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Effect of food plants on *Spodoptera litura* (Lepidoptera: Noctuidae) larvae immune and antioxidant properties in response to *Bacillus thuringiensis* infection

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ABSTRACT

The larvae of *Spodoptera litura* (Fabricius) were reared on five host plants, *Brassica oleracea*, *Nicotiana tabacum*, *Ricinus communis*, *Gossypium hirsutum*, and *Arachis hypogaea*. The larvae were immunized with *Bacillus thuringiensis* to observe the immune response. The results of total and differential hemocyte count were increased in *B. oleracea*, *N. tabacum*, and *R. communis* fed *S. litura* larval hemolymph. Similar results were observed in the parameter of nodulation, melanization, and phenoloxidase. Total protein was higher in *R. communis* fed larvae. Antioxidant levels like Catalase (CAT), Superoxide dismutase (SOD), Glutathione S- transferase (GST), Peroxidase (POX), Lipid peroxidase (LPO), and Esterase (EST) was found in moreover all plant-feeding insect. High CAT activity was observed 2–6 h in *R. communis*, *G. hirsutum*, and *A. hypogaea* fed *S. litura* larval midgut and fatbody samples. Increased SOD activity in both midgut and fatbody at 2–12 h of *B. oleracea*, *G. hirsutum*, and *A. hypogaea* fed. GST activity was increased initially 2–6 h in *G. hirsutum* and *A. hypogaea*. Increased POX activity was observed initially in all treated groups. Highest LPO observed at 6 h in *N. tabacum* in both midgut and fatbody. Whereas increased EST activity was observed in *N. tabacum* and *B. oleracea*. The results of the present study shows that nature of food influence the immunity against Bt infection. This information can be very useful for incorporating biological control program for insect pest.

1. Introduction

Insects are consumed varies types of host plant species and have varied types of nutrient quality in their food [1]. These plant nutrients play a vital role in insect development, reproductive, fecundity and also improve the immune defense mechanisms against their microbial infections [1,2]. In previously reported the relationship between host plant food and insect immunity has concentrated on the quality and quantity of food nutrients [3,4].

Insect immune system is aimed at eliminating the invading pathogens and parasites by the response of cellular and humoral immunity [5]. This activates the several immune parameters against the microbial infections like alteration of hemocytes, coagulation, microaggregation, increasing phenoloxidase, and formation of melanin surrounding with toxic particles [6,7]. Humoral responses like, antimicrobial peptide production from fatbody cells have an effective role in killing the toxicants [8]. Including, the antioxidant enzymes as part of an immune response against the pathogens [9,10]. This enzymes to control the ROS (Reactive oxygen species) generation from biotic and abiotic stress in insects [11,12]. ROS including, free radicals, oxygen ions, and organic, inorganic molecules. These molecules are increased when the exposure to pathogens and damage the cell structure [13–15]. In many studies, the antioxidant system shows a defense mechanism against ROS production induced by pathogens [16,17]. The major antioxidant enzymes in insects are catalase, glutathione-S-transferase, superoxide dismutases, peroxidases, and esterase [18–22]. These enzymes are mostly found in lepidopteran larvae on infection with pathogens [23].

Spodoptera litura (Lepidoptera: Noctuidae) is a notorious insect pest it causes severe economic loss in crop fields [24]. Insect pest management has become increasing difficult nowadays and most insecticides are ineffective. Insect control has mainly relied on chemical insecticides for decades, which had led to the development of insecticide resistance. Using microbes and their metabolites are cheap and safe methods for control the insects for example, *Bacillus thuringiensis* (Bt). Bt is spores forming gram-positive bacteria that produce several insecticidal activities [25]. This biopesticide is mostly used to control lepidopteran insect

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larvae [26]. Recently, the few studies reported that lepidopteran insects getting resistant to Bt by improving their immune status [27–29].

The ability of an insect to survive against any insecticide depends on its ability to produce a structural modification in receptors and to produce detoxification enzyme which can sequester/metabolize the toxins [30,31]. For this, the insect diet may play a vital role in the supply of nutrients required for increased protein synthesis [32]. The composition or the types of diet may influence an insects ability to survive the infection. A further immune response is also related to improving the defense mechanism of the individual. Hence the present study understands the influence of different plant diet on the immunological and physiological mechanisms in a Bt challenged *S. litura* larvae.

2. Materials and methods

2.1. Maintenance of insect

The tobacco cutworm, *S. litura* eggs were purchased from National Bureau of Agricultural Insect Resources (NBAIR), Bangalore, India. Purchased eggs were transferred into the plastic tray (8 cm in diameter and 10 cm in height) and allow to hatching. The newly hatched larvae transfer on another plastic tray (30 cm in diameter and 55 cm in height). Caster leaves were provided daily to larvae until the larvae reached pupae. Pre-pupae were collected and transfer into a big plastic tray (30 cm in diameter and 55 cm in height) containing soil for the complete pupal stage. Male and female pupae were identified to transfer into another plastic tray for adult formation. One male has two female pupae ware provide in a tray and covered with net cloth and allow to complete mating. The adults were fed with sugar solution through cotton balls and placed on top of the tray. Complete mating, the female adult were transferred to another plastic tray mentioned above and provide a 10 % sugar solution. The female adults produce the eggs masses were collected daily and hatched first instar larvae from this generation as used for testing. Newly hatched larvae were maintained with different plant leaves (*Brassica oleracea* (cabbage), *Nicotiana tabacum* (tobacco), *Ricinus communis* (castor), *Gossypium hirsutum* (cotton), and *Arachis hypogaea* (peanut)) up to 3 generations. Each treated plants have 30 larvae and each 3 replicates. The maintaining procedure of *S. litura* was mentioned above. Same procedure were applied to control experiment (artificial diet containing, chickpea flour (200 g), yeast powder (30 g), ascorbic acid (3.5 g), sorbic acid (1 g), formaldehyde solution (2.5 ml), methyl-p-hydroxybenzoate (2 g), agar (14 g) and distilled water (500 ml)).

