



Pathogenic fungi associated with *Ageratina adenophora* (Spreng.) King & H. Rob and *Parthenium hysterophorus* L. in Nepal

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Abstract: This study was carried out in Kathmandu valley, Nepal to document the fungal pathogens associated with the worst invasive species *Ageratina adenophora* and *Parthenium hysterophorus*. Pathogen infected leaves were collected in the fields and were cultured in the PDA media. The pathogenic fungi were isolated and identified. Eleven fungal pathogens, namely *Alternaria* sp., *Fusarium* sp., *Cercospora partheniicola*, *Colletotrichum capsici*, *Curvularia* sp., *Passalora ageratinae*, *Chaetomium anguipilium*, *Cladosporium cladosporoides*, and *Puccinia abrupta* var. *partheniicola* were found on *Parthenium hysterophorus*. In contrast, *Passalora ageratinae* and *Cladosporium cladosporoides* were found on *Ageratina adenophora*. *Curvularia lunata* was seen on both the invasive plant species.

Keywords: Invasive species; pathogens; culture; fungi; PDA media

Introduction

Invasive Alien Plant Species (IAPS) are introduced from another geographical range intentionally or accidentally, which tends to spread rapidly in the introduced range causing damage to the novel environment, economy, or human health (Tiwari *et al.* 2005). Two species viz. *Parthenium hysterophorus* L. & *Ageratina adenophora* (Spreng.) King & H. Rob. are the worst IAPS spreading throughout Nepal's tropical and subtropical parts (Shrestha 2016). Both the species belong to the family Asteraceae. They are known to alter community composition, soil quality, and growth of native species (Timsina *et al.* 2011; Thapa *et al.* 2017).

Control and manage of these IAPS have been a challenging issue. Among the several methods of control and management, the application of specific fungal pathogens is one of the best options as a biocontrol method (Aggarwal *et al.* 2014; Ellison & Barreto 2004). Therefore, the studies on the pathogenic fungi associated with these IAPS have great importance. This study aims to document the associated fungal pathogens in the invasive *P. hysterophorus* and *A. adenophora* in Kathmandu valley, Nepal.

Materials and Methods

Collection of infected leaves of IAPS

Infected leaves of *P. hysterophorus* and *A. adenophora* were collected from March to May 2018 from Chobhar, Champadevi, and Tribhuvan University (TU) campus areas in Kathmandu valley, Nepal. The leaves having symptoms like leaf spot, rust, anthracnose, blight, etc. were collected in a sterile zip-seal bag and brought to the plant pathology lab at Central Department of Botany, Tribhuvan University, Kathmandu, Nepal.

Culture, isolation and identification of the pathogens

The infected leaf surface was washed with sterile distilled water to remove dust and adherent soil particles. Small pieces of the infected part of the leaves were surface sterilized with 70% ethanol and rinsed with sterile distilled water. The infected leaf piece was then transferred to the petriplates containing Potato Dextrose Agar (PDA) medium (potato - 200g, agar-agar - 20g, dextrose - 20g, distilled water - 1000 ml). The media was supplemented with streptomycin sulphate as an antibiotic to inhibit bacterial

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growth. The petriplates were incubated at 25 ± 2 °C. The rust-infected leaves were cultured without washing. The fungi were isolated and pure cultured. They were identified based on morphological characteristics of the mycelium, conidia, and their fruiting bodies following Barnett (1960), Gilman (1975), and Watanabe (2010).

Results

A total of 9 fungal species were identified from both the invasive weed *Parthenium hysterophorus* and three fungi from *Ageratina adenophora*. One of the fungus *Curvularia lunata* was found in both the weeds. Most of them belong to the group fungi imperfecti (Deuteromycetes). *Passalora ageratinae* belongs to Ascomycetes and *Puccinia abrupta* var *partheniicola* belongs to Basidiomycetes (Table 1). The isolated fungal species, disease symptoms, and the characters of culture colony are as follows:

1. *Alternaria* sp.

