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Effect of different density of lac insect (*Kerria lacca*) infestation on host plant *Flemingia macrophylla* growth and photosynthetic pigments

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Abstract
Lac insect life cycle is entirely dependent upon host plants. Its production relies on host plant sustenance of the insect to harvest economic yield. However insect attachment causes fitness cost to host plants. The present study identified that at any crop stage the insect settlement density that is at level III or more is damaging to the plant and is not suitable for lac crop. Leaf biomass decreased by 22-37% at density levels I and II. Total plant biomass was highly affected at early stage of 30 days when density level I and II showed 46.2±8.2 g and 35.2±8 g when control plants had 51.5±16.2 g. At later stages 45 days and 120 days the decline in biomass was less than at 30 days. Level II plants showed increased leaf area of 902 cm2 when control were 898 cm2 at 45 days suggesting that this density level is causing an over compensatory response. Both chlorophyll and carotenoid content showed decline with increasing density and at all stages. In summary, it has been observed that a density of up to level II is suitable for both balanced growth and lac production. However, densities beyond this level significantly disturb the growth of the host plants. Additionally, around 120 days after infestation, plants become less susceptible to chlorophyll damage and show improved growth, highlighting the critical vulnerability of early stages. Therefore, maintaining optimal environmental conditions during these stages is crucial to ensure a successful lac crop.

Keywords: Lac insect, host plants, stem herbivory

Introduction

Having an export share of 2.9% among the few natural resins and gums which amounts to a total of 7.3 thousand tonnes in 2019-20 *lac* is one and only insect origin resin that finds its application in several small and large-scale industries (Yogi *et al*., 2021) [32]. Its specialty materials- resin, wax, dye have applications in paint and varnishes and also in food, pharmaceutical industries (Srivastava *et al*., 2017) [29] and the constituting chemical aleuritic acid has immense applications in perfume industries and bioactive compounds (Sharma *et al.*, 2016) [28]. Its production is concentrated in a handful of countries across South, East, and Southeast Asia, such as India, Thailand, China, Indonesia, Bangladesh, Myanmar, Laos, and Vietnam. India holds the top position globally in lac production, with Thailand following closely behind. India being the largest producer and having large cultivation base has high potential of increased production which is yet underutilized. Therefore understanding and identifying the yield of lac encrustation is imperative.

Lac is an obligate plant colonizer that derives its complete nutrition from the phloem sap of host plants by inserting their proboscis into the stem of the plant. Traditionally lac is cultivated on host trees- *Schleichera oleosa, Butea monosperma, Zizyphus mauritiana.* Morphological characterization of good host plants have been carried out by Prasad *et al*., 1965 [22]. A complete and exhaustive list is also available (Sharma *et al*., 1997; Mishra *et al*., 2001) [26, 20]. This is a highly specific plant-insect interaction that changes with the host plant, insect strain and co-regulated with the environmental complexes. Therefore a plant-based approach to understand the effect of lac insect infestation needs to be understood to harness the full potential.

Recently there has been an upsurge on usage of bushy host shrubs to enable the cultivation practices at ease (Sharma *et al.*, 2016) [28]. *Flemingia* species mainly *Flemingia macrophylla* and *Flemingia semialata* have shown over 80% host preference for lac insect as insects reared on them had increased cell weight and resin output per female (Sharma *et al.*, 2007) [27]. These members of Leguminoseae family are currently being exploited for commercial production in India. *Cajanus cajan* another legume is also a host plant that can sustain lac insect but the infestation causes decrease in seed protein quality and seed yield (Lohot *et al.*, 2018) [16]. Host-species mediated differentiation of lac insects into apterous male is high in *F. macrophylla* as compared to that on *C. cajan* and *A. lucida* (Ramani 2003) [24]. While most of the studies have been carried out to decipher the effect of host plant on lac and its production limited studies are available that investigated the effect of lac infestation on host plants.

