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Association of *Zeuzera conferta* Walker on agarwood formation in *Aquilaria malaccensis* Lamk.

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ABSTRACT

Agarwood (Aquilaria malaccensis Lamk.) is a one of the important non-timber forest species of North East India which on microbial infection produces fragrant resinous wood and agar oil, of tremendous market value. Agarwood and agar oil is extensively used in perfumery and pharmaceutical industries. So far, the research on A. malaccensis has been mostly on the role of microbes in agar production, specially the process of conversion of the healthy, non-infected trees to infected agar oil bearing tree. However, no concerted efforts have been made so far to study the role of Zeuzera conferta, a borer insect often found to be associated with the phenomena of agarwood production. In the present study, the association of this important insect on agarwood formation has been established. The borer insect (Z. conferta) is found to be habitually associated with the agarwood trees (23.93 per cent) where agar formation takes place and 69.91 per cent of harvested trees with agar formation of good quality. A new area has been opened up through this study and research efforts needs to be focused on bio-ecology of Z. conferta and its use for possible artificial infestation and agar production.

Key words: *Aquilaria malaccensis* Lamk., agar oil formation, *Zeuzera conferta* Walker.

INTRODUCTION

In India, agarwood tree (*Aquilaria malaccensis* Lamk.) is distributed in all the North Eastern States [1]. Agarwood is the myth of perfumery world. Agar and agar oil are the most exalted perfumery and fragrance raw materials obtained from the infected wood of agarwood tree (*Aquilaria malaccensis* Lamk. Syn. Name *Aquilaria agallocha* Roxb. of Thymelaeceae family), known locally in North East India as "Sanchi Plant". *Aquilaria malaccensis* is a medium to large tree found in Eastern Himalayas from Bhutan to North Eastern India up to altitude of 1200 meter [2] [11]. This is an important species in commerce and valued for its resinous dark coloured wood known in trade as agar which is used as incense [3]. Apart from the perfumery and fragrance value, agar and agar oil are widely used in preparations of various cosmetic products and in Unani & Ayurvedic medicines [4]. The black resinous solid product is developed as a result of fungal infection of agarwood trees in its whole life span [5-9]. The process of agarwood formation is very slow and production of agar as compared to the market demand is very less. Because of availability of the *Aquilaria* species in natural stands, agarwood trade in North east India is presently a fascinating industry. Professional agarwood collectors are experienced and able to identify the agar bearing tree whereas unskilled personnel cut the trees indiscriminately placing the species at a threatened category [10]. The agar oil (the essential oil), the products of this wonder tree are still regarded as the invaluable gift of nature since the tree does not contain any perfumery oil glands or cells like any other essential bearing plants, but the products are formed when infected with microbes or falls sick (diseased) or injured. The percentage of such diseased agarwood tree in natural plantation is very less (<30%). There is an important role of a borer insect called *Zeuzera conferta* Walker in inducing disease and formation of resin in agarwood [8], [11], [12]. In nature, the infection of fungi in agarwood plant is only observed when the tree is bored & infested by the trunk borer, *Zeuzera conferta* Wlaker. The larvae of *Z. conferta* make vertical tunnels in the tree which is the initial site of infection of fungi and gradually moves

upward spreading the infection and accumulating the oleoresins in the infected area. In absence of the borer, *Z. conferta*, mechanical injury to the plant also cause infection in the injured part and oleoresinous substances are accumulated but could not spread further beyond the mechanically injured portion of the tree [13]. Although, many efforts have been made to artificially inoculate the fungi in the tree for getting infection [14], [15], [16], the quality of agar produced from artificial inoculation is not at par with the naturally occurring agar. Therefore, along with the intensive efforts made on artificial induction of fungi in agarwood plant in many parts of the world, it is also equally important to study about the role of *Z. conferta* in disease infection and formation of agar in *A. malaccensis.* In the present study, efforts have been made to survey the agar (resin) formation in *A. malaccensis* in relation to the tree borer, *Z. conferta* infestation in North Eastern region of India.