The infected portions of *P. hysterophorus* leaves were similar to *A. alternata*. The colony of this species on the culture plate was greenish-black with whitish margins. The mycelium was branched and septate. Conidiophores were solitary and few are in groups and septate. The conidia were dark to light brown in the colour (Figure 6).

2. *Alternaria alternata*

The infected parts in *P. hysterophorus* were blackish-brown spots scattered on the leaves. The spots were irregular in shape with varied size. The colony on the culture plate was dark grey with white margins which became black at maturity (Figure 4).

3. *Cercospora partheniicola*

The symptom caused by this fungus in *P. hysterophorus* leaves was greyish brown spots. The colour of the colony was cottony white at an immature stage and became blackish at maturity. The septate mycelia were well-

branched. The conidiophores were hyaline, few were dark brown and septate (Figure 7).

4. *Cladosporium cladosporoides*

The symptoms of fungus were small dark spots on the leaves of *A. adenophora*. The colony colour in the culture plate was greyish black. The conidia were dark brownish. They were formed in branched chains. The conidiophores were erect, straight, and branched.

5. *Curvularia lunata*

The leaves of both *P. hysterophorus* and *Ageratina adenophora* had brownish black leaf spots. The colony in the media was shiny, velvety grey and fluffy growth on the colony surface. The conidiophores were erect, having dark brown, septate, conidia with a rounded base (Figure 11).

6. *Colletotrichum capsici*

The soft and white colony appeared on the culture media. Light yellowish anthracnose appeared on the leaves of *Parthenium hysterophorus*. Hyaline and ellipsoidal conidia were seen under the microscope (Figure 1).

7. *Fusarium oxysporum*

The symptoms of this fungus were the large brown spots coalesced by small spots in *P. hysterophorus* leaves. The colony colour was whitish creamy in the culture plates. Aerial hyphae were white and pinkish in the colour at maturity (Figure 3).

8. *Fusarium solani*

The leaf spots of this fungus in *P. hysterophorus* leaves were similar to *F. oxysporum*. The fungal colonies were white, cottony, and slimy. The colony turned brownish at maturity. The conidiophores were branched, and the hyaline conidia were slightly curved, having 3 to 4 septa (Figure 9).

Table 1. Fungi isolated from *P. hysterophorus* and *A. adenophora* infected leaves.

S.No.	Fungal pathogens	Class	Host plant	Symptoms	Colony characters	Pathogenicity
1	<i>Fusarium oxysporum</i> Schlecht.	Deuteromycetes	<i>P. hysterophorus</i>	Brown leaf spots	Creamy white	++++
2	<i>Alternaria sp.</i> Nees	Deuteromycetes	<i>P. hysterophorus</i>	Dark grey spot-on leaves	Greenish black colour	+++
3	<i>Alternaria alternata</i> (Fr.) Keissier	Deuteromycetes	<i>P. hysterophorus</i>	Dark grey spot-on leaves	Dark grey	++++
4	<i>Fusarium solani</i> (Mart.) Sacc.	Deuteromycetes	<i>P. hysterophorus</i>	Brown leaf spot	White cottony	+++
5	<i>Curvularia lunata</i> Wakker	Deuteromycetes	<i>P. hysterophorus</i>	Brownish black	Velvety grey	+++
6	<i>Curvularia lunata</i> wakker	Deuteromycetes	<i>A. adenophora</i>	Brownish black	Velvety grey	++++
7	<i>Cercospora partheniicola</i> Chupp.	Deuteromycetes	<i>P. hysterophorus</i>	Greyish brown leaf spot	Cottony white/blakish	++
8	<i>Colletotrichum capsici</i> (Syd.) E.J. Butler	Deuteromycetes	<i>P. hysterophorus</i>	Anthracnose	White to brown	+++
9	<i>Passalora ageratinae</i> Crous and A.R. Wood	Ascomycetes	<i>A. adenophora</i>	Leaf spot	White	
10	<i>Cladosporium cladosporoides</i> (Fresen.) G.A. de Vries	Deuteromycetes	<i>A. adenophora</i>	Dark spots	Greyish black	+++
11	<i>Chaetomium anguipilium</i> L.M. Ames	Deuteromycetes	<i>P. hysterophorus</i>	Leaf colour light yellowish	Olive green	++
12	Winter rust (<i>Puccini abrupta</i> var. <i>partheniicola</i>) (Jackson) Parmelee	Basidiomycetes	<i>P. hysterophorus</i>	Brown rust	-	

