

## Growth disruptive effects of ferulic acid against *Spodoptera litura* (Fabricius) and its parasitoid *Bracon hebetor* (Say)

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### ABSTRACT

We studied the effects of ferulic acid (5, 25, 125, 625, 3125 ppm concentrations) in artificial diet on *Spodoptera litura* (Fabricius) and its parasitoid *Bracon hebetor* (Say) on 6-days old larvae of *S. litura*. Higher concentrations of ferulic acid increased the mortality of *S. litura* larvae, decreased adult emergence and delayed the developmental period. The higher concentration (LC<sub>50</sub>= 2573.33ppm) of ferulic acid also decreased the growth of *B. hebetor* (Say) larvae. However, the adverse effects were less at lower concentration (LC<sub>30</sub>=25.92 ppm). The ferulic acid significantly reduced the nutritional indices (RGR, RCR, ECD and ECI) and immune response in *S. litura* larvae. Our findings suggested that lower concentrations of ferulic acid may help in controlling insect-pest population and conserve the parasitoid's fitness.

**Keywords:** *Bracon hebetor*, Ferulic acid, haemocyte, larval, nutritional indices, Phenols, *Spodoptera litura*, tritrophic.

### INTRODUCTION

Insect-pests cause huge losses in crops despite too much use of chemical pesticides (38). Botanical insecticides as an alternative to organic pesticides are being considered for pest management due to their low impact on non-target organisms, minimal residual environmental effects, fast degradation in field conditions and low toxicity for humans (27,39). Thus, recently there is an increased interest in their development especially in the developing countries (India, China and Brazil) and other emerging economies (15). Plants synthesize allelochemicals that are feeding deterrent to insects and thereby regulate the insect population (24,53). Plant phenolics, hydroxybenzoic acids viz., gallic acid, hydroxyl-cinnamic acids found in grape juice are used by plants for pigmentation, growth, reproduction, resistance to pathogens and insect herbivores etc. (23). Plant secondary metabolites such as ferulic acid, chlorogenic acid, quercetin and rutin are inhibitory to insect pest, *Spodoptera litura* (Fabricius) (22,50). The lepidopteran pest, *S. litura* is a polyphagous pest widely responsible for damage to many important crops (31,47). The economic losses caused by it range from 25.8-100 % depending on crop stage and its infestation level (12). The unjudicious use of chemical pesticides and insecticide resistance has resulted in its frequent outbreak (44,52).

*Bracon hebetor* (Say) (Hymenoptera: Braconidae) is a cosmopolitan, gregarious, ectoparasitoid that attacks the larval stage of various species of lepidopterans. The parasitoid has been used to control the population of cotton bollworm, *Helicoverpa armigera* (Hubner) and the Indian meal moth, *Plodia interpunctella* (Hubner) (7,21).

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One of the central themes in ecology is the interaction between the population of consumers (predators, parasitoids) and resources (prey, host). The importance of nutritional suitability of the hosts of the phytophagous insects for the successful development of parasitoids has now received an increased attention (1,29,32). The feeding, growth and development of the pest population greatly affects the tritrophic interactions between plants, herbivores and their natural enemies and can be altered by the quality of host plant (13,16,34). In the ecological system, allelochemicals in food chain not only affect the parasitoids, pathogens and predators but also influence the capability of these natural enemies to impact and alter the herbivore fitness and population growth. There are very few studies determining the effects of botanical compounds on parasitoids in the third trophic level. Plant allelochemicals in the diet of host not only adversely affects the growth of phytophagous insect but also impacts the parasitoid's growth and survival (5,40). Thus, before considering these compounds for pest management, it is essential to study their effects not only on the insect pest but also on its parasitoids as the latter play a key role in limiting insect pest population. Therefore, this study aimed to investigate the effects of ferulic acid (a plant allelochemical) on the tobacco cutworm, *S. litura* and to explore its potential in pest management programmes and also on its parasitoid *B. hebetor*.

## MATERIAL AND METHODS

The study was done from April 2019 to June 2019 in Department of Zoology, Guru Nanak Dev University, Amritsar, Punjab, India (longitude 31.6340° N, and latitude 74.8723° E, Annual rainfall: 703 mm, minimum temp.: 3.9 °C and maximum temp: 43 °C). The cultures of *S. litura*, *B. hebetor* and rice moth, *Corcyra cephalonica* (Stainton) were maintained in laboratory at standard conditions [25±2°C temperature, 65±5% relative humidity (RH) and 12:12 (D: L) photoperiod].