Stem herbivory occurs in several scale insect and aphid species such as *Coccus hesperidum* on Citrus and fern species (Golan *et al.*, 2015) [6]. It reduces plant survivability by over 60% and causes more than 30% reduction in reproduction and almost 15% reduction in biomass (Stephens and Westoby, 2014) [30]. This is mostly due to the defense response mounted by the host plants in response to insect load. Insect herbivory reportedly causes decrease in growth of infected plants but reports exist that aphid feeding can occur without affecting the growth and fitness of plant (Meyer and Whitlow, 1992) [18]. The deliberate infection of host plants with lac crawlers is to ensure that host survives and lac yield is maximized. But to identify this optimal insect load and plant stage, the effect of lac infection on host plant growth and photosynthetic capacity needs investigation. Insect preference and its inhabitation on host plant was investigated in which phloem distance from stem exterior was found non-significant (Kaushik *et al.*, 2012) [12]. The phytochemical constituents of lac host plant barks were found non-significant in determining the insect preference for host plants (Pushker *et al.*, 2011) [23]. In order to develop a good insect density and host plant combination it is necessary to understand what density of insects should be loaded onto host plants and understand their effect on plant fitness. Therefore, the main objective of this study was to determine the growth as well as defense responses of lac host plants on lac inoculation. This helped us in identifying the best host-plant and density of lac insect combination.

Materials and Methods

Experimental location, plant material and insect

The entire experiment has been conducted inside the greenhouse and polyhouse facility available at the Institute Research Farm of ICAR-NISA, Ranchi. *Flemingia macrophylla* plants were raised from seeds sourced from the IRF and grown in nethouse with best package of practices. Six months old plants raised in 6-inch diameter pots filled with field soils. Visually good-looking *kusmi* strain broodlac loaded with bright red lac cells free of any pest were harvested from Kusum trees present at IRF. They were sized into 5cm logs and tied on host plant stem keeping a distance of 2 cm from soil with cotton plugs placed at the top and bottom portion of stem as followed by Ghosal *et al.*, 2000. This was then removed after two weeks when the emergence from the broodlac stopped and the surface of stem were inhabitated by lac crawlers showing no movement. It was made sure that crawlers have settled with their proboscis inserted indicated by their non-movement and they have assembled evenly on the defined length of the stem without conglomerating at one place. Inoculated host plants were then categorized into four groups based on lengthwise area of insect settled on stem surface measured as percentage of the entire stem height. Plants were thus categorized such that their stem height has 25%, 50%, 75% and complete stem (100%) length is inhabited by insect and were categorized as Class I, Class II, Class III and Class IV respectively along with a control. Control plants did not have inoculation. Data was collected with 4 replicates each at 30 days, 45 and 120 days post inoculation.

Physiological parameters

Leaf area (LA) was recorded manually on graph paper by using third leaf from top of 4 different plants pooled together to make one replicate and three such replicates were used. Total fresh weight was taken for leaf and stem separately using same plant and four such plants were taken as replicates for 30 days, 45 days and 120 days post insect inoculation. The same was repeated for dry weight measurements after drying in oven at 70 degree C for one week.

Biochemical parameters

Pigments namely Chlorophyll a, Chl b and total Chl was measured by non-maceration method using DMSO following protocol by Hiscox and Isrelstam (1979) [10] carotenoids by Arnon, 1949 and calculations as per Lichtenthaler and Wellburn (1983) [15]. Obtained data were analyzed by two factor analysis method using Wasp2.0 maintained by ICAR-CCARI and test was conducted at $p \leq 0.05$ level of significance

Statistical Analysis

Data collected was analyzed using the standard techniques of 2-Factor Analysis of Variance (ANOVA) with the assistance of computer software Wasp2.0 maintained by ICAR-CCARI. "F" test with significance at a 5% level of significance ($p<0.05$) is reported using critical difference (C.D) calculated to determine the significance of differences between two treatment means.

Results and Discussion

Most of the plant-insect interaction studies have focused on interpreting the defense or resistance response mechanism induced in the host and majority are carried out using foliage herbivores. However a metaanlysis has identified that stem herbivory causes 22% loss in photosynthetic performance than uninfested plants (Stephens and Westoby, 2014) [30]. While defense responses are result of gene expression changes that occur in response to feeding guild their long-term response is manifested in plant physiological performance. Therefore changes in growth characteristics of host plants influenced by insect feeding and specifically lac insect which is associated with the plant throughout its life cycle is imperative to understand. The effect of density of insects on plant physiological aspects over the duration of insect attachment would lead us to decide what amount of broodlac must be inoculated in the host plant to get economic benefit from lac crop without damaging the host plant physiology.

