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Assessing the temporal fatty-acid dynamics in *Flemingia semialata* Roxb. ex W. T. Aiton during lac insect infestation

Atul Raikwar[1], Ashish Kumar Choudhary[2], Anand Kumar Pushker[3], Sandeep Kaushik[1*]

[1]Department of Environmental Science, Indira Gandhi National Tribal University, Amarkantak, India.
[2]Department of Botany, Deshbandhu College, University of Delhi, New Delhi, India.
[3]Department of Botany, Ramjas College, University of Delhi, New Delhi, India.

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ABSTRACT

Fatty acids and lipid-derived signaling molecules are crucial in plant-insect interactions; however, their dynamic changes during lac insect infestation are inadequately understood. The present preliminary investigation revealed distinct temporal shifts in the fatty acid profiles of *Flemingia semialata* Roxb. ex W.T. Aiton bark following infestation by the Indian lac insect *Kerria lacca* (Kerr), as determined by Gas Chromatography-Mass Spectrometry analysis. Comparative analysis with non-infested controls across 3 time points (7, 14, and 21 days) indicated progressive biochemical alterations associated with insect infestation. In association with the pronounced reduction of major saturated fatty acids in plants, in particular palmitic and stearic acids, an increased abundance of myristic acid and a large amount of polyunsaturated fatty acids, especially linoleic and α-linolenic acids, was observed following lac insect infestation of the bark. The results indicate that lac infestation may reflect a robust lipid-based response in the host plant *F. semialata* bark, mediated by coordinated modulation of polyunsaturated fatty acid levels, oxylipin precursor concentrations, and fatty acid ratios. The present study shows a biochemical framework for lipid-induced host responses in the context of lac insect infestation. Further studies that combine metabolomic and functional analyses will provide a better understanding of the impact of fatty acid remodeling on plant defense capacity and the long-term interactions between host plants and lac insects.

1. INTRODUCTION

The plant-insect interaction is one of the most fundamental and extensively studied biotic interactions in terrestrial ecosystems, which significantly influences the patterns of biodiversity, community structure, and ecosystem stability [1]. Interestingly, plants are the primary producers, supplying everything from food to shelter and even oviposition sites for most of the arthropods. Insects are key drivers of plant reproduction through pollination regimes, in turn regulating the population and evolutionary changes [2]. These mutual interactions are the result of long-term coevolutionary processes that have produced remarkable diversity in insect feeding strategies and highly specialized plant defense systems [3]. Both plants and insects have undergone active evolution in adaptive associations over evolutionary time [4]. Insect infestation can also significantly change plant physiology, leading to changes in metabolic pathways, affecting lipid metabolism, and adding another dimension to primary metabolic reprogramming. Certain fatty acids and lipid-derived metabolites extend their functions beyond signaling by directly impacting insect feeding and growth, diminishing tissue palatability or nutritional quality [5,6]. These primary metabolic alterations frequently trigger and influence the activation of secondary defense mechanisms. After insect infestation, plants undergo extensive changes in primary metabolism, in which fatty acids become key regulators of both defense and signaling [7]. Insect infestation induces rapid remodeling of membrane lipids and fatty acid pools to maintain cellular integrity, allocate energy, and adapt to stress [8–11]. Polyunsaturated fatty acids (PUFA), primarily linoleic ($18{:}2\Delta^{9,12}$) and α-linolenic acid ($18{:}3\Delta^{9,12,15}$), serve as vital precursors for oxylipin biosynthesis and are catalytically converted in the lipoxygenase pathway [12]. α-Linolenic acid is the direct precursor for jasmonic acid biosynthesis; linoleic acid may contribute indirectly after desaturation [13,14]. Previous studies have reported significant changes in plant fatty acid composition in response to whitefly and leafminer infestation [15]. This study was conducted to assess how infestation by the Indian lac insect (*Kerria lacca*) alters the fatty acid profile of its primary host plant, *Flemingia semialata*. It is a perennial, shrub-like legume belonging to the Fabaceae family (subfamily Faboideae) and is found throughout tropical and subtropical areas of India and Southeast Asia, and is also one of the most significant host plants for the lac insect [16]. The lac insect, *K. lacca*, is an economically important resin-producing scale insect from the order Hemiptera, superfamily Coccoidea, and family Kerriidae. It