### *S. litura* culture

The culture of *S. litura* larvae was maintained on fresh castor (*Ricinus communis* L.) leaves, in Insect Physiology laboratory. The pupae were kept in pupation jars (15 cm×10 cm) having 4-5 cm of moist sand covered with filter paper until the adult emergence. The adults were transferred to oviposition jars (15 cm×10 cm) lined with filter paper to facilitate egg laying, and a cotton swab soaked with water and honey solution (4:1) as food. All the jars were covered with muslin cloth, secured with rubber band to check their escape.

### *B. hebetor* culture

The culture of parasitoid, *B. hebetor* was maintained on 5<sup>th</sup> instar larvae of *C. cephalonica*. The culture of *C. cephalonica* was reared on partially crushed sorghum grains at standard conditions (25±2 °C, 65±5 % RH). The freshly emerged adult parasitoids in ratio 1:2 (male:female) were transferred to glass chimneys having a cotton swab soaked in water and honey (4:1) solution to serve as food. Healthy 5<sup>th</sup> instar larvae of *C. cephalonica* were provided to the parasitoid for parasitization and parasitized larvae were observed for egg laying under microscope. The parasitized larvae were then kept inside tissue papers in plastic petri plate (90 mm×15 mm) until cocoon formation. The tissue papers having cocoons were shifted into sterile solo cups (4 cm × 6 cm) and were daily checked for

emergence, and the emerged adults were allowed to mate for 24 h. These newly emerged *B. hebetor* adults were used for bioassay studies.

#### Chemical used

Ferulic acid with  $\geq 95$  % purity was obtained from Sigma Aldrich Pvt. Ltd., India and used in this study.

#### Bioassays with *S. litura*

Stock solution of ferulic acid (4000 ppm) was prepared in 0.5 % ethanol, which was diluted to get the experimental concentrations of 5, 25, 125, 625 and 3125 ppm. The antibiosis effects of ferulic acid on *S. litura* were recorded in 6-days old larvae fed on diet amended with 5, 25, 125, 625 and 3125 ppm of ferulic acid. Diet without ferulic acid was taken as control. The artificial diet was prepared according to protocol of Koul *et al.* (19). The larvae were kept in sterilized plastic containers (1 larva per container) containing treated diet and were observed daily for different parameters viz., larval period, total development period, larval mortality, pupal weight and adult emergence. There were 5-test larvae for each replicate and each experiment had six replicates. The experiment was repeated twice. The  $LC_{50}$  and  $LC_{30}$  calculated on the basis of larval mortality were 2573.33 ppm and 25.92 ppm concentrations, respectively.

#### Nutritional assays with *S. litura*

A 3-days experiment was done to find the effects of ferulic acid at 5, 25, 125, 625 and 3125 ppm concentrations with control (water) on nutritional physiology of *S. litura* larvae using 6-days old larvae as per the protocol of Koul *et al.* (16). The pre-weighed larvae were released into sterilized plastic containers with treated and control diet. The larvae were fed on treated and control diet for 72 h and the final weight of larvae, diet left and fecal matter (mg) was recorded. Their dry weights were also taken after incubating for another 72 h at 60 °C inside an incubator. The dry weight readings served the purpose of water loss under controlled conditions. The data obtained was also used to calculate the nutritional indices on dry weight basis after 3 days feeding as per Waldbauer (55). Each concentration including control was replicated 6 times with 5 larvae in each replicate and the experiment was repeated twice.

$$RGR = \frac{\text{Change in larval dry weight/day}}{\text{Starting larval dry weight}}$$

$$RCR = \frac{\text{Change in diet dry weight/day}}{\text{Starting larval dry weight}}$$

$$ECI = \frac{\text{Dry weight gain of insect}}{\text{Dry weight of food ingested}} \times 100$$

$$ECD = \frac{\text{Dry weight gain of insect}}{\text{Dry weight of food ingested} - \text{Dry weight of frass}} \times 100$$

$$AD = \frac{\text{Dry weight gain of insect} - \text{Dry weight of frass}}{\text{Dry weight of food ingested}} \times 100$$

Where, RGR: Relative growth rate, RCR: Relative consumption rate, ECI: Efficiency of conversion of ingested food, ECD: Efficiency of conversion of digested food, AD: Approximate digestibility.

