

# Host Specific Phytohistological And Fitness Responses In Papaya Mealybug *Paracoccus marginatus* Under Divergent Selection

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## Abstract

This study presents the phytohistological investigation of papaya mealybug PMB (*Paracoccus marginatus* Williams and Granara de Willink) across six host plants papaya, tapioca, cotton, mulberry, brinjal, and hibiscus highlighting their varied responses under divergent natural selection. Leaf samples from healthy and PMB-infested plants were examined for anatomical and biochemical changes. Significant structural alterations were observed in infested leaves, including distortion of abaxial and adaxial epidermal layers, disorganization of mesophyll tissues, enlargement of xylem and phloem cells, crystal body formation, and irregular cell arrangements, particularly in the midrib. Biochemical analysis revealed elevated tannin and phenol content, coupled with a decline in total carbohydrates, reducing sugars, and proteins in infested tissues. Susceptibility to PMB was pronounced in papaya and cotton, where severe histological damage and substantial reductions in primary metabolites were noted. On the other hand, tapioca showed resistance, as seen by higher secondary metabolite levels and less tissue damage. These results support the idea that diverse natural selection shapes resistance mechanisms by indicating that host plants respond to PMB infection with varying defense measures. This study can direct the creation of resistant crop types and offers insightful information on host-pest interactions.

**Keywords:** Phytohistology, fitness response, papaya mealybug, *Paracoccus marginatus*, divergent selection, biochemical, resistance, antioxidants

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## INTRODUCTION:

The papaya mealybug PMB *Paracoccus marginatus* Williams & Granara de Willink is a sap-sucking, highly polyphagous insect infests more than 60 plant genera, including tapioca, jatropha, pigeon pea, teak, castor, eggplant, mulberry, hibiscus, and papaya and prospering across a range of agroecosystems. It had been a significant threat to both wild and cultivated plants Finch et al. (2021). Chlorosis, leaf curling, stunted growth, the development of sooty mold from honeydew secretion, and in extreme infestations, plant death are among the harm produced by PMB. Although it has a broad host range, different plant species exhibit different levels of infestation and damage, suggesting that host plant characteristics affect resistance or susceptibility. In order to protect themselves from insect herbivory, plants naturally produce secondary metabolites such as tannins and phenols. By serving as poisons, enzyme inhibitors, or feeding deterrents to invasive pests, these substances are essential to plant defense He et al. (2021). Abiotic variables may also have an impact on the accumulation of these metabolites, which are frequently brought on by an adaptive reaction to biotic stressors like insect infestation. By identifying resistant cultivars, an understanding of these plant-pest interactions at the anatomical and biochemical levels can help guide pest management tactics and offer important insights into natural resistance mechanisms Zhao et al. (2023).

The current study assessed the relative susceptibility or resistance of six host plant species to infestation by the papaya mealybug (*Paracoccus marginatus*): papaya (*Carica papaya*), cotton (*Gossypium* spp.) tapioca (*Manihot esculenta*) mulberry (*Morus* spp.) brinjal (*Solanum melongena*) and hibiscus (*Hibiscus rosa-sinensis*). The goal of the study was to evaluate the histological alterations and biochemical traits in response to PMB attack. The chosen host plants responded differently to infestation, as evidenced by changes in the primary and secondary metabolite profiles and the morphology of the leaf tissue. Plants that were susceptible, like cotton and papaya, displayed significant histological changes as well as a notable decrease in biochemical components like proteins, reducing sugars, and total carbohydrates. In contrast, despite PMB feeding pressure, resistant species such as tapioca showed a rise in defensive secondary

metabolites, especially phenols and tannins, and retained comparatively intact tissue integrity. The dynamic and continuous evolutionary arms race between insect herbivores and host plants is highlighted by these findings Zhao and Wang (2024). While pests like PMB develop strategies to get past these protections, host plants try to fend off insect attack by strengthening anatomical barriers and upregulating biochemical defenses. In order to choose resistant cultivars for cultivation and create sustainable pest control plans, this work adds to our understanding of plant resistance mechanisms and provides insightful information about the nature of host-pest interactions.

## MATERIALS AND METHODOLOGY

The experiment was carried out in the Department of Entomology Laboratory at SRM College of Agricultural Sciences, Chengalpattu. *Paracoccus marginatus* was first mass-cultured on potato sprouts, and the cultivated insects were then evenly dispersed onto the studies chosen potted host plants, which included papaya, cotton, tapioca, mulberry, brinjal, and hibiscus. Following that, biochemical investigations were carried out utilizing normal procedures on both healthy and PMB- infested leaves. Light microscopy was then used in phytohistological investigations to look at anatomical alterations in the midrib and leaf lamina areas of both healthy and infected samples. Below is a full description of the methods used for each experimental component.

