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Table: 1, Figs : 6

Interactions between herbivore *Leucinodes orbonalis* G. and its host plant *Solanum melongena* L.: A study on insect induced direct plant responses

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ABSTRACT

In laboratory assays, we studied the directly induced defence mechanisms and plant pest relations between the host eggplant (*Solanum melongena* L.) and its specific pest *Leucinodes orbonalis* G. The plant induced nutritional changes, quantitative differences in anti-oxidative enzymes and secondary metabolites were analysed in the pest attacked plants and compared with normal (non-attacked) eggplants. The nutritional qualities (carbohydrate, protein and amino acid content) of the leaves of fruit infested eggplants increased significantly than normal leaves. The chlorophyll content of leaves increased in the infested plants. The increased production of reactive oxygen species [(ROS), indicator of activation of plant defences] further increased the lipid peroxidation. The total phenols, total flavonoids and phenylalanine ammonia lyase (PAL) activities were significantly enhanced in infested *S. melongena* plants. The phenols in infested leaves were quantified and their role in plant resistance was observed. Gallic acid, caffeic acid, hydroxybenzoic acid, chlorobenzoic acid, epicatechin and vanillic acid contents increased, but chlorogenic acid content was decreased. Along with these phenols, 8 unidentified phenols were also found. Amount of total antioxidants (measured by the scavenging capacity of leaf extract) was considerably increased in infested plants than normal plants. Additionally, activities of oxidative enzymes (catalase and peroxidase) were strikingly induced in *L. orbonalis* infested eggplants. We demonstrated that the oxidative damage due to pest feeding elevated the levels of biochemicals, phenolic acids, and enzymes which may play major role in plant defence.

Keywords: Egg plant, herbivory, *Leucinodes orbonalis*, lipid peroxidation, oxidative enzymes, phenols, ROS, *Solanum melongena* .

INTRODUCTION

Insect - plant interactions are always the subject of interest to researchers Worldwide. Plants upon attack by leaf feeding insects, sucking pests, pathogenic microbes or even stem borers induce their defense systems to avoid the damage which results in accumulation of chemicals (25, 56). These defense mechanisms include constitutive defences (trichomes, cuticle, secondary cell walls and toxic metabolites) and directly induced defenses [foliar enzymes (PPO, SOD, CAT, PAL and POD) secondary metabolites: alkaloids, phenols, tannins, flavonoids (28)]. Indirectly induced defences

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against insects are mediated by the release of a blend of volatiles that specifically attract natural enemies of herbivores and/or by providing food (e.g., extra floral nectar) and housing to enhance the effectiveness of the natural enemies (6). Understanding the host plant resistance, plays major role in insect pest management, resulting in reduced losses due to herbivores, less insecticide use, better crop yields and safer environment (62).

Feeding stress caused by insects result in the physiological, nutritive and oxidative changes in plants. Such differences in various chemicals or enzymes in the pest damaged plants are important to determine the susceptibility of the plant to the insect attack. Hence it is necessary, to investigate the differences in biochemical and nutrient constituents in plants and study the effects of herbivore attack on these chemicals (58,34). Primary metabolites (involved in fundamental plant physiological processes) are rarely considered the major determinants of host-plant resistance but they elicit many signaling pathways upon insect herbivore attack (67). Plant's primary metabolites, proteins and their constituent amino acids are the nutrients, most influenced by insect herbivory as the nutritional barrier between these two species is responsible for utilizing plants as food (31). Carbohydrates and proteins are used by the herbivores for their growth and development (4). Secondary metabolites have been studied as the mediators of direct defense, however, there is also need to reveal the unidentified or emerging signaling pathways. After the induction by herbivores, the phenols and flavonoids get accumulated in large amounts in various tissues to reduce the pest's further feeding by acting as feeding deterrents and antioxidants and thus are responsible for effective dir ect defences (37).

One of the immediate responses triggered upon herbivory is the burst of reactive oxygen species (ROS). This leads to the release of harmful unstable intermediates of lipid peroxidation namely hydrogen peroxide (H_2O_2), hydroxyl radical (OH^-) and superoxide radical (O^{2-}) (41). H_2O_2 molecules are associated with systemic acquired resistance and OH^- ions lead to the induction of injurious mechanisms namely lipid peroxidation, DNA and protein oxidation (16). Oxidative enzymes (catalases, peroxidases) and anti-nutritive enzymes (polyphenol oxidases and lipoxygenases) act as effective defences against insect herbivores reduce the ROS amounts (21). Hence these can be studied as biochemical markers to study the induced responses caused by herbivory. The herbivory attack leads to accumulation of malondialdehyde (MDA), the main product of lipid peroxidation. Thus the amount of increased MDA can be used to measure the membrane damage caused by insect feeding (39). Phenolics and flavonoids being antioxidant in nature have potential to reduce the ROS, ultimately reducing the lipid oxidation. Thus the amount of total antioxidants is a reliable measure to determine the scavenging capacity of plants against the harmful effects of ROS.