#### **Immune response of *S. litura***

To evaluate the effects of ferulic acid on immune response of *S. litura*, larvae were fed on diet supplemented with LC<sub>30</sub> (25.92 ppm) and LC<sub>50</sub> (2573.33 ppm) concentrations for 24, 48, 72, 96 and 120 h. The treated larvae and the larvae fed on control diet were kept at standard conditions of temperature and humidity (25±2 °C and 65±5 %, respectively). For each treatment interval the haemolymph was collected using a sterile needle from randomly selected 10-larvae by piercing the prothoracic leg. The haemolymph collected was pooled and used to study the total haemocyte count (THC) and deformities in haemocytes as per the protocol of Tauber and Yeager (51) and Arnold and Hinks (3), respectively. All the experiments for immune response were replicated twice.

#### **Bioassays with *B. hebetor***

To study the effects of ferulic acid on parasitization and fecundity of *B. hebetor*, newly emerged parasitoids were allowed to mate for 24 h. The adults in the ratio 2:1 (female: male) were then transferred to a glass chimney. The larvae of *S. litura* were reared on diet supplemented with LC<sub>30</sub> (25.92 ppm) and LC<sub>50</sub> (2573.33 ppm) concentrations of ferulic acid as well as on control (unamended diet) until it reached the third instar stage. A single treated 12-days old larva was then introduced for parasitization in the chimney for 24 h and then transferred to petri plates (90 mm×14 mm) on tissue paper. The paralyzed larvae were observed daily under stereo microscope (Magnus) at 40X magnification to observe the parasitoid eggs for recording various parameters. In this way, *S. litura* larvae were fed artificial diet containing various concentrations of ferulic acid till the female died in each treatment. The effects of ferulic acid on development of *B. hebetor* was evaluated by introducing 2-days old mated females in the glass chimneys (2-wasps per chimney) and allowed to oviposit on larvae of *S. litura* reared on artificial diet amended with different concentrations of ferulic acid. After 24 h, the parasitized larvae were removed from the chimneys and fresh hosts were given daily to the wasps. The parasitized larvae were transferred individually in petri plates and were checked daily for development of parasitoid larvae. Cocoons of the parasitoid were inspected daily to assess time of emergence of wasps. On emergence, the number and sex ratio of the progeny were recorded. There were 6-replications with two females per replication and a total of 60 larvae were exposed to parasitoid wasps in each treatment and control.

#### **Statistical analysis**

The data were represented as their means ± SE. The one-way analysis of variance (ANOVA) along with Tukey's test at  $p \leq 0.05$  was done to check the differences in means. SPSS software for windows version 16.0 (SPSS Inc, Chicago), Microsoft office Excel 2007 (Microsoft Corp., USA) and ASSISTAT software were used to perform the statistical analysis.

## RESULTS AND DISCUSSION

### EFFECTS ON *S. LITURA*

#### (i). Growth and development

The effects of ferulic acid on the growth, development and nutritional physiology of *S. litura* larvae are shown in Figures 1 and 2. The mortality was high in treated larvae and maximum mortality was observed at 3125 ppm, (36.66 % increase in larval mortality over control) (Fig. 1). Similarly, high mortality was also observed at higher concentrations of quercetin in Egyptian cotton leafworm, *Spodoptera littoralis* (Boisd.) (26). A significant inhibition in adult emergence occurred in all treatments (Fig. 1). Another phenolic compound, gallic acid too increased the larval mortality and reduced the adult emergence of melon fruit fly, *Bactrocera cucurbitae* (Coquillett) with increase in concentration (46). The treatments significantly prolonged the larval period and the total development period when compared with control. Maximum prolongation was noticed at the highest concentration (Fig. 1).

