Research Article

First Report of *Fusarium circinatum* Associated with Declining *Pinus wallichiana* Forests in Kumrat Valley, Dir Kohistan Division

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Abstract | The study reports the occurrence of *Fusarium circinatum*, the causal agent of Pine Pitch Canker, in *Pinus wallichiana* forests in Kumrat Valley, Pakistan. The disease was first observed in early 2023, leading to widespread damage in affected forest areas. The pathogen caused cankers, crown dieback and root decay in *Pinus wallichiana*. Field surveys were conducted and samples from symptomatic trees were collected for laboratory analyses. The presence of *Fusarium circinatum* was confirmed through morphological identification. Pathogenicity tests conducted on two years old seedlings of *Pinus wallichiana* and *Pinus roxburghii* showed that *Pinus wallichiana* is more susceptible to the fungus by exhibiting larger lesions compared to *Pinus roxburghii*. The study underscores the growing threat of the pathogen to *Pinus* species in the region, raising concerns about the long-term sustainability of these forest ecosystems.

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Introduction

Pinus wallichiana A.B. Jackson, commonly known as blue pine, is one of the most important *Pinus* species native to the Himalayan, Karakoram, and Hindu Kush mountain ranges. The tree grows at an altitude of 1800 to 4300 m, thriving extensively along the Himalayan ranges from eastern Afghanistan through Pakistan, India, Nepal, Bhutan, Myanmar, and China (Ghimire *et al.*, 2010). It is one of the three naturally occurring pine species in Pakistan, alongside *Pinus roxburghii* and *Pinus gerardiana* (Rahman *et al.*, 2020), and is a key component of both dry and moist temperate forests in Pakistan (Khan *et al.*, 2014). *Pinus wallichiana* is commonly preferred as a source of timber and fuel wood in regions where it grows, largely because of its easy availability and the scarcity of other related species, such as *Cedrus deodara*.

Pine forests are prone to various insects and pathogens, which can severely affect the productivity of individual trees as well as the entire forest stands or plantations (Huang *et al.*, 2008; Chavarriaga *et al.*, 2007). Among these, *Fusarium circinatum* has emerged as a significant concern, causing substantial damage to the *Pinus* species worldwide (Nirenberg and O'Donnell, 1998). The pathogen is responsible for causing Pine Pitch Canker disease in more than

60 *Pinus* species (Wingfield *et al*., 2008), affecting the cortical and sub-cortical tissues of the tree trunk, causing reduced growth and timber loss (Storer *et al*., 1994). The characteristic symptom of the disease is resin-soaked cankers on the main trunk and branches of the affected pine tree. Besides cankers, affected trees show crown dieback and wilting (Barnard and Blakeslee, 1980; Barrows-Broaddus and Dwinell, 1985). Root infection causes brown discoloration and decay of the root cortex (Zamora-Ballesteros *et al*., 2019). Severe infection can lead to high levels of pine tree mortality in some regions (Dwinell *et al*., 1985). However, the severity of the disease and the appearance of symptoms are greatly associated with the host and existing biotic and abiotic factors (Wingfield *et al*., 2008).

Pine Pitch Canker was first observed in the Southeastern United States (Hepting and Roth, 1946). Since then, the disease has been reported from other parts of the world, including California (McCain *et al*., 1987), Haiti (Hepting and Roth, 1953), Mexico (Santos and Tovar, 1991), Chile (Wingfield *et al*., 2002), South Africa (Viljoen *et al*., 1994), Japan (Muramoto and Dwinell, 1990), and Spain (Landeras *et al*., 2005). However, the disease is known to be absent in Asia except for Japan and South Korea (Kobayashi and Muramoto, 1989; Lee *et al*., 2000). Recently, an outbreak of a serious disease was observed in *Pinus wallichiana* forests in Kumrat Valley, Dir Kohistan Division, which resembled Pine Pitch Canker. This study aimed to survey declining *Pinus wallichiana* forests in Kumrat Valley, Dir Kohistan Division, to identify the cause of the disease and evaluate the pathogenicity of *Fusarium circinatum* against two *Pinus* species.

Materials and Methods

Area of study

Kumrat Valley of the Dir Kohistan region, Khyber Pakhtunkhwa (KPK), is situated on the banks of the Panjkora River, between the latitude and longitude of 35°32'11.44" N and 72°13'45.01" E (Figure 1). The elevation of this valley ranges from 2,439 to 3,048 m, with an annual precipitation varying between 1,200 and 600 mm.

