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First report of *Rhizoctonia solani* causing sudden wilting and mortality of agar (*Aquilaria malaccensis* Lamk.) seedlings in Assam

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Abstract
Aquilaria malaccensis Lamk. is one of the most economically important, medicinal and aromatic tree species of North-East India. It is naturally grown and commercially cultivated in different districts of Assam. Locally known as *Xanchi* or *Agar*, the Upper Assam region is known for the natural belt of agarwood formation. The principal use of agarwood is in perfume industries. Besides, the essential oil from agarwood is valued for preparation of several pharmaceutical and cosmetic products and its cost is extremely high depending on the oleoresin content of the wood. Due to high demand for agarwood, people are getting engaged in large scale cultivation of this species in this region. But, due to unscientific cultivation practices, new diseases are emerging in the nursery. Unknown fungal infection of *A. malaccensis* with grey to brown spots on leaves leading to sudden wilting and mortality was observed in seedlings raised in polybags at Rain Forest Research Institute (RFRI), Jorhat, Assam in 2017. The fungal infection causes 89.71% damage to the seedlings at 45 days after transplanting. The pathogen was primarily identified as *Rhizoctonia solani* ITCC No. 10.693.17 on the basis of morphology. Aqueous suspension of whitish brown growth of the fungus sprayed on leaves produced typical symptoms in about 15 days followed by wilting and death of *Aquilaria* seedlings. The fungus was highly pathogenic on artificial inoculation. This is the first report of *R. solani* affecting seedlings of *A. malaccensis*. Soil drenching and foliar spray of 0.1% Bavistin was found effective in checking further spread of the disease.

Keywords: *Xanchi*, *Rhizoctonia solani*, agarwood, *Aquilaria malaccensis*, soil drenching and Bavistin

Introduction
Aquilaria malaccensis Lamk, locally known as *agar* or *xanchi*, belonging to the Thymelaceae family, is a precious floral wealth of North-East India. The tree is known to produce dark coloured resinous wood known as agar wood or eaglewood formed in the xylem of the tree as a result of host- fungus interaction. Tree is evergreen, medium to
large in size, which can grow up to 40 m in height and reach a diameter of 60 cm., leaves simple, 5-9 cm long, oblong, lance late, elliptic to obovate-lanceolate. Flower bell shaped, of white or green or dirty yellow coloured, Fruit capsule, 3.5-5 cm long, obovoid, acuminate bearing 1-2 dark brown coloured seeds. Agar wood is valued in high class perfumery as a fixative and is much priced by European perfumer for mixing their best grade scents Agarwood and its products are also described as aphrodisiac, alternative anodyne, antidiarrhoeal, antiasthmatic, astringent, carminative, cordial diuretic, laxative stomachic and tonic and enter into the preparation of several pharmaceutical and cosmetic products.
Today, the demand for agar wood far exceeds supply. Indeed, agar wood is reputed to be the most expensive wood in the world and traders even quote prices as high as US $ 50,000 for one liter pure agar wood oil. Population of *A. malaccensis* has markedly decreased in natural forests of north eastern India due to unsustainable harvesting of mature trees for trade, forcing the government to take action to bring the international trade within sustainable limits. Hence, the species is included in Appendix II of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES 1994) [5]. The species was listed as 'Vulnerable' globally (IUCN-1998), but at present 'Critically Endangered' in India (IUCN 2018) and almost 'Extinct in Wild' in Assam (Anonymous 2003) [1]. The EXIM policy (2009-2015) published by the Directorate General of Foreign Trade, Government of India, permits the export of plant portions of wild or cultivation origin of species specified under Appendix II or III of CITES but the export has to be supported with the production of certificate of legal possession in favour of the exporter, issued by the Divisional Forest Officer (DFO) having jurisdiction where the exporter is situated and also as per the provisions of CITES. Therefore, there is an urgent need to develop sustainable regeneration strategies to augment its natural regeneration and conservation.

Because of its high demand, thousands of people have been engaging themselves in large scale cultivation of this species. It has been noticed that rural economy of three districts of Upper Assam viz., Jorhat, Golaghat and Sivsagar are mostly dependent on Agar cultivation. But, due to unscientific cultivation practices, new diseases are emerging in the nursery and causing large scale mortality with consequent loss of revenues. To mention a few, Borah *et al*., (2012) [4] reported occurrence of leaf spot disease caused by *Corynespora cassiicola* while Das and Dubey (2000) [6] reported the occurrence of root rot caused by *Phytophthora parasitica* and damping off diseases of seedling in nursery by *Pythium aphanidermatum* (Tabin *et al*., 2009) [7] in *Aquilaria malaccensis*.

During the routine survey of nursery seedlings of *A. malaccensis* raised in Rain Forest Research Institute (RFRI), Jorhat, Assam, sudden wilting and mortality of Agar seedlings were noticed. So, considering the importance of the plant, the disease was further investigated and reported in this study.

Materials and Methods

Survey: Seedlings of *Aquilaria malaccensis* infected with disease symptoms in RFRI nursery were observed and the infected seedlings were collected**.** Symptoms were recorded and diseased seedlings were brought to laboratory for further investigation.

Isolation and Identification: Isolation was done from 100 small root pieces, cut from adjoining areas of diseased and healthy areas of the plants. Root pieces were washed under tap water for about 30 minutes to remove any dirt or soil particle. The root pieces were dipped in 0.01% HgCl2 for about 2 minutes and then passed from two washes of distilled sterile water for 2-3 minutes each. The treated root pieces were dried completely and then transferred to Petri dishes containing sterilized potato-dextrose agar medium with five pieces per plate. All the plates were kept at 25 ± 1ºC for 7 days. The fresh growth of the fungi was transferred to freshly prepared potato-dextrose agar medium for sub culturing. The fungal culture was sub-cultured whenever needed during the entire study. The fungus isolated was identified by studying their typical mycelial growth produced on the potato dextrose agar medium and conidial morphology using standard diagnostic keys and sent to Indian Type Culture Collection (ITCC), Indian Agricultural Research Institute (IARI), New Delhi for confirmation.

