

RESEARCH ARTICLE :

Phytohystology of papaya mealybug *Paracoccus marginatus* Williams and Granara de willink *vis-a-vis* divergent natural selection

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SUMMARY : Phytohystology of papaya mealybug PMB *Paracoccus marginatus* Williams and Granara de Willink was studied for the first time in this experiment and that is based on divergent natural selection process. Histology of healthy and mealybug infested leaves of different host plants *viz.*, papaya, tapioca, cotton, mulberry, brinjal and hibiscus examined for the biochemical changes and the results were correlated with phytohystological changes. Changes were noticed in the abaxial and adaxial leaf surfaces and mesophyll regions. Spongy parenchyma region and crystal bodies, enlargement of xylem and phloem cells, irregular arrangement of cells were noticed due to PMB attack in the leaves and midrib region of host plants. And, these were also accounted for increase in tannin and phenol content and reduction in total carbohydrate, reducing sugars and protein in leaves. Papaya and cotton leaves showed susceptible to PMB attack and the biochemicals and secondary metabolites were drastically reduced, whereas tapioca showed the resistance against PMB attack and the secondary metabolites were increased.

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BACKGROUND AND OBJECTIVES

The papaya mealybug PMB, *Paracoccus marginatus* (Williams & Granara de Willink) (Hemiptera: Pseudococcidae) is a small polyphagous sucking pest that attacks several genera of host plants like hibiscus, mulberry, eggplant, castor, teak, pigeon pea, tapioca, jatropha and many weed hosts, including parthenium (Miller *et al.*, 1999). It also damages economically

important crops like tropical fruits, vegetables and ornamentals. However, the efficiency of PMB attack may vary in different host plants. The plants has natural tendency to resist the pest attack by showing the increase in secondary metabolites like phenol and tannin. They were produced as a defense mechanism to protect from invaders (biotic stress) or stress factors (abiotic stress) and are believed to be an adaptive response to the altered conditions (El-Akkad, 2004). In the present

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study, different host plants *viz.*, papaya, cotton, tapioca, mulberry, brinjal and hibiscus were assessed for the effectiveness of PMB by studying their biochemical characteristics and histology. The host plants show variance in the infestation level by showing the increase and reduction in the biochemical contents and changes in the leaf cell anatomy due to susceptibility and resistance ability of them. The arms race between host plants and PMB was discussed here under.

RESOURCES AND METHODS

The experiment was carried out in the Insectary and Laboratory, Department of Agricultural Entomology, Tamil Nadu Agricultural University, Coimbatore. For this research, mass culturing of *Paracoccus marginatus* was done on potato sprouts at first and uniformly released into the pot cultured plants papaya, cotton, tapioca, mulberry, brinjal and hibiscus taken for study. Secondly, biochemical changes of healthy and PMB infected leaves were analysed using different methods quoted. Then finally, phytohistology experiment was undertaken under the light microscope in both healthy and infected leaf lamina and mid rib of the leaves. The methodology for all the experiments were discussed below.

Collection and mass culturing of *Paracoccus marginatus* :

Potato sprouts was used as an alternate food source for rearing mealybugs. Mass culturing of potato sprouts (Fig.1a.) was done in line with the reference of Serrano and Laponite, (2002). Papaya mealybugs collected from different host plants like papaya, tapioca, cotton, mulberry, brinjal and hibiscus were released on potato sprouts using camel hair brush at the rate of 3 to 5 ovisacs per potato and mealybugs *en masse* were obtained within 25 to 30 days of release. Mass culturing was also carried out in above said host plants and used for further experiments (Amarasekare *et al.*, 2008) Fig. (1b to 1g).

Phytohistology of plants in relation to resistance :

This investigation is to characterize the histologic changes resulting from the feeding of *P. marginatus* on leaves of different host plants using light microscopy. To describe the findings with confirmation, the biochemical changes were also studied in all the host plant leaves. The biochemical contents *viz.*, total carbohydrate was analysed by Anthrone method (Hedge and Hofreiter,

1962). Reducing sugar was determined by the method of Somogyi (1952). The protein content was estimated by the method suggested by Lowry *et al.* (1951). Total free amino acid was determined using the method followed by Moore and Stein, 1948. And for the secondary metabolites, Method described by Malik and Singh (1980) was followed for the estimation of phenol. Tannin content in the leaf samples was estimated following the Folin-Dennis method described by Oberbacher and Vines (1963).

The leaf bits and the midrib portion of different infestation gradience at different places of lamina from infested as well as non – infested leaves from the pot cultured plants were cut and fixed in Formal – Acetic acid- Alcohol (Ethanol) (FAA solution). They were dehydrated in Butanol + Ethanol series and impregnated with paraplast. Section of 10 μm to 15 μm were made serially using rotary microtome and mounted on a slide. After defarrafination the tissues were stained using Saffranine and Fast green (Johansen, 1940 and Jensen, 1962). Then they were observed under bright field and florescence microscopes.

OBSERVATIONS AND ANALYSIS

The results obtained from the present study as well as discussions have been summarized under following heads :

Biochemical analysis of host plants of Papaya mealybug Paracoccus marginatus :

Insect resistance in plants is also due to the quality and quantity of chemical constituents. These chemicals occur within certain parts of the plant or in specific stages of plant growth. The herbivore behaviour and adaptation to the host plant are influenced by the chemical composition of host plant. Hence, the biochemical constituents responsible for susceptibility or resistance to mealybug namely carbohydrates, reducing sugars, protein, total free amino acid, secondary metabolites like phenol and tannin were estimated.

Estimation of the soluble protein revealed that in all the host plants, a significant difference in the protein content was observed (Table 1). Infested plants recorded a lower amount of protein than the mealybug free plants. The highest amount of protein was observed in the papaya (13.01mg/g). The tapioca leaves recorded 6.09 per cent reduction from its healthy plants, while papaya showed

54.11 per cent reduction. Similar results were noticed by Mollema and Cole (1996) who found that low amount of total leaf proteins were correlated with a reduction in damage by insects. They concluded that higher concentrations of aromatic amino acids in plant proteins are important for successful pest development. The present finding explains that infested plants had shown significant decrease in protein content.

The carbohydrate content was higher in the healthy leaves than mealybug infested leaves. Among the healthy leaves, papaya recorded higher amount of 68.79 mg/g, while lower amount of 46.19 mg/g was present tapioca. The infested leaves showed reduction in the carbohydrate content, the highest per cent reduction being 55.82 per cent in papaya and the lowest in tapioca (6.08 %). The infested leaves of the poorly preferred plants possessed a higher amount of carbohydrate almost double than the highly preferred host. The results indicate that mealybug did not prefer high amounts of carbohydrates. The findings were in line with Janaki (2010) who reported the 30 per cent reduction in the concentration of total carbohydrates in brinjal leaves by feeding with papaya

mealybug. Similar results were reported by the earlier workers (Muruges, 1997) in brinjal. Rockstein (1978), Ananthkrishnan (1990) and Jayaraj and Uthamasamy (1990) reported that the primary metabolites including carbohydrates are exploited by the herbivores for their growth and development. Kalode and Pant (1967) reported that insect susceptible plant parts had higher concentration of sugars which acted as feeding stimulant in the susceptible varieties.

