

## Allelochemicals in castor (*Ricinus communis* L.) plants and their impact on pest larval feeding as anti-herbivore defensive

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### ABSTRACT

Among the chemical defensive strategies developed by the plant, leaf phenolics generated due to insect herbivory play a major role in controlling the herbivore damage. This study deals with the quantitative and qualitative changes in castor (*Ricinus communis* L) plant leaf phenolics due to the feeding of its major pests, the castor semilooper [*Achaea janata* (L.)], tobacco caterpillar [*Spodoptera litura* (Fabricus)] and the capsule borer [*Dichocrocis punctiferalis* (Guenee)]. The foliar phenolics extracted from the pest damaged leaves were compared between the pests having different modes of feeding. These experiments revealed the presence of elevated levels of phenolic acid in insect infested plants than uninfested plants. Seven individual phenolic acids were monitored by high performance liquid chromatography (HPLC) in pest damaged and normal healthy castor plant leaves and an increase in vanillic, coumaric, ferulic and syringic acids was noted in the insect infested plants. The impact of increased phenolics on pest feeding behaviour and the growth were analysed through antifeedant assays. A significant difference in the modification of phenolic acids between specialist and generalist leaf feeders was found.

**Key words:** *Achaea janata* L, antifeedant, castor, *Dichocrocis punctiferalis*, herbivory, HPLC, phenolics, , *Ricinus communis*, *Spodoptera litura*.

### INTRODUCTION

Plants employ different defensive strategies (including chemical and physical defences) to protect themselves from their attack or to deter the herbivore activities. Many of these have been developed as natural insecticides or have served as templates from which synthetic pest control agents have been derived (9). Among the anti-herbivore chemicals produced by plants, phenolics has to attained main status in recent years as they are often referred to as resistance compounds (5). The term 'phenolics' describes structurally diverse plant secondary metabolites that possess one or more hydroxy groups (OH) bound to an aromatic 6-carbon ring. A correlation of phenolics in plants with herbivory or herbivore performance is a strong evidence of their important role in antiherbivore defence and it has been studied intensely in past.

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Insect herbivory induces many changes in the metabolism of their target plants, which include biosynthesis of plant defense chemicals (14,18), generation of reactive oxygen species (ROS), hydroxy radical (OH<sup>•</sup>) (3), and increased phenolic content (24). Plants accumulating higher phenolics concentrations tend to repress herbivore development (15). Many evidences suggest that phenolic acids limit the herbivore damage based on their activity as deterrents and toxins (20). Applications of pure phenolics on leaves reduced the growth and survival of caterpillars indicating the antifeedant activity of phenolics and also their role in chemical defence (2,12,37). They can inhibit herbivore digestion by producing free radicals, which bind to consumed plant proteins and interferes in their absorption (4).

Castor [*Ricinus communis* (L.) (Euphorbiaceae)], is major crop in dry land areas of Andhra Pradesh, India. The seeds are used in many pharmaceutical preparations, thus having tremendous export value. Castor oil and its derivatives have more than 700 industrial uses (e.g. in paints and varnishes for surface coatings, nylon type synthetic polymers, resins, lubrication for aviation engines, cosmetics textile dyeing, insecticides, and leather industry). Castor semilooper [*Achaea janata* L. (Noctuidae: Lepidoptera)] is regular and serious pest on castor during July-October and the tobacco caterpillar [*Spodoptera litura* F.], a major defoliator of castor is highly polyphagous and occurs throughout India (36). Castor capsule borer [*Dichocrocis punctiferalis* (G.)] is also one of the most destructive pests of castor crop in India (13). The borer attacks various plant parts like shoots, inflorescences and capsules, causing considerable yield losses (28). Pest feeding induces defensive responses affecting the plant physiology and biochemical composition of plants. There are evidences that leaf phenolics increase due to insect-herbivore damage in tomato (*Lycopersicon esculentum* Mill) (11,30). All these lepidopteron pests cause different extents of feeding damage and castor plants responds variable as per the type of herbivore feeding on them, though all are phytophagous in nature (34). Feeding by larvae of *S. litura* and *A. janata* on castor induces polyphenol oxidase activity, which is involved in the formation of phenolics (33).

