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RESEARCH ARTICLE

Assessing the infestation-induced response on the plant host by the Indian lac insect

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Abstract

Lac, the only natural resin of animal origin is produced by the Indian lac insect- *Kerria lacca*. It is the by-product of the complex natural interaction between the lac insect and its host plant. Despite several studies on the perspectives of the chemistry of lac and its production, very little work has been carried out to understand the biology of lac and its associated plant taxa. The present work has been designed to understand the preliminary response if any, of the host plant against lac insect infestation. Structural studies and metabolic profiling such as the determination of total phenolics, flavonoids, antioxidants, FTIR, and GC-MS were carried out. The anatomical investigations revealed coagulation/deposition of metabolites in the infested sites. The infested bark showed higher phenolic, flavonoid content, and antioxidant activity in comparison to non-infested bark which was corroborated by Fourier-transform infrared spectroscopy (FTIR) and GC -MS biochemical analysis. This preliminary study will shed some light on understanding the lac plant host's physiological response and the putative mechanism used by the lac insect in overcoming the plant response.

Keywords

FTIR; GC-MS; lac host bark; plant secondary metabolites; TFC

Introduction

Insects are a remarkably diverse group of organisms, and nearly half of the approximately one million known insect species are known to feed on plants (1). The plant-insect relationship dates back as far as 350 million years and this virtually simple but complex relationship has played an important role in shaping terrestrial ecosystems on Earth (2). During the course of their evolution, land plants and insects have developed complex relationships at various levels ranging from individual organisms to populations and entire communities. There is a continuous co-evolutionary struggle between herbivorous insects and plants, where the herbivores attempt to consume the plants, and the plants strive not to be consumed (3). This struggle has not only contributed to the remarkable diversity of plant metabolism but has also influenced the genetic diversity of plants, insects, and the associated biota. As a result, research on plant-herbivore interactions has become a highly multidisciplinary pursuit within plant biology (4). In the complex interplay between plants and herbivorous insects, both parties invest their metabolic machinery in defense and

adaptation mechanisms. The causal mechanisms behind these interactions vary and involve induced plant morphology, allelo-chemistry, nutrition, altered natural-enemy attack, and plant or insect-associated microbiotic flora (5). A range of allelochemicals such as defensive proteins, phenolics, terpenoids, alkaloids, etc. induced in the plants by insect feeding can affect the performance of other herbivores which play a major role in shaping the dynamics of plant-insect interactions (6-10).

The Indian lac insect *Kerria lacca* (Kerr.) is known to infest more than 400 plant species. The scale insect *Kerria lacca*, is a hemipteran, sap-sucking insect. The insect possesses specialized mouthparts that help in piercing the plant tissues (epidermis, cortex, and vascular tissues) to access the phloem cells to consume the plant sap (11). Once the insect can successfully penetrate the host tissue it undergoes a significant metamorphic change and remains sedentary for the rest of its life cycle. Subsequently, insects entirely depend on the host plant for its nourishment and keep on secreting lac throughout its body surface, a unique resin of animal origin (12). The lac is composed of several chemical constituents and holds immense economic importance. A fascinating aspect of the relationship between Lac insects and their hosts is the selection of host plants at an early stage of their life cycle and the mechanisms of overcoming the plant host response if any in good hosts. Like other hemipteran sap-sucking insects, the lac insect penetrates the plant bark to reach the phloem tissues, which are known to contain a wide variety of plant metabolites such as carotenoids, polyphenols, significant phytochemical subgroups including phenolic acids, flavonoids, stilbenes and lignans (13, 14). Several investigations have been carried out on the taxonomy of the lac insect, chemistry of lac, biology of lac, lac production, etc., but studies on the physiology of the lac-infested plant host are lacking. The present study was aimed at (i) anatomically mapping the initial plant response to lac insect infestation (ii) Quantitative estimation of total phenol content, flavonoids, and antioxidants in the bark of plant host in response to lac insect infestation (iii) drawing a comparison between lac insect-infested and non-infested plant metabolites using analytical techniques viz FTIR and GC-MS.

Materials and Methods

Anatomical investigations:

The fresh stem (infested with lac) was fixed in the F.A.A. till further processing of the material. Longitudinal sections of the infested stem/petiole of *Zizyphus mauritiana* with still-feeding insects were cut and observed under a fluorescent microscope (Nikon eclipse E200 system) to investigate the feeding sites and initial plant response if any induced during the infestation. Image acquisition and analysis were made using the Imaging software NIS Elements (Nikon).

Sample collection and crude extract preparation

The lac host (*Ziziphus mauritiana*) plant selected for this experiment was inoculated/infested with the kusumi strain of *Kerria lacca* while the non-infested plant was treated as a control. After three days of infestation, the bark of the host plant (infested-Z.I. as well as non-infested-Z.N.I.) was peeled away and gently washed with tap water to remove undesired filth. In the case of infested samples, the insects were removed using forceps and brush. Subsequently, the bark of both the samples (Z.I. and Z.N.I.) was air dried under shade. A mechanical grinder was used to grind samples into a fine powder at room temperature (RT). The powdered samples (10 g) were macerated in 100 mL of 80% (v/v) methanol using a magnetic stirrer for three days. The phytoconstituents were entirely extracted by re-macerating the residues 3 times. It was subjected to filtration using Whatman No. 1 filter paper and the extract obtained was then concentrated to dry mass at RT. The dried residue was collected, put into airtight containers, and kept at 4 °C in a refrigerator for further use. The yield % was calculated following the standard method (15).

