5-12x2.5-3.5 µm while macro conidia is fusiform that is slightlycurvedwith 3-5 septa that size ranges 24-45x3-5 µm.



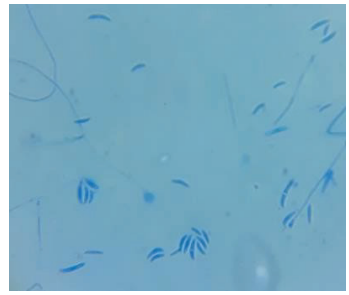

Fig. 4. **(a) Plant infected with root rot** **(b) Colony of *Fusarium oxysporum*** **(c) Conidia of *F. oxysporum***

Bipolaris sorokiniana

Cultural and morphological characteristics

Colony is irregular and flat. The obverse view of fungus is grayish black while reverse view is grayish black with dark brown ridges. The fungus produces septate and hyaline mycelium which bears unbranched conidiophores. The conidia is ellipsoid, dark brown, smooth walled with ends rounded which is broad at middle. It bears 3-10 distosepta, which size ranges 40-100x17-23µm (Fig. 5).


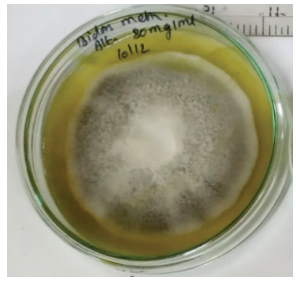
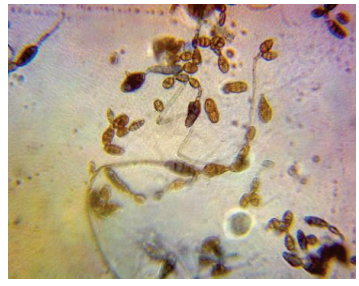

Fig. 5. **(a) Plant infected with leaf spot** **(b) Colony of *Bipolaris sorokiniana*** **(c) Conidia of *B. sorokiniana***

Trichoderma harzianum

The disease specimen was collected from the stem of the plant and browning of stem and wilting of stem were observed.

Cultural and morphological characteristics:

Colony is flat, filamentous with entire margin. The colony consists of patches of green conidia. At obverse view, the colony is pale yellow and at reverse view, the colony is creamy white with green patches (Fig. 6).

The mycelium of the fungus is hyaline and septate where conidiophores are highly branched. The conidia are hyaline, ovoid, one celled borne in small terminal cluster which is of 3 to 5 µm in diameter.

Fig. 6. **(a) Plant infected with wilting** **(b) Colony of *Trichoderma harzianum*** **(c) Conidia of *T. harzianum***

Botrytis cinera

Cultural and morphological characteristics

Colony is flat, cottony with entire margin. At obverse view, the colony is which to grey in colour and at reverse view; the colony is creamy white with dark grey patches (Fig. 7).

Conidia (macroconidia) which are unicellular,hyaline to slightly colored, smooth, ovoid to ellipsoid, and measure 10-12x 8-10 µm. Conidia are produced on short sterig-mata on the swollen tips of aerial, free, branched conidiophores.

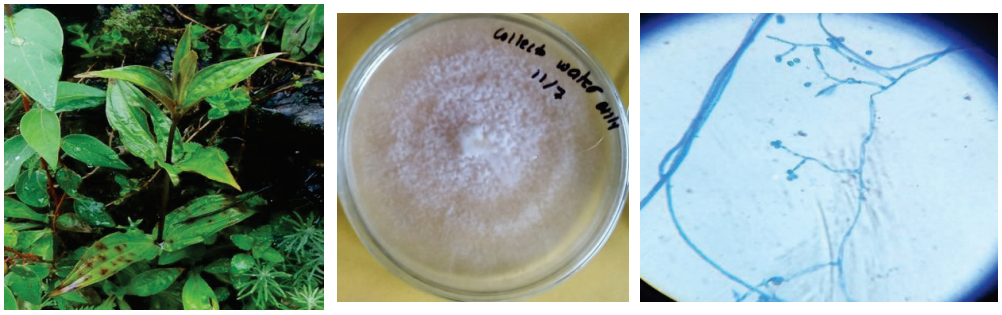

Fig. 7. **(a) Plant infected with leaf spot** **(b) Colony of *Botrytis cinera*** **(c) Conidia of *Botrytis cinera***