The total carbohydrate and total sugars content (Table 1) were in positive relationship in all the host plants. The quantity of reducing sugars was vary accordingly with host plants. The tapioca had minimum amount of reducing sugars (2.46 mg/g) whereas, papaya had maximum amount of reducing sugars (8.2 mg/g). The results are in conformity with Palanisamy (1984), Baby Rapheal (1991) and on brinjal and sorghum, respectively. The reduction was noted in the infested leaves against healthy leaves. Lowest per cent reduction was noted in hibiscus (13.90 %), whereas highest in brinjal (22.20 %). The reducing sugar, which is considered to be an essential component in insect nutrition and plays a vital role in

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)		
	Healthy	Infested	Per cent Reduction	Healthy	Infested	Per cent Reduction	Healthy	Infested	Per cent Reduction
Papaya	13.01 ^a	5.97 ^c	54.11 ^a	68.79 ^a	30.39 ^f	55.82 ^a	8.2 ^a	6.38 ^a	22.20 ^a
Cotton	11.15 ^b	6.06 ^{bc}	45.65 ^b	63.86 ^b	33.52 ^e	47.51 ^b	5.9 ^b	5.02 ^b	14.92 ^d
Tapioca	6.73 ^f	6.32 ^a	6.09 ^f	46.19 ^e	43.38 ^a	6.08 ^f	2.46 ^e	2.07 ^e	15.85 ^c
Mulberry	9.99 ^c	6.21 ^{ab}	37.84 ^c	55.16 ^c	38.41 ^c	30.37 ^d	5.2 ^c	4.39 ^c	15.58 ^c
Brinjal	8.76 ^d	6.25 ^{ab}	28.65 ^d	54.87 ^c	36.2 ^d	34.03 ^c	5.12 ^c	4.27 ^{cd}	16.60 ^b
Hibiscus	7.33 ^e	6.39 ^a	12.82 ^e	50.02 ^d	40.58 ^b	18.87 ^e	4.82 ^d	4.15 ^d	13.90 ^e
S.E. ±	0.1591	0.1015	0.5749	0.9315	0.6097	0.5898	0.0898	0.0754	0.2724
C.D. (P=0.05)	0.3466	0.2210	1.2526	2.0296	1.3284	1.2850	0.1956	0.1642	0.5935

*Mean of four replications

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

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

Host plants	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 ^a	8.31 ^a	8.90 ^f	4.8 ^e	5.59 ^f	14.13 ^f	2.31 ^e	2.69 ^e	14.13 ^d
Cotton	6.2 ^b	6.9b ^c	10.14 ^e	5.68 ^c	7.01 ^e	18.97 ^e	2.69 ^d	3.49 ^d	22.92 ^c
Tapioca	4.9 ^e	6.53 ^d	24.96 ^a	5.25 ^d	10.98 ^a	52.19 ^a	3.81 ^a	6.01 ^a	36.61 ^a
Mulberry	5.89 ^c	6.91 ^{bc}	14.76 ^d	6.01 ^b	8.25 ^d	27.15 ^d	2.72 ^d	3.55 ^d	23.38 ^c
Brinjal	5.83 ^c	7.1 ^b	17.89 ^c	6.28 ^a	9.67 ^b	35.06 ^c	2.95 ^c	3.92 ^c	24.74 ^b
Hibiscus	5.43 ^d	6.81 ^c	20.26 ^b	5.33 ^d	9.06 ^c	41.17 ^b	3.17 ^b	4.22 ^b	24.88 ^b
S.E. ±	0.0988	0.1175	0.2795	0.0922	0.1398	0.5549	0.0484	0.0662	0.4149
CD. (P=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



Mass culturing of papaya mealybug *Paracoccus marginatus* on potato sprouts



a) Papaya



b) Cotton



c) Tapioca



d) Mulberry



e) Brinjal



f) Hibiscus



g) Potato sprouts

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

host selection by phytophagous insects.

In present investigation, the total free amino acid content vary with different host plants. The difference in the quantity of amino acid among the host plants and between healthy and infested leaves was significant (Table 2). Total free amino acids was more in the infested plants than in healthy plants. Papaya had comparatively higher amounts (7.57 mg/g), than the other host plants and it was 4.9 mg/g in tapioca. The same plants had 8.31 and 6.53 mg/g of amino acids, respectively among the infested plants. Among the infested plants, the highest increase of amino acid to a tune of 24.96 per cent was recorded in tapioca, while papaya exhibited 8.9 per cent increase over its healthy plants. Amino acids are known to have a greater role in insect nutrition and reproduction. It would be reasonable to conclude that these variations in amino acid content contributed substantially in the antibiosis mechanism of host plants.

Secondary plant metabolites play a key role in the defense against insect herbivore as they act as insect repellents, feeding inhibitors and/or toxins and thus protecting plants at different phases of growth (Sadasivam and Thayumanavan, 2003). Phenols are known to be physiological inhibitors against herbivore attack and induced after herbivory. In the present investigation, the quantity of phenols was significantly different among the selected host plants and also between the healthy and infested leaves (Table 2). The phenol content was higher in brinjal (6.28 mg/g) with lowest per cent increase (35.06 %) than tapioca and lower in papaya (4.8 mg/g). The per cent increase was higher in tapioca (52.19 %) among all the plants, whereas it was only 14.13 per cent in papaya. The result of present study is supported by Soundararajan and Baskaran, (2001). Goodman (1986) reported that the cassava variety with a high phenolic acid level in its extracellular fluids was less preferred by *P. manihoti*. This result is also in line with Gopalan *et al.* (1987) who reported that feeding injury by mealybugs resulted in a marginal increase in total phenolic content in rice leaves.

Tannin, an important biochemical factor of resistance was also analysed and correlated with mealybug injury and the resistance. The tannin quantity examined in six host plants showed a significant difference in the healthy as well as mealybug infested plants (Table 2). Polymeric phenols act primarily as digestibility reducers and play a role in plant defense

against herbivory. In the present study there was a substantial increase in the quantity of polymeric phenols as a result of feeding by mealybugs. The mealybug induced 14.13 – 36.61 per cent increase in tannins in the host plants. Papaya showed comparatively less increase in tannin from 2.31 to 2.69 mg/g, while tapioca recorded almost a two fold increase from 3.81 to 6.01 mg/g with 36.61 per cent increase. The increased quantity of tannin led to reduction in subsequent feeding of inducers themselves, as they are highly toxic to most of the phytophagous insects.

