0971-4693/94 US $ 5.00
Allelopathy Journal 24 (1): 131-142 (2009) *International Allelopathy Foundation* 2009
Table: -, Fig s: 3

Effects of herbivore feeding on biochemical and nutrient profile of castor bean, *Ricinus communis* L. plants

JYOTHSNA YASUR, KAPIL MATHUR and PATHIPATI USHA RANI*

Biology and Biotechnology Division
Indian Institute of Chemical Technology, Tarnaka, Hyderabad-500 607, India.
E. Mail: purani@iict.res.in, usharani65@yahoo.com

(Received in revised form: May 5, 2009)

ABSTRACT

We studied the effects of four major pests [capsule borer, *Dichocrocis punctiferalis* (Guenée), semilooper, *Achaea janata (L)*, tobacco cutworm, *Spodoptera litura* (Fabricius) and sucking pest, *Empoasca flavescens* (Fabricius)] feeding on the castor *Ricinus communis* (L) plants, on primary metabolites and biochemical constituents of leaves. We measured the primary metabolites (amino acids, glutamine, urea, ammonia, carbohydrates, proteins, phenols and oxidative and hydrolyzing enzymes) involved in the synthesis of secondary metabolites. The quantities of biochemical constituents (except glutamine) were increased by the feeding of leaf eating insects than sucking pests or mechanically damaged plants. All the enzymes activities enhanced in the semilooper, tobacco cutworm and capsule borer damaged plants. We concluded that the defence mechanism of plant differs with the type of attacker pest and were related to the enhanced enzymatic activities.

Keywords: *Achaea janata* L. Castor *Ricinus communis* L., *Dichocrocis punctiferalis* G., *Empoasca flavescens* F., insect feeding, Oxidative enzymes, *Spodoptera litura* F.

INTRODUCTION

Plants contain several chemicals that play defensive role against herbivorous insects and recently it has become an area of intensive research, to understand the insect-plant interactions. Most plants are very complex, dynamic mosaics of various chemical compounds and nutrients, which govern the acceptance and suitability of plant to feeding insects (11). The nutritional disorders can affect (i) the plant growth, (ii) plant's susceptibility to pests and (iii) host plant resistance (8). These primary metabolites as insect nutrients can have profound effects on insect behavior and physiology. Many aspects of nutrient-allelochemical interactions are probable key factors in the suitability of a given plant species as a host for a particular insect. As Reese (41) suggested, many deleterious physiological effects of plant allelochemicals may be due primarily to various interactions between these allelochemicals and essential nutrients. The nutritive value and morphology of plant for its insect pests appears to play an important role in determining the susceptibility of the plant to insect attack.

[1] Correspondence author

Berenbaum (8) stated that nutritional defenses could be expected to arise most frequently in those cases in which the nutritional needs of plant and animal diverge most dramatically. Plants require proteins, lipids, carbohydrates, vitamins and micronutrients to grow and reproduce. The feeding stress caused by herbivore often might result in changes in these contents affecting the plant growth or causing changes in its physiology. 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. However, the effects or differences of these nutritional compounds in the pest damaged plants is reported in a very few cases (45). Zangerl *et a*l. (48) found adverse effects of caterpillar feeding on photosynthesis, which extend well beyond the areas of the leaflet in which caterpillars removed tissues.

Proteins and their constituent amino acids are among the primary metabolites most likely to be influenced by insect herbivory. Increased herbivore damage resulted in higher proteinase inhibitor (PI) activity, a Jasmonate-regulated defensive protein (40). A more dispersed pattern of caterpillar damage altered the expression of induced responses (40). Insect feeding often results with a hypersensitive response in plants, characterized by a rapid oxidative burst of reactive oxygen species (ROS): hydrogen peroxide, the hydrogen radical and the super oxide radical (42). ROS play different roles in plant defence against pest as well as pathogen attack (31) and the pest feeding (wounding) has been shown to trigger the production of ROS in plant tissues (12). We made a thorough investigation on the defensive chemicals and biochemicals that are induced due to the herbivory by castor pests.

The castor, *Ricinus communis* (L.) (Euphorbiaceae) is major crop in Southern India and its oil has great medicinal value. Several pests attack this plant at various stages of growth. In this study, the impact of herbivory on the degree of variation in plant primary metabolism, the effects of plant's nutritional and biochemical stress, the changes in foliar nutritional quality due to herbivore feeding and the oxidative defence was determined.

MATERIALS AND METHODS

I. Plants

The Experiment consisted of 4 herbivores [jassid, *Empoasca flavescens* F. (Homoptera: Jassidae), the capsule borer, *Dichocrocis punctiferalis* Guenee (Lepidoptera: Pyralidae), the semilooper, *Achaea janata* L. (Lepidoptera: Noctuidae), and the tobacco cutworm, S*podoptera litura* Fab. (Lepidoptera: Noctuidae)] feeding on Castor, *Ricinus communis* L. plants. The treatments were replicated thrice in randomized block design. The study was conducted in laboratory conditions at temperature 25±2ºC and relative humidity 65±5%. Castor, *Ricinus communis* L. 'Aruna' (highly susceptible to the attack of both leaf feeding pests, i.e. *Achaea janata* L. and *Spodoptera litura* F) plants were used in this study. The seeds were planted in garden potting soil mix in plastic pots (10 x 15 cm dia) kept in green house (12h light/ 12 h dark, 25±2ºC and relative humidity 65±5%). The study was carried out during July - September 2007. Two seeds were planted in each pot and 15d old plants having 5 leaves were used for the experiments.

