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Secondary metabolites of lac host plants and their impact on lac resin and dye production by the female Indian lac insect *Kerria lacca* (Kerr.)

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Abstract
The study reveals that *Kerria lacca* utilizes the host plant's secondary metabolites, especially terpenoids to make the resinous encrustation surrounding itself, probably as a protective device. The rate of lac resin secretion largely depends on the plant terpenoids concentrations. The concentration of anthraquinone dyes present in the lac-encrustation is higher in *Butea monosperma* in comparison to *Ziziphus mauritiana*. The plant phenolics can also affect the rate of lac-resin production. It is also evident that *K. lacca* depends on the concentration of host plant phenolics to make its anthraquinone dye. *K. lacca* thriving on *B. monosperma* can produce greater amount of dye in which the concentration of phenolics is also higher in comparison to *Z. mauritiana*. The statistical analysis support the present studies also.

Keywords: *Kerria lacca*; lac insect; lac; encrustation; secondary metabolites; terpenoids; phenolics

1. Introduction
Kerria lacca is economically important for its resinous endoglandular secretions, consisting of two types of sesqui-terpenoids (shellolic acid, jalaric acid), a long chain fatty acid (aleuritic acid) and a type of anthroquinone dye (Bose 1963) [1]. This unique type of insect, especially its female morph, loses all effective organs (locomotory and sensory appendages) during the course of post-embryonic development. They protect themselves under its hard resinous encrustation from predation, which is commercially known as lac (Ghorai 1995) [2]. Several studies are known on monarch butterfly and sawfly larvae in which they store plant defensive compounds (alkaloids, terpenoids) in their body as a defense mechanism against their predators (Matthews and Matthews 2010) [3]. This study is unique in the sense that female *K. lacca* may utilize its host plant produced secondary metabolites (terpenoids and phenolics) and consequently secreting lac to make a hard protective resinous encrustation around itself, minimizing its chances of predation as an adaptive survival trait. The objective of this chapter is to ascertain whether the population density of lac insect as well as total lac resin and dye production depend upon the amount of secondary metabolites (terpenoids and phenolics) of their respective host plants or not.

2. Materials and Methods
i) Study specimen
The Indian lac insect *Kerria lacca* (kerr.) is a unique form of coccide having prominent sexual dimorphism (Varshney, 1977; Kaushik *et al.*, 2012) [4, 5]. During their development, the adult males are winged and female are wingless and globular in shape losing all kinds of locomotary and sensory appendages (Fig. 1). The insect, usually thrive on some prominent host plants like *Butea monosperma*, *Ziziphus mauritiana*, *Flemingia semialata* and *Schleichera oleosa*. The thriving strain on host plant *Butea monosperma* in the months of Nov.-Dec. to May-June and May-June to Nov.-Dec. are known as Baisakhi and Kartiki crop of Rangeeni strain respectively and the strain thriving on host plant *Schleichera oleosa* and *Flemingia semialata*in the month of Dec.-Jan. to June-July and June-July to Dec.-Jan. are known as Aghani and Jethui crop of Kusmi strain respectively. Importantly, the female makes a colony and becomes sedentary in nature. As a protective device, they secrete a resinous encrustation over their colony, which is known as lac encrustation. The study was conducted between October 2013 June 2014 only on the Baisakhi crop of Rongeeni strain of *K. lacca*. Two different host plants had been selected viz. *Butea monosperma* (Family- Fabaceae), *Ziziphus mauritiana* (Lam.) [=*Ziziphus jujuba* (Mill.)] (Family-Rhamanaceae) on which commercial production of lac in West Bengal, India is mostly dependent (Varshney and Teotia, 1967; Varshney, 1968; Sabbarayudu and Ram, 1997; Sharma *et al.*, 1997; Pushkar *et al.*, 2011) [6, 10]. Nine to ten months old branches emerged after pruning (a common practice for traditional lac culture) were chosen for the inoculation in this study.

ii) Study area

Major host plants of *K. lacca* are available in the region of three districts in West Bengal, India viz. Bankura, Purulia and Paschim Midnapure due to their favorable environmental conditions. Two farms were considered namely Tarkajor state brood lac culture farm (23□09'25.33"N, 86□59'01.04"E) at block Bankura-I and Gananando Saraswati Ashram (23□20'17.19"N, 86^{0}54'32.44"E), Chakaltatal more, Dalpur at Block Chhatna, in Bankura District.

