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Fungal diseases of economically important tree species in plantation forest of Arjam, Myagdi district, Nepal

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This paper deals with the fungal diseases of important tree species, which have enormous economic value, i.e. *Melia azedarach, Celtis australis and Toona ciliata.* These tree species are used for timber, fuelwood, fodder and for infrastructure development. A number of devastating fungal diseases were prevalent among the tree species in plantation forest of Myagdi District. For Isolation and identification of pathogen infected samples were cut into small pieces, washed, sterilized with 70% ethanol and transferred to Petri plates containing potato dextrose agar (PDA) media. Then, incubated at 25 ± 2ºC and after few days when fungal colonies developed observed in microscope. These fungal pathogen causing different disease were *Erysiphe kusanoi* (powdery mildew), *Colletotrichum gloeosporioides* (anthracnose), *Pestalotia neglecta* and *Fusarium* sp. (canker) and *Alternaria alternata* (blight). It has been concluded that to moderate the damages caused by these pathogens, it is must to identify them early in the infection process.

Keywords: *Celtis australis*, diseases*, Melia azedarach, Toona ciliata*

Forests cover 40.36% of the country's total land area in Nepal (Paudel, *et al*., 2021). The rate of deforestation in Nepal has been declining (Oli & Shrestha, 2009). However, pressure on natural forests for lumber, fuelwood, fodder, and infrastructure development has increased dramatically in recent years, negatively affecting the delivery of forestry goods and services to people (Pokharel, 2019). Establishment of plantation forests reduces logging pressure on natural forests by offering alternative sources of these supplies (Cossalter & Pye–Smith, 2003).

Some of tree species that are being planted in Nepal are *Melia azedarach, Celtis australis, Toona ciliata* and so on. Besides being good quality timber species these tree species are used in multiple ways. For example, leaves of *Celtis australis* are used as fodder in dry season (Gautam, 2014) and extract from the trees are used to treat edema, headache and boils (Hocking 1993; Singh 1982). Similarly, *Melia azedarach* leaf based products are used as botanical insecticides in agriculture in Asia and the Middle East (Thacker, 2002). Traditionally, *Melia azedarach* based products are used as anthelmintic, antilithic diuretic, astringent and stomachic drugs (Warrier *et al*., 1995). Likewise, the bark of *Toona ciliata* is used as astringent and antiperiodic drugs, and in the treatment of chronic infantile dysentery and ulcers (Singh & Plant 1995).

Several pathogenic fungi cause plant diseases such as anthracnose, leaf spot, rust, blight, gall, canker, mildew, etc. (Jain *et al*., 2019). *Nigrosora sphaerica* that causes leaf spot on *Celtis australis* (Gautam, 2014) and *Rhytisma acerinum* that causes tar spot on *Toona ciliata (*Chandel & Kumar 2017) are some of the pathogenic fungi recorded for the study tree species. Fungal pathogens play crucial roles in producing diseases. Forest diseases are causing significant losses in plantation forests

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of Nepal (Malla & Pokharel, 2018). However, due to limited research, proper documentation of fungal pathogens causing diseases on planted tree species has not been done so far in Nepal. The main aim of this study was to identify the fungal pathogen to mitigate the damages caused by these fungal pathogens.

Materials and methods

Study site

The study was conducted in Arjam plantation forest located at Beni municipality 1, Myagdi district, Gandaki Province, Nepal (Figure 1). Geographically, it is located at 28º 19' 13" N to 28º 19' 8" N latitude and 83º 33' 55" to 83º 34' 56" E longitude and at an elevation of 1,400–m.a.s. l. The study area has subtropical climate.

Collection of disease sample

The diseased parts of the selected tree species were collected from the study plantation forest in November 2020. Before collecting the infected parts, photographs were taken with their host plants. The collected samples were then placed in paper bags and store in icebox for long – term preservation. Especial care was taken while cutting infected parts from the trees not to damage the samples and trees. Thereafter, the samples were brought to the laboratory of Central Department of Botany, Kirtipur, Kathmandu for isolation and identification of causal organisms.