In this study we evaluated the changes in phenolics profile of castor plant leaves in response to herbivory by three different pests: a specialist, a generalist and a borer pest. The correlation between quantitative and qualitative changes with the mode of feeding as well as the extent of feeding damage by the pests was investigated. A total of seven common phenolic acids were determined using HPLC in pest damaged plants and compared to normal healthy castor plants. Also the impact of ingestion of individual phenolic acids on the feeding herbivore through antifeedant as well as toxicity studies was investigated. In our past studies we reported on the herbivore induced phenolic compounds in castor plants due to the feeding damage by two major pests of castor, *S. litura* and *A. janata* (35). Yet information on individual phenolic acid on physiology and behaviour of the insect herbivores and their role in limiting the herbivore damage is unknown.

## MATERIALS AND METHODS

### I. Castor plants Cultivation

Castor seeds of variety 'Kranthi' were sown in plastic pots (30× 40 cm) at 2 seeds per pot. Pots were arranged in completely randomized experimental design. The

plants were placed in a glass chamber at  $34 \pm 2^\circ\text{C}$  temperature and were fertilized with Osmocote (N/P/K = 14: 14: 14) for the efficient growth. Ideal moisture content in pots was kept by irrigation. Healthy plants of  $60 \pm 5$  d old were used for the experiments.

## II. Insect rearing

The lepidopteran pests, *S. litura* and *A. janata* were from our laboratory colony. The culture has been continuously maintained on fresh castor bean leaves at  $25 \pm 2^\circ\text{C}$  temperature,  $65 \pm 5\%$  relative humidity and 16:8 h photoperiod. Healthy third instar larvae of *A. janata* and *S. litura* were used for the experiments. The cultures of the capsule borer (*D. punctiferalis*) were maintained in the lab fields. The infested leaves were collected from the plants grown in our laboratory campus.

## III. Chemicals

Phenolic acids such as vanillic (4-hydroxy-3-methoxybenzoic acid), *p*-hydroxybenzoic, syringic (*O*-methylated trihydroxybenzoic acid), cinnamic (phenylacrylic acid), ferulic, 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). The water used was treated in a Milli-Q water purification system (Millipore, Bedford, MA).

## IV. Pest feeding and phenolic acid extraction

Ten randomly selected castor plants (2 months old) were brought to the laboratory and a pre-starved healthy 3<sup>rd</sup> instar larvae of *A. janata* and *S. litura* was released separately on terminal leaf of each plant and confined at the point of release by enclosing it in a muslin bag. They were allowed to feed on the leaves for 6 h and the remaining leaf portions after feeding (50-60%) were used for the phenolics assay. The insects were removed after feeding and then the infested leaves were collected at 0, 24, 48, and 72 h after pest feeding. In all the experiments, fresh leaves from the normal and healthy castor plants (without any pest infestation) of similar age were used as controls for comparison. The phenolic extractions were done as per the method described earlier by Jyothsna and Usha Rani (24) with minor modifications. 1 g leaf was weighed and extracted in 95% methanol for 3 d under continuous shaking condition. The solution was filtered and evaporated into dryness by rotavapor. The dried material was re-suspended in 2 mL of HPLC grade methanol and was taken for colorimetric and HPLC analysis.

## V. Determination of Total Phenolics

The amount of total phenolics in leaf extracts was estimated as per the method of Singleton and Rossi, (29). Briefly, 100  $\mu\text{L}$  samples were introduced into test tubes and the volume was made up to 1 mL with distilled water. Then 0.25 mL of Folin-Ciocalteu reagent (1:1 with water) and 2.5 mL of sodium carbonate solution (20%) were added sequentially into each tube. Soon after vortexing the reaction mixture, the tubes were incubated at room temperature for 1 h. The absorbance of the coloured reaction product was measured at 765 nm (TECHCOMP vis 7200 visible spectrophotometer). The total phenolic content was compared with gallic acid as standard and expressed in terms of Gallic Acid Equivalent (GAE).