Solanum melongena L. (Solanaceae) is most common vegetable crop in India. This plant is one of top ten foods able to absorb the oxygen radicals due to high contents of fruit phenolic constituents (14). Brinjal fruit and shoot borer (BFSB) *Leucinodes orbonalis* causes economic losses upto 50-70% and affects the crop from early seedling transplanting stage till fruits harvest. Thus a detailed study of the induced defences in the host plant was required to understand the role of induced chemicals that help in combating the pests. Exploitation and manipulation of these chemical pathways can help in developing varieties resistant to this pest. Farmers indiscriminately spray many poisonous pesticides to control this pest and one promising alternative to this is, by increasing the resistance of plants for better management of pests. This study aimed to (i). Determine the

oxidative potential of phenols produced systemically in Eggplant leaves in combating the oxidative damages induced by the feeding of specialist pest *Leucinodes orbonalis* (G.) (Lepidoptera: Pyralidae), (ii). Evaluate the induced defense mechanisms in form of biochemical and enzymatic changes and (iii). Total oxidative status in *L. orbonalis* infested brinjal (egg plant) leaves and to correlate with the intact undamaged plants.

MATERIALS AND METHODS

The seedlings of eggplant variety-'Gulabi' were collected from the Nursery, Vegetable Section, Acharya N. G. Ranga Agricultural University (ANGRAU), Rajendra Nagar, Hyderabad. This study was conducted in year 2013 during August, September and October months when the growth of plants and insects was optimum. The healthy seedlings were planted in plastic trays (20 x 40 cm) and after 2 weeks, the seedlings of 2-3 leaf stage were removed and planted in earthen clay pots (18 cm x15 cm dia). No botanical/chemical pesticide was applied on the plants. Plants were maintained in green house (photoperiod 12:12 (L: D), temperature 28 ± 2 ºC and relative humidity 65 ± 5 %). Each experiment consisted of 5 plants per replicate and a total of 12 replicates per treatment.

I. INSECT REARING

Leucinodes orbonalis (G.) (Pyralidae: Lepidoptera) is serious pest of *S. melongena* in India. They are small moths and active fliers. Each female lay about 150-200 eggs in its life time. *L. orbonalis* larvae were segregated from the infested fruits and were reared on eggplant fruits till pupation under laboratory conditions (16 L: 8D, 28 ± 2^0 C and 65±5% RH). Pupae were collected in Petri dishes and placed the nylon mesh cages (45×45×45 cm) having soil at the bottom. Adults were reared in glass cylinder (30 x 15 cm) lined inside with a layer of purple colour coarse paper. A 4-weeks old eggplant was placed inside the cylinder and the lower end of the cylinder was closed by a Petri plate. For oviposition, 2-3 pairs of freshly emerged adults were released into the cylinder. A cotton swab dipped in 10% honey solution served as food for adults. The top of cylinder was covered with nylon net cloth held in place with a rubber band. The set-ups were checked after 4-6 days for the eggs laid on paper and lower parts of eggplant leaves.

II. PEST FEEDING

Healthy and well established eggplants having minimum of 5-fruits per plant (120-140 d old) were separated from the rest. Third instar *L. orbonalis* larvae were released on the fruits at single larva per fruit and five larvae per plant. The larvae settled on the fruits within 5-10 min after their release and bore holes into fruits, where they started feeding. These plants were termed as treated plants. The insects were allowed to feed for 3 days, which result in symptoms like withering of stem and leaves. The infested leaves were collected and used for further analysis of biochemicals. The normal healthy un-infested eggplants were termed as control plants.