Biomass

The fresh weight of leaf is an indicator of plant growth. In the present study, it was found that during early stages at 30 days post infestation the leaf weight decreased significantly with increase in insect density. At level IV the growth was almost one-third of the growth in control plants. While with increasing densities the growth declined gradually but significantly by 22%, 37% at density I and II while drastic reduction occurred by 62% and 69% at levels of III and IV density respectively. An even higher level of reduction in fresh leaf weight by 39%, 61%, 66% and 72% occurred at density levels I, II III and IV. This suggests that lac insect feeding reduces the plant resources allocation to growing parts and potentially diminishing the plant growth potential as is reported by grass bug feeding on rye by Hansen and Nowak, 1988. At later stages of insect inhabitation and feeding similar but less prounounced decline in growth occurred. At the highest level of infestation only 52% decrease is recorded while at lower densities the damage ranged from 19% to 37% between levels I-III. This is suggestive of the ameriolation of damage impacted by feeding lac insect occurs by regaining the photosynthetic potential of *Flemingia* plants at later stages probably like an over compenstaory response as is reported in several other crops such as oat-cherry aphid feeding on barley (Marlott and Davy, 1978). Environmental responses play key role in regulating the photosynthetic potential of plants which must not be undermined in such scenarios.

Feeding damage by herbivory causes alteration in balance between source and sink capacity thereby causing changes in photosynthetic potential of host plants. There exist a reconfiguration of primary metabolism towards secondary metabolic pathways (Schwachtje and Baldwin, 2008) [25]. Phloem-feeders have been reportedly known to be an additional sink for photosynthates during their infestation in the host plant (Meyer and Whitlow, 1992) [18]. Total biomass of entire plant was measured to understand the photosynthetic responses manifesting into growth upon lac feeding. Control plants rapidly accumulated photosynthates and showed increased biomass but the infected plants showed reduction in biomass accumulation over time that varied with density. Evidently it is found that after density level II, the level III and IV were devastating for host plants and they could not maintain the biomass accumulation with high insect load. While level I showed 46.2±8.2 g at 30 days, the control plants had 51.5±16.2 at same time; level II had 35.2±8.3 but level III and IV accumulated only 27±2.3 and 25±4.5 g of biomass. At 45 days, level I slightly higher 49.2±87.7 g biomass but level IV had no significant increase in its biomass from 30 days growth while the control plants at 45 days displayed 63±12.2 g biomass. Thus it is evident that insect feeding is diminishing biomass accumulation and however the growth is not reduced but is retired severely at increasing densities and is almost nil growth at level IV. Decrease in growth with increasing insect density is also reported in trees like poplar that varied with different nutrient levels in the host (Glynn *et al*., 2003) [5]

Table 1: Total Biomass of *F. macrophylla* at four different insect density levels inoculated for 30 days, 45 days and 120 days

	Total Plant Biomass (g)		
	30 days	**45 days**	**120 days**
Control	51.5±16	63±4.9	98±12.3
Level I	46.2±10.7	49±8.2	64±8.2
Level II	35.2±8.3	37±9.1	61±13.7
Level III	27±2.3	32±10.8	52±6.0
Level IV	25±11.1	29±7.2	38±7.8