Isolation and identification of pathogen

The collected infected samples were cut into small pieces and washed in sterile distilled water for removing dust and adherent soil particles. These pieces were sterilized with 70% ethanol and washed with sterile distilled water. Then, the pieces were transferred to sterilized Petri plates containing potato dextrose agar (PDA) media. The Petri plates were then incubated at 25 ± 2ºC. After few days, the fungal colonies were developed. The pure cultures were obtained by inoculation pieces of respective fungal mycelia. Powdery mildew was observed directly in microscope. While, preparing slide for powdery mildew, a piece of sticky tape

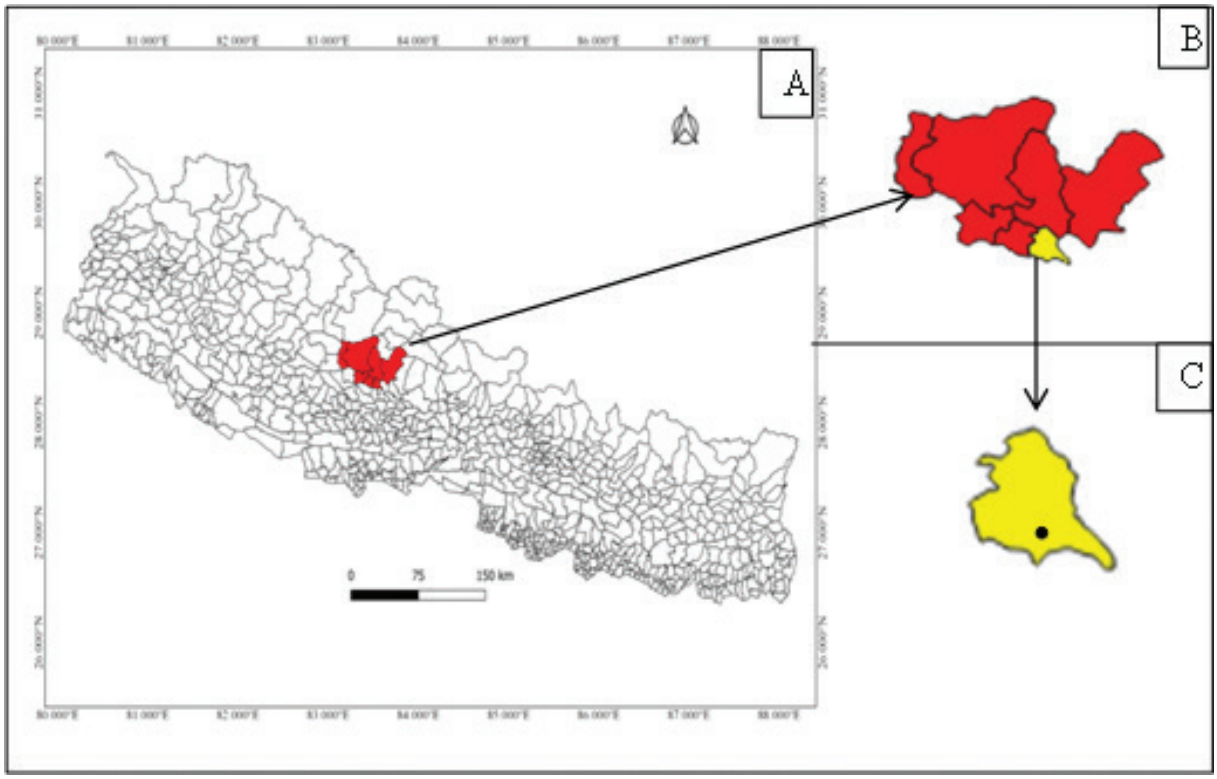

Figure 1. Study area map showing A) location of study municipality within Nepal B) location of study municipality within Myagdi district and C) location of study plantation forest with Beni Municipality

was placed on infected leaves, stripped off and placed on a slide with 1–2 drop of cotton blue. Lacto–phenol or cotton blue was used as staining agent while preparing slides for microscopic examination of fungi. Fungi were identified based on morphological characteristics such as colony morphology, conidial septation pattern and shape and size of the conidia (Barnett, 1960).

Results

Celtis australis, Melia azedarach and Toona ciliata were the economically important tree species planted in the study area**.** Five species of fungi causing four fungal diseases were isolated and identified. Out of four identified fungal disease, three were foliage diseases and one was stem diseases (Table 1). The description of the identified fungal diseases and the causal organisms Figure (2–7) and plant pathogen with their colony size and spore size is given at Table 2.

1. Leaf blight of *Melia azedarach* L.

Causal organism– *Alternaria alternata* (Fr.) Keissl.

Symptoms: Brown– lesions towards the tip of leaves. At later stage, dark brown lesions extended to the midribs and entire leaves showing blighted appearance and, curling inwards.

