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Biological control of *Heortia vitessoides* Moore, the most serious insect defoliator of *Aquilaria malaccensis* Lamk., a commercially important tree species of Northeast India

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ABSTRACT

Agar tree (*Aquilaria malaccensis* Lam., Family- *Thymeleaceae*) has drawn unique position due to the production of world's most expensive essential oil used as perfume. This paper deals with the ease & problems associated with large scale propagation of agar plant and biological control of *Heortia vitessoides* Moore (Pyrilidae: Lepidoptera), a serious insect pest that severely damages its seedlings very often. Germination test performed with 4500 seeds for consecutive 7 days showed 93%, 75%, 44.8%, 40.6%, 33%, 29.6% and 14.4% germination with 73.61, 53.37%, 49.60%, 31.52%, 23.66%, 31.80% and 35.70% survival respectively. After three months, average height (15.83 ± 1.45 cm to 25.83 ± 1.71 cm) and average numbers of leaves (8.33 ± 0.88 & 12.5 ± 0.28) of seedlings were measured. Overall, only 42.75% seedlings could survive due to flood followed by grasshopper attack and *H. vitessoides* infestation during the study period. Biological control approach was adopted to manage this insect-pest using concentrated chilly, garlic and onion extracts @ 1kg/10 liter and fungal spore suspensions (*Trichoderma viride*, *Aspergillus flavus*, *Metarhizium anisopliae* *Beauveria bassiana*) @ approximately $2.2-2.4 \times 10^6$ spores/ml. Chilly, *M. anisopliae* and *B. bassiana* could control insect population above 90%. Onion/garlic were less effective (40-50%), *Aspergillus flavus*/*Trichoderma viride* had least (>20%) or no effect at all Chilly may be used as effective, eco-friendly and economic bio-control agent in the field.

Keywords: *Aquilaria malaccensis*, large-scale propagation, insect pest, biological control

INTRODUCTION

Agar (*Aquilaria malaccensis* Lamk., family *Thymelaeaceae*) is an exceptional tree species that is known in the world due to the presence of high quality and world's most expensive essential oil extracted from its resinous heartwood. As a result of defense mechanism to send off pathogens, *Aquilaria* species develop agarwood which can be used for incense, perfume, and traditional medicines. Agarwood, eaglewood, gaharu, aloe wood are just a few local names used for the resinous, fragrant and highly valuable heartwood produced by *Aquilaria malaccensis* and other species of the Indo-Malaysian tree genus *Aquilaria*. The natural habitat of this species mainly confined to South and Southeast Asia. One of the earliest supplies of agarwood is believed to have originated from the Indian hills of Assam, sourced from *Aquilaria agallocha* since soils of upper Assam are found to be most suitable for production of quality agar products [1]. The high prices demanded for agarwood has led to the rapid depletion of *Aquilaria* trees from the natural forests. The search for agarwood has spread from one country to another. At

present, Indonesia and Papua New Guinea are the main suppliers of agar products. Due to degradation of natural forests at an alarming rate and excessive exploitation, agar plants are losing their natural habit and habitats. This has brought agar plant under endangered species [2] whereas categorized critically endangered in India but at the same time its cultivation has increased many folds especially in upper Assam districts and Arunachal Pradesh of NE India as people realized the market strength of this species.

Agar products from the Northeast India are considered to be the best in the world market and fetch highest price [3]. It is also considered as sacred plant and for its very high price, it is termed presently in trade as 'liquid gold' or 'Woods of the God' [4]. It can grow on a wide range of soils. Seedlings need a lot of shade and water. Trees grow fast and start producing flowers and seeds as early as seven years old. The tree is valuable only after its heartwood is infected by certain group of fungi [5]. The increasing demand and easy propagation have tempted many people of northeast India to take up a promotional agar planting in the private sector on large scale. In future, agarwood plant industry may be the most profitable rural industry in northeast India especially in Arunachal Pradesh. *Aquilaria* seeds are recalcitrant in nature and lose viability within few days hence investigations were carried out to standardize propagation timeframe and understand associated ease/problems for large scale production of agar seedlings in the state of Arunachal Pradesh, India.