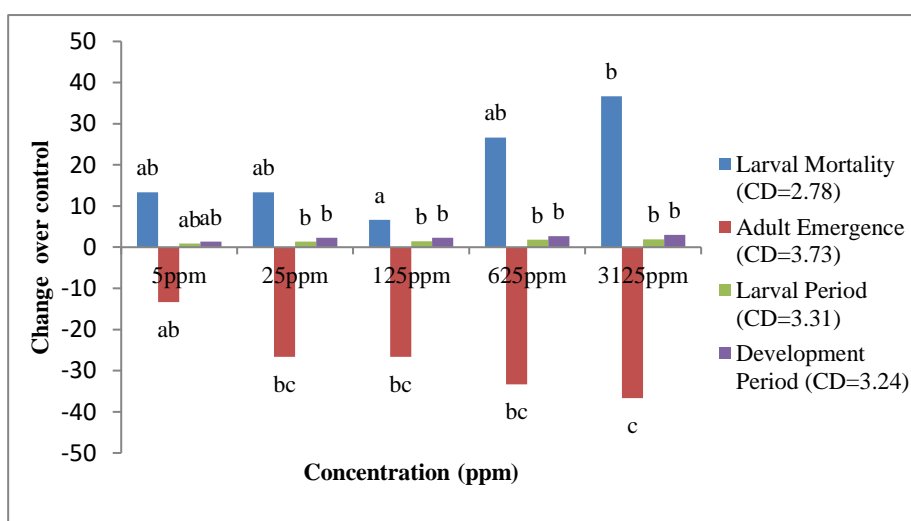


Figure 1. Effects of ferulic acid diet on the 6-days old instar larvae of *S. litura* on larval mortality, adult emergence, larval period, and total developmental period with respect to control. CD= Critical difference at 1%.

A concentration dependent increase in larval mortality, larval period and total development period of *S. litura* larvae was also reported with pyrogallol (8). Similar findings were reported by Alves *et al.* (2) in fall army worm, *Spodoptera frugiperda* (J.E. Smith) treated with the phenolic acids (gallic acid, catechin, gallic acid, epicatechin, ellagic acid, and salicylic acid) isolated from jaboticaba, *Myrciaria cauliflora* (Mart.). Phenolic compounds (hydroquinone, chlorogenic acid, gallic acid and syringic acid) present in the seed coat extract of red gram too suppressed the growth and development of hairy caterpillar, *Creatanotos gangis* (L.) (9). Phenolic compounds can act as gut toxins causing mortality in

insects. Steinly and Berenbaum (49) reported that tannin forms degenerative lesions in the gut of swallowtail caterpillar, *Papilo polyxenes* (F.). Lindroth and Peterson (20) also reported that the southern armyworm, *Spodoptera eridania* (Stoll) larvae fed on phenols (salicortin and tremulacin) developed degenerative lesions in the tissues of midgut.

### (ii). Nutritional physiology

The ferulic acid significantly affected the nutritional physiology of *S. litura* larvae (Fig. 2). The relative consumption rate significantly declined with all concentrations of ferulic acid and maximum decline (36.15 %) occurred at 125 ppm compared to control (Fig. 2). The RGR (relative growth rate) of treated larvae slightly declined in comparison with control. Ganter *et al.* (14) had also reported low level acceptance of filbert aphid, *Myzocallis coryli* Goetze for the leaves of hazel (*Corylus* L.) cultivars rich in phenolic acids (gallic acid and caefferic acid). In ferulic acid treated larvae, the ECI (Efficiency of conversion of ingested food) and ECD (Efficiency of conversion of digested food) were higher than control at all concentrations except at 3125 ppm, where they were significantly lower than control. On the other hand, the AD (approximate digestibility) was reduced at all concentrations except at 3125 ppm, where it was higher than control (Fig. 2). These findings indicated that ferulic acid was not toxic to insect at lower concentrations but the highest concentration induced post ingestive toxicity in larvae. ECI is the measure of the food consumed, that is metabolized into biomass and its decreased value indicates that only limited amount of food is converted into biomass and rather it might have been metabolized for energy (10). The decreased value of ECD suggested the diversion of energy from producing biomass into detoxifying the compound (11). Wang *et al.* (57) reported negative impact of gramine and

