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Effect of arbuscular mycorrhizal fungi on damping-off disease in *Aquilaria agallocha* Roxb. seedlings

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Abstract: Damping-off disease of *Aquilaria agallocha* seedlings caused by the pathogenic fungus (*Pythium aphanidermatum*) results in poor regeneration under natural conditions and in the nursery. In the present study, *Glomus fasciculatum*, an arbuscular mycorrhizal (AM) fungus was examined for its ability to reduce the rotting incidence of *Aquilaria* seedlings. Dual inoculations (AM+pathogen) restricted the progression of the pathogen in the root tissues of *Aquilaria* seedlings. Mycorrhizal inoculation not only reduced the percentage of disease incidence, but also significantly increased host plant height, total biomass and dry matter. Our results suggest that inoculation of AM fungi in the rhizosphere soil of *Aquilaria* improves plant-soil interaction by enhancing nutrient status, plant growth, promote co-existence of other microbes and protect the host against pathogens. It could be suggested that the AM inoculation may be viable for commercial plantation of *Aquilaria*, the economically important tree species.

Resumen: La enfermedad de la podredumbre de las plántulas de *Aquilaria agallocha* causada por el hongo patógeno (*Pythium aphanidermatum)* provoca una regeneración pobre tanto en condiciones naturales como de vivero. En el presente estudio se examinó la habilidad de *Glomus fasciculatum*, un hongo micorrícico arbuscular (MA), para reducir la incidencia de la podredumbre de las plántulas de *Aquilaria*. Inoculaciones duales (MA+patógeno) restringieron la progresión del patógeno en los tejidos radiculares de las plántulas de Aquilaria. La inoculación micorrícica no sólo redujo el porcentaje de la incidencia de la enfermedad, sino que también incrementó significativamente la altura, la biomasa total y la materia seca del hospedador. Nuestros resultados sugieren que la inoculación de hongos MA en el suelo de la rizósfera de *Aquilaria* mejora la interacción planta-suelo al mejorar el estatus nutricional y el crecimiento vegetal, y al promover la coexistencia de otros microbios y proteger al hospedador de los patógenos. Se puede sugerir que la inoculación de MA puede ser viable para la plantación comercial de *Aquilaria*, la especie arbórea económicamente importante.

Resumo: O "damping-off" em plântulas de *Aquilaria agallocha* causadas pelo fungo patogénico (*Pythium aphanidermatum*) resultou sob condições naturais e no viveiro numa regeneração pobre. Neste estudo, a *Glomus fasciculatum*, um fungo micorrízico arbuscular, foi examinado quanto à sua aptidão em reduzir a incidência de podridões nas plântulas de *Aquilaria*. Inoculações duais (AM+patogénio) restringiu a progressão do patogénio nos tecidos radiculares das plântulas de *Aquilaria*. A inoculação micorrízica não só reduziu a percentagem da incidência da doença, mas também aumentou, significativamente, a altura da planta hospedeira, a biomassa total e a matéria seca. Os resultados sugerem que a inoculação do fungo AM na rizosfera do solo da *Aquilaria* melhorou a interacção planta-solo aumentando o satus nutricional, o crescimento das plantas, promove a co-existência de outros micróbios e protege as plantas hospedeiras contra patogénios. Pode sugerir-se que a inoculação com AM pode ser viável para plantações comerciais de *Aquilaria* e espécies arbóreas economicamente importantes.

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Key words: *Aquilaria agallocha*, biological control, *Pythium aphanidermatum*, *Glomus fasciculatum*.

Introduction

Aquilaria agallocha Roxb., popularly known as agarwood, aloe wood or eagle wood, belongs to the family Thymelaeaceae and is one of the world's most expensive essential oil yielding tree species. The oleoresin contained in the wood is widely used in the perfume industry and in medicine (Sumadiwangsa 1997). Most of *Aquilaria* species are found under natural population in Southeast Asia (Donovan & Puri 2004). *Aquilaria agallocha* and *A. khasiana* have been reported to grow naturally in the plains, foot-hills of the north-east India (Hajra 2000). Damping-off disease caused by the soil-borne pathogen *Pythium aphanidermatum* has been reported to cause losses of plants in the nursery as well as in the field during early stage after transplantation (Kavita *et al*. 2003).