Data represented as Mean + Standard Deviation

Leaf Area

Aphid feeding have been shown to be non-effective in growth parameters of the plant however contrastingly the over compensatory photosynthetic responses of plants by increasing photosynthetic rates and leaf area is frequently reported to insect feeding is also reported (Meyer, 2000) [19]. Leaf is the seat of photosynthesis and leaf expansion, number or leaf area is often highly affected by both biotic and abiotic stress imposed on plants. In the present study it is found that leaf area reduced with increased density levels at early stages. At 30 days, the leaf area was 931 cm^2, 832 cm^2 and 854 cm^2 in Control, level I and II plants. While at 45 days the level II plants had 902 cm^2 area while control plants had 898^2 and later at 120 days the leaf area of level II was 1133 cm^2 while control plants had 1236 cm^2. Level III and IV plants had almost 50% or even less leaf area than control plants at all three time stages showing drastic reduction in leaf area. It thus indicates this density is an overload of insect on the host plant which is unable to sustain and balance its growth with the load. Similar leaf area metrics is displayed by level III density levels. Increased leaf area with decreased leaf weight suggests increased photosynthetic rate (Specific leaf area) at these two levels II and III as is shown by Huang *et al*., 2014 [11] on Brassica species. This over compensatory response of increased net photosynthetic is reportedly involved in generating defense response to the insect damage by producing secondary metabolites in several plants (Koricheva *et al.*, 2004) [13]. Stem herbivory often associated with photosynthetic overcompensation by decline in the leader performance but simultaneously supplementing the lateral growth (Stephens and Westoby, 2014) [30]. Such compensatory growth response to low aphid pressure is also demonstrated in a field study on Soybean by soybean aphid herbivory. Plants with low cumulative aphid days had higher yields than control plants in this study (Kucharik *et al*., 2016) [14].

Photosynthetic pigments

Chlorophyll catabolism is associated with different stressors such as depleted soil nutrition, insect damage and other abiotic stresses (Ni *et al.*, 2002) [21]. Chlorophyll is the premium pigment involved in photosynthesis and is located in the thylakoid membranes of the plastid. Decrease in chlorophyll content in *Flemingia* upon lac inoculation is reported by Ghosh *et al.*, 2018 [4] wherein the decrease was higher during initial stages but was insignificant at later stages of lac infestation. Similar results are evident from the current study. Chlorophyll a as well as total chlorophyll all decreased with increased duration and density levels of lac insect than non-infested plant at all times (Figure 1a, c). Chlorophyll b had less decline at all stages but chl a showed ~3 fold decrease than control plants at 120 days an at level IV density (Figure 1b). Decrease in chlorophyll content with aphid feeding and increasing duration of infestation is reported in multiple legumes (Golawska *et al.*, 2010) [7] and Brassica as well (Huang *et al.*, 2014) [11].

Carotenoids

At early stage when control plants had 0.8mg/gfr wt carotenoids F. macrophylla plants had 0.4 mg/gfw which is almost 50% of the control and this was similar at all density levels. At 45 days, the similar 50% reduction is evident at level I and II but severe reduction at level III and IV. At 120 days the decline was less steep and only 16% of that of Control at level II (Figure 1d). Total chlorophyll content is negatively correlated with phenolics-a secondary metabolite involved in resistance responses in woody species such as Eucalyptus in response to herbivory due to photoinhibition effect rather than resource allocation (Close *et al.*, 2003) [2]. Phloem feeding by aphids leads to reduction in luminal or stromal pH thereby affecting the zeaxanthin-violaxanthin system of regeneration rejecting the hypothesis of photoinhibition (Golawska *et al*, 2010) [7]. They suggested aphid-feeding induced chlorophyll degradation being traded off with the cost of defense metabolic intermediated production which can be a suggestive reason for reduced pigments in our study but which needs further detailed study.

