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Effects of Indian coral tree, *Erythrina indica* lectin on eggs and larval development of melon fruit fly, *Bactrocera cucurbitae*

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Abstract: *Present study was undertaken to investigate the influence of D-galactose binding lectin from Erythrina indica Lam. on the eggs and second instar larvae (64-72 hr) of melon fruit fly, Bactrocera cucurbitae (Coquillett). The lectin from E. indica seeds was extracted and purified by affinity chromatography using asilofetuin linked porous amino activated silica beads. The effects of various concentrations (0, 125, 250, 500 and 1000 µg ml^{-1}) of lectin were studied on freshly laid eggs (0-8 hr) of B. cucurbitae which showed non-significant reduction in percent hatching of eggs. However, the treatment of second instar larvae (64-72 hr) with various test concentrations (0, 25, 50, 100 and 200 µg ml^{-1}) of lectin significantly reduced the percent pupation and percent emergence of B. cucurbitae depicting a negative correlation with the lectin concentration. The LC_{50} (81µg ml^{-1}) treatment significantly decreased the pupal weight. Moreover, the treatment of larvae had also induced a significant increase in the remaining development duration. The activity of three hydrolase enzymes (esterases, acid and alkaline phosphatases), one oxidoreductase (catalase) and one group transfer enzyme (glutathione S-transferases) was assayed in second instar larvae under the influence of LC_{50} concentration of lectin for three exposure intervals (24, 48 and 72 hr). It significantly suppressed the activity of all the enzymes after all the three exposure intervals except for esterases which increased significantly.*

Key words: *Lectin, Erythrina indica, Bactrocera cucurbitae, Development, Esterases, Phosphatases, Catalase, Transferases*
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Introduction

Plant lectins, a heterogenous group of proteins or glycoprotein's, are classified on the basis of their ability to recognize and specifically bind to carbohydrate ligands (Goldstein and Poretz, 1986). In addition to storage function, the plant lectins have been associated with the important role of defence in plants (Murdock and Shade, 2002). These are widely used as tools in the study of different biological processes such as detection, isolation, characterization of glycoproteins and glycolipids and also as anti-cancer and anti-fungal agents. Another possible but important biological application can be their anti-insect activity, as some plant lectins have already been reported to have detrimental effects on some insect species (Gatehouse *et al*., 1996; Goldstein and Poretz, 1986; Rao *et al*., 1998; Bandyopadhyay *et al*., 2001). The significance of studying insecticidal lectins lies in the possibility of cloning and transferring the lectin genes into plants susceptible to insects, thereby conferring resistance in them. Already a few plant species have been thus transformed, such as rice, O*rzya sativa* L. (Rao *et al*., 1998), wheat, *Triticum aestivum* L. (Stoger *et al*., 1999), potato, *Solanum tuberosum* L. (Gatehouse *et al*., 1996), and tobacco, *Nicotiana tobacum* L. (Wang and Guo, 1999) with gene from snowdrop, *Galanthus nivalis* L. Transgenic potato plants expressing Concanavalin A lectin from jackbean, *Canavalia ensiformis* (L.) retarded the development of tomato moth, *Lacanobia oleracea* L. and also decreased its larval weight (Gatehouse *et al*., 1999). Consequently lectins are attracting attention of scientists and gaining significance day by day as potentially useful agents of insect resistance when introduced into transgenic plants.

Previous studies have shown that legume lectins have negative effects on the growth and development of several insects. The kidney beans (*Phaseolus vulgaris* L.) and pea, (*Pisum sativum* L.) were found to be toxic to cowpea weevil (*Callosobrucus maculatus* F.) larvae fed on treated artificial diet (Boulter *et al*., 1986). The lectin from soybean (*Glycine max* L.) also showed detrimental effects on the larval growth of *Manduca sexta* (Johanson) when incorporated into the diet at 1% level. There was a significant weight difference between control and treated larvae at the end of 8 days (Shukle and Murdock, 1983). Another legume lectin having specificity for D-galactose, from Indian Coral tree, *Erythrina indica* Lam. (Synonym of *E. variegata* L.) has already been isolated from seeds and leaves, purified and characterized but its biological activity in various systems is yet to be explored and realized in detail (Konozy *et al*., 2002).