Agar tree easily can be propagated by its seeds however rapid loss of viability is reported as a result of drop in moisture contents of seeds below 20% [6]. The seeds of *A. malaccensis* are very sensitive to heat, moisture and soil conditions. The young seedlings of agar plant are prone to insect and pest attack and attacked by *Heortia vitessoides* Moore (Pyralidae: Lepidoptera), a major defoliator which damage its leaves, vegetative buds and tender shoots causing heavy loss. *H. vitessoides* is a moth of Lepidoptera family Crambidae. This insect is distributed in South-east Asian countries, including India, Fiji, Hong Kong, Taiwan, Thailand and northern Queensland in Australia. Its larvae have been recorded feeding on leaves of *Rhus* and *A. malaccensis* plants. In northeast region of India, it has been established as a serious insect pest and leaf defoliator of agar plant. They live jointly in a shelter made by joining a number of leaves together with formation of silk and cause serious damage to *Aquilaria* plant especially in young stages. Sometimes, it kills the *Aquilaria* seedlings by feeding on all leaves and young twigs of the seedlings.

Literature survey reveals that the information regarding this insect pest is very poor. At times, grasshoppers also cause severe loss to this plant especially to young ones. Therefore an attempt has been made to manage *H. vitessoides* by means of biological control. The results of large scale propagation and biological control of *H. vitessoides*, major leaf defoliator of *A. malaccensis* are presented and discussed in this paper.

MATERIALS AND METHODS

Seeds of agar tree were collected during the months of July, 2012 from the agar tree growing home gardens/plantations of Hojai and Golaghat districts of Assam and from Itanagar capital complex of Arunachal Pradesh, India. The seeds were propagated in the forest nursery of North Eastern Regional Institute of Science and Technology (NERIST), Nirjuli, Arunachal Pradesh. A total of 4500 viable seeds (checked through floating test) were used of which 1000 seeds each were sown on 3rd & 4th day and 500 seeds each were sown on 5-9th days after collection to check the germination percentage. Seedlings were transferred into poly bags within 30 days of sowing. Their growth rates were recorded by randomly selecting 100 seedlings from each seed beds for the period of three months consecutively at an interval of one week. In the months of August and September, 2012, heavy infestation of *H. vitessoides*, an insect pest and specific defoliator of agar plant was observed. To control this pest, biological control approach was adopted by the application of concentrated plant extracts and fungal spore suspensions. Green chili (*Capsicum annum*), garlic flakes (*Allium sativum*) and peeled onion bulbs (*Allium cepa*) extracts (grinding 1kg material to obtain 10L extract) and spore suspensions of *Trichoderma viride*, *Aspergillus flavus*, *Metarhizium anisopliae*, *Beauveria bassiana* ($2.2-2.4 \times 10^6$ spores/ml) were applied as bio-control agents. Sterilized water was used as control. Chili, garlic and onion are well known for their antimicrobial activities, *Trichoderma viride*, a well known bio-control fungus, *Beauveria bassiana* and *Metarhizium anisopliae*, best known entomopathogenic fungi, were tested (Table I). All bio-control agents/materials were tested on triplicates of 25 infected seedlings. The application of vegetable extracts/fungal suspensions were applied in the nursery at an interval of three weeks in 3-4 consecutive applications and results were recorded.

RESULTS

Germination took place between 6th–21st days in total 4500 *Aquilaria* seeds sown in the forest nursery of NERIST, Arunachal Pradesh, India. The germination percentage decreased sharply with seed's storage period and approximately 93%, 75%, 44.8%, 40.6%, 33%, 29.6% and 14.4% germination from 3rd to 9th days after collection respectively was recorded (**Figure I**). Seedlings were transferred into poly bags when they developed 2-3 leaves. Growth records after three months period showed average height of seedlings between 15.83±1.45-25.83±1.71cm (**Table II**). The seeds sown on 3rd day showed highest growth in height (25.83±1.71cm) as compare to 4th day (21.43±1.53cm) and 5th day (20.30±2.28cm) respectively with least height growth in seeds sown on 9th day (15.83±1.45cm). Maximum average number of leaves was recorded (12.5±0.28) in case of seeds sown on 3rd day followed by 4th day (11.66±0.33) and likewise the minimum numbers of leaves were documented in the seeds sown on 9th day (9.83±1.09).