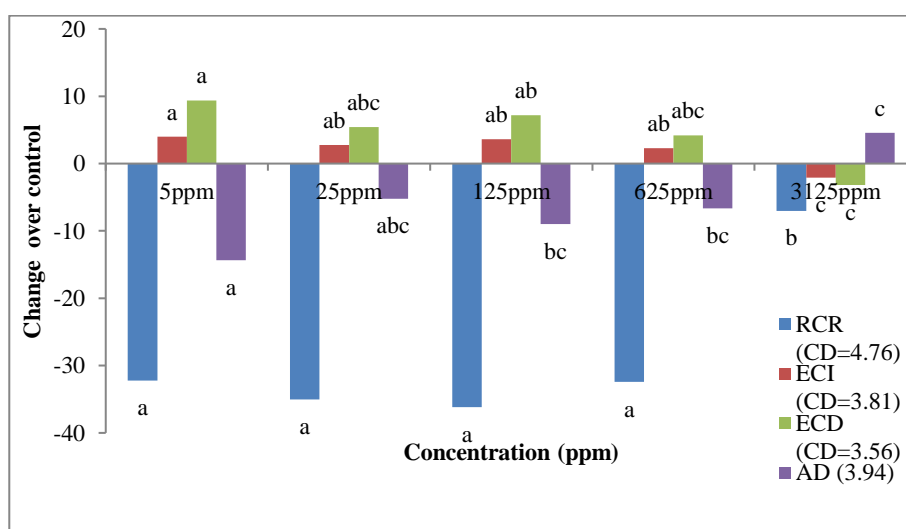


Figure 2. Effects of ferulic acid diet on the 6-days old instar larvae of *S. litura* on RCR (Relative growth rate), ECI (Efficiency of conversion of ingested food), ECD (Efficiency of conversion of digested food), AD (Approximate digestibility).  
CD= Critical difference at 1%.

ferulic acid on the nutritional physiology of the cotton bollworm as the RGR, AD and ECD declined with treatment. Similar findings were reported in southern armyworm, *Spodoptera eridania* (Cramer) larvae treated with tremulacin (20) and in white marked tussock moth, *Oryzia leucostigma* (L.) treated with condensed tannins obtained from paper birch, *Betulla papyrifera* (Marshall) (18). Reitz and Trumble (41) too had reported that linear furanocoumarin increased the mortality in cabbage looper, *Trichoplusia ni* (Hubner) and parasitoid *Copidosoma floridanum* (Ashmead) along with decreased RCR of *T. ni*. The decreased AD values suggest poor digestibility of insect.

### (iii). Immune response

The total haemocyte count in *S. litura* larvae fed on diet amended with LC<sub>30</sub> and LC<sub>50</sub> concentrations of ferulic acid declined significantly than control in all treatment intervals (Fig 3a). At LC<sub>50</sub> concentration, the haemocyte count was much lower than at LC<sub>30</sub> concentration in all treatment intervals. The percent haemocyte deformities calculated at different time intervals from 24 h to 120 h were significantly increased over control (Fig. 3b). Various types of morphological deformities (change in haemocyte shape, aggregation, multinucleated cells, vacuolization, necrosis etc.) were observed (Fig. 4a, b).

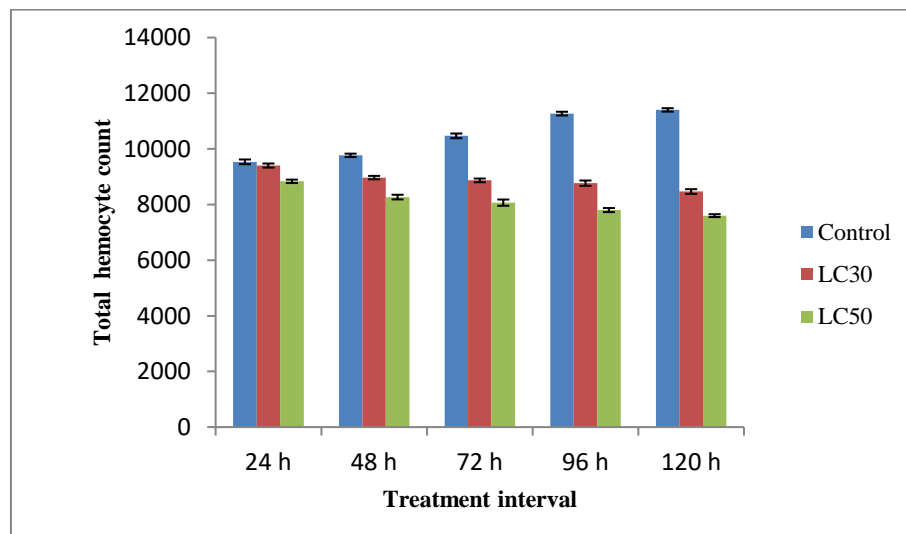


Figure 3a. Influence of ferulic acid on total haemocyte count (Means±S.E.) of *S. litura* larvae.

