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## Preliminary investigation on terpenoids present in the bark of major host plants, and lac-encrustations made by the Indian lac insect *Kerria lacca* (Kerr.) with respect to host plant selections

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### Abstract

Indian lac insect, *Kerria lacca* used to settle on the bark of host plants to take the plant sap after penetrating its rostrum. Plants accumulate a great variety of secondary metabolites in their bark tissues possibly to resist herbivorous invaders. Near about hundred plant species are known as host plant of *K. lacca*. The endoglandular secretion of this insect is a commercial resin commonly known as lac. In the present study data obtained from genomic sequences of 5.8S r DNA have shown that the studied host plants are not taxonomically closely related, though *Butea monosperma* and *Ziziphus jujuba* are more close than *Schlischeria oleosa*. But gas chromatographic analysis reveals the presence of same kind of terpenoid molecules among the studied host plants viz. *B. monosperma*, *Z. jujuba*, *S. oleosa*, as well as in the lac encrustations secreted by lac insect thriving on these host plants. Present study focuses solely on the possibilities of host plant terpenoids as one of the major key factors of host plant selection by *K. lacca*.

**Keywords:** *Kerria lacca*, Lac, Lac resin, Terpenoids, Secondary metabolites, Host plants

### Introduction

The Indian lac insect, *Kerria lacca* belongs to the order Hemiptera, Family Tachardiidae. Insects belong to this order are characterized by mouth parts modified into proboscis, sheathed within a modified labium to form a rostrum. The mouthparts are modified typically for piercing the plant tissues and sucking out the plant sap by the homopteren insects<sup>[1]</sup>. The crawling nymphal stage of *K. lacca*, within a few hours of its emergences from the shell of mature female lac insects, are able to successfully penetrate the host plant tissues and access the phloem tissue sap<sup>[2]</sup>. Unlike the other homopterans, the lac insect once inserts its proboscis into the plant tissue remains sedentary for rest of the life<sup>[3]</sup>. The insect starts secreting lac, the only known commercial resin of animal origin<sup>[4]</sup>, which acts as a protective coating for the vulnerable, sedentary insect body<sup>[3]</sup>. Lac has a great value in economy, since large numbers of commercially important compounds are obtained from it<sup>[5]</sup>. More than hundred plants have been described as host plants of *K. lacca* which belong to different families<sup>[6, 7]</sup>. The large scale production of lac in India depends on three major host plants viz. *Butea monosperma*, *Ziziphus jujuba* and *Schlischeria oleosa*. Selection of the appropriate host plant by the insects is one of the most intriguing aspects of lac insect-host plant interactions<sup>[4]</sup>.

The factors behind host plant selection of *K. lacca* had been investigated mainly based on the differences of pH values of sap of host plants<sup>[8]</sup>. Various classes of phytochemicals are known to be present within the bark of the plants<sup>[9]</sup>. The present study has been conducted with an objective to understand the importance of phytochemicals present in the bark of respective host plants in host selection. Terpenoid compounds present in the encrustation of lac and in bark tissues of three major host plants have been analyzed in this study also.

### Materials and Methods

The entire study was conducted between the months of June, 2014 to December, 2015.

- **Collection of Samples**

The bark from three major host plants viz. *Butea monosperma*, *Ziziphus jujuba* and *Schleichera oleosa* were collected from the Tarkajor Brood Lac Culture Farm, BLOCK- Bankura I, District-Bankura, West Bengal, India for the purpose of study. To study the terpenoid compounds present in the encrustation of lac, the encrustations were collected from three studied host plants from Tarkajor brood lac culture form, BLOCK-Bankura I, District-Bankura, West Bengal, India.

- **Preparation of plant extracts**

Isolated bark samples of three studied host plants were air dried and crushed with mixture grinder. Solvent Chloroform was used for the purpose of phytochemical extraction in Soxhlet. Soxhlet phytochemicals had been concentrated from solvent CHCl<sub>3</sub> using the rotary evaporator (Buchi, model no. R3). Column chromatography (CHCl<sub>3</sub>: Ethyl Acetate = 8:1) was performed using Silica gel (mesh, 230-400) to purify it. Activated charcoal was used to remove the necessary pigments from column derived products.

- **Preparation of the extracts from the encrustation of lac**

To expose the inner surface of the lac, it was crushed. The crushed lac was sieved through the proper meshes. The bigger ones were separated mechanically. Washing of lac was done by putting the crushed sieved lac into cup shaped stone vats, mixing with double distilled water. Same process was repeated twice. After washing the dust form of lac encrustation it was air dried for overnight. Powder form of lac encrustations were extracted with solvent chloroform, overnight at room temperatures. Generally 10 gm. of dust of lac encrustations were dissolved in 100 ml of solvent CHCl<sub>3</sub>. Extracted chemicals were further concentrated from solvent CHCl<sub>3</sub> using the rotary evaporator (Buchi, Model No. R3). Column chromatography (CHCl<sub>3</sub>: Ethyl Acetate = 8:1) was performed using Silica gel (mesh, 230-400) to purify it.