iii) Study on population density of *K. lacca*

Population density of *K. lacca* had been measured twice during entire study period, initially after 10 days from the date of stick brood lac tagging at suitable branches and finally at the end of study period. During on the first day of study, population density of lac insect was estimated using a 2mm graph paper and total number of insects had been recorded per 16mm^2 areas. At the end of the study, to measure the population densities of insect total amount of produced lac on16mm2 areas (using a 2 mm graph paper) was removed mechanically and dissolve in 20 ml of absolute Ethanol for 36 hrs. And then the total number of insects had been recorded. On both cases total thirty biological replicates had been taken randomly.

iv) Estimations of month wise total concentration of plant phenolics and terpenoids

Month wise total concentration of host plant phenolics and terpenoids had been recorded at the 3rd week of each month on inoculated branches during entire study period following the methods of Ainsworth *et al.,* (2007) [11] and Ghorai *et al.,* (2012) [12] respectively.

v) Study on month-wise total production of lac

Month wise total production of lac on both two studied host plants had been measured using a 2mm graph paper and recorded at 16mm^2 areas on inoculated branches simultaneously with the total concentration of host plant phenolics and terpenoids on each month. Sampling had been carried out randomly on different inoculated branches of those two studied host plants and total ten biological replicates were taken on each month.

vi) Estimation of anthraquinone dye, present in lac encrustations

Total concentration of anthraquinone dye present in the lac encrustation was estimated calorimetrically (λ-560nm) at the end of study. The protocol had been standardized after the reduction of anthraquinone dye, Alizarin red S (Purchas from Sigma–Aldrich catalogue no.- A5533) with sodium hydroxide (Sigma, catalogue no.- S8045) and zinc dust (Sigma, catalogue no.- 209988) in which brilliant yellow color was converted to purple red. Generally zinc dust can reduce anthraquinone dye in any strong alkali medium (like NaOH solution) and afford a purple red color (Ghosh1998) [13]. Concentration of anthraquinone dye was estimated by computing the absorbance of unknown sample at the standard curve equation. Total incubation time to develop the reaction was 1hr at 37^{0}C temperature.

vii) Statistical analysis

Correlation study between the month wise total production of host plant terpenoids and phenolics with the month wise total production of lac by *K. lacca* was done using "R-3.0.1" software. Confidence of co-relation had been calculated for their possible range of correlation coefficient. Difference of month wise average production of lac by *K. lacca* and month wise average production of terpenoids and phenolics by their host plants were statistically tested by "R-3.0.1" using the command *t.test* and their statistical significance were conducted using command *cor.test* with *p-values* which shows their significance. Necessary graphical representation also had been done by "R" using plot command.

3. Results

The total concentration of host plant terpenoids and phenolics increases gradually on both studied host plants after the inoculation of *K. lacca* (Fig. 2). Statistical analyses revealed that host plant *Z. mauritiana* can accumulate a greater amount of terpenoids in its tissues earlier and even after the development of inoculation in comparison to *B. monosperma* in which the concentrations of phenolics are much more (Fig. 2A). Production rate of lac are higher by *K. lacca* on host plant, *Z .mauritiana* rather than on *B. monosperma* (Fig. 3). Although there is no statistically significant difference ($p > 0.05$) of the population density of *K. lacca* on both studied host plants (Fig. 4). Total production of lac gradually increases on both host plants corresponding to the production of terpenoids and phenolics of its respective host plants. Month wise total production of lac by *K. lacca* is statistically correlated with the production of total terpenoids and phenolics of their respective host plants (Fig. 5, Table 1). Values of correlation are higher between the month-wise production of host plant terpenoids and lac encrustation in comparison to plant phenolics (Table 1). We run the Regression equation of production of lac on the production of phenolics and terpenoids of both two studied host plants were worked out. For *Z .mauritiana* regression equation is powerful since, R^2 is 0.9799 with a *P* value 0.000056 and regression co-efficient corresponding phenolics production is more effective, but not significant ($\neq 0$) but for terpenoids, regression co-efficient is significantly more than 0 at the 5% level of significance. Hence, the production of lac by *K. lacca* depending on the production of host plant terpenoids is remarkable. For host plant *B. monosperma*, regression on production of lac on the production of host plant terpenoids and phenolics is linearly dependent on R^2 (0.9674). The regression equation which is also highly significant with *F* statistics 74.10 and *P* value 0.00019. Regression co-efficient corresponding to production of host plant terpenoids is 0.2092 that is statistically different from 0 at the 1% level of significance.