III. CHEMICALS

Standard enzymes, thio barbituric acid, quercetin, gallic Acid, 1, 1-Diphenyl-2-picrylhydrazyl radical (DPPH) were purchased from Sigma- Aldrich Chemicals Pvt. Ltd.,

India. Phenolic acids such as vanillic (4-hydroxy-3-methoxybenzoic acid), *p*-hydroxybenzoic, epicatechin, chlorogenic acid, chlorobenzoic acid, caffeic (3, 4-dihydroxy-cinnamic acid) and coumaric acids were purchased from Sigma Aldrich (purity~99%) and were used as standards for comparison. The solvents were of HPLC grade and the reagents used for estimations were of 99% purity (Merck chemicals, Darmstadt, Germany). Water used was treated in a Milli-Q water purification system (Millipore, Bedford, MA).

IV. CHLOROPHYLL CONTENT

The chlorophyll content of eggplant leaves was determined as per the method of Knudson *et al.* (36). Fresh eggplant leaf (0.5 g) was kept in dark with 2 ml of 95 % ethanol for 24 h and the extracted solution was analysed. The amounts of chlorophyll a and b were determined spectrophotometrically (Molecular Devices- SpectraMax M3), by reading the absorbance at 665 and 649 nm. The chlorophyll content was calculated as per the formulae given by Knudson *et al.* (36) and concentrations were expressed as unit's µg per gram-fresh weight (µg g^{-1} FW).

V. BIOCHEMICAL ESTIMATIONS OF PRIMARY METABOLITES

To analyze the biochemical and nutritional changes in leaves from the pest infested *S. melongena* plants, the following methods were employed. The total concentrations of amino acids were estimated by Moore and Stein (43), total carbohydrates by Dubois *et al.* (20), cell bound phenolics by Hori (32), proteins by Lowry *et al.* (40) and total flavonoids by Nabavi *et al.* (45) with slight modifications. The flavonoids are described as quercetin equivalents/ g FW and total phenols as gallic acid equivalents/ g FW.

VI. LIPID PEROXIDATION

Lipid peroxidation was determined by measuring malondialdehyde (MDA), a major thiobarbituric acid reactive species (TBARS) and the main product of lipid peroxidation (30). Fresh samples (0.2 g) were ground in 3 ml of trichloroacetic acid (0.1 %, w/v). The homogenate was centrifuged at 10,000 x g for 10 min and 1ml of the supernatant fraction was mixed with 4 ml of 0.5 % thiobarbituric acid (TBA) in 20 % TCA. The mixture was heated at 95^0 C for 30 min, chilled on ice and then centrifuged at 10,000 x g for 5 min. The absorbance of the supernatant was measured at 532 nm on a Molecular devices- MicroMax M3 Multimode reader. The value for non-specific absorption at 600 nm was subtracted. The amount of MDA was calculated using the extinction coefficient of 155 mM^{-1} cm^{-1} and expressed as n mol g^{-1} F.

VII. DPPH RADICAL SCAVENGING ACTIVITY

The free radical scavenging capacity of the extracts was determined as per method of Hasan *et al.* (29) using DPPH free radical. Freshly prepared DPPH solution (0.004% w/v in 95% methanol) was taken in test tubes and *S. melongena* extracts were added followed by serial dilutions (100 µg to 1000 µg) to each test tube so that the final volume was 2 mL. After 15 min incubation in dark condition, the absorbance (A_{15}) was read at 515 nm (SpectraMax M_3 Multimode reader, Molecular devices, USA). Quercetin (1 mg/mL in methanol) was used as a reference standard. Controls (A_0) were prepared containing the same volume of DPPH without any extract. % scavenging of the DPPH free radical was measured using the following equation: absorbance of the control (A_0) minus absorbance of the test sample (A_{15}) divided by absorbance of the control (A_0) multiplied by 100. Experiments were conducted in triplicate.

IC_{50} values were obtained by probit analysis (60). IC_{50} values denote the concentration of sample, which is required to scavenge 50% of DPPH free radicals. 95% methanol was used as blank.

VIII. INDIVIDUAL PHENOLIC COMPOUNDS BY HPLC

The identification and quantification of specific phenolic compounds in the plant samples were carried out by using the Gilson GX-271 semi-preparative HPLC system. The plant sample extraction was done as per the method of Usha Rani and Jyothsna (59) with minor modifications. 1g leaf was extracted in 95 % methanol (20 ml) by continuous shaking for 3 days. Later, the sample was filtered and evaporated using a rotary evaporator (Heidolph Instruments, Germany). For dried sample, 1 ml of HPLC-grade methanol was added and filtered using 0.2-µm membrane. This filtered sample was further injected into the HPLC column (C18 2.5 x 30 cm). The separation of phenolic compounds was done at room temperature (28 ± 1 ^{0}C). The mobile phase consisted of solvent A with TFA in deionized water (pH 2.5) and absolute methanol as solvent B. The conditions used were 0-50 % solvent B (0-20 min), 50-60 % solvent B (20-30 min) and 60-0 % solvent B (30-40 min). The flow rate of mobile phase was set at 1.0 mL/min, with 20 µl sample injection into the column. The monitoring of phenolic compounds was carried out at 280 nm (63) and peak area was used for quantification using external standard calibration curves. Identification of the unknown phenolics was based on matching their retention times with those of pure standards of phenolics and the amounts were expressed as µg/g sample.

