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Effect of plant extracts on biochemistry of *Bactrocera cucurbitae* (Coquillett)

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ABSTRACT
The present study was to postulate hypothesis to generate vegetable crops with increased level of bioactive compounds against pests thus modifying the antioxidant capacities. To accomplish this, the expression of some antioxidant and detoxifying enzymes in *Bactrocera cucurbitae* (Coquillett) larvae after treatment with plant (*Acacia auriculiformis* A. Cunn.) derived fractions was analyzed. The increased concentrations of these fractions in artificial diet of larvae showed altered activities of enzymes. Artificial diet incorporated with these fractions showed improved antioxidant capacity; however, there is a complex relationship between antioxidant capacity and selected phytochemical content, suggesting the role of these compounds in antioxidant potential of plants.

Keywords: *Acacia auriculiformis,* antioxidant, *Bactrocera cucurbitae,* Biochemical, detoxifying, Phytochemicals.

1. Introduction
The interactive relationship between plants and insects has resulted in the evolution of diverse chemical substances which are not only involved in essential metabolic and photosynthetic processes of plants, but also constitute an important component of their defense arsenal. The bioprospection of these compounds for their anti-insect potential could lead to the development of new compounds to control insect pests. Irrational use of broad-spectrum insecticides was recognized with its hazardous effects on environment and mankind soon after their introduction [1]. They can harm many non-target and beneficial insects, leading to outbreaks of secondary pests and may bioaccumulate in the food chain [2]. The global outburst of population needs a continuous food supply. However, the problems associated with insecticide resistance and environmental contamination cannot be ignored. These concerns have promoted the search for environment-friendly pest control strategies. The demand for natural bioactive products has intensified many folds in recent years because of their biologically active and rapidly degrading nature; these bioactive compounds are being sought for use in alternative and sustainable integrated pest management programs [3]. Phytochemicals may contain active compounds with insecticidal properties and can be developed as inexpensive and eco-friendly insecticides. Herbivorous insects possess various physiological traits that enable them to exploit chemical substances, which are potentially damaging to their cellular processes. The antioxidant and detoxification enzymes of insects are important in detoxification of reactive oxygen species produced by plant allelochemicals such as furanocoumarins, lignans, phenolics, quinines etc. [4 & 5]. Prooxidant phenolic compounds are often transformed to semiquinone radicals [6], which in turn react with O_2 to generate superoxide radicals and consequently hydrogen peroxide (H_2O_2) and hydroxyl radicals. These radicals may react with biomolecules such as DNA, RNA, proteins and lipids causing alterations within their structures. For phytophagous insects, lipid peroxidation is especially harmful because it changes cell membrane permeability and disturbs specific functions of juvenile hormones and pheromones [7]. Keeping in mind the importance of phenolic compounds in development of resistance in plants, the present study was carried out to examine the biochemical activity of plant fractions of *A. auriculiformis* against the melon fruit fly. In order to gain some insight into the metabolic adaptations occurring at biochemical level, it was also envisaged to investigate the activity of some antioxidant and detoxifying enzymes of *B. cucurbitae* under the influence of partitioned fractions of *A. auriculiformis.* In the present study, acute toxicity and mode of action of plant-derived compounds against the melon fruit fly *Bactrocera cucurbitae* (Coquillett) was evaluated. Three plant fractions (A1, A2 and A3) were

analyzed for their lethal and sublethal effect against melon fruit fly. The resultant LC_{50} concentrations of these phytochemical fractions were used as test concentration for enzyme assays to evaluated the biochemical mechanisms underlying the activity of these phytochemicals and the basis of their toxicity to the insect. These phytochemicals affect the biotransformational capacity of the insect to detoxify the compounds and acts as naturally occurring enzyme inhibitors playing an important role in development of genetically modified crops and pesticidal discovery program.

2. Material and methods

The present study was carried out in the Insect Physiology and Biochemistry Laboratory of Department of Zoology, Guru Nanak Dev University, Amritsar, Punjab (India). The findings of the present study will postulate a basis for development of genetically modified vegetable crops with enhanced expression of biotoxic phytochemicals against fruit fly and the development of cucurbitaceous crop without pesticide residue.