II. Insects

The cultures of castor semilooper, *Achaea janata* (L) and the cluster caterpillar, *Spodoptera litura* (Fab) were maintained in our laboratory. The diet consisted of bouquets of fresh castor leaves, with their petioles immersed in glass conical flasks (100 ml capacity) containing tap water to keep the leaves fresh for longer periods. Two such bouquets were placed inside a big plastic tub (15 l capacity) and covered with fine muslin cloth after releasing 20 larvae in each tub. The leaves were replaced every morning with fresh castor leaves. Larvae pupated at the bottom of the plastic tub or on the leaves. Pupae collected in glass Petri dishes (3" dia), and placed inside a big nylon mesh cage (30 x 30 x 45 cm) with a Zinc bottom. A bouquet of castor leaves was offered to the emerging adults for oviposition.

The cultures of the Capsule borer, *Dichocrocis punctiferalis* Guenee and jassids, *Empoasca flavescens* were maintained in the lab fields. The infested leaves were collected from the plants grown in the laboratory campus.

III. Pest Feeding

Castor plants 45 day old were brought to the laboratory and one 3rd instar larva was released on terminal leaf of each plant and confined to the point of release by enclosing it in a muslin bag. *S. litura* larvae were released during the early hours of the day. Both the insects fed on the leaves for 6 h and the leaf portions remaining after feeding by caterpillars were used for the enzyme analysis. Larvae usually consumed approximately 30-50% of the leaf during this time.

Leaves of castor plants having capsules infested with *Dichocrocis punctiferalis* for about 60 days and the 45 day old castor plants infested with jassids for about 10 days were selected from the field and the leaves plucked at the bottom of the petiole. For obtaining mechanically damaged leaves, the leaves from 45 d old castor plants were damaged mechanically by making holes and cuts on the leaf surface using a surgical blade. Each response was analyzed after 24 h. In all experiments, fresh leaves from the normal and healthy castor plants were used as controls for comparison.

IV. Biochemical estimations

Standard methods were employed to estimate amino acids (38), glutamine (16), ammonia (10), urea (39), total carbohydrates (19), proteins (32) and total phenols (24). For extracting the biochemicals, the leaves were excised at the upper end of petiole followed by macerating them in a tissue grinder. The amino acids are expressed as Teq/gm fresh weight and the other biochemicals were expressed in µg/gm fresh weight (FW).

V. Enzyme methods

Catalase (CAT) activity was assayed colorimetrically as per Aebi (2) and the Peroxidase (POX) activity by Kar and Misra (27) with slight modifications. Assay mixture containing 1.0 ml of 0.1 M phosphate buffer (pH 7.0), 0.5ml of 1% H_2O_2 and 1.0 ml of 0.05 M pyrogallol was measured at 420 nm. The estimation of Superoxide dismutase enzyme (SOD) activity was done by Beauchamp and Fridovich (5) method. In this a 1-ml reaction mixture contained 30µl enzyme extract and a 970 µl solution of: 2 µM riboflavin, 0.1 mM EDTA, 75 µl 4-nitro blue tetrazolium chloride (NBT) and 13 mM methionine. Irradiation was performed by placing the sample 30 cm below two fluorescent lamps (2 x

40 W) for 30 min in an aluminium foil-covered box. A sample without irradiation was used as control during the measurement of absorbance at 560 nm. Glucanase activity was assayed using the method of Abeles and Forrence (1) with laminarin (*Lanunaria digitata,* Sigma) as the substrate. Reactions were run at pH 5 at 50°C.

To determine the Chitinase activity, the amount of reducing sugars produced from chitin were measured using dinitrosalicylic acid (DNS) (37). The reaction mixture contained 0.5 ml of 0.1 M potassium phosphate buffer (pH 6.75), 0.4 ml of 1% colloidal chitin, and 0.1 ml of the sample material. The mixture was incubated at 50°C for 30 min. The activity was described as change in absorbance per 30 min per gm FW.

The reaction mixture for PAL activity consisted of 6-µM L-Phenyl alanine, 0.5 M Tris-HCl buffer (pH 8.0), and 200 µL of plant extract. After 60 min at 37°C, the reaction was terminated by the addition of 0.05 mL of 5 N HCl and the activity was assessed by measuring the cinnamic acid extracted in toluene at 290 nm and is expressed as change in absorbance per hour per gm FW, similar to the procedure described by Beaudoin-Eagan and Thorpe (6).

The polyphenol oxidase was measured by the method of Thaler *et al.* (44). Assay mixture contained 10-30 µl of enzyme extract, 1ml of 2.92mM Caffeic acid in pH-8 phosphate buffer was added and absorbance measured at 470nm.