The prerequisite studies in order to ascertain the anti-insect activity of any compound include the investigations regarding its influence on the growth, development and toxicity on some insect model. For this purpose, melon fruit fly, *Bactrocera cucurbitae* (Coquillett) belonging to family Tephritidae and order Diptera was selected. This fly is a serious pest of fruits and vegetables especially of Cucurbitae family in tropical countries where it has defied the conventional control technology repeatedly and sometimes its damage has reached up to 100% (Gupta *et al*., 1978). Moreover, this fly is of large size and can be cultured in the laboratory. Therefore, it was proposed to study the influence of *E. indica* lectin on various developmental parameters such as egg hatching, developmental duration, percent pupation, percent emergence and subsequently

on some important enzymes involved in digestion, growth, development, metabolism and ageing of the melon fruit fly as a first step in the process to explore the potential of this lectin in the management strategies of insect pests.

Materials and Methods

Plant material: Seeds of Indian coral tree, *E. indica* were purchased from Dehradun.

Insect rearing: The stock cultures of melon fruit flies were reared using the procedure described by Gupta *et al.* (1978) in wire mesh cages (Rescholar equipment; L 45 x B 45 x H 50 cm). The adult flies were provided with proteinex (Pfizer India) and 20% sugar solution as food along with pieces of pumpkin fruit, *Cucurbitae moschata* Dusch for oviposition. The cultures of flies were maintained in insect culture room with regulated temperature (25±2°C), relative humidity (70-80%) and photophase (10:14 LD).

Extraction, isolation and purification of lectin from seeds of *E. indica*: Lectin from *E. indica* seeds was extracted with 0.01 M phosphate buffered saline (PBS) pH 7.2 (1:5 w/v). The mixture was allowed to stand overnight at 4°C. After centrifugation at 20,000 rpm for 30 min, the clear supernatant obtained was dialyzed against 0.01 M PBS, pH 7.2 at 4°C to remove any low molecular weight substances, which may interfere in lectin activity. The dialyzed crude extract was applied to affinity column of asilofetuin-linked amino activated silica beads (pore size: 1000 Å, diameter: 100 µ) equilibrated with 0.01 M PBS, pH 7.2. The column of asilofetuin-linked amino activated silica beads was prepared as described in Shangary *et al*. (1995). The bound lectin was eluted with 0.1 M glycine–HCl buffer, pH 2.5 and the eluted fractions were neutralized immediately with 2 M Tris–HCl buffer, pH 8.8.

Hemagglutination assay: This assay was done to check the activity of lectin rich fractions out of the total eluted fractions collected. It was performed in 96 well polystyrene microtitre plate having U-shaped wells. Thirty µl of 2% human O-blood group erythrocytes suspension was dispensed in each well containing the same amount of test lectin. The plate was incubated for 1h at 37°C. The agglutination was observed with naked eye (Kaur *et al*., 2002). The active fractions were pooled and extensively dialyzed against 0.01 M PBS at 4°C to bring the purified lectin in physiological buffer and to remove Tris ions, which interfere in protein estimation.

Native PAGE: To check the affinity purity of purified lectin preparations, native PAGE at pH 4.5 was carried out using 7.5 percent tube gel by the method of Reisfeld *et al*. (1962).

Protein estimation: Protein estimation was done in crude and purified lectin preparations by the method of Lowry *et al.* (1951) using bovine serum albumen as standard, for preparing various test concentrations for performing experiments with eggs and larvae.

Experiment with eggs: The eggs of melon fruit fly (0-8 hr) were harvested from egg-charged pumpkin pieces which were kept in mesh cages for 1-8 hr, having 100 gravid females. Twenty eggs were placed on a small triangular piece of filter paper and 20 µl of solution having required concentration of lectin (0, 125, 250, 500 and 1000 µg ml^{-1}) was poured on it. After one minute the excess of lectin solution was drained and the filter paper piece (having treated eggs on it) was shifted to a vial containing artificial culture media suggested by Srivastava (1975) for this fly. The vials were kept in culture room and observed at intervals of 24 hr for hatching of larvae. There were 20 eggs in each vial with six replications for each concentration and the experiment was repeated twice.