2.1 Procurement and extraction of plant material

A. auriculiformis found in the campus of the Guru Nanak Dev University, Amritsar was identified by comparing it with the specimen available in herbarium (Voucher number 6422). The bark was procured, washed with tap water (thrice), dried and ground to fine powder. Dried bark powder (2 kg) was suspended in 1500ml of ethyl acetate and kept on shaker for 24 hours at room temperature. After 24 hours, the suspended solid was filtered off through Whatman No.1 filter paper and the filtrate was collected. The solid residue was again suspended in ethyl acetate (1500 ml) and the mixture was allowed to stand again for 24 hours. This procedure was repeated thrice to obtain three filtrates of ethyl acetate. The filtrates were combined and solvent was removed under vacuum using rotary evaporator. This extract was subjected to column chromatography to isolate different phytochemical rich fractions.

2.2 Laboratory Rearing of Melon Fruit Fly: The wild culture of melon fruit fly was procured from the infested bitter gourds, *Momordica charantia* collected from the kitchen gardens of university campus and vegetable market of Amritsar city. The freshly emerged flies from the infested bitter gourds were identified on the basis of its taxonomic characters given by Kapoor [8]. The flies were reared in the laboratory as per the requirement on natural and artificial diet in insect culture room/B.O.D under controlled temperature (25±2 ºC), relative humidity (70-80%) and photoperiod (10 Light: 14 Dark).

2.2.1 Insect Rearing on Natural Food: The methodology of Gupta and Verma [9] was used for rearing the larvae of melon fruit flies on natural food under controlled laboratory conditions.

2.2.2 Insect Rearing on Artificial Diet: Standardized methodology given by Srivastava [10] was used to rear fruit fly larvae on artificial diet.

2.3 Biochemical assay: The biochemical assay was carried out in second instar larvae (64-72 hours old) fed on artificial diet incorporated with LC_{50} concentration of fraction A1, A2 and A3.

The *ad libitum* feeding to these larvae was given thrice at regular intervals of 24 hours (24, 48 and 72 hours) on treated diet as well as untreated diet. Estimations were done for analyzing the activity of four antioxidant enzymes viz; ascorbate peroxidase, peroxidase, superoxide dismutase (SOD), catalase and two detoxification enzymes, esterases and glutathione S-transferases (GSTs), involved in metabolism and detoxification mechanisms. There were six replicates under each treatment and control for each time interval. The experiments were repeated twice.

2.3.1 Ascorbate peroxidase: The method given by asada [11] was used for estimating the activity of ascorbate peroxidase.

2.3.2 Peroxidase: Peroxidase activity was assayed by the method of Kar and Mishra [12] as modified by Kumar and Khan [13].

2.3.3 Superoxide dismutase (SOD): Superoxide dismutase (SOD) activity was estimated according to the methodology of Kono [14].

2.3.4 Catalase: The methodology of enzyme extraction and estimation followed was given by Bergmeyer [15].

2.3.5 Esterases: The methodology of Katzenellenbogen and Kafatos [16] was used to extract and estimate esterases.

2.3.6 Glutathione S-transferases (GSTs): The method of Chien and Dauterman [17] was followed for extraction and estimation of glutathione S-transferases (GSTs).

2.4 Statistical analysis: The data was computed and statistically analyzed for Student's 't' test by using SPSS software and the following tests were employed according to the requirements of the experiments.

3. Results

After giving oral treatment of different concentrations of fraction A1, A2 and A3 to the second instar larvae of melon fruit fly in their artificial diet, the values of LC_{50} calculated with probit analysis were 32.28 ppm, 46.77 ppm and 89.13 ppm for fractions A1, A2 and A3 respectively. Now the biochemical assay with these concentrations was performed.