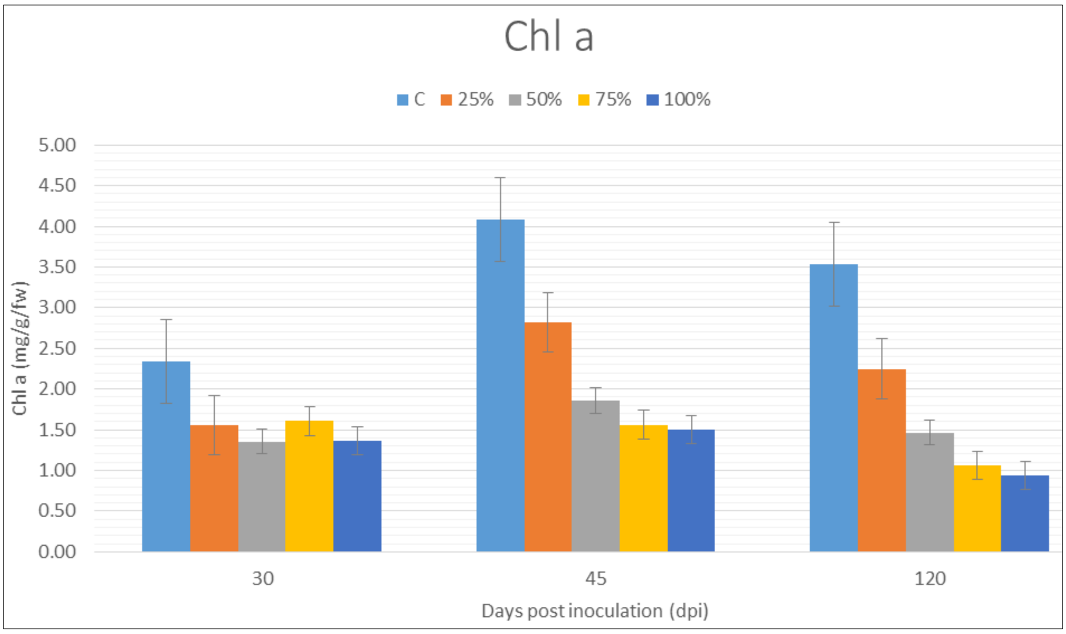

Fig 1a: Chlorophyll a content of *F. macrophylla* Control and infect plants at different density levels measured at 30, 45 and 120 days post inoculation

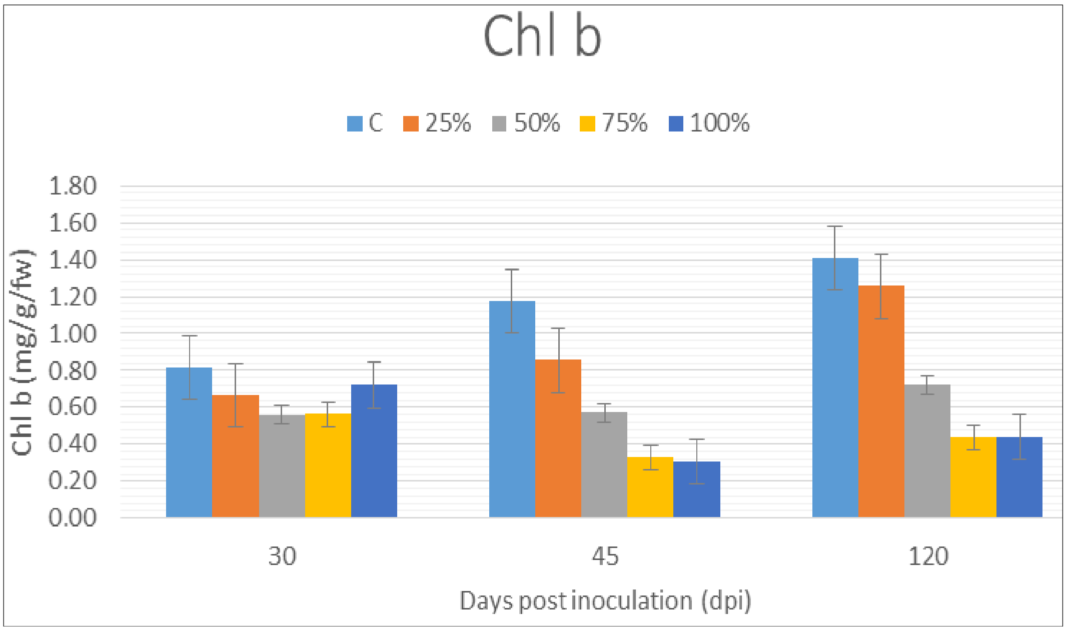

Fig 1b: Chlorophyll b content of *F. macrophylla* Control and infect plants at different density levels measured at 30, 45 and 120 days post inoculation