Anatomy of healthy plant leaf :

The general histology of healthy leaf lamina was described earlier by the scientists Ahmed *et al.* (2013), Albert *et al.* (2011), Pecinar *et al.* (2006), Kennedy (1995)

Phytohistology of mealybug infested leaf lamina of all the host plants :

Due to mealybug infestation in papaya, both adaxial and abaxial epidermal cells were found to be disorted with a reduction in the size of epidermal cells. Poor differentiation of palisade and spongy mesophyll was observed with formation of vacuoles in mesophyll region. Deposition of tannin was observed in the spongy mesophyll area. There was a reduction in the thickness of leaf lamina might be due to loss of turgidity (Fig. 2).

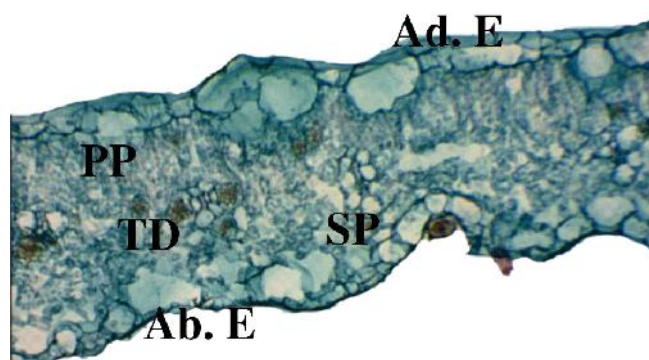


Fig. 2 : Cross section of mealybug infested papaya leaf lamina

There was not much change observed in the adaxial epidermal layer due to the mealybug feeding in cotton leaves. Palisade and spongy parenchyma cells were retained (Fig. 3). Even they did not suffer much alteration, but far few crystal cells in palisade parenchyma and accumulation of phenols observed in spongy

mesophyll area. The periclinal divisions (Fig. 3) were seen in spongy mesophyll area with accumulation of phenols in it. In tapioca, due to feeding of mealybug, individual epidermal cells were transformed into thick walled rectangular cells. Scattered accumulation of tannin was observed in palisade parenchyma cells. There was not much change in palisade parenchyma but for, in spongy parenchyma there was a reduction in number of layers (5 to 6 layers) was observed without any vacuoles. Periclinal divisions were observed in spongy mesophyll area with highest phenol accumulation in it. The abaxial epidermis gave rise to short and closely packed unicellular cells (Fig. 4). In mulberry, the adaxial epidermis were given rise to large sized vacuoles in between cells extended upto the palisade parenchyma. Palisade and spongy mesophyll seemed to be compact with poor differentiation. There was a reduction in size of palisade parenchyma cells due to the mealybug feeding in leaf was observed. Distortion of both adaxial and abaxial epidermal cells resulted in thick walled and irregular shaped cells (Fig. 5).

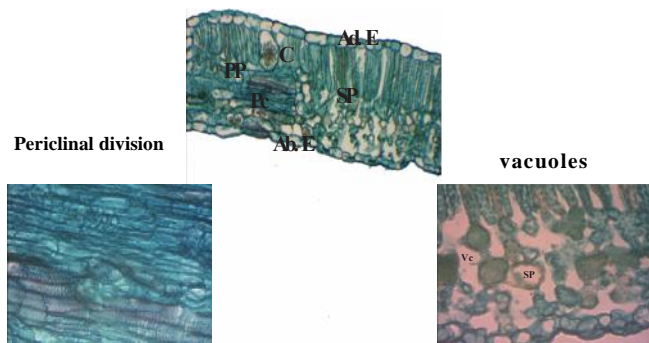


Fig. 3 : Cross section of infested leaf lamina of cotton

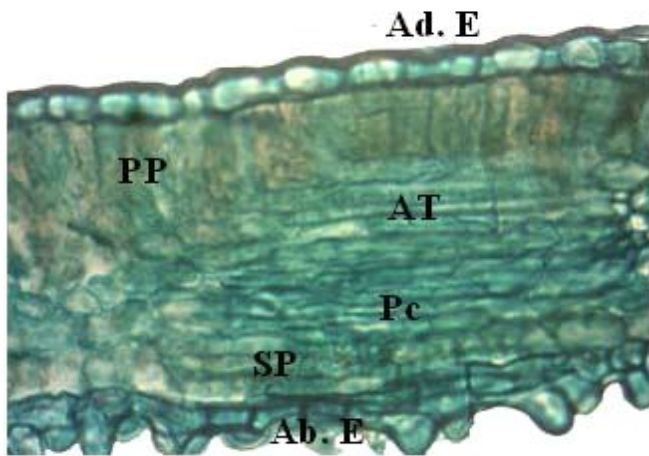


Fig. 4 : Cross section of mealybug infested leaf lamina of tapioca

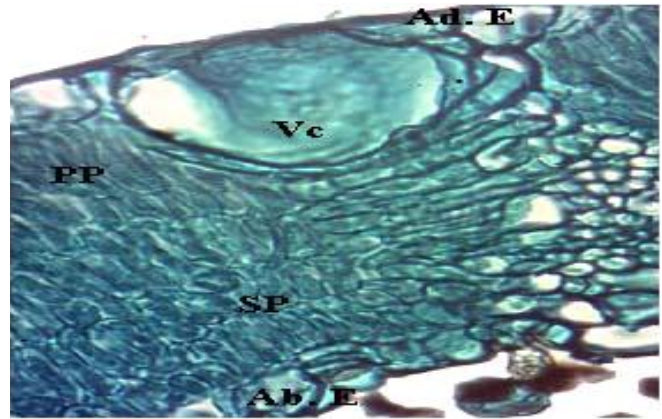


Fig. 5 : Cross section of mealybug infested leaf lamina of mulberry

There was no profound change in upper epidermal layer in brinjal. Deposition of tannins was observed in palisade parenchyma. Spongy mesophyll cells were disorganized with numerous large sized vacuoles in between them. Deposition of tannin and star shaped crystals were seen in spongy mesophyll area due to mealybug infestation. There was complete distortion of abaxial epidermis with reduction in their cell size was observed (Fig. 6). The adaxial epidermal cells were not uniform in their size and shape and the epidermal cells were thickened due to mealybug feeding in hibiscus. They given rise to numerous number of vacuoles extended upto the palisade parenchyma due to mealybug feeding in the leaf. Structure of palisade parenchyma was distorted with tannin deposit in it. And the infestation lead to numerous vacuolar space in spongy mesophyll area and between the epidermal cells (Fig. 7).

