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Explicating the impact of phloem sap sucking lac insect (*Kerria lacca* kerr.) On phytochemistry of Kusum (*Schleichera oleosa* Oken.) Host

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Abstract
Kusum (*Schleichera oleosa* Oken.) is a major host plant for Indian lac insect (*Kerria lacca* Kerr.). This lac insect produces a resin known as lac via complex lac insect-host plant interaction. This resin acts as a protective covering over its body which was secreted by sucking the phloem sap from the stem of the host plant. This resin has several commercial applications ranging from paint, varnishes to aleuritic acid. This insect-plant interaction has been exploited by several communities especially tribals residing around the forest areas of Jharkhand, Chhattisgarh, Madhya Pradesh, West Bengal, and Odisha states of India. To elucidate the impact of lac insect feeding on kusum host, *Kusmi* strain of Indian lac insect was inoculated (infested) and compared with non infested condition (control). From the pooled data over two years it was observed that total sugar, soluble protein and free phenol level were increased in the leaves of lac insect infested host plant as compared to control. Proline also recorded an abrupt increase in level as compared to control whereas non significant increase was observed in malondialdehyde level. Superoxide radical, hydrogen peroxide and ascorbic acid level were also elevated upon lac insect feeding on host plant Kusum as compared to control. Thus, the study reveals that a dialogue between insect and host plant occurs at cellular level when lac insect feeds on host plant Kusum, thus significantly altering primary and secondary metabolism of host plants to ensure its survival along with host plant.

Keywords: Lac insect, *Kerria lacca*, kusum, *Schleichera oleosa*, phloem sap, phloem feeders

1. Introduction

Green plants are the source of food material for many phytophagous insect species. A variety of interactions was developed with plants by insects during their long period of association. The insects obtained their food and nutrition either by chewing or sucking sap from various plant parts [1-3]. Not all the insects are threat to plants. There are many insects found on land those are not threat to the ecosystem but beneficial to the humans in different aspects, as natural enemies, pollinators, productive insects, scavengers, weed killer and soil builders [4]. The sap feeders like aphids and whiteflies impose severe damage to the plants by ingesting the phloem sap [5]. Yet some sap feeders are productive insects which are beneficial to human beings. In this category lac insects find a prominent place which was known to humans for its commercial value since time immemorial. Indian lac insect (*Kerria lacca* Kerr.) belonging to the family Tachardiidae (=Kerriidae), a specialized group in Superfamily Coccoidea (Hemiptera: Sternorrhyncha) is an exclusive stem phloem feeder. In order to protect its soft body it secretes a resin as a protective hard shell around its body called 'lac'.

The lac insects been exploited by several communities especially tribals residing around the forest areas of Jharkhand, Chhattisgarh, Madhya Pradesh, West Bengal, and Odisha states of India. Kusum (*Schleichera oleosa*), Ber (*Ziziphus mauritiana*) and Palas (*Butea monosperma*) are the major host plants for lac culture. Exploitation of lac resin is attributed to its many commercial applications [6-9]. In response to sucking of phloem sap which was intended for growth and development of host plants, insects triggers variety of biochemical mechanisms in the host plants [10, 11]. Such herbivore-induced plant responses influence the behavior and growth of both the insect and the host-plant [12, 13]. Due to sessile nature, lac insects spend its entire life cycle and future generations on the same host plant thereby reducing the life span and potentiality of host plant over the years. Moreover, the slow growth of traditional hosts limits the lac cultivation potential [14,15]. In addition deforestation of host trees raises concern over the sustainable lac production. Therefore, maintaining the sustainable lac production along with the health of host plants, one must understand the dialogue between lac insect and their host plant. Thus, the present study was attempted to analyze phytochemical response of Kusum (*Schleichera oleosa*) to lac insect feeding. Such an understanding would help in explicating the basics of lac insect-host plant interaction and would provide the basis for enhancing lac yield in sustainable manner.