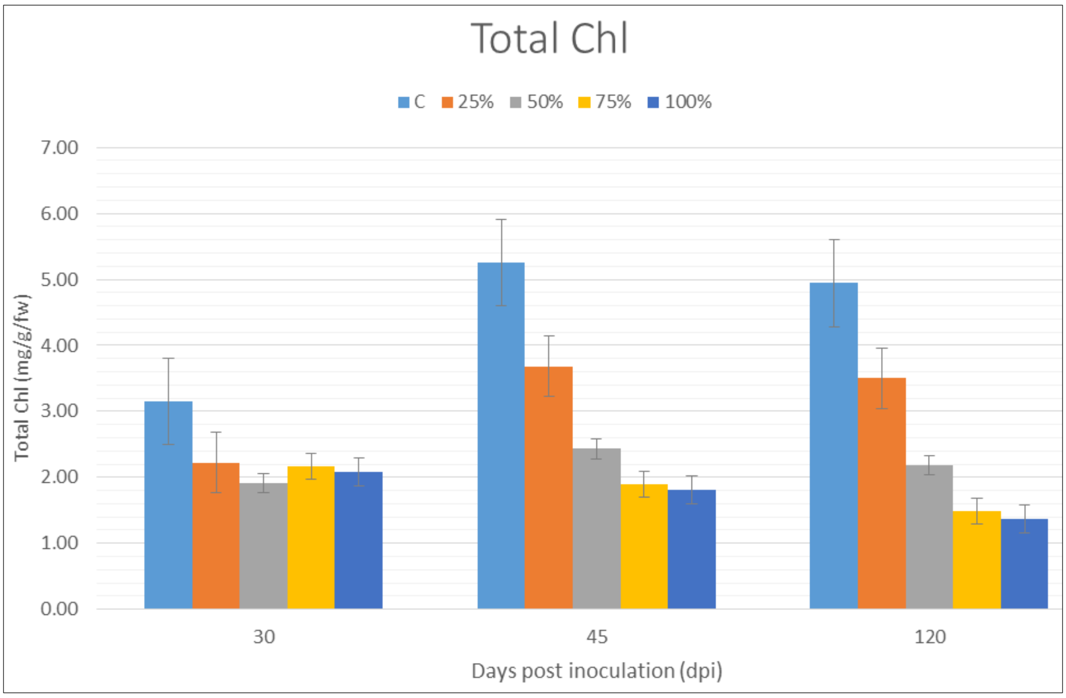

Fig 1c: Total Chlorophyll content of *F. macrophylla* Control and infect plants at different density levels measured at 30, 45 and 120 days post inoculation