### Collection and mass culturing of *Paracoccus marginatus*

Papaya mealybugs were raised using potato sprouts as a substitute food supply. Mass culture was performed on potato sprouts (Fig. 1a) using the procedure outlined by CABI review (2024). Using a camel hair brush, *Paracoccus marginatus* individuals were put onto potato sprouts at a rate of three to five ovisacs per sprout. These individuals were obtained from a variety of host plants, such as papaya, tapioca, cotton, mulberry, brinjal, and hibiscus. Within 25 to 30 days of inoculation, a substantial population of mealybugs developed. Additionally, mass culturing was simultaneously conducted on the aforementioned host plants to maintain uniform infestation levels for subsequent experimental studies (Zheng et al. (2022)) (Fig. 1b to 1g).

### Phytohistology of plants in relation to resistance

This study aimed to examine the histological changes caused by *Paracoccus marginatus* feeding on the leaves of various host plants using light microscopy. To validate these anatomical observations, biochemical analyses were also conducted on both healthy and infested leaves. Total carbohydrates were estimated using the Anthrone method, while reducing sugars were quantified following Somogyi's method. The Lowry method was used to determine the protein content, and the Moore and Stein procedure was used to evaluate the total free amino acids. The Folin-Dennis method was used to measure the tannin concentration and the phenol content was examined (Sharma et al. (2022) Khanum et al. (2023) in accordance with for secondary metabolites. In order to provide a thorough understanding of host plant defense mechanisms against PMB infestation, these biochemical measures were utilized to connect the degree of metabolic changes with histological responses. Samples of non-infested leaves and leaf segments, such as the midrib and distinct lamina areas showing varying degrees of *Paracoccus marginatus* infection, were taken from pot-cultured host plants for histological examination. In order to retain cellular architecture, the samples were preserved in a solution of formalin, acid, and alcohol (FAA). Tissues were embedded in paraplast for sectioning after being dried using a graded sequence of ethanol and butanol after fixation. A rotary microtome was used to create serial slices that were 10  $\mu\text{m}$  to 15  $\mu\text{m}$  thick. These sections were then placed on sterile microscope slides. After dewaxing, the sections were stained with Safranin and Fast Green according to standard protocols described by Johansen (1940) and Jensen (1962) BMC Plant Biology (2020) Pramayudi et al. (2022). The stained sections were examined under both bright-field and fluorescence microscopes to assess anatomical changes induced by PMB infestation across different host plant species.

## RESULTS AND DISCUSSION

### Biochemical analysis of host plants of Papaya mealybug *Paracoccus marginatus*

Insect resistance in plants is also influenced by the quality and quantity of chemical constituents. These chemicals are often localized in specific plant parts or appear during particular growth stages. The

chemical composition of the host plant significantly affects herbivore behavior and adaptation. Therefore, biochemical constituents responsible for plant susceptibility or resistance to mealybug infestation namely carbohydrates, reducing sugars, protein, total free amino acids, and secondary metabolites such as phenol and tannin were estimated. Estimation of soluble protein content revealed significant differences between infested and healthy plants across all host species (Table 1). Infested plants consistently showed lower protein levels compared to their healthy counterparts. Papaya leaves recorded the highest protein content in healthy conditions (13.01 mg/g) but exhibited a substantial 54.11% reduction upon infestation. In contrast, cassava leaves showed only a 6.09% decrease. These findings align with Chuai et al. (2022) who reported that, reduced protein levels correlate with lower insect damage and emphasized the role of aromatic amino acids in supporting pest development. This study confirms that mealybug infestation significantly reduces protein content. Carbohydrate content was consistently higher in healthy leaves compared to mealybug-infested leaves across all host plants. Among the healthy samples, papaya recorded the highest carbohydrate content (68.79 mg/g), while cassava had the lowest (46.19 mg/g). Upon infestation, a marked reduction in carbohydrate levels was observed, with papaya showing the highest percentage reduction at 55.82%, whereas cassava exhibited only a 6.08% decrease. Interestingly, the infested leaves of less preferred host plants retained higher carbohydrate levels almost double than those of highly preferred hosts, suggesting that *Paracoccus marginatus* does not favor carbohydrate-rich plants. These results are consistent with Janaki (2010), who reported a 30% reduction in carbohydrate concentration in brinjal following mealybug infestation. Similar observations were made by Mwanauta et al. (2021) in brinjal. Earlier researchers, including Chuai et al. (2022) Mwanauta et al. (2023) and Mamahit et al. (2024) emphasized that herbivores exploit primary metabolites like carbohydrates for growth, while Zheng et al. (2022) found sugars act as feeding stimulants in susceptible varieties. The total carbohydrate and reducing sugar contents (Table 1) showed a positive correlation across all host plants. The quantity of reducing sugars varied by species, with cassava recording the lowest (2.46 mg/g) and papaya the highest (8.2 mg/g). These findings align with earlier studies by Palanisamy (1984) and Baby Raphael (1991) on brinjal and sorghum. A noticeable reduction in reducing sugars was observed in infested leaves compared to healthy ones. The lowest percentage reduction was in hibiscus (13.90%), while the highest was in brinjal (22.20%). Reducing sugars are essential for insect nutrition and influence host plant selection by phytophagous insects. In the present study, total free amino acid content varied significantly among different host plants and between healthy and infested leaves (Table 2). Infested leaves consistently showed higher amino acid levels than their healthy counterparts. Papaya recorded the highest amino acid content among healthy plants (7.57 mg/g), followed by cassava (4.9 mg/g). In infested leaves, the amino acid content increased to 8.31 mg/g in papaya and 6.53 mg/g in cassava. The highest percentage increase (24.96%) was observed in cassava, while papaya showed an 8.9% increase. These findings suggest that amino acids play an important role in the nutritional requirements and reproductive success of phytophagous insects. The observed variation in amino acid levels may significantly contribute to the antibiosis resistance mechanism of host plants, influencing the extent of *Paracoccus marginatus* infestation and plant susceptibility or resistance to the pest. Secondary plant metabolites play a crucial role in plant defense against insect herbivores by functioning as repellents, feeding deterrents, or toxins, thereby protecting the plant throughout its growth stages Chuai et al. (2022) Phenolic compounds, in particular, act as physiological inhibitors and are often induced in response to herbivory. In this study, phenol content varied significantly among the selected host plants and between healthy and infested leaves (Table 2). Brinjal exhibited the highest phenol concentration (6.28 mg/g), although it showed a lower percentage increase (35.06%) upon infestation. In contrast, cassava displayed the greatest percentage increase (52.19%) in phenol content, while papaya recorded the lowest phenol level (4.8 mg/g) and only a 14.13% increase. These findings are consistent with Chuai H.Y. et al. (2022) who reported that cassava varieties with higher phenolic acid levels were less preferred by *P. manihoti*. Similar observations were made by Mwanauta et al. (2021) in mealybug-infested rice plants.