VI. Protease and protease inhibitor assay

Total protease activity in the extracts was determined as per previously described procedure (36). The assay mixture contained 35µl leaf extract, 5ml of 0.5mg/ml (w/v) Trypsin dissolved in 50mM Tris pH 8.0. After 30-min incubation at 37°C, 80 µl of 2% (w/v) azocasein (1 mg protein) in assay buffer was added to the enzyme/inhibitor solution, and the complete mixture was incubated for 3 h at 37°C. After proteolysis, 300 µl of 10% (w/v) trichloroacetic acid was added to the mixture and residual azocasein was removed by centrifugation at 13 000×*g* for 5 min. The supernatant (350 µl) was added to 400 µl of 1 N NaOH and the absorbance (*A*) was measured at 440 nm using a spectrophotometer.

All enzymatic measurements were made in sets of three and each set consisting of 5 replicates, thus for a single sample there were 15 replicates measured. A spectrophotometer (Molecular Devices Soft Max Pro) was used for the measurement of enzyme activities, which was performed at 25˚C.

Statistical analysis: All the enzymatic activities were expressed as change in absorbance per min per gm FW. The differences in the activities between pest infested/mechanically damaged and normal non-infested plants (controls) were analyzed using paired t- test at $P< 0.05$. All the statistical analysis was performed and the figures were plotted using the software Origin (version 7.5).

RESULTS AND DISCUSSION

The results of the investigations revealed the quantitative differences in various biochemicals and nutrients in pest infested and normal (uninfested) castor plants. Changes occurred in different biochemical and nutritional profiles of the plant, which depend on the mode of feeding on the plant i.e., leaf surface or the internal tissue of the capsule.

Statistical analysis showed significant differences in biochemical constituents of castor plants fed by the four pests, *A. janata, S. litura, E. flavescens* and *D. punctiferalis*; irrespective of the fact that whether they were resistant or susceptible to the above pest infestation (Figs. 1, 2, 3). Changes in "primary metabolites" may change the efficacy of secondary metabolites. For example herbivore attack increases the titer of ROS and reduces the level of antioxidants such as ascorbic acid (12). These changes should combine to the increase the oxidative damage directly to herbivores and to leaf nutrients that herbivores use.

A reduction in the amino acid content was observed in pest-infested plants than the control plants in all the treatments except that of *A. janata* infested plants (Fig. 1). It is interesting to note that, though both *A. janata* and *S. litura* has similar feeding modes and even the same feeding extent, the differences observed in the amino acid content denotes possible involvement of other factors such as role of salivary enzymes or any other unknown factor. Although proteins are not secondary metabolites, certain types of proteins have functions similar to secondary metabolites, including protection of the plant against insects (7). There was an enhancement in protein content of wounded plants. However, these changes were not found in sucking pest, *E. flavescens* infested leaves and mechanically damaged plants. Earlier reports states that the proteins accumulate in apoplasts in plants are induced by insect wounding (25) and are known to involve in the plant defense mechanism (14).

Amino acids, vitamins, sterols and other herbivore nutrients are chemical constituents of plants that are synthesized sequestered or metabolized as alkaloids, coumarins and secondary metabolites (8)**.** Considerable evidence exists that they play an essential role in determining host-plant suitability for herbivores such analysis of primary metabolism stands to make sustained contribution to understanding the chemical bases of for host-plant choice in insects and the evolutionary responses of plants to herbivory.

There was a significant decrease in carbohydrates due to sucking pest, borer insect attack and mechanical damage. The damage caused by the leaf chewing insects- *A. janata* and *S. litura* resulted in the increased carbohydrate content (Fig. 1). The induction is assumed to be caused due to the mobilization of the carbohydrates to the wounded sites as plant might be attempting to cope with the loss. Similar observations were made in the Arabidopsis plant also (35). Plants require nutrients to grow and reproduce as the reduction in the amounts of these constituents may comprise a plant's physiological capabilities at the same time may decrease the attractiveness to herbivores (8). These primary metabolites- carbohydrates and proteins are exploited by the herbivores for their growth and development (3).

A slight increase in the quantities of ammonia and urea were recorded in the leaf eating and borer infested plants and decrease in the jassids and mechanically damaged plants when compared to the normal plants (Figure 1). It is also interesting to note that the amount increased depending on the mode of feeding and is specific to the pest insect. The content of Glutamine decreased in *E. flavescens, D. punctiferalis, A. janata* and mechanically damaged plants, while a slight increase was found in the case of *S. litura* fed leaves in comparison with normal healthy castor plants (Figure 1). Considerable increase in the quantities of urea was observed in the case of *A. janata* infested castor plants while in *E. flavescens, S. litura* and mechanically damaged plants a decrease was noted (Fig. 1).