Hence, in *B. monosperma* the production of lac depends more on production of terpenoids than the production of plant phenolics. Intercept term of the regression equation for the production of lac by *K. lacca* on the production of plant terpenoids and phenolics is negative and statistically significant for host plant *Z. mauritiana*. In host plant *B. monosperma* (Table 3) the scenario is different; production of lac starts from the very beginning and significantly depends on terpenoids level. Production of lac will depend both on terpenoids and phenolics level for both of the studied host plants, but they are complementary to each other which is also revealed from correlation coefficient (0.95 for *Z. mauritiana* and 0.99 for *B. monosperma*) (Table 1). The multi co-linearity effects of regression coefficients corresponding to phenolics are statistically insignificant for both the host plants. So, the

role of phenolics is necessary and essential which also is evident from the regression pattern of lac production by phenolics.

The effects of multi co-linearity is so severe that, in the presence of terpenoids, regression coefficients corresponding to phenolics for both the host plants are insignificant, and negative is remarkable for the host plant *B. monosperma*, in spite of the highly positive correlation between lac and phenolics. Total concentration of anthraquinone dye in the encrustations is higher in lac produced on host plant *B. monosperma* in which the total concentration of phenolics is relatively more than *Z. mauritiana* (Fig. 6). The month-wise ratio between the production of plant phenolics and terpenoids (phenolics: terpenoids) has shown that values of ratio are always higher in case of *B. monosperma*, because the production rate of phenolics is higher than *Z. mauritiana* (Fig. 7). During the first three months of inoculation the values of ratio increases in case of host plant *Z. mauritiana* which marks the higher rate of accumulation of plant phenolics than terpenoids.

4. Discussion and Conclusion

The present study has established a correlation between the productions of secondary metabolites by host plants with the inoculation of *K. lacca*. Present study has revealed a trend that concentration of terpenoids and phenolics on both the host plants gradually increases with the increased rate of production of lac encrustation by *K. lacca* (Fig. 2, Table 1). Notably, there is no statistically significant difference between the production of plant terpenoids and phenolics with the production of lac encrustations by *K. lacca* on both studied host plants during the last two months of its life cycle (Fig. 2, 3 and 5). Among two host plants, the total concentration of plant terpenoids has been always higher in the *Z. mauritiana*. (Fig. 2B), on which the production rate of lac encrustations is higher than on another host *B. monosperma* (Fig. 3). There is no statistical difference in population density of *K. lacca* in both the studied host plants (Fig. 5). Total amount of dyes present in lac encrustations are higher on plant *B. monosperma* in comparison to *Z. mauritiana* (Fig. 6). More-over, it has been established from the present study that host plant *B. monosperma* can accumulate greater amounts of phenolics in its bark than the host plant *Z. mauritiana* (Fig. 2A.). Correlation matrix has shown a significant relationship between the production of encrustation of lac and the accumulation rate of host plant phenolics and terpenoids on both studied host plants (Table 1). Dependency of *K. lacca* to make its encrustation both on host plant phenolics and terpenoids as revealed through regression analysis is worthwhile. The same is evident from the result of *F* statistics, which reveals that total production of lac is dependent on plant terpenoids than phenolics (Table 3). Though the total density of insect population is same on both host plants, yet the insect can produce a greater amount of lac on host plant *Z. mauritiana* in which the accumulation rate of terpenoids is higher. Correlation matrix represents the result that in both cases production of lac