Tannin, a key biochemical component associated with resistance, was analyzed to assess its correlation with mealybug injury. The tannin content showed significant variation among the six host plants in both healthy and mealybug-infested conditions (Table 2). Polymeric phenols, such as tannins, function primarily as digestibility reducers and contribute to plant defense against herbivores. In this study,

mealybug feeding triggered a notable increase in tannin levels, ranging from 14.13% to 36.61%. Papaya exhibited a modest rise from 2.31 to 2.69 mg/g, whereas tapioca showed a significant increase from 3.81 to 6.01 mg/g (36.61%). This elevated tannin content likely contributed to reduce feeding by mealybugs due to its toxic effects on phytophagous insects.

#### **Anatomy of healthy plant leaf**

The general histological structure of healthy leaf lamina has been previously documented by several researchers, including Langer, Speck & Speck (2021) Ramírez-Díaz, Gutiérrez-Gallegos & Terrazas (2024) Lv et al. (2023) George & Hari (2021) Fajri et al. (2024)

#### **Phytohistology of mealybug infested leaf lamina of all the host plants**

Mealybug infestation in papaya caused distortion and size reduction of both adaxial and abaxial epidermal cells. Palisade and spongy mesophyll layers showed poor differentiation with vacuole formation. Tannin deposition was noted in the spongy mesophyll, and leaf lamina thickness reduced, likely due to turgor loss (Fig. 2). In cotton leaves, mealybug feeding caused minimal changes in the adaxial epidermal layer. The palisade and spongy parenchyma cells remained largely intact (Fig. 3), with only a few crystal cells appearing in the palisade parenchyma. Notable accumulation of phenols was observed in the spongy mesophyll region, along with periclinal cell divisions (Fig. 3). In tapioca, feeding led to the transformation of individual epidermal cells into thick-walled, rectangular forms. Scattered tannin accumulation was evident in the palisade parenchyma, which otherwise remained unchanged. However, a reduction in the number of spongy parenchyma layers (from 5 to 6) without vacuole formation was noted. Prominent periclinal divisions and phenol accumulation were seen in the spongy mesophyll, while the abaxial epidermis formed compact, short unicellular cells (Fig. 4). In mulberry leaves, large vacuoles appeared in the adaxial epidermis, extending into the palisade region. Both mesophyll layers appeared compact with poor differentiation, and the palisade parenchyma cells were reduced in size. The adaxial and abaxial epidermal cells were distorted, becoming thick-walled and irregularly shaped due to mealybug damage (Fig. 5). In brinjal, the upper epidermal layer remained largely unaffected by mealybug infestation. However, tannin deposits were noted in the palisade parenchyma. The spongy mesophyll cells appeared disorganized, containing numerous large vacuoles, along with tannin deposits and star-shaped crystals in the mesophyll region. The abaxial epidermis showed complete distortion with a reduction in cell size (Fig. 6). In hibiscus, the adaxial epidermal cells exhibited irregular size and shape, with cell wall thickening due to mealybug feeding. Numerous vacuoles extended into the palisade parenchyma, which also showed structural distortion and tannin deposition. Spongy mesophyll areas exhibited extensive vacuolation, with vacuolar spaces also occurring between the epidermal cells, indicating significant cellular disruption from mealybug infestation (Fig. 7).

