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BIOCHEMICAL RESPONSE OF *FLEMINGIA SEMIALATA* (ROXB.) TO SAP SUCKING LAC INSECT *KERRIA LACCA* KERR

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ABSTRACT

Lac insect *Kerria lacca* (Kerr), an exclusive stem phloem feeder, produces a resinous secretion known as lac, over its body for protection which has several commercial applications. By sucking phloem sap from certain host plants, lac insect exerts stress. To elucidate its feeding effect on the host plant biochemical response, *Flemingia semialata* was infested with lac insect. From the pooled data over months and years it was observed that total sugar, soluble protein, free phenol, total chlorophyll and carotenoid contents increased in the leaves of lac insect infested host plant. A reverse trend was observed with leaf starch, and it decreased under lac insect infested condition. The study reveals that lac insect feeding significantly alters primary and secondary metabolism of host plants to ensure its survival as well that of its host plant.

Key words: *Kerria lacca*, *Flemingia semialata*, phloem feeder, response, biochemical constituents, total sugar, soluble protein, free phenol, starch, carotenoid

Higher plants face biotic and abiotic stress throughout their life cycle due to sessile nature. More than one million phytophagous insect species use plants as a source of their food and obtain their nutrition either by chewing or sucking sap from various plant parts (Mithofer and Boland, 2008; Dubey et al., 2013). Sap sucking insects like aphids and whiteflies belonging to the order Hemiptera inflict damage to the plants by inserting specialised tube like structures called stylets and ingest phloem sap. These insects cause severe damage to crop plants grown in temperate and tropical regions (Couldridge et al., 2007).The sucking of phloem sap (meant for growth and development of host plants) exerts stress on the hostplants; thereby triggering variety of biochemical and physical defense mechanisms that can deter feeding of insect (Dicke and Baldwin, 2010; Mithofer and Boland, 2012). Such herbivore-induced plant responses affect the behaviour and growth of the attacker and thus influence host plant suitability (Ohgushi, 2005; 2008). Herbivory can severely impair plant growth or even kill plants due to redirection of energy and matter from primary metabolism to defense (Zvereva et al., 2010; Zust and Agrawal, 2017).

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 but occasionally feeds on leaf petioles also. It is a soft bodied insect, producing a resin called 'lac' as a protective hard shell around its body by adult female. Due to its sessile nature it had an association with host plants throughout its lifecycle which extends from four to eight months. Lac, a unique product of 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. Lac cultivation is also found in Bangladesh, Myanmar, Thailand, Laos, Vietnam, and parts of China. Tree species like kusum (*Schleichera oleosa*), ber (*Ziziphus mauritiana*) andpalas (*Butea monosperma*) are the major host plants for lac culture. A bushy host plant, *Flemingia semialata* Roxb. of Papilionaceae family, has been identified for intensive lac cultivation for winter season. Exploitation of lac resin is attributed to its many commercial applications (Krishnaswami, 1962; Ramani, 2011; Sarkar, 2011; Ahmad et al., 2012). Lac insect details with host plant *F. semialata* are given in Fig. 1.

Host plants, which are the closest biotic associates of lac insect, influence its growth and development. Continuous lac insect life cycle on host plant reduces its life span and potentiality over the years by exerting stress. The slow growth of traditional hosts prevents