IX. BIOCHEMICAL ESTIMATIONS OF ENZYME ASSAYS

To measure all enzymes activities, the leaf materials from the plants infested with pests and the normal plants were collected and 1 g leaf material was homogenized using suitable buffer. The POD, CAT, SOD, PAL and PPO activities were measured using Kar and Mishra (35), Aebi (1), Beyer and Fridovich (9), Dickerson *et al.* (18) and Thaler *et al.* (55) methods respectively.

XI. STATISTICAL ANALYSIS

Quantitative differences in biochemical and enzymatic changes of *L. orbonalis* infested and normal *S. melongena* plants were measured and compared using t-test. All the data were presented as mean ± SE. The statistical analysis was performed and the figures were plotted using the software Origin (Ver. 8.0).

RESULTS AND DISCUSSION

Plant nutritional and physiological variances

In the current study, we examined herbivore induced damage on eggplants and its subsequent effects on the plant biochemical, oxidative and enzymatic changes in the form of quantitative changes. As reviewed from many reports by Schwachtje and Baldwin (50), hundreds of genes upregulated during the plant-herbivore interactions have been analyzed with help of microarray studies and almost all aspects of metabolism were represented, with a significant part coming from primary metabolism. The increase in chlorophyll pigments as well as primary metabolites perhaps is to compensate the nutrient losses that could have incurred in

the fruits due to the insect feeding. There was significant physiological change in terms of chlorophyll contents, where the amounts of chlorophyll pigments a and b were synthesized in much higher amounts in *L. orbonali*s infested eggplant leaves (102.06 µg/ g FW) compared to the uninfested plant. Increased photosynthetic rates could also be due to the changes that occurred in source-sink relationships which resulted from the imposition of increased demand for energy and carbon (C)-based resources for the production of defensive compounds (~~7~~8).

Enhanced biochemical content in form of total aminoacids, total carbohydrates and total proteins was found in eggplants infested with *L. orbonalis*. In this study amino acid concentration increased from 28.189 µg/ g FW in normal plants to 45.78 µg/ g FW in infested plants (Fig. 1).. In normal *S. melongena* plants the concentrations of proteins and carbohydrates were 668.72 and 65.05 µg/ g FW which rose significantly to 1254.46 and 326.03 µg/ g FW, respectively, in infested plants ($p < 0.001$). The effects of variability in the amino acid and protein concentrations have already been well established in many works (13). Weibull (61) demonstrated that *Hordeum vulgare* resistance to *Rhopalosiphum padi* is related with variation in content of glutamic acid in sense that low glutamic acid levels are associated with reduced aphid weights. An increase in the protein content in aphid infested *Brassica juncea* and *Spodoptera litura* feeding on *Ipomea batata* plants was observed in earlier reports (52, 48). Jyothsna *et al.* (34) also reported the increased levels of carbohydrate concentrations in *Ricinus communis* when infested by *Achaea janata* and *S. litura*..

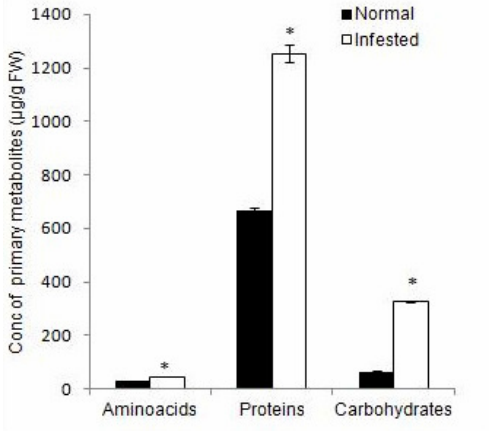

Figure 1. Quantitative changes in the leaf biochemicals, carbohydrates, amino acids and proteins (µg/g FW) of *S. melongena* leaves in response to *L. orbonalis* infestation. Bars with standard error are significantly different at ($P < 0.001$), are indicated by asterisks (paired t-test) between the control and stress induced plant (N = 12). FW- Fresh Weight of leaves.