by insect is highly correlated with the month-wise production of plant terpenoids than phenolics (Table 1). On both the host plant productions of lac are highly correlated on plant terpenoids rather than phenolics which indicate the dependency of *K. lacca* to form its encrustations on plant terpenoids predominantly. Since regression co-efficient corresponding to phenolics is negative, which is probably masked by the production of plant terpenoids it appears that terpenoids dominate the lac production. But plant phenolics seem to be essential which is evident from regression on lac production of plant phenolics. Negative values of estimate for regression analysis on concentration of phenolics of host plant *B. monosperma* indicates that concentration of host plant phenolics can play a major role to mask the production of host plant terpenoids (Table 3A). Among two studied host plants the month-wise ratio of the production of host plant phenolics and terpenoids are gradually decreasing in case of host plant *B. monosperma* which indicates that accumulation rate of terpenoids is higher than host plant phenolics (Fig. 7). In case of host plant *Z. mauritiana* during the initial stages of insect inoculation the ratio increases that denote the higher accumulation rate of plant phenolics than terpenoids. An estimate of the intercept values is negative on host plant *Z. mauritiana* instead of *B. monosperma* which indicates that production of lac by *K. lacca* has been started earlier than host plant *Z .mauritiana* (Table 2). The accumulation rate of phenolics is initially higher in the host plant *Z. mauritiana* instead of *B. monosperma* that may be the reason for delay to produce lac by *K. lacca* as because the production of lac are highly co-related to the production of plant terpenoids. The total concentration of phenolics is higher in host plant *B. monosperma*, where the production rate of terpenoids is low in comparison to *Z. mauritiana*. In case of host plant *Z. mauritiana* initially during the first three months, after the inoculation of insects, accumulation rate of phenolics is higher which may play an important role to make a hindrance to start the production of lac. All of these findings have corroborated the negative values of estimate of regression analysis on the concentration of host plant phenolics of plant *B. monosperma* where the higher accumulation rate of phenolics may be responsible to suppress the production of host plant terpenoids (Table 2, Fig. 7). Regression co-efficient, corresponding to the concentration of plant terpenoids are significant for both the host plants. Which indicates that production rate of lac depends largely on the production of plant terpenoids. The statistical analyses *viz.* correlation, regression analysis, *F* statistics, and from population density of insects it has been also evident that *K. lacca* utilizes the plant's secondary metabolites especially, terpenoids to secret its hard resinous encrustations. Concentration of anthraquinone dyes, present in lac encrustations is higher in case of plant *B. monosperma* which can also accumulate a greater amount of phenolics during the course of inoculation. Several isomers of erytholaccin and laccic acid also have been reported from lac encrustation (Bose *et al*. 1963, Ghorai 1995) [1, 2]. Present study has also confirmed that *K. lacca* depends upon host plant phenolics to produce its dyes (Fig. 2 and 6). This might be caused due to several detoxifying enzyme *viz.* mixed function oxidase (Després *et al*. 2007) [14], with the help of such enzymes, *K. lacca* may oxidizes the plant phenolics into anthraquinone dyes. Plant phenolics and terpenoid are known as the secondary metabolites which they use to defense against their herbivorous predators, can accumulate at the site of infestation after infection (Muller and Borger 1940) [15]. It has been evident from the present study that Indian lac insect *K. lacca* utilizes their host plants' secondary metabolites to make its hardy resinous encrustation after losses the locomotary activities during the courses of post embryonic development.