Materials and Methods

Kusum (*Schleichera oleosa*) host plant was taken for study to explicate the impact of lac insect feeding on host-plant phytochemistry. The plants were infested (inoculated) with lac insect (*Kerria lacca* Kerr.) during the month of February end and study continued till lac insect completes its life cycle i.e. up to July. Lac insect details with host plant *Kusum* is presented in plate 1.Plants with no lac insect infestation served as control. The standard cultural/ package of practices for lac cultivation were followed. The study was carried out for two years, 2016 and 2018 at ICAR-Indian Institute of Natural Resins and Gums, Namkum, Ranchi (JH). Leaf samples from both the conditions were taken during morning hours for biochemical analysis. Total sugar, soluble protein, free phenol, proline, malondialdehyde (MDA), superoxide radicals, hydrogen peroxide and ascorbic acid were determined from the leaves of host plant kusumduring different months in replications over two years and expressed on fresh weight (fr. wt.) basis. The total sugar was extracted using 80% hot alcohol [16] and determined by phenol method [17, 18]. Soluble protein was estimated by Lowry's method [19] and free phenol was determined using Folin-Ciocalteu reagent method [20]. Proline was estimated by sulphosalicylic acid method [21]. Malon dialdehyde (MDA) was estimated by thiobarbituric acid [22]. Superoxide radicals were determined by NBT method [23]. Hydrogen peroxide was determined by titanium reagent method [24]. Ascorbic acid was determined by TCA method [25]. The data was pooled over months and years. The absorbance of all the biochemical constituents had recorded using spectrophotometer (Shimadzu-UV-1700 E 23 OCE) at specific wavelength for a particular constituents and comparing them with standard curve prepared by known amounts of that particular constituents. RBD analysis was done with standard statistical packages to draw conclusive result.

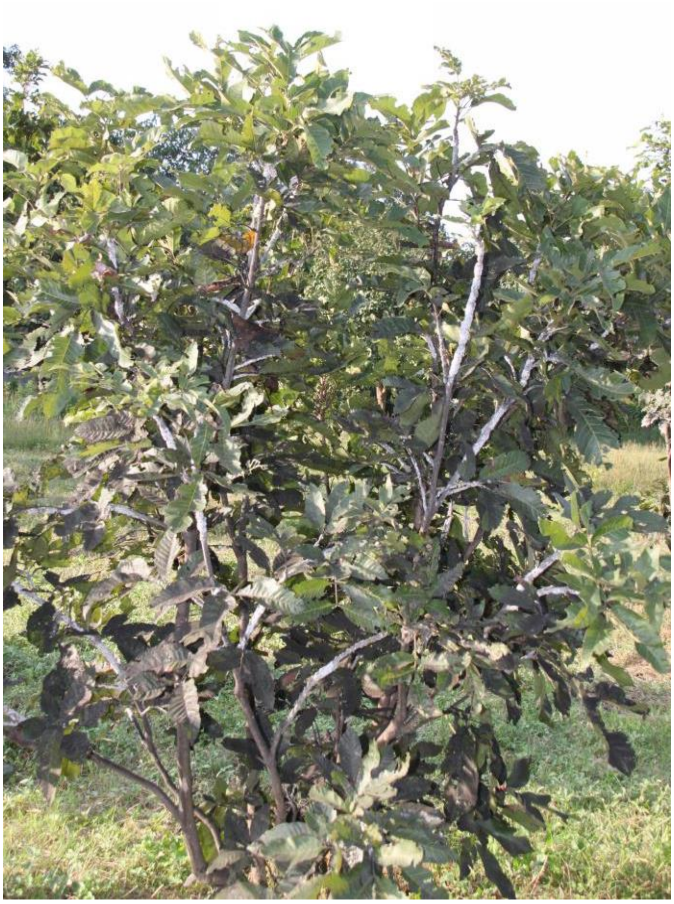

Plate 1: *Kusum* tree inoculated with *kusmi* strain of lac insect (*Kerria lacca* Kerr.)