*Corresponding Author
Sandeep Kaushik, Department of Environmental Science, Indira Gandhi National Tribal University, Amarkantak, India.
E-mail: sskaushik2002@gmail.com

feeds on plant phloem sap and infests more than 400 plant species [17]. Interestingly, lac resin is primarily composed of polyesterified hydroxy fatty acids and sesquiterpenic acids. Thus, the fatty acid fraction can play a structural and functional role in resin formation, as the insect, being sedentary/immobile, survives on the plant phloem as its sole source of nutrition, and can also play a major role in defense mechanisms. As lac resin is rich in polyesterified hydroxy fatty acids and the insect relies exclusively on host phloem sap [18], host fatty acid remodeling may simultaneously reflect defense activation and supply essential precursors for resin biosynthesis, thereby shaping the plant–insect interaction.

2. MATERIAL AND METHODS

2.1. Sample Collection

The Kusumi strain of *K. lacca* (Kerr) was used to inoculate the lac host plant *F. semialata* Roxb. ex W.T. Aiton for the present investigation. In natural field conditions, approximately 80 g of broodlac was inoculated on each plant to ensure uniform infestation. All host plants selected for the study were of uniform age and maintained under identical field conditions, receiving the same nutritional and agronomic management to minimize environmental variability. A non-infested *F. semialata* plant maintained under the same conditions served as the control. Following infestation, bark samples were collected at four time intervals: 1 (control- non-infested), 7, 14, and 21 days post-infestation with three biological replicates at each sampling point. Bark tissues were excised from young, green, and tender shoots, immediately frozen in liquid nitrogen, and stored for subsequent biochemical and Gas Chromatography-Mass Spectrometry (GC-MS) analysis.

2.2. Sample Preparation

The stored samples were lyophilized to enhance stability for subsequent analytical procedures. Lyophilized powdered materials (200 mg) were thoroughly mixed with 600 µl of cold methanol (GC-MS grade) using a vortex. The mixture was chilled on ice for 10 minutes, then vortexed again. The material was then centrifuged at 15,000 g for 10 minutes. The supernatant was transferred to a new Micro Centrifuge Tube, and the extraction process was repeated. The same tube was used to combine the obtained supernatant.

2.3. Derivatization Process

To the dried oil extract, 300 µl of acidified methanol was added. The mixture was stirred for 2 minutes using a vortex and heated at 70°C for 1 hour. After that, the sample was cooled down to room temperature for 10 minutes. Then, 300 µl of NaCl solution was added, followed by 300 µl of hexane. The mixture was agitated in a vortex for 1 minute to ensure complete mixing. Finally, the material was centrifuged at 3,000 g for three minutes.

2.4. GC-MS Analysis

Following centrifugation, 100 µl of the hexane layer was carefully transferred into a GC-MS vial. After that, the vial was sealed and kept cold until it was injected into the GC-MS apparatus. The prepared plant sample was analyzed using GC-MS to identify the compounds. This procedure followed the methodology described in [19], with minor adjustments. The GC-MS analysis was conducted using a Shimadzu GC-MS-QP2010 system (Shimadzu, Kyoto, Japan) with an auto-injector (AOC-20i), a headspace sampler (AOC-20s), and a silica capillary column (Rtx-5). The oven

Table 1. The changes in fatty acid composition of *Flemingia semialata* during *K. lacca* infestation at different sampling days (Day 1, 7, 14, and 21). Values are presented in mean percentage ± SE ($n = 3$).