Yield % = weight of the dry extract/ Weight of the dry plant material × 100.

The crude extract/samples were further subjected to quantitative assays.

Quantitative Phytochemical Analysis

Total Phenol Content (TPC)

The total phenol content (TPC) was determined using a spectrophotometer and the Folin-Ciocalteu methodology with minor modifications (16). First, a 300 µg/mL concentration of methanolic solution was prepared and subsequently, combined with 7.5% Na_2CO_3 (2.5 mL) followed by 10 percent Folin–Ciocalteu reagent (2.5 mL). The complete solution was vortexed for 2 min, then left in dark incubation at RT for 45 min. Absorbance was measured at 750 nm using a spectrophotometer. The Gallic acid calibration curves were constructed in the range of 20-100 µg/mL. Finally, the concentrations of phenolics were represented in gallic acid equivalent terms (mg GA/g DW).

Total Flavonoids Content (TFC)

The total flavonoid content (TFC) was determined using the $AlCl_3$ colorimetry method (17). Plant extracts were diluted with methanol until their concentration reached 1mg/mL and a calibration curve was drawn using 20-100 µg/mL of quercetin dissolved in methanol. In methanol, 2.0 mL of diluted extracts/quercetin were dissolved with $AlCl_3$ [0.1 mL of 10% (w/v)] and CH_3COOK (0.1 mL of 0.1 mM) to form a solution. After 30 min of incubation at 25 °C, absorbance was measured at 415 nm. Furthermore, TOC was expressed as mg quercetin equivalent per g dry weight of extracts (mg QE/g DW).

Total Antioxidant Activity (TAA)

The total antioxidant activity (TAA) of the crude extract was determined using a modified version of the standard method (18, 19). In brief, methanol solution was used to dilute the crude stock to 1 mg/mL, and 0.2 mL aliquot of each extract was added with 1.8 mL of the reagent (0.6 M H_2SO_4, 28 mM Na_3PO_4, and 4 mM $(NH_4)_6Mo_7O_{24}$). The reaction mixture was then incubated in a water bath at 90 °C for 90 min and subsequently cooled to RT. Using a UV-VIS

spectrophotometer, the sample's absorbance was measured at 695 nm. The total antioxidant activity was used as the standard (20-100 µg/mL) and the TAA result was calculated as the equivalent amount of ascorbic acid per g of plant extract.

FTIR Spectral Analysis

The initial variation in the functional groups (if any) in the dried extract of *Ziziphus mauritiana* (Infested and non-infested bark) were identified using FTIR (Fourier-transform infrared spectroscopy) performed on a Thermo Nicolet iS5 instrument (Thermo Scientific, Madison, WT, United States). All readings were taken at ambient temperature. The instrument consists of deuterated triglycine sulfate (DTGS) and KBr, which function as a detector and beam splitter, respectively. The instrument was connected to the OMNIC software, which records and analyzes FTIR spectra output. All spectra were collected between 4000-500 cm^{-1} at 4 cm^{-1} resolution and background air spectra were subtracted to optimize the actual spectra. After each reading, the ATR (attenuated total reflectance) crystal (sample spotting area) was routinely cleaned with 70% ethanol.

Gas Chromatography-Mass Spectrometry (GC–MS) Analyses

The plant extract was examined with GC-MS for compound identification using the methodology with a few modifications (20). A GC-MS instrument (GCMS-QP2010, Shimadzu, Kyoto, Japan) containing an auto-injector (AOC -20i), a headspace sampler (AOC-20s), and a silica capillary column (Rtx-5) was utilized. The oven temperature was initially set to 50 °C and then gradually increased to 280 °C. One microliter of extract (1 mg/mL) was injected into the column as an equal mixture of crude extract. Helium (purity 99.99%) gas was chosen as the carrier gas (1.2 mL/ min). The MS detector detected the presence of a unique peak. However, the compounds were identified based on RT (retention time) and mass was measured under similar GC-MS conditions. The peak area was used to measure the quantity of the substance. Finally, the obtained spectral data of the peaks were compared with mass spectral library data (NIST14 and WILEY8). The relative % of each constituent was compared through the peak area normalization.

Statistical Analysis

Each experiment was replicated three times and the results were presented as mean ± standard deviation (SD). Microsoft Excel 2016 was used to calculate the sample's data. The Student T-test was used to determine significant variation between samples. GraphPad Prism 9.3.3 was used for statistical analysis and graph design. FT-IR data was analyzed in OriginPro 2021.