3.1 Effect of A1 on enzymes of melon fruit fly: Investigations pertaining to the activity of six enzymes got specifically influenced with the treatment whereas GSTs showed insignificant effect of treatments (Table 1, Figure 1). The ascorbate peroxidase showed inconsistent activity during normal course of development, increased significantly at all treatment intervals (Table 1, Figure 1). All other enzymes *i.e.* peroxidase, SOD, catalase and esterase showed alterations during normal development. Peroxidase activity decreased significantly after 24 hours and 72 hours of treatment but remained same as control at 48 hours (Table 1, Figure 1). SOD showed increased activity after 24 hours of treatment at 5% level of significance whereas an insignificant decrease after 48 hours and significant ($p<0.01$) decrease after 72 hours of treatment was observed (Table 1, Figure 1).

Table 1: Enzyme activity in melon fruit fly treated with fraction A1.

Enzyme	C T F-Value	0 Hours Mean ± S. E.	24 Hours Mean ± S. E.	48 Hours Mean ± S. E.	72 Hours Mean ± S. E.
APox (mM/min./g tissue)	C T F-Value	0.01±0.00 - -	0.02±0.00 0.04±0.00 11.4*	0.01±0.00 0.02±0.00 11.5*	0.01±0.00 0.02±0.00 11.8*
Pox (µM/min./g tissue)	C T F-Value	0.07±0.00 - -	0.07±0.00 0.03±0.00 11.7*	0.06±0.00 0.06±0.00 0.4^{NS}	0.09±.00 0.03±0.00 15.0*
SOD (Units/µl)	C T F-Value	0.05±0.00 - -	0.04±0.00 0.07±0.01 3.0**	0.06±0.00 0.05±0.00 2.3^{NS}	0.06±0.00 0.04±0.00 7.7*
Cat (mM/min./g tissue)	C T F-Value	0.16±0.00 - -	0.15±0.00 0.14±0.00 2.6**	0.09±0.00 0.11±0.00 9.6*	0.01±0.00 0.07±0.00 40.5*
Est (mM/min./g tissue)	C T F-Value	0.02±0.00 - -	0.04±0.00 0.03±0.00 2.6**	0.04±0.00 0.05±0.00 0.2^{NS}	0.04±0.00 0.05±0.00 4.4*
GST (mM/min./g tissue)	C T F-Value	0.08±0.00 - -	0.06±0.00 0.05±0.00 1.8^{NS}	0.07±0.00 0.08±0.00 1.8 ^{NS}	0.08±0.01 0.08±0.00 0.4 ^{NS}

**Significant at 1% level, *Significant at 5% level, ^{NS} Non Significant

A significant ($p<0.05$) decrease in activity of catalase was reported after 24 hours of treatment but the activity was significantly ($p<0.01$) increased after 48 hours and 72 hours of treatment (Table 1, Figure 1). At three different treatment intervals, enzyme esterase showed different activity. After 24 hours of treatment, a significant decrease at 5% level of significance, after 48 hours, an insignificant increase and after 72 hours, significant ($p<0.01$) increase in enzyme activity was reported (Table 1, Figure 1).

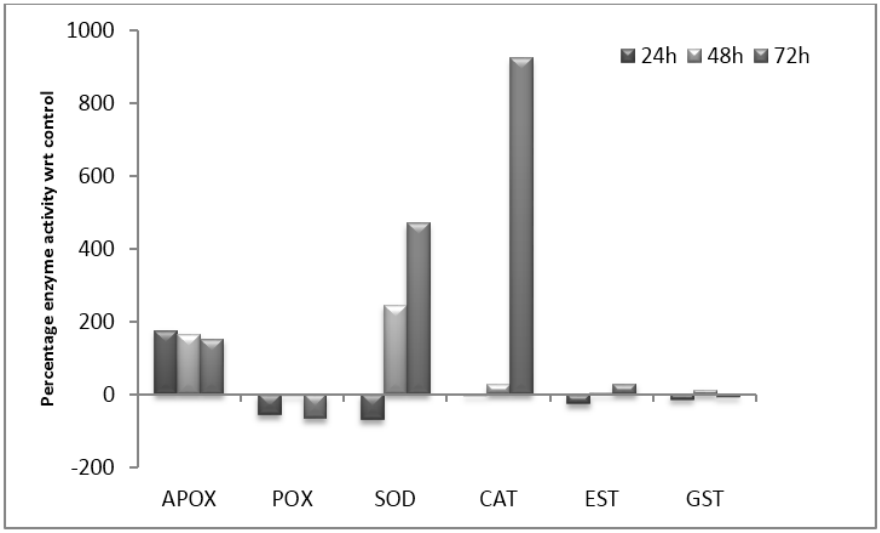

Fig 1: Percentage enzyme activity w.r.t. control in melon fruit fly treated with fraction A1.