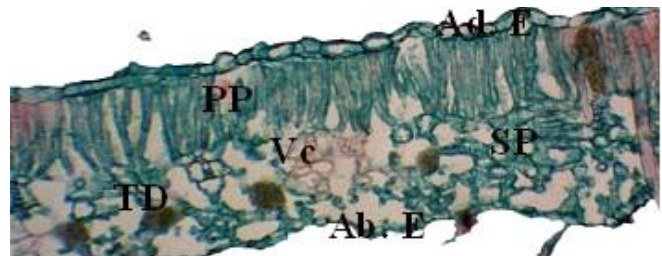


Fig. 6 : Cross section of mealybug infested brinjal leaf lamina

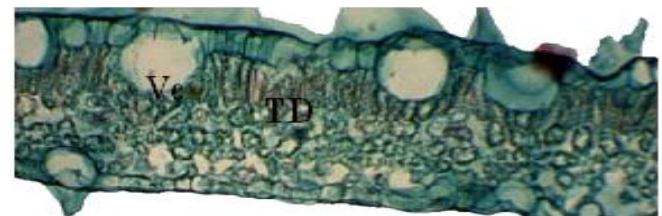


Fig. 7 : Cross section of mealybug infested leaf lamina of hibiscus

Phytohystology of mealybug infestation on midrib :

The figures (8 to 14) inferred that the uninfested midrib of all the host plants had comprised of abaxial and adaxial epidermis, cortical parenchyma and a ring of vascular bundles ranging from eight to ten in a crescent form. The rectangular dorso ventral cells in adaxial epidermis was observed. The xylem cells in vascular bundles were distinctly visible in rows. Abaxial cortical parenchyma were interspersed with large secretory cells, which were apparently distinct because of its larger size compared to the adjacent cells. The cortex consists of nearly seven layers of parenchyma cells, some of which are filled with tannin. The vascular bundles are collateral with inner xylem and outer phloem and a large resin canal occurring outside at the centre of the latter. Epidermal cells are small, barrel-shaped and filled with tannins.

The mealybugs mostly fed near the midrib of leaves. So, it was necessitated to study the anatomical changes taken place in midrib of the host plant leaves. The figures (8 to 14) inferred that, the epidermal layer of the infested midrib is highly modified. The palisade cells become enlarged due to hyperplasia condition (Fig. 8), resulting in loss of turgidity in the leaves. The increase in the space occupied by the abnormally inflated cells, lead to shrinkage in vascular bundles, which indirectly reduced the translocation of nutrients and water conductivity through xylem. The point of attack or the feeding pathway revealed clearly in infested papaya leaves (Fig. 8) and lysis of cells (Fig. 9). Deposition of tannin was observed in the palisade cells and cortex cells of midrib area of host plants (10). The hyperplasia condition of cells was distinctly observed in infested brinjal and hibiscus leaves. Deposition of tannins and accumulation of phenols was seen in infested tapioca and hibiscus leaves. There was not much change observed in palisade cells of mulberry and tapioca (Fig. 11 to 14).

Infestation initiated with periclinal and anticlinal divisions in the epidermis of leaf lamina and the hypodermal layer below it. Repeated divisions and an increase in the number of cells broke the epidermal layer. The cells formed were anticlinally elongated and oriented in radially arranged rows. The epidermal cells lose their tanniniferous content and undergo division. After oviposition the insect probably secreted a secretion to protect the egg. The larvae might be moved by piercing and feeding on the epidermal cells, thereby destroying them. The larvae that hatched from the eggs deposited on the leaflets might have developed into the plant tissue

and reached the centre of the midrib or settled down in the outer parenchymatous region. Following this, the cells surrounding the pathway show great alterations. Some of the cells become necrotic, while certain others above them show dense contents and yet others show degeneration along the pathway. Some of the cells show meristematic activity, which is probably due to the stimulus of wounding. This stimulus involves wound healing along the feeding path, hypoplasia of ground tissue and extensive hyperplasia of neighbouring cells (Fig. 8 to 14)

The investigations confirmed a considerable impact of the mealybug on all host plants at the anatomical level. Changes of leaf anatomy were conspicuously evident between insect infested and non-infested plants (Ahmed *et al.*, 2013; Albert *et al.*, 2011; Pecinar *et al.*, 2006; Kennedy, 1995). The observed anatomical deformations of leaves were the consequences of feeding activity of mealybug. Feeding of mealybug and the presence of dense mealybug population on the lower leaf surface initiated leaf curling and distortion and/or undulation of the leaf blade, thus provoking deformations of lower mesophyll tissues. In these grooves, the mesophyll cells became compromised and disorganized in various ways, leaving large intercellular spaces among them (Pecinar *et al.*, 2006). These findings were supported by Rancic (2003 and 2006), who reported the similar damages of mesophyll layers in feeding interaction of

Aceria anthocoptes with the leaves of *Cirsium arvense*. And Royalty and Perring (1996) reported such changes in mesophyll cells of citrus leaves infested with the mite *Phyllocoptruta oleivora*. The most striking anatomical change induced by mealybug feeding in infested plants, was the enlargement of epidermal cells, primarily on the lower leaf side. Similar alterations were reported by Mihajlovic (1996), that alterations were present in epidermal cells of lower leaf side of *Euphorbia seguierana* infested with *Aculops euphorbiae*. Thus, it was inferred that, there was alterations in leaf lamina and midrib after mealybug feeding. It is supported by Raman (2007), who reported that the insect activates a perturbation in growth mechanisms and alters the differentiation processes in the host plant, modifying plant architecture to its advantage.

Due to mealybug feeding, there was also changes like thickened epidermal cells, deeper vascular bundles (xylem & phloem), in tapioca and hibiscus. These plants poorly preferred by mealybug for feeding, due to some