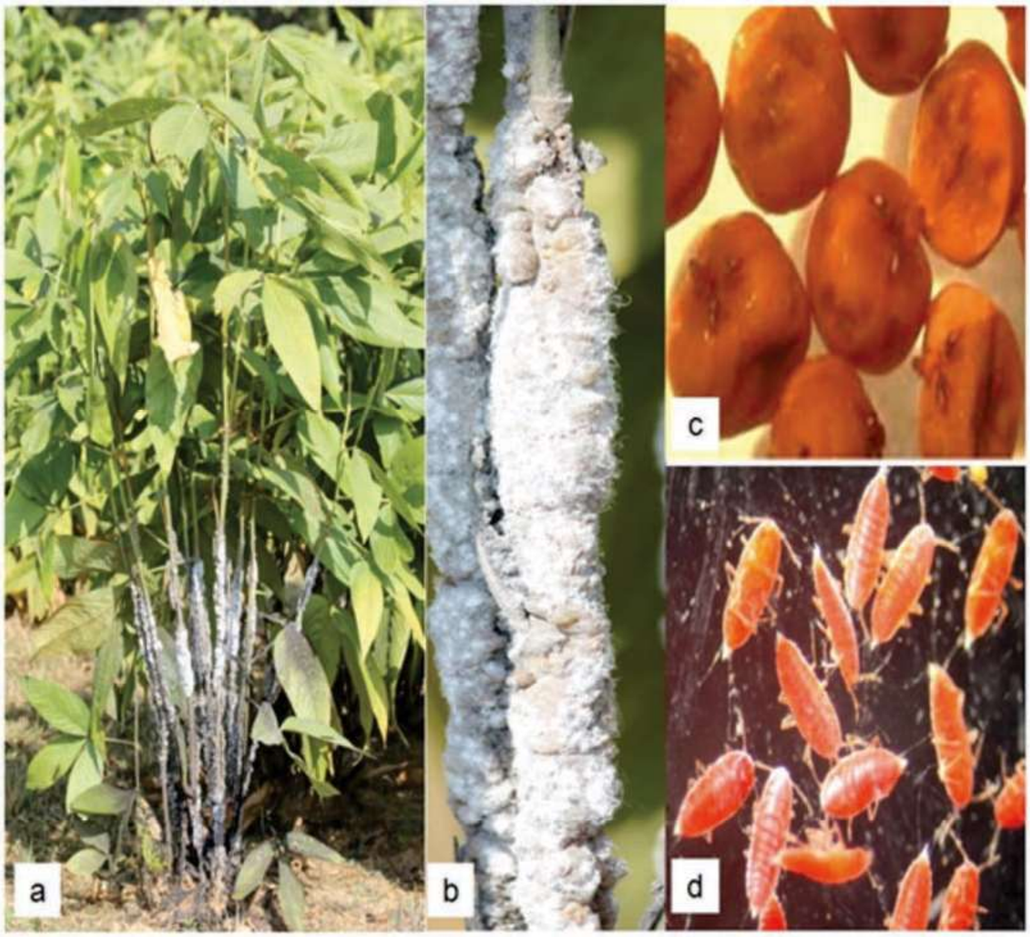

Fig. 1. a. Host plant *Flemingia semialata* with lac insect encrustation around stem; b. lac insect (*K. lacca*) adult female with lac resin covering; c. naked lac insect (*K. lacca*) adult female*; (d) lac insect in nymph stage*. *microscopic view

the growers from establishing new plantation (Ghosh et al., 2017). With dwindling number of host trees due to deforestation raises concern over the sustainable lac production. Therefore, maintaining the health of host plants one must understand the interaction between lac insect and their host plant. Thus, the present study attempts to analyze the influence of lac insect feeding on biochemical response of *F. semialata*. Such an understanding would help in elucidating the fundamentals of lac insect-host plant interaction and would provide the basis for enhancing lac yield in sustainable manner.

MATERIALS AND METHODS

To elucidate the effect of lac insect feeding on host-plant biochemistry, *F. semialata*, a bushy host plant was taken for study. The plants were infested (inoculated) with lac insect (*K. lacca*) during middle of July and study continued till lac insect complete its life cycle i.e. upto January of next year (approx. six months life cycle). 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 in 2013 and 2014 at the ICAR-Indian Institute of Natural Resins and Gums, Namkum, Ranchi. Leaf sample from both the conditions were taken during morning hours for biochemical analysis. Total sugar, starch, total chlorophyll, carotenoid, soluble protein and free phenol were determined from the leaves of *F. semialata* in each month starting from August till January in three replications over two years and expressed on fresh weight basis. The total sugar was extracted using 80% hot alcohol (McCready et al., 1950) and determined by Nelson's arseno-molybdate method using improved copper reagent of Somogyi (Nelson,1944; Somogyi, 1952). Total chlorophyll and carotenoid content was determined by DMSO method as described by Hiscox and Israelstam (1979) and values were calculated as per the equations given by Arnon (1949) and Litchtenthaler and Wellburn (1983). Soluble protein was estimated by Lowry's method (1952) and free phenol was determined using Folin-Ciocalteau reagent method as described by Bray and Thorpe (1954). Starch was determined by the anthrone method as described by McCready et al., 1950 and Scott and Melvin (1953). Starch content was calculated by multiplying the glucose values by 0.9 (Pucher et al., 1948). The data was pooled over months and years. Factorial analysis was done with standard statistical packages.