Results

Anatomical investigations

The host plant stem displayed a range of surface characteristics, encompassing trichomes, cuticular surfaces, and thorns. Interestingly these surface features did not inhibit the process of stem penetration, despite their presence. Remarkably, an examination of the stem morphological attributes revealed a non-uniform appearance. Feeding crawlers exhibited a distinct orientation positioning themselves with their heads directed downward, toward the bark surface. While the insect showed a propensity for settling on young shoots it was observed to settle at various locations along the stem with a notably higher frequency at the junctions where petioles met the stem or at the intersections of lateral branches with the main stem. This behavior strongly suggested a preference for specific regions of the plant. In the early stages of infestation, morphological indicators in the form of scar marks were observed on the stem bark. Evaluation of longitudinal sections (L.S.) of the infested stem observed under fluorescent microscopy revealed scattered feeding sites on the plant's surface encompassing both the bark and epidermal tissues. However, a lack of a specific settlement pattern was observed in the uneven and dispersed feeding sites within the L.S. of the infested stem. Notably, the microscopic studies revealed that cortical cells surrounding these feeding sites displayed an accumulation of coagulated cytoplasmic material potentially resulting from the plant's secretion of primary and secondary metabolites in response to insect infestation. Moreover, areas traversed by the insect mouthparts exhibited fluorescence, indicating the presence of insect secretions, plant responses, or a combination of both, as compared to similar non-infested cellular structures (Fig. 1). Additionally, fluorescent studies revealed a reduction in chlorophyll content in the areas adjacent to the infestation site when compared to normal cells. This observation implies a potential disruption of the plant's photosynthetic activity and capabilities resulting from the insect's feeding activities (Fig. 1).

Total Phenol, Flavonoid and Antioxidant Concentration

Two major secondary metabolites namely phenol, flavonoids, and total antioxidant content were estimated in infested and non-infested stem parts. Both phenol and flavonoids showed an increase in the infested plant as compared to the non-infested plant. Phenolic content in infested plants was about 67.93 ± 2.03 mg GAE g^{-1} DW which is significantly higher than in non-infested plant (51.51 ± 1.69 mg GAE g^{-1} DW) with $p<0.001$ (Fig. 2). However, flavonoid content was observed in the infested plant showed nearly two-fold increase in infested stem parts (51.85 ± 2.11 mg QE g^{-1} DW) than in non-infested stem parts (24.82 ± 0.71 mg QE g^{-1} DW) (Fig. 2). Similarly, total antioxidants were significantly higher in the infested plant (72.86 ± 1.85 mg AAE g^{-1}) than in non-infested (55.81 ± 1.10 mg AAE g^{-1} DW) plants and that could be due to higher phenol and flavonoid content. Overall, *Z. mauritiana* stem was found to significantly induce the production of higher phenolic compounds, flavonoids, and antioxidant content in the parts infested with lac insects (Fig. 2).