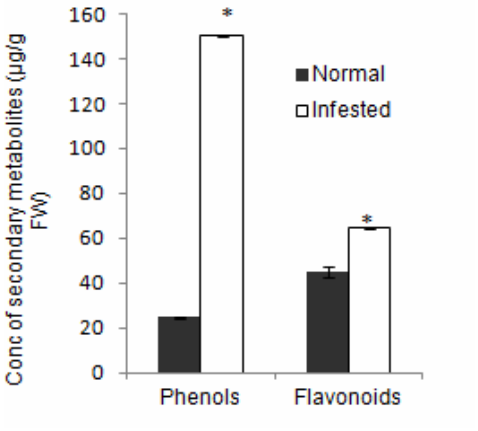

Figure 2. Quantitative changes in the leaf secondary metabolites- phenols (GE/g FW) and flavanoids (QE/g FW) of *S. melongena* leaves in response to *L. orbonalis* infestation. Bars with standard error are significantly different at ($P < 0.001$), are indicated by asterisks (paired t-test) between the control and stress induced plant (N = 12). FW- Fresh Weight of leaves.

Also primary metabolites are known to play an essential role in determining host-plant suitability for herbivores as well as for conferring resistance against herbivores in presence of toxic secondary metabolites (6). Secondary metabolite concentrations in context of both total phenols and flavonoids are significantly increased due to *L. orbonalis* infestation. Total flavonoid concentration was increased from 44.0 µg QE/g FW in normal uninfested plants to 64.516 µg QE /g FW in infested plants and total phenolic concentration increased from 24.64 µg GE /g FW in uninfested eggplants to 150.33µg GE / g FW in *L. orbonalis* infested eggplants (Fig. 2). These flavonoids can have negative effects on non-adopted insects or may reduce the nutritive value of their food as described by Treutter (54). They may behave as antifeedants, as digestibility reducers and also act as toxins. Whereas, polyphenols scavenge reactive oxygen species and can also inhibit lipid peroxidation by using its ability to trap the lipid alkoxyl radical. Phenolics render various significant roles in plant- insect and/or environment interactions by attracting pollinators and fruit dispersers, by absorbing harmful ultraviolet radiation and also act as mechanical support in the plants besides contributing to the plant defence by repelling feeding herbivores and inhibiting their enzymes (15). Michalak (44) has reported few evidences of induction of phenolic metabolism in plants as a response to multiple stresses. In our study it is observed that total phenolic content has increased six fold times compared to control eggplants. There are a number of examples ~~of~~ where role of phenolics in defence against insect herbivores has been observed. Wheat cultivars containing higher concentrations of phenolics are much less attractive to *Rhopalosiphum padi* (cereal aphid) compared to varieties with lower total phenol concentrations (38). Similar results were obtained in *I. batata* plants upon feeding by *S. litura* (48). Thus secondary metabolites are enhanced significantly in our present study indicating a very important role of these molecules in the induced defences.

Herbivory-altered plant phenolic compounds

To overcome the damages imposed by biotic stress, plant modifies its chemical constituents accordingly. Phenolic compounds play main role in this phytochemical change with respect to the specific pest feeding. The early community and clade- based studies conducted by Agarwal and Weber (5) on plant chemicals involved in defences, trait- herbivory correlations further strongly supported that secondary metabolites provide plant defence, including resistance to specialist herbivores. The individual phenolic compounds present in the plant leaf tissue of both infested and normal control plants were identified with HPLC (Table 1). Along with 8 common phenols identified, there were 8 more phenols observed as very small peak areas in the chromatograms(Fig. 3(a) and 3(b)). Among the eight phenolic acids which have been identified and quantified using HPLC, caffeic acid, coumaric acid, epicatechin, gallic acid exhibited radical increase in individual phenol concentrations followed by hydroxybenzoic acid, chlorobenzoic acid and gallic acid in *L. orbonalis* infested plants compared to control plants (Table-1). Chlorogenic acid is the only phenol which reduced in infested plants, though the reduction noted is very less. Chlorobenzoic acid and p-hydroxy benzoic acid though present in very minute quantities, increased significantly in infested plants. Individual HPLC profiles showed a vivid radical change in the infested plants indicating their obvious role in plant defense mechanisms. Jyothsna *et al.* (34) and Felton *et al.* (23) have found that increased