Magnitude: +++++ = Heavy, +++ = Moderate, ++ = low, + = very low, - = no culture

Table 2. Colony size of fungal pathogens

S.No.	Pathogens	Colony diameter on PDA media (cm)
1	<i>Alternaria alternata</i>	6.29±0.21
2	<i>Fusarium oxysporum</i>	7.00±0.04
3	<i>Fusarium solani</i>	5.78±0.10
4	<i>Curvularia lunata</i>	5.10±0.09
5	<i>Colletotrichum capsici</i>	6.71±0.03
6	<i>Alternaria sp.</i>	5.67±0.09
7	<i>Chaetomium anguipilium</i>	5.00±0.22
8	<i>Cladosporium cladosporoides</i>	5.97±0.04
9	<i>Cercospora parthenicola</i>	5.6±0.09
10	<i>Passalora ageratinae</i>	6.5±0.08
11	<i>Puccinia abrupta</i> var. <i>partheniicola</i>	5.66±0.09

Table 3. Spore size of fungal pathogens

S.No.	Pathogens	Spore size (µm) Length x Breadth
1	<i>Alternaria alternata</i>	16-27 x 9-11.76
2	<i>Fusarium oxysporum</i>	24-29 x 3-5.25
3	<i>Fusarium solani</i>	65-66.4 x 4-6
4	<i>Curvularia lunata</i>	21.5-29 x 9- 14.98
5	<i>Colletotrichum capsici</i>	3.24-4.65 x 21.44-10.55
6	<i>Alternaria sp.</i>	7-10 x 23-26
7	<i>Chaetomium anguipilium</i>	3-5 x 79.88-100
8	<i>Cladosporium cladosporoides</i>	2-62 x 40-99
9	<i>Cercospora parthenicola</i>	4-69 x 10-88.56
10	<i>Passalora ageratinae</i>	9-14.34 x 27-64.88
11	<i>Puccinia abrupta</i> var. <i>partheniicola</i>	Uredino spores size (18- 25 x 22 - 27)

9. *Puccinia abrupta* var. *partheniicola*

Brown rust appeared during late winter in the leaves of *P. hysterophorus*. Brown colour rounded spores were observed under microscope. Rust appeared on *P. hysterophorus* leaves was also observed under the microscope without culture (Figure 8).

10. *Passalora ageratinae*

This fungus was cultured in media and black colonies were seen. The greyish dead spots caused by this fungus were seen in the leaves of *A. adenophora*. The pale brown spores observed were cylindrical and straight. The spores were single to three celled and were in chains (Figure 10).

11. *Chaetomium anguipilium*

Light olive-green colonies appeared on PDA. The old colony become grey to olive in colour. Septate hyphae and the perithecia were large, hair-like, brown to black setae and had ostioles (small rounded openings) and contained asci and ascospores (Figure 2).