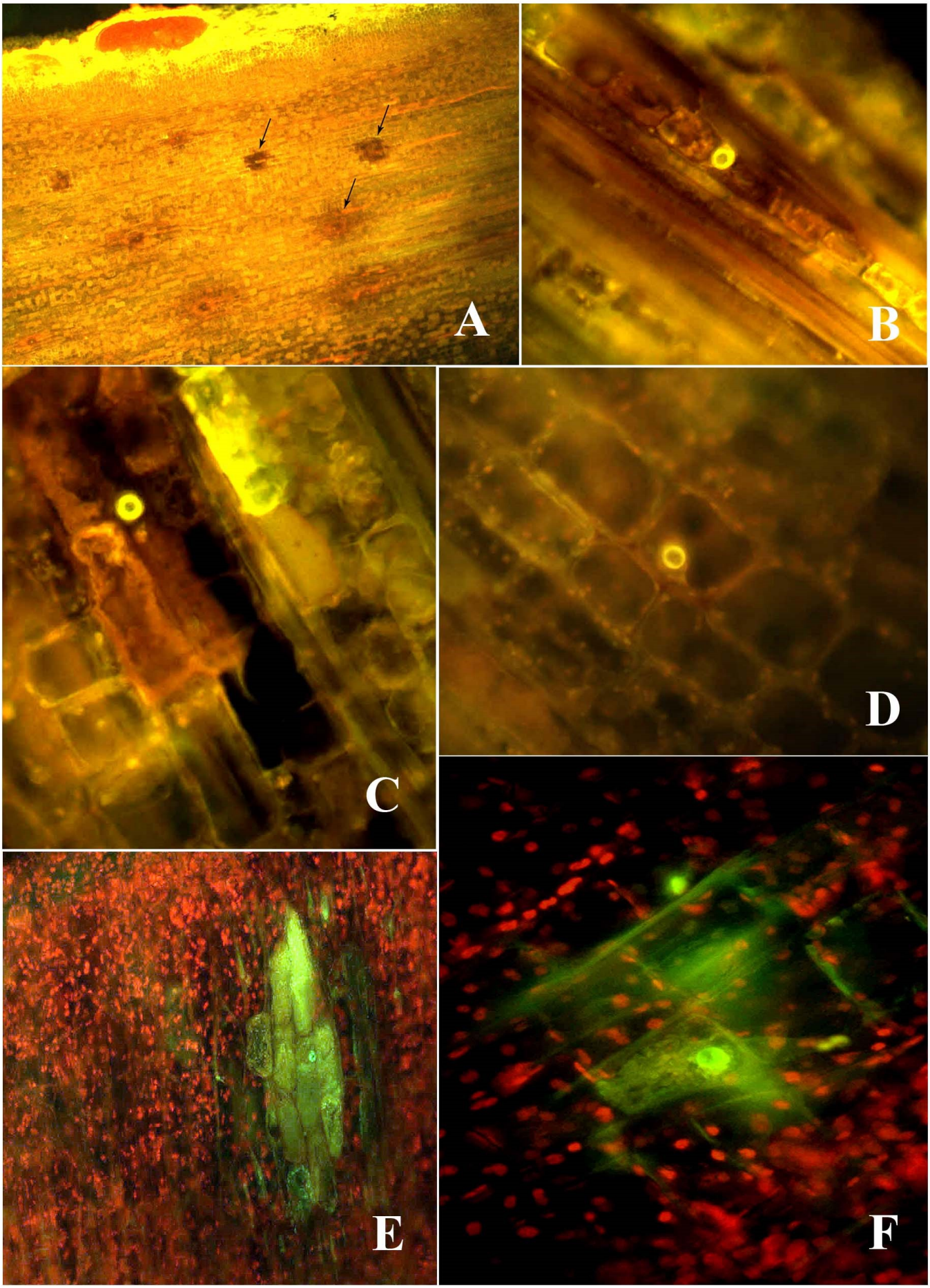

Fig. 1. Longitudinal sections of lac-infected *Zizyphus mauritiana* stem (observed under the fluorescent microscope) showing feeding sites (A) Arrows indicate the feeding sites on the surface of the stem as chlorotic spots; (B-C) Salivary sheath (section) depicted by autofluorescence indicated by the arrow; (D) Intercellular penetration of styles depicted by a salivary sheath (E) Deposition of clogging material as a plant response in the cortical cells adjacent to feeding sites (fluorescing) (F) loss of chlorophyll adjacent to feeding sites.

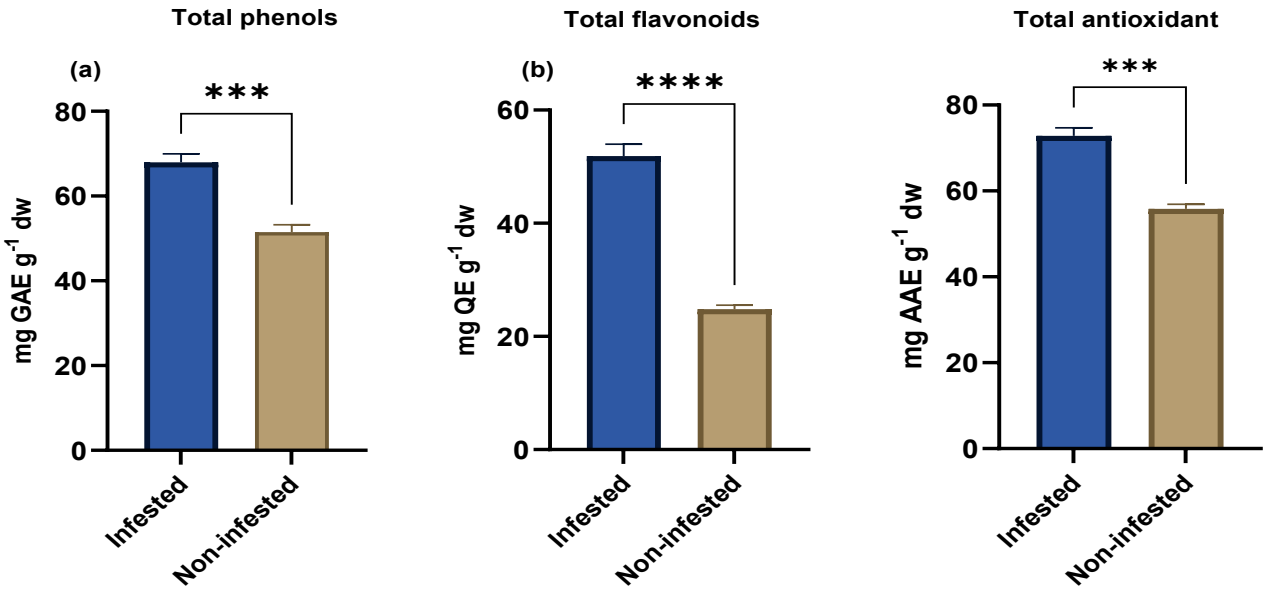

Fig. 2. Analysis graph of the total phenols, total flavonoids, and total antioxidants present in the infested and non-infested bark sample of lac host. All data points are means ± SD (n = 3). ***, and **** represent p<0.001 and 0.0001 respectively (Student T-test).

