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A new Heart rot disease in *Ailanthus excelsa* Roxb. caused by *Navisporus floccosus* (Bres.) Ryvarden

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ABSTRACT

A wood decay fungus was defined based on the zone of tree that invades. *N. floccosus* causing heart rot was recorded in living trees of *A. excelsa* for the first time. The cultural characters of *N. floccosus* were described for the first time. Heart rot fungus i.e. *N. floccosus* decayed mostly heartwood of living tree. It colonized in the central portion of tree and begins decaying of wood which ultimately lead to death of *A. excelsa* tree was recognized for the first time. When decay proceed to the top of tree, the fruiting bodies grown in large size at butt region. During July 2007 i.e. monsoon months, due to the high wind velocity the weak tree was up rooted, which indicates that the fungus severely decayed heartwood of *A. excelsa* tree. After falling the tree, fungal hyphae in heart wood were very active so it produced fruiting bodies outside the trunk. On the malt agar plates containing tannic acid, it showed a positive reaction for oxidase and laccase and negative reaction for tyrosinase. Peroxidase test was positive with growth rate of >70 mm in 7 days.

***Keywords*:** Heart rot; *Navisporus floccosus*; Ailanthus excelsa; cultural characters; Disease cycle

1. INTRODUCTION

Ailanthus excelsa Roxb. was a multipurpose tree species of arid region because of its ability to grow well at low rainfalls (from 400 to 1900 mm) and in strong light conditions (Bhimya et al, 1963). It play an important role in management of land resources and securing livelihoods of economically poor people through agro-forestry system to meet the increasing demand for food, fodder and fuel wood (Jat et al, 2011). Stem bark of this tree was bitter in taste but it was used as astringent, febrifuge, stomachic, antihelmintic, antispasmodic, expectorant and used for the treatment of bronchitis, cold, cough, skin diseases, trouble of rectum, diarrhoea, dysentery, dropsy, fever due to tridosha, guinea- worms, snakebite and also used as contraceptive (Kumar et al, 2010). An antifungal-activity containing two new dammarane-type triterpenes, namely ailexcelone and ailexcelol was isolated from heartwood of *A. excelsa* (Srinivas, et al 2006).

The wood was white and very light in weight. The timber can be used especially for making catamarans for fishing, packing cases, sword sheaths and matchboxes. It produces grade-III commercial and grade-IV moisture proof plywood. It also supplies the wood to meeting the demand of plywood, match stick, toys and packing material Industries of Rajasthan (Jat et al. 2011). The xylem of Ailanthus was porous with indistinct growth rings in both normal and combined air pollutants trees. In affected trees, the vessels were arranged in radial or tangential multiples (Rajput and Rao 2005).

The major diseases occured in Ailanthus was Verticillium wilt caused by *Verticillium albo-atrum*, A root rot caused by *Armillaria mellea* and white rot causing fungi *Inonotus hispidus* (Bull.) P. Karst. delignifing the wood of *A. excelsa* (Koyani et al 2010). Earlier the fungus was reported from east Africa but *N. floccosus* (Bres.) Ryv. was new report for India (Arya et al 2008). In present paper the *A. excelsa* living tree was infected by *Navisporus floccosus* (Bres.) Ryvarden. and it cause a heart rot disease was studied. The fungus was isolated from the wood samples. Its identity, cultural characters and disease cycle was studied.

2. MATERIALS AND METHODS

2. 1. Identification

Materials were collected in clean polythene bags from different locations and brought to the laboratory. Basidiomes were studied using macroscopic (eg. size, colour, number of pores/mm, length of tubes) and microscopic (presence/absence of structures, dimensions, vegetative and reproductive characters (Ryvarden 1991).

To observe basidia, basidiospores and cystidia free hand sections of pore surface were taken. For the clear observation of context, skeletal hyphae, and generative hyphae lactophenol cotton blue was used as staining and mounting medium. Xanthochoric reaction was also tested using potassium hydroxide solution.

Measurements were made from slide preparations by using cameralucida. Specimens were identified to species level using (Stalper 1978), CBS Aphyllophorales database, (www.cbs.knaw.nl/databases/) New Zealand Fungi database, and Species Fungorum. Certain specimens were sent to The Forest Research Institute, Dehradun for final confirmation. These fungi are kept in fungal collection of Botany Department of The M. S. University of Baroda, India.