Table I: Medicinal properties/activities of agents used for bio-control of *H. vitessoides* Moore

Name of bio-control agent	Classified under		Medicinal uses/properties	Part used
	Division	Family		
A. Plants used				
<i>Capsicum annuum</i> (Common name: Chilly, Mirch)	Dicotyledons	Solanaceae	Powerful stimulant, antibacterial, carminative, used in flatulent dyspepsia, lumbago, neuralgia, rheumatism	Fruits
<i>Allium sativum</i> (Common name: Garlic, Lahsun)	Monocotyledons	Alliaceae	carminative, gastric stimulant, antibacterial, antiseptic and used in blood pressure, cough, fever, rheumatism, skin diseases, intestinal disorders,	Bulbs
<i>Allium cepa</i> (Common name: Onion, Pyaz)	Monocotyledons	Alliaceae	Stimulant, antibacterial, expectorant, aphrodisiac, used in flatulence and dysentery	Bulbs
B. Fungi used				
<i>Aspergillus flavus</i>	Ascomycota	Trichocomaceae	Antagonistic fungi, antibacterial	Spore - suspension
<i>Beauveria bassiana</i>	-do-	Cordycipitaceae	Entomo-pathogenic fungi	-do-
<i>Metarhizium anisopliae</i>	-do-	Clavicipitaceae	Entomo-pathogenic fungi	-do-
<i>Trichoderma viride</i>	-do-	Hypocreaceae	Bio-control agent	-do-

Table II: Effect of storage period on growth characteristics of seedlings after 3 months

Sowing day	Average height (cm) of seedlings	Average number of leaves/seedling
3 rd	25.83±1.71	12.5±0.28
4 th	21.43±1.53	11.66±0.33
5 th	20.30±2.28	11.33±0.88
6 th	20.10±0.20	10.83±0.16
7 th	17.93±1.27	10.66 ±0.72
8 th	17.26±1.63	8.33 ±0.88
9 th	15.83±1.45	9.83±1.09

Values in the parenthesis indicates mean ± SE of 3 replicates

Table III: Treatment of Chili, *A. sativum* (garlic), *A. cepa* (onion), *Trichoderma viride*, *A. flavus*, *M. anisopliae*, *B. bassiana* and on control of *Heortia vitessoides* Moore. in laboratory conditions

	Chili	<i>Allium cepa</i>	<i>Allium sativum</i>	<i>Trichoderma viride</i>	<i>Aspergillus flavus</i>	<i>Metarhizium anisopliae</i>	<i>Beauveria bassiana</i>	Control
No. of <i>H. vitessoides</i>	10	10	10	10	10	10	10	10
Survivality	All killed	4 killed	5 killed	No effect	2 killed	9 killed	All killed	No effect
Survival %	100%	40%	50%	0%	20%	90%	100%	0%