## VI. HPLC Analysis of individual phenolic acids

Phenolic acids were analysed using HPLC according to the method described by Tuzen (32). The separation of phenolic compounds was accomplished on a Gilson GX-271 semi preparative HPLC system. The column was C<sub>18</sub> (2.5 x 30 cm Gilson apparatus) and a liquid handler with auto injector was employed. For phenolic acid analysis a gradient elution programme was applied and elution was done with solvent A (acetic acid/water (2:98 v/v)) and solvent B (acetic acid: acetonitrile: water (2:30:68 v/v)) as mobile phase. Initial condition was programmed as 100% A; 0–5 min, changed to 100% B; 25–35 min., with a flow rate of 1.0 mL/min and the sample injection volume was 100 µL. The signals were detected at 254 nm. Retention times for the standard compounds and the major peaks in the extract were recorded. Identification and determination of the separated compounds were made by comparison of retention time and structure of UV spectra of the separated compounds and standards. All the experiments were performed in five independent replicates.

## VII. Individual phenolic acids effect on insect feeding behaviour

To understand how the individual phenolic acids may affect the relationship between the foliar quality and herbivore performance as well as to explain the leaf phenolic role in larval feeding, we conducted several antifeedant and toxicity bioassays. Classical no-choice leaf disc assay described earlier by Akhtar *et al.* (1) with slight modifications was employed to determine the antifeedant activity of pure phenolic compounds. Clean glass petri dishes (15 cm dia) were used for study. Fresh leaf discs (10.6 cm<sup>2</sup> area) were cut from foliage of greenhouse-grown castor plants with a cork borer. Different doses (50, 75, 100, 125, 150 and 200 µg/ cm<sup>2</sup> of leaf) of the test materials were sprayed uniformly on both sides of leaf discs at 100 µL per disc. A pre-starved (for about 3 h) actively feeding early 3<sup>rd</sup> instar larvae of *S. litura* and *A. janata* were placed individually into the petri dishes containing the treated and pre weighed castor leaf discs. The control leaf discs sprayed with 100 µL absolute methanol. A small piece of moistened cotton placed inside the petri dish provided the adequate humidification and prevented drying of leaf. The experimental containers were kept in an illuminated growth chamber at 28±2°C. There were 10 replicates for each treatment and all the treatments were repeated on 3 different days to avoid day to day variation if any. The food consumption by each larva was recorded by measuring the leaf area consumed after 24 h of treatment using leaf area meter (Area meter AM 300, ADC Bioscientific Ltd). Uneaten leaf material was also measured in the similar manner.

The antifeedant index (%) was calculated as under:

$$\text{Antifeedant index (\%)} = [(C - T) / (C + T)] \times 100$$

Where, C: Area of leaf discs consumed in control and T: Area of leaf discs consumed in treatment.

To determine the toxicity, oral feeding bioassays were done by the method described above for antifeedant activity. Except that the larvae were allowed to feed on the treated disc for 24 h and the mortality of the larvae and the toxic effects were recorded.

### VIII. Statistical analysis

The differences in the phenolic acid levels between pest infested and normal uninfested plants were calculated from HPLC chromatograms and were analyzed using paired t-test at  $p < 0.05$ . All the data were presented as mean  $\pm$  SD

## RESULTS

Quantitative analysis of the phenolics showed significant variation among the plants damaged by pest attack and normal healthy plants. The pest feeding resulted in statistically significant increases in the concentrations of total phenolic components and individual phenolic acids. The pure phenolic compounds showed significant impact on the larval physiology and feeding behaviours, a few of them being antifeedant while some of them were extremely toxic to the insect herbivores.