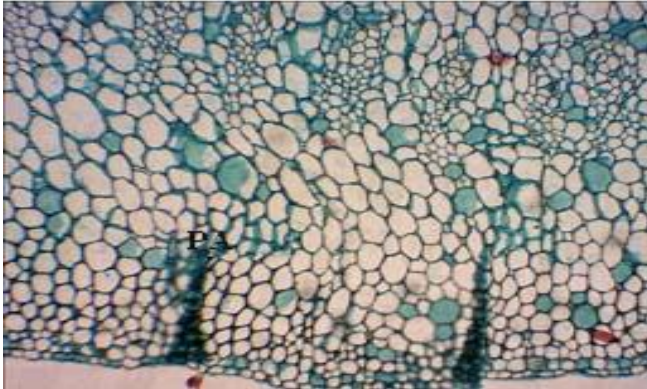


Fig. 8 : Cross section of infested midrib of papaya

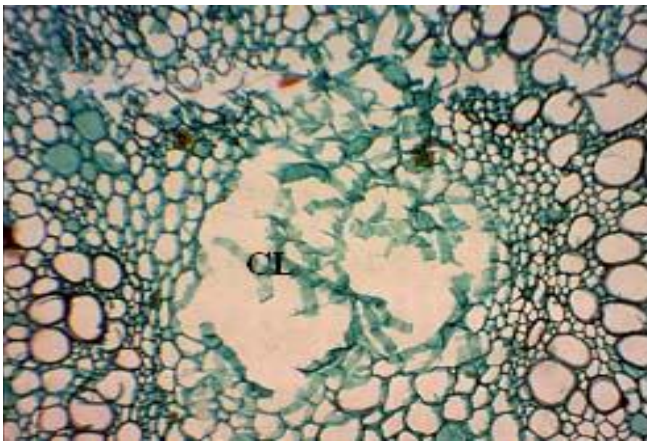


Fig. 9 : Cell lysis in midrib of cells

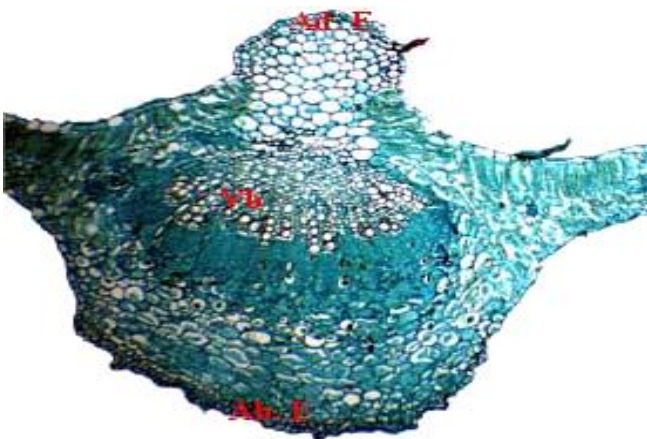


Fig. 10 : Cross section of infested midrib of cotton

*Ad. E - Adaxial epidermis, Ab.E - Abaxial epidermis, VB- Vascular bundles, PP - Palisade parenchyma, SP - Spongy parenchyma, Hp- Hyperplasia of cells, TD- Tannin deposit, PA- Phenol accumulation, PA- Point of attack, CL- Cell lysis, Vc- Vacuoles, AT - Accumulation of tannin, Pc- Periclinal division

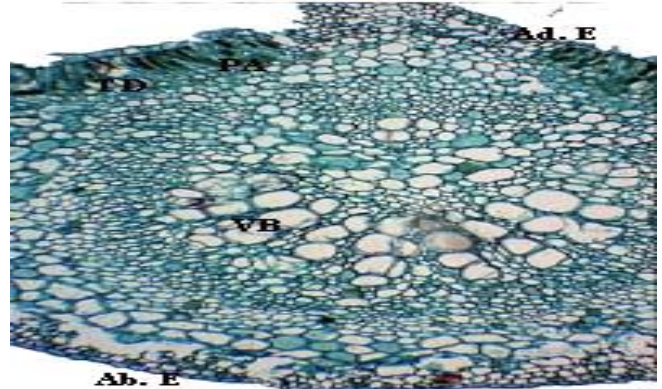


Fig. 11 : Cross section of infested midrib of tapioca

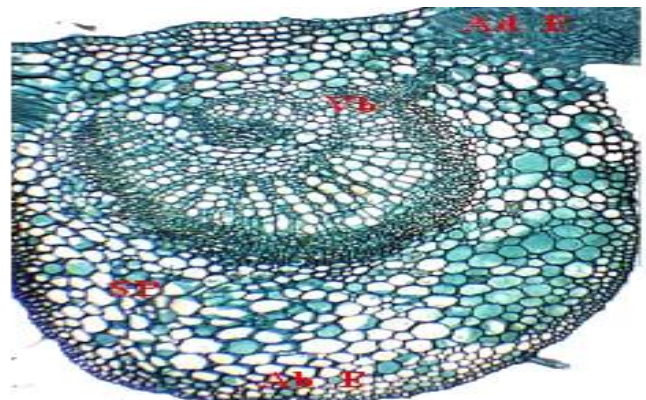


Fig. 12 : Cross section of infested midrib of mulberry

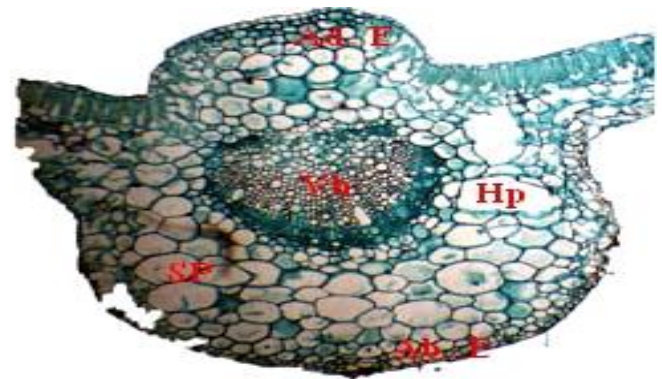


Fig. 13 : Cross section of mealybug infested midrib of Brinjal

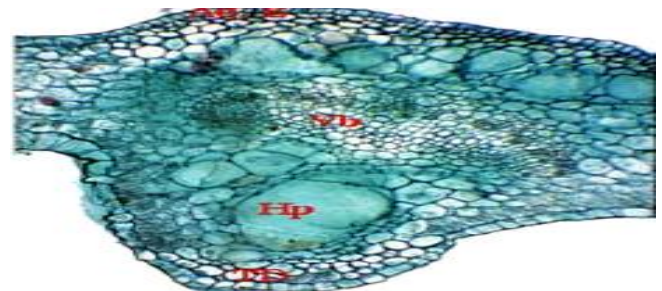


Fig. 14 : Cross section of mealybug infested midrib of Hibiscus

resistance mechanisms created by leaf tissues. This is supported by Reda Bakr *et al.* (2009) and Radwan (2003), who reported the changes in mango leaves infested by scale insects. Insects attack plant tissues resulting in the alteration of the subcellular environment and the placement of those tissues in a state of chemical shock. This shock evokes osmotic changes in the cells attacked. To neutralize the stress arising consequent to osmotic changes, aggravated by wounding, the plant responded by developing usually one, sometimes 2-3, metaplasied cells (Albert *et al.*, 2011). Metaplasia is the transition of one type of tissue into another type of tissue. Localized metabolic changes diffuse from the metaplasied cells, but not throughout either the involved plant organ (e.g. leaves) or the plant (Rohfritsch, 1992). The anatomical injury of infested plants, extended to epidermal cells on both leaf surfaces and to those of deeper mesophyll layers. This is in agreement with findings of Rancic *et al.* (2006), who reported the same anatomical injury induced by the eriophyid mite *Aceria anthocoptes* on the leaves of *Cirsium arvense*. The periclinal divisions were seen in the mealybug infested leaves of tapioca (heavy periclinal activity) and cotton. It is in line with the report of Albert *et al.* (2011), the periclinal divisions of the cells give rise to neoformed tissue due to insect infestation in leaves.