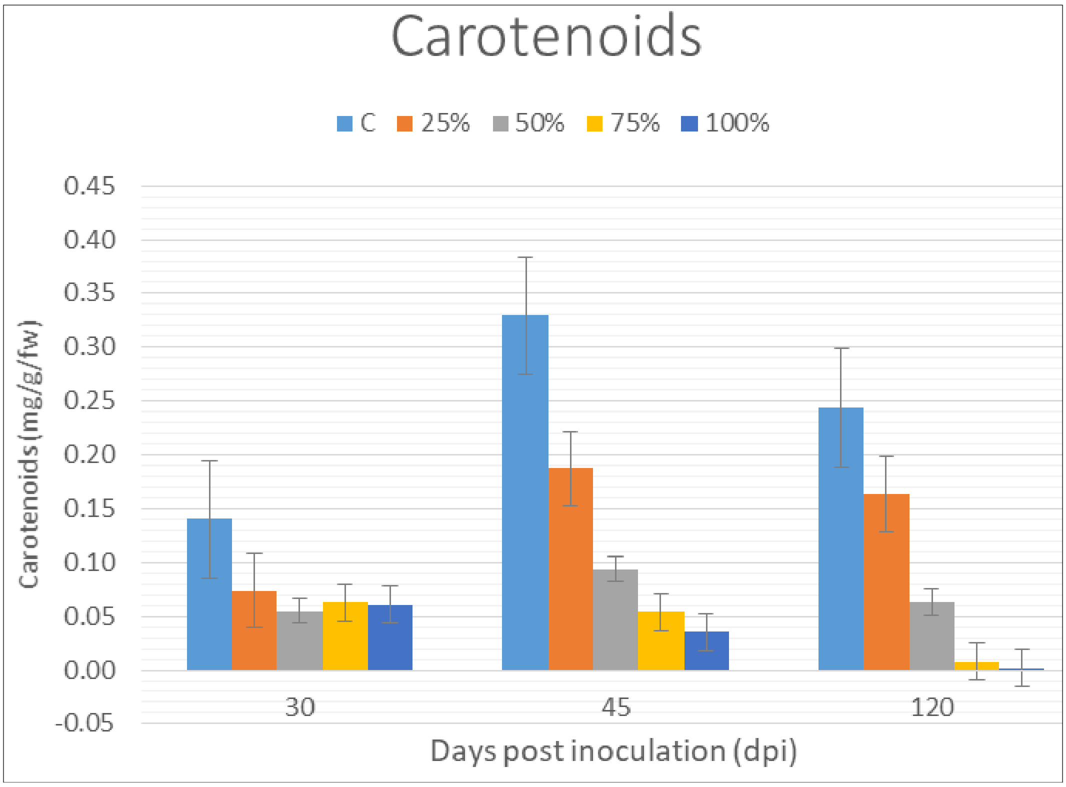

Fig 1d: Carotenoid content of *F. macrophylla* Control and infect plants at different density levels measured at 30, 45 and 120 days post inoculation

Conclusion

The study thus finds out that insect feeding causes negative impact on plant growth as well as pigment concentration. The damage varies with the insect density levels as well as the stage of plant infested with lac and is similar to that found in Citrus species when scale insects feed on them at different densities (Golan *et al.*, 2015) [6]. Overall it could be found that a density of up to level II is permissible in terms of both balanced growth as well as lac production as density beyond that led to drastic perturbation of host growth. Besides at about 120 days post infestation the plants were less vulnerable to chlorophyll damage and growth suggesting the early stages are more vulnerable and must be maintained with care providing the optimum environmental conditions to ensure a good lac crop. Also an optimum level of insect settlement without hampering growth drastically is when crawlers have inserted their proboscis at approximately half of the stem length dispersed evenly on the entire length. Any densities more than that would reduce growth drastically.