Use of biological agents as an alternative to synthetic pesticide application to control plant diseases has gained importance in recent years. In this context, the importance of AM fungi colonization for growth and survival of forest seedlings has been widely acknowledged **(**Harley & Smith 1983; Hood *et al*. 2004). The symbiotic association of various genera of AM fungi (e.g. *Acaulospora, Glomus, Gigaspora* and *Entrophospora*) with medicinally important plants **(**Babu & Manoharachary 2003) and their role as biofertilizers for promoting plant growth through increased uptake of water, phosphorus and other nutrients and as a biological control of soil-borne diseases is recognized as well. The present study was conducted to determine the role of AM fungi as biofertilizers for increasing the plant growth and its potential for use as a biocontrol agent against damping-off disease of *Aquilaria agallocha*.

Materials and methods

A. agallocha tree density was measured using 100 x 100 m quadrats. Rhizosphere soil samples were collected from five surveyed locations, *viz*. Balijan, Itanagar, Namdapha, Nirjuli A and Nirjuli B of Arunachal Pradesh, all in northeast India (Fig. 1). The vegetation type of Itanagar and Namdapha were sub-tropical evergreen forest, while Balijan, Nirjuli A and B were of tropical evergreen forest. Plant roots with adhering soil were carefully removed and placed in sterile polythene bags and brought to the laboratory. Rhizosphere soil properties were analyzed following the method of Gupta (2004) and AM fungal propagules were isolated by wet-sieving and decanting (Gerdemann & Nicolson 1963). Spores were collected and counted as given in Reddy *et al*. (2006) and identified up to species level with the help of standard keys (Schenck & Perez 1990). The fungal pathogen *Pythium aphanidermatum* was isolated from the base of stem and soil of infected *Aquilaria* seedlings according to Schmitthener (1962) and Yella *et al*. (2006). The above fungal species, along with aggregated chlamydospores of *Glomus fasciculatum* from pot cultures, were used as inocula.

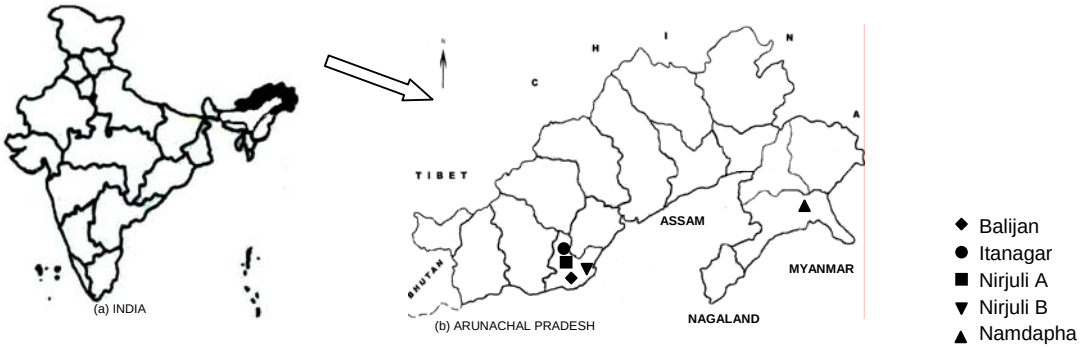

Fig. 1. **(**a) Map of India and (b) of Arunachal Pradesh indicating the study sites.

The experiment was conducted in pots of pasteurized soil in the forest nursery of the North Eastern Regional Institute of Science & Technology, Nirjuli, Arunachal Pradesh. *Glomus fasciculatum* inocula were propagated in pot cultures of maize seedling to develop pure culture in roots. Maize seedlings were grown under greenhouse conditions natural light without temperature or humidity control. After two months, spores and colonized roots of *G. fasciculatum* were examined and 10 g of AM inocula soil and 1 mm size of pathogen inoculum cut by using cob-borer from a juvenile culture were either transferred individually or in combination to the rhizosphere of *Aquilaria* seedling in plastic pots and placed 5 cm below the soil surface. The four treatments (control, only AM fungus [10 spores g^{-1} soil], only pathogen, and AM fungus + pathogen) were replicated five times. Ten *Aquilaria* seedlings were used for studying growth parameters in each of the treatments. No fertilizer was applied to the soils. The pots were irrigated with sterile water and kept at a soil moisture level of 20-30% in a mist chamber. Plants were uprooted on the 45th and 90th days after sowing and evaluated for individual or combined effect of mycorrhizal colonization and pathogenic infection.