These findings suggested the negative impact of ferulic acid on *S. litura*'s immune response. A lower immune response weakens the insect's defense against its parasitoids and pathogens. Haemocyte deformities as well as dose dependent decrease in total haemocyte count of *S. litura* larvae occurred after treatment with essential oil of sweet flag, *Acorus calamus* L. rhizomes (45). Azadirachtin caused 20-25 % decrease in total haemocyte count in last instar nymphs of American cockroach, *Periplaneta americana* L. (37). It also declined (31-41 %) the total haemocyte count in final instar female nymphs of brown spotted locust, *Cyrtacanthacris tatarica* L. (32). Zibae and Bandani (59) also reported decrease in

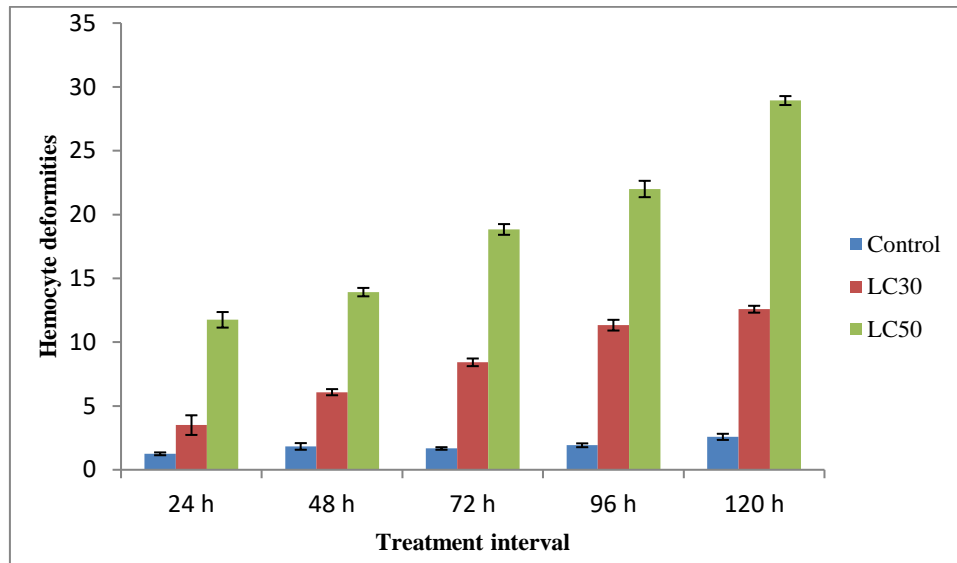


Figure 3b. Influence of ferulic acid on morphological deformities (Means  $\pm$  S.E.) in *S. litura* larvae.

the total and differential haemocyte count in adult Sunnpest, *Eurygaster integriceps* (Puton), when treated with *Artemisia annua* L. extract. Crude methanolic extract of *Pergularia tomentosa* (L.) also decreased the haemocyte count and caused cell lysis in migratory locust, *Locusta migratoria* (L.) (26).

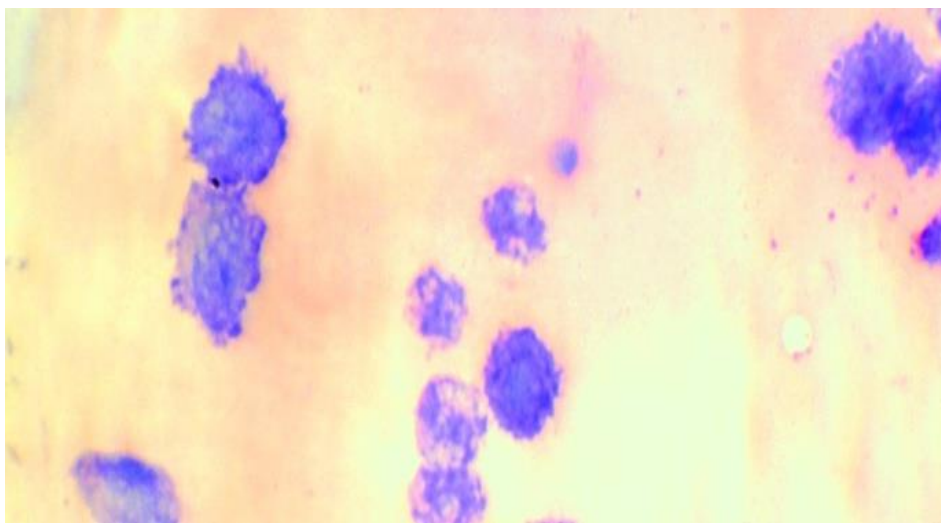


Figure 4a. Vacuolization and disintegration of plasma membrane in haemocytes in ferulic acid fed larvae.