2. 1. Isolation

The fungi associated with the samples were isolated. Wood chips measuring 5 mm × 5 mm × 1 mm were aseptically removed from the samples and transferred to Petri plates containing 2 different cultural media: 2 % malt extract agar amended with 250 µg streptomycin sulphate per mL and PDA medium amended with 250 µg streptomycin sulphate per mL.

The first medium was intended to isolate Basidiomycetes fungi and the second medium to specifically isolate total fungi. Eight pieces of wood were removed from each disc and placed in 2 plates. The plates were incubated at 25 °C for 7days. Once fungal colonies formed in the agar plates, each colony was transferred to a new agar slant to grow as a pure culture. The cultural and growth characters were described based on Stalpers (1978) and Nobles (1965).

3. RESULTS AND DISCUSION

3. 1. Taxonomy

The sporophore of *N. floccosus* were annual, sessile, aplanate, light in weight, 46.5 – 15 cm x 30.5 – 8 cm x 5 – 3.5 cm thick; upper surface smooth, glabrous, cream to pale yellow, broad base attached to the host, lower surface was grayish-yellow to violet-brown, pores round, irregular, visible to the naked eye, 2 – 4 pores per mm; context duplexed with upper hard, woody layer with creamy white and lower smooth, fibrous layer with pale yellow. Each year concentrically layers were added in context. sporophores were dimitic, skeletal hyphae were dextrinoid, pale yellow, hyaline, unbranched, soft in texture, 9.3 μm thick. Generative hyphae hyaline thinwalled, aseptate, branched 3.12 μm thick with clamp connection. Gleocystidia present, hyaline to pale yellow, 43.4 x 9.3 μm in size. Basidia clavate, 15.5 x 6.3 μm in size. Basidospores are large navicular, non-dextrinoid, thinwalled, pale yellow, oval to ellipsoidal 12.3 x 6.25 μm in size.

Habitat: Kavadia colony, Narmada District and Arboretum of Botany Department, Baroda (Gujarat); Collected by N. Praveen Kumar and Prof. Arun Arya; Accession No: MSU Bot. 66 and 67; 5-9-2005 and 25-11- 2006.

3. 2. Cultural characters

The fungal culture was purified and identified as *N. floccosus* based on the cultural characters and basidiocarp. The pure culture was grown on Malt Extract Agar medium to study the cultural characters. The culture characters of *N. floccosus* were as follows.

3. 2. 1. Growth characters

On the malt agar plates containing tannic acid, it showed a positive reaction for oxidase and laccase and negative reaction for tyrosinase. Peroxidase test was positive with growth rate of >70 mm in 7 d, In KOH, it turned to brown colour, marginal hyphae raised, and distant of marginal hyphal tips densed. The outline of colony was wavy, aerial mycelium silky, colony colour cream, azoneate, odour absent, reverse bleached and clamps present.

3. 2. 2. Hyphal characters

Advancing zone: hyphae hyaline, thin walled, with simple septa 3.12 μ diameter. Aerial mycelium a) hyphae as in advancing zone, with walls irregularly thickened and with small projections along the walls b) fiber hyphae numerous, with thick walls and lumina apparently lacking, unbranched, interwoven to form though pellicle 6.25 μ in diameter.

3. 3. Heart rot disease

Heart rot fungi were specialized organisms that attack the heartwood of living trees. They colonize in the central portion of tree and begin the decay process of wood, which ultimately leads to death of trees. Infection occurs when basidiospores were released from the hymenium surface of basidiocarps (Plate I Fig. A, B). They accumulate in the butt region of host tree. Favorable moisture and temperature conditions permit the spores to germinate. The fungus grows slowly into the vulnerable wood tissues of the trunk. The Butt rot fungus i. e. *N. floccosus*, initially entered into the roots to produce decay and spreads into the butt portion of *A. excelsa* tree (Plate I Fig. C). It produced heart rot by invading only the tree's central column of heartwood. It spread more in a vertical direction than in a horizontal plane from the place of infection zone showing an hole in the fallen tree trunk (Plate I Fig. D).

Plate I

Illustration of Photographs

Plate I

Fig. A. Large sized sporphore of the *Navispora floccose*.

Fig. B. T.S. of pore layer showing the basidiospores.