3.2 Effect of A2 on enzymes of melon fruit fly: The activity of hydrolases, oxidoreductase and transferase was assayed at three time intervals (24, 48 and 72 hours) under the influence of LC_{50} (46.77 ppm) of gallic acid in the second instar larvae of *B. cucurbitae*. Among the phosphatases, the activity of ascorbate peroxidase significantly increased at 24 hours and 72 hours but decrease significantly at 48 hours during normal course of larval development (Table 2, Figure 2). Peroxidase showed significantly consistent decrease in enzyme activity at all the three assessed developmental time intervals (Table 2, Figure 2). Value of SOD activity increased significantly ($p<0.05$) after 24 hours of treated diet feeding whereas an insignificant unchanged enzyme activity was observed at 48 hours and 72 hours (Table 2, Figure 2). Catalase induced a continuously decreased enzyme activity at all the three developmental durations under investigation ($p<0.01$). The activity of esterase usually involved in digestion and GSTs (transferases) insignificantly decrease at 24 hours whereas significantly ($p<0.01$) decrease at 48 hours and 72 hours (Table 2, Figure 2).

Table 2: Enzyme activity in melon fruit fly treated with fraction A2.

Enzyme	C T F-Value	0 Hours Mean ± S. E.	24 Hours Mean ± S. E.	48 Hours Mean ± S. E.	72 Hours Mean ± S. E.
APox (mM/min./g tissue)	C T F-Value	0.01±0.00 - -	0.02±0.00 0.05±0.00 6.9*	0.01±0.00 0.02±0.00 8.7*	0.01±0.00 0.03±0.00 19.4*
Pox (µM/min./g tissue)	C T F-Value	0.08±0.00 - -	0.08±0.00 0.05±0.00 12.3*	0.08±0.00 0.06±0.00 3.1**	0.08±0.00 0.05±0.00 7.7**
SOD (Units/µl)	C T F-Value	0.06±0.00 - -	0.05±0.00 0.06±0.01 5.3**	0.05±0.00 0.05±0.00 2.2^{NS}	0.06±0.00 0.06±0.00 0.9^{NS}
Cat (mM/min./g tissue)	C T F-Value	0.15±0.01 - -	0.15±0.00 0.11±0.00 10.2*	0.09±0.00 0.01±0.00 51.3*	0.01±0.00 0.00±0.00 5.4*
Est (mM/min./g tissue)	C T F-Value	0.03±0.00 - -	0.04±0.00 0.05±0.00 1.3^{NS}	0.06±0.00 0.03±0.00 4.9*	0.05±0.00 0.02±0.00 10.5*
GST (mM/min./g tissue)	C T F-Value	0.04±0.00 - -	0.09±0.02 0.10±0.00 0.04^{NS}	0.10±0.00 0.03±0.00 13.9*	0.09±0.01 0.05±0.00 5.5*