Colonies fast growing. White cottony to black green in colour. Conidiophores arise singly or in small groups and are pale to golden brown in colour. Conidia in branched chains of up to 15–20, sometimes separated by a short secondary conidiophore. Conidia obpyri form in shape with long beak, obclavate with rounded at the apex. Average conidial length of three spores 32.24µm and width 10.64µm and conidiophore 10.83µm in length.

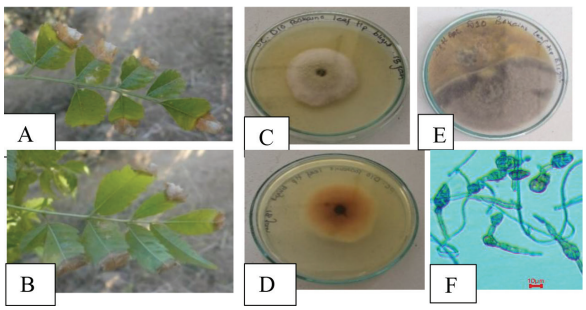

Figure 2: (A–B) Front and back view of infected leaf. (C–D) Pure culture on PDA. (E) First culture. (F) *Alternaria alternata*. Scale bar: 10 µm

2. Canker of *Melia azedarach* L.

Causal organism – *Pestalotia neglecta* Thüm.

Symptoms – Elongated, slightly discolored brown to reddish wound in the tree trunk.

Colonies white to whitish from the edge to the center of colony and cottony. Colonies gets, darker with age. Conidia smooth, five–celled, four septa, curved, relatively short apical appendages.

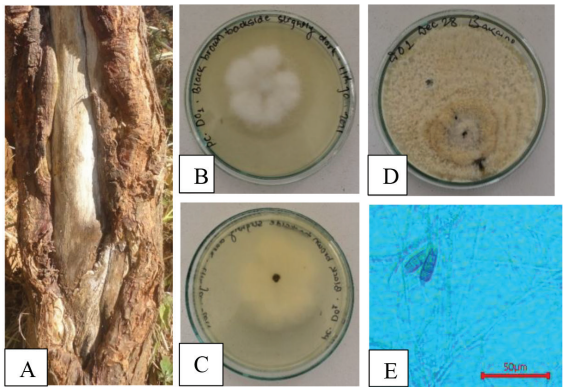

Figure 3: (A) Infected trunk of *M. azedarach*. (B–C) Pure culture on PDA. (D) First culture. (E) Conidia of *Pestalotia neglecta*. Scale bar: 50 µm

3. Powdery mildew of *Celtis australis* L.

Causal organism – *Erysiphe kusanoi* (Syd. & P. Syd.) U. Braun & S. Takam.

Symptoms – White mycelia on the surface of leaves with embedded small black to brown spherical ascomata and in severe case the white powdery mass on the backside of leaves.

Powdery mildew fungi grow superficially or epiphytically on plant surfaces. It can be observe directly in microscope without culture in media. Chasmothecia black, scattered, with about 7–22 appendages, equatorial, stiff or mostly somewhat flexuous, coiled or hooked at the tip. Asci 3–7, obovoid–saccate, short stalked.

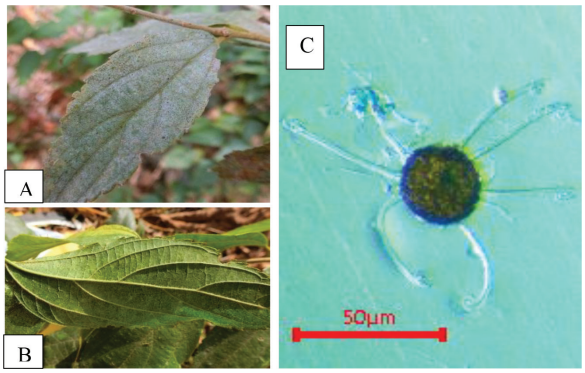

Figure 4: (A–B) Front and back side of infected leaf. (C) Chasmothecia with coil appendages. Scale bar: 50 µm

4. Leaf blight of *Celtis australis* L.

Causal organism – *Alternaria alternata* (Fr.) Keissl.

Symptoms – Irregular, brown–black lesions in the leaf of the infected plant. These appear on the tips and margins of the leaves. As a disease progresses, leaves turn brown, curl up and die. Affected leaves shrivel and dry up.