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References
1. Arnon DI. Copper enzymes in isolated chloroplasts. Phenoloxidase in Beta vulgaris. Plant Physiol. 1949 Jan;24:1-15.
2. Close D, McArthur C, Paterson S, Fitzgerald H, Walsh A, Kincade T. Photoinhibition: a link between effects of the environment on eucalypt leaf chemistry and herbivory. Ecology. 2003 Nov;84(11):2952-2966.
3. Ghosal S, Sharma KK, Bhattacharya A, Kumar KK. Broodlac and sticklac yield as affected by site of inoculation, shoot length coverage and irrigation levels.
4. Ghosh J, Lohot VD, Ghosal S, Singhal V, Sinha NK. Drought resilient *Flemingia semialata* Roxb. for improving lac productivity in drought prone ecologies. Indian J Genet. 2017 Mar;77(1):153-159.
5. Glynn C, Herms DA, Egawa M, Hansen R, Mattson WJ. Effects of nutrient availability on biomass allocation as well as constitutive and rapid induced herbivore resistance in poplar. Oikos. 2003 Aug;101(2):385-397.
6. Golan K, Rubinowska K, Kmieć K, Kot I, Górska-Drabik E, Łagowska B, *et al*. Impact of scale insect infestation on the content of photosynthetic pigments and chlorophyll fluorescence in two host plant species. Arthropod-Plant Interact. 2015;9:55-65.
7. Goławska S, Krzyżanowski R, Łukasik I. Relationship between aphid infestation and chlorophyll content in Fabaceae species. Acta Biol Cracov Bot. 2010;52:76-80.
8. Hansen JD, Nowak RS. Feeding damage by *Irbisia pacifica* (Hemiptera: Miridae): effects of feeding and drought on host plant growth. Ann Entomol Soc Am. 1988;81:599-60.
9. Heng-Moss TM, Ni X, Macedo T, Markwell JP, Baxendale FP. Comparison of chlorophyll and carotenoid concentrations among Russian wheat aphid (Homoptera: Aphididae)-infested wheat isolines. J Econ Entomol. 2003 Apr;96:475-481.
10. Hiscox JD, Israelstam GF. A method for the extraction of chlorophyll from leaf tissue without maceration. Canad J Bot. 1979;57(12):1332-1334.
11. Huang TI, Reed DA, Perring TM, Palumbo JC. Feeding damage by *Bagrada hilaris* (Hemiptera: Pentatomidae) and impact on growth and chlorophyll content of Brassicaceous plant species. Arthropod-Plant Interact. 2014;8:89-100.
12. Kaushik S, Pushker AK, Lakhanpaul S, Sharma KK, Ramani R. Investigations on some of the important host plants of *Kerria lacca* with reference to phloem distance. Eurasian J Biosci. 2012;6:32-38.
13. Koricheva J, Nykänen H, Gianoli E. Meta-analysis of trade-offs among plant antiherbivore defenses: are plants jacks-of-all-trades, masters of all? Am Nat. 2004 Apr;163(4).
14. Kucharik CJ, Mork AC, Meehan TD, Serbin SP, Singh A, Townsend PA, *et al*. Evidence for Compensatory Photosynthetic and Yield Response of Soybeans to Aphid Herbivory. J Econ Entomol. 2016 Jun;109(3):1177-1187.
15. Lichtenthaler HK, Wellburn AR. Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. Biochem Soc Trans. 1983;11:591-592.
16. Lohot VD, Ghosh J, Ghosal S, Thamilarasi K, Gunjan SK. Effect of lac culture on seed quality traits of Pigeonpea [*Cajanus cajan* (L.) Millsp.]. J Entomol Zool Stud. 2018;6(2):2293-2298.
17. Mallott PG, Davy AJ. Analysis of effects of the bird cherry-oat aphid on the growth of barley: unrestricted infestation. New Phytol. 1978;80(1):209-218.
18. Meyer GA, Whitlow TH. Effects of leaf and sap feeding insects on photosynthetic rates of goldenrod. Oecologia. 1992;92:480-489.
19. Meyer GA. Effects of insect feeding on growth and fitness of goldenrod (*Solidago altissima*). Recent Res Dev Entomol. 2000;3:29-41.
20. Mishra YD, Sushil SN, Bhattacharya A, Chakrabarty PK, Kumar S, Mallick A. Lac productivity rating of different lac insect on conventional and promising host plants. Final Report on the A.P. Cess Funded Ad-hoc project. Indian Lac Res Inst, Namkum, Ranchi, India; 2001.
21. Ni X, Quisenberry SS, Heng-Moss T, Markwell J. Dynamic change in photosynthetic pigments and chlorophyll degradation elicited by cereal aphid feeding. Entomol Exp Appl. 2002;105:43-53.
22. Prasad UN. A phylogenetic-cum-artificial analysis of the host plants of the lac insect, *Laccifer lacca* (Kerr) with a view to determine host preference. Indian Forester. 1965;91(5):297-308.
23. 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. Bot Res Int. 2011;4(3):48-51.
24. Ramani R. Genetic basis of deviations from bilateral symmetry in insects and its application, with special reference to lac insects. Ph.D. Thesis. Ranchi University, Ranchi; c2003.
25. Schwachtje J, Baldwin IT. Why does herbivore attack reconfigure primary metabolism? Plant Physiol. 2008;146(3):845-851.
26. Sharma KK, Ramani R, Mishra YD. An additional list of the host plants of lac insects, *Kerria* spp. (Tachardidae: Homoptera). J Non-Timber For Prod. 1997;4:151-155.
27. Sharma KK, Kumari KKK, Lakhanpaul SSL. Host plant mediated variations in resin producing efficiency of Indian lac insect, *Kerria lacca* (Kerr) (Homoptera: Coccoidea: Tachardiidae). Entomon. 2007;32(3):203-207.
28. Sharma KK, Md M, Mohanasundaram A, Ramani R. Beneficial Insect Farming: Benefits and Livelihood Generation. ICAR-Indian Institute of Natural Resins & Gums, Ranchi, India; c2016. p. 1-194.
29. Srivastava SA, Thombare NA. Safety assessment of shellac as food additive through long term toxicity study. Trends Biosci. 2017;10(2):733-40.
30. Stephens AE, Westoby M. Effects of insect attack to stems on plant survival, growth, reproduction and photosynthesis. Oikos. 2015;124(3):266-273.

31. Thakur AK. Comparative study of lac-hosts with special reference to Acacia catechu and Cassia florida. Bull Indian Lac Res Inst. 1932;9:1-8.
32. Yogi RK, Kumar Nirmal, Sharma KK. Lac, Plant resins and Gums statistics 2020: At a glance. ICAR-Indian Institute of Natural Resins and Gums, Ranchi, India. Tech Bull No. 2/2022:15.