MATERIALS AND METHODS

The study was carried out during 2012-14 in North Eastern part of India and divided in to two categories as follows.

Incidence of *Z. conferta* in Agarwood plantations

A survey was conducted in all the seven states under North Eastern Region of India *viz.* Arunachal Pradesh, Assam, Meghalaya, Manipur, Mizoram, Nagaland & Tripura in natural forest and manmade plantations for prevalence of *Z. conferta*. Survey sites were selected based on the experienced harvesters' ethnobotanical knowledge along with information on historical patterns of harvest and trade. Harvested and unharvested populations are considered equally relevant, and both standing and felled trees are therefore sampled for the incidence of *Zeuzera conferta*. The records were also made on plantation age, time of infestation and stand composition (pure and mixed species) to find out possible relationship with infestation of *Z. conferta*. All the surveyed trees were divided in to three groups' *viz.* below 8 years, 8-16 years and above 16 years.

Evidence of *Z. conferta* infestation in harvested agarwood trees

The studies on evidence of *Z. conferta* infestation was made on harvested *A. malaccensis* plants in agar processing centre in four locations (Hojai, Naharoni, Tilikiam and Namti) in the state of Assam. The study could not be attempted in other states owing to non-availability of such major agar processing centre in Arunachal Pradesh, Meghalaya, Manipur, Mizoram, Nagaland and Tripura. The harvested agarwood trees brought for processing were carefully examined after splitting the stems lengthwise. Although age of the plants could not be ascertained, the plants were divided in to three groups *i.e.* below 8 years, 8-16 years and above 16 years, based on the best knowledge of agarwood collectors. These harvested trees are either from natural vegetations or from cultivated areas. The observations and records were made on the bore holes of the insect *Z. conferta*, presence of larvae of the insect and or evidence of distinct tunnels made by the borer insect, which were considered as positive indication of the infestation of *Z. conferta.* Formation of resinous lesions around the tunnels and from other injuries on the stem was carefully examined and noted. The numbers of plants having resinous lesions with insect injuries and without insect injuries are recorded. The quality of agarwood depends on the duration of fungal infection and infestation of *Z. conferta* which was ascertained with the experienced agarwood processor, in the respective processing centre.

RESULTS AND DISCUSSION

A total numbers of 3824 *A. malaccensis* Lamk. trees were examined and observation were made on the incidence of *Z. conferta* in mixed and pure plantation, and in harvested trees brought for processing to agarwood processing centres.

Incidence of *Z. conferta* in Agarwood plantations

The observations made in seven different states of North Eastern India on agarwood plantations in respect to the infestation of *Z. conferta* are presented in Table 1 with respect to each state (locality).

Arunachal Pradesh : *A. malaccensis* Lamk. grows normally on the foothills of Kameng, Lower Subansiri, Siang, Changlang, Lohit and Tirap districts of Arunachal Pradesh. The natural density of this plant was 0.73-0.77% which has been diminishes at a very high rate [17]. In the present study we have surveyed only 125 plants of *A. malaccensis* Lamk. and 8.8% of the standing trees were found to be infected with *Z. conferta.* 4.80 per cent infested trees are aged between 8 – 16 years and 4.00 per cent trees were of above 16 years of age. However, within the group, the infestation percentages of *Z. conferta* are 8.70 and 50, respectively. There was no tree below 8 years found in the forest of Arunachal Pradesh in present study.

Assam : The per cent of infested trees in the state were recorded to be 26.46 out of which 83.84 per cent belongs to the age group of 8-16 years. The incidence of *Z. conferta* within the group of 8-16 years is 37.05 per cent. The population of trees which attained more than 16 years is low and only 12.26 per cent trees are infested with *Z.*

conferta when calculated amongst group. It was also revealed that, 9.38 per cent of trees below 8 years are also got infested by the insect.