- **Qualitative analysis of alkaloids, terpenoids and phenolics**

Several Qualitative analyses were done on the semi purified product derived from the silica-gel column chromatography, to establish the nature of the chemical present. All of the semi qualitative analyses were done after the methods of Pushkar. *et al* [4].

- **Gas Chromatographic (GC) analysis**

TG-5MS (5% Phenyl Methylpolysiloxae) column was used as stationary phase for gas chromatography with the Helium (He) gas (flow rate 1ml/min) as mobile phase (Thermo Scientific, model name- Trace 1300/Gas Chromatography). During GC the initial temperature of the column was set at 150 °C up-to 240 °C at the acceleration rate of 20 °C/min and then increased up-to 310 °C at the rate of 5 °C/min. Total run time was 18 minutes. Sample was loaded 1µl in both studies and the concentrations were 1mg/ml. HPLC grade hexane was used for dilution of samples and for injections.

- **Molecular Phylogenetic analyses of Studied Host Plants of *K. lacca***

To analyze the phylogenetic relationship among three different studied host plants, the necessary genomic information (5.8S r DNA) had drawn from the NCBI database [10]. Software "PHYMLIP" was used to make a relationship after the analysis of dendrogram and distance matrix [11].

## Results and Discussion

Qualitative estimations derived from Silica-gel column chromatography have shown the terpenoids in each case. Further analyses of gas chromatography have also confirmed the presence of same terpenoid moiety obtaining from their respective three different studied host plants (The most notable peaks are *Rt* 16.55, 15.36, 14.33, 13. 10, 10. 81, 8.61, 7.62, 7.29 and 5.83). (Fig. 1 A<sub>1</sub>, B<sub>1</sub>, C<sub>1</sub>) Results after the analyses of GC on the semi-purified terpenoids moiety of lac encrustations from three different host plant species have revealed the presence of same molecule with the respective plant species (Fig. 1). Presence of same terpenoid molecules among encrustations of lac and bark of respective host plants has been evident in the study (Fig. 1). To explore the factors determining the host preferences, sap of the all host plants had pH ranging from 5.8 to 6.2 and the density in between 0.14 to 0.17 [8]. All of three major host plants which have selected for studies belong to different taxonomic Family (Table. 1). Data obtained from the genomic sequences of 5.8S r DNA have shown that they are neighbors but belong to different branches of plant molecular phylogeny (Fig. 2). Results obtained from the distance matrix and dendrogram based on 5.8S r DNA sequences establish the higher degree of similarities between host plant *B. monosperma* and *Z. jujuba* in comparison to *S. oleosa* (Table-2, Fig- 2). Although the three studied host plants belong to different taxonomic Families with enough genomic distances yet bearing the same terpenoid moiety, which in turn is utilized by *K. lacca* to make an encrustation as a protective device (Fig. 1). This may be a tenable determining factor of the host plant selection by Indian lac insect. Plant can store their secondary metabolites in its bark and other parts of its body but the rate of accumulation increases at the site of infestation after infection [12]. Several studies have done on the adaptation of insects against plant defenses [13, 14, 15, 16]. Insects can defend against the toxic metabolites of its host plant by synthesizing several detoxifying enzymes or antioxidant compounds [15, 17]. A great variety of terpenoids is known among different host plants [18]. The present study reveals that all of the terpenoids are common among three studied host plants as well as in their respective encrustations of lac (Fig.1). Gas Chromatographic analyses establish that *K. lacca* utilizes the defensive compounds of its host possibly to make a protective device wrapping itself with the lac-encrustations. Several studies have also shown that during larval stages monarch butterfly and saw fly used to store the secondary metabolites of its host plants in its body to deter predators [19]. It is tenable that sedentary adult female morph of *K. lacca* is unique in adapting the same device to minimize its chances of predation.

## Conclusion

Data obtain from genomic sequences of 5.8S r DNA have shown that the studied host plants are not taxonomically closely related, though *Butea monosperma* and *Ziziphus jujuba* are more close than *Schleichera oleosa*. The terpenoids present in the bark tissues of host plants are also the major sources of lac resin which is commercially known as lac. Present study reveals that possibly host plant terpenoids are one of the major determining key factors of host plant selections by Indian lac insect, *K. lacca*.