Field surveys and sampling

Surveys were conducted in three areas of Kumrat Valley: Chanishai Dab, Jaye Kit, and Sari Mayai, on 25/07/2023. Plants were visually observed for the presence of disease symptoms.

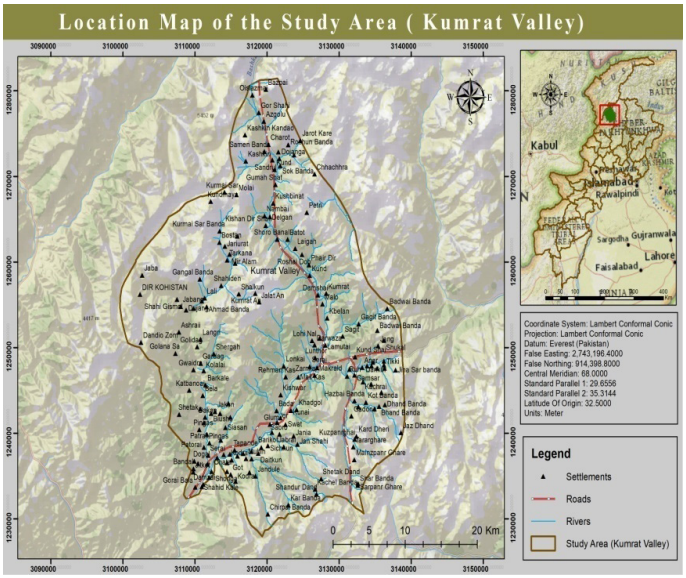

Figure 1: *Study area where diseased Pinus wallichiana forests were surveyed and samples were collected.*

To identify the disease-causing agent, samples were collected from shoots, needles, stem cankers, bark, and roots of healthy, progressively declining, and diseased trees, where:

Healthy trees= no stem cankers and branch dieback, progressively declining= fewer than three cankers on the main stem and minor branch dieback and diseased trees= maximum cankers on the main stem, severe shoot dieback, and wilting.

For the stem cankers, bark was removed, and the area around the margin of the lesion was excised using a sterile blade. Each collected sample was placed in a polyethylene bag, labeled, and transported to the Forest Pathology Laboratory, PFI, Peshawar.

Fungal isolations

Tree needles, stems, bark, and roots were cut from the margins of healthy and diseased tissues into 1- to 2-cm-long pieces, sterilized with 0.1% mercuric chloride for 30 seconds, and rinsed with sterile distilled water. Samples were then dried on a sterile paper towel and placed on Petri plates containing autoclaved Potato Dextrose Agar (PDA) media amended with ampicillin and rifampicin at the rate of 250 mg and 10 mg per liter, respectively (Leslie and Summerell, 2008). Petri plates were incubated for 7 days at 25–30°C. Fungal mycelium was re-cultured on fresh PDA media to isolate the causal fungi.

Morphological identification

Fungal cultures were grouped together and identified based on cultural characteristics. Small fragments of growing mycelium were taken with a sterile scalpel, mounted on a glass slide containing 100% lactic acid, and observed under a light microscope at various magnifications. The isolation frequency of fungi from roots, stems, needles, and bark was calculated using the formula: IF = Ni/Nt x 100, where Ni is the number of fragments obtained from needles, stems, bark, and roots from which fungi were isolated, and Nt is the total number of cultured fragments (Rodríguez and Meneses, 2005).

Pathogenicity test

Two-years old *Pinus wallichiana* and *Pinus roxburghii* seedlings were tested for pathogenicity against *Fusarium circinatum* as described by Roux *et al.* (2007). Twenty-five seedlings of each *Pinus* species were planted in loamy soil in a greenhouse at the Bhurban Field Station of the Pakistan Forest Institute, Peshawar. Plants were watered periodically and maintained under screen-house conditions for 2 to 3 months. Physically healthy and well-growing seedlings were selected for the pathogenicity test. The surfaces of seedlings were disinfected with 85% ethanol, and bark was removed with the help of a sterile, sharp blade to expose the cambium. A 4-mm plug was taken from the margins of *Fusarium circinatum* mycelium growing on PDA media and inoculated into each seedling. The mycelium was covered with cotton soaked in sterile distilled water and sealed with the parafilm. *Pinus wallichiana* seedlings inoculated with sterile PDA plugs were used as controls. The length of lesions produced by fungi was measured every two weeks to assess the pathogenicity of the isolated fungi on each *Pinus* seedling. Reisolation of the disease-causing fungi was performed to satisfy Koch's postulates.