Experiments with larvae: About 100 gravid females were released in mesh cages having fresh pumpkin pieces for 8 hr and these charged pumpkin pieces were dissected in saline water for harvesting the larvae (64-72 hr old), after 64 hr of the removal of the fruit flies. The harvested larvae were shifted to culture vials (25 mm Dx100 mm L) containing treated medium of various concentrations (0, 25, 50, 100 and 200 µg ml^{-1}) of *E. indica* lectin. The experimental vials were kept in culture room and observed daily for various parameters such as percent pupation, percent emergence and developmental period. There were six replications with 20 larvae each for each concentration and the experiments were repeated twice.

Another experiment was set up to adjudge the influence of LC_{50} concentration (81µg ml^{-1}) of *E. indica* lectin on pupal weight of larvae. The larvae (64 -72 hr old) were permitted ad-libitum feeding on lectin treated medium till pupation and then pupal weights were measured. There were 10 larvae in each vial and six replications were used each for control and treatment.

Biochemical analysis: The second instar (64 -72 hr) larvae were released on both the treated and control diet for periods of 24, 48 and 72 hr. The larvae were harvested after specified treatment period and were assayed for activity of five enzymes *i.e.* three hydrolases (esterases, acid and alkaline phosphatases) one oxidoreductase (catalase) and one group transferase (glutathione S-transferases). The estimations of various enzymes were done on the fresh weight basis by taking 10 larvae for preparing the required concentration of homogenate. There were six replications for each experiment. The methodology given by Katzenellenbogen and Kafatos (1971) was followed for extraction and estimation of esterases activity. The catalase activity was measured according to the protocol given by Bergmeyer (1974). Activity of phosphatases (acid and alkaline) was determined by following the method given by Mac Intyre (1971). Glutathione S-transferases (GSTs) activity was estimated as given by Chein and Dauterman (1991).

Statistical analysis: The data were subjected to statistical analysis by applying ANOVA (one way), least significant difference (LSD), and Student's 't' test. Probit line was drawn for emergence in order to calculate LC_{50}. All these tests were carried out with the help of SPSS computer program.

Results and Discussion

The present study describes the effects of affinity purified *E. indica* lectin on the eggs and larval development of *B. cucurbitae.*

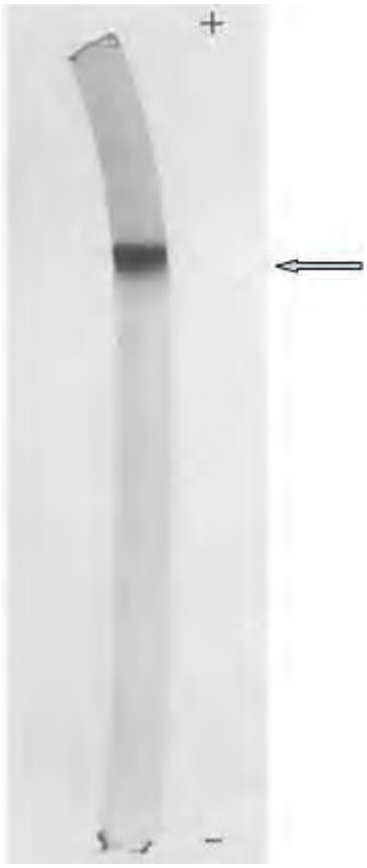

Fig. 1 Discontinuous PAGE of *E. indica* lectin at pH 4.5 using 7.5% tube gel (running time 8 hr at constant 150 V). Eighty micrograms of protein loaded on each tube gel

Table 1. Percent hatching of *B. cucurbitae* eggs after treatment with lectin from *E. indica*

Lectin concentration (µg ml^{-1})	Percent hatching (Mean ± SE)
0 (Control)	88.33±1.68
125	86.70±2.20
250	86.67±2.12
500	83.33±2.10
1000	80.00±2.58
F (df=4, 20)	2.50^{NS}