The percentage of damping-off disease was assessed as described by Horsfall & Barrat (1945), while percentage of mycorrhizal colonization was calculated by collecting lateral roots from inoculated treatments of *Glomus* and *Pythium* separately or combination of both *Glomus* + *Pythium* in compare to uninoculated (control). The roots were gently washed under tap water, cut into 1 cm sized pieces (25 nos), stained and examined for mycorrhizal colonization using a light microscope (Phillips & Hayman 1970). The percentage of AM infection was estimated by the root slide technique (Read *et al.* 1976). All infected and non-infected segments were counted. The percentage of infection was calculated as

$$\text{AM infection (\%)} = \frac{\text{No. of infected segments}}{\text{Total no. of segments examined}} \times 100$$

Results and discussion

Soil samples were collected from the rhizospheric zone of *A. agallocha* growing in field conditions in Namdapha and Balijan and their physico-chemical properties were analysed (Table 1). In general, slightly acidic soils (pH 6.0 to 6.3) had significantly ($p < 0.05$) greater number of AM propagules, whereas the soils with pH 5.3 - 5.7 had fewer propagules. More spores (613 100 g^{-1} of soil) were recorded in Nirjuli B, which had a 24% soil moisture, and fewer spores (417 100 g^{-1} of soil) at 30% soil moisture level at Namdapha. Over all, it appears that 20-25% soil moisture content is favourable for AM association in the sub-tropical soils of Arunachal Pradesh. Nevertheless, determination of AM fungi present in the rhizosphere soils of *Aquilaria* tree in different regions showed numerous AM fungal species (Table 2). A total of nine AM fungal species belonging to genus *Acaulospora, Gigaspora, Glomus* and *Sclerocystis* were recorded, with the genus G*lomus* being predominant over others. Schalamuk *et al.* (2006) also reported similar results while working with a wheat cultivation system. Only *Gigaspora margarita, G. fasciculatum* and *G. aggregatum* were present in all sites. Analysis of physico-chemical properties of soils of *A. agallocha* seedlings grown under greenhouse conditions under different treatments (Table 3) did not differ much from natural soils collected from different locations (Table 1).

Table 1. Density (nos ± SE; n=5) of *A. agallocha*, abundance of AM propagules and physico-chemical properties of the rhizosphere soil of *Aquilaria* seedlings collected from different sites in northeast India.

Parameters	Sites				
	Namdapha	Balijan	Itanagar	Nirjuli A	Nirjuli B
Density of *A. agallocha*(plants ha^{-1})	2.1±2.59^{a}	0.3±0.00^{b}	0.1±0.00^{bc}	0.4±0.58^{bd}	0.9±2.12^{e}
Moisture content (%)	30.1±1.48^{a}	13.4±0.75^{b}	12.4±1.13^{bc}	22.7±1.65^{e}	24.1±2.35^{f}
pH	5.3±0.27^{a}	5.5±0.33^{b}	5.7±0.32^{c}	6.0±0.63^{d}	6.3±0.31^{e}
Electrical conductivity(dS m^{-1})	0.01± 0.002^{a}	0.03±0.02^{ab}	0.03±0.01^{ac}	0.07± 0.03^{d}	0.09±0.07^{de}
Organic C (g kg^{-1})	16.6±0.72^{a}	13.1±0.68^{b}	12.9±0.52^{bc}	17.5±1.67^{d}	18.0±0.64^{e}
Total N (g kg^{-1})	6.2±0.14^{a}	4.4±0.18^{b}	4.2±0.11^{bc}	6.4±0.20^{ad}	6.5±0.22^{ae}
C/N	2.68±0.58^{a}	2.97±0.50^{b}	3.07±0.41^{c}	2.69±0.47^{d}	2.77±0.42^{e}
Available P (g kg^{-1})	15.4±0.50^{a}	9.0±0.24^{b}	9.0±0.17b^{c}	13.6±0.97^{ad}	13.8±0.46^{ae}
Available K (g kg^{-1})	28.3±1.94^{a}	30.7±2.61^{b}	27.5±2.69^{c}	36.5±2.07^{d}	.5±1.72^{e}
Abundance of AM spores (no. 100 g^{-1} rhizosphere soil)	417±6.11^{a}	520±7.48^{ab}	525±7.13^{ac}	588±9.80^{d}	613±10.91^{de}