Table 1. Individual phenolic acid profile extracted from the leaves of eggplant, *S. melongena* infested by *L. orbonalis*

S.No	Phenolic acid	R.T	Control (µg/ml/GFW)	Infested (µg/ml/GFW)
1.	UD	3.16	+	+
2.	UD	3.36	+	+
3.	UD	4.42	+	+
4.	UD	4.60	+	+
5.	Gallic acid	9.39	5.76 [a]	26.51[b]
6.	UD	12.60	+	+
7.	UD	13.58	+	+
8.	Caffeic acid	13.92	8.41 [a]	78.15 [b]
9.	UD	14.70	+	+
10.	Chlorogenic acid	15.26	85.80 [a]	80.35[b]
11.	*p*-Hydroxy benzoic acid	15.98	1.64 [a]	13.70[b]
12.	Chlorobenzoic acid	16.23	1.06 [a]	6.72[b]
13.	Vanillic acid	16.69	6.50 [a]	29.74[b]
14.	Coumaric acid	18.50	27.60[a]	42.91[b]
15.	UD	18.93	+	+
16.	Epicatechin	19.90	20.90 [a]	30.86[b]

Values are mean ± standard error for five replicates. Mean values for each compound in the same row followed by different superscript letters are significantly different ($p<0.001$, paired t-test). UD: Unidentified phenols +: Unidentified phenols

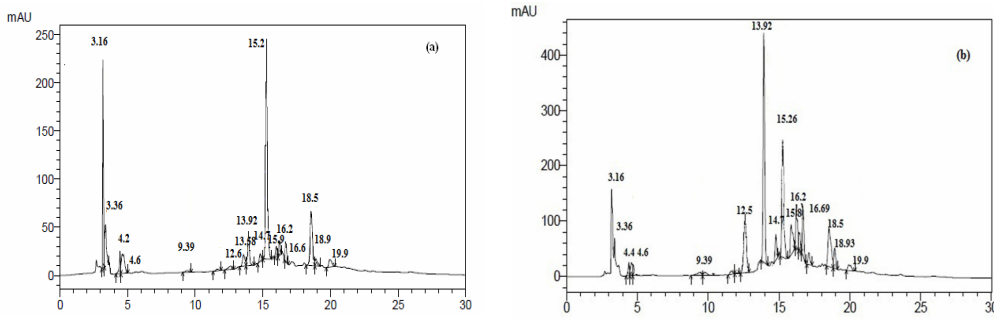

Figure 3. (a) HPLC profiles of a control and (b) *L. orbonalis* infested Eggplant leaf samples. Identified and unidentified (U.D) phenols with their Retention times.

concentration in phenolic compounds is according to the extent of tissue damaged by feeding insects or due to pathogen infection. HPLC results suggest that increased coumaric and caffeic acids form central intermediates of lignin biosynthesis (12). Lignin and other phenolics can be anti-nutritional by strengthening of the cell walls (49).The summing up of results of this study show that the leaf phenolics are modified quantitatively due to pest feeding and further these enhanced phenolics have profound effects on feeding herbivore. For example, the cotton plants infested with *Helicoverpa zea* exhibited a rise in the levels of gallic acid contents (11). Phenolic acids such as vanillic acid and p-coumaric acid were

also found to increase in the *Scirpophaga incertulas, Cnaphalocrosis medinalis and Nilaparvata lugens* infested rice plants (59).

Summers and Felton (51) proposed that the induction of oxidative stress may be an important component of phenolic toxicity in lepidopteran larvae. Induced accumulation of phenyl propanoids in wheat tissues, especially *p*-coumaric acids in response to insects feeding by *Sitodiplosis mosellana* has been reported (19). Chlorogenic acid inhibits early larval growth of the fruit worm, *H. zea* when added to its artificial diet (33). When the tomato fruit worms *H. zea* feed on tomato foliage, a considerable amount of the ingested chlorogenic acid is oxidized to chlorogenoquinone, a highly reactive electrophile by PPO in the insect gut. The reduction in larval growth is expected to result from reduction in the nutritive quality of foliage by the alkylation of amino acids or proteins by o-quinones. Role of epicatechin in combating feeding stress was reported earlier by Pratyusha and Usha Rani (47). They observed that epicatechin levels were increased in *Aproaerema modicella* and *Spilosoma oblique* infested groundnut plants. Similarly in the present study, higher biosynthesis of phenolic acids in eggplant leaves after insects feeding was observed. Thus it can be suggested that feeding of the herbivorous insects induce antibiosis based on accumulation of the phenolic acids in eggplant leaves.