^{NS} = Non significant

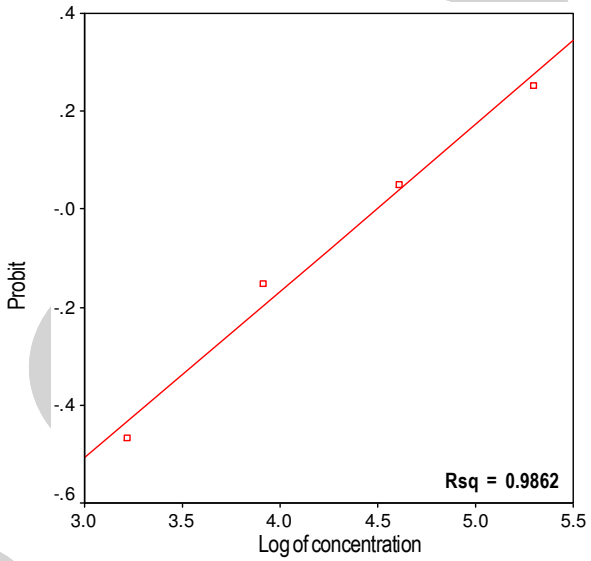

Fig. 2: Probit line response curve of percent emergence of *B. cucurbitae* under the influence of various concentrations of *E. indica* lectin

The lectin gave a single band in native PAGE at pH 4.5 assuring the purity of lectin preparations (Fig. 1 arrow). The treatment of freshly laid eggs with various concentrations (from 0 to 1000 µg ml^{-1}) of lectin resulted in statistically non-significant reduction in the hatching potential (Table 1). The failure of the lectin to bind the chorion of the egg during the short embryonic period (20-24 hr) and then to interfere in development of the embryos might be the cause for its non-significant ovicidal influence.

The treatment of second instar larvae (64-72 hr) induced more severe effect as compared to that of eggs, on percent pupation, percent emergence of adult flies and on the remaining developmental duration. The treatment of the larvae lowered the percent pupation significantly ($p<0.01$). It showed negative correlation with increase in concentration of lectin. The pupation got reduced only to 50% at 100 µg ml^{-1} concentration as compared to control with $LSD_{0.01}$ 11.80%. The intensity of the influence of this lectin was manifested further with a significant ($p<0.01$) and negatively correlated reduction in emergence of adult flies from these pupae. The emergence at 100 µg ml^{-1} concentration was less than 48% of that in control with $LSD_{0.01}$ 8.94% between concentrations (Table 2). A very low concentration 81µg ml^{-1} was found to be the LC_{50} as per probit analysis (Fig. 2).

The remaining developmental duration got prolonged significantly as compared to control (Table 2). The treatment of the larvae with LC_{50} concentration reduced the pupal weight by being 6.30±0.33 mg per pupae as compared to control where it was 7.00±0.10 mg per pupae and the difference was statistically significant at 5% ($p<0.05$).

Highly significant inhibitory effect demonstrated by *E. indica* lectin both on the pupation and emergence which could be either due to its anti-feedant property or due to anti-metabolic effect. The assumption of anti-feedant effect gets support from the fact that remaining developmental duration had also prolonged significantly and the pupal weight got significantly ($p<0.05$) reduced when larvae were treated with LC_{50} concentration (81 µg ml^{-1}) of the lectin. Also, recently in our laboratory asialofetuin binding tetrameric lectins extracted from tubers of two other species of *Arisaema i.e. A. helleborifolium* Schot and *A. jacquemontii* Blume belonging to Araceae family had shown significant inhibitory effect both on the pupation and emergence of melon fruit fly by having 32 and 34 µg ml^{-1} LC_{50} (Kaur *et al.,* 2006a,b). Corroboratory results to the present findings with D-galactose binding legume lectin (*Soybean agglutinin*) had been reported in literature by Shukle and Murdock