Pathogenicity test: The pathogenicity test of fungus was done by mixing the fresh inoculum of the fungus with 1kg of steam sterilized soil in plastic pots. Eighty *Aquilaria* seeds were sown per pot containing infested soil. Number of healthy and infected seedlings and disease incidence were recorded after 15 and 30 days of sowing in the pots containing infested and un-infested soil.

Effect of different fungicides on damping-off disease: Twenty *Aquilaria* seeds treated with 0.01% HgCl2 were grown in pots containing steam sterilized soil. The soil was infested with fresh culture of the fungus and then treated with different fungicides *viz*., Neem pesticides, Bavistin and Mancozeb by drenching. The seeds grown in infested and untreated soil served as control. The disease infection was recorded after 7, 15, 30 and 45 days of sowing.

Results and Discussion

Symptoms: The damping-off occurred on seedling stage. In early stage, seedlings became stunted with typical discoloration on the collar region and formed small lesions on roots near to soil level and grey to brown spots on leaves were also recorded. The spots coalesced together and formed large areas (Fig.1). In severe infection, the entire root was found to be rotted and mortality of the seedlings occured.

Fig 1: Photographs of typical symptoms recorded during the investigation

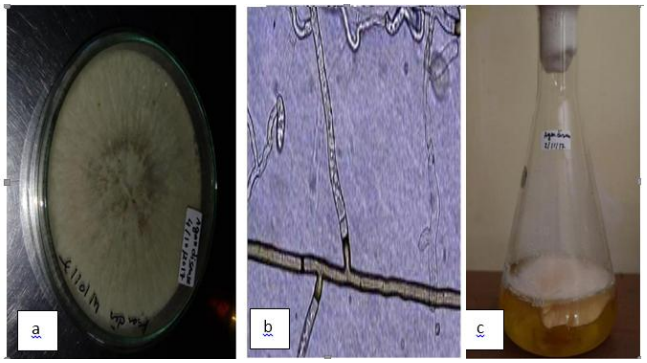

Fig 2: Growth of *Rhizoctonia solani in vitro*

1. on PDA media
2. Microscopic observation
3. growth in liquid media

Isolation of damping-off fungi from infected *Aquilaria* seedlings: Isolation from root portion of *Aquilaria* plants was done on large scale. The fungi isolated were identified on their typical colony characteristics. The pathogen was primarily identified as *Rhizoctonia solani* ITCC No. 10.693.17 on the basis of morphology (Fig. 2). *Rhizoctonia solani* produces white to deep brown mycelium when grown on artificial medium. A septum near each hyphal branch and a slight constriction at the branch (Fig. 2).

Pathogenicity test: Pathogenicity test of *Rhizoctonia solani* was conducted under laboratory conditions by artificially inoculating the steam sterilized soil (Table 1). The maximum number of infected plants was obtained after 30 days of transplanting (DAT) as compared to 15 days transplanting from the infested soil with the disease incidence of 63.63% as compared to un-infested soil where disease incidence was 13.33% (Table 1). Aqueous suspension of whitish brown growth of the fungus sprayed on *Aquilaria* seedlings produced typical symptoms in about 15 days followed by wilting and death of seedlings. The fungus was highly pathogenic on artificial inoculation. Anderson (1985) [3] conducted

pathogenicity of *Rhizoctonia solani* and observed typical symptoms of the fungus on root and lower stem of mungbean.

Table 1: Pathogenicity test of *Rhizoctonia solani* on *Aquilaria* seedlings.

Treatment	Healthy plants		Infected plants		Incidence %)
Infested soil	15 DAT	30 DAT	15 DAT	30 DAT	30 DAT
	35	20	20	35	63.63
Un-infected	59	65	16	10	13.33

Effect of different fungicides on damping-off disease

Different fungicides such as Bavistin, Mancozeb and neem pesticides were applied as soil drench. In the present investigation results showed that the seeds treated with Bavistin (0.1%) gave highest control against the pathogen with 30.65% incidence followed by Mancozeb with 57.81% incidence at 45 days after sowing (Table 2). The highest disease incidence was recorded in control (89.71%). This study was conformed to the findings of Taha *et a*l., (1988) [8-*/7] who suggested that Topsin-M and Bavistin as the most effective fungicides in laboratory test against *Rhizoctonia*.

Table 2: Effect of different fungicides on damping-off disease

	Treatments	% Disease Severity			
		7 DAT	15 DAT	30 DAT	45 DAT
T1	Control	26.11	46.56	69.09	89.71
T2	Neem pesticides 0.2%	11.67	31.89	57.66	78.02
T3	Mancozeb 0.1%	0.00	18.11	39.89	57.81
T4	Bavistin 0.1%	0.00	07.33	18.90	30.65
SEd±		0.268	0.205	0.574	0.258
CD0.05		0.461	0.348	0.986	0.439

Conclusion

Success of any plantation programme depends on production of quality seedlings in nursery. So, occurrence of sudden wilting and mortality of agar seedlings incited by *R. solani* posing potential threat to agar plantation resulting in significant economic loss to the farmers. The outcome of the control experiment against the disease revealed that soil drenching and foliar spray of 0.1% Bavistin as an effective remedy in controlling the disease.

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