Oxidative response in plants

One of the important components of induced resistance involves a significant shift in the oxidative status of the host plant. Rapid generation of ROS which is referred to as oxidative burst is a common phenomenon in plants due to biotic stress. These ROS molecules play signaling functions and can also act directly as toxins (41). The level of lipid peroxidation is used as an indicator of ROS mediated cell membrane damage as it increases oxidative stress by producing lipid-derived radicals which react with and damage proteins and DNA (17). The content of thiobarbituric acid reactive species, mainly MDA concentration increased significantly from 5.49 nmol mL^{-1} in normal plants to 7.60 nmol mL^{-1} in *L. orbonalis* infested plants indicating the progress of reactive species outburst leading to membrane degradation (Fig. 5). Thus increased MDA level in *L. orbonalis* infested eggplants can be implicated in increased damage to the plant cell membrane. Similar results were observed by Zhang *et al.* (64) where MDA concentration was increased by *Bemisia tabaci* infestation in cucumber seedlings. Phenolics and flavonoids possess high numbers of hydroxyl groups which involve in scavenging reactive oxygen species rendering them inactive, hence the ability to scavenge DPPH free radicals (2). In our study, a positive correlation was noticed between total phenolic content, total flavonoid content and DPPH scavenging ability. Total radical scavenging activity was calculated from IC_{50} values of antioxidants which were found to be 380.0 µg/ml for un-infested *S. melongena* extract, compared to 320.0 µg/ml in infested plants. The IC_{50} of standard quercetin calculated was 59.05 µg/mL (Fig. 4). These results are in agreement with the results of Gallegos-Infante *et al.* (26) which show that scavenging of DPPH free radicals was relevant to polyphenolic content. Surveswaran *et al.* (53) reported that increase in flavonoid contents increased the DPPH activities in many plant extracts. These results indicate that the increase of secondary metabolites upon insect infestation are not only useful for defending herbivory but can also help to reduce oxidative damage to the cell membrane.

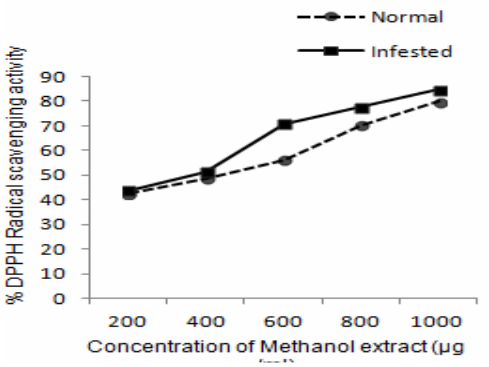

Figure 4. DPPH radical scavenging activity of normal and *L. orbonalis* infested *S. melongena*. Values are the average of ten replicates and represented as mean ± standard error. The radical scavenging capacity of control and infested plants are significantly different (P = <0.001) (paired t-test).

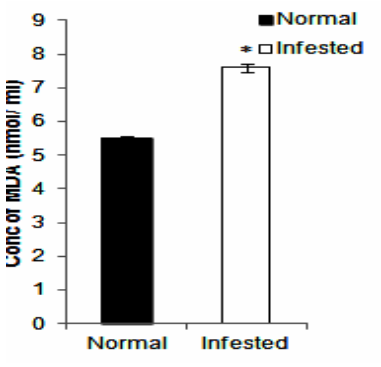

Figure 5. Quantitative changes in the Malondialdehyde (MDA) contents (nmol/ ml) of *S. melongena* leaves in response to *L. orbonalis* infestation. Bars with standard error are significantly different at ($P < 0.001$), are indicated by asterisks (paired t-test) between the normal and stress induced plant (N =10). FW: Fresh Weight of leaves.

Plant Defense enzymes

The importance of antioxidant system in plant defences also depends on the activity of multicomponent anti-oxidative enzymes. Activities of anti-oxidative enzymes like catalase, polyphenol oxidase and peroxidase were significantly induced in *L. orbonalis* infested plants (Fig. 6(b) and 6 (a)). Similar results were reported in *Gossypium hirsutum* plants where catalase and peroxidase concentrations increased significantly upon herbivory by *S. litura* (57). In general POD participates in the integrated defense responses of plants to a variety of stresses through cell wall toughening and production of toxic secondary metabolites. Increase in POD activity in response to insect attack can also be attributed to the participation of these enzymes in lignification, suberization and wound healing, as well as, defense against biotic and abiotic stresses (3). SOD is crucial for the dismuting of O^{2-} into water and H_2O_2. But in eggplant they do not contribute to defence mechanisms as no significant increase or decrease is observed in superoxide dismutase contents of infested plants compared to the control plants.