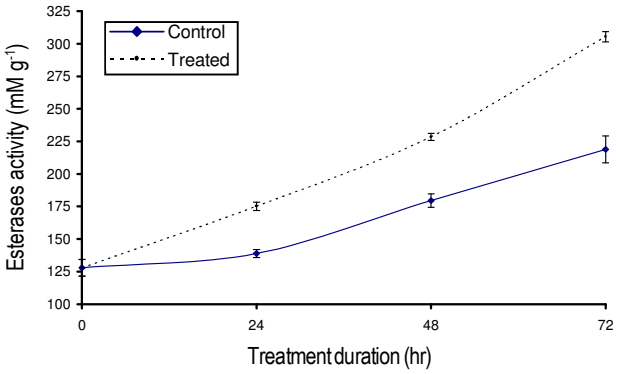

Fig. 3a: Esterase activity of *B. cucurbitae* larvae under the influence of *E. indica* lectin

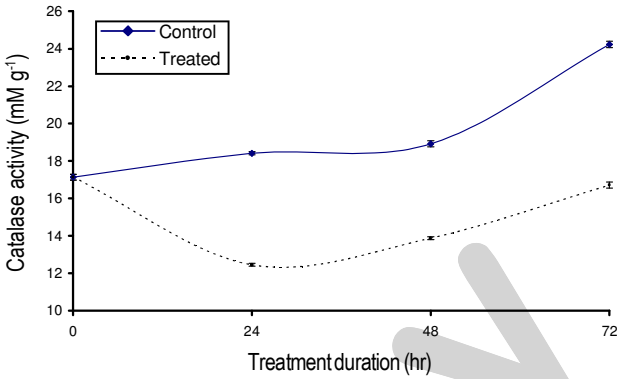

Fig. 3d: Catalase activity of *B. cucurbitae* larvae under the influence of *E. indica* lectin

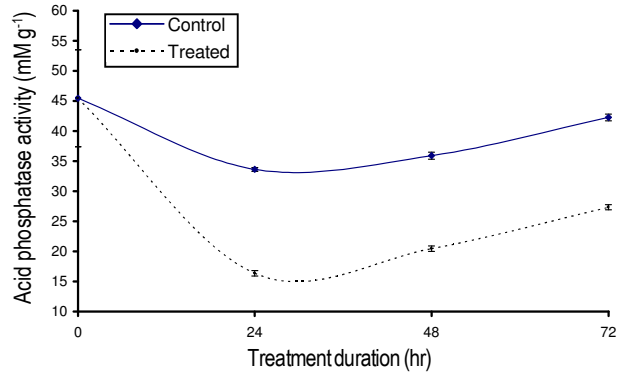

Fig. 3b: Acid phosphatase activity of *B. cucurbitae* larvae under the influence of *E. indica* lectin

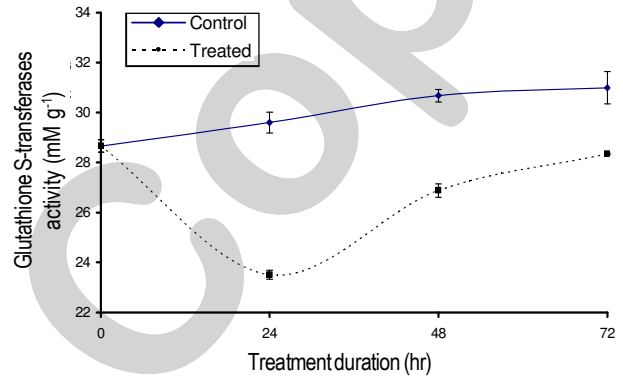

Fig. 3e: Glutathione S-transferases activity of *B. cucurbitae* larvae under the influence of *E. indica* lectin

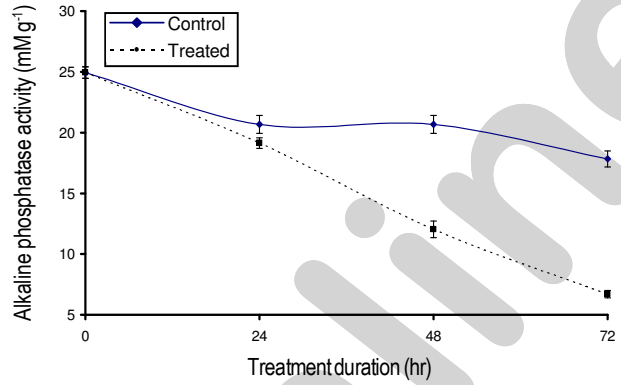

Fig. 3c: Alkaline phosphatase activity of *B. cucurbitae* larvae under the influence of *E. indica* lectin