Results and Discussion

Total sugar

Plants infested with lac insect resulted in significant increase in totalsugar content in the leaves (Table1 & Fig. 1a). Leaf total sugar in lac insect infested plants showed high content (18.37 mg/g fr. wt.) than control (14.21 mg/g fr. wt.). The lac insect infected plants observed 29.32 % increase in total sugar over control. It was reported that genes involved in sugar remobilization in celery were induced by aphid infestation [26]. It was suggesting that phloem-feeding insects may enhance thephotosynthetic rates in their hosts [27-29].While studying the transcriptomes in *Gossypium hirsutum* in response to sap sucking insects aphids and whitefly genes like phosphate translocator, and transketolase were found to be induced by whiteflies infestation [3]. Phosphate translocator, including triose phosphate translocator, was responsible for increase the source of carbon in the form of sugar. While studying the biochemical response of *Flemingia semialata (Roxb.)* to sap sucking lac insect, total sugar content in leaves of lac insect infested host plant was found to increase over non infested plants [15]. Present study also observed the increase in the total sugar content of kusum plant leaves infested with lac insect over control. This finding seems to be an important way by which sap-sucking insects increase their sugar concentration in sap. This indicates activationof sugar metabolism in the phloem sap and it may have activated multiple pathways to control transcription, translation, protein stability and enzymatic activity.

Soluble protein

Infestation with lac insect had significantly increased the level of leaf soluble protein than the non infested control plants

(Table1 & Fig. 1a). The protein content in lac insect infested plants was 25.47 mg /g fr. wt. as compared to plants with no lac insect infestation (16.03 mg/g fr. wt.). There was 58.88 % increase in protein in lac insect infested plants as compared to control. Recent studies established that protein has role of protein-based defense in plants against herbivore. These defense proteins may be anti-nutritional or toxic. The role of protease inhibitors (PIs) against herbivore are well established in many plants [11, 30]. In response to herbivorous insect attack, plant produces antinutritive and toxic proteins that interfere with oviposition, feeding, digestion, and absorption of essential nutrients by the insects [31-34]. The sap sucking insects like aphids and whiteflies up regulate the amino acid metabolism significantly in cotton plants [3]. This increase in protein potentially influences the insect-plant interaction. The study conducted on host plant *F. semialata* also shows that lac insect feeding activity increases the soluble protein content as compared to control [15]. In the present study the observed increase in the leaf protein in the infested condition suggests that lac insect feeding activates the protein synthesis pathway in the plant to produce more protein for defense as well for development of both, lac insect and plant.

Free phenol

Lac insect feeding on host plant resulted in significant increase in the amounts of phenolic compounds as compared with the non-infested control plants (Table1 & Fig. 1a). Freephenol from the leaves of lac insect infested plants was as high as 29.49 mg/g fr. wt. as compared to control (19.48 mg/g fr. wt.). Lac infested kusum plant showed 51.43 % increase in free phenol over control (Table1& Fig. 8). The role of secondary metabolites like phenolics in defensive mechanism of host plants after herbivore attack was examined and reviewed thoroughly by many workers [31, 35, 30]. The increase in free phenol content in lac insect infested kusum plant over control was also reported in *F. semialata* host plant of lac insect [15]. The same result obtained in the present kusum plant indicates that lac insect suppresses or evades the defense mechanism of the host plant and establishes a compatible relationship which continues successful life cycle of lac insect. The breaching of defensive mechanism of host plant by lac insect needs further through investigation.

Oxidative stress

Oxidative radicals play an important role in plants during various stresses, including the biotic stress such as insect infestation [36]. In our study it was found that superoxide radical and hydrogen peroxide level significantly increased in the host plant when infested with lac insect as compared to host plant with no lac infestation (Table1 & Fig. 1c). Superoxide radicals ($O_2^{.-}$)content in the leaves of lac insect infested kusum plant showed high content (2.30 mg/g fr.wt) in the leaves than tree with no lac inoculation (1.75 mg/g fr.wt). There was 31.92 % increase in superoxide radicals content was observed in lac infested condition over control. Higher hydrogen peroxidecontent in the lac infested tree was observed (2.18 mg/g fr wt) in the leaves of control tree (1.58 mg/g fr wt). There was 37.97% increase in hydrogen peroxide content in lac infested condition over control. Reactive oxygen species (ROS) and H_2O_2 levels known to increase during biotic stresses and are mainly involved in signaling. Their level is finely modulated by the plants to avoid tissue damage. They play a role as a secondary messenger and modifying protein structures thereby activating defense genes [37, 31, 38-41]. Our results also indicate that lac insect may modulate the expression of scavenging enzyme for reactive oxygen species (ROS) and thereby increases the concentration of ROS and H_2O_2 and acts as signals or messengers.