#### **Phytohistology of mealybug infestation on midrib**

Figures 8 to 14 depict the midrib anatomy of uninfested leaves across all host plants, revealing a consistent structural pattern. The midrib is composed of both adaxial and abaxial epidermal layers, followed by cortical parenchyma and a crescent-shaped arrangement of 8 to 10 vascular bundles. The adaxial epidermis is made up of rectangular, dorsiventral cells, while the xylem vessels within the vascular bundles are aligned in organized rows. The abaxial region features cortical parenchyma interspersed with large secretory cells, which are easily distinguishable by their relatively larger size. The cortex includes approximately seven layers of parenchymatous cells, with some containing tannin deposits. The vascular bundles are collateral, with inner xylem and outer phloem, and a large resin canal is positioned externally near the phloem. The epidermal cells in these regions are typically barrel-shaped and densely packed with tannins, suggesting a structural role in the plant's innate defense system. Mealybugs predominantly fed near the midrib region of the leaves, prompting a detailed examination of the anatomical changes in this area. Figures 8 to 14 reveal significant alterations in the epidermal layer of infested midribs, with notable cell modifications. In particular, hyperplasia of palisade cells was observed (Fig. 8), leading to their enlargement and a subsequent loss of leaf turgidity. This abnormal expansion of cells caused compression and shrinkage of the vascular bundles, thereby impairing the translocation of nutrients and water through the xylem. In infested papaya leaves, feeding sites and cell lysis were clearly evident (Figs. 8 and 9). Tannin deposition was prominent in both palisade and cortical cells of the midrib (Fig. 10). Hyperplasia was distinctly observed in brinjal and hibiscus, while notable phenol and tannin accumulation occurred in

tapioca and hibiscus. However, mulberry and tapioca showed minimal structural changes in palisade cells (Figs. 11 to 14), suggesting potential resistance mechanisms. Periclinal and anticlinal cell divisions in the leaf lamina's epidermis and underlying hypodermal layers marked the beginning of the mealybug invasion. The epidermal layer finally broke due to constant cell divisions and an increase in the number of cells. The resultant cells were distributed radially and had an anticlinally elongated appearance. Epidermal cells rich in tannins began to divide after losing their contents. The insect most likely secreted a protective material over the eggs after oviposition. Upon hatching, larvae pierced and fed on the epidermal cells, damaging them and advancing into the plant tissue, either toward the midrib center or settling in the outer parenchymatous region. This feeding path triggered significant cellular alterations. Some surrounding cells turned necrotic, while others exhibited dense cytoplasmic content or signs of degeneration. Certain cells became meristematic, likely responding to the wounding stimulus. This led to wound healing responses such as hyperplasia of surrounding cells, hypoplasia in ground tissues, and restructuring of the feeding path (Figs. 8 to 14). The study clearly demonstrated that mealybug infestation significantly altered the anatomical structure of leaves across all examined host plants. Marked differences were observed between healthy and infested tissues, supporting earlier findings (Langer, Speck & Speck (2021) Ramírez-Díaz, Gutiérrez-Gallegos & Terrazas (2024) Lv et al. (2023) George & Hari (2021) Fajri et al. (2024)). The damage was directly linked to the feeding behavior and dense colonies of mealybugs, particularly on the abaxial surface of leaves, which triggered curling, distortion, and irregular undulations in the leaf blade. These changes were accompanied by disruption of the mesophyll structure, with affected cells losing their integrity and becoming disorganized, forming large intercellular spaces. Similar patterns of mesophyll degradation have been documented during interactions of *Aceria anthocoptes* with *Cirsium arvense* (Takano et al. 2023) and in citrus leaves attacked by *Phyllocoptruta oleivora* (Veershetty et al. 2023). One of the most prominent symptoms observed was the enlargement of epidermal cells, especially on the lower side of the leaf an effect also noted by Sassaloue et al. 2024 in *Euphorbia seguierana* infested by *Aculops euphorbiae*. Moreover, changes were not limited to the lamina; midrib structures also underwent significant modification. These anatomical disruptions suggest that mealybug feeding induces abnormal cellular activity and growth responses. Ahmed et al. 2023 also noted that certain insect pests can alter plant development by interfering with growth and differentiation pathways, thereby reshaping host architecture to benefit the pest. Mealybug feeding caused notable anatomical alterations in host plants, particularly in tapioca and hibiscus. These changes included thickened epidermal layers and more deeply embedded vascular bundles comprising xylem and phloem. These species are less suitable for mealybug feeding because of these structural changes, which are probably a component of the plant's defense mechanism. This observation is consistent with research by Takano et al. (2023), which showed comparable alterations in mango leaves after infestations by scale insects. Insect attacks cause a type of chemical stress or shock by upsetting the subcellular environment in plant tissues. In reaction to injury, plants produce specialized cells, usually one or more metaplasied cells, as a result of the shock-induced osmotic imbalances in the impacted cells (Ahmed et al. 2023). One tissue type changing into another is called metaplasia, and it frequently occurs locally. Sassaloue et al. 2024 noted that these metabolic changes are confined to specific regions and do not spread throughout the entire organ or plant. Additionally, epidermal cells on both adaxial and abaxial surfaces, as well as deeper mesophyll tissues, displayed clear signs of damage. Veershetty et al. 2023 reported similar injury patterns in *Cirsium arvense* leaves attacked by *Aceria anthocoptes*. Notably, pronounced periclinal cell divisions were observed in mealybug-infested leaves of tapioca and cotton, forming new tissues consistent with Ahmed et al. 2023, who reported neotissue development in infested leaves. The present study suggests that cell lysis in papaya leaves may result from salivary secretions injected by mealybugs into the upper epidermis and palisade cells. This irritation triggered pronounced hyperplasia and elongation of palisade parenchyma cells, accompanied by anticlinal cell divisions. The mealybug's four nymphal stages, from egg to adult female, may employ strategies that limit water loss from host tissues. Raval et al. 2023 noted such protective mechanisms in sap-sucking insects. Takano et al. 2023 observed that certain psyllids remain active in humid conditions, while others secrete wax to minimize dehydration. Similarly, Arduin and Kraus (2001) reported that in *Piptadenia gonoacantha* and *Guarea macrophylla*, palisade proliferation and spongy mesophyll division reduce intercellular spaces under stress. This study identified salivary pathways and cell lysis in papaya leaves