Manipur : During the present investigation, only 45 numbers of trees could be traced and out of which 6.67 per cent of trees in the age group of 8-16 years are infested with *Z. conferta*. The infestation percentage of the infested trees within the age group is observed to be only 9.38.

Meghalaya : Out of the 65 tress examined in the state, the incidence borer, *Z. conferta* is recorded only in 7.69 per cent of trees with age group of 8-16 years (11.76%) and above 16 years (6.25%).

Mizoram : During the study, only 32 numbers of agarwood plants were surveyed for the availability of *Z. conferta*. It was observed that, the borer is available in the state and 9.38 per cent of trees are infested by the insect in the age group of 8-16 years. The infestation percentage within the trees of the group 8-16 years is 18.75 per cent.

Nagaland : In the present study, 52 numbers of trees were examined for the incidence of *Z. conferta* and observed that 13.46 per cent of trees are infested with the borer insect. Out of the infested trees, 13.51 per cent of trees belong to age group of 8-16 years and 28.57 per cent of above 16 years of age.

Tripura : 255 trees were examined in the state and 10.20 per cent of trees found to be infested with *Z. conferta*. 73.08 per cent of infested trees are of 8-16 years of age. The infestation percentages within the group are 19.79 and 30.43 in 8-16 years and above 16 years, respectively.

Infestation of *Z. conferta* in relation to different age group of *A. malaccensis* Lamk.

A very young plant of *A. malaccensis* Lamk., below 5 year was not found to be infested by *Z. conferta*. The plant attaining an age of 5 years or so may get infested with the borer. The percentage of infestation of *Z. conferta* in different age group of plantation has been presented in Figure 1. It is revealed from the investigation that only 7.14 per cent of tree below 8 years have been infested by the insect. The maximum infestation (34.13%) of borer was observed in the examined trees of age group 8-16 years. 13.40 per cent of trees which attained above 16 years are also have evidence of insect infestation.

Table 1. Infestation of *Zeuzera conferta* Wlk. in *A. malaccensis* Lamk. in North Eastern States of India

State	Sample size	Total Infestation (%)	Age group wise Infestation of *Z. conferta* (%)		
			< 8 years	8-16 years	> 16 years
Arunachal Pradesh	125	8.80 (17.26)	0.00 (00.00)	8.70 (17.16)	50.00 (45.00)
Assam	3250	26.46 (30.98)	9.38 (17.85)	37.05 (37.52)	12.26 (20.44)
Manipur	45	6.67 (15.00)	0.00 (00.00)	9.38 (17.85)	0.00 (0.00)
Meghalaya	65	7.69 (16.11)	0.00 (00.00)	11.76 (20.09)	6.25 (14.54)
Mizoram	32	9.38 (17.85)	0.00 (0.00)	18.75 (25.70)	0.00 (0.00)
Nagaland	52	13.46 (21.56)	0.00 (00.00)	13.51 (21.56)	28.57 (32.33)
Tripura	255	10.20 (18.63)	0.00 (00.00)	19.79 (26.42)	30.43 (33.46)
Mean		**19.63**	**2.55**	**23.76**	**20.82**
SD (±)		**5.42**	**6.75**	**7.04**	**17.24**
Probability output (percentile)			**16.67**	**83.33**	**50.00**

- *The infestation percentages shown against different age groups are within the group.*
- *The figures within parenthesis are Arc Sine transformation values of percentages.*
- *Analysis of variance shows significant ($F=7.09^{**}$) difference in incidence of Z. conferta between the three age groups of plants.*
- *Analysis of variance shows non-significant (F=0.64) difference in incidence of Z. conferta between the states (locality).*

The present study also revealed that, there is no significant difference in incidence of *Z. conferta* between the localities but a significant difference has been observed (Table 1) between the three age group under consideration.

Relative infestation of *Z. conferta* in pure and mixed plantation of *A. malaccensis* Lamk.