### Total phenolic content assay

The quantitative estimation of total phenolic content was done by colorimetric assay and is expressed as GAE. A significant difference was observed in the levels of total foliar phenolics, which were amplified in herbivore damaged castor plants. A maximum increase of 0.986 mg/mL GAE was recorded in 48 h duration in *A. janata* infested plants (Figure 1), thereafter a slight decrease in 72 h post feeding. In *S. litura* infested plants, an increase of 0.358 mg/mL GAE was observed in 24 h after damage (Figure 1) and there was a decrease in phenolic acid content after 24 and 48 h respectively. However the increase in total phenolic content was comparatively less in the castor leaves infested with *S. litura* than that of *A. janata* damaged leaves. This quantitative difference in phenolics has also occurred due to capsule borer, *D. punctiferalis* infestation (Fig.1). In healthy and uninfested castor plants, 0.7635 mg/mL GAE of foliar phenolic content was observed, whereas in *D. punctiferalis* infested plants; the level of leaf phenolics has been increased to 1.124 mg/mL GAE.

### HPLC analysis of individual phenolic acids

The leaf extracts of castor plants damaged by 3 different herbivore pests were analysed by HPLC for the changes in seven common phenolic acids and were compared with the standard chemicals for confirmation. A significant difference in quantities of certain phenolic acid contents was recorded due to herbivory by all the 3 castor pests tested and this depended on the type of feeding by that particular pest.

A quantitative enhancement of the ferulic, vanillic, syringic and coumaric acids were recorded while a decrease in cinnamic acid was evident in the castor plant leaves damaged by *A. janata* larvae. It is interesting to note that the caffeic acid has increased until 24 h and *p*-hydroxybenzoic acid until 12 h after *A. janata* feeding, but disappeared 48 h and 24 h after the commencement of feeding by the larvae respectively. Individual phenolic acid profile at different intervals after *A. janata* feeding showed that coumaric and ferulic acids increased up to 48 h followed by a slight decrease before returning to the normal levels while vanillic and syringic acids increased till 72 h (Table 1).

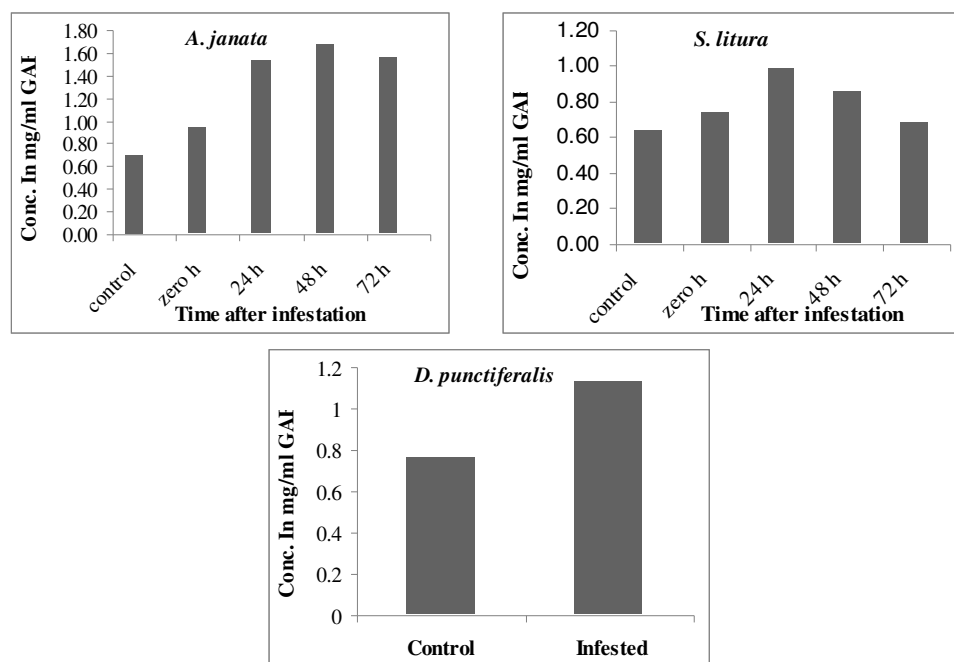


Figure 1. Total phenolic content in castor leaves in response to insect feeding by three major pests