In the present study, it is revealed the cell lysis of papaya leaves might be due to salivary secretion in upper epidermis and palisade cells. This stimulus brought about intense hyperplasia and elongation of palisade parenchyma, which has undergone division by anticlinal division. The mealybug had four nymphal instars, from eclosion of the egg to the adult female, might have mechanisms that protect them from loss of humidity in leaves. It is supported by the report of Gullan and Martin (2003) and Raman (2007). Free feeding psyllids are more active during periods of higher humidity, while others produce wax as a strategy to avoid water loss (Arduin *et al.*, 2005). This condition of conspicuous palisade parenchyma proliferation, cell hyperplasia and the division of the spongy parenchyma lead to smaller intercellular spaces (Arduin and Kraus, 2001), in *Piptadenia gonoacantha* (Mart.) and *Guarea macrophylla* subsp. *tuberculata* (Vell.).

In the present study, salivary path and lysis of cells were observed in infested papaya leaves. Albert *et al.* (2011), reported the cell lysis in *Alstonia scholaris* induced by the insect *Paurosylla tuberculata*

(*Psyllidae*). Hemipteran insects exhibits a sucking feeding habit. These insects introduce stylets into the tissue and, they take some time exploring the tissue and injecting viscose substances forming a salivary sheath (Arduin *et al.* 2005). This salivary sheath is considered to facilitate the penetration of the insect proboscis or prevent the plant unleashing hypersensitivity reactions to combat the damage inflicted upon it (Fernandes, 1990 and Milles, 1999). Raman (2007) considered the penetration to be intercellular with the dissolution of middle lamella of the adjacent cells, though Spiller *et al.* (1985) and Tjallingh and Hogen Esch (1993) revealed that this always was not the case, because the stylet can penetrate between the wall and the plasmatic membrane and may even display an intracellular path.

The shrinkage of vascular bundles was observed due to the mealybug infestation in the midrib. The insects are known to extract nutrients from the phloem, xylem or non-conducting plant cell (Meyer, 1987). This might be the reason for shrinkage of xylem and phloem cells. The neoformed parenchyma resulted from hypertrophy and hyperplasia of the spongy parenchyma cells initially but later the palisade also undergone hyperplasia condition. Therefore the spongy parenchyma cells responded more rapidly to the insect stimulus than the preferred sites of insect attack which is at the abaxial surface of the leaflet, so the cecidogenic field is nearer to the spongy parenchyma. This is supported by Ahmed *et al.* (2013), who reported hypersensitive reaction and anatomical changes of young tea leaf *Camellia sinensis* during feeding by tea mosquito bug *Helopeltis theivora*.

These findings of the changes in leaf anatomy due to mealybug feeding are in agreement with some earlier reports. New leaves may become larger due to increase in cell size via mesophyll elongation (Kolodny-Hirsch and Harrison (1986) and Satoh *et al.* (1997)) or increased cell division (Morton and Watson, 1948) expansion may be influenced by chloroplast enlargement due to starch accumulation or via increased turgor pressure (Milthorpe and Moorby, 1979). Turgor pressure provides the force for cell expansion, which declines if the leaf water content falls below 90%, and ceases entirely below 70%. Thus, partial defoliation during periods of water stress may improve the water potential of remaining foliage and result in cell expansion (Trumble *et al.*, 1993).

Biochemical results in the present study, showed variations in the content due to mealybug infestation in leaves. The reduction in the chlorophyll content of infested

leaves was observed. This might be due to the loss of palisade tissues, disappearance of chloroplast and modifications of spongy mesophyll. This loss of chlorophyll is responsible for the decolourisation of the area of the leaf where egg was laid in *Ficus* leaves (Moghe, 1980). Present domestication of biochemical changes, revealed higher concentration of total carbohydrate and sugars in the papaya and cotton leaves. Higher amount of carbohydrates present in the leaves might also lead to paralysis of cells. This is supported by Laila (2002), who reported the larger amount of glutamine amino acids can cause paralysis in cells of leaves.

In the present study, a reduction in the protein content of infested leaves was noticed. But in tapioca and hibiscus, mealybug lead to minimum per cent reduction of protein was noticed than other host plants. It was confirmed from earlier findings that, plants that were poorly preferred by mealybugs. This had marginal level protein concentration observed in the infested leaf tissues and it corroborates the observations of Mehalingam (2012) and Scareli-Santos & Varanda (2003). It is also in line with Arora & Patni (2001) and El-Akkad (2004). Synthesis of diverse plant proteins are believed to be of importance in defence is also known (Reinbothe *et al.*, 1994). Defensive proteins that block the action of proteolytic enzymes from herbivores are found in legumes, tomatoes and other plants. These proteins, known as proteinase inhibitors, rapidly seem to accumulate throughout plants that are being fed upon by insects and even accumulate in undamaged areas of plants that are far from the initial feeding site (Ananthkrishnan, 2001). So it can be concluded that when plants are attacked by insects, they generate signals and one of these signals is the initiator of expression of certain polypeptides that may be useful in providing the basis for new crop protection strategies.

An increase of phenol and tannin content in the mealybug infested leaves occurred compared to the uninfested leaves. Induction of phenol and tannin indicates that this has been produced due to the stress. They were produced as a defence mechanism to protect from invaders (biotic stress) or stress factors (abiotic stress) and are believed to be an adaptive response to the altered conditions. It is supported by the report of El-Akkad (2004). This also might be the reason for poor preference of tapioca and hibiscus, which had the higher

accumulation of phenol and tannin content in their leaves, while feeding by mealybug. According to Cornell (1983), presence of phenolics indicates a chemical defence mechanism because they are thought to inhibit the feeding of herbivorous insects. Phenolics are a large class of secondary metabolites produced by plants to defend themselves against pathogens and insects (Lattanzio *et al.*, 2006).

Ahmed *et al.* (2013) reported the discolouration of the cells and formation of a dark brown coloured halo around the dead cells in a lesion indicates the role of an inducible defence mechanism with possible involvement of oxidation of phenolic compounds through polyphenol oxidase (PPO) enzymes, and these compounds are normally found in tea leaves may reveal the actual mechanism of host resistance in *Helopeltis theivora* herbivory. Likewise, this might be the reason for resistance showed by tapioca and hibiscus leaves to mealybug, since they were found to be the poorly preferred host plants in the current study. These alterations reveal the plasticity of plant tissues, which may be controlled by insects. According to Taf and Bissing (1988), presence of the inducer leads to a rupture of the cellular process of the host, and also results in an active redirection of the existing ontogenetic patterns, to the benefit of the inducer. Schonrogge *et al.* (2000) detected similar proteins in seeds and in gall tissue suggesting gall formation might involve the ectopic expressions of genes of other structures of the host plant. However it confirms with concept put forward by Arduin *et al.* (2005), *i.e.* pathological tissues reveal a potential not manifested in healthy plant tissue.