infested by mealybugs. Similar cell breakdown was noted by Ahmed et al. 2023 in *Alstonia scholaris* attacked by *Pauropsylla tuberculata*. Hemipteran insects feed by inserting stylets into plant tissues, pausing to inject a viscous secretion that forms a salivary sheath Veershetty et al. 2023. This sheath is believed to assist in stylet penetration and potentially suppress the plant's hypersensitive defense responses (Sassalou et al. 2024). He also suggested that stylet insertion often occurs between cells, dissolving the middle lamella. However, Takano et al. 2023 reported that stylets can also penetrate between the cell wall and plasma membrane or follow an intracellular route, depending on the species. Mealybug infestation led to noticeable shrinkage of vascular bundles in the midrib region. This deformation likely results from the insect's extraction of nutrients from the phloem, xylem, or non-conductive tissues, as noted by Sassalou et al. 2024. The xylem and phloem cells showed reduced structure, possibly due to this feeding behavior. Initially, hypertrophy and hyperplasia occurred in the spongy parenchyma, eventually extending to the palisade layer. Spongy parenchyma cells appeared more sensitive and responded earlier to insect-induced stress, aligning with the insect's preference for the lower leaf surface. These anatomical disruptions resemble those documented by Raval et al. (2023) in *Camellia sinensis*, where feeding by *Helopeltis theivora* triggered significant cellular changes and hypersensitive reactions. The anatomical changes in leaves caused by mealybug feeding align with previous studies. According to Langer, Speck & Speck (2021) Ramírez-Díaz, Gutiérrez-Gallegos & Terrazas (2024) Lv et al. (2023) George & Hari (2021) Fajri et al. (2024) new leaves may grow larger due to mesophyll cell elongation, while Takano et al. 2023 attributed it to increased cell division. Leaf expansion can also result from chloroplast enlargement due to starch buildup or higher turgor pressure. Since turgor drives cell growth, a drop in water content below 90% impedes expansion, while stress-related defoliation may enhance water availability and promote cell expansion (Veershetty et al. 2023). The biochemical analysis in this study revealed noticeable changes in leaf composition following mealybug infestation. A significant decrease in chlorophyll content was detected, likely due to damage to palisade tissues, chloroplast degradation, and alterations in spongy mesophyll cells. Such chlorophyll loss contributes to leaf discoloration, especially around egg-laying sites, as noted in *Ficus* leaves by Takano et al. (2023). Additionally, infested papaya and cotton leaves showed elevated levels of total carbohydrates and sugars. The accumulation of carbohydrates may be linked to cellular dysfunction or paralysis. This observation aligns with Raval et al. (2023) who found that high glutamine concentrations can impair normal cell function, leading to paralysis in leaf tissues. The current investigation revealed a noticeable decline in protein content in leaves infested by mealybugs. However, among the studied host plants, tapioca and hibiscus exhibited the least percentage reduction in protein levels, suggesting they were less favored by the pest. This observation aligns with earlier studies by Sultana et al. (2022) and Bhat and Sreewongchai (2018), who also noted minimal protein loss in plants with lower infestation preferences. Similarly, findings from Rathore et al. (2018) support this trend. Previous research has emphasized that plants synthesize a variety of defensive proteins in response to insect attacks Zhao and Wang (2024). Notably, proteinase inhibitors defensive compounds that inhibit digestive enzymes in herbivores are known to accumulate in plants like legumes and tomatoes. These proteins can build up not only at the feeding site but also in distant, unaffected plant tissues. Singh et al. (2024) highlighted this systemic response, indicating a plant-wide defense mechanism triggered by insect feeding. Therefore, it can be inferred that insect attacks prompt the activation of specific protein signals, potentially offering insights into developing novel strategies for crop protection through induced resistance pathways. The present study showed that phenol and tannin levels significantly increased in leaves infested by mealybugs compared to healthy leaves. This accumulation is likely a stress-induced defense response triggered by insect feeding. Such compounds are commonly synthesized under biotic or abiotic stress to enhance plant resistance, as noted by Zhang et al. (2024). The elevated levels of phenols and tannins in tapioca and hibiscus may explain their lower susceptibility to mealybug attacks. Sultana et al. (2022) suggested that phenolic compounds serve as chemical defenses by deterring insect feeding. These secondary metabolites interfere with insect digestion and development. Zhao et al. (2023) also reported that phenolics play a critical role in protecting plants against both insect herbivores and microbial pathogens. The formation of a dark brown halo surrounding necrotic cells in tea leaves by Ito et al. (2023) demonstrated how insect-induced tissue damage can cause major physiological alterations in host plants. Since polyphenol oxidase (PPO) oxidizes phenolic molecules during herbivore attacks, this discolouration, which is connected to