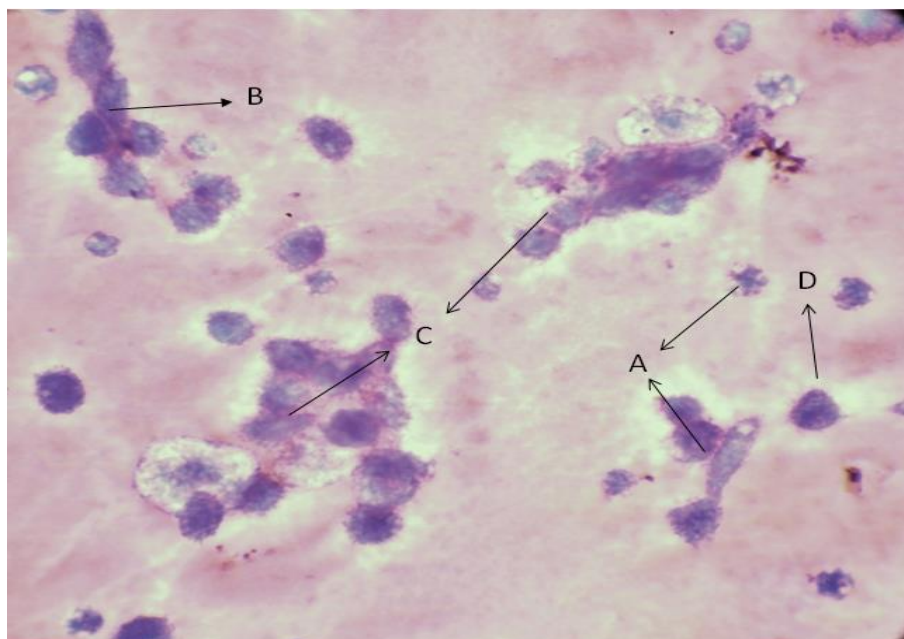


Figure 4b. Haemocyte deformities in *Spodoptera* larvae. A. Deformed haemocytes B. Change in shape of haemocytes C. Clustering of haemocytes D. Normal haemocyte

#### EFFECTS ON *B. HEBETOR*

The LC<sub>50</sub> concentration of ferulic acid adversely affected the various growth parameters of *B. hebetor*, while LC<sub>30</sub> concentration had much less effect than control. The mortality of *B. hebetor* larvae at LC<sub>50</sub> concentration was significantly higher than at LC<sub>30</sub> concentration as compared to control (Fig. 5). Significantly less number of larvae hatched at LC<sub>50</sub> than at LC<sub>30</sub> in comparison to control. At the higher concentration (LC<sub>50</sub>), the adult emergence was reduced by 13 % and the female emergence was reduced by 20.36 % than control (Fig. 5). The cocoon period, larval period and the total development period of *B. hebetor* larvae was not much affected with treatment. There was no significant effect of ferulic acid on percent parasitization and the fecundity of *B. hebetor* but the eggs laid per host declined significantly with increase in concentration than control (Fig. 5).

Plant allelochemicals in the diet not only influence the fitness of host but also affect the development of parasitoid (48). The present study revealed that the parasitization, fecundity, cocoon period and the larval as well as total development period was not much affected in the parasitoid, *B. hebetor* when provided with host *S. litura* treated with LC<sub>50</sub> (2573.22ppm) and LC<sub>30</sub> (25.92 ppm) concentrations of the test compound. On the other hand, larval mortality, egg hatching and emergence were significantly affected with ferulic acid treatment. Barbosa *et al.* (4) reported that plant alkaloids (nicotine and hordenine) in the diet of tobacco horn worm negatively affected its survival, increased larval mortality,