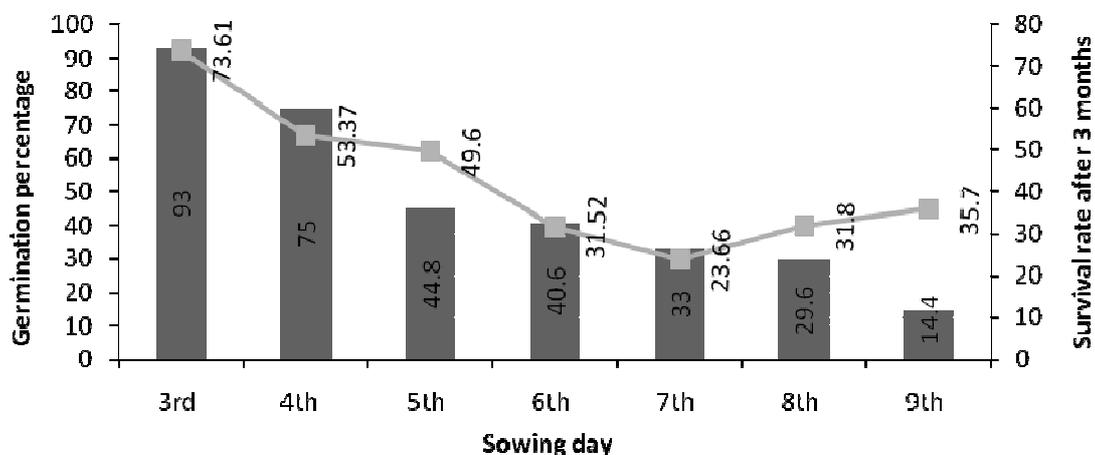


Figure I: Effect of storage periods on seed germination characteristics of *Aquilaria malaccensis* Lamk.



Plate I: Photographs showing infected seedlings and leaves with *H. vitessoides* Moore and healthy seedlings in nursery

When seedlings were about one month old, heavy loss was caused to them due to rains followed by grasshopper's attack during study period. In addition, seedlings were also attacked by *H. vitessoides*, an insect pest and defoliator of *Aquilaria malaccensis* (Plate I). As a result, only 42.75% seedlings could survive but surprisingly seedlings grown from comparatively longer storage period of 7th-9th days could sustain flood condition and insect attack better. The survival percentage per seed bed was recorded as 35.7%, 31.8% and 23.66%, 31.52%, 49.60%, 53.37% and 73.61% respectively (Figure I).

An attempt was made to manage *H. vitessoides* through biological control. Biological activity of plant extracts and their application as ecologically harmless bio-pesticide is gaining importance in the management of plant diseases. Concentrated green chili, garlic and onion extracts (1kg fruits/bulbs grounded to make 10 liter extract) and fungal spores suspensions of *Trichoderma viride*, *Aspergillus flavus*, *Metarhizium anisopliae* and *Beauveria bassiana* ($2.2-2.4 \times 10^6$ spores/ml each) were used. The application of chili extract showed highly affirmative results by controlling 100% insect pest population under laboratory conditions (Plate II) where as 90.75% seedlings were found free of insects up to next 15-20 days after first spray in the nursery conditions. Application of garlic and onion extract was also effective (50 and 40% respectively) to control *H. vitessoides* (Table III). Chili extract application could be able to control *H. vitessoides* population in the lab and nursery initially but they again infected the seedlings after 20 days of application in the nursery. The treatments repeated consecutively for 3-4 times (as required) could check the further infestation of this pest completely as it was not seen until next rainy season.