Proline

Proline content in the leaves of lac infested tree showed high content (0.57 mg/g fr wt) as compared to control (0.10 mg/g fr wt). There was an abrupt increase (475.29 %) in proline content of lac infested plants over control (Table1 & Fig. 1c). Osmotic adjustment is one of the important components of drought resistance in crop plants, this involves accumulation of compatible solutes and thus a decreased osmotic potential as well as increased pressure potential [42]. Osmotic adjustment helps the plants in maintaining a high relative water content (RWC) at a low leaf water potential, thereby sustaining the growth [43]. It is an acclimation that enhances dehydration tolerance. Osmotic adjustment enables the plant to extract more of this tightly held water. Proline accumulation is an indication of disturbed physiological condition, triggered by biotic or abiotic stress condition. Continuous feeding on host plants and profuse honey dew secretion by lac insect imposes biotic stress leading to abiotic stress condition such as moisture stress inside the plants. The high proline content in the leaves shows that water content in the plant was highly influenced by sap sucking lac insect.

Malondialdehyde

There was no significant increase in malondialdehyde content in the leaves of lac insect infested plants and control (Table1 & Fig. 1c). It is an important bye product of lipid oxidation involved in signaling the plant defense against a variety of stresses [44]. Lipid peroxidation encourages green leaf to emit volatiles in reaction to herbivory attack that attract the natural enemies of the herbivores [45]. The level in the content of malondialdehyde needs further investigation.

Ascorbic acid

The data indicate the significant differences in ascorbic acid content between leaves of lac insect infested plants and control (Table1 & Fig. 1d). The lac insect infested plants showed high content of ascorbic acid (6.08 mg/g fr wt) in the leaves than tree with no lac inoculation (5.51 mg/g fr wt). An increase of 10.22% was observed in ascorbic acid content in lac insect infested plants over control. It was reported that *H. armigera*- infested groundnut plants showed significantly greater activity over control [46]. Increase in the level of this scavenging enzyme indicates that host plant controls oxidative stress. This suggests that lac insect controls oxidative stress inside the host plant by triggering the production of scavenging enzyme.

The sessile nature, dependency of entire life cycle and its future generations on the single host plant makes lac insect unique phloem sap feeder alike whitefly and aphid sap feeder. The bond between lac insect and its host plant resulted in beneficial product the ‘lac resin’. Thus, it can be concluded from the above study that lac insect also manipulates many biochemical processes differently than the other phloem sap feeders to ensure its survival as well as host plant. In depth studies are needed on host plant responses to lac insect feeding at molecular to gain a better understanding of signal transduction, coevolution between host plants and lac insects, and the mechanisms of plants support to lac insects and use this information for sustainable lac production.

Table 1: Impact of lac insect (*K. lacca* Kerr.) feeding on leaf biochemical constituents of host plant Kusum (*Schleichera oleosa*)

Biochemical Parameters	Plants with lac insect	Plants without lac insect (Control)	CD-5%	% increase/ decrease over control
Total Sugar (mg/gfr. wt.)	18.37±0.34	14.21±0.32	0.37	29.32
Soluble Protein (mg/gfr. wt.)	25.47±0.43	16.03±0.33	1.21	58.88
Free Phenol (mg/gfr. wt.)	29.49±0.53	19.48±0.33	1.78	51.43
Proline (mg/gfr. wt.)	0.57±0.006	0.10±0.005	0.02	475.29
Malondialdehyde (mg/gfr. wt.)	1.63±0.02	1.61±0.01	NS	1.15
Superoxide Radicals (mmol/min/g fr. wt.)	2.30±0.032	1.75±0.32	0.12	31.92
Hydrogen Peroxide (mmol/min/g fr. wt.)	2.18±0.03	1.58±0.02	0.06	37.97
Ascorbic Acid (mg/gfr. wt.)	6.08±0.08	5.51±0.1	0.26	10.22