Polyphenol oxidases are a class of anti-nutritive enzymes. Phenols and flavonoids are major substrates for this enzyme. This enzyme activity is highly increased in *L. orbonalis* infested plants compared to control plants. Felton *et al.* (22) found reduction in growth of the tomato fruit worm *H. zea* when PPO levels in tomato leaves were increased. The reduced growth was also positively correlated with the amount of the plant phenolic compounds. Phenols also play an important role in reduction of reactive oxygen species (ROS) such as along with activation of defensive enzymes through a cascade of reactions (41). Oxidation of phenols catalyzed by polyphenol oxidase (PPO) and peroxidase (POD) is an efficient defense mechanism in plants against herbivorous insects. Quinones formed by oxidation of phenols inhibit the protein digestion in herbivores by covalently binding to

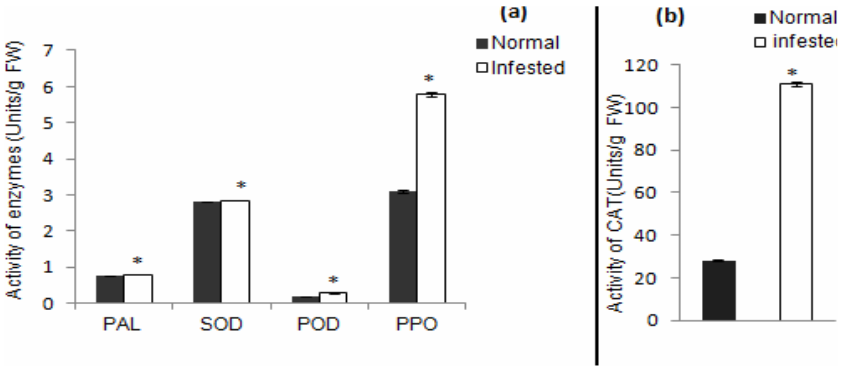

Figure 6. Quantitative changes in the enzyme activities (U/g FW) of PAL, SOD, POD, PPO **(a)** and CAT **(b)** of *S. melongena* leaves in response to *L. orbonalis* infestation. Bars with standard error are significantly different at ($P < 0.001$), are indicated by asterisks (paired t-test) between the normal and stress induced plant (N = 12). FW: Fresh Weight of leaves.

leaf proteins (10). Thus increase in total flavonoid and total phenol concentrations positively correlates to the increased PPO content and also implies directly to induced enzymatic defences in *S. melongena* that are mainly conferred by catalase, peroxidise and polyphenol oxidases.In other studies performed on different cultivars of eggplants, amino acids, crude protein and sugar content (total and reducing sugars) showed a high positive and poly phenol oxidase, phenylalanine ammonia lyase, peroxidase, glycoalkaloids and lignin content showed a highly negative correlation with shoot and fruit borer infestation (46,42).

Induced defence responses in infested *S. melongena* plants by cell wall strengthening:

Another component of induced resistance involves lignification and cell wall strengthening (27). Phenylalanine ammonia lyase is the enzyme responsible for lignification of cell walls through phenyl propanoid pathway and in current study this enzyme is shown to be increased significantly in *S. melongena* plants after infestation (Fig. 6). The by-products of this pathway attribute to alter the susceptibility and palpability of plants to insects (24). The H_2O_2 that is required for the oxidation is supplied by POD activity which produces phenolic radicals that condense to form lignin (27). The strengthened cell wall caused by lignification and insoluble cell wall proteins may serve as a defensive barrier by reducing the digestibility of cell wall carbohydrates and proteins.

CONCLUSIONS

The directly induced defences in eggplants include induction of the enzymatic, nutritional quality and biochemical changes in *L. orbonalis* infested plants showed that the induced responses were not only restricted to areas affected but were also induced systemically. As the survival of plants depends largely on their own defensive strategies, hence, these coordinated responses of directly induced defences represent a multicomponent mechanism of plant defences against the attack of herbivores. This study formed the basis for application of natural defences for reducing the yield losses in *S. melongena*.

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