During the present investigation, it was observed in all the state that the incidence of *Z. conferta* in *A. malaccensis* varies with the stand composition (Pure or Mixed plantation) as presented in the Table 2 and Figure 2. The infestation in mono-cultured plantation (pure species) is found to be higher (25.09%) compared to 22.28 per cent in

mixed plantation of *A. malaccensis* with other tree species. However, the effect of mixed and pure plantation on incidence of *Z. conferta* in agarwood trees is not-significant.

Table 2. Relative infestation of *Z. conferta* in pure and mixed plantation of *A. malaccensis* Lamk

Plants stand composition	Total sample size	Infestation of *Z. conferta* (%)			
		Age Group of *A. malaccensis*			
		< 8 years	8 – 16 years	> 16 years	Total infestation (%)
Mixed	1580	3.77 (11.24)	33.33 (35.24)	10.41 (18.81)	22.28
Pure	2244	9.06 (17.56)	34.68 (36.09)	16.31 (23.81)	25.09

- *The figures within parenthesis are Arc Sine transformation values of percentages.*
- *F-test for two tailed probability (F=0.7424) showed that the incidence of Z. conferta in pure and mixed plantation is not significantly different.*
- *t-Test: Paired Two Sample for Means exhibited non-significant positive correlation (Pearson Correlation : 0.9997*

Table 3. Incidence of resinous agar lesions with and without *Z. conferta* infested tunnels

Name Agarwood processing centre	Sample size	Resinous agar lesions		Age group wise resinous agar lesions in harvested trees					
				< 8 years		8-16 years		> 16 years	
		With *Z. conferta* infested tunnels (%)	Without *Z. conferta* infested tunnels (%)	With insect infestation (%)	Without insect infestation (%)	With insect infestation (%)	Without insect infestation (%)	With insect infestation (%)	Without insect infestation (%)
Hojai	850	67.41 (55.18)	32.59 (34.82)	2.40 (8.91)	1.87 (7.92)	14.22 (22.14)	8.22 (16.64)	8.84 (17.26)	2.22 (8.53)
Naharoni	575	74.78 (59.87)	25.22 (30.13)	1.56 (7.27)	1.42 (6.80)	9.56 (18.05)	4.09 (11.68)	8.00 (16.43)	0.93 (5.53)
Tilikiam	280	66.07 (54.39)	33.93 (35.61)	1.69 (7.49)	1.16 (6.29)	5.16 (13.18)	2.27 (8.72)	1.38 (6.80)	0.80 (5.13)
Namti	545	70.64 (56.79)	29.36 (32.83)	3.02 (9.98)	1.06 (6.02)	11.33 (19.64)	4.53 (12.25)	2.76 (8.72)	1.51 (7.04)

- *The figures within parenthesis are Arc Sine transformation values of percentages.*
- *F-test for two tailed probability (F=0.0984) showed that the incidence of Resinous agar lesions with and without Z. conferta infested agarwood trees are significantly different.*
- *t-Test: Paired Two Sample for Means exhibited significant negative correlation (Pearson Correlation : - 0.99698) on incidence of resinous agar lesions with and without insect infestation*
- *t-Test: Paired Two Sample for Means exhibited significant positive correlation (Pearson Correlation : 0.8489) and P (T<=t) one tail value is 0.1799396, P (T<=t) two tail value is 0.359879 in respect of age groups.*

Figure 1. Infestation of *Z. conferta* in relation to different age group of *A. agallocha* Roxb