2.2. Microbial culture

Bacillus thuringiensis (Bt) 4D1 strain was obtained from plant and microbial biotechnology laboratory, Periyar University, Salem. The culture was maintained on Luria Broth (LB) at 37 °C in a 50 ml conical flask with shaking at 200 rpm. 50 µl of mother culture transfer into 50 ml fresh LB medium and incubate at 37 °C for several hours. 10 ml of log-phase culture was centrifuged for 10 min at 7000 rpm in 4 °C. Collected bacterial pellet washed with 10 ml of cold sterile 5 mM phosphate buffer saline (PBS). After a wash the pellet was dry for 10 min and stored at −20 °C for further use.

2.3. Treatment with Bt cells

The 3rd generation, the 5th instar *S. litura* larvae were used for experimental study. Larvae were immunized with an injection of Bt 4D1 cells. The thin-micro syringe needle was dipped in bacterial pellet and injected to larval prolegs. Then the larvae were kept in 30 °C in dark and the hemolymph, midgut, and fatbody were collected at 2–24 h of exposure. The collected hemolymph sample were used for immunological assay and tissue samples, midgut and fatbody were used enzyme activity. The same procedure was applied for the control experiment except it was injected with PBS without bacteria. The experiments conducted by 3 replicates and each replicates having 10 larvae from the each treated plant groups.

2.4. Immunological assay

2.4.1. Total hemocyte count (THC)

The hemocytes were counted by using a hemocytometer, neubauer chamber. Collected hemolymph sample form 2–24 h was diluted twenty times with phosphate buffer saline (PBS) containing, 3.8 g of Na_2HPO_4, 5.47 g of K_2HPO_4, making at 1 L of distilled water, with pH 6.6. 20 µl of hemolymph sample was placed on coverslip edge of hemocytometer and counted four corners ruled squires under phase contrast microscope (PCM) at 40× magnification. The total hemocyte was counted by the method of Jones [33].

Formula:

$$\text{THC (Cells/mm}^3) = \frac{\text{X} \times \text{dilution factor x depth factor}}{\text{Number of squares counted}}$$

X = Total number of cells count,
20= Dilution factor,
10 = Depth factor,
4= Number of squares count

2.4.2. Differential hemocyte count (DHC)

Collected hemolymph sample was fixed on glacial acetic acid for 2−3 min and the addition of PBS for 15 min to neutralize the hemocyte content. A drop of hemolymph sample was placed on a sterile glass slide to making a smear and added a few drops of Giemsa staining for 5 min. DH was counted under PCM with 40X magnification. The identification of DH count followed by Gupta [34].

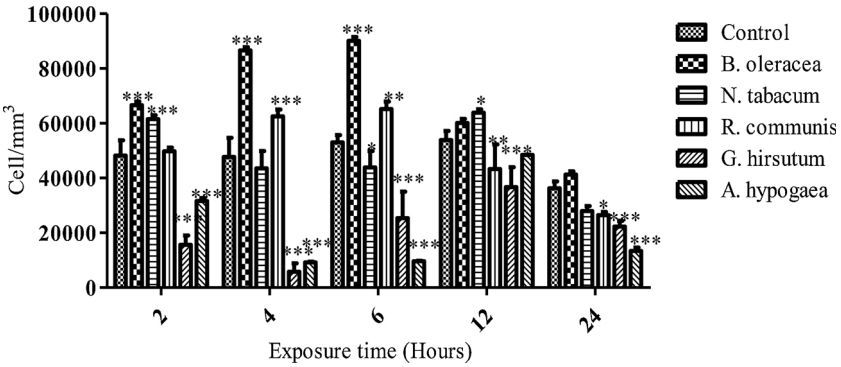

Fig. 1. Total hemocyte count of *Bacillus thuringiensis* challenged *Spodoptera litura* larvae. The values are expressed as mean (±S.D) analyzed by Two-Way ANOVA. Asterisk (*) indicates significant difference among treatments with respect to control. (*) indicates ($p < 0.05$), (**) indicates ($p < 0.001$), (***) indicates ($p < 0.0001$).

Table 1
Number of nodule formation counted in *S. litura* larval hemolymph after injection of with *B. thuringiensis*.

Experimental Groups	Time (Hours)				
	2h	4h	6h	12h	24 h
Control	6.3 ± 2.5	6.3 ± 2.5	8.6 ± 3.5	–	–
B. oleracea	64.33 ± 3.5***	94.6 ± 2.5***	**125 ± 5.0*****	**130.6 ± 6.0*****	84.3 ± 1.1***
N. tabacum	31.66 ± 2.8***	61 ± 3.7***	74.3 ± 3.5***	84.6 ± 5.5***	50 ± 1.1***
R. communis	35.33 ± 3.5***	60 ± 1.1***	98 ± 2.6***	**117.6 ± 2.5*****	62.6 ± 1.1***
G. hirsutum	13.33 ± 3.5	36 ± 2.6***	47.33 ± 2.8***	31 ± 4.5***	26 ± 1.7***
A. hypogaea	24.33 ± 3.5***	50.33 ± 3.5***	64 ± 4.6***	85 ± 4.5***	61 ± 1.7***

The values are expressed as mean (±S.D) analysed by Two-Way ANOVA. Asterisk (*) indicates significant difference among treatments with respect to control. Note: (***) indicates ($p < 0.0001$). No nodules were found in the control treatment at 12 h and 24 h.

2.4.3. Microaggregation (nodulation)

Nodulation assay was followed by Franssens et al. [35]. A drop of collected hemolymph sample was immediately placed on hemocytometer for nodule/microaggregation count and the dark melanized nodules were observed under PCM with 40× magnification.

2.4.4. Melanization activity

Collected 2−24 h of hemolymph sample was immediately centrifuged at 5000 rpm for 2 min to separate the plasma from the hemolymph. 100 µl of melanized plasma was added into 96 well plates and measured at 450 nm. 2.5 mM of phenylthiourea (PTU) was added into collected hemolymph and centrifuged. The PTU supplemented plasma used as a negative control. The melanization activity was calculated by Freitak et al. [36].