Discussion

The invasive *P. hysterophorus* and *A. adenophora* are the worst invasive alien species invading mainly on roadsides, fallow lands, crop fields, and forest margins in Nepal (Thapa *et al.* 2015; Balami and Thapa 2017; Balami *et al.* 2017, 2019). They are responsible for replacing native species, affecting species composition and diversity, plant growth and development, and soil nutrient dynamics (Thapa *et al.* 2016, 2017, 2020; Timsina *et al.* 2011). As these alien species are naturalized since a long time ago in the invaded range, their control and management have become a challenging issue. The conventional methods of their control and management are extremely difficult (Aggarwal *et al.* 2014). As another option, the chemical methods are harmful to the non-target species, soil, and environment. Therefore, the biological control methods are considered safe, eco-friendly and cost-effective. There are several studies regarding the effect of IAPS on natural ecosystems and crop fields as well as the diversity and distributions of these species are also highly focused by several researchers, but the issues regarding the control and manage the IAPS have been still neglected (Yan *et al.* 2001; Gnanavel and Natarajan 2013; Niu *et al.* 2007). In this context, searching biological control agents to control these IAPS would have great significance.

This study revealed that dozens of fungal species are associated with these invasive species (Table 1). The fungi isolated (*Alternaria sp.*, *A. alternata*, *C. partheniicola*, *C. capsici*, *C.*

anguipilium, *C. lunata*, *F. oxysporum*, *F. solani*, and *P. abrupta* var. *partheniicola*, *P. ageratinae*, and *C. cladosporoides*) were the pathogenic fungi. This preliminary record of the fungal pathogens would be highly valuable for further studies for confirmation and probabilities of using them as the biocontrol agents.

Aggarwal *et al.* (2014) isolated and tested the pathogenicity of seven fungi in India and proposed them as the biocontrol agents. The pathogens were *Alternaria* sp., *A. alternata*, *Curvularia* sp., *Fusarium solani*, *Fusarium* sp., *Torula* sp. and *Trichoconellia padwickii*. The species such as *Alternaria* sp., *A. alternata*, *Curvularia* sp., *Fusarium* sp., *Chaetomium* sp., *Cercospora* sp., *Cladosporium* sp., are also found in our study but two of the species *Torula* and *Trichoconellia padwickii* were not reported. Similar to our study the *Colletotrichum* sp. was also reported as the pathogen by Aggarwal *et al.* (2014). A rust pathogen *P. abrupta* var. *partheniicola* in *P. hysterophorus* and *P. ageratinae* in *A. adenophora* were the most common fungi reported in Nepal (Shrestha 2012; Winston *et al.* 2014; Shrestha *et al.* 2016).

Overall, this study reveals that dozens of pathogenic fungi are associated with the invasive *P. hysterophorus* and *A. adenophora* in Nepal. This report is probably the first in Nepal's context except *P. ageratinae* from *A. adenophora* and *P. abrupta* var. *partheniicola* from *P. hysterophorus*. These fungi can be utilized as the biocontrol agents of such problematic invasive species. Further studies about confirmation and potential use for biological control of invasive species are recommended.

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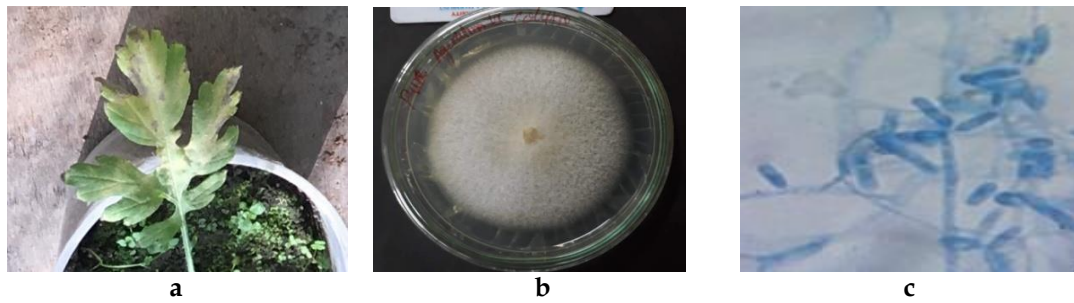
Fungal pathogens identified from infected leaves of invasive alien plant species.