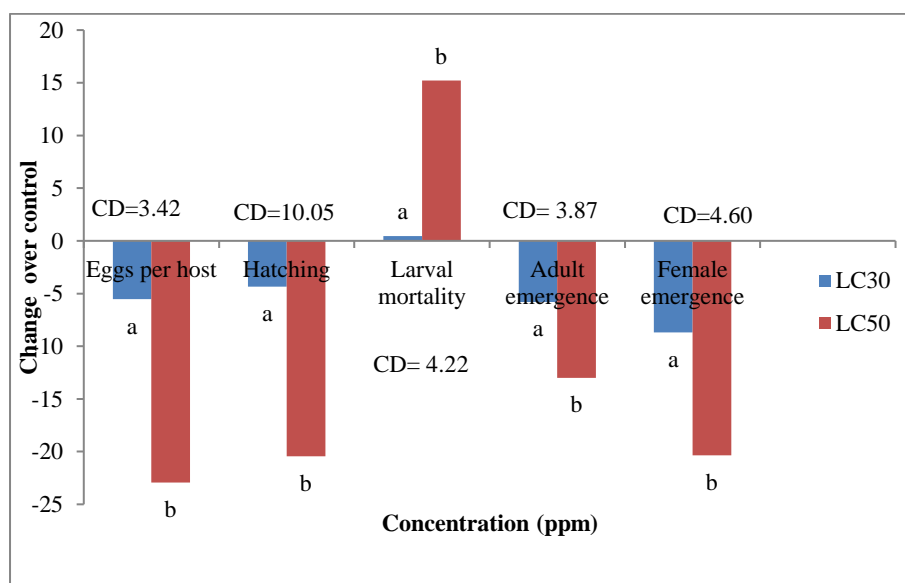


Figure 5. Effects of ferulic acid diet on development and survival of *B. hebetor* larvae emerged from *S. litura* larvae over control  
CD= Critical difference at 1%.

parasitoid survival, adult mass, and development time of its parasitoid, *Cotesia congregata* (Say). Bloem and Duffey (6) reported that changes in artificial diet negatively impacted the growth of parasitoid *Hyposoter exiguae* (Viereck). Traugott and Stamp (54) also reported that the performance of predator spined soldier bug, *Podisus maculiventris* (Say) was negatively affected, when its prey tobacco hornworm, *Manduca sexta* (L.) was fed on chlorogenic acid and tomatine. Roth *et al.* (43) had reported that tannic acid and phenolic glycosides incorporated in the diet of gypsy moth, *Lymantria dispar* (L.) not only reduced its growth, decreased pupal weight and increased mortality but also affected the development time of its parasitoid *Cotesia melanoscela* (Ratzeburg). The increased level of CO<sub>2</sub> increased the total phenolic content of *Medicago truncatula* (Gaertn.) that affected the growth and survival of pink colour genotype of pea aphid, *Acyrtosiphon pisum* (Harris) and also decreased the parasitization rate of its parasitoid *Aphidius avenae* (Haliday) (58). Plant allelochemicals have negative effects on herbivore fitness along with reduced survival, clutch size, body size and fecundity of parasitoid (30).

The parasitoid provided with *S. litura* larvae fed on higher concentration (2573.33 ppm) of ferulic acid was more affected than those fed on lower concentration (25.92 ppm) of ferulic acid than control (Fig. 5). Similarly, plant secondary metabolites such as ellagic acid and gallic acid negatively impacted the survival of *S. litura* and the parasitoid *B. hebetor* (35,36). Increasing concentration of linear furanocoumarin increased the mortality of both beet armyworm, *Spodoptera exigua* (Hubner) and its parasitoid, *Archytas marmoratus* (Townsend). Reudler *et al.* (42) had also reported that increase in concentration of iridoid glycosides not only reduced the fitness of herbivore, the wood tiger

moth, *Parasemia plantaginis* (L.) but also provided defence against predators and parasitoids. Wang *et al.* (56) also reported that higher dose of gossypol prolonged the development period of *H. armigera* larvae and the parasitoid *Compoletis chloridae* (Uchida) but lower dose of gossypol had no significant effects.

## CONCLUSIONS

The natural enemies of insects have an essential role in regulating the population of insect pests and therefore, the understanding of their tritrophic interactions is crucial for designing the appropriate strategies for pest control. Our findings have revealed that ferulic acid at higher concentrations (2573.33 ppm) adversely affected the development of both *S. litura* as well as the parasitoid, *B. hebetor*. While at lower concentration (25.92 ppm), it only disrupted the development of *S. litura* but not that of *B. hebetor*. Thus, ferulic acid holds considerable promise in pest control strategies and the findings can help in breeding plants resistant to insect pests. However, the efficacy of the compound needs to be determined under natural field conditions.

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## CONFLICT OF INTEREST

The authors announce that they have no conflict of interest.

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