Table 1. Individual phenolic acid profile extracted from the leaves of castor plant infested by *A. janata*

Phenolic compounds	Hours after infestation				
	Control	0	24	48	72
<i>p</i> -Hydroxy benzoic acid	1.40±0.15	1.02±0.15	*	*	*
Vanillic acid	3.84±0.29	5.61±0.36	10.76±0.45	13.72±0.12	16.13±0.52
Caffeic acid	0.61±0.11	1.16±0.14	1.72±0.20	*	*
Coumaric acid	9.66±0.39	12.99±0.40	22.34±0.41	25.63±0.47	21.62±0.40
Cinnamic acid	9.64±0.55	6.22±0.34	6.17±0.40	5.57±0.43	4.22±0.07
Ferulic acid	1.49±0.27	2.39±0.21	7.47±0.34	16.73±0.26	9.79±0.26
Syringic acid	1.33±0.23	2.91±0.36	5.37±0.37	6.16±0.40	9.53±0.34

Data in the table are compound weight in µg/gram fresh weight of leaf. \* indicates the compound was not detected.

Similar study with the *S. litura* larval feeding indicated a rapid defensive response from the castor plant causing a highest increase of phenolic content at 24 h after infestation. Cinnamic and *p*-hydroxybenzoic acids decreased with increase in time after *S. litura* damage, whereas vanillic and coumaric acids were maximum at 24 h after *S. litura* feeding. However, syringic and ferulic acids were increased at 48 h after feeding by *S. litura* larvae, while no significant changes were observed in caffeic acid levels (Table 2).

Table 2. Quantitative differences in individual phenolic profiles of leaf extracts infested by *S. litura*.

Phenolic compounds	Hours after infestation				
	Control	0	24	48	72
<i>p</i> -Hydroxy benzoic acid	1.44±0.23	0.685±0.04	0.486±0.06	*	*
Vanillic acid	3.564±0.031	4.041±0.37	6.470±0.047	6.460±0.046	6.480±0.042
Caffeic acid	1.472±0.30	0.754±0.08	0.880±0.08	0.852±0.50	0.745±0.14
Coumaric acid	7.579±0.32	9.755±0.43	13.207±0.47	12.056±0.56	8.567±0.48
Cinnamic acid	8.157±0.39	4.522±0.45	5.096±0.43	2.511±0.33	1.788±0.24
Ferulic acid	1.214±0.18	1.464±0.10	4.420±0.46	5.864±0.45	3.872±0.34
Syringic acid	1.790±0.28	2.131±0.30	3.091±0.28	3.094±0.40	3.618±0.51

Data in the table are compound weight in µg/gram fresh weight of leaf. \* indicates the compound was not detected.

The assessment of individual phenolic acids in uninfested leaves of castor plants infested by capsule borer, *D. punctiferalis*, it was observed that caffeic, coumaric and cinnamic acid levels decreased, while those of vanillic, syringic and ferulic acids increased (Fig. 2). The quantities of *p*-hydroxybenzoic acid remained constant in *D. punctiferalis* infested plants where they are comparable with that of the quantities of normal healthy plants.

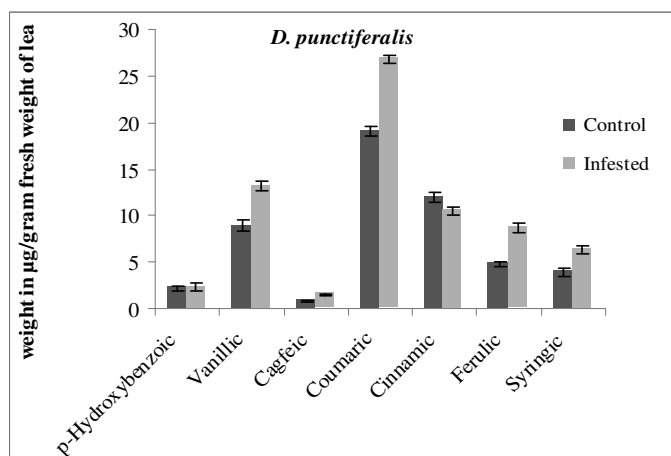


Figure 2. Individual phenolic acid profile in uninfested leaf extracts of castor plant infested by *D. punctiferalis*