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REFERENCES

- Ahmed P, Prasenjit Bhagawati, Kr. Saurabh Das, M.C. Kalita and D. Sudripta. 2013. Hypersensitive reaction and anatomical changes of young tea leaf (*Camellia sinensis*, clone TV1) during feeding by tea mosquito bug (*Helopeltis theivora* Waterhouse: Hemiptera: Miridae). *Int.J.Curr.Microbiol.App.Sci.*, 2(8): 187-195.
- Albert, S. A. Padhiar, D. Gandhi and P. Nityanand. 2011. Morphological, anatomical and biochemical studies on the foliar galls of *Alstonia scholaris* (Apocynaceae). *Revista Brasil. Bot.*, 34(3): 343-358.

- Albert, S. A. Padhiar, D. Gandhi and P. Nityanand. 2011. Morphological, anatomical and biochemical studies on the foliar galls of *Alstonia scholaris* (Apocynaceae). *Revista Brasil. Bot.*, **34**(3): 343-358.
- Amarasekare, K. G., C. M. Mannion., L. S. Osborne and N. D. Epsky. 2008. Life history of *Paracoccus marginatus* (Hemiptera: Pseudococcidae) on four host plant species under laboratory conditions. *Environ. Entomol.*, **37**: 630–635.
- Ananthkrishnan, T.N. 2001. Phytochemical defence profiles in insect–plant interactions. *In: Insects and plant defence dynamics* (T.N. Ananthkrishnan, ed.). Science Publishers, Enfield, p.1-21.
- Ananthkrishnan, Ô. Í. 1990. Facets of chemical ecology in insect–plant interactions:
An overview; *Proc. Indian Acad. Sci., (Anim. Sci.)* **99**: 177–183.
- Arduin, M. and J. E. Kraus. 2001. Anatomia de galhas de ambrosia em folhas de *Baccharis concinna* e *Baccharis dracunculifolia* (Asteraceae). *Rev. Bras. Bot.*, **24**:63-72.
- Arduin, M., G.W. Fernandes and J.E. Kraus. 2005. Morphogenesis of gall induced by *Baccharopelma dracunculifoliae* (Hemiptera: Psyllidae) on *Baccharis dracunculifolia* (Asteraceae) leaves. *Braz. J. Biol.*, **65**:559-571.
- Arora, D. K. and V. Patni. 2001. Localization of metabolites and enzymes in insect induced rachis gall and normal tissues of *Prosopis cineraria* (Linn.) Druce. *J. Phytol. Res.*, **14**:179-181.
- Baby Rapheal, N.V. 1991. Screening of sorghum entries for resistance to mite – *Oligonychus indicus* Hirst. (Unpublished) M.Sc., Thesis, Tamil Nadu Agric. Univ., Coimbatore (Tamil Nadu: India).112p
- Cornell, H. V. 1983. The secondary chemistry and complex morphology of galls formed by the Cynipinae (Hymenoptera): why and how?. *Am. Midland Naturalist*, **110**:225–234.
- El-Akkad, S.S. 2004. Biochemical changes induced in *Populus nigra* leaves by galling aphids *Pemphigus populi*. *Int. J. Agric. Biol.*, **6**:659-664.
- Fernandes, G.W. 1990. Hypersensitivity: A neglected plant resistance mechanism against insect herbivores. *Environ. Entomol.*, **19**:1173-1182.
- Goodman, R. N. 1986. Cell-wall composition and metabolism. *In: The Biochemistry and Physiology of Plant disease*, R. N. Goodman (Ed.), University of Missouri Press, Columbia, USA, pp. 105-149.
- Gopalan, M., N. C. Radja and G. Balasubramanian. 1987. Biochemical changes in rice plants infested with mealybug. *International Rice Research Newsletter (Philippines)*. **12**(4): 45.
- Gullan, P.J. and J.H. Martin. 2003. Sternorrhyncha (jumping plant-lice, whiteflies, aphids and scale insects). *In: Encyclopedia of insects*. V. Resh & R. Cardé, (Eds.). Academic Press, Amsterdam p.1079-1089.
- *Hegde, J.E. and B.T. Hofreiter. 1962. *In: Methods in Carbohydrate Chemistry*. R.L. Whistler and J.N. Be Miller (eds). Academic Press, New York. **17**.
- Janaki, I. 2010. Varietal resistance and management of papaya mealybug in brinjal. Tamil Nadu Agric. Univ., Coimbatore. (Tamil Nadu: India).160p.
- Jayaraj, S. and S. Uthamaswamy. 1990. Aspects of insect resistance in crop plants: *Proc. Indian Acad. Sci. (Anim. Sci.)*, **99**: 211–224.
- Jensen, W. A. 1962. Botanical histochemistry. W. H. Freeman and Co., San Fransisco, U.S.A., 408 PP.
- Johansen, D.A. 1940. Plant microtechnique. McGraw- Hill Publications, New York, U.S.A., pp. 523.
- Kalode, M. B. and N. C. Pant. 1967. Studies on the amino acids, nitrogen and moisture content of maize and sorghum varieties and their relation to *Chilo zonellus* (Swin) resistance. *Indian J. Entomol.*, **29**(2): 139-144.
- Kennedy, J. S.1995. Functional ecology of false spider mite, *Brevipalpus Phoenixicus* (Geijskes). D.Sc. Thesis, University Catholique De Louvain. Louvain-la-Neuve. 406p.
- Kolodny-Hirsch, D. M., J. A. Saunders and F.P. Harrison. 1986. Effects of simulated tobacco hornworm (Lcpidoptera: Sphingidae) defoliation on growth dynamics and physiology of tobacco as evidence of plant tolerance to leaf consumption. *Environ. Entomol.*, **15**: 1137-44.
- Laila, H. S. 2002. Toxicological and biochemical studies on the effect of Admiral (IGR) and nuclear polyhedrosis virus (SNPV) on *Spodoptera littoralis* (Bois D.) larva. *J. Egypt Acad. Soc. Environ. Develop. (A. Entomology)*, **2**(1):15-29.
- Lattanzio V., Lattanzio V.M.T., Cardinali A., 2006. Role of phenolics in the resistance mechanisms of plant against fungal pathogens and insects. *Phytochemistry: Advances in Research*. Kerala, India, Research Signpost: 23–67.
- Lowry, O. H., N. T. R. Brough, L. A. Fair and R. J. Randall. 1951. Protein measurement with folin phenol reagent. *J. Biol. Chem.*, **193**: 265-275.
- Malik, P. and M.B. Singh. 1980. Extaction and estimation of total phenols. *In: Plant enzymology and histo-enzymology*. Kalyani Publishers, New Delhi, 286p.
- Mehalingam, P. 2012. Morphological and Anatomical Studies of the Ovary Galls of *Sesamum indicum* l. Induced by the Gall