5. Acknowledgements
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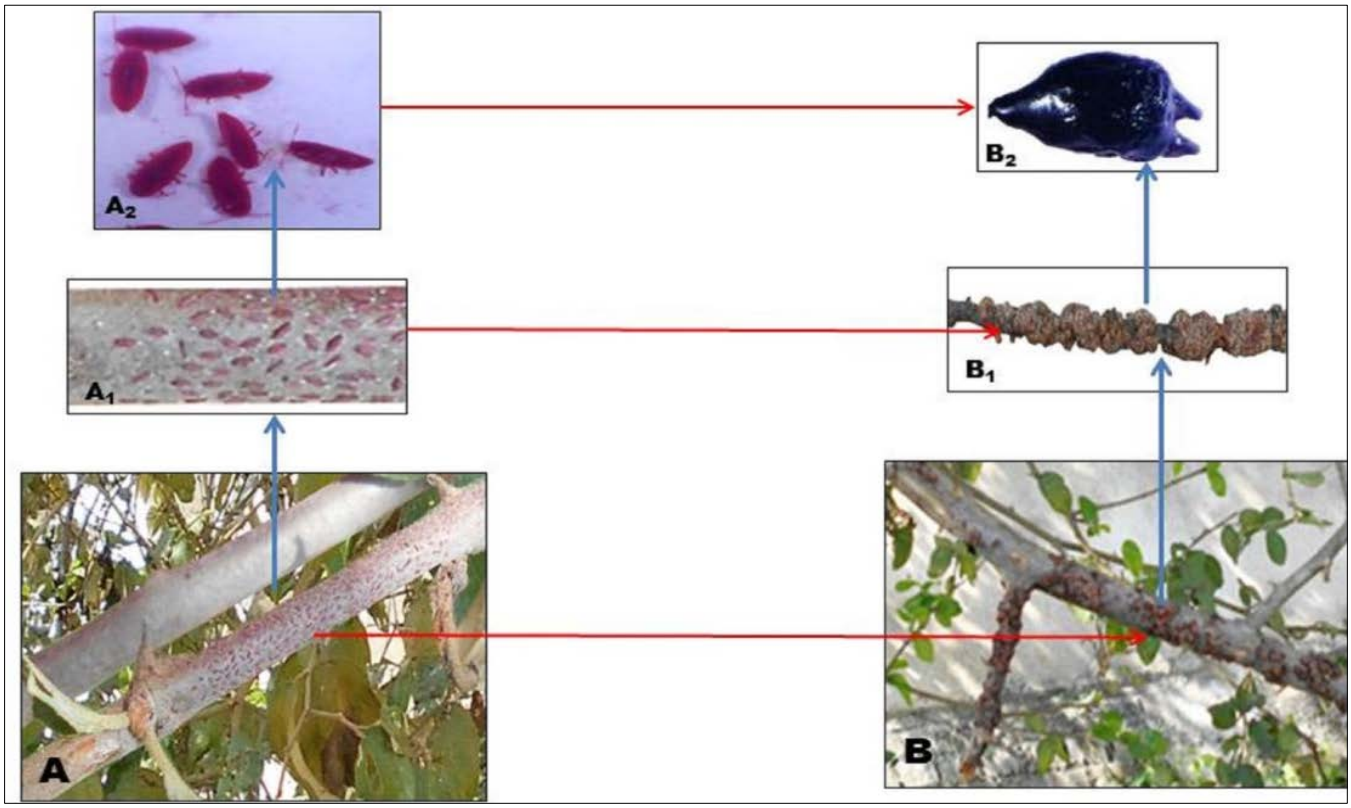

Fig 1: A. Inoculation of *K. lacca* on host plant *Z. mauritiana* at its early nymphal stage. A1 Enlarge views of nymph of *K. lacca*. A2 Microscopic views of *K. lacca* at early nymphal stages with locomotory appendages and antennae (250X). B. Encrustations of lac host plant *Z. mauritiana* at the late phase. B1. Enlarged view of lac encrustations. B2. Enlarged view of adult female lac insect, without locomotory and sensory appendages (25X), present inside the lac encrustation. [Blue arrows indicate the enlarged view and the red arrows indicate the stage of development, from early nymph to adult female].

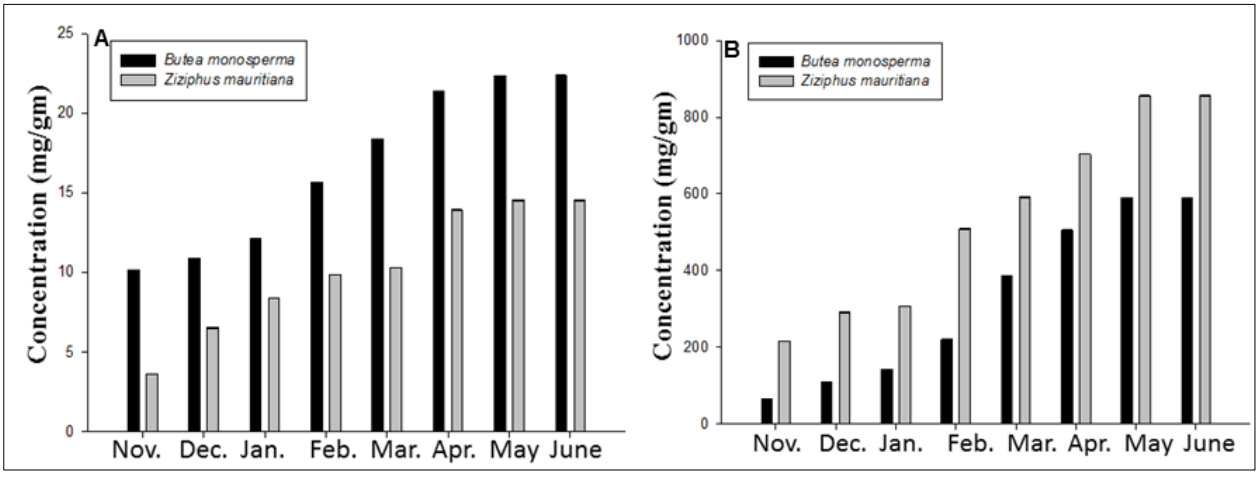

Fig 2: Month wise total concentration of secondary metabolites of two studied host plants. A. total concentration of plant phenolics. B. total concentration of plant terpenoids (n=4).