**Significant at 1% level, *Significant at 5% level, ^{NS} Non Significant

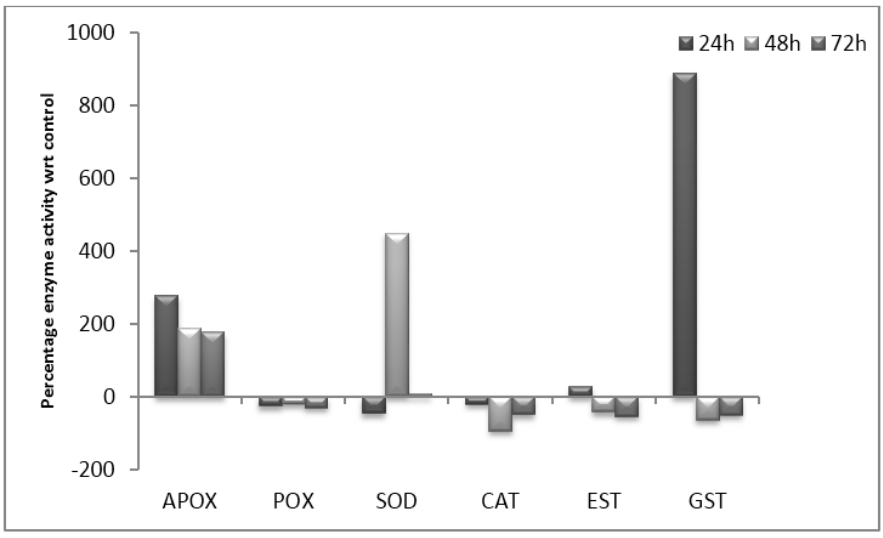

Fig 2: Percentage enzyme activity w.r.t. control in melon fruit fly treated with fraction A2.

3.3 Effect of A3 on enzymes of melon fruit fly: 89.13 ppm of A3 was fed to larvae for assessment of enzyme activity. A consistence and significant increase in activity of ascorbate peroxidase was reported. Peroxidase showed increased activity after 24 and 48 hours and decrease in activity at 72 hours of treatment. The insignificant increase and similar activity of SOD was observed after 24 and 72 hours of treatment. After 48 hours, a significant ($p<0.01$) decrease was reported. Catalase activity showed consistent and significant ($p<0.01$) values at all the three developmental levels whereas GSTs showed continuous decrease in enzyme activity with normal developmental pattern. The activity of esterase significantly increased at 24 hours, insignificantly decreased at 48 hours and significantly decreased at 72 hours interval (Table 3, Figure 3).

Table 3: Enzyme activity in melon fruit fly treated with fraction A3.

Enzyme	C T F-Value	0 Hours Mean ± S. E.	24 Hours Mean ± S. E.	48 Hours Mean ± S. E.	72 Hours Mean ± S. E.
APox (mM/min./g tissue)	C T F-Value	0.01±0.00 - -	0.01±0.00 0.07±0.01 4.3*	0.01±0.00 0.02±0.00 10.0*	0.01±0.00 0.02±0.00 12.7*
Pox (µM/min./g tissue)	C T F-Value	0.08±0.00 - -	0.05±0.00 0.04±0.00 5.4*	0.05±0.00 0.04±0.00 3.2**	0.04±0.00 0.01±0.00 14.3*
SOD (Units/µl)	C T F-Value	0.05±0.00 - -	0.05±0.00 0.06±0.00 1.7^{NS}	0.06±0.00 0.05±0.00 3.6*	0.06±0.00 0.06±0.00 1.7^{NS}
Cat (mM/min./g tissue)	C T F-Value	0.16±0.01 - -	0.15±0.00 0.07±0.00 31.3*	0.09±0.00 0.08±0.00 8.6*	0.01±0.00 0.00±0.00 8.6*
Est (mM/min./g tissue)	C T F-Value	0.03±0.00 - -	0.05±0.00 0.06±0.00 3.8*	0.06±0.00 0.05±0.00 2.0^{NS}	0.06±0.00 0.03±0.00 6.2*
GST (mM/min./g tissue)	C T F-Value	0.04±0.00 - -	0.16±0.01 0.09±0.00 10.7*	0.10±0.00 0.07±0.01 3.3**	0.09±0.01 0.02±0.00 10.9*