cell death, has been linked to an inducible plant defense system. Similar resistance patterns were seen in the current study in hibiscus and tapioca, which mealybugs preferred less. This is probably because to increased PPO activity and phenolic accumulation. Plant tissues' dynamic character and capacity to react to outside stimuli are demonstrated by these defense-related reactions. According to Zhao et al. (2023), when plants reroute developmental pathways in response to insect stimuli, such reactions represent a disruption in normal cellular processes. Zhang et al. (2023) discovered that aberrant expression of genes typically active in other plant tissues, such as seeds, may contribute to gall formation. These structural alterations imply that insect damage might trigger dormant developmental potentials in plants that are not expressed in typical, healthy circumstances, as determined by Kaur and Kaur (2023).

## CONCLUSION

An infestation of *Paracoccus marginatus* causes notable morphological and chemical changes in the leaves of the host plant, according to the results of the phytohistological and biochemical analyses. The presence of crystal structures and disturbed cellular organization were noted, as were notable alterations in the vascular bundles, mesophyll tissues, spongy parenchyma, and epidermal layers. A decrease in proteins, carbs, and sugars was linked to these structural alterations, along with an increase in phenol and tannin. Papaya and cotton were the most susceptible of the host plants under investigation, but tapioca showed significant resistance, as seen by higher levels of defensive metabolites, suggesting that PMB may not favor it as a host.

## Acknowledgement

The present study was supported by the Department of Entomology, SRM College of Agricultural Sciences, SRM Institute of Science and Technology, Chengalpattu, Tamil Nadu, India.

## Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

## Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish, or preparation of the manuscript.

## Informed consent

All animal procedures for experiments were approved by the Committee of Experimental Animal care and handling techniques were approved by the University of Animal Care Committee.

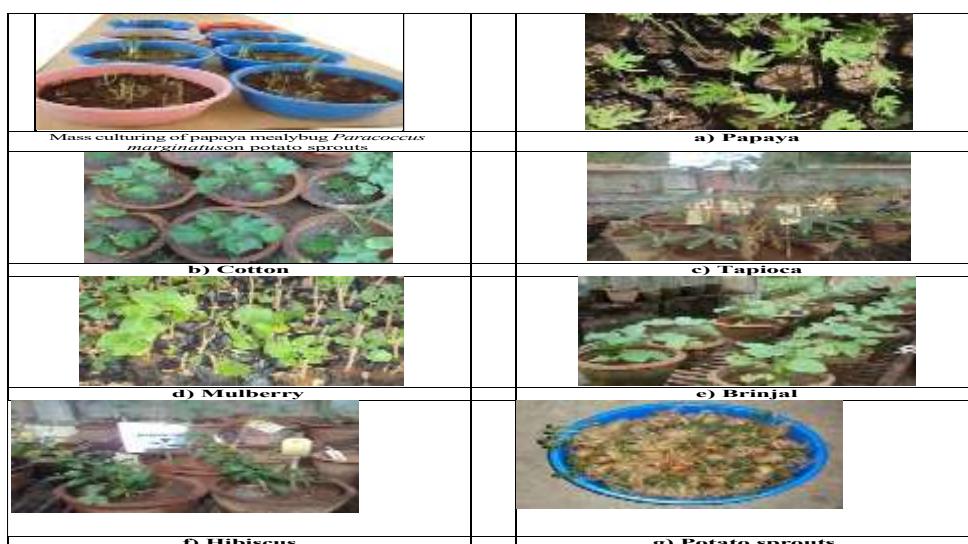


Fig. 1. Mass culturing & Host plants raised in pots for studying the phytohistology of Papaya mealybug *Paracoccus marginatus* on different host plants