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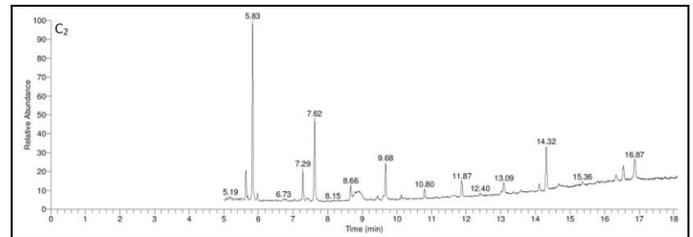
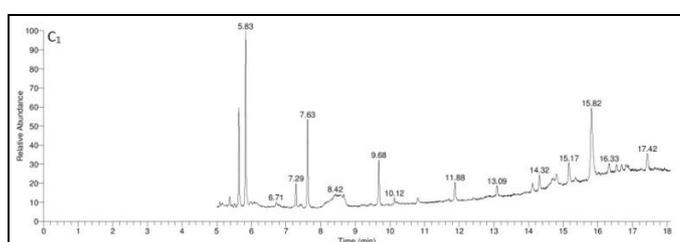
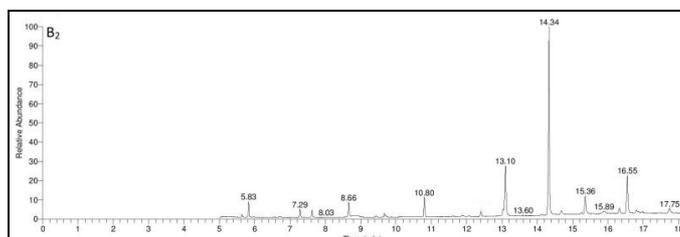
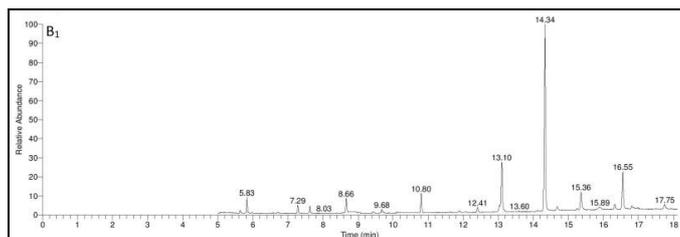
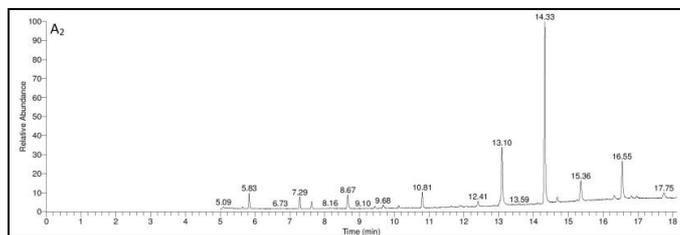
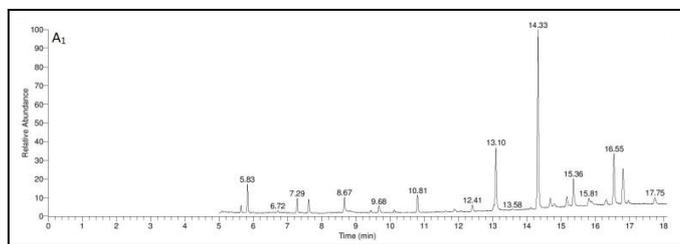
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**Table 1** Taxonomic position of three different studied host plants.

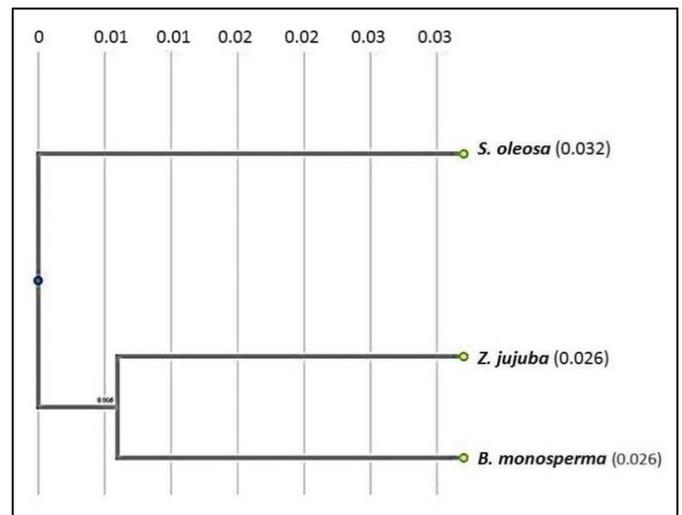
	<i>B. monosperma</i>	<i>Z. jujuba</i>	<i>S. oleosa</i>
Order	Fabales	Rosales	Sapindales
Family	Fabaceae	Rhamnaceae	Sapindaceae

**Table 2:** Distance matrix of three different studied host plants based on 5.8S r DNA sequences.

	<i>Z. jujuba</i>	<i>B. monosperma</i>	<i>S. oleosa</i>
<i>Zizyphus jujuba</i>	0	0.051565	0.075516
<i>Butea monosperma</i>	0.051565	0	0.052291
<i>Schleichera oleosa</i>	0.075516	0.052291	0



**Fig 1:** Gas Chromatographic analysis of terpenoid moiety present in the bark of host plant and the encrustation of lac. A<sub>1</sub>, B<sub>1</sub>, C<sub>1</sub> represents the chromatogram (GC) of terpenoids present in the bark of host plant, *B. monosperma*, *Z. jujuba* and *S. oleosa* respectively. A<sub>2</sub>, B<sub>2</sub>, C<sub>2</sub> shows the chromatogram of terpenoid molecules present in the encrustation of lac on respective host plant species as studied.



**Fig 2:** Dendrogram showing the phylogenetic relationship of three studied host plants of *K. lacca* based on 5.8S r DNA sequences.

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