2.4.5. Phenoloxidase activity assay (PO)

PO activity followed by the method of Harizanova et al. [37]. 10 µl of hemolymph sample was diluted in 1 ml of ice-cold PBS and frozen at 48 h. PO activity was measured by a frozen hemolymph sample was thawing at 37 °C for 5 min and centrifuged at 5000 rpm for 5 min. Collected the 100 µl of supernatant was added into 200 µl of 3 mM DL–dihydroxyphenylalanine (DL-DOPA) and incubated at 15 min in dark. The enzyme activity was measured at 490 nm for 45 min, using a spectrophotometer with dopamine as a substrate and enzyme expressed as unit/min/mg of protein.

2.4.6. Total protein

The protein was estimated by using the method of Lowry et al. [38].

2.5. Antioxidant and detoxification enzyme assay

2.5.1. Sample preparation

The collected tissue samples (midgut and fatbody) were homogenized in 1 ml of ice-cold 50 mM PBS with 10 % glycerol. Sample mixtures centrifuge at 8000 rpm for 15 min in 4 °C. The collected supernatant was used for enzyme and metabolite assays.

2.5.2. Catalase activity (CAT)

CAT activity was followed by the method of Luck [39]. The enzyme mixture contained, 0.4 ml of 10 mM H_2O_2, 2.6 ml of 50 mM ice-cold PBS, and 40 µl of an enzyme. Measurement of enzyme reaction at 240 nm using a spectrophotometer. CAT enzyme expressed as 1 µmol of H_2O_2 decomposition/min/mg of protein.

2.5.3. Superoxide dismutase (SOD)

SOD enzyme activity was determined by using the method of Marklund and Marklund [40]. 3 ml of enzyme mixture contains, 2.8 ml of Tris-EDTA buffer, pH at 8.2 and 50 µl of enzyme extract. The enzyme mixture makes at final volume 2.9 ml of Tris-EDTA buffer and added 100

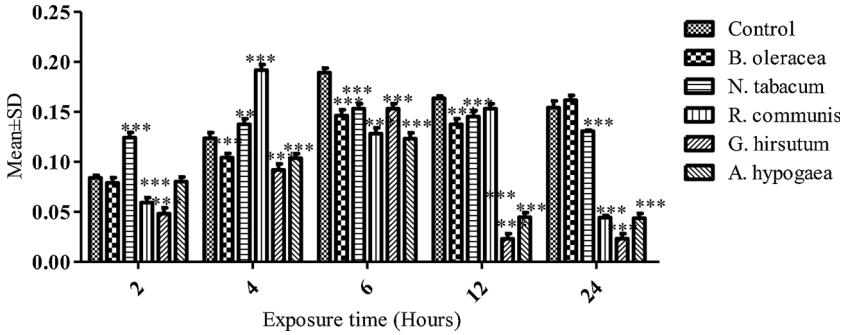

Fig. 2. Melanization rate of *Bacillus thuringiensis* challenged *Spodoptera litura* larvae. The values are expressed as mean (±S.D) analyzed by Two- Way ANOVA. Asterisk (*) indicates significant difference among treatments with respect to control. (*) indicates ($p < 0.05$), (**) indicates ($p < 0.001$), (***) indicates ($p < 0.0001$).

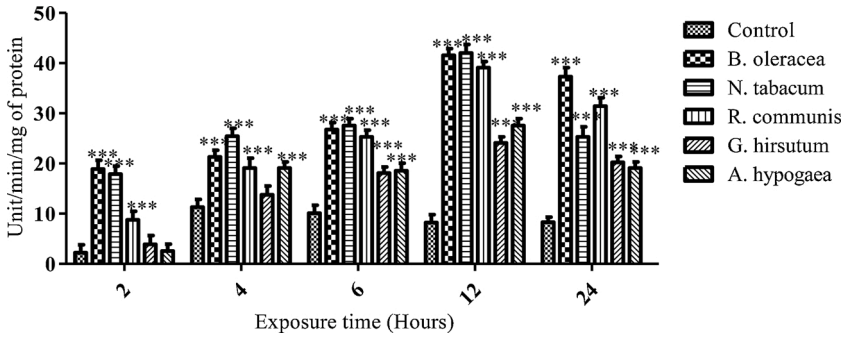

Fig. 3. ProPO rate of *Bacillus thuringiensis* challenged *Spodoptera litura* larvae. The values are expressed as mean (±S.D) analyzed by Two-Way ANOVA. Asterisk (*) indicates significant difference among treatments with respect to control. (*) indicates ($p < 0.05$), (**) indicates ($p < 0.001$), (***) indicates ($p < 0.0001$).

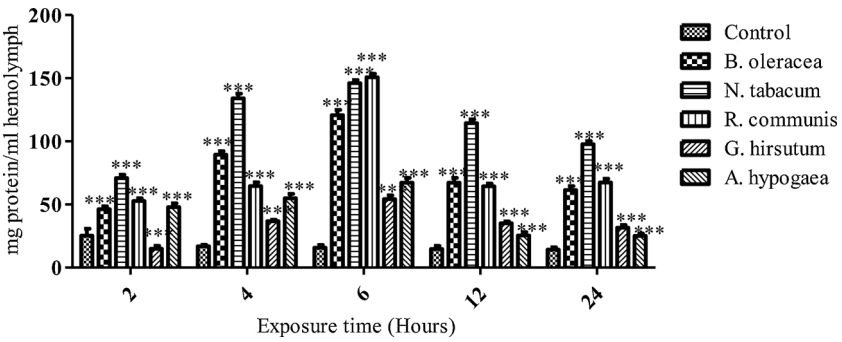

Fig. 4. Total protein of *Bacillus thuringiensis* challenged *Spodoptera litura* larvae. The values are expressed as mean (±S.D) analyzed by Two-Way ANOVA. Asterisk (*) indicates significant difference among treatments with respect to control. (*) indicates ($p < 0.05$), (**) indicates ($p < 0.001$), (***) indicates ($p < 0.0001$).