**Significant at 1% level, *Significant at 5% level, ^{NS} Non Significant

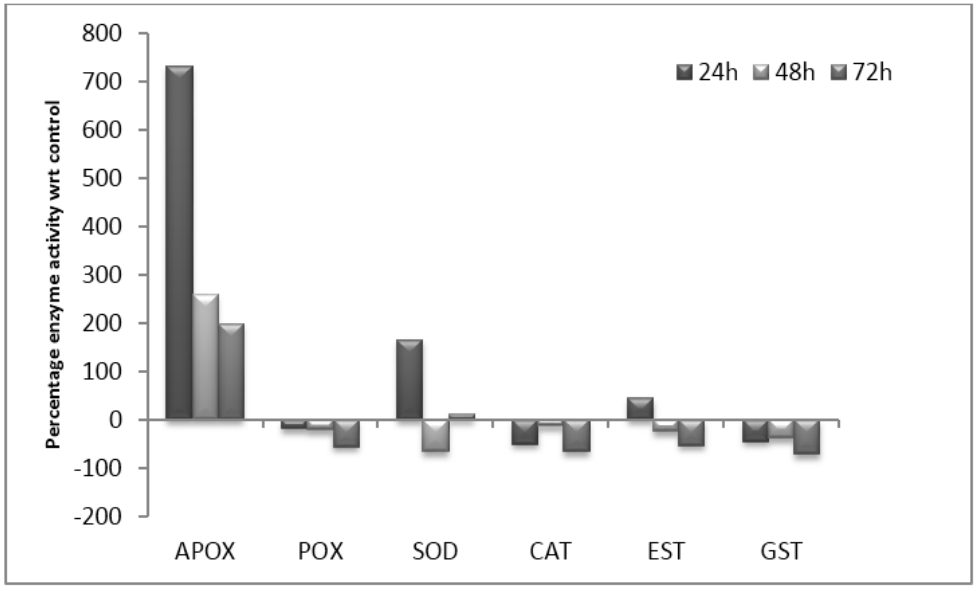

Fig 3: Percentage enzyme activity w.r.t. control in melon fruit fly treated with fraction A3.

4. Discussion

4.1 Effect on ascorbate peroxidase activity: Herbivorous insects possess ascorbate peroxidase that destroys H_2O_2 [18]. Ascorbate peroxidase plays a crucial role in protecting herbivores against H_2O_2 toxicity when catalase is inefficient at removing low concentrations of H_2O_2 [19]. The observations for ascorbate peroxidase activity in the larvae of *B. cucurbitae* showed that the enzyme activity was induced at most of the treatment intervals with all the three fractions, clearly indicating a significant role of this enzyme in scavenging H_2O_2.

4.2 Effect on peroxidase activity: Peroxidases play an important role in neutralizing plant phenols. This has been demonstrated in the spotted alfalfa aphid, *T. trifolii maculata* [20]. The present findings also showed reduction in peroxidase activity in the larvae of *B. cucurbitae* treated with all three fractions, indicating a little involvement of peroxidases in oxidizing plant allelochemicals. Chrzanowski *et al.* [21] had perceived an induction in peroxidase

after 24 hours of feeding the grain aphid, *S. avenae* on phenolic rich plant extract but prolonged feeding time resulted in decrease in enzyme activity.

4.3 Effect on SOD activity: The primary defense against the damage that can be caused by superoxide radicals and by its reactive progeny is the SODs [22]. SOD, which catalyzes the dismutation of superoxide radical, was induced in the larvae of *B. cucurbitae* after treatment for 48 and 72 hours with all three fractions. Consistent with our findings, Krishnan and Kodrik [23] had reported a significant increase in SOD activity in midgut tissue of *S. littoralis* larvae fed on potato leaves containing high concentration of allelochemicals.

4.4 Effect on catalase activity: The rapid induction of SOD activity leads to conversion of superoxide radicals into H_2O_2 and a greater intracellular concentration of H_2O_2 induces catalase activity [24]. In the present findings, an induction in catalase activity was observed at all treatment intervals in the larvae fed on A1 fraction treated diet but in A2 and A3 treated diet, reduction in enzyme activity was observed at all the treatment levels. These findings suggest that the intracellular concentration of H_2O_2 might not have been sufficient at some treatments to induce the catalase activity in the A2 and A3 treated larvae. Lukasik [18] had also observed an inhibition in catalase activity in *S. avenae* and *R. padi* fed on O dihydroxy phenols (caffeic acid, quercetin and chlorogenic acid). The induction in catalase activity in larvae of *B. cucurbitae* could either be an insect specific response to the flavonoids rich fractions or could be due to an antagonistic effect of the compounds.