Figure 1: a. infected leaf of *Parthenium hysterophorus*, b. *Colletotrichum capsici* in culture plate, c. mycelium and conidia

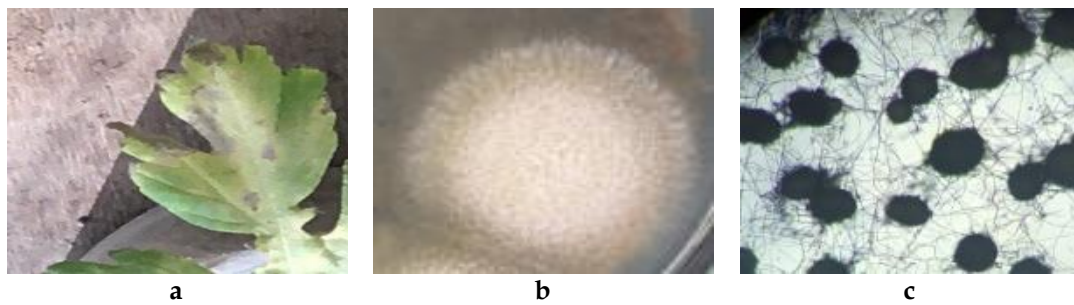


Figure 2: a. infected leaf of *P. hysterophorus*, b. *Chaetomium anguipilium* in culture plate, c. *Chaetomium* ascoma in pure culture

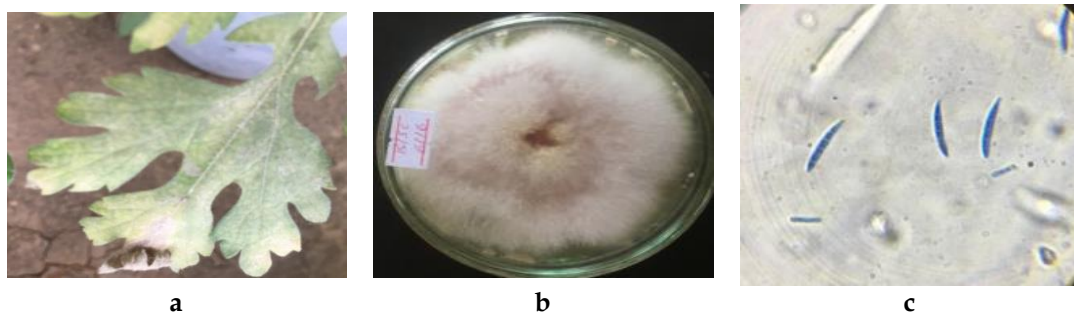


Figure 3: a. infected leaf of *P. hysterophorus*, b. *Fusarium oxysporum* in culture plate, c. hyphae and conidia

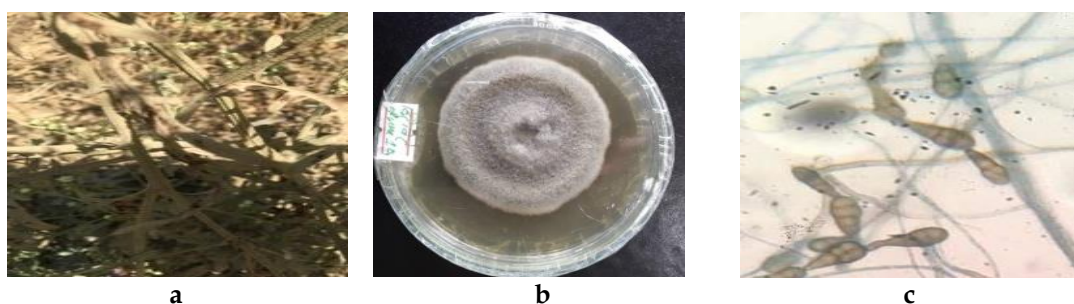


Figure 4: a. infected leaf of *P. hysterophorus*, b. *Alternaria alternata* in culture plate, c. hyphae and conidia