FTIR Spectrum Analysis for Functional Group of Z. mauritiana Extract

The FTIR investigation was conducted to reveal the chemical fingerprints of the infested and non-infested bark of *Ziziphus mauritiana* extract (Fig. 3). The absorbance bands were examined at a resolution of 500 to 4000 cm^{-1} (Fig. 3). Predominantly, the data signifies, free hydroxyl (OH) stretch at 3662.44, (OH) stretch at 3309.73, H bonded, 2983.73 (CH) stretching, (CH) stretching at 2901.96, (N-H) bend at 1618.78, 1388.16 (C=O) stretching, 1245.32 (C-H) stretch, 1055.40 (CN components) stretch and 892.61 (C-H "oop") cm^{-1} have been specified to a variety of potential functional groups, including Alcohols, phenols, alkanes, primary amines, carboxylic acids, esters, ethers, alkyl halides, aliphatic amines, compounds are identified. The variable peaks in the infested and non-infested samples indicated that there lies an initial plant response to lac insect infestation. The same is indicated by the sharp peak fingerprints of the infested samples as compared to the non-infested ones. These functional groups indicate the presence of many secondary metabolites including phenols and flavonoids. However, FTIR could not identify all functional groups of active ingredients contained in the crude extract and thus, GC-MS investigation was also carried out.

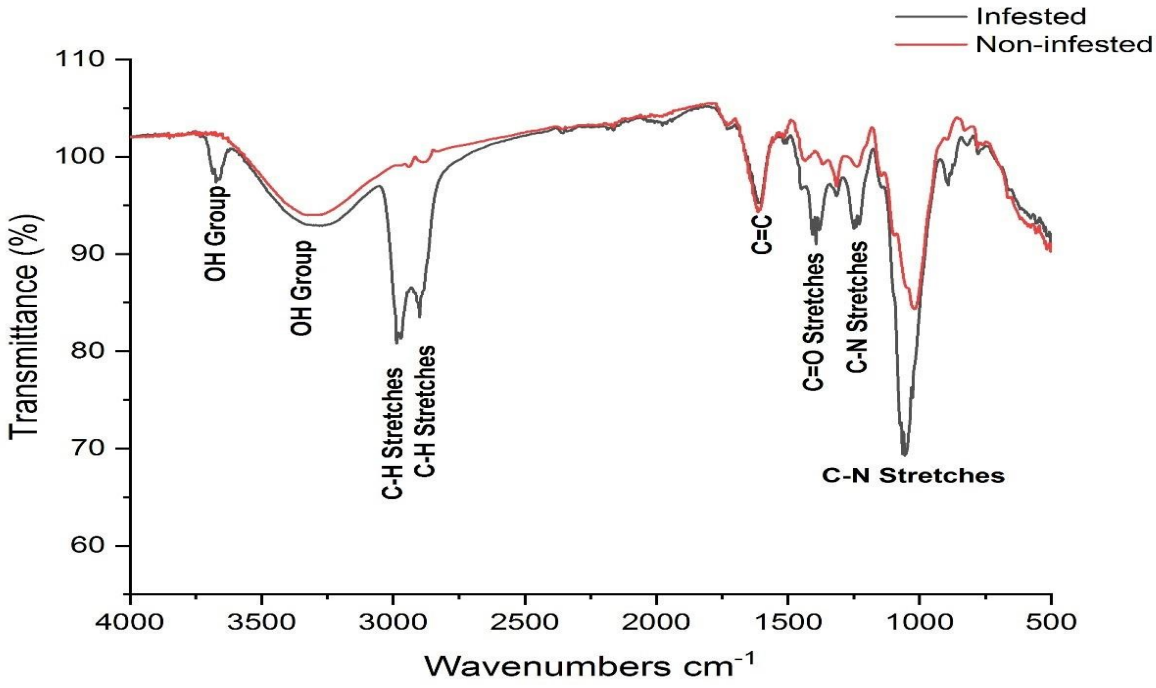

Fig. 3. FTIR Analysis graph of functional groups present in the infested and non-infested bark sample of lac host.

GC-MS analysis for Metabolites detection

As Fig. 4 shows, metabolite profiling using Gas Chromatography-Mass Spectrometry (GC-MS) was conducted on plant samples, both infested with insects and non-infested control samples. The analysis revealed a total of 28 volatile organic compounds which were further classified into distinct categories such as alcohols, sugar alcohols, sugars, amines, phenolics, fatty acids, and organic acids. To determine the significance of metabolite induction, a T-test with a significance threshold of P<0.05 was applied.

The results unveiled notable differences in the metabolite profiles between infested and non-infested plants. Specifically, infestation with insects led to a reduction in the levels of alcohol. Additionally, significant reductions in pyridine 3-hydroxy and 1,3-propanediol in infested plants were observed. Similarly, sugar alcohol levels were significantly lower in infested plants compared to non-infested ones. Interestingly, only myo-inositol exhibited a significant increase (p<0.01) in infested plants (Fig. 4).

Regarding sugars, 2 sugars, xylose, and glucose, were highly induced in non-infested plants, while the level of galactose was significantly higher in infested plants. Among the amine compounds, hydroxylamine exhibited a significant decrease (p=0.04) in infested plants (Fig. 4).