Colonies fast growing, white cottony at margin while black at center. Conidiophores arising singly or in small groups, pale to golden brown in colour and up to 50 µm long, 3–6 µm thick with one or more distinct conidial scars. Conidia in chain, long chain more than 4 conidia, pale brown to light brown, obclavate, ovoid or ellipsoidal, short conical beak at the tip or beakless. 1–7 (commonly 3) transverse septa, 0–2 longitudinal septa.

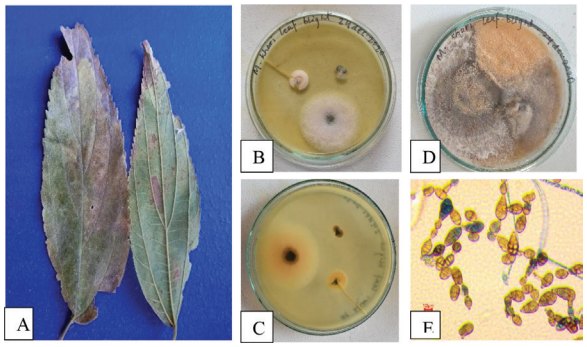

Figure 5: (A) Infected leaves. (B–C) Pure culture on PDA. (D) First culture on PDA. (E) Conidia of *Alternaria alternata* in chain. Scale bar: 10 µm

5. Anthracnose of *Toona ciliata* M. Roem.

Causal organism – *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc.

Symptoms – Appears first as small, irregular brown spots and patches. These spots darken as they age and develop sunken lesions on leaves. These symptoms are inclined to be located on edges of the leaves and between veins.

Colony on PDA flat, irregular margin, first white in colour later turning grey to black. Conidia are hyaline, ovoid to oblong, one–celled, slightly curve or dumbbell shaped 13 µm in length and 6 µm in width.

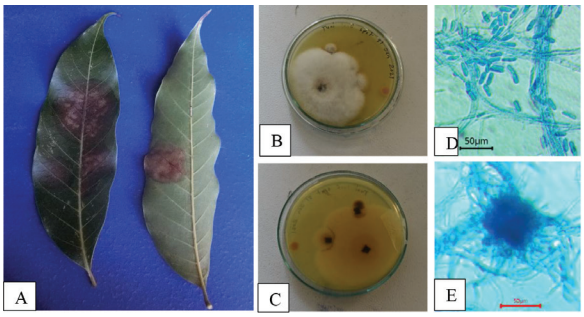

Figure 6: (A) Front and back view of infected leaves of *T. ciliata* (B–C) First culture on PDA. (D) *Colletotrichum gloeosporioides* conidia. (E) Ascocarps. Scale bar: 50 µm

6. Disease – Canker of *Toona ciliata* M. Roem.

Causal organism – *Fusarium* sp.

Symptoms – Affected area appears cracked, swollen and discoloured.

Colonies fast growing, white in colour. Conidia are fusiform to ovoid, straight to curved, one or two celled and hyaline

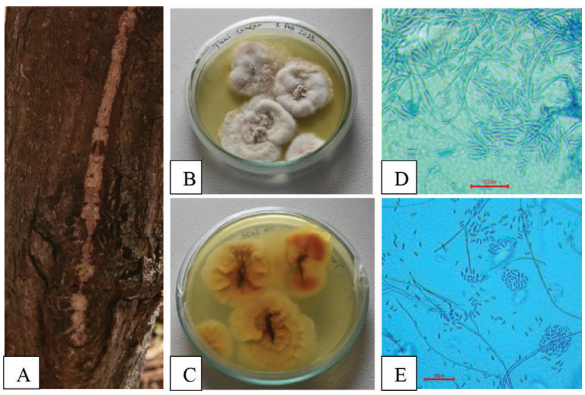

Figure 7: (A) Infected trees (B–C) First culture on PDA. (D–E) Conidia of *Fusarium* sp. Scale bars: 50 µm

Table 1. List of fungal diseases with their host plants and causal organisms

SN	Diseases	Host plant	Plant Pathogens	Class	Colony character
1.	Leaf blight	*Melia azedarach* L.	*Alternaria alternata* (Fr.) Keissl.	Dothideomycetes	White cottony to black green
2.	Canker	*Melia azedarach* L.	*Pestalotia neglecta* Thüm.	Sordariomycetes	White to whitish
3.	Powdery mildew	*Celtis australis* L.	*Erysiphe kusanoi* (Syd. & P. Syd.) U. Braun & S. Takam.	Leotiomycetes	Direct observed
4.	Leaf blight	*Celtis australis* L.	*Alternaria alternata* (Fr.) Keissl.	Dothideomycetes	White cottony at margin black at center
5.	Anthracnose	*Toona ciliata* M. Roem.	*Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc.	Sordariomycete	White to black
6.	Canker	*Toona ciliata* M. Roem.	*Fusarium* sp.(Link)	Sordariomycete	White