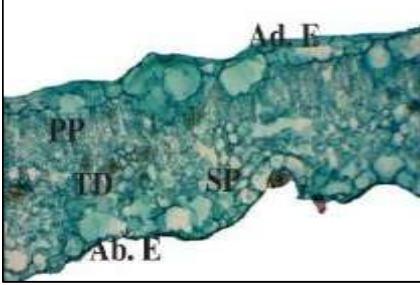
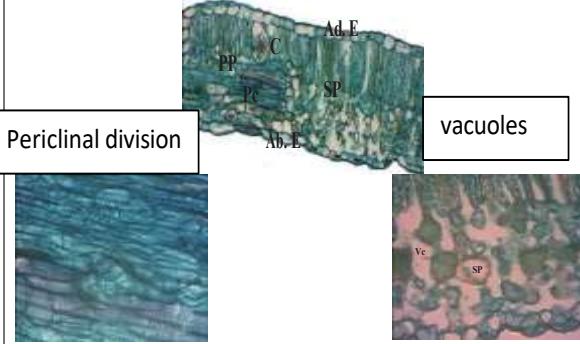
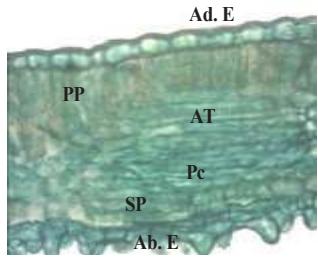
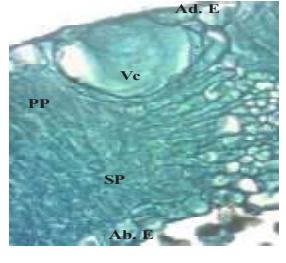
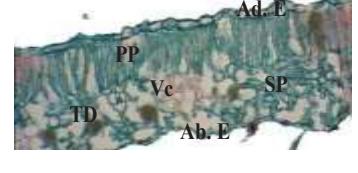
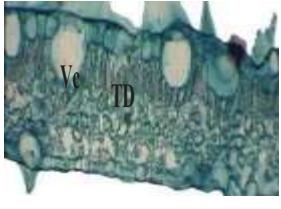
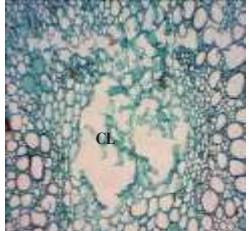
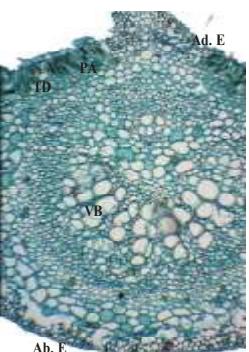
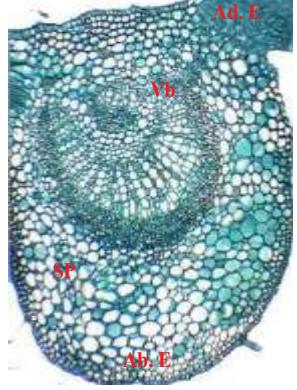
<b>Fig.2. Cross section of mealybug infested papaya leaf lamina</b> 	<b>Fig. 3. Cross section of infested leaf lamina of cotton</b> 	
<b>Fig. 4. Cross section of mealybug infested leaf lamina of tapioca</b> 	<b>Fig.5. Cross section of mealybug infested leaf lamina of mulberry</b> 	<b>Fig.6. Cross section of mealybug infested brinjal leaf lamina</b> 
<b>Fig. 7. Cross section of mealybug infested leaf lamina of hibiscus</b> 	<b>Fig.8. Cross section of infested midrib of papaya</b> 	<b>Fig.9. Cell lysis in midrib of cells</b> 
<b>Fig.10. Cross section of infested midrib of cotton</b> 	<b>Fig.11. Cross section of infested midrib of tapioca</b> 	<b>Fig.12. Cross section of infested midrib of mulberry</b> 

Table 1. Soluble protein, total carbohydrates and reducing sugar contents on the leaves of host plants of *Paracoccus marginatus*