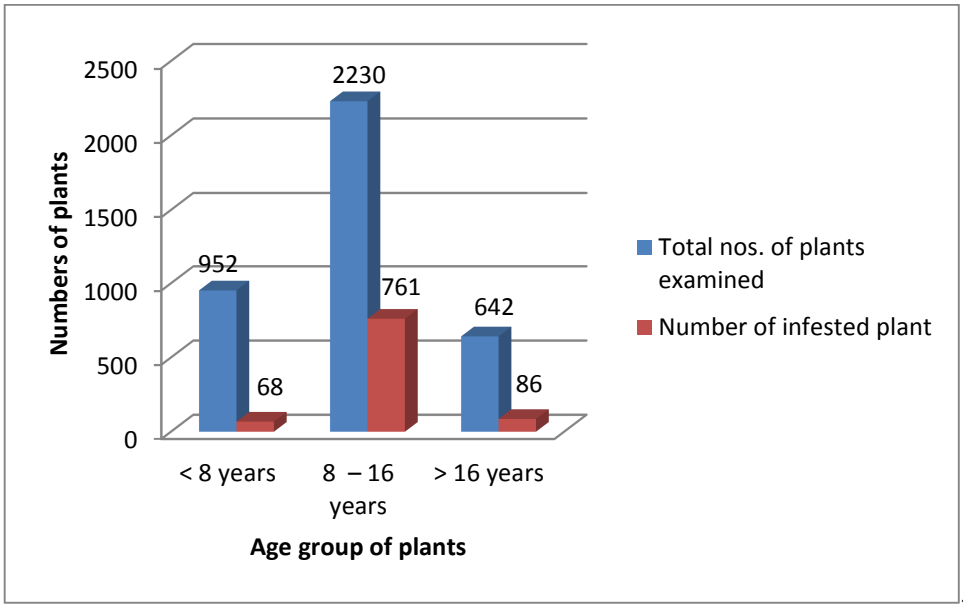
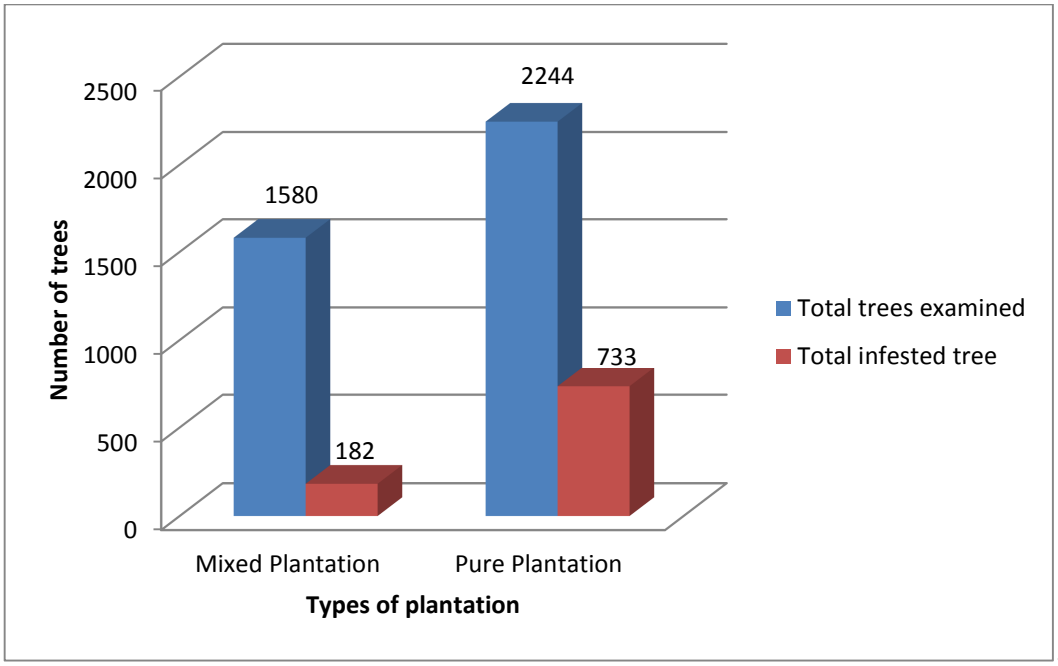


Figure 2. Relative infestation of *Z. conferta* in pure and mixed plantation of *A. agallocha* Roxb