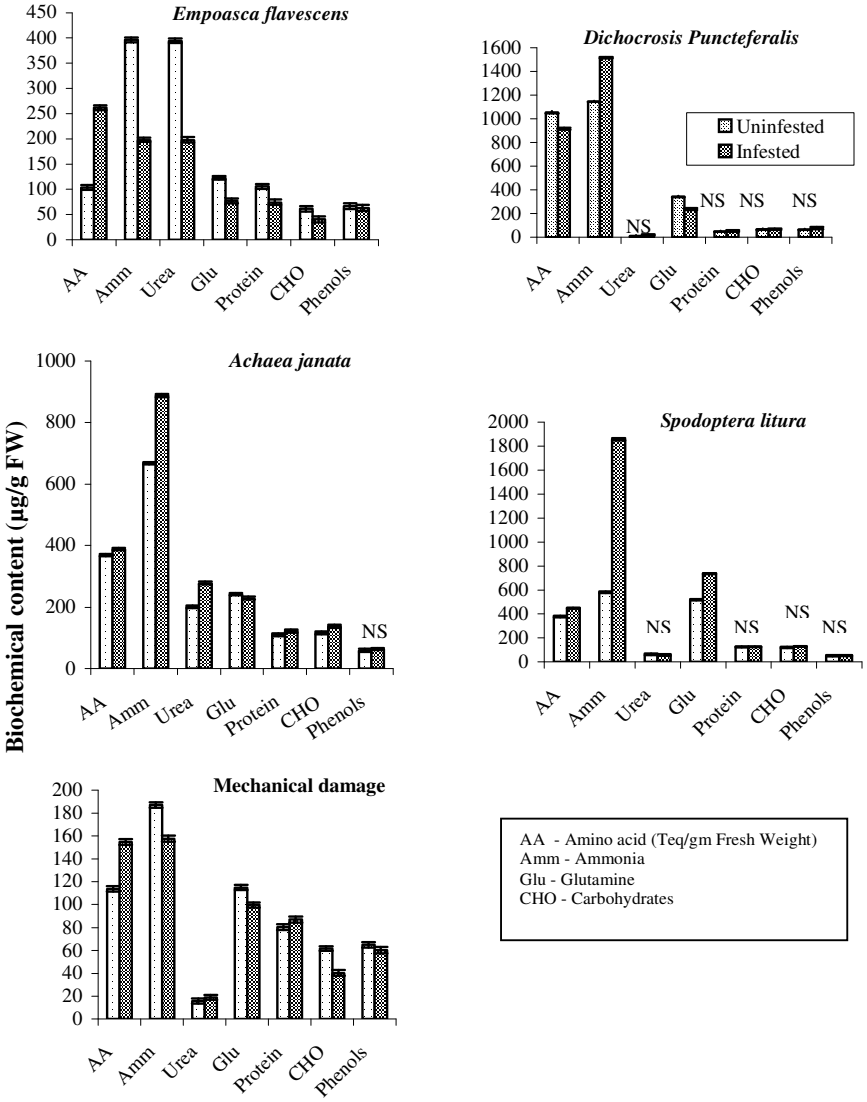

Figure 1. Biochemical changes in Castor leaves in response to insect feeding by four major pests: and mechanical damage. The biochemical contents in control (uninfested) and infested plants are significantly different ((P< 0.05) (paired t-test), NS= not significant. (N=15 replicates of each sample).

An increase in the phenol content was recorded due to damage inflicted by *A. janata*, *S. litura* and capsule borer *D. punctiferalis* on *R. communis* leaves (Fig,. 1). A decrease was also observed in the mechanically damaged and jassid infested plants, suggesting that the loss of larger areas of the leaf biomass is stimulating the plant to enhance the phenolic contents. It is previously reported that high phenol contents reduce the performance of the larval feeding. Cotton bollworm, *Heliothis armigera* (Hubner), feeding on cotton, *Gossypium hirsutum* increased phenolics in the plants, which was detrimental to the conspecifics (30). Possibly for similar function the phenols in castor plants are also increased. Many of the secondary metabolic pathways are inducible by herbivory. Enhanced availability of common precursors within these inducible pathways may result incidental overproduction of primary metabolites (48). It is also suggested that these compounds may contribute to the defense of the plants against herbivores by reducing digestibility or palatability.

Feeding by different insects on castor plants increased the activity of the catalase enzyme as found in *Helicoverpa zea* larval feeding on cotton foliage (13) and alfalfa (*Medicago sativa* L.) with the spotted alfalfa aphid, *Therioaphis maculata* B. (18). There was a significant increase in peroxidase activity in *D. punctiferalis, A. janata* and *S. litura* infested plants when compared to their respective control plants suggesting the cause being the extent of feeding damage (Fig. 2). Past reports are available on Peroxidase activity, which was shown to be increased in foliage damaged by two-spotted spider mite (23), and *H. zea* (12). However, it is interesting to note a decreased activity of POX in *E. flavescens* and mechanically damaged plants (Fig. 2). Perhaps the reason for these quantitative changes could be the lesser amounts of damage. A number of reports have suggested that peroxidases play an important role in herbivore resistance in crop plants (15, 17).

The castor plants infested with capsule borer and semilooper induced the antioxidant superoxide dismutase activity (Fig. 2). The result similar to this was also found in the herbivore wounded lima bean plants (33), and gall insect infestation in eucalyptus (29). However, the activity of this enzyme was reduced in *E. flavescens*, a sucking pest infested castor plants, and a similar response was found in the cabbage plants due to the infestation of the phloem sucking aphids, *Brevicoryne brassicae* L (28). It appears that the reduction in the SOD enzyme activity seems to be related to that of the sucking mode of feeding, and we suspect the involvement of sight of wounding also playing role in this type of response. No change was recorded in the mechanically damaged plants.