µl of 15 mM pyrogallol solution. The rate of SOD was measured at 440 nm using a spectrophotometer and the total SOD was expressed as units/min/mg of protein.

2.5.4. Glutathione-S-transferase (GST)

GST activity was followed by the method of Habig et al. [41]. Reaction mixture contains, 20 µl of an enzyme, 50 µl of 50 mM CDNB (1-chloro-2, 4-dinitrobenzene), 150 µl of 50 mM GSH (reduced glutathione) and 2.78 ml of PBS. The mixtures was incubated for 2-3 min at 20 °C and OD at 340 nm using a spectrophotometer. The rate of GST was calculated by 1 µmol of GSH with CDNB/min/ mg of protein.

2.5.5. Peroxidase activity (POX)

POX estimation was carried out using the method of Reddy et al. [42]. 0.5 µl of enzyme added into 2 ml of PBS, 1 ml of pyrogallol solution, 1 ml of 0.005 M hydrogen peroxide. The reaction mixture was incubated for 5 min at 25 °C and the enzyme reaction terminated by the addition of 1 ml sulphuric acid with 2.5 normality. The enzyme reaction was observed at 420 nm in a spectrophotometer and the rate of POX was calculated by Unit/min/mg of protein.

2.5.6. Lipid peroxidase (LPO)

LPO activity was estimated by the method of Esterbauer and Cheeseman [43]. The reaction mixture contains, 0.1 ml of an enzyme, 1.9 ml of PBS was incubated at 37 °C for 1 h and addition of 10 % trichloroacetic acid for precipitating the sample. The mixture was centrifuged at 5000 rpm for 15 min and collected supernatant into 1 ml of 1 % thiobarbituric acid (TBA). The samples allow to the boiled water bath for 10 min and after that, the collected supernatant allows it to cool. The formation of malondialdehyde (MDA) concentration was measured at 532 nm in a spectrophotometer and MDA concentration expressed as 1 µmol/min/mg of protein.

2.5.7. Esterase activity (EST)

EST activity was determined by using the method of Kranthi [44]. The reaction mixture contains, 0.2 ml of an enzyme into 0.1 ml of 0.3

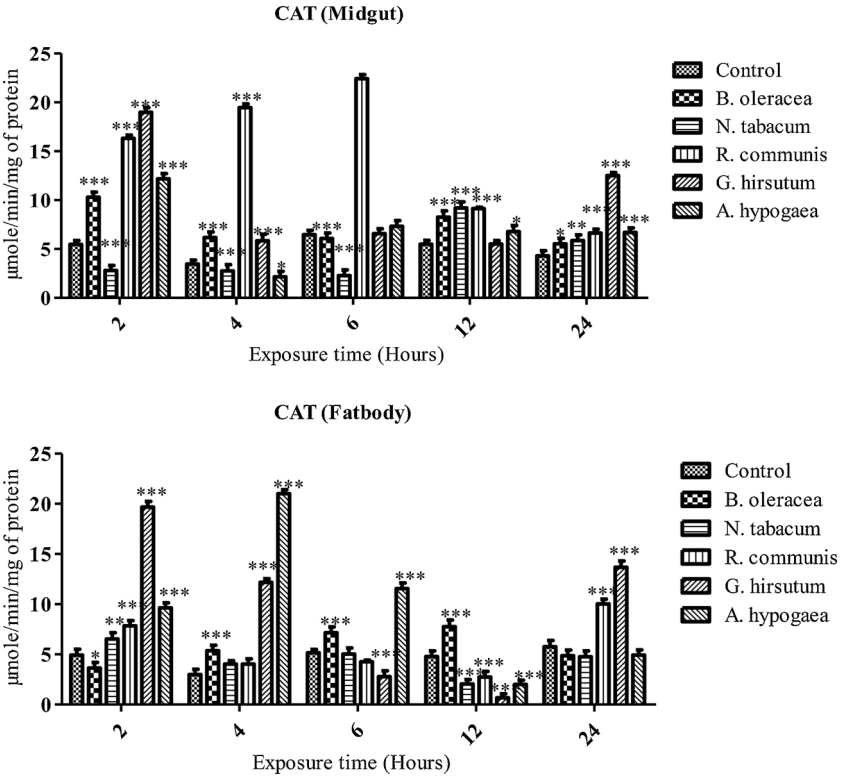

Fig. 5. Catalase enzyme levels in *Bacillus thuringiensis* challenged *Spodoptera litura* larvae. The values are expressed as mean (±S.D) analyzed by Two-Way ANOVA. Asterisk (*) indicates significant difference among treatments with respect to control. (*) indicates ($p < 0.05$), (**) indicates ($p < 0.001$), (***) indicates ($p < 0.0001$).

mM a-naphthyl acetate was incubated for 20 min in 30 °C dark. The mixture sample added with 1.0 ml of fast BB salt and sodium dodecyl sulfate (SDS) solution at a 2:5 ratio. EST activity was measured at 590 nm using a spectrophotometer. The enzyme activity expressed as 1 μmol/min/mg of protein

2.6. Statistical analysis

All experimental assays were performed using three replications, and the obtained assays data were analyzed by Bonferroni post-test (two-way ANOVA) using PRISM 5 software. The P-value (<0.05) was considered significant.

3. Results

3.1. Immunology assays

3.1.1. THC and DHC count

Increased total hemocyte count was found in *B. oleracea* and *N. tabacum* fed insects after 6 h and thereafter the hemocyte level was reduced. *R. communis* fed larval hemocyte level was increased in 4 and 6 h of exposure and after that, the hemocyte level can decrease has come back to normal. Total hemocyte level was low in *G. hirsutum* and *A. hypogaea* fed larvae (Fig. 1).