Fig. C. Decay of Sap and Heart wood of the *Ailanthus excelsa* due to the growth of *N. floccosa* at bunt region.

Fig. D. Fallen *A. excelsa* trunk with hole created due to the decay of heartwood.

Fig. E. T. S. of the trunck showing the barrier zone between the decayed wood to undecayed sap wood.

Fig. F. Decayed surface of the bole with mycelial cords.

The first symptoms indicative of fungal infection were discoloration of heartwood. It becomes dark brown, sapwood becomes brown. Incipient decay in heartwood was difficult to detect but wood colour was intermediate between that of healthy wood and decayed wood, i.e. it was usually of darker colour than normal heartwood. The cross section of infected *A. excelsa* stem shows a barrier zone line indicating that almost all of the heartwood was infected and only a little portion of healthy wood was left (Plate I, Fig. E). The dark brown zone indicates that the wood was in advanced stage of decay with only remnants of fibers in hole. The decay extended upwards from a basal end to a distance of ten meters in 5 years since no active resistance was provided by dead tissue, in this condition the growth of fungus was very easy.

This heart rot fungus was a typical white rot pathogen. Developing fungus degrades both cellulose and lignin, finally leaving behind a yellowish-white, spongy mass. With advancement of decay the wood tissues were accumulated with bundles of hyphae whereas remnants of fibers were present in the center (Plate I, Fig. F). As the decay proceeds to the top of the tree, the fruiting bodies develop at butt region. During July 2007 (Monsoon months) due to the high wind velocity the weak tree was up rooted. This indicates that the fungus severely decayed the heartwood (Plate I, Fig. D). The fungal hyphae were very active to produce the fruiting bodies outside the trunk. The small pieces of wood samples when placed on the PDA medium pure culture of the fungus was obtained which indicated that the fungus was in active state.

The wood was perishable and subject to insect attack and stain. The fungi causing leaf spot disease were *Cercospora glandulosa* and *Alternaria* sp. (Orwa et al 2009). *Inonotus hispidus* produced a typical pattern of soft rot decay even though it was grouped with white rot basidiomycetes (Koyani et al 2010). In the present paper the *N. floccosus* produced typical white rot decay. *I. hispidus* was often known as heart rot fungus (Nutman 1929). In the present paper *N. floccosus also* causing severe heart rot. The fruiting bodies were also observed on relatively young branches of 5-7 inch in diameter (Koyani et al 2010). In the present paper the fruiting bodies were observed at Butt region.

In Ailanthus infection by *I. hispidus* was initiated during the monsoon when moisture levels were very high and the cut branch stubs were exposed to rain (Koyani et al 2010). But in the present paper the fungi infection was started during monsoon, grown extensively and form large basidiocarps on living tree in later stages. In Gujarat State it was one of the most important species cultivated under agro-forestry and social forestry programmes. Besides *I. hispidus*, *Polyporus* sp., *Ganoderma* sp., *Fomes* sp., etc. are other rot fungi that attack this tree

(Koyani et al 2010). In the present study another white rot fungi like *N. floccosus* was causing heart rot in wood. They require further study to understand the pattern of delignification by these species. A mechanical injury made on a healthy plant does not produce an immediate response by the formation of cavities in the wood. However, traumatic cavities and vascular occlusions develop in the secondary xylem in response to injury and fungal infection (Shah & Babu, 1986). In the present paper the entry of the fungi was through roots so there was no cavity formation for this fungal infection.

4. CONCLUSIONS

N. floccosus causing heart rot was recorded for the first time in living trees of *A. excelsa*. Based on the biochemical reactions the *N. floccosus* shows a positive reaction for oxidase and laccase and negative reaction for tyrosinase. Peroxidase test was positive so the cultural characters like growth characters and hyphal characters of *N. floccosus* were described for the first time. Besides *I. hispidus*, *Polyporus* sp., *Ganoderma* sp., *Fomes* sp., etc. are other heart rot fungi that attack this tree but another heart rot fungus i.e. *N. floccosus* decayed mostly heartwood of living tree was described. It colonized in the central portion of tree and begins decaying of wood which ultimately lead to death of *A. excelsa* tree was recognized for the first time. The disease cycle of heart rot of *Alianthus* living tree was described for the first time.

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