The present study was focused on fungal pathogens responsible for causing diseases in *Swertia chirayita*, and identified six fungal diseases caused by *Fusarium oxysporum, Colletotrichum gloeosporioides, Alternaria alternata, Botrytis cinera, Trichoderma harzianum* and *Bipolaris sorokiniana.* The diseases identified were leaf blight disease

caused by *Colletotrichum gloeosporioides,* leaf spot caused by *Alternaria alternata, Botrytis cinera,* and *Bipolaris sorokiniana, Fusarium* wilt caused by *Fusarium oxysporum* and *rot disease* caused by *Trichoderma harzianum.* Leaf spot disease caused by *Colletotrichum gloeosporioides* and Fusarium wilt caused by *Fusarium oxysporum* (DPR, 2015) and leaf spot caused by *Alternaria alternata* in *S. chirayita* (Baskey *et al*., 2018) were also reported in previous studies.

Occurrence of *Alternaria alternata, Colletotrichum gloeosporioides* and *Fusarium oxysporum* in various medicinal plant was also found by Ma *et al*. (2014), Rahman *et al*. (2019), Pati *et al*. (2008), Kalra *et al*. (2005) and other researchers. In the northern region of Bangledesh, *Alternaria alternata* caused Aswagandha leaf blight, *Collettotrichum gloeosporioides* caused Kalmegh leaf spot, *Fusarium oxysporum* caused Satamuli stem rot, and Tulsi root rot (Rahman *et al*., 2019). *Fusarium* was soil inhabiting pathogen, attacking large number of host plants including oilseeds, pulses, vegetables and ornamentals (Mani & Sethi, 1968; Bazalar & Delgadi, 1981; Kumar *et al*., 1983). *Fusarium* caused wilting of leaves, tips and loss of turgidity, followed by yellowing and drooping of leaves, and underground stems became dry, brown, and peeling of epidermis, resulting in reduced yield. Roots became fragile, watery, and the vascular bundle browns (Gangopadhyay, 1984).

Similarly, *Hylocereus undatus*, a Chinese medicinal food, found to be suffered from Anthracnose, caused by *Colletotrichum gloeosporioides* and contained reddish-brown, sunken lesions with pink masses of spores in the center on young stems and leaf spot was identified to be the most prevalent disease in *Withania somnifera* (Ma *et al*., 2014). The association of the fungal pathogen *Alternaria alternata* was detected by repeated isolations from affected leaf tissues and pathogenicity tests (Pati *et al*., 2008). Kalra *et al*. (2005) also found leaf spot disease caused by *Alternaria alternata* on Mints (*Mentha* sp.), a widely used essensial oil producing plant.. *Fusarium* sp. and other fungi caused the damping off of *Papaver somniferum*, a medicinally essential herb. Thee infected seedlings develop yellowing signs and die prematurely (Alam *et al*., 1996).

Medicinally important plants are facing serious problems due to fungal pathogens which cause leaf spot and blight diseases where the symptoms appear on the leaves

in the form of leaf spot followed by shrinking of the dead area and separation from the surrounding healthy tissues with varying lesion shapes (Thaung, 2008; Bhandari *et al.,* 2014). It may be harmful to the human body while using these infected parts as a medicine (Chavan & Korekar, 2011). Leaf necrosis and stem dieback lead to a reduction in the effective photosynthetic surface area of the crop, and epidemics that commence prior to or during tuber formation can result in yield losses in excess of 85% (Green, 1994).

Swertia chirayita is one of the popular cash crops of hill farmers of eastern region of Nepal. The disease incidence in the cultivated field of Madi gaupalika, Sankhuwasabha district was found. Leaf spot caused by *Alternaria alternata, Botrytis cinera* and *Bipolaris sorokiniana,* leaf blight caused by *Colletotrichum gloeosporiodes* and *Fusarium* wilt caused by *Fusarium oxysporum* and rot disease caused by *Trichoderma harzianium* are the fungal pathogen causing the devastating disease in *Swertia chirayita*.

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