From the results on antifeedant bioassays, it is obvious that though both *A. janata* and *S. litura* are leaf tissue chewing insects, the larval responses towards the phenolics differed. The castor leaves surface treated with gallic, caffeic, *p*-hydroxybenzoic and chloro-benzoic acids showed good antifeedant activity to the 3<sup>rd</sup> instar larvae of semilooper pest, *A. janata*. Whereas, vanillic and coumaric acids were less active as antifeedants. The syringic and cinnamic acids were toxic to these larvae resulting in their

death. Antifeedant index of pure phenolic compounds against *A. janata* and *S. litura* were shown in Figure 3. On contrary, vanillic, caffeic, syringic and cinnamic acids exhibited high antifeedant activity to the tobacco caterpillar, *S. litura* larvae, while chloro-benzoic, *p*-hydroxybenzoic and gallic acids were less active and the coumaric acid exhibited moderate antifeedancy to *S. litura*. Only caffeic acid was an effective feeding deterrent to both the leaf feeding pests. It is interesting to note that *A. janata* caterpillar was able to tolerate high dosage (200 $\mu$ g/cm<sup>2</sup> of leaf) of pure phenolics than *S. litura* larvae (100  $\mu$ g/cm<sup>2</sup> of leaf).

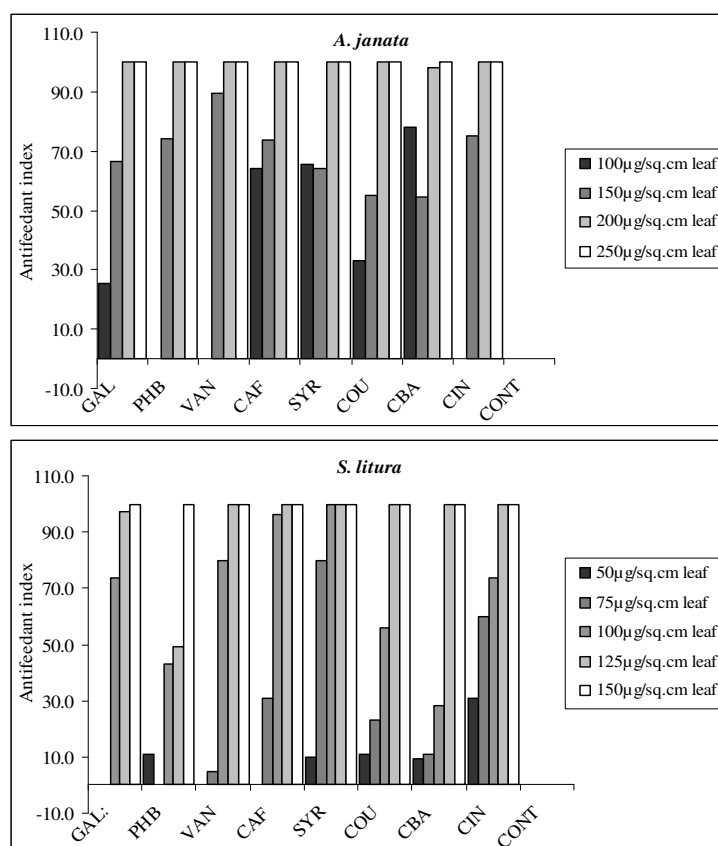


Figure 3. Antifeedant index of pure phenolic acids against two major pests of castor, i.e. *A. janata* L. and *S. litura* F. (GAL: gallic acid, PHB: *p*-hydroxybenzoic acid, VAN: vanillic acid, CAF: caffeic acid, SYR: syringic acid, COU: coumaric acid, CBA chloro-benzoic acid, CIN: cinnamic acid and CONT: control)