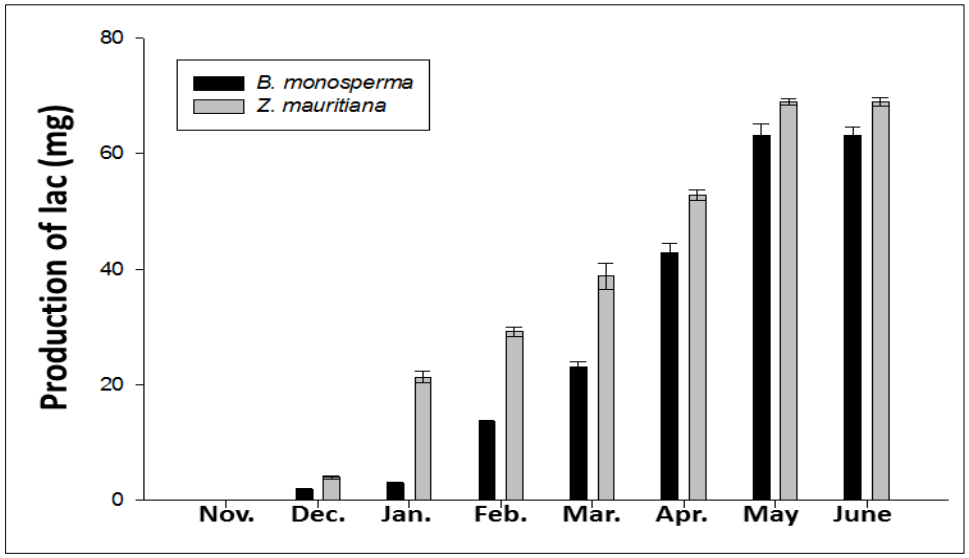

Fig 3: Month-wise total production of lac on two studied plants during the entire life cycle of the *Baisakhi* crop of *Rangeeni* strain. (n = 10).

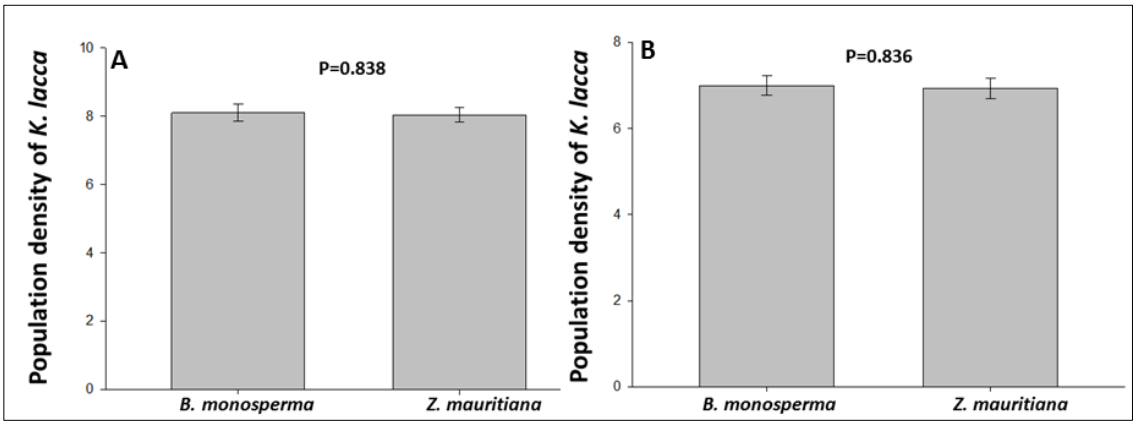

Fig 4: Population density of insects on two studied host plants. **A.** During insect settlement. **B.** During crop maturation. (n = 30).