- Midge, *Asphondylia sesami* Felt.
J. Ornamental. Hort. Plants, **2** (3): 191-200.
- Meyer, J. 1987. Plant galls and gall inducers. Gerbruder Borntraeger, Berlin.
- Mihajlović, N. 1996. Morfo-anatomske promene kod *Euphorbia seguireana* ssp. *seguirana* (Neck.) nastale pod uticajem grinje *Vasates euphorbiae* (Pet.), Biološki fakultet, Beograd.
- Miller, D. R., D. J. Williams and A. B. Hamon. 1999. Notes on a new mealybug (Hemiptera: Coccoidea: Pseudococcidae) pest in Florida and the Caribbean: the papaya mealybug, *Paracoccus marginatus* Williams and Granara de Willink. *Insecta Mundi*, **13**: 179-181.
- Milles, P.W. 1999. Aphid saliva. *Biol. Rev.*, **74**:41-85.
- Milthorpe, F. L. and J. Moorby. 1979. *An Introduction to Crop Physiology*. Cambridge: Cambridge Univ. Press. 2nd ed.
- *Moghe, M. 1980. Studies on the insect gall of *Ficus racemosa* Linn. Ph.D. Thesis, The Maharaja Sayajirao University, Vadodara (Gujarat: India).
- Mollema, C. and R. A. Cole 1996. Low aromatic amino acid concentrations in leaf proteins determine resistance to *Frankliniella occidentalis* in four vegetable crops. *Entomol. Exp. Appl.*, **78**(3): 325-333.
- Moore, S. and W.H. Stein. 1948. Methods in Enzymol. (Eds.) Colowick, S.P. and N.D. Kalpan. Academic press, New York. 468p.
- Morton, A. G. and D. J. Watson. 1948. A physiological study of leaf growth. *Ann. Bot. New Ser.*, **12**:22-52.
- *Murugesh, T. 1997. Ecology and Management of shoot and fruit borer, *Leucinodes orbonalis* Guen. and spotted leaf beetle, *Henosepilachna vigintioctopunctata* (Fabricius) of brinjal. M.Sc. (Ag.) Thesis, Tamil Nadu Agric. Univ., Killikulam. (Tamil Nadu: India).
- Oberbacher, M.F. and H.M. Vines. 1963. Response of oxidation and phosphorylation in *Citrus* mitochondria to arsenate. *Nature*, 206: 19-320.
- Palanisamy, S. 1984. Ecology and host resistance of carmine spider mite, *Tetranychus cinnabarinus* (Boisduval) in brinjal. Ph.D., Thesis, Tamil Nadu Agric. Univ., Coimbatore. (Tamil Nadu: India). 148p.
- Pečarić, I., B. Stevanović, B. G. Rector and Radmila Petanović. 2006. Anatomical injuries caused by *leipotrix dipsacivagus* petanovic & rector on cut-leaf teasel, *Dipsacus laciniatus* L. (Dipsacaceae). IV Balkan Botanical Congress, Sofia. Book of Abstracts, 1 139.
- *Radwan S.A. 2003. Toxicological studies on some scale insects infested mango and guava trees. Ph. D., Thesis. Faculty of Agriculture. Cairo University.
- Raman, A. 2007. Insect-induced plant galls of India: unresolved questions. *Curr. Sci.*, **92**:748-757.
- Ranèić, D. 2003. Morpho-anatomical alterations of vegetative and reproductive organs of the plant species *Cirsium arvense* (L.) Scop. caused by the eriophid mite *Aceria anthocoptes* (Nal.). M. Sc., Thesis. Faculty of Biology, University of Belgrade (in Serbian).
- Ranèić, D., B. Stevanović and R. Petanović. 2006. Anatomical injury induced by the eriophid mite *Aceria anthocoptes* on the leaves of *Cirsium arvense*. *Exp. Appl. Acarol.*, **38**: 243-253.
- Reda Bakr, F. A., R. M. Badawy, S. F. Mousa, L.S. Hamooda and Sahar A. Atteia. 2009. Ecological and taxonomic studies on the scale insects that infest mango trees at Qaliobiya governorate. *Egypt. Acad. J. biolog. Sci.*, **2**(2): 69- 89.
- Reinbothe, S., R. Mollenbauer, R. and C. Reinbothe. 1994. JIPs and RIPs: the regulations of plant gene expression by jasmonates in response to environmental cues and pathogens. *Plant Cell*, **6**:1197-1209.
- Rockstein, Ā. 1978. Biochemistry of insects, New York: Academic Press.
- Rohfritsch, O. 1992. Patterns in gall development. In: Biology of insect induced galls J.D. Shorthouse & O. Rohfritsch, (Eds.). Oxford University Press, New York, pp.60-86.
- Royalty, R.N. and T. M. Perring. 1988. Morphological analysis of damage to tomato leaflets by tomato russet mite (Acari: Eriophyidae). *J. Econ. Entomol.*, **81** (3): 816-820.
- Sadasivam, S. and B. Thayumanavan. 2003. *Molecular Host Plant Resistance to Pests*. Marcel Dekker, New York. p. 479.
- Satoh, M., P. E. Kfiedeman and B.R. Loveys. 1977. Changes in photosynthetic activity and related processes following decapitations in mulberry trees. *Physiol. Plant.* **41**:203-10.
- Scareli-Santos, C. and E.M. Varanda. 2003. Morphological and histochemical study of leaf galls of *Tabebuia ochracea* (Cham.) Standl. (Bignoniaceae). *Phytomorphology*, **53**:207-217.
- Schonrogge, K., P. Walker, P. and M. J. Crawley. 2000. Parasitoid and inquiline attack in the gall of four alien, cynipid gall wasps: host switches and the effect on parasitoid sex ratios. *Ecol. Entomol.*, **25**: 208-219.
- Serrano, M. S. and S. L. Laponite. 2002. Evaluation of host plants and a meridic diet for rearing *Maconellicoccus hirsutus* (Hemiptera: Pseudococcidae) and its parasitoid *Anagyrus kamali* (Hymenoptera: Encyrtidae). *Florida Entomol.*, **85**: 417-425.

- Somogyi, N. 1952. Determination of reducing sugars. *J. Biol. Chem.*, **200**: 245-252.
- Soundararajan, R. and P. Baskaran. 2001. Mechanisms of resistance in brinjal (*Solanum melongena* L.) to whitefly (*Bemisia tabaci* Gen.). *Madras Agric. J.*, **88**: 657-659.
- Spiller, N. J., F. M. Kimmins and M. Llewellyn. 1985. Fine structure of aphid stylet pathways and its use in host plant resistance studies. *Entomol. Experiment. Appl.*, **38**:293-295.
- Taf, J.B. and D.R. Bissing. 1988. Developmental anatomy of the horned oak gall induced by *Callirhytis cornigera* on *Quercus palustris* (pin oak). *Am. J. Botany*, **75**:26-36.
- Tjallingh, W.F. and T. Hogen-Esch. 1993. Fine structure of aphid stylet routes in plant tissues in correlation with EPG signals. *Physiol. Entomol.*, **18**: 317-328.
- Trumble J. T. 1993. Plant Compensation For Arthropod Herbivory. *Annu. Rev. Entomol.*, **38**:93-119.