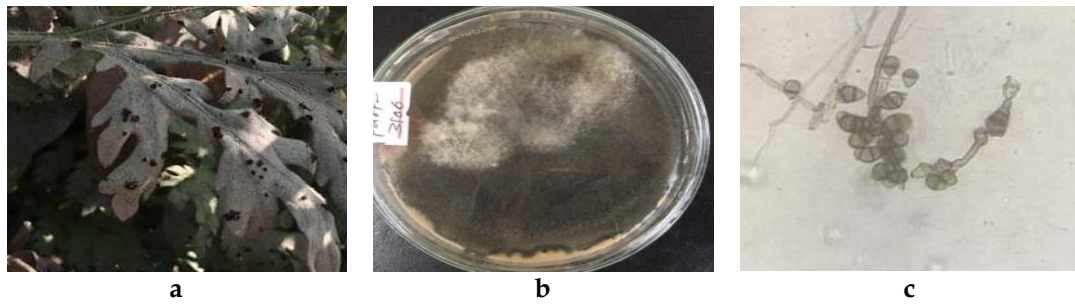


Figure 5: a. infected leaf of *P. hysterophorus*, b. *Curvularia lunata* in culture plate, c. conidiophore and conidia

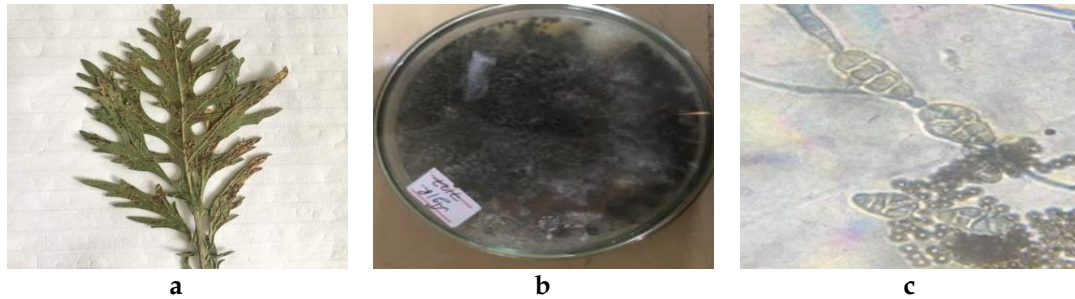


Figure 6: a. infected leaf of *P. hysterophorus*, b. *Alternaria* sp. in culture plate, c. conidia

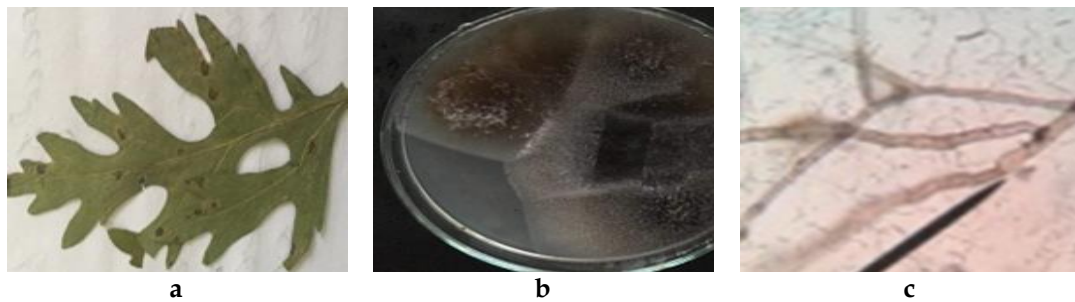


Figure 7: a. infected leaf of *P. hysterophorus* leaf infected, b. *Cercospora partheniicola* in culture plate, c. mycelium