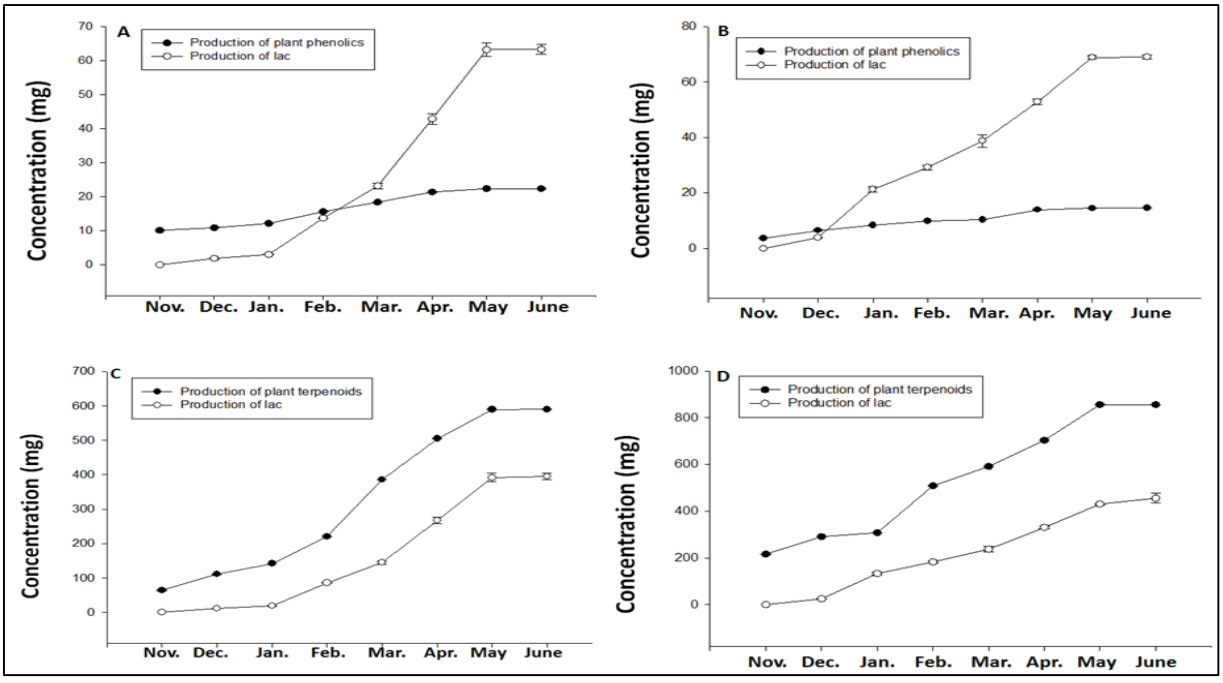

Fig 5: Correlation between the total concentration of phenolics (A, B) and total concentration of terpenoids (C, D) of inoculated branches with total amount of lac production. A. and C. *B. monosperma*. B. and D. *Z. mauritiana*.(for graph A and B total amount of lac production has been measured at 16mm^2 area and for C and D total amount of production of lac has been converted into production at 1cm^2 area mathematically).

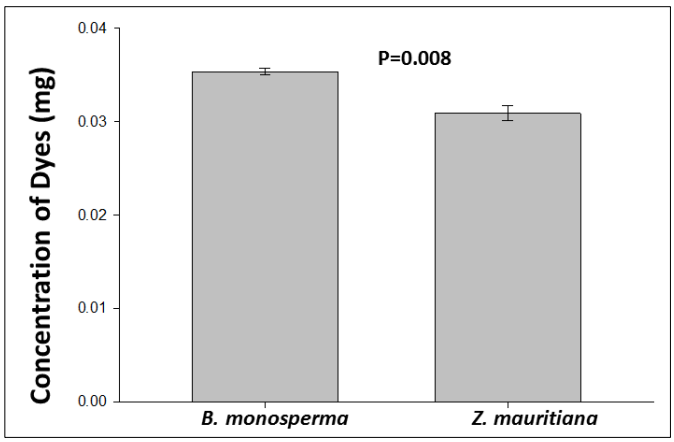

Fig 6: Amount of anthraquinone dye present in lac encrustation in both two studied host plants (mg/g).

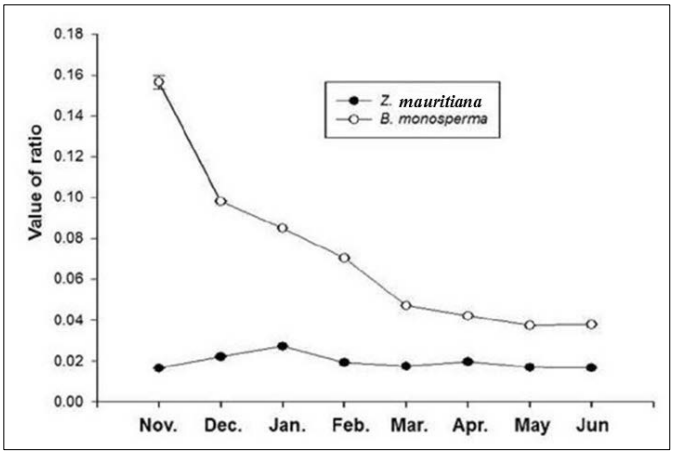

Fig. 7 Ratio between the month-wise production of plant phenolics and terpenoids (phenolics: terpenoids) of two different host plants.