4.5 Effect on esterase activity: The activity of esterases, which have been implicated in resistance in insects to toxic plant allelochemicals [25], was induced in the larvae of *B. cucurbitae* fed on A1 incorporated diet only after prolonged feeding, whereas in larvae fed with A2 and A3 treated diet, an induction was observed at 24 hours of the treatment interval, indicating a greater toxic effect of the fraction on the larvae, and consequently a greater need to metabolize the plant phenolics. Congruent to the present findings, an increase in esterases activity has also been perceived in mosquitoes, *A. detritus, A. cataphylla* and *A. rusticus* when fed on alder leaf litter containing polyphenols [26]. An induction in esterases activity has also been reported in the gypsy moth, *L. dispar* after they were fed on a diet of aspen leaves supplemented with phenolic glycosides [27].

4.6 Effect on GSTs activity: The role of GSTs, a group of multifunctional enzymes, in metabolizing various toxic allelochemicals has been well documented [28]. However in the present study, GSTs after showing an induction during the initial treatment interval, declined when the larvae were fed on both A2 fraction incorporated diet for 24 hours treatment interval, indicating that the phenolic rich fractions might have interfered with GST mediated detoxification in the melon fly. Many flavonoids have been observed to be potent inhibitors of GSTs, with apigenin reported as the most effective inhibitor [29]. With A1 and A3 treatments, reduction in enzyme activity was noticed at most of the treatment intervals. Lee [5] too had perceived an inhibitory effect of plant phenols viz. quercetin, ellagic acid and juglone on GST activity in *T. ni* and *P. polyxenes*.

5. Conclusion

It was perceived from the present study that at biochemical level, fractions A1, A2 and A3 showed an alterations in all the enzymes under investigations when second instar larvae (64-72 hours old) were fed on treated diet.

The adverse effect of *A. auriculiformis* fractions observed on the biochemistry of *B. cucurbitae* revealed that plant and its phenolic constituents hold considerable potential for use in the management of the insect pest. Moreover, these studies could also form the basis to carry on further research on transgenic plants having higher levels of phenolics which could significantly influence plant insect interactions.

6. References

1. Carson R. Silent Spring. Houghton Mifflin. Boston, USA, 1962.
2. Repetto R, Baliga S. Pesticides and the Immune System: The public health risks. World Resources Institute, Washington, D.C. 1996.
3. Rattan RS. Mechanism of action of insecticidal secondary metabolites of plant origin. Crop Protection 2010; 29(9):913-920.
4. Felton GW, Duffey SS. Protective action of midgut catalase in lepidopteran larvae against oxidative plant defenses. Journal of Chemical Ecology 1991; 17(9):1715-1732.
5. Lee K. Glutathione S-transferases activities in phytophagous insects: Induction and inhibition by plant phytotoxins and phenols. Insect Biochemistry 1991; 21(4):353-361.
6. Ahmad S. Biochemical defence of pro-oxidant plant allelochemicals by herbivorous insects. Biochemistry, Systematics and Ecology 1992; 20(4):269–296.
7. Downer RGH. Comprehensive insect physiology, biochemistry and pharmacology. In: Endocrinology of insects (Eds. Kerkut GA and Gilbert LI) Pergamon Press, Oxford, UK, 1986, 77-113.
8. Kapoor VC. Indian fruit flies (Insects: Diptera: Tephritidiae), Oxford and IBH publishing Co. Pvt. Ltd. New Delhi, 1993:1-228.
9. Gupta JN, Verma AN. Screening of different cucurbit crops for the attack of the melon fruit fly, *Dacus cucurbitae* Coquillett (Diptera: Tephritidae). Haryana Journal of Horticultural Sciences 1978; 7(1):78-82.
10. Srivastava BG. A chemically defined diet for *Dacus cucurbitae* (Coq.) larvae under aseptic conditions. Entomology News Letters 1975; 5(1):24-27.
11. Asada K. Chloroplasts: formation of active oxygen and its scavenging. Methods in Enzymology 1984; 105(1984):422-429.
12. Kar M, Mishra O. Catalase, peroxidase and polyphenol oxidase activities during rice leaf senescences. Plant Physiology 1976; 57(2):315-319.
13. Kumar KB, Khan PA. Age related changes in Catalase (EC 1.11.1.6) and peroxidase (1.11.1.7) activities in the excised leaves of *Eleusine coracana* Gaerth.cv. PR–202 during senescence. Experimental Gerontology 1983; 18(5):409-417.
14. Kono Y. Generation of superoxide radical during auto-oxidation of hydroxylamine and an assay for superoxide dismutase. Archive of Biochemistry and Biophysics 1978; 186(1):189-195.
15. Bergmeyer HU. Reagents for enzymatic analysis, Methods of Enzymatic Analysis. Eds. Bergmeyer HU and Gawehn K. Verlag Chemie, Weinheim, 1974, 1-438.
16. Katzenellenbogen B, Kafatos FC. General esterases of silk worm moth moulting fluid: Preliminary characterization.