Samples	Lauric acid (12:0)	Myristic acid (14:0)	Palmitic acid (16:0)	Stearic acid (18:0)	Nonadecanoic acid (19:0)	Heneicosanoic acid (21:0)	Docosanoic acid (22:0)	Tricosanoic acid (23:0)	Pentacosanoic acid (25:0)	Hexacosanoic acid (26:0)	Oleic acid ($18:1\Delta^{9}$)	Linoleic acid ($18:2\Delta^{9,12}$)	Alpha-Linolenic acid ($18:3\Delta^{9,12,15}$)
Non-infested (day 1)	0.164 ± 0.026	0.615 ± 0.01	22.392 ± 0.184	10.71 ± 0.182	0.167 ± 0.028	0.103 ± 0.007	0.805 ± 0.008	0.435 ± 0.005	0.531 ± 0.004	0.323 ± 0.006	0.473 ± 0.019	14.897 ± 0.461	5.554 ± 0.561
Infested (day 7)	0.247 ± 0.038	2.731 ± 0.015	21.255 ± 0.204	10.376 ± 0.152	0.123 ± 0.021	0.079 ± 0.006	0.796 ± 0.006	0.439 ± 0.008	0.462 ± 0.006	0.02 ± 0.004	0.491 ± 0.021	14.965 ± 0.742	10.259 ± 0.568
Infested (day 14)	0.172 ± 0.018	3.172 ± 0.019	20.956 ± 0.182	9.602 ± 0.193	0.116 ± 0.029	0.086 ± 0.008	0.832 ± 0.006	0.335 ± 0.007	0.438 ± 0.009	0.353 ± 0.006	0.531 ± 0.017	17.161 ± 0.456	11.066 ± 0.581
Infested (day 21)	0.203 ± 0.036	2.728 ± 0.01	19.652 ± 0.154	8.213 ± 0.106	0.117 ± 0.018	0.087 ± 0.006	0.644 ± 0.005	0.35 ± 0.005	0.517 ± 0.006	0.464 ± 0.006	0.60 ± 0.014	18.213 ± 0.591	14.108 ± 0.608

temperature was originally set at 50°C and gradually increased to 280°C. 1 microliter of the plant extract at 1 mg/ml was loaded onto the column in crude form. The carrier gas was helium (99.99% purity), circulating at 1.2 ml/min. The mass spectrometer detected unique chromatographic peaks, and compound identification was accomplished using retention time (RT) and mass spectra collected under standardized GC-MS conditions. Compounds were quantified using peak area. The peak spectral data were compared with mass spectral libraries, specifically NIST14 and WILEY8, to identify chemicals. The relative fraction of each ingredient was calculated using peak area normalization.

2.5 Statistical Analysis

For the current study, the experiment was carried out with biological triplicates ($N = 3$). The final value was determined as the average and SE derived from these triplicates. The IBM SPSS version 21.0 software was used for one-way ANOVA with Duncan post hoc analysis at a 95% confidence level ($p < 0.05$) to compare temporal intervals. The data were graphically presented in Microsoft Word.

3. RESULT AND DISCUSSION

The present study revealed that infestation by the lac insect, *K. lacca*, resulted in significant temporal changes in the fatty acid profile of *F. semialata*. These changes in fatty acids align with typical herbivore-induced lipid signalling responses in plants and may be associated with the activation of the classical herbivore-mediated lipid pathway [20]. The GC-MS analysis of the infested and non-infested young bark of the tender shoots of *F. semialata* identified thirteen fatty acids, comprising saturated (lauric 12:0, myristic 14:0, palmitic 16:0, stearic 18:0, nonadecanoic 19:0, heneicosanoic 21:0, docosanoic 22:0, tricosanoic 23:0, pentacosanoic 25:0, hexacosanoic 26:0), monounsaturated (oleic 18:1Δ^{9}), and PUFAs (linoleic 18:2$\Delta^{9,12}$ and α-linolenic 18:3$\Delta^{9,12,15}$) (Table 1; Supplementary Fig. 1). A distinct temporal shift in fatty acid profiles was evident when comparing non-infested plant samples (day 1) with lac-infested plants after 7th, 14th, and 21st day of the infestation. Most saturated fatty acids (SFAs) showed declining or fluctuating patterns during infestation. Palmitic acid (16:0), the dominant SFA, decreased steadily from 22.39% in non-infested bark to 21.25%, 20.95%, and 19.65% on Day 7, Day 14, and Day 21, respectively. Stearic acid (18:0) followed a similar pattern, declining from 10.71% to 10.37%, 9.60%, and 8.21% across the same period (Fig. 1A and Table 1). Lauric acid (12:0) showed minor fluctuations (0.16–0.24%), while nonadecanoic acid (19:0), heneicosanoic acid (21:0), docosanoic acid (22:0), tricosanoic acid (23:0), and pentacosanoic acid (25:0) exhibited slight reductions or remained relatively stable. Hexacosanoic acid (26:0) decreased sharply at Day 7 (0.02% compared to 0.32% in controls) but subsequently recovered to 0.35% and 0.46% by Days 14 and 21. In contrast to these general declines, myristic acid (14:0) showed a pronounced increase from 0.61% in non-infested bark to 2.73%, 3.17%, and 2.72% during infestation (Fig. 1A and Table 1). The decrease of these major SFAs, such as palmitic acid (16:0) and stearic acid (18:0), may reflect redistribution of membrane lipid carbon toward unsaturated fatty acid pools involved in defense signalling [21]. On the other side, the significant increase of myristic acid (14:0) may suggest that de novo fatty-acid synthesis is more pronounced in the case of cell wall reinforcement and wound healing, as was reported with herbivores during wound response [22,23].