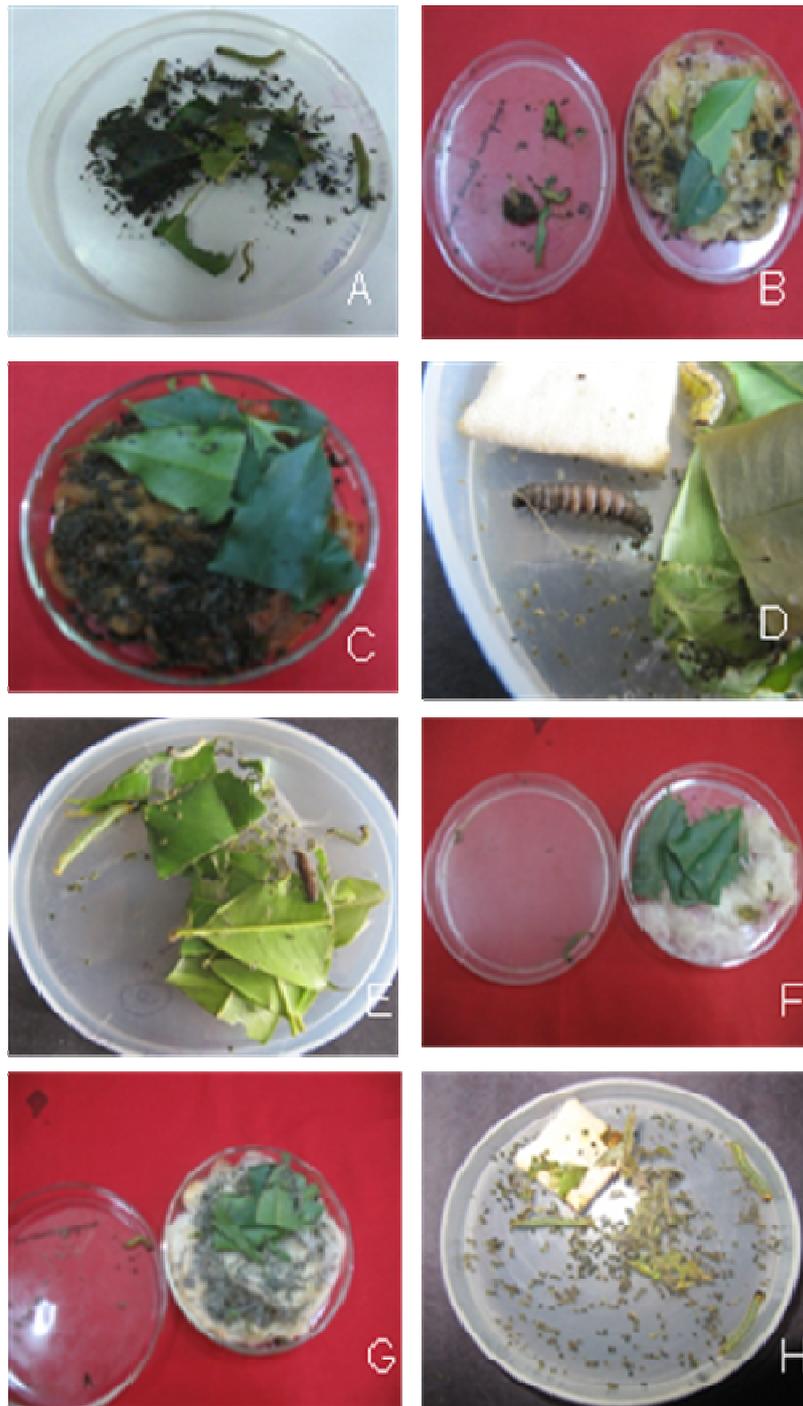


Plate II: Effect of different entomopathogenic fungi and plant extracts on control of *H. vitessoides* Moore. A. *Trichoderma viride*; B. *Aspergillus flavus*; C. Chili (*Capsicum annum*); D. *Metarhizium anisopliae*; E. *Beauveria bassiana*; F. Garlic (*Allium sativum*); G. Onion (*Allium cepa*) and H. Control

Under laboratory conditions, the spray of fungal spore suspension of entomopathogenic fungi *B. bassiana* and *M. anisopliae* was also found very effective as bio-control agents against *H. vitessoides* with 100% and 90% (**Table III**) control respectively. *Aspergillus flavus*, an antagonistic fungus appeared as a weak (20%) bio-control agent against this particular insect pest whereas *Trichoderma viride* could not control it at all. Two entomopathogenic

fungi also tested in the nursery gave 84% and 76% control respectively. After 3-4 consecutive applications at the intervals of three weeks, no caterpillars were noticed in the treated seedlings.

The morphological features and life cycle of *H. vitessoides* Moore was also studied in brief (Plate 1 & 2). The caterpillars of this species were pale green in color with a broad knobby black line along each side. Their head was brown. The caterpillars drop on silk threads, if disturbed. When mature, the caterpillars descend and pupate in the soil. Adults showed a striking pattern of black or pale yellow on the forewings. The hind-wings were white with a broad black margin. Its moths had a yellow and black banded abdomen. The wingspan was measured about 3 cm. Their eggs were yellowish-green in color and flattened in nature. They lay eggs in an overlapping cluster like tiles on a roof.