Identify the five types of differential hemocytes based on morphology (Fig. S1). Prohemocyte (PR) is a small cell with a large central nucleus and thin cytoplasm (Fig. S1(A)). Plasmatocyte (PL) is large and varies in size, with have a small nucleus and few granules in the cytoplasm (Fig. S1(B)). Granulocyte (GR) is the ellipsoidal shape and large nucleus and a high granule present in the cytoplasm (Fig. S1(C)). Oenocyte (OE) is an oval shape with a large unconventional nucleus within the cytoplasm (Fig. S1(D)). Spherule cell (SP) has a circular cell which have several spherules in the cytoplasm (Fig. S1(E)).

Differential hemocyte count from 2–24 h shows, Prohemocyte were increased after 12 h of *B. oleracea*, *N. tabacum*, and *R. communis* fed larvae. While decreased count was observed in *G. hirsutum* and *A. hypogaea* fed larvae when compared to the control. Increased Plasmotocyte were observed in 2, 12 and 24 h of *B. oleracea* and *N. tabacum* fed larvae. 2–24 h of increased plasmatocyte were observed in *R. communis*. Increased Granulocytes were found in 2–6 h of *R. communis* fed larvae and after 6 h the hemocyte levels were reduced and come back to normal.

Oenocyte count was increased in 2 h of *R. communis* after that there was a reduction level observed in till 12 h. The increased spherule cells were observed in 2 and 24 h of *R. communis* fed larvae (Fig. S2).

3.1.2. Nodulation

B. oleracea and *R. communis* fed larvae were found to induce maximal nodule formation in 6 h and 12 h after the injection (Fig. S3). Whereas the control group nodule formation was very low and after 6 h there were no nodules were found (Table 1).

3.1.3. Melanization

Melanization was significantly increased from the 2 h infection in *N. tabacum* and 4 h infection *B. oleracea* and *R. communis*, while *G. hirsutum* and *A. hypogaea* showed the low level of melanization when compare to the control (Fig. 2).

3.1.4. Phenoloxidase

Increased PO activity was observed between 2–12 h of infection and

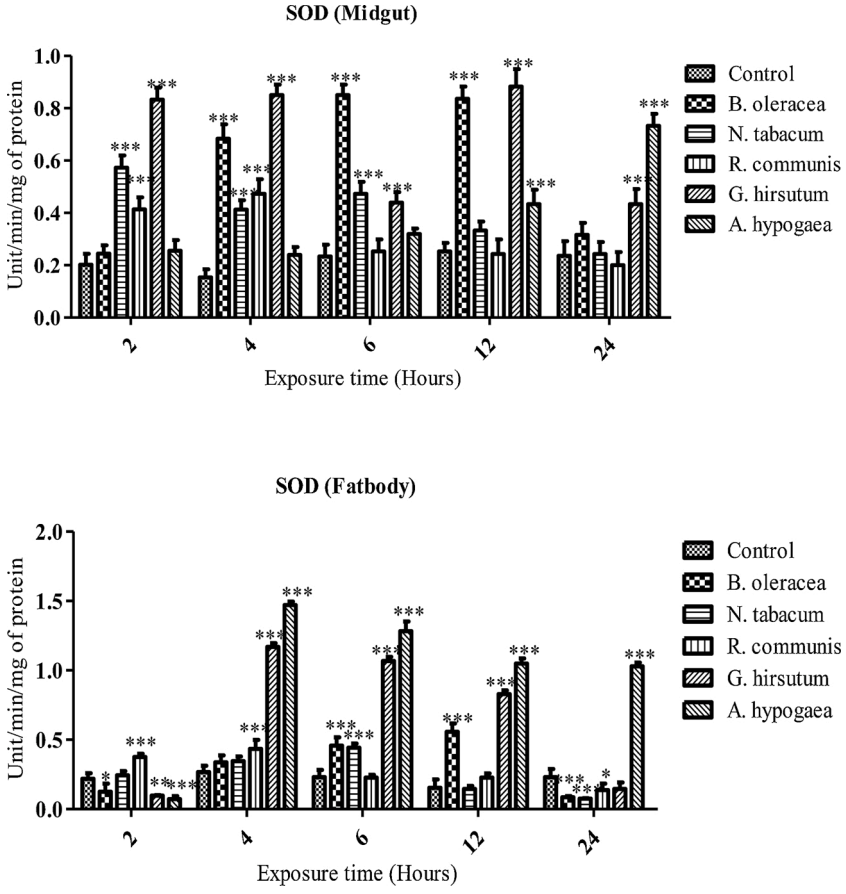

Fig. 6. SOD enzyme levels in *Bacillus thuringiensis* challenged *Spodoptera litura* larvae. The values are expressed as mean (±S.D) analyzed by Two-Way ANOVA. Asterisk (*) indicates significant difference among treatments with respect to control. (*) indicates ($p < 0.05$), (**) indicates ($p < 0.001$), (***) indicates ($p < 0.0001$).

decreased activity was observed in after 12 h in all treatment groups (Fig. 3).

3.1.5. Total protein

Total protein content was observed after Bt exposure in 2–24 h of *S. litura*. Increased protein level was observed after 2–6 h of all treated groups. Total protein content was highest in *N. tabacum* and *R. communis* (Fig. 4).

3.2. Antioxidant and detoxification enzymes assay

Increased CAT activity in *R. communis*, *G. hirsutum*, and *A. hypogaea* fed *S. litura* in 2–6 h of midgut and fatbody sample (Fig. 5). SOD activity increased at 2–12 h of *B. oleracea* and 2, 4 and 12 h of *G. hirsutum* in midgut sample, whereas decreased activity was found in *R. communis*, *N. tabacum*, and *A. hypogaea* fed larvae. In fatbody, increased SOD activity was found in 4–12 h of *G. hirsutum* and 4–24 h of *A. hypogaea* when compare to other treatment groups (Fig. 6).

Increased GST activity in midgut, 2–24 h of *G. hirsutum* and *A. hypogaea*, while other treatments observed the decreased activity. In fatbody, increased activity was observed in all treated groups initially 2–4 h and after that, it decreased activity was observed (Fig. 7). POX activity initially increased in 2–4 h of all treated groups and increased till 6 h of *B. oleracea* and *G. hirsutum*. After that, there was decreased in all groups. In fatbody, increased activity was in 6 h only of *B. oleracea*, *N. tabacum* and *A. hypogaea* (Fig. 8).