RESULTS AND DISCUSSION

Total sugar and starch: Plants infested (inoculated) with lac insect and plants without lac insect (control) had significant difference on total sugar content in the leaves in August to January (Table 1). Total sugar in infested plants showed more (12.9 mg/g fr. wt.) compared to control (12.2 mg/g fr. wt.), but this trend was inconsistent across the month (Fig. 1). It increased upto October in general and then decreased till maturity of the lac crop, with exceptionally higher amount in December in both the condition. Overall lac insect infected plants observed 5.5% increase in total sugar over control.

In plants, sugars control metabolism, growth, stress responses, and development from embryogenesis to senescence. Plants respond to stress (biotic or abiotic) by generating different sugar signals via photosynthesis to modulate growth and development (Rolland et al., 2006). It has been reported that sap feeders which suck the phloem sap from leaves (mesophyll feeders) cause reduction of photosynthesis (Welter, 1989; Nabity et al., 2009; Zangerl et al., 2002; Zvereva et al., 2010). Studies conducted by Crawley (1989), Collins et al. (2001) and Retuerto et al. (2004) suggest that phloem-feeding insects may also increase the photosynthetic rates in their hosts. However, in our study, increase in total sugar content in leaves of lac insect infested host plant was observed over control. This indicates activation of sugar metabolism in the phloem sap and it may have

Table 1. Influence of *K. lacca* feeding on biochemical constituents of *F. semialata*

Biochemical parameters (mg/gfr. wt.)	With lac insect	Without lac insect	CD, 5%	% increase/ decrease over control
Total sugar	12.91 ± 0.22	12.24 ± 0.22	0.64	5.5
Starch	22.42 ± 0.42	28.47 ±0.42	2.13	-21.78
Total chlorophyll	6.90±0.13	6.58±0.13	0.37	4.70
Total soluble protein	72.62 ± 0.39	65.44 ± 0.39	1.2	11.00
Total (free) phenol	13.9 5± 0.06	11.54 ± 0.06	0.17	20.62
Carotenoid	6.37±0.11	5.90±0.11	0.33	8.0

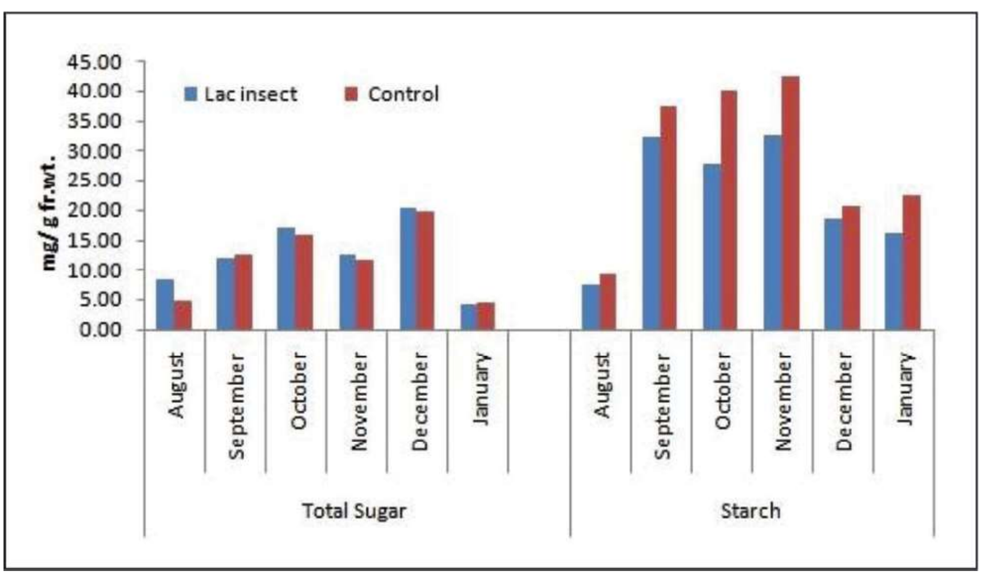

Fig. 2. Effect of *K. lacca* feeding on total sugar and starch

activated multiple pathways to control transcription, translation, protein stability and enzymatic activity

The starch content in the leaves of lac insect infested plants was invariably lower than control, over month and year (Table 1) and it was overall as low as 22.4 mg/g fr. wt. in lac insect infested plants vis-à-vis control (28.5 mg/g fr. wt.). Further the difference in starch content was wider under active growth period of lac insect in the month of September, October, and November. However, the differences were rather narrow in December but again wide at lac insect maturity time in January (Fig. 2). Overall decrease in starch was 21.8 % in lac insect infested condition over control.