Host plants	Soluble protein (mg/g)			Total Carbohydrates (mg/g)			Reducing sugar (mg/g)		
	Health y	Infested	Per cent Reduction	Health y	Infested	Per cent Reduction	Health y	Infested	Per cent Reduction
Papaya	13.01 <sup>a</sup>	5.97 <sup>c</sup>	54.11 <sup>a</sup>	68.79 <sup>a</sup>	30.39 <sup>f</sup>	55.82 <sup>a</sup>	8.2 <sup>a</sup>	6.38 <sup>a</sup>	22.20 <sup>a</sup>
Cotton	11.15 <sup>b</sup>	6.06 <sup>bc</sup>	45.65 <sup>b</sup>	63.86 <sup>b</sup>	33.52 <sup>e</sup>	47.51 <sup>b</sup>	5.9 <sup>b</sup>	5.02 <sup>b</sup>	14.92 <sup>d</sup>
Tapioca	6.73 <sup>f</sup>	6.32 <sup>a</sup>	6.09 <sup>f</sup>	46.19 <sup>e</sup>	43.38 <sup>a</sup>	6.08 <sup>f</sup>	2.46 <sup>e</sup>	2.07 <sup>e</sup>	15.85 <sup>c</sup>
Mulberry	9.99 <sup>c</sup>	6.21 <sup>ab</sup>	37.84 <sup>c</sup>	55.16 <sup>c</sup>	38.41 <sup>c</sup>	30.37 <sup>d</sup>	5.2 <sup>c</sup>	4.39 <sup>c</sup>	15.58 <sup>c</sup>
Brinjal	8.76 <sup>d</sup>	6.25 <sup>ab</sup>	28.65 <sup>d</sup>	54.87 <sup>c</sup>	36.2 <sup>d</sup>	34.03 <sup>c</sup>	5.12 <sup>c</sup>	4.27 <sup>cd</sup>	16.60 <sup>b</sup>
Hibiscus	7.33 <sup>e</sup>	6.39 <sup>a</sup>	12.82 <sup>e</sup>	50.02 <sup>d</sup>	40.58 <sup>b</sup>	18.87 <sup>e</sup>	4.82 <sup>d</sup>	4.15 <sup>d</sup>	13.90 <sup>e</sup>
SED	0.1591	0.1015	0.5749	0.9315	0.6097	0.5898	0.0898	0.0754	0.2724
CD (0.05)	0.3466	0.2210	1.2526	2.0296	1.3284	1.2850	0.1956	0.1642	0.5935

Table 2. Total free amino acid, phenol and tannin contents on the leaves of host plants of *Paracoccus marginatus*

Host plant s	Total free amino acid (mg/g)			Phenol (mg/g)			Tannin (mg/g)		
	Healthy	Infested	per cent increase	Healthy	Infested	Per cent increase	Healthy	Infested	Per cent increase
Papaya	7.57 <sup>a</sup>	8.31 <sup>a</sup>	8.90 <sup>f</sup>	4.8 <sup>e</sup>	5.59 <sup>f</sup>	14.13 <sup>f</sup>	2.31 <sup>e</sup>	2.69 <sup>e</sup>	14.13 <sup>d</sup>
Cotton	6.2 <sup>b</sup>	6.9 <sup>b</sup> <sup>c</sup>	10.14 <sup>e</sup>	5.68 <sup>c</sup>	7.01 <sup>e</sup>	18.97 <sup>e</sup>	2.69 <sup>d</sup>	3.49 <sup>d</sup>	22.92 <sup>c</sup>
Tapioca	4.9 <sup>e</sup>	6.53 <sup>d</sup>	24.96 <sup>a</sup>	5.25 <sup>d</sup>	10.98 <sup>a</sup>	52.19 <sup>a</sup>	3.81 <sup>a</sup>	6.01 <sup>a</sup>	36.61 <sup>a</sup>
Mulberry	5.89 <sup>c</sup>	6.91 <sup>b</sup> <sup>c</sup>	14.76 <sup>d</sup>	6.01 <sup>b</sup>	8.25 <sup>d</sup>	27.15 <sup>d</sup>	2.72 <sup>d</sup>	3.55 <sup>d</sup>	23.38 <sup>c</sup>
Brinjal	5.83 <sup>c</sup>	7.1 <sup>b</sup>	17.89 <sup>c</sup>	6.28 <sup>a</sup>	9.67 <sup>b</sup>	35.06 <sup>c</sup>	2.95 <sup>c</sup>	3.92 <sup>c</sup>	24.74 <sup>b</sup>
Hibiscus	5.43 <sup>d</sup>	6.81 <sup>c</sup>	20.26 <sup>b</sup>	5.33 <sup>d</sup>	9.06 <sup>c</sup>	41.17 <sup>b</sup>	3.17 <sup>b</sup>	4.22 <sup>b</sup>	24.88 <sup>b</sup>
SED	0.0988	0.1175	0.2795	0.0922	0.1398	0.5549	0.0484	0.0662	0.4149
CD (0.05 )	0.2152	0.2560	0.6091	0.2009	0.3045	1.2091	0.1055	0.1443	0.9039

\* Mean of four replications

Means followed by the same alphabets are not significantly different at 5 % level by DMRT

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