## DISCUSSION

Phenolic acids are structurally diverse class of phytochemicals, which play major roles in plants protection against herbivore attack (17). In regard to insect-pests, phenolics act as digestion inhibitors, antioxidants and reacting oxygen species by producing free radicals (4). Total amount of phenolic acids (presented as sum of phenolic compounds) was the lowest in control plants and the increase was observed after insect's infestation. A similar result of low level free phenolics was observed in control soybean plants and the increase in their content after exposure to stress factors (23)

From the quantitative analysis of foliar phenolics, an increase was observed in *A. janata* infested plants. In these plants a gradual increase in phenols was recorded in 24 h to 48 h followed by decline after 72 h. On the other hand a maximum increase in foliar phenolics was observed 24 h after *S. litura* feeding indicating a rapid response. The increase in quantity of total phenols might be attributed to defense mechanism (22). The increase in phenolics in relation to resistance was reported in *Brassica* by Singh, (27). In our earlier studies we reported the higher peroxidase activity in castor plant leaves fed by the third instar larvae of *A. janata* (24). Increased peroxidase activity is associated with the resistance reaction which could be due increased phenolic acid concentration.

*A. janata* popularly called as castor semilooper was specific pest on castor. Third and fourth instar larvae voraciously fed on castor leaves and tender branches causing extensive defoliation (37). By comparing the increase in level of foliar phenolics in case of all the three pests, it can be demonstrated that the feeding of *A. janata* induced higher amount of foliar phenolics i.e. early 1.5 times more than the levels recorded in control plants whereas in case of *S. litura* infested plants 0.5 times increase was observed. It was only 0.47 times increase in case of *D. punctiferalis*. From the analysis of total phenolics, it was observed that increase in phenolic acids is comparatively higher in case of *A. janata* infested plants than *S. litura* and *D. punctiferalis* infested plants. Perhaps the reason might be the plant had increased responses towards toward the specific pest. *S. litura* is a polyphagous pest that can feed on any plant, and *A. janata* being monophagous has restricted feeding on a limited species of plants (34).

It is interesting to note that the induction of higher phenolic production was greater in leaf feeding larvae than the fruit borer. This can be correlated to the direct leaf tissue damage by both *A. janata* and *S. litura*, whereas, the tissue damage to the leaf does not occur due to *D. punctiferalis* attack, as it feeds only on the tissue of castor plant capsules/fruit. It is known that fruit or stem borer pests too induce systemic chemicals in the entire plants on which they feed which cause changes in the uninfested leaves of the borer infested plants.

Earlier reports indicate that mode of feeding plays an important role in inducing plant responses (33). The difference in the increased levels of phenolic acids in case of three insects might be due to the difference in their feeding modes. *A. janata* is avid feeder on castor plant and specific pest on castor plant whereas *S. litura* is a polyphagous pest that feeds on castor on availability. On the other hand *D. punctiferalis* is a capsule borer and damages the capsules. From the results it is noted that the uninfested leaves from the capsule borer infested plants also brings changes in leaf phenolics, but the change was less when compared with the control. The variation in the mode of feeding by each of 3 insects

caused a variation in plants response too and the results were obvious. Also the distance between the wound area (herbivore damage) and the plant response (leaves) is greater in case of *D. punctiferalis*. This also might be one of the reasons.

Duration of feeding by pest also has profound impact on phenolic production. Though the duration of feeding or the damage extent is same in both the leaf feeders, the differences in time of production and availability of phenolic acids in the leaf tissue were observed. Phenolic acid levels in *A. janata* infested leaf extracts were maximum at 48 h after infestation and remained above the control levels for 72 h of post infestation. In case of *S. litura* damaged leaf extracts, foliar phenolics were recorded maximum at 24 h after feeding and decreased nearly to control levels at 72 h after damage.