Values with different superscripts across each row are significantly different (Oneway ANOVA; $p < 0.05$).

Table 2. Presence (+) and absence (-) of AM fungi species in the rhizosphere soils of *Aquilaria* seedlings from different sites in northeast India.

AM fungal species	Collection sites				
	Namdapha	Balijan	Itanagar	Nirjuli A	Nirjuli B
Acaulospora bireticulata	–	+	–	+	+
Gigaspora margarita	+	+	+	+	+
Glomus aggregatum	+	+	+	+	+
Glomus aureum	–	–	–	+	+
Glomus constrictum	+	+	–	–	–
Glomus fasciculatum	+	+	+	+	+
Glomus geosporum	+	–	–	+	+
Glomus macrosporum	–	–	–	+	+
Sclerocystis clavispora	–	–	–	+	+
Total no. of species	5	5	3	8	8

Table 3. Physico-chemical properties (± SE; n=5) of pot soil of *Aquilaria* seedlings 45 and 90 days after treatment.

Soil property	Treatments							
	Control		*Glomus*		*Pythium*		*Glomus* + *Pythium*	
	0	45	45	90	45	90	45	90
pH	6.0±0.1a	5.3±7.0^{-2}b	5.2±7.0^{-1}c	5.7±0.1d	5.4±0.1e	5.9±0.2f	5.6±0.1g	5.6±0.2h
EC (ds m^{-1})	0.01±2.6^{-3}	0.06±7.1^{-3}	0.08±8.4^{-3}	0.06±1.5^{-2}	0.06±7.1^{-3}	0.03±8.9^{-3}	0.05±8.4^{-3}	0.07±9.5^{-3}
Org C (g kg^{-1})	17.6±1a	14.6±0.6b	12.8±1bc	16.2±0.7ad	15.9±0.5e	16.8±0.9af	16.0±0.4ag	17.4±0.7ah
Total N (g kg^{-1})	9.6±0.7a	6.2±0.7b	4.4±0.5c	7.3±0.4bd	6.8±0.2be	8.5± 0.6af	7.0±0.7bg	9.1±1.2ah
C/N (g kg^{-1})	1.9±0.2a	2.5±0.2ab	3.1±0.3c	2.2±0.5^{-1}ad	2.3±0.7^{-1}ae	2.0±0.1af	2.4±0.3ag	2.1±0.3ah
Avail P (g kg^{-1})	11.2±1a	13.2±0.8b	10.0±1ac	11.7±0.5ad	10.8±0.7ae	11.6±0.5af	11.6±0.8ag	11.2±0.3ah
Avail K (g kg^{-1})	28.3±0.7a	35.4±1.1b	27.4±1.9ac	31.4±1.2d	36.1±1.1be	30.5±1.1df	31.2±1.7dg	31.4±1.6dh

Values with different superscripts across each row are significantly different (one way ANOVA; $p < 0.05$).