Table 2. Plant pathogen with their colony size (expressed as mean ± standard deviation) and spore size (length and breadth)

SN	Plant Pathogens	Colony diameter on PDA media (cm)	Spore Length × Breadth (µm)
1.	*Alternaria alternata* (Fr.) Keissl.	6.63±0.42	25.15 –36.09 × 7.22–12.56 µm
2.	*Alternaria alternata* (Fr.) Keissl.	6.26±0.20	20.3 –37.1× 7.1–11.6 µm
3.	*Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc.	5.80±0.10	10.5–14.6 × 5.5–6.5 µm
4.	*Erysiphe kusanoi* (Syd. & P. Syd.) U. Braun & S. Takam.	Direct observed	28–35 × 11–16 µm
5.	*Fusarium* sp.(Link)	3.96±0.51	20.3 –50.8 × 3.3–5.1 µm
6.	*Pestalotia neglecta* Thüm.	8.60±0.17	25–27 × 6–8 µm

Discussion

(Espinoza *et al*., 2008) identified *Pestalotiopsis clavispora*, *P*. *neglecta* and *P*. *angustata* (*Pestalotiopsi*s = *Pestalotia*) are associated with canker and twig dieback of blueberry in Chile for the first time. Here, study found that *P*. *neglecta* is also responsible for causing canker on *Melia azedarach*

The leaf blight disease caused by *A*. *alternata* was first observed in 1996, and it was reported as one of the most severe and common diseases among crop plants (Mmbaga & Sheng, 1997; Mmbaga *et al*., 2005). Later different researchers (Hubballi *et al*., 2010; Maurya *et al*., 2016) reported that *A*. *alternata* is also responsible for causing leaf blight in different plants such as *Morinda citrifolia* & *Aegle marmelos*. In our case, we found that *A*. *alternata* is causing causing leaf blight on *Melia azedarach.* To our knowledge, this is the first study to report *A*. *alternata* causing leaf blight on *M*. *azedarach*. Similarly, we also found that *A*. *alternata* is causing leaf blight on *Celtis australis*.

We confirmed *Erysiphe kusanoi* as a causal agent of powdery mildew on *C*. *australis* based on morphological and microscopic characteristics such as Conidia, Chasmothecia, its appendages, asci and ascospores (Barun & Cook 2012), which has also been reported by (Gautam, 2014). However, (Ahmad *et al*., 1995; Adhikari, 2018) found *Pleochaeta indica* as causative organism of powdery mildew of *C*. *australis*.

(Zhou *et al*., 2016) described two novel *Fusarium* species that caused canker disease in *Zanthoxylum bungeanum* in northern China and stated that different *Fusarium* species can cause canker on woody plants. Based on the structure of microconidia and other morphological character (Singha *et al*., 2016) we also confirmed that *Fusarium* sp. as causal agent of canker on *Toona ciliata*.

Colletotrichum gloeosporioides has been reported as a causal agent of leaf anthracnose on *Euonymus japonicas* by (Huang *et al*., 2016). Here, we found that *C. gloeosporioides* is also responsible for causing leaf anthracnose on *T. ciliata.* This suggests that although the majority of fungal pathogens are host specific (Li *et al*., 2020), some of them are not.

Conclusion

Some fungal pathogens are becoming prevalent among the economically important tree species in the plantation forest of Myagdi district and are likely to reduce their quality and productivity, causing morbidity and mortality. The tree species like *Melia azedarach* were found to be infected by pathogens like *Alternaria alternata* and *Pestalotia neglecta,* associated with leaf blight and canker. Similarly, fungal diseases found in *Celtis australis* were powdery mildew and leaf blight. The responsible fungi were *Erysiphe kusanoi and A*. *alternata*. In addition*, Toona ciliata* was found to be infected by diseases like anthracnose and canker*,* caused by pathogenic fungi like *Colletotrichum gloeosporioides* and *Fusarium* sp. To moderate the damage caused by these pathogens, it is necessary to identify them early in the infection process. Furthermore, effective management and control measures are needed to reduce the incidence of disease in these economically important tree species.

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