During the day, starch is accumulated in the chloroplasts and degraded in the subsequent night to sucrose to support respiration and growth as well as metabolic processes generating plant defenses (Chapin et al., 1990; Smith et al., 2005). The decrease in the starch content of lac insect infected host plant leaves may be attributed to the fact that lac insect feeds passively on the plants which continues day and night. The extra demand for sucrose posed by lac insect feeding during the period of darkness may be fulfilled by increasing the degradation of starch. In fact, the morning leaf sample analyzed was the sample from where the starch was already exhausted during the preceding dark period. However, starch content at the end of the day in lac infested leaves needs investigation to establish its role during sap feeding. Thus, lac insect may induce the starch degradation pathway during dark period to ensure constant supply of sucrose.

Total chlorophyll and carotenoid: The plants with lac insect showed higher total chlorophyll content in leaves compared to control except in the month of August and at the time of maturity i.e. in the month of January (Fig. 3). Overall the total chlorophyll content was significantly higher (7.8 mg/g fr. wt.) in lac infested plants as compared to control (6.6 mg/gfr. wt.) over months (Table 1). There was 4.7% higher total chlorophyll content in lac infested plants as compared to control. Higher carotenoid content was found in the leaves of lac insect infested plant compared to control over entire life cycle of lac insect (August to January) (Fig. 3). About 8.0% higher carotenoid was recorded in leaves of lac insect infested plants over control. It was 6.4 mg/g fr. wt. in lac insect infected plants and 5.9 mg/g fr. wt. in control (Table 1). Ability of carotenoids to detoxify reactive oxygen species (ROS) is very well known (Taiz and Zeiger 1998). Hessian fly (*Mayetiola destructor*) larvae attack showed a rapid elevation of ROS in wheat (Liu et al., 2010). Dubey et al. (2013) also found an increase in the concentration of ROS and H_2O_2 in aphid and whitefly insect-infested leaves. The increase in the carotenoid content in the lac insect infested leaves indicates that ROS may be generating during its feeding.

Soluble protein and free phenol: Soluble protein content from the leaves of lac insect infested plants was significantly higher than control (Table 1) and