Aquilaria agallocha reportedly has a higher probability of poor regeneration due to susceptibility of germinating seeds and young seedlings to damping-off disease caused by soil-borne fungi (Nelsi 2004). In this regard, the predominant AM fungus, *G. fasciculatum*, was examined for its potential to enhance nutrient status and growth, and to protect the host plant against the pathogens. AM fungi penetrate the root system, help uptake of plant nutrition from soil, and change the anatomy and structure of the root system. These changes, together with the activation of the plant defense mechanisms, seem to be responsible for disease reduction (Pozo *et al.* 2002). Recently, Manoharachary (2000) reported the potential of AM fungi as biofertilizers with regard to their capacity to commercial and large scale production of inoculum. A culture of *P. aphanidermatum*, isolated from infected seedlings**,** was used as pathogen inoculum, with maize pot culture of the most dominant AM fungi species, *G. fasciculatum,* serving as biocontrol inoculum. We observed variations in colonization percentage on different days after inoculation in individual colonization, such that there was little variation in the percentage of dual colonization (Table 4). Earlier studies (e.g**.** Manka 1998; Hedge & Rai 1984; Mosse 1973) have indicated that mycorrhizal colonization induces chemical, physiological and morphological alterations in the host plant that may increase host resistance. However, Kaye *et al.* (1984) also observed that adequate contents of plant nutrients (nitrogen, phosphorus and potassium) in the soil were favourable for mycorrhizal colonization; on the other hand, the rich nutrients in soil are not conducive for the growth of soil-borne pathogens.

The seedlings inoculated with mycorrhiza alone and dual inoculations showed a gradual increase in the percentage of root colonization by *G. fasciculatum* with seedling age. The seedlings were unequally colonized at both the stages of plant growth, i.e. 45th and 90th days. The root colonization with individual mycorrhiza and dual

inoculum was 73% and 53.8%, respectively, 90 days after inoculation (Table 4). On the other hand, the seedlings inoculated only with the pathogen showed heavy incidence of damping-off (0% control). In the dual inoculated seedlings, the progress of the pathogen was suppressed (65% control), consequently reducing the severity of the damping-off disease by 35%. Sullivan (2004) stated that AM fungi protect plant roots from diseases in several ways: by providing a physical barrier, producing antibiotics and other toxins, changing the amount and type of plant root exudates against the invading soil-borne pathogens. Inoculation with AM fungi not only reduces soil-borne pathogens but also stimulates seedling growth (Chakravarty & Mishra 1986). Recently, Reddy *et al*. (2006) reported that inoculation of fungal symbionts in the rhizosphere zone of tomato plants exhibited increased resistance to damping-off disease. The potential of AM fungi for biological suppression of soil-borne pathogens gives a better option to develop an alternative strategy for host plant disease management with biological agents.

Table 4. Percentage (± SE; n=5) of root colonization by *Glomus fasciculatum* in *Aquilaria* seedlings.

Treatments	Days after inoculation	
	45	90
Mycorrhiza	51.0±12.58[a]	73.0±13.40[b]
Mycorrhiza+*Pythium*	40.8±7.63[a]	53.8±11.01[b]

Values with different superscripts across each row are significantly different (oneway ANOVA; $p < 0.05$).

The plant height, biomass and dry matter of seedlings inoculated with *Glomus* were 27.45 cm, 7.6 g & 5.5 g respectively; likewise, the data for the *Glomus+Pythium* inoculated seedlings recorded 26.69 cm, 6.4 g & 5.1 g respectively (Table 5). Evidently, there were significant ($p < 0.05$) differences in the effect of microbial incoluation in *A. agallocha* seedlings. Although we employed a limited number of replicates, our results show that mycorrhiza not only act as a good biocontrol agents but also improve plant nutrition status, growth and development of *A. agallocha*. This is in accordance with the findings of Moora *et al*. (2004), who reported that seedlings of species *Pulsatilla* inoculated with AM fungi showed better growth and development. Therefore, the introduction and consequent management of symbiotic AM fungi colonization in the roots of economically important tree seedlings could be employed for effective controlling the root disease by biological means, however, after validating the AM fungus-host-pathogen combination.

Table 5. Effect of microbial inoculation on plant height, fresh and dry weight of *Aquilaria* seedlings (± SE; n=5).

Treatments	Plant height (cm)	Fresh biomass (g)	Dry weight (g)
Pythium	24.66±4.97[a]	4.6±1.10[a]	3.5±1.22[a]
Glomus	27.45±1.06[b]	7.6±1.03[b]	5.5±1.08[b]
Glomus+ *Pythium*	26.69±1.33[bc]	6.4±0.96[bc]	5.1±0.59[bc]
Control	24.98±1.88[ad]	5.3±0.43[d]	4.3±0.84[d]

Values having different superscripts across each column are significantly different at $p < 0.05$.

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