Results and Discussion

Field survey

The problem started in early spring and progressed rapidly from June, 2023 onwards. The disease was more severe in relatively plain areas of the valley compared to nearby elevated areas and was found to be more severe on mature pine trees. Affected trees had dried brown needles that remained hanging on the tree. The branches of the trees showed dieback symptoms. Cankers were observed on the main stem of older trees, and upon the removal of bark, black and white colored fungal growths were observed invading the vascular tissues of the trees. The roots of infected trees appeared water-soaked and decayed. On severely affected trees, white- and green-colored fungal mycelium was observed on the surface of branches and roots. Overall, the diseased trees appeared brown and wilted (Figure 2).

Figure 2: *(A) wilting of Pinus wallichiana stands in Kumrat valley; (B) stem cankers caused by Fusarium circinatum in mature Pinus wallichiana trees.*

Fungal isolation and identification

Fungal cultures isolated from diseased *P wallichiana* roots, needles, stem cankers, and bark were similar to those of *Fusarium circinatum* as described by Nirenberg and O'Donnell (1998) and Britz *et al.* (2002). The fungus was predominant in the root samples of *P wallichiana* trees, including asymptomatic healthy trees. Cankers on the stem also yielded the maximum isolation frequency of the pathogen. Overall, *Fusarium circinatum* was found in varying degrees in all the trees, i.e., healthy, progressively declining, and diseased, from which the pathogen was isolated (Table 1).

Table 1: *Percentage Isolation frequency of Fusarium circinatum isolated from Pinus wallichiana trees.*

Tree specimen	No of sample	Needle	Stem cankers	Bark	Root
Healthy	10	–	–	–	20
Progressively declining	10	–	40	20	60
Diseased	10	40	80	30	80

On PDA media, the fungus grew as off-white-colored fibrous or ropy mycelial colonies with a light orange tinge in the center. Upon microscopic examination, monophialidic and polyphialidic branched conidiophores were observed. Microconidia were aseptate, obovoid in shape, and aggregated in false heads, while macroconidia were boat-shaped

and septate. A number of sterile coiled hyphae were seen in the fungal mycelia, which differentiated the isolated fungus (*Fusarium circinatum*) from other closely related species (Figure 3).

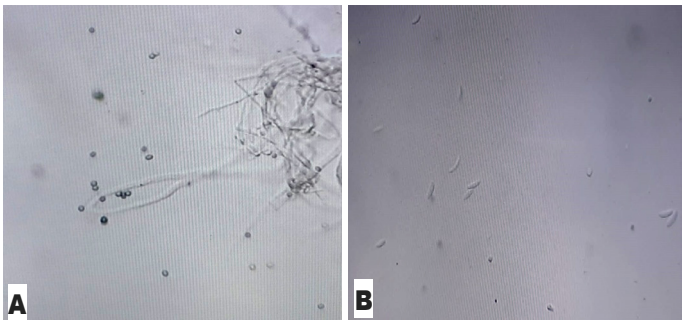

Figure 3: *Microscopic features of Fusarium circinatum (A) sterile coil hyphae on PDA media; (B) obovoid micro-conidia and curved macro-conidia on PDA medium.*

Pathogenicity test

Both *Pinus* species, i.e., *Pinus wallichiana* and *Pinus roxburghii*, exhibited cankers and branch wilt. Upon removal of bark from the cankered areas, brown discoloration and lesions corresponding to external injuries were observed. Symptoms were more severe on *P wallichiana*, with a high lesion length of 40 mm on the 48th day post-inoculation (Table 2). There was no significant difference in mean lesion lengths between the post-inoculation days. The control seedlings, which were mock-inoculated, showed minor injuries that healed completely over the time. *Fusarium circinatum* was isolated and identified from both *Pinus* seedlings through culturing on PDA media and microscopic examination.