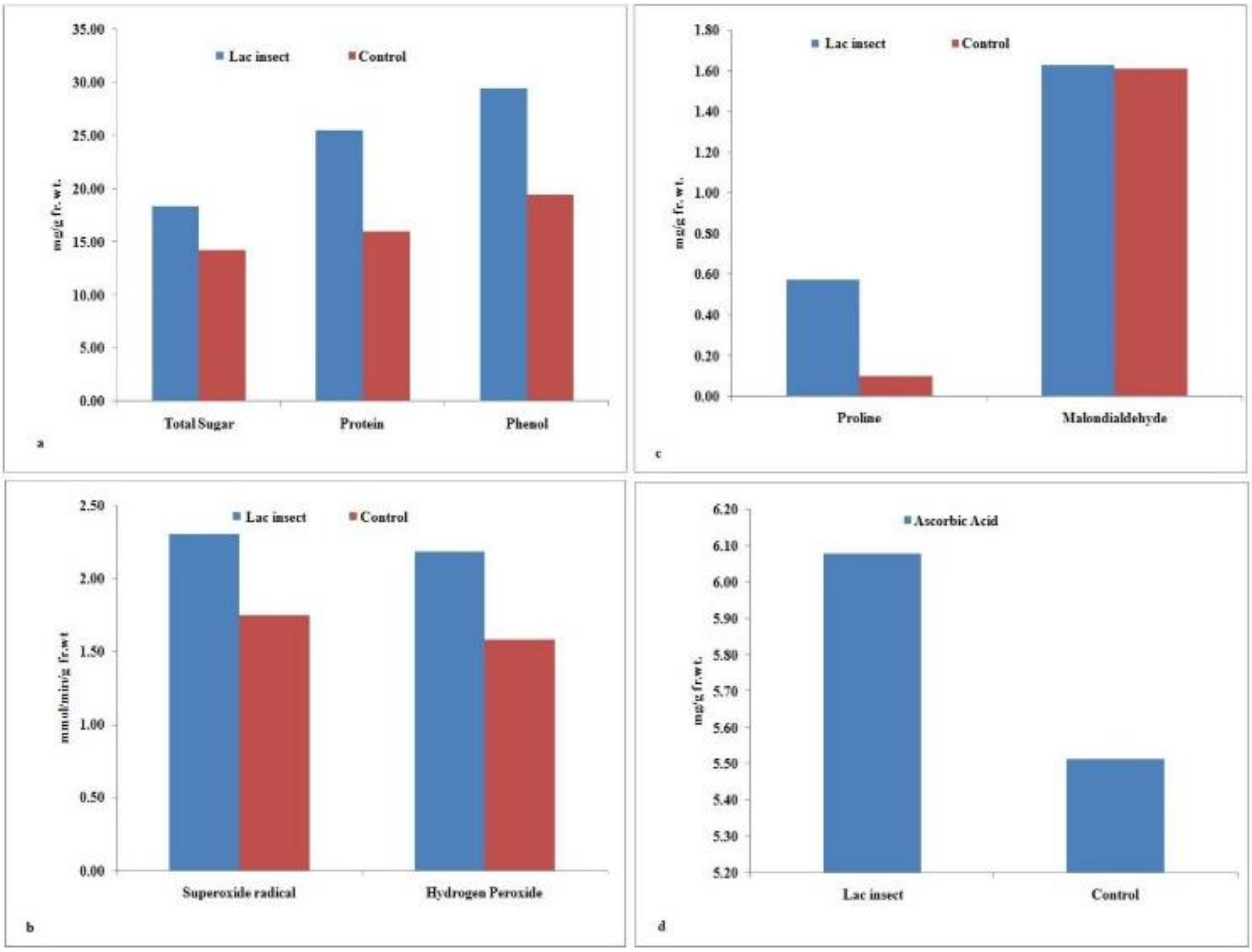

Fig 1: Impact of lac insect (*K. lacca* Kerr.) feeding on different leaf biochemical constituents of host plant Kusum (*Schleichera oleosa*)

Declaration
The authors declare no conflict of interest

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