(1983) where they observed that 1% concentration of lectin inhibited larval growth of tobacco hornworm, *Manduca sexta* (L.). Habibi *et al.* (1993) also observed that other D-galactose binding lectins from lentil, *Lens culinaris* Medk. and horse gram, *Dolichos bifloris* L. significantly reduced survival of sap sucking potato leafhopper, *Empoasca fabae* (Harris) compared to control at the dietary level of 0.2-1.5%. Recently, Machuka *et al*. (2000) had found that another D-galactose binding lectin from African yam beans (AYB), *Sphenostylis stenocarpa* (A. Rich) Harms inhibited the development of cowpea weevil, *C. maculatus* and also increased the larval mortality from 30 to 80%, however, the same lectin failed to induce any derogatory effect in the larvae of legume pod-borer, *Maruca vitrata* (Fab.).

The activity of five enzymes (esterases, acid and alkaline phosphatases, catalase and glutathione S-transferases) was assayed under the influence of lectin from *E. indica* in the second instar larvae of *B. cucurbitae*. The activity of esterases which are usually involved in digestion and hydrolysis in metabolism, increased with the maturation of larvae from 72 hr age to 144 hr age during the normal course of development. The increase in activity was 1.7 times as it was 127.95±6.45 mM g^{-1} at 72 hr age and 218.96±4.00 mM g^{-1} at the age of 144 hr. The treatment given to larvae with affinity purified lectin from *E. indica* at LC_{50} (81 µg ml^{-1}) concentration resulted in a significant ($p<0.01$) increase in esterases activity at all the three time intervals compared to controls, for example the increase after 72 hr of treatment (144 hr old larvae) was 2.42 times by being 305.45±10.4 mM g^{-1} (Fig. 3a) compared to 1.7 times in controls. The role of esterases in development of resistance and in sequestration of xenobiotics has been well established (Devorshak and Roe, 1999; Rup *et al*., 1999; Yu, 2004). But there is hardly any literature available related to the influence of lectins on the enzyme system of the insects except with lectins from snowdrop, *Galanthus nivalis* L. and jackbean, *Canavalia ensiformis* (L.) DC. as reported by Fitches and Gatehouse (1998) who observed that these lectins affected the activities of soluble

Table - 2: Percent pupation, emergence and remaining development duration of *B. cucurbitae* after treatment of second instar larvae with lectin from *E. indica*

Lectin concentration (µg ml^{-1})	Percent pupation (Mean ± SE)	Percent emergence (Mean ± SE)	Remaining development duration (in days) (Mean ± SE)
0 (control)	100.00	100.00	15.07±0.08
25	69.90±3.07	67.98±3.07	15.64±0.13
50	52.38±4.94	55.98±3.33	15.85±0.18
100	50.00±2.24	47.99±2.58	15.90±0.18
200	38.10±4.22	40.00±4.28	16.38±0.20
F(df=4, 20)	32.74*	19.26*	8.07*
$LSD_{0.01}$	11.80	8.94	0.61
$LSD_{0.05}$	8.65	6.55	0.44

* = Significant at 1%

and brush border membrane enzymes (α-glucosidase and alkaline phosphatase) in the midgut of *Lacanobia oleracea* L. larvae. The increase in the plateau of esterase activity of treated larvae in the present experiment suggests that esterases might be playing a significant role in detoxification of *E. indica* lectin and the increase in activity could be attributed to positive feedback response.