In the metabolite screening, 2 phenolic compounds phloroglucinol and quinic acid were detected in both infested and non-infested plants. Phloroglucinol showed a reduction (log2 fold change: -0.72) in infested plants, whereas quinic acid levels were notably higher (log2 fold change: 1.01) (Fig. 4).

Regarding fatty acids, octadecanoic acid levels were significantly higher in non-infested plants. Out of the nine detected organic acids, most of them were substantially reduced in infested plants, except for 2-butanedioic and malic acid (Fig. 4).

As depicted in Fig. 4, we observed a significant decrease (log2 fold change: -0.678) in the infested bark compared to the non-infested bark for pyridine 2-hydroxy (p-value: 0.045). Chlorohexanol, although not statistically significant (p-value: 0.075), displayed a decreasing trend in

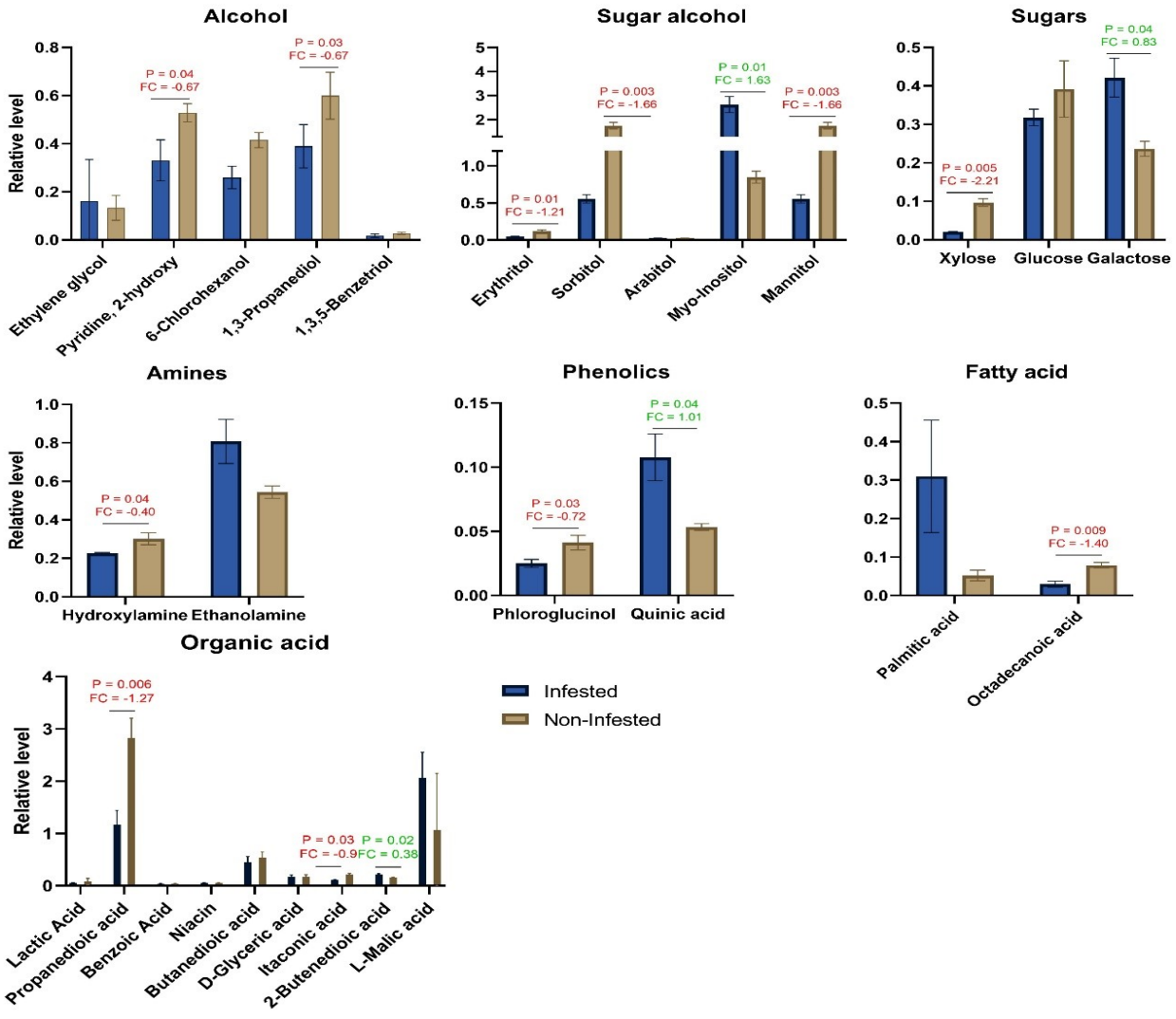

Fig. 4. GC-MS-based metabolites profiling of lac-infested and non-infested *Ziziphus* plant. Metabolites are categorized into different groups based on their nature. All metabolites are expressed as relative levels of ribitol (internal standard), n=3 ± SD. Student T-test and log2 fold change (FC) were calculated between similar metabolites (p<0.05). P value and FC in green color indicate significant increment in the infested plant while values in red color are vice-versa.

the infested bark compared to the non-infested bark. 1,3-Propanediol exhibited a significant decrease (log2 fold change: -0.623) in the infested bark (p-value: 0.034). In contrast, the infested bark had a significantly higher concentration of 4-Pyridinol (p-value: 0.020). Several other metabolites (1-Pentanol, 1,2,3-Butanetriol, meso-erythritol, etc.) also demonstrated significant differences between infested and non-infested bark samples. However, metabolites like ethylene glycol, lactic acid, stearic acid, and others did not show statistically significant differences.