Evidence of *Z. conferta* infestation in harvested agarwood trees

Incidence of agar lesions in harvested *A. malaccensis* was observed in relation to the presence or absence borer tunnel of *Z. conferta*. Normally the agarwood trees are harvested on the basis of visual symptoms like (i) a poor crown, decayed branches, and uneven bole (ii) swelling or depression and cankers on the bole (iii) the appearance of hordes of ants in the fissures (iv) a distinctly yellowish to brownish tinge in the wood under the outer bark (v) signs of ill-health particularly a die back symptoms of the top and outer branches and (vi) inspection by driving screw augurs. In the present study, the incidence of agar lesions with symptoms of borer tunnels in harvested agarwood have been recorded as high as 69.91 per cent and agar lesions without symptom of borer tunnels is 30.09 per cent. Amongst the four Agarwood Processing Centers under consideration, Naharoni in Golaghat district of Assam recorded to be the highest number of agar lesions (74.78%) with *Z. conferta* injury followed by Namti in Sivasagar district (70.64%), Hojai in Nagaon district (67.41%) and Tilikiam in Jorhat district (66.07%) (Table 3). It is also observed in the present investigation that, 40.27 and 20.98 per cent of agar lesions found in *Z. conferta* infested plant are in the age group of 8-16 years and above 16 years, respectively. The farmers also harvested and sold young plants to the processors where agar lesions have been developed (8.67%). The agar lesions were also observed in 30.09 per cent of harvested agarwood trees having other visual symptoms.

The present study showed that incidence of resinous agar lesions with and without insect infested agarwood trees are significantly different. There is no significant difference of incidence of *Z. conferta* infested lesions among the locations but significant in different age group of trees harvested for processing agarwood.

Agarwood and agar oils are regarded as pathological products. The mechanism of formation of oleoresins in agarwood tree is not yet fully understood. The fragrant resinous wood of *A. malaccensis* used in fragrance industry is reported to be formed only when the tree is infected by microorganisms or injuries caused by boring insects or even manmade wounds [5], [6], [8], [11]. Many researchers [18], [19], [14], [16] [20] have reported their works on microbial involvement in inducing agar in A*quilaria* species from different parts of the world, however, no concerted efforts have been made so far to study the role of the borer insect, *Zeuzera conferta* in agar formation. In the present study, it is observed that 60.91 per cent of harvested *a. malaccensis* tree having agar lesions or resinous wood with the borer infestation. It was reported that the soil borne fungus, *Cystosphera mangifera*e Died., which is responsible for infection of *A. agallocha* Roxb. is transported by the borer insect directly to the trunk [8]. Many of agar dealers of the state of Assam claim that an insect is instrumental in inducing agar formation. The *Kolagachi mal* (one particular grade of agarwood product in trade) fetches very highest price due its high quality obtained due to infestation of borer insect, *Zeuzera conferta*. The products from 30.09 per cent of harvested trees having agar lesions without borer insect tunnel are considered to be the second grade (*Dum mal*) with lower market price. The borer found to infest the agarwood tree starting from 5 years of its age, although higher percentage of infestation was observed in the plant age group of 8-16 years. The present study reveals the importance of *Z. conferta* in inducing disease and formation of resin in agarwood as was doubted by researchers & traders in this line.

CONCLUSION

The north east region of India is very rich in natural resources and globally recognized as mega biodiversity hot spot. *A. malaccensis* Lamk., the wonder tree of this region has been recognized as the valuable plant wealth. Agarwood products of this region have made a special place in the international scenario because of its unique quality. Agarwood plant being the non-timber tree species, the non-infected and healthy trees has no value as such in trade. Therefore, there is an urgent need to develop suitable and viable technologies to convert each and every agarwood tree commercially utilizable. In this study, it can be concluded that, besides important microbial role, the trunk borer insect *Z. conferta* may have a significant catalytical role in developing pathogenicity and agar (resins) in *A. malaccensis.* The exact role of *Z. conferta* is required to be find out which needs further study.

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