Individual phenolic profiles from *A. janata* infested leaf extracts suggest that the quantities of vanillic, coumaric, ferulic and syringic acids increased, while cinnamic acid decreased. On the other hand *p*-hydroxybenzoic acid was noticed only in control and in infested plants at zero h. While caffeic acid increased up to 24 h after feeding signifying its role in biosynthesis of lignin, a major component of cell wall and it was not present at 48 and 72 h after feeding. Interestingly, in case of *S. litura* infested plants, the presence of caffeic acid was recorded even after 72 h after infestation. Among the seven phenolic acids monitored coumaric acid was maximum followed by ferulic acid in case of *A. janata* as well as in *S. litura*. According to Santiago *et al.*, (25) the amount of free *p*-coumaric acid was correlated with the resistance level in the important pest of maize, *Sesamia nonagrioides* (Lefebvre).

HPLC results suggest that there is a rapid decrease in cinnamic acid and increase in other acids like ferulic, vanillic and coumaric acids. Thus it can be proposed that cinnamic acid produced in response to insect herbivory is converted into other phenolic acids like coumaric, ferulic and vanillic acids and are employed in controlling the herbivore activity. Cabrera *et al.* (8) stated that ferulic acid used as a resistance factor or incorporated into artificial diets decreased aphid survival and reduced reproductive index of the green bug (*Schizaphis graminum*). Caffeic acid is one of the phenolic acids, which play a major role in biosynthesis of lignin (6) and is a precursor to ferulic acid. Coumaric, caffeic and ferulic acids are central intermediates of lignin biosynthesis. Individual phenolic acid profiles showed an increase in ferulic acid and approximately constant levels of caffeic acid. Thus increase in caffeic acid will be correlated with lignin biosynthesis. Lignin and other phenolics can strengthen cell walls and therefore can be anti-nutritional (7, 26)

It is interesting to study the effects of different phenolic acids on the feeding herbivore growth, mortality and behaviour. The results on antifeedant bioassays indicated a significant interaction between the plant feeding and herbivore performance. Herbivory modified the leaf chemical structure thus affecting the leaf suitability to larvae. Ingestion of gallic, caffeic, *p*-hydroxybenzoic acids deterred the feeding of the lepidopteran *A. janata*. The pure phenolic acids, vanillic, caffeic, syringic and cinnamic acids too modified the feeding behaviour of the bioassay larvae of *S. litura* when exposed to the treated leaf disc. It is noteworthy that compounds with less toxicity and having antifeedant properties like PHB, vanillic, and coumaric acids were increased due to infestation by *A. janata* larvae. Furthermore it can be noticed that cinnamic acid which produced toxicity at a lower dosage was maximum at zero h after infestation. Summers and Felton (31) proposed that the induction of oxidative stress may be an important component of phenolic

toxicity in lepidopteran larvae. Ferulic acid has the strongest oviposition deterrent to the spruce budworm, *Choristoneura fumiferana* (Lepidoptera: Tortricidae) (16). Induced accumulation of phenyl propanoids in response to insects feeding was reported, especially for the ferulic and *p*-coumaric acids in wheat tissues as a response to *Sitodiplosis nonagrioides* (10).

Chlorogenic acid and rutin, major phenolic constituents inhibit early larval growth of the fruit worm, *Heliothis zea* when added to an artificial diet for this insect (21). When the tomato fruit worm *Heliothis zea* or the beet army-worm *Spodoptera exigua* feed on tomato foliage, a substantial 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 proposed to result from the alkylation of amino acids or proteins by o-quinones and subsequent reduction in the nutritive quality of foliage. The cinnamic acid derivatives like chlorogenic acid and rutin represent model phenolics in the study of plant antiherbivore defense due to their ubiquitous occurrence among terrestrial plants and well documented-toxicity to insect herbivores. (21, 19)

The results presented in this work show much higher biosynthesis of phenolic acids in castor plant leaves after insects feeding. Thus it can be suggested that feeding of the herbivorous insects induce antibiosis based on accumulation of the phenolic acids in studied castor plants. In summary, the 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 larval performance and mortality.

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