Our comprehensive metabolite profiling analysis revealed significant alterations in the metabolic profiles of infested plants compared to their non-infested counterparts. These findings provide valuable insights into the biochemical responses of plants to insect infestation and highlight specific metabolites that may play key roles in plant-insect interactions. Additionally, we identified two major phenolic compounds, salicylic acid, and hydroquinone, in infested plants, suggesting their potential involvement in the plant's defense mechanisms against insect pests.

Discussion

This study aimed to investigate the response of the host plant, *Ziziphus mauritiana*, to lac insect infestation using various techniques such as fluorescence-based anatomical studies and GC-MS-based analysis of the secondary metabolic profiles of infested and non-infested samples. The results obtained revealed that while *Z. mauritiana* is considered a good host for lac insects it exhibits an intriguing initial deterrence to their infestation. The presence of this initial deterrence highlights the significance of bark phytochemicals in influencing the settlement patterns of lac crawlers. The initial insect settlement density also varies among different host plants a factor that ultimately impacts lac production during later stages (11). However, what makes this interaction (lac insect-plant host) particularly intriguing is that despite the initial deterrence the sedentary lac insects which are known to exhibit sedentary behavior for extended periods often exceeding five months can extract uninterrupted plant sap and simultaneously produce resin (11). An essential aspect of the lac insect's lifecycle, encompassing molting, mating, reproduction, and crawler discharge, all occurs within the same location. This life cycle demands a continuous and uninterrupted source of nutrient-rich sap, highlighting the intricate nature of this plant-insect interaction (21). This behavior distinguishes them from aphids, suggesting that lac insects establish a harmonious and prolonged relationship with their host plant.

The qualitative and quantitative variations of different plant secondary metabolites such as carotenoids, polyphenols, anthocyanins, flavones, flavanones, isoflavones, and flavonols, hold significant promise for understanding the early settlement patterns of lac insects and may play pivotal roles in shaping the behavior and preferences of lac crawlers during the initial stages of colonization. These responses lead to changes in metabolite concentrations within the plant, reflecting its attempt to defend itself against lac insect infestation.

In response to insect infestation, plants activate a series of defense mechanisms aimed at protecting themselves from the stress caused by the infestation. These defense responses often involve the production of secondary metabolites some of which have been identified in our dataset. These changes in metabolite concentrations can be seen as the plant host strategy to defend itself against lac insect infestation. However, systemic responses are part of the plant's adaptive strategy to cope with the stress caused by infestation, involving changes in enzymatic activities, gene expression, and metabolic pathways (2, 22). It is important to note that the production of secondary metabolites is largely dependent on the reservoir of primary metabolites, primarily sugar compounds (23). In our study, we observed significant changes in the metabolite profile of infested plants, with a notable reduction in metabolites belonging to various groups, including alcohols, sugar alcohols, and sugars.

Interestingly, the induction of myo-inositol in infested plants suggests its potential role as a major signal -transducing metabolite initiating the defense process. Myo-inositol is well-known for its function as a signaling molecule during stress conditions and is involved in the biosynthesis of essential components such as the cell wall and ascorbic acid. It also plays a role in regulating cell death processes (24). Moreover, myo-inositol is associated with various stress-related pathways, including those regulated by its derivatives such as phosphatidylinositol isoforms and associated enzymes, kinases, and phosphatases. These pathways appear to work in parallel to coordinate growth and stress responses, further emphasizing the importance of myo-inositol in the plant's defense mechanisms (25). The identification of quinic acid as a major phenolic compound in plants infested with insects suggests its role in defense responses. Quinic acid is a precursor to chlorogenic acid, an ester known for its anti-herbivore activity. This activity has been demonstrated against various pests, including the tomato fruit worm and several beetle species (26-29).

Another noteworthy phenolic compound detected in our study is salicylic acid, known as one of the chief hormones involved in plant defense against herbivores. Plant immunity against pests involves complex regulatory systems that rely on two key hormones: Salicylic acid (SA) and Jasmonic acid (JA), which act as defense signals (30). SA induction can have detrimental effects on attacking herbivores, acting as a deterrent and even as a toxin to insect herbivores (31). The presence of SA in infested plants aligns with previous findings of SA-induced defense responses against herbivore feeding and egg deposition in Arabidopsis (32).