LPO activity was increased at 6 h in *N. tabacum* in both midgut and fatbody. After that there was a decreased activity was observed (Fig. 9). Midgut, EST activity was increased in till 12 h of exposure in *B. oleracea* fed larva. Fatbody, increased in 2−6 h of *N. tabacum* (Fig. 10).

4. Discussion

Lepidopteran insects are usually pests of crops. *S. litura* lepidopteran insect is a polyphagous pest feeding on several crops. In the present study, the *S. litura* larvae maintained on five different plants (diet) was analyzed after Bt exposure. Larval defense mechanism was assessed based on immunological, antioxidant and detoxification enzymes.

Plant diet plays a vital role in the physiological mechanisms of vertebrate and invertebrate and also improve immunity [45,46]. The quality and quantity of plant diet determine insect fitness. Insects have a robust immune system comprising of hemocytes, AMPs, phenoloxidase, lysozyme, and other mechanisms that are activated in response to infection [5,7]. In this study, we found an increased THC in *B. oleracea*, and *N. tabacum*, and *R. communis* fed larvae when compared with control and other plant groups. This may the reason of food plants have sufficient nutrient value and high utilization of food. Therefore enhance the immunity of *S. litura* when compare to the other two plants, *G. hirsutum* and *A. hypogaea* (Fig. 1). In other reports, the fluctuating hemocytes have seen in *G. mellonella* [47], *Reticulitermes flavipes* [48], *Oxya japonica*, *Eurygaster integriceps* [49] and *Chilo suppressalis* [50] after microbial exposure. While decreased THC was observed in *Aspergillus flavus* exposure on *S. litura* [51].

There is also a change in DH composition in insect hemolymph following microbial exposure. Identification of five hemocytes, prohemocyte, plasmatocyte, granulocyte, oenocyte, and spherule cells are usually found in lepidopteran insects [52,53]. These hemocytes having a different functions in insect immunity, prohemocyte forms a putative stem cell population [54], plasmatocyte participate in nodulation, encapsulation, and in wound healing [55], granulocyte activates cell-mediated immunity. Oenocyte is involved in the melanization process [56]. Whereas spherule cells are not much involved in immune

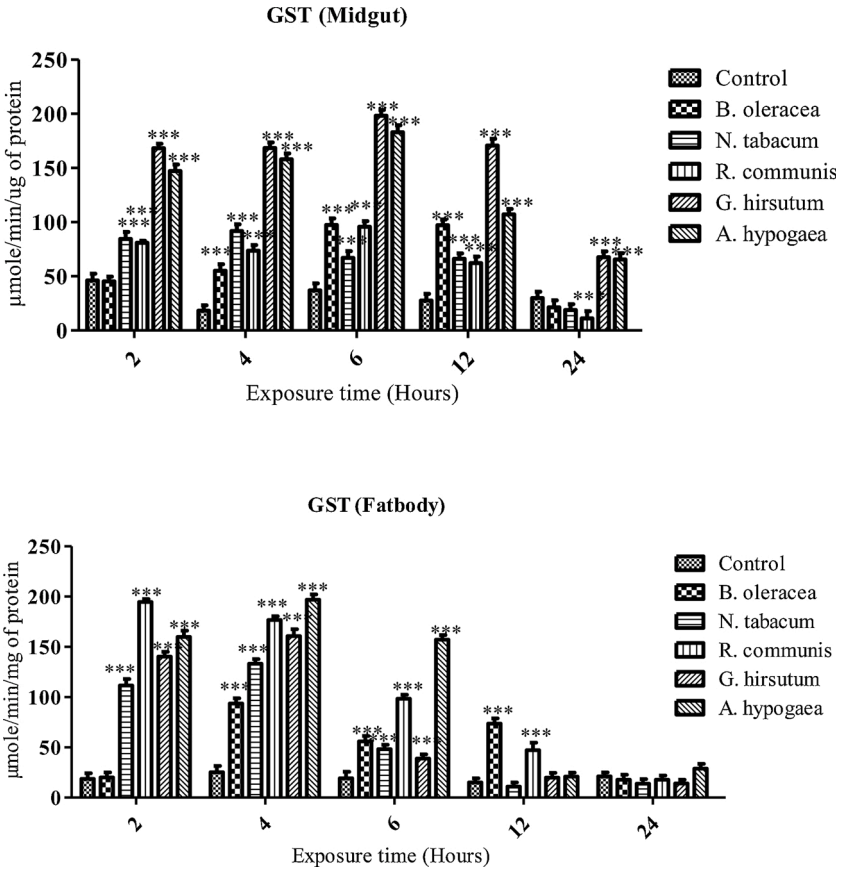

Fig. 7. GST enzyme levels in *Bacillus thuringiensis* challenged *Spodoptera litura* larvae. The values are expressed as mean (±S.D) analyzed by Two-Way ANOVA. Asterisk (*) indicates significant difference among treatments with respect to control. (***) indicates ($p < 0.0001$).