Journal of Insect Physiology 1971; 17(6):1139-1151.
17. Chien C, Dauterman WC. Studies on Glutathione-S-transferases in *Helicoverpa* (*Heliothis*) *zea*. Insect Biochemstry 1991; 21(8): 857-864.
18. Lukasik I. Changes in activity of superoxide dismutase and catalase with in cereal aphids in response to plant *o*-dihydroxyphenols. Journal of Applied Entomology 2007; 131(3):209-214.
19. Mathews MC, Summers CB, Felton GW. Ascorbate peroxidase: A novel antioxidant enzyme in insects. Archive of Insect Biochemistry and Physiology 1997; 34(1):57–68.
20. Jiang Y. Oxidative interactions between the spotted alfalfa aphid (*Therioaphis trifolii maculata*) (Homoptera: Aphididae) and the host plant *Medicago sativa*. Bulletin of Entomological Research 1996; 86(5):533-540
21. Chrzanowski G, Leszczynski B, Sempruch C, Sytykiewicz H, Sprawka I. Effect of phenolics from woody plants on activity of grain aphid oxidases. Pesticides 2009; 1(4):63-70.
22. Zenkov NK, Lankin VZ, Men'shchikova EB. Oxidation Stress: Biochemical and Patophysiological Aspects. State Publishing House of Biochemistry and Biology Literature, Moscow, Russia, 2001:1-343.
23. Krishnan N, Kodrik D. Antioxidant enzymes in *Spodoptera littoralis* (Boisduval): Are they enhanced to protect gut during oxidative stress? Journal of Insect Physiology 2006; 52(1):11-20.
24. Lukasik I, Golawska S, Wójcicka A. Antioxidant defense mechanisms of cereal aphids based on ascorbate and ascorbate peroxidase. Biologia 2009; 64(5):994-998.
25. Lindroth RL. Differential esterase activity in *Papilio glaucus* subspecies: absence of cross-resistance between allelochemicals and insecticides. Pesticide Biochemistry and Physiology 1989; 35(2):185-191.
26. David JP, Rey D, Marigo G, Meyran JC. Larvicidal effect of a cell-wall fraction isolated from alder decaying leaves. Journal of Chemical Ecology 2000; 26(4):901-913.
27. Hemming JDC, Lindroth RL. Effects of phenolic glycosides and protein on gypsy moth (Lepidoptera: Lymantriidae) and forest tent caterpillar (Lepidoptera: Lasiocampidae) performance and detoxification activities. Environmental Entomology 2000; 29(6):1108-1115.
28. Yu SJ, Hsu EL. Induction of detoxification enzymes in phytophagous insects: role of insecticide synergists, larval age, and species". Archive of Insect Biochemistry and Physiology 1993; 24(1):21-32.
29. Yu SJ, Abo-Elghar GE. Allelochemicals as inhibitors of glutathione S-transferases in the fall armyworm. Pesticidal Biochemistry and Physiology 2000; 68(3):173-183.