Table 2: *Mean lesion length on two Pinus species artificially inoculated with Fusarium circinatum.*

***Pinus* specie**	**Mean lesion length (mm)**		
	14 DPI*	**28 DPI**	**48 DPI**
Pinus wallichiana	35	38	40
Pinus roxburghii	25	26	29
Control	7	7	7

** Days post inoculation.*

In the present study *Fusarium circinatum* was isolated from diseased *P. wallichiana* trees in Kumrat valley, Dir Kohistan division. Symptoms on affected trees significantly resembled pine pitch canker disease caused by same pathogen. The pathogen was identified using morphological features and confirmed to be pathogenic to two *Pinus* species i.e., *P. wallichiana* and *P. roxburghii* through pathogenicity test.

With the ever-increasing global temperatures leading to climate change and extreme weather conditions, the incidence of plant diseases is also escalating (Marcott *et al.*, 2013; Dale *et al.*, 2001; Schiermeier, 2011). Pakistan has also faced the consequences of climate change (You *et al.*, 2024) and the extreme weather events that occurred in 2022 are one of the possible major contributing factors to the onset of *P wallichiana* disease caused by *Fusarium circinatum* in the Kumrat Valley. Temperature and moisture are key climatic factors responsible for the distribution, spread, and symptom development of *F circinatum*, which thrives best in warm and humid climates (Gordon, 2006; Drenkhan *et al.*, 2020). The devastating floods of 2022 were followed by an outbreak of *P wallichiana* disease in the subsequent year, highlighting the possible association of *Fusarium circinatum* with the altered climatic conditions of the area. Mycelial growth of the fungus is favored by an optimum temperature between 20 to 25 ˚C with high relative humidity, whereas suboptimal growth is observed below 5 ˚C or 10 ˚C (Inman *et al.*, 2008; Mullett *et al.*, 2017). This accounts for disease gaining the status of an epidemic during summer in the *P wallichiana* forests of the area.

Root infection of *P wallichiana* trees particularly those which apparently looked healthy suggests that the fungus can reside in roots without causing visible disease symptoms. Swett *et al.* (2016) also reported that the fungus is capable of colonizing roots of pine host and remains symptomless up to 52 weeks from initial entry into the host roots.

F circinatum is primarily a wound pathogen and enters the plants through mechanical injuries associated with silvicultural practices, insect feeding holes and weather-related injuries caused by hail or wind in the trunk and branches of the tree (FAO, 2017; Dwinell *et al.*, 1985). Rubble carried by fast-moving floodwaters damaged the tree trunks, resulting in injuries that potentially contributed to the incidence of disease.

The fungus has been reported to affect various pine species worldwide (Perez-Sierra *et al.*, 2007). Inoculation of *Pinus wallichiana* and *Pinus roxburghii* seedlings confirmed susceptibility to the pathogen, as measured by lesion lengths of 40 mm and 29 mm, respectively. *P wallichiana* was more susceptible to the pathogen, as confirmed by natural infection of mature trees in forest stands and artificial inoculation of

seedlings in greenhouse experiments. This is the first report of the pathogenicity of the fungus on these *Pinus* species. The occurrence of natural infections in *P. wallichiana* forests, combined with artificial inoculation experiments raises concerns about its potential to cause greater harm to forest ecosystems and underscores the importance of continued research and effective disease management.

Conclusions and Recommendations

The study reports the first occurrence of *Fusarium circinatum* causing pine pitch canker in *Pinus wallichiana* forests in Kumrat Valley, Pakistan. The pathogen was confirmed through field surveys, laboratory isolation and pathogenicity tests, showing its ability to cause significant damage to both *Pinus wallichiana* and *Pinus roxburghii*. The findings highlight the potential threat this disease poses to the region's forest ecosystems, especially under changing climatic conditions. Continued research and effective disease management strategies are recommended to mitigate the spread and impact of this destructive pathogen.

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Novelty Statement

This study provides the incidence of *Fusarium circinatum* on *Pinus wallichiana* forests in Kumrat valley, Pakistan, highlighting the previously unreported threat to these forests. These findings are crucial for understanding the emerging risks posed by this fungal specie and the need for management strategies to ensure the sustainability of P wallichiana forests in the region.

Author's Contribution

Mahnoor Baloch: Data curation, formal analysis, investigation, methodology, visualization, writing original draft, writing review and editing.
Sanam Zarif Satti: Supervision.

Conflict of interest

The authors have declared no conflict of interest.

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