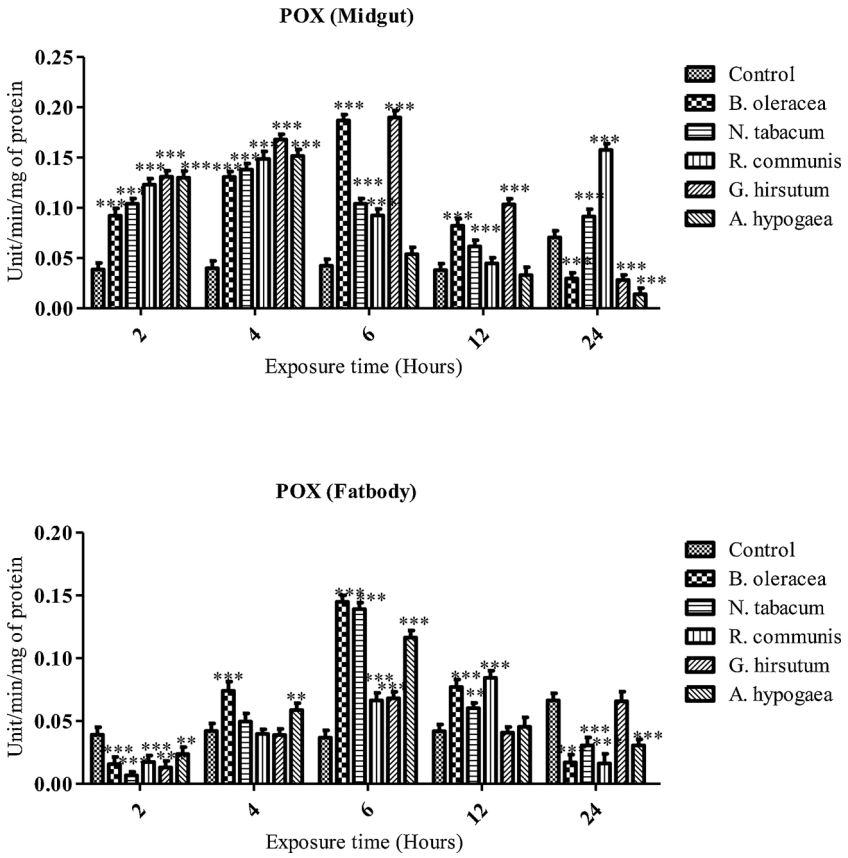

Fig. 8. POX enzyme levels in *Bacillus thuringiensis* challenged *Spodoptera litura* larvae. The values are expressed as mean (±S.D) analyzed by Two-Way ANOVA. Asterisk (*) indicates significant difference among treatments with respect to control. (**) indicates ($p < 0.001$), (***) indicates ($p < 0.0001$).

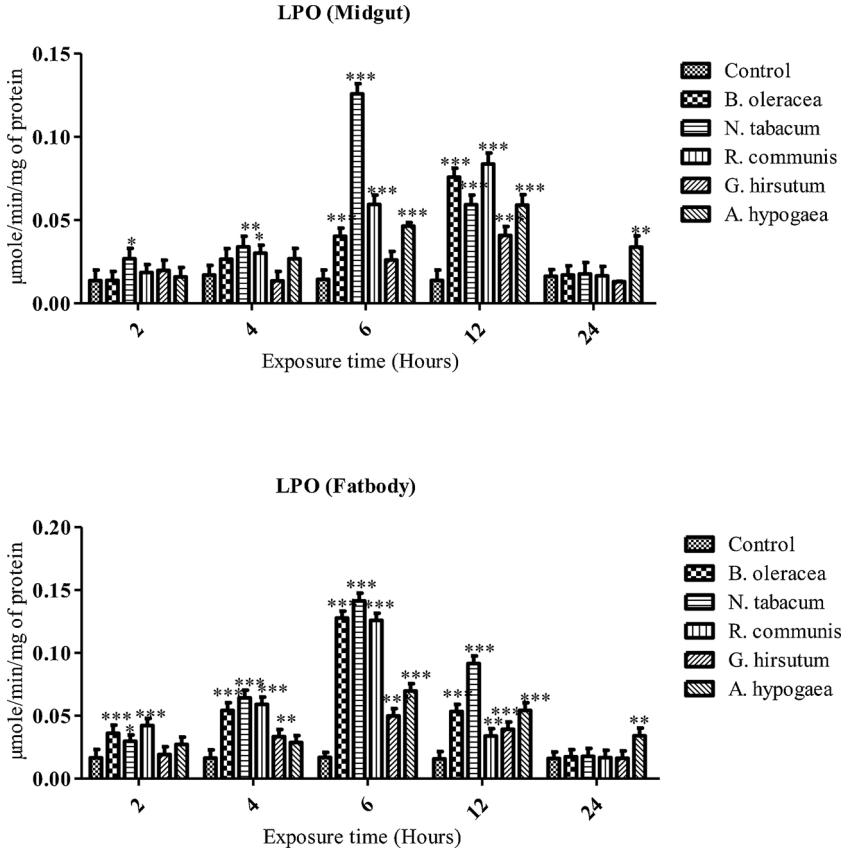

Fig. 9. LPO enzyme levels in *Bacillus thuringiensis* challenged *Spodoptera litura* larvae. The values are expressed as mean (±S.D) analyzed by Two-Way ANOVA. Asterisk (*) indicates significant difference among treatments with respect to control. (*) indicates ($p < 0.05$), (**) indicates ($p < 0.001$), (***) indicates ($p < 0.0001$).