Oleic acid (18:1Δ9), the only MUFA detected, showed a moderate but consistent increase from 0.47% in non-infested bark to 0.49%, 0.53%, and 0.60% on Days 7, 14, and 21. This increase in oleic acid (18:1Δ^{9}) concentrations towards compensatory stability and repair of the membrane during infestation, with MUFAs frequently serving as a buffer to oxidative damage, may be associated with promoting bilayer maintenance in stress conditions [21]. The PUFAs showed pronounced, progressively increasing trends during infestation (Fig. 1C, D). Linoleic acid (18:2$\Delta^{9,12}$) increased from 14.89% in non-infested bark to 14.96%, 17.16%, and 18.21% on Day 7, 14 and 21, respectively (Fig. 1C). Alpha-linolenic acid (18:3$\Delta^{9,12,15}$) showed a sharper rise, increasing from 5.55% in the non-infested sample to 10.25%, 11.06%, and 14.10% over the same period (Fig. 1D and Table 1). By Day 21, the total PUFA fraction had increased by approximately 58% relative to non-infested bark (Fig. 1C, D and Table 1). Several studies have documented the elevation of free and esterified PUFAs following herbivory infestation as an initial response to the activation of the octadecanoid pathway [10]. These may also be associated with the enhancement of green leaf volatiles (GLVs) and oxylipins, which can modulate both direct and indirect defenses [13,24]. The observed steady rise in PUFAs in *F. semialata* might improve the preparation of distal tissues for a more rapid and systemic defensive response away from the local

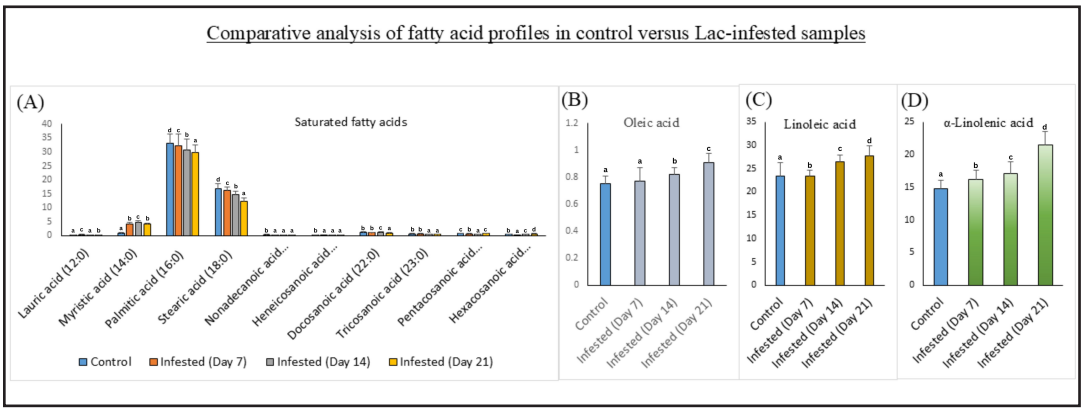

Figure 1. Comparative analysis of fatty acid profiles in *Flemingia semialata* bark under control (non-infested) and *K. lacca* infested conditions. (A) Changes in saturated fatty acids (C12:0–C26:0) in control and infested samples at 7, 14, and 21 days post-infestation. (B–D) Temporal variation in major unsaturated fatty acids: (B) oleic acid, (C) linoleic acid, and (D) α-linolenic acid in control and infested bark. Data are presented as mean ± SE of three independent biological replicates. Lowercase letters indicate significant differences between days ($p < 0.05$, one-way ANOVA, Duncan's post hoc test).

feeding site. However, the lac insect can still successfully colonise the host, despite these biochemical defense responses.