Table 1A Correlation matrix of production of host plant phenolics, terpenoids and production of lac by K. lacca in case of A. *Z. mauritiana*, B. *B. monosperma*

Table 1A	Production of phenolics	Production of terpenoids	Production of lac
Production of phenolics	1.00000	0.958787	0.9719964
Production of terpenoids	0.958787	1.00000	0.9852229
Production of lac	0.9719964	0.9852229	1.00000

Table 1B

Table 1B	Production of phenolics	Production of terpenoids	Production of lac
Production of phenolics	1.00000	0.9903324	0.95444684
Production of terpenoids	0.9903324	1.00000	0.9781798
Production of lac	0.95444684	0.9781798	1.00000

Table 2A: Summary of regression result in case of A. *Z. mauritiana*. B. *B. monosperma*

Table 2A	Estimate	Std. Error	t value	Pr (> [t])
Intercept	-24.92042	4.77292	-5.221	0.00341**
Phenolics	2.23124	1.46641	1.522	0.18861
Terpenoids	0.06974	0.02355	2.961	0.03147*

Signif. Code: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
Residual standard error: 4.495 on 5 degree of freedom. Multiple R-square: 0.9799, Adjusted R-squared: 0.9719. F- statistics: 122.2 on 2 and 5 DF, p- value: 0.00005693

Table 2B

Table 2A	Estimate	Std. Error	t value	Pr (> [t])
Intercept	21.79884	27.30291	0.798	0.4609
Phenolics	-3.8224	3.00365	-1.273	0.2591
Terpenoids	0.20924	0.07115	2.941	0.0322*

Signif. Code: 0 '***' 0.001 '**' 0.01 '*' 0.05 ' .' 0.1 ' ' 1
Residual standard error: 5.706 on 5 degree of freedom. Multiple R-square: 0.9674, Adjusted R-squared: 0.9544. F- statistics: 74.18 on 2 and 5 DF, p-value: 0.0001919

6. References

1. Bose PK, Sankaranarayanan Y, Gupta SC. Chemistry of Lac. Indian Lac Research Institue, Namkum, Ranchi, Bihar, India. 1963, 1-116.
2. Ghorai N. Lac-Culture in India. International Books and Periodicals Supply Services, New Delhi. 1995, 6-117.
3. Matthews RW, Matthews JR. Insect Behaviour. John Wiley and Sons, Inc., Springer, United Kingdom. 2010, 185-215.
4. Varshney RK. Taxonomic studies on lac insects of India. Orient. Insects. Suppl. 1977; 5:97.
5. Kaushik S, Pushkar AK, Lakhanpaul S, Sharma KK, Ramani R. Investigations on some important host plants of *Kerria lacca* with reference to phloem distance. EurAsian Journal of BioSciences. 2012; 6:32-38.
6. Varshney RK, Teotia TPS. A supplementary list of the host plants of lac insects. Journal of Bombay Natural History Society. 1967; 64:488-511.
7. Varshney RK. Further data on host plants of lac insect (Homoptera: Tachardiidae). Journal of Bombay Natural History Society. 1968; 65, 249-251.
8. Subbarayudu BB, Ram RL. Distribution of host plants of the lac insect, *Kerria lacca* (Kerr). Journal of Entomological Research. 1997; 21:187-192.
9. Sharma KK, Ramani R, Mishra YD. An additional list of the host plants of lac insects, *Kerria* spp. Tachardiidae: Homoptera. Journal of Non-Timber Forest Products 1997; 4:151-155.
10. Pushker AK, Kaushik S, Lakhanpaul S, Sharma KK, Ramani R. Preliminary Phytochemical Investigation on the Bark of Some of the Important Host Plants of *Kerria lacca* The Indian Lac Insect. Botany Research International. 2011; 4:48-51.
11. Ainsworth EA, Gillespie KM. Estimation of total phenolic content & other oxidation substrates in plant tissue using Folin - Ciocalten reagent. Nature protocol. 2007; 2:875-877.
12. Ghorai N, Chakraborty S, Gucchait S, Saha SK, Biswas S. Estimation of total Terpenoids concentration in plant tissues using a monoterpene, Linalool as standard reagent. Protocol Exchange 2012; doi:10.1038/protex.2012.055.
13. Ghosh SK. Advance General Organic Chemistry. New Central Book Agency Private Limited, Kolkata, India. 1986, 1-180.

14. Després L, David JP, Gallet C. The evolutionary ecology of insect resistance to plant chemicals. Trends in Ecology and Evolution. 2007; 22(6):298-307.
15. Muller K, Borger H. Experimentelle untersuchungen uber die Phytophthoraresistance der kartoffel. Arbeiten Biologischen Anst. 1940; 23:189-231.