In several instances plant resistance to herbivores has been correlated with an enhanced oxidative state of plant tissue (12), which involves in generation of ROS. Wounding has been found to rapidly increase the activity of lipoxygenases (13,43) Peroxidase (44) and Polyphenol oxidase (17,44). Since oral secretions from caterpillars, dramatically increase oxidative enzymes (12, 43) and trigger the jasmonate cascade (34) plants may be responding specifically to the damage caused by caterpillar feeding. The enzymes are involved in few defense related events that occur in extra cellular matrix. These include the strengthening by lignification and the formation of intermolecular cross links, suberin formation and the production of ROS which is associated with eliciting and signaling events as well as direct defense (14).

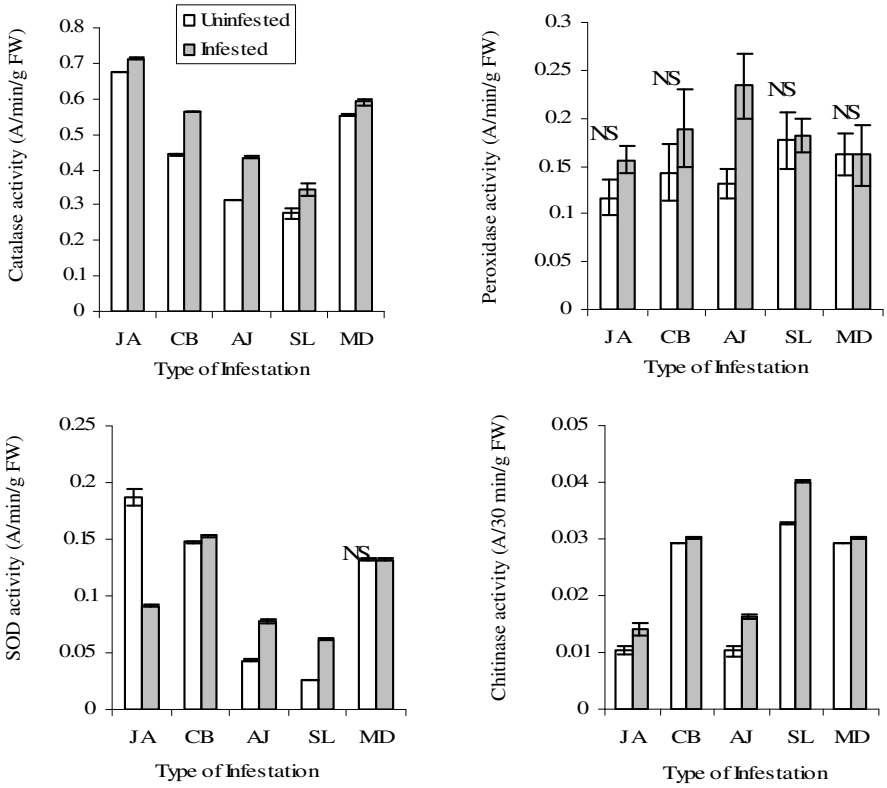

Figure 2. Changes in the activities of defence-related enzymes of castor upon infestation with four insect pests. JA: *Empoasca flavescens*, CB: *Dichocrocis punctiferalis*, AJ: *Achaea janata*, SL: *Spodoptera litura* and MD: Mechanically damaged plants: (a) Catalase (b) peroxidase, (c) superoxide dismutase and (d) chitinase. Bars indicate Mean ± SE. Differences between the undamaged and damaged plants are significant ($P < 0.05$) (Paired t-test), NS= Not significant. (N=15 replicates of each sample).

Chitinase activity increased in all the four pests infested and mechanically damaged plants as also being reported in tomato plant due to aphid infestation (Fig. 2). All kinds of wounding including mechanical disturbance of the leaf structure appear to trigger the chitinase activity. These enzymes are known to act as amylase inhibitors and interfere in the digestion of the plant parts by insect gut enzymes (4). Hence, the induction of this enzyme might also affect the insect development, feeding and growth, and may finally cause death (47).

The activity of β-1, 3-glucanase was more in *D. punctiferalis*, *A. janata* and *S. litura* infested plants (Fig. 3). The increased glucanase activity was also reported previously in the silver leaf whitefly (*Bemisia* Sp.) infested plant (26). The suppressed