Additionally, the total SFAs decreased by approximately 9% from Day 1 to Day 21, while unsaturated fatty acids (MUFA + PUFA) increased substantially. Consequently, the SFA:UFA ratio declined from 1.73 in non-infested bark to 1.00 at Day 21, which may suggest a substantial shift toward higher unsaturation. The PUFA: MUFA ratio increased throughout infestation, driven by stronger induction of linoleic ($18:2\Delta^{9,12}$) and α-linolenic ($18:3\Delta^{9,12,15}$) acids relative to oleic acid ($18:3\Delta^{9}$). Furthermore, the 18:2/18:3 ratio declined from 2.68 on Day 1 to 1.29 on Day 21, reflecting proportionally greater accumulation of α-linolenic acid over linoleic acid, which may be associated with membrane remodelling resulting from biotic stress and has previously been associated with increased desaturase activity and enhanced membrane fluidity, which have been linked to signal transduction in previous studies. In addition to insect infestation, other factors such as plant developmental stages, wound responses, changes in carbon allocation during phloem sap feeding, and insect nutritional needs are also known to impact fatty acid composition [11,21,25]. Thus, changes in fatty acid composition should be viewed as multifactorial responses rather than the outcome of a single influencing factor.

These findings may be associated with mechanisms observed in other plant-insect systems, in which herbivores are recognised as affecting plant defensive responses. [26,27]. Interestingly, insects with a piercing-sucking behaviour, such as scale insects, also cause comparatively minimal tissue damage, compared to chewing herbivores, as reported in previous studies [28]. Furthermore, various hemipterans have been reported to detoxify or neutralise lipid-derived defense molecules through gut peroxidases or symbiotic microbes, further compromising the plant's defense mechanisms [29,30]. The lac insect infestation caused the following major changes in the fatty acid profiles of host plant: (i) progressive decreases in major SFAs (16:0 and 18:0), (ii) a marked increase in PUFAs, especially α-linolenic acid ($18:3\Delta^{9,12,15}$), (iii) moderate increases in oleic acid ($18:1\Delta^{9}$), and (iv) significant reductions in the saturated to unsaturated fatty acid (SFA: UFA) and the $18:2\Delta^{9,12}/18:3\Delta^{9,12,15}$ ratio. These findings demonstrate substantial remodelling of fatty acid composition throughout the 21-day infestation period.

4. CONCLUSION

These findings suggest that *K. lacca* infestation may be related to lipid profile changes associated with defensive responses in *F. semialata,* such as the accumulation of PUFA, oxylipin precursor activation, membrane remodeling, and alteration in certain fatty acid ratios. Nevertheless, the lac insect's adaptations to stylet feeding, salivary inhibition and biochemical detoxification may help the insect adapt to host plant responses and maintain successful colonization. This interaction highlights a complex relationship in which temporal changes in lipid profiles occur during infestation, while the insect continues to colonize the host successfully. Such metabolic adaptability in the plant, combined with effective counter-defense strategies in the lac insect, likely underlies their long-term compatibility. Understanding this balance may provide novel insights into host compatibility and the sustainable management of the host plant–lac insect system.

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6. AUTHOR CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be author as per the International Committee of Medical Journal Editors (ICMJE) requirements/guidelines.

7. CONFLICT OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

8. ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

9. DATA AVAILABILITY

All the data is available with the authors and shall be provided upon request.

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12. SUPPLEMENTARY MATERIAL

The supplementary material can be accessed at the journal's website: https://jabonline.in/admin/php/uploadss/1491_pdf.pdf

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