Additionally, our study detected another phenolic compound, hydroquinone, known for its antioxidant properties. Plants producing antioxidant metabolites are better equipped to counteract the generation of reactive

oxygen species induced by stress factors, including insect infestation (33, 34). Furthermore, the metabolite screening in our study revealed the induction of important phenolic compounds like quinic acid and signaling metabolites such as myo-inositol. The presence of salicylic acid and hydroquinone in infested plants further suggests their role in mounting an effective defense response against insect infestation. In contrast to these phenolic compounds, the metabolite Pyridine, 2-hydroxy exhibited a significant decrease in concentration in infested bark compared to non-infested bark. Pyridine derivatives are known for their antimicrobial and insecticidal properties implying a potential role in plant defense against insects (35). Similarly, metabolite 1,3-Propanediol showed a significant decrease in infested bark. Although its specific role in plant defense is not extensively studied, 1,3-Propanediol has been reported to possess antifungal properties, suggesting its potential defensive function (36). Unlike the aforementioned metabolites, 4-Pyridinol exhibited a significant increase in concentration in infested bark. While its exact role in plant defense remains unclear, it might be involved in defense responses or signal transduction pathways triggered by insect infestation. Additionally, salicylic acid, a well-known signaling molecule, plays a pivotal role in activating defense genes and inducing systemic acquired resistance (SAR) against pathogens (37). Similarly, L-malic acid has been associated with defense against pathogens, acting as an intermediate in defense-related metabolic pathways and participating in the production of secondary metabolites involved in plant defense mechanisms (38). Hydroquinone, on the other hand, maybe a component of the phenylpropanoid pathway, responsible for synthesizing phenolic compounds such as lignin and flavonoids. It could also be involved in the shikimate pathway, which produces aromatic amino acids (39).

This study has illuminated a fascinating interplay between *Z. mauritiana* and lac insects. Despite being initially deterred, these insects exhibit impressive adaptability allowing them to successfully colonize and complete their life cycle within the same location. This intricate relationship highlights the role of bark phytochemicals and metabolite variations in shaping the outcome of plant-insect interactions. Further research into the specific phytochemicals involved and their mechanisms of action will deepen our understanding of these complex ecological dynamics. Our study provides insights into the dynamic responses of *Z. mauritiana* to lac insect infestation, with a focus on the metabolic changes that occur as part of the plant's defense mechanisms. The observed alterations in metabolite concentrations, particularly the induction of myo-inositol, underscore the complexity of plant-insect interactions and highlight the role of specific metabolites in initiating and regulating defense responses. Further research into the mechanisms underlying these metabolic changes will enhance our understanding of how plants adapt and respond to insect infestation. It is important to note that the metabolites discussed are primarily non-volatile and include organic acids, sugars, alcohols, and derivatives. However, it is worth considering that volatile compounds, including terpenes, aldehydes, and aromatic compounds, also play significant roles in plant defense and communication. These findings collectively highlight the complexity of plant-insect interactions and the multifaceted nature of plant defense mechanisms against insect infestation.

Conclusion

Lac insects, known for their sap-feeding behavior and dependency on host plant nutrition, have demonstrated their ability to successfully colonize host plants despite the presence of various phytochemical defenses. This observation suggests that lac insects possess physical or chemical cues that enable them to overcome the plant's defensive responses.

Several putative mechanisms may explain this phenomenon:

1. ***Formation of Salivary Flanges and Sheath*:** Lac insects may employ the strategy of forming salivary flanges and a salivary sheath as protective barriers during feeding. These structures could prevent air contact with injured or infested plant cells, thereby minimizing the plant's response to their feeding activities (12).
2. ***Production of Specific Enzymes*:** Lac insects may produce specific enzymes capable of breaking down secondary metabolites found in host plants. By converting these compounds into simpler molecules, the insects may avoid the harmful effects of plant defenses and enhance their ability to thrive on the host.
3. ***Evolutionary Adaptations*:** Over time, lac insects may have evolved mechanisms to bypass or inhibit plant defense pathways, such as the Jasmonate pathway, which is known to be involved in plant responses to insect attacks.
4. ***Microbial Partnerships*:** Lac insects may host specific gut microbiota or endosymbionts in addition to known endosymbionts, like *Wolbachia*, Wolbachia Phage, and YLS (40, 41). These microorganisms could play a role in neutralizing or modulating the plant's response to infestation, acting as partners in the insect's adaptation to its environment.

Additional studies, such as functional assays, metabolomics analyses, gene expression studies, or genetic analyses, can provide deeper insights into the defense pathways and interactions between these metabolites and lac insects. Furthermore, investigating the chemical profiles of various host plants and their impact on lac crawler settlement patterns could yield essential information for optimizing lac production and enhancing our understanding of the intricate interactions between lac insects and their host plants. These results might contribute to a more logical assessment of the Z. *mauritiana* host plant's potential as a good host as despite an increase in the concentration of these secondary metabolites the insect can invade the plant tissues

successfully. This ongoing research may lead to the development of strategies for improving lac production and management practices in lac cultivation. It will also contribute to a better understanding of the co-evolutionary dynamics between lac insects and their host plants, shedding light on insect-plant interactions and potentially offering valuable applications for lac production and management.

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Authors' contributions

PS and AR carried out all the experimental works and writing of the manuscript. AM participated in the Quantitative and qualitative data analysis. AR carried out the writing assistant. SK and SS participated in the design of the study and AM performed the statistical analysis. AK and SL conceived the study and participated in its design and coordination. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None.

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