DISCUSSION

In our mass scale propagation experiment with *Aquilaria* seeds, only 21 days was required to complete germination of 4500 seeds as compare to 35 days [7], 40 days [8] and 45 days [9] recorded earlier. The germination of *Aquilaria* seeds was highly influenced by storage period of seeds after collection even if delayed by one day. In the present studies, germination decreased sharply (**Figure I**) from 4th to 7th day's stored seeds (53.37% – 23.66%) as compared to 3rd day (73.61%). *Aquilaria* seeds are highly prone to desiccation. Seed storage directly influenced growth parameters (height and leaf number) in the growing seedlings (**Table II**). Decreased seed viability is mainly attributed to decreased seed moisture whereas seed germination and establishment may be correlated to light intensity [7, 8]. These workers reported recalcitrant nature of *Aquilaria* seeds with maximum germination percentage in fresh sown seeds (92.0 %) followed by 5days (39.6 %) and 10 days (4.0 %) stored seeds but no germination in 15 days stored seeds. Its young seedlings are sensitive to abiotic and biotic factors such as heat, light, moisture, flood, drought, insect pests and fungal attacks [10, 11] hence greatly affect survival and establishment. Most of these factors badly affected our trial and resulted with 42.75% survival after three months. However, an increased survival % in the seedlings developed from 8th & 9th days stored seeds (**Figure I**) could be due to hardiness developed by the seeds to combat decreased moisture contents during storage hence were able to protect themselves better during stress period.

Use of plant extracts and entomogenous fungi as bio control agents are effective tools (**Table 1**) to prevent insect pests and diseases; however, their effectiveness depends on dosage and exposure periods. *Aspergillus flavus* was tested as another antagonistic fungus reported to control pathogenic bacteria [12] earlier. *H. vitessoides*, a specific defoliator of Agar plant had caused heavy infestation and loss to the seedlings during study period (**Plate I**). The application of chilies showed positive response up to 20 days after first spray and could control *H. vitessoides* completely in further 3-4 applications (**Table III, Plate II**). The recurrence of insect infection in the nursery may be due to the availability of insect pest in the locality on other individuals already growing in the neighborhood and favorable weather. The preference of *Aquilaria* plant by *H. vitessoides* has been well established [10]. Similar results were also obtained by earlier workers [13]. Application of garlic and onion extract was also effective up to some extent in control of insects.

Entomogenous fungi are known to be more specific target organisms under field condition, especially during epizootic [14] and this specificity evidently reduces the level of hazard to non-target organisms; however, the differences in the response by the different insect pest species may be attributed to the morphological and behavioral differences between the insects [15]. A study on pathogenicity of entomopathogenic fungi *Beauveria bassiana* on *H. vitessoides* of *Aquilaria agallocha* was done earlier [16]. Spray of *Metarhizium anisopliae* and *Beauveria bassiana* spores in the laboratory showed best results to control *H. vitessoides* caterpillar whereas, *Aspergillus flavus* had appeared as a weak agent against it. The *Trichoderma viride* spores concentration used in the experiment might be inappropriate ($2.2- 2.4 \times 10^6$ spores /ml) to control this insect hence need more trials.

From these investigations, it may be concluded that germination percentage of *Aquilaria* seeds and survival rate of seedlings depend upon various factors like heat, moisture contents of seed, soil conditions, soil moisture and also on insect pest attack. Agar seedlings are highly susceptible to *H. vitessoides*, a specific leaf defoliator. This insect pest of Agar plant may effectively be controlled through simple biological control approaches i.e. by the application of green chili extract at the intervals of 15-20 days in 3-4 applications during infestation period even in the field conditions. The fresh green chilies are available during this period in plenty and easily affordable in terms of money. Botanical extracts are effective as protectant without hampering non target organisms. Spray of microbial pesticides

also showed satisfactory results in controlling the insect pest. The experimental results proved that the bio-pesticides i.e. plant extracts and microbial formulations can be used as an alternate control method in controlling this serious insect pest of *A. malaccensis* effectively.

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