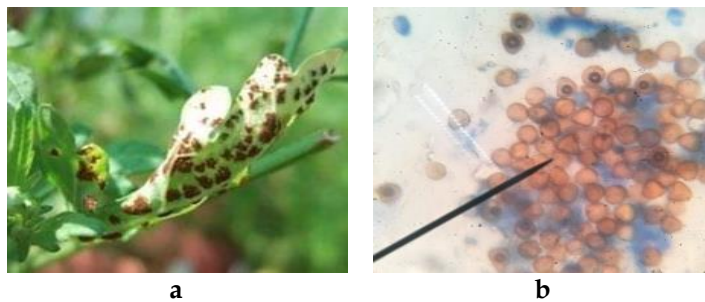


Figure 8: a. infected leaves of *P. hysterophorus*, b. conidia of *Puccinia abrupt* var *partheniicola*)

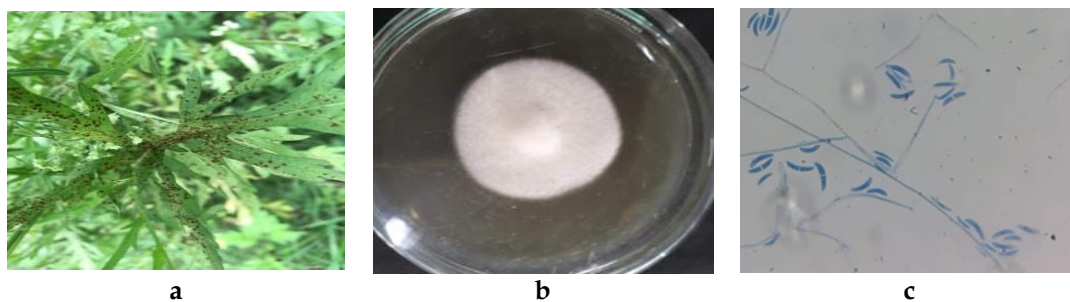


Figure 9: a. infected leaf of *P. hysterophorus*, b. *Fusarium solani* in culture plate, c. hyphae and conidia

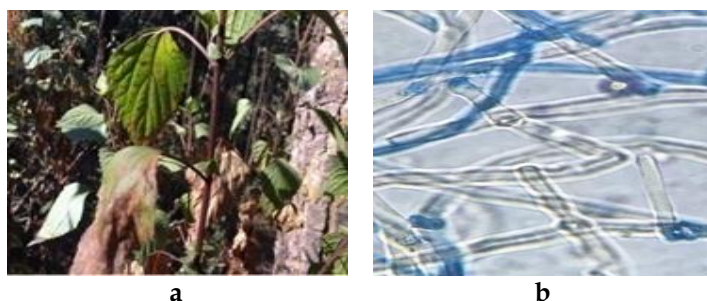


Figure 10: a. infected leaf of *A. adenophora*, b. *Passalora ageratinae*

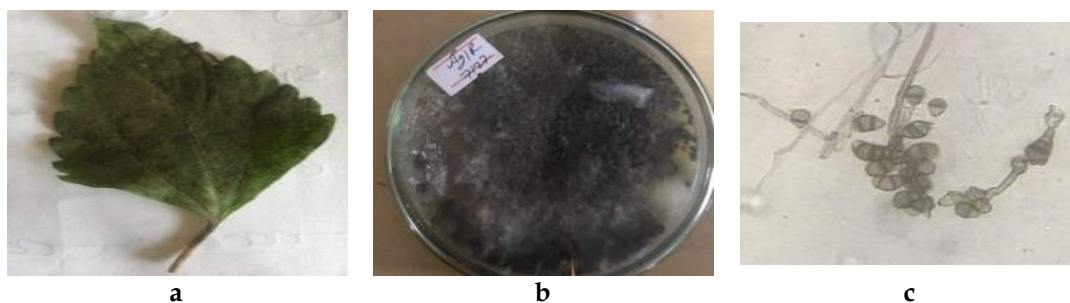



Figure 11: a. infected leaves of *A. adenophora*, b. *Curvularia lunata* in culture media, c. conidia

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