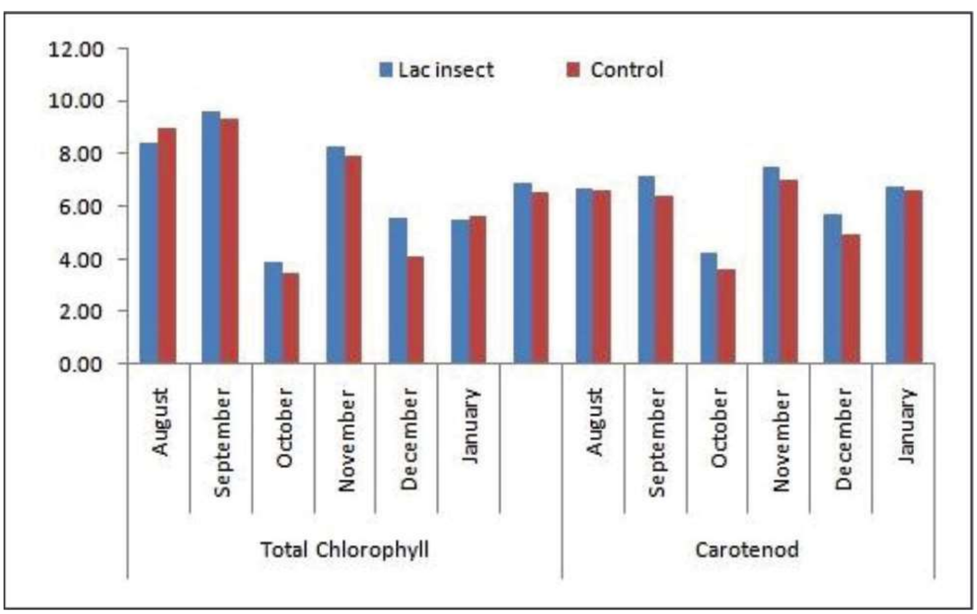

Fig. 3. Effect of *K. lacca* feeding on total chlorophyll and carotenoid

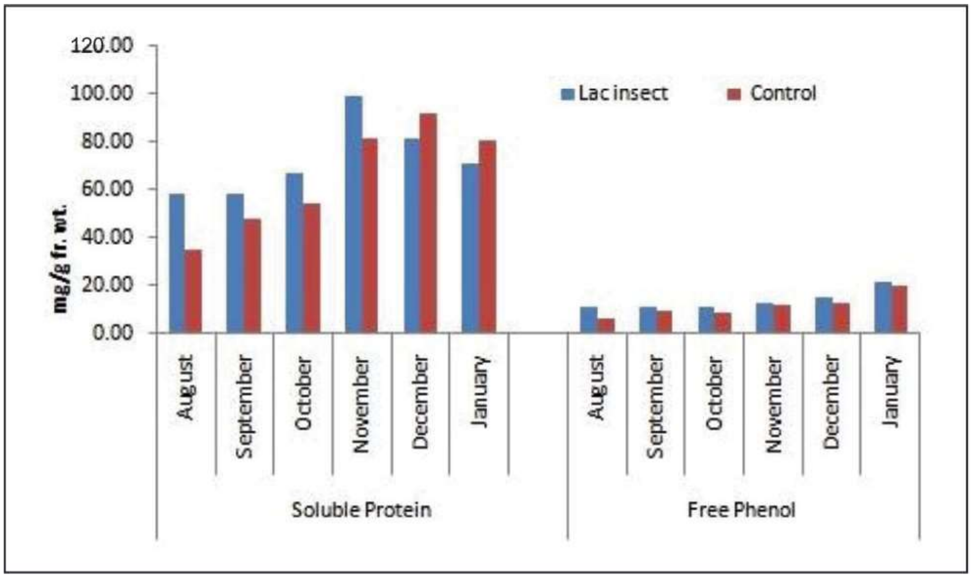

Fig. 4. Effect of *K. lacca* feeding on Soluble protein and free phenol

trend was consistent over year and month. (Fig. 4). The protein content over months in lac insect inoculated plants was 76.6 mg/g fr. wt. as compared to plants with no lac insect infestation (65.4 mg/g fr. wt.). Overall there was 11 % increase in protein in lac insect infested plants as compared to control. Novel approaches like microarray and proteomics changed the traditional view of role of proteins and established that it has role of protein-based defense in plants resistance 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 (Mithofer and Boland, 2012; Schuman and Baldwin, 2016). The sap sucking insects like aphids and whiteflies up regulate the amino acid metabolism significantly (Dubey et al., 2013). This increase in protein potentially influences the insect-plant interaction.

Parallel to protein content in the leaves, free phenol content from the leaves of lac insect infested plants was significantly higher than the control and it was constantly increased up to lac insect maturity (Fig. 4). Freephenol from the leaves of lac insect infested plants was as high as 13.9 mg/g fr. wt. as compared to control (11.5 mg/g fr. wt.). The lac infested plant showed 20.6 % increase in free phenol over control (Table 1). Primary metabolites contribute to the building blocks for defense production and provide resistance in response to attack. The defensive role of secondary metabolites like phenolics in plants after herbivore attack was reviewed thoroughly by many workers (Howe and Jander 2008; Stam et al., 2014; Schuman and Baldwin 2016). This result revealed that sap-sucking insects in our case the lac insect also breaches the integrity of plant and establishes a compatible relationship by evading all the defensive responses of host plants as evident from its continuous, successful life cycle of 4-8 months.

The immobility, longer life cycle and prolonged stem phloem sap feeding are the features that distinguish the lac insect from whitefly-plant and aphid-plant interaction. 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. However, there is a need to gather more information on manipulation of biochemical processes by lac insect feeding on other host plants also.

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