Among the phosphatases the activity of acid phosphatase decreased at the age of 96 hr and then it gradually increased and came near to that of control at 72 hr of age during normal course of larval development. The treatment with *E. indica* lectin showed a similar trend of activity but there was a significant suppression ($p<0.01$) compared to control at all the three time intervals assayed (Fig. 3b). The other phosphatase enzyme, alkaline phosphatase showed a slight but gradual decrease as the larval development progressed. The treatment with *E. indica* lectin showed a significant suppression ($p<0.01$) in the activity of alkaline phosphatase in all the three treatment durations as compared to controls. The difference between activities of controls and treatments increased as the larvae advanced in age so much so that after 72 hr (144 hr ages) of treatment the activity was about 37.5% of that in control at that age (Fig. 3c). The suppression of hydrolases (acid and alkaline phosphatases) indicated that both acid and alkaline might not be playing any significant role in the detoxification of lectin from *E. indica* in *B. cucurbitae* and that *E. indica* lectin might be interfering in the feedback biomechanism of these enzymes during their synthesis.

The catalase enzyme which is usually involved in decomposition of hydrogen peroxide and in the detoxification of xenobiotics showed a gradual increase in activity from 17.13±0.16 mM g^{-1} to 24.23±0.17 mM g^{-1} as the larval development progressed. Catalase activity in treated larvae showed a significant suppression ($p<0.01$) at all the three age groups assayed. (Fig.3d). The suppression in catalase activity with the application of *E. indica* lectin indicated that some alternative enzymes are involved in the detoxification of oxygen radicals generated by the application of lectin in *B. cucurbitae* and this lectin is having a toxic effect on the synthesis of this enzyme.

The activity of GSTs showed a slow and gradual increase as the larvae matured. The activity of GSTs under the influence of lectin was also significantly suppressed ($p<0.01$) at all the three time intervals compared to control and suppression was maximum after 24 hr (96 hr age) of treatment by being 23.51 mM g^{-1} in treated larvae as compared to control where it was 29.60 mM g^{-1}. (Fig. 3e). The group transferases (GSTs) generally play a central role in detoxification of endogenous and xenobiotic compounds in insects and are also involved in biosynthesis of hormones, intracellular transport and against oxidative stress (Enayati *et al*., 2005). But this does not seem to be a universal phenomenon as in many insects the activity of GSTs gets suppressed under the influence of xenobiotics. In congruent to the present finding, a number of allelochemicals suppressed GSTs activity *e.g.* in cabbage looper, *Trichoplusia ni* (Hb) and oligophagous black swallow tail, *Palilio poyxenes* (Fab.) with plant phenols (Lee, 1991); in the blood sucking bug, *Tritoma infestans* Klug with flavonoid, quercitin and gossypol (Sivori *et al*., 1997) in the fall armyworm, *S. frugiperda* with flavonoids, phenols and isothiocynates *etc.* (Yu and Abo-Elghar, 2000) and in aphid, *Rhopalosiphum padi* (L.) with hydroxamic acid (Mukanganyama *et al*., 2003). All these workers inferred that these chemicals interfered in the GSTs mediated detoxification of xenobiotics by suppressing GSTs activity. Results analogous to the findings of present experiment were also perceived by Singh *et al*. (2006) when they treated *B. cucurbitae* larvae with D-galactose binding *Glycine max* lectin.

The mechanisms by means of which lectins exercise their toxic effects in insects are not clearly understood, but one possible way suggested by Czapla and Lang (1990) is that the lectins may bind to the peritrophic membrane (PM) in the midgut region and block the bidirectional movement of the nutrient or prevent the formation of the membranes itself. Other possibility is that the lectin molecules first have to bind to receptors on the midgut epithelium, resulting in subsequent systemic effects (Eisemann *et al.,* 1994).

From the present finding it could be concluded that lectin from *E. indica* has great potentiality as anti-insect compound not only due to its ability to reduce percent pupation and adult emergence but also due to its efficacy in influencing the normal growth, development and metabolism of the fruit fly. Therefore, there is a need to explore the mechanism of action of this lectin in detail, identification of the genes involved in synthesis of lectin and subsequently transformation of this gene for making transgenic plant. These studies will be beneficial in development of pest

resistance in important crops attacked by fruit flies specifically and other insects in general.

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