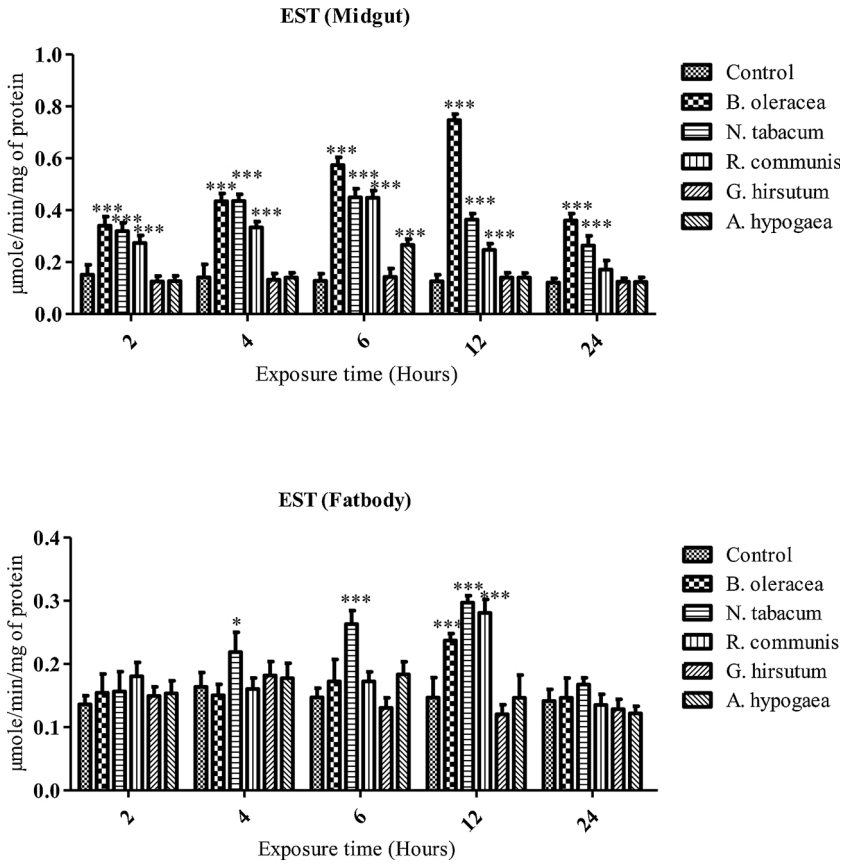

Fig. 10. Esterase enzyme levels in *Bacillus thuringiensis* challenged *Spodoptera litura* larvae. The values are expressed as mean (±S.D) analyzed by Two-Way ANOVA. (*) indicates significant difference among treatments with respect to control. (*) indicates ($p < 0.05$), (***) indicates ($p < 0.0001$).

functions [53]. In our previous study reported the five hemocyte types are a response to pesticide exposure on *S. litura* larvae [57]. In this study, identify the DH type against Bt exposure on *S. litura* larvae. Increased granulocyte and plasmatocyte levels in *B. oleracea*, *N. tabacum*, and *R. communis* when compared with *G. hirsutum*, and *A. hypogaea* fed larvae which suggest an increased cell-mediated immunity and nodulation and encapsulation. Other previous studies was reported prohemocyte, granulocyte, and oenocyte were observed in the pea aphid, *Acyrthosiphon pisum* against parasite exposure [58].

Nodulation is one of the cellular responses of insects against microbes [47]. Bt toxin initiates nodule formation in *S. litura* larvae, the highest nodulation was observed in *B. oleracea* and *R. communis* fed larvae and no nodules were found in control group after 12 h and 24 h of treatment. A similar result was found in fungal pathogen exposure to *Chilo suppressalis* larvae [49]. Less number of the nodule was found in *G. hirsutum*, and *A. hypogaea* fed larvae, it may the reason of reduced THC. Gillespie et al. [59] has well established about the correlation between THC and nodule formation against pathogen infection.

PO and melanization is a part of immune the response against the wounding/infection of invading microbes [60]. These enzymes hydrolyze the tyrosine to L-dihydroxyphenylalanine and oxidize the o-diphenol to form a quinone [61]. The conversion of melanin inhibits the Bt by forming of encapsulation was observed in *S. exigua* and *Heliothis virescens* [62]. In our experiments, the highest PO and melanization were observed in *B. oleracea*, *N. tabacum*, and *R. communis* fed larvae when compare to the control and other treated groups (Figs. 2 and 3), which corresponds to the increasing of hemocyte and nodulation. These results may suggest that melanin deposition also is complementary to nodule formation. Therefore, in insects, PO, melanization, and nodulation are important immune functions against microbial toxins [63–65,47,48].

Based on the immune response the total protein level was higher in *N. tabacum* and *R. communis* fed larvae. These host plants have rich nutrients that enhance the protein content in the larval hemolymph.

CAT enzyme is involved in oxidative stress and H_2O_2 scavenging mechanism in insects [66]. Here, the highest CAT activity was observed in *R. communis*, *G. hirsutum*, and *A. hypogaea* fed larvae because this plant leaves may be containing high antioxidants levels when compared to the other plant leaves (Fig. 5). In previous study, increased CAT activity was observed in silkworm (*Bombyx mori*) fat body [67], and other lepidopteran insects, *G. mellonella* [17], *Helicoverpa armigera* [68], *S. litura* [50], and including other hemiptera insect, *Bemisia tabaci* [69].

SOD and GST pay a protective role from oxidative stress [70–73]. In this study, the highest SOD and GST activity was observed in *G. hirsutum* and *A. hypogaea* (Figs. 6 and 7). Similar results, the increasing SOD activity were observed in *S. litura* larvae [50] and increasing GST was seen in *G. mellonella* [67]. Therefore the results suggest that both enzymes play a vital role in the elimination of ROS.

POX is an important antioxidant enzyme involved in the H_2O_2 break down in insects [74]. Our study, the POX activity initially increased at all treated groups in midgut sample when compare to the control (Fig. 8). Similar increased POX activity were observed in *Apis mellifera* against microbial exposure [75].

LPO is involved in the protection of cellular damage from toxin. In the present study, LPO activity was increased in 6–12 h of all treatment (Fig. 9). Therefore, the insect suffers from cellular damage within 6 h of Bt exposed. These similar results were observed in other species, *Bactrocera dorsalis* [73], and *Scapharca broughtonii* [76].

Insect EST is metabolized a wide range of xenobiotic. EST activity was increased in *B. oleracea* and *N. tabacum* fed larva in both midgut and fatbody when compare to the control (Fig. 10). Similar results were observed in *E. integriceps* species with microbial exposure [77]. From this study, we suggest that esterase had an important role in the metabolism or detoxification of microbes in the insect.

5. Conclusions

In this study, the effect of Bt on *S. litura* fed with five host plant were evaluated. *R. communis*, *B. oleracea*, and *N. tabacum* fed larvae *S. litura* improve their defense mechanisms against the microbial infection when compared with other plant treatment *G. hirsutum*, and *A. hypogaea* including control. Therefore the host plant and their nutrients may be regulating *S. litura* survival ability to overcome pathogenic infections. The presence of host plants in the field, the condition needs to be considered to avoid plant pest infecting species.

Author contributions

Designed and performed the experiments, G. V.; Analysed the data, G.V.; M.A.; Manuscript correction, M.S.S.

Declaration of Competing Interest

The authors report no declarations of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.toxrep.2020.10.005.

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