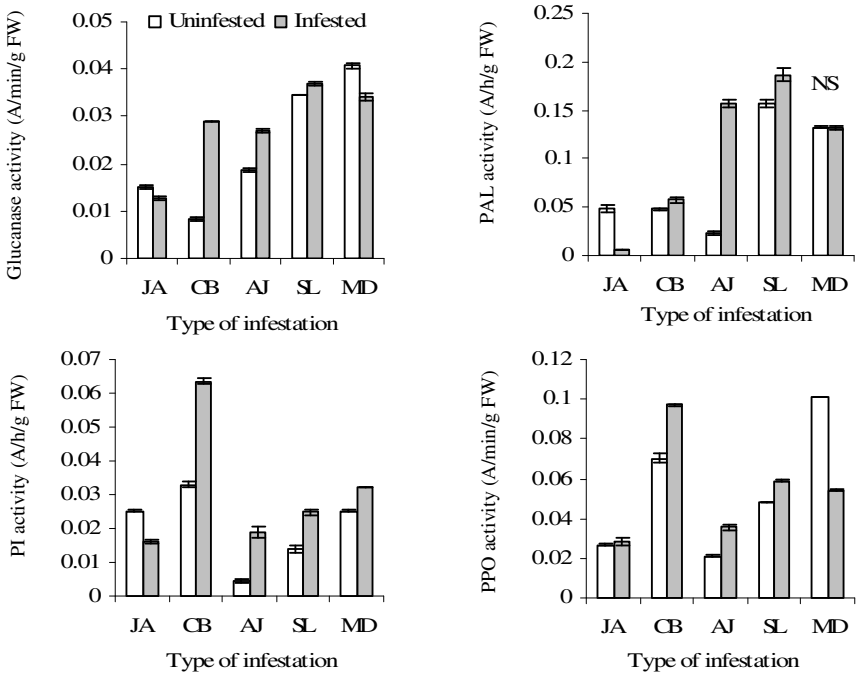

Figure 3. Changes in the activities of defence-related enzymes of castor upon infestation with four insect pests. JA: *Empoasca flavescens*, CB: *Dichocrocis punctiferalis*, AJ: *Achaea janata*, SL: *Spodoptera litura* and MD: Mechanically damaged plants: (a) b- 1,3-glucanase, (b) PAL- phenylalanine ammonia lyase (c) PI- Protease Inhibitor and (d) PPO- Polyphenol oxidase. Bars indicate Mean ± SE. Differences between the undamaged and damaged plants are significant ($P<0.05$)(Paired t-test), NS= Not significant. (N=15 replicates of each sample).

activity of the glucanase was identified in the jassids and mechanically damaged plants. β-1, 3-glucanase enzyme is generally known to be induced by the fungal pathogen infection (46). A possible mechanism for the β-1, 3-glucanase action is to release oligosaccharides from the plant cell wall, molecules, which are known to trigger other defense reactions in plants (9).

The feeding damage caused by the capsule borer, castor semilooper and tobacco cutworm induced the activity of PAL enzyme in the plants as found in the birch foliage on caterpillar damage and artificial damage (22). The byproducts of the enzyme in the phenyl propanoid pathway are attributed to alter the palatability and suitability of the plant to an insect (20). There was a decline in the activity of PAL in *E. flavescens* and mechanically damaged plants (Fig. 3), which can be attributed to the lesser feeding damage. In the past it has been reported that the plants with suppressed PAL have low levels of salicylic acid but can have an effective grazing-induced resistance to *Heliothis virescens*, whereas in those

plants in which PAL has been over expressed there is a decrease in grazing-induced resistance (21).

The PPO activity was induced by the capsule borer and castor semilooper insect (Figure 3) and was also described in the chewing and leaf-eating insects previously (43). Felton *et al.* (21) have found a strong negative correlation between PPO activity and the growth of *Heliothis zea*. The decreased activity of the Polyphenol oxidases is seen in the *D. punctiferalis*, *S. litura* and mechanically damaged plants when compared to the normal plants. Polyphenol oxidases are the enzymes involved in the lignin formation, oxidation of a variety of phenolic compounds as well as help in quinine formation thus having direct role in resistance.

In general, the herbivory by the insects causing maximum damage like *D. punctiferalis*, *A. janata and S. litura* increased the activities of catalase, peroxidase, superoxide dismutase, chitinase, glucanase, phenylalanine ammonia lyase, polyphenol oxidase and Proteinase inhibitor enzymes while reduced activities were observed for the sucking pest, *E. flavescens* and mechanically damaged plants. There were increased activities of catalase, peroxidase and chitinase enzymes in pest-damaged plants (Fig. 2). The enhanced activities of these enzymes may increase the scavenging capacity for free oxygen species. Similar results have been recorded by Stout *et al.* (43); Bi *et al.* (13) and Constabel, (17) in different plant species.

The survival of plants through evolutionary time is due largely to their own defensive strategies. It is necessary to develop crop plants resistant to insect infestation to avoid or reduce the quantity of harmful pesticides that are responsible for environmental hazards. It has become clear that secondary compounds and the relative concentrations of primary nutrients in plant tissues will help in restricting the feeding and growth of herbivorous insects. Thus the study of biochemical changes and enzymatic changes in castor plants infested with insect pests suggest their role in the defense mechanism.

ACKNOWLEDGEMENTS

Authors are grateful to Dr. G. Bhaskar Rajan, Horticulture Department, Indian Institute of Chemical Technology, Hyderabad, India, for the excellent maintenance of experimental plants, Department of Science and Technology, New Delhi for the research grant, Dr. J. S. Yadav, Director, Indian Institute of Chemical Technology, Hyderabad, India for the encouragement and facilities and Dr. Prakash Diwan, Head, Pharmacology Division, IICT.

REFERENCES

1. Abeles, F.B. and Forrence, L.E. (1970). Temporal and hormonal control of β-1-3-glucanase in *Phaseolus vulgaris*. *Plant Physiology* **45**: 395-400.
2. Aebi, H. (1984). Catalase. In: *Methods in Enzymology*, (Ed., L. Packer) pp. 121-126, Academic Press, Orlando.
3. Ananthakrishnan T. N. (1990) Facets of chemical ecology in insect–plant interactions: An overview. *Proc.Indian Acad. Sci.* (*Animal Science*) **99:** 177-183.
4. Ary, M.B., Richardson, M. and Shewry, P.R. (1989). Purification and characterization of an insect a-amylase inhibitor/endochitinase from seeds of Job's tears (*Coix lachryma-jobi*). *Biochimica et Biophysica Acta* **913**: 260-266.

5. Beauchamp, C.O. and Fridovich, I. (1971). Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. *Analytical Biochemistry* **44:** 276-287.
6. Beaudoin-Eagan, L.D. and Thorpe T.A. (1985). Tyrosine and phenylalanine ammonia lyase activities during shoot initiation in tobacco callus cultures. *Plant Physiology* **78**: 438-441.
7. Bennett, R. and Wallsgrove, R. (1994). Secondary metabolites in plant defense mechanisms. *New Phytologist* **127**: 617-633.
8. Berenbaum, M.R. (1995). Turnabout is fair play: Secondary roles for primary compounds. *Journal of Chemical Ecology* **21**: 925-940.
9. Bergey, D.R., Orozco-Cárdenas, M.L., de Moura, D.S., and Ryan, C.A. (1999). A wound- and systemic-inducible polygalacturonase in tomato leaves. *Proceedings, National Academy of Sciences* USA **96**: 1756-1760.
10. Bergmeyer, H.U. (1974). *Methods of Enzymatic Analysis.* I. Vol **4**: 1802-1806. II Ed. Academic Press. New York.
11. Bernays, E.A. and Chapman R. (1994). *Host Plant Selection by Phytophagous Insects.* pp. 312, Chapman & Hall, New York.
12. Bi, J.L. and Felton, G.W. (1995). Foliar oxidative stress and insect herbivory: Primary compounds, secondary metabolites, and reactive oxygen species as components of induced resistance. *Journal of Chemical Ecology* **21**: 1511-1530
13. Bi, J.L., Murphy J.B., and Felton, G.W., (1997). Antinutritive and oxidative components as mechanisms of induced resistance in cotton to *Helicoverpa zea*. *Journal of Chemical Ecology* **23**: 97-117
14. Bowles, D.J. (1990). Defense-related proteins in higher plants. *Annual Review of Biochemistry* **59**: 873-907
15. Chittoor, J.M., Leach, J.E. and White, F.F. (1999). Induction of peroxidase during defense against pathogen. In: *Pathogenesis Related Proteins in Plants*. (Eds., S. K. Datta and S. Muthukrishnan) pp. 171-193. CRC, Boca Raton, FL.
16. Colowick, S.P and Kaplan, N.O. (1967). Determination of amide residues by chemical methods In: *Methods in Enzymology*, (Ed., C.H.W. Hirs). Vol **2**: 63-65. Academic Press, New York.
17. Constabel, C.P. (1999). A survey of herbivore-inducible defensive proteins and phytochemicals. In: *Induced Plant Defenses Against Pathogens and Herbivores-Chemistry, Ecology and Agriculture* (Eds., A.A. Agrawal, S. Tuzun and E. Bent). pp. 137- 166, APS. Press. The American Phytopathological Society, St. Paul, MN.
18. Dillwith, J.W., Berberet, R.C., Bergman, D.K., Neese, P.A., Edwards, R.M. and McNew, R.W. (1991). Plant biochemistry and aphid populations: studies on the spotted alfalfa aphid, *Therioaphis maculata*. *Archives of Insect Biochemistry* **17:** 235-251.
19. Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A. and Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Analytical Chemistry* **28**: 350-356.
20. Felton, G.W., Korth, K.L., Bi, J.L., Wesley, S.V., Huhman, D.V., Mathews, M.C., Murphy, J.B., Lamb, C. and Dixon, R.A. (1999). Inverse relationship between the systemic resistance of plants to microorganisms and to insect herbivory. *Current Biology* **9**: 317-320.
21. Felton, G.W., Donato, K., Del Vecchio, R.J. and Duffey, S.S. (1989). Activation of plant foliar oxidases by insect feeding reduces nutritive quality of foliage for noctuid herbivores. *Journal of Chemical Ecology* **15**: 2667-2694.
22. Hartley, S.E. and Firn, R.D. (1989). Phenolic biosynthesis, leaf damage, and insect herbivory in birch (*Betula pendula*) *Journal of Chemical Ecology* **15**: 275-283
23. Hildebrand, D.F., Rodriguez, J.G., Brown, G.C., Luu, K.T. and Volden. C.S. (1986). Peroxidative responses of leaves in two soybean genotypes injured by two spotted spider mites (Acari: Tetranychidae). *Journal of Economic Entomology* **79**: 1459-1465.
24. Hori, K. (1974). Studies on the feeding habits of *Lygus disponsi* L. (Hemiptera: Miridae) and the injury to its host plant Vs Phenolic compounds, acid phosphatase, and oxidative enzymes in artificially infested tissue of the sugar beet leaf. *Applied Entomology* and *Zoology* **9**: 225-230.
25. Ignatius, S.M.J., Chopra, R.K. and Muthukrishnan, S. (1994). Effects of fungal infection and wounding on the expression of chitinases and β-1,3-glucanases in near-isogenic lines of barley. *Physiologia Plantarum* **90**: 584-592
26. Inbar, M., Doostdar, H., Leibee G.L. and Mayer, R.T. (1999). The role of plant rapidly induced responses in asymmetric interspecific interactions among insect herbivores. *Journal of Chemical Ecology* **25**: 1961-1979.
27. Kar, M. and Mishra, D. (1976). Catalase, peroxidase, and polyphenoloxidase activities during rice leaf senescence. *Plant Physiology* **57**: 315-319.

28. Khattab, H. (2007). The Defense Mechanism of Cabbage Plant Against Phloem-Sucking Aphid (*Brevicoryne brassicae* L.). *Australian Journal of Basic and Applied Sciences* **1**: 56-62.
29. Khattab, H.I. and Khattab, S.A. (2005). Responses of Eucalypt trees to the insect feeding (Gall forming Psyllid). *International Journal of Agriculture and Biology* **7**: 979-984.
30. Kogan, M. and Paxton, J. (1983). Natural inducers of plant resistance to insects. In: *Plant Resistance to Insects*. (Ed., P.A. Hedin) ACS Series **208**: 153-171. American Chemical Society, Washington, DC.
31. Levine, A., Tehnaken, R., Dixon, R. and Lamb, C. (1994). H_2O_2 from the oxidative burst orchestrates the plant hypersensitive disease resistance response. *Cell* **6**: 427- 37.
32. Lowry, O.H., Rosenbrough, N.J., Farr A.L. and Randall, R.J. (1951). Protein measurement with Folin phenol reagent. *Journal of Biological Chemistry* **193:** 265-275.
33. Maffei, M.E., Mithofer, A., Arimura, G.I., Uchtenhagen, H., Bossi, S., Bertea, C.M., Cucuzza, L.S., Novero, M., Volpe, V., Quadro, S. and Boland, W. (2006). Effects of feeding *Spodoptera littoralis* on lima bean leaves. III. Membrane depolarization and involvement of hydrogen peroxide. *Plant Physiology* **140**: 1022-1035
34. McCloud, E.S. and Baldwin, I.T. (1997) Herbivory and caterpillar regurgitants amplify the wound-induced increases in jasmonic acid but not nicotine in *Nicotiana sylvestris. Planta* **203**: 430-435
35. McConn, M., Creelman, R.A., Bell, E., Mullet, J.E., and Browse, J. (1997). Jasmonate is essential for insect defense in *Arabidopsis. Proceedings of National Academy of Sciences* USA **94:** 5473-5477
36. Michaud, D., Faye, L. and Yelle, S. (1993). Electrophoretic analysis of plant cysteine and serine proteinases using gelatin-containing polyacrylamide gels and class-specific proteinase inhibitors. *Electrophoresis* **14**:94-98.
37. Miller, G.L. (1959) Use of dinitrosalicylic acid for estimation of reducing sugar. *Analytical Chemistry* **31**: 426-434.
38. Moore, S. and Stein W.H. (1954). A modified ninhydrin reagent for the photometric determination of amino acids and related compounds. *Journal of Biological Chemistry* **211:** 907.
39. Natelson, S. (1971). *Techniques of Clinical Chemistry*, (Ed., G. Charless). pp. 365. Spring Field, Illinois, USA.
40. Qubbaj, T., Reineke, A. and Zebitz, C.P.W. (2005). Molecular interactions between rosy apple aphids, *Dysaphis plantaginea* and resistant and susceptible cultivars of its primary host *Malus domestica*. *Entomologia Experimentalis et Applicata* **115**: 145-152.
41. Reese, J.C., Chan, B.G. and Waiss, A.C.J. (1982). Effects of cotton condensed tannin, Maysin (corn) and Pinitol (soybeans) on *Heliothis zea* growth and development. *Journal of Chemical Ecology* **8**: 1429-1436.
42. Ruuhola, T. and Yang, S. (2006). Wound-induced oxidative responses in mountain birch leaves. *Annals of Botany* **97**: 29-37
43. Stout, M.J., Fidantsef, A.L., Duffey, S.S. and Bostock R.M. (1999). Signal interaction interactions in pathogen and insect attack: Systemic plant - mediated interactions between pathogens and herbivores of tomato, *Lycopersicon esculentum. Physiological and Molecular Plant Pathology* **54**: 115-130.
44. Thaler, J.S., Stout, M.J., Karban, R. and Duffey, S.S. (1996). Exogenous jasmonates simulate insect wounding in tomato plants, *Lycopersicon esculentum*, in the laboratory and field. *Journal of Chemical Ecology* **22**:1767-1781.
45. Van Dam, N.M., Raaijmakers, C.E., and van der Putten, W.H. (2005). Root herbivory reduces growth and survival of the shoot feeding specialist *Pieris rapae* on *Brassica nigra*. *Entomologia Experimentalis et Applicata* **115**: 161-170.
46. Van Loon, L.C. (1997). Induced resistance in plants and role of pathogenesis-related proteins. *European Journal of Plant Pathology* **103:** 753-765.
47. Wang, X., Ding, X., Gopalakrishnan, B., Morgan, T., Johnson, L., White, F.F., Muthukrishnan, S. and Kramer, K. J. (1996). Characterization of a 46 kDa insect chitinase from transgenic tobacco. *Insect Biochemistry and Molecular Biology* **26**: 1055-1064.
48. Zangerl, A.R., Hamilton, J.G., Miller, T.J., Crofts, A.R., Oxborough, K., Berenbaum, M.R., and De-Lecia, E.H. (2002). Impact of folivory on photosynthesis is greater than the sum of its holes. *Proceedings, National Academy of Sciences* USA **99**: 1088-1091.