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Research Paper

BIOEFFICACY OF FLUORIDE, COBALT AND ZINC ON LARVAL TRIACYLGLYCEROL ESTER HYDROLASE ACTIVITY OF *PAPILIO DEMOLEUS* (LINNAEUS)

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Abstract

Partial characterization of larval triacylglycerol ester hydrolase (EC.3.1.1.3) of *P. demoleus* (Linnaeus) revealed pH 7.8, substrate concentration 5% and Michaelis-Menten constant 0.1735×10^{-2} mM. The gradual increase in triacylglycerol ester hydrolase activity was observed from 6-day to 9-day, decrease from 9-day to 12-day and maximum activity in 9-day in control, fluoride treated, cobalt treated and zinc treated larvae of *P. demoleus* (Linnaeus). The triacylglycerol ester hydrolase activity of 9-day control larvae is 1.2384, 1.1333 and 1.0625 times as compared to 9-day fluoride, cobalt and zinc respectively treated larvae of *P. demoleus* (Linnaeus). The larval triacylglycerol ester hydrolase of *P. demoleus* (Linnaeus) inhibition noted in the order fluoride > cobalt > zinc with percent value 19.26 %, 11.77 % and 5.89 % respectively. Arithmetic mean and standard deviation were computed to 0.2972, 0.2535, 0.2667, 0.2802 and 0.03698, 0.02538, 0.03006, 0.03350 µ-Equiv. free fatty acids /100mg /25 minutes respectively of 6 to 12-day control, fluoride treated, cobalt treated and zinc treated larval triacylglycerol ester hydrolase activity of *Papilio demoleus* (Linnaeus). The inhibitory role of fluoride, cobalt and zinc on larval triacylglycerol ester hydrolase of *Papilio demoleus* (Linnaeus) has been attempted in the present paper.

Key words: *Triacylglycerol ester hydrolase, larva, fluoride, cobalt, zinc, P. demoleus (L.).*

INTRODUCTION

Insecticides now may be regarded as a "normal" environmental hazard for insects, survival over which has been achieved through natural selection of resistant strains. Insects have various methods for detoxifying potentially harmful substances, many of which parallel those found in vertebrates. Hydrolysis, hydroxylation, sulfation, methylation, acetylation and conjugation with cysteine, glycine, glucose, glucoronic acid or phosphate are examples of the methods employed. In a few species, resistance seems to have developed as a result of an increase in the quantity or decrease in the sensitivity of the enzyme normally affected by the insecticide, specifically cholinesterase in the nervous system [5]. Many insects are scavengers and convert the dead plant and animal tissues into humus and enrich the soil. Insects as a group of the animal kingdom clash directly with the interests of man for survival. However, all the insects are not enemies of man for survival. The great majorities are harmless and they play a salient role in maintaining the balance of nature; some fed on other plants and animals and some are eaten by others [2]. The citrus butterfly, *P. demoleus* (Linnaeus) causes damage to citrus plants. Larvae feed for about 14 days in summer [18]. On an agricultural basis, we are concerned when the crop damage caused by insects leads to a loss in yield or quality, resulting in a loss of profits by the farmer. When the yield loss reaches certain proportions (5-10 % loss) the pest can be defined as an economic pest. Obviously a loss of 10 % of the plant stand in a cereal or sugarcane field is not particularly serious, whereas the loss of a single mature tree of citrus or mango is important [11]. When the action of digestive enzymes is inhibited, insect's nutrition is impaired, growth and development are retarded and eventually death occurred due to starvation. For this reason, synthetic and plant origin inhibitors are widely regarded as the potential candidates for improved pest control in transgenenic plants. Therefore, combination of the inhibitors targeting digestive enzymes will likely be more efficacious in promoting ant-biosis in host plants [26].

Some attempts have been made to note triacylglycerol ester hydrolase activity in insect species [14; 21; 23; 26; 16].

In the present paper, an attempt has been made to reveal bioefficacy of fluoride, cobalt and zinc on larval triacylglycerol ester hydrolase activity during larval development of *Papilio demoleus* (Linnaeus) which is mainly concerned with release of energy for their active life.

MATERIALS AND METHODS

The rearing of citrus butterfly, *P. demoleus* (Linnaeus) was attempted in the laboratory on the natural food of citrus leaves. The larval stages from 6 to 12-day larvae were taken for study. Efficacy of fluoride ion, cobalt and zinc on larval triacylglycerol ester hydrolase activity of *P. demoleus* (Linnaeus) have been noted according to [19; 3; 26]. In the present study, to examine the sublethal concentration of ammonium fluoride, cobalt sulphate and zinc sulphate 6-day old larvae were treated at various concentrations. Each concentration was replicated 3 times and ten larvae were used in each replication. The death data was subjected to compute LC_{50} values for 24 hours to the ammonium fluoride, cobalt sulphate and zinc sulphate. There was no mortality at 15 mg/L ammonium fluoride, 17 mg/L cobalt sulphate and 25 mg/L zinc sulphate, said concentrations selected as sublethal concentration for the study. The ammonium fluoride, cobalt sulphate and zinc sulphate of desired concentrations were prepared in water. Prior to experiments, larvae of *P. demoleus* (Linnaeus) were starved for two hours as per the method of [13]. Leaves of citrus plants treated with 15 mg/L

ammonium fluoride, 17 mg/L cobalt sulphate and 25 mg/L zinc sulphate were provided to different larval stages from 6-day to 12-day larvae and after 24 hours larvae were taken for triacylglycerol ester hydrolase activity. Larvae of 6-day to 12-day age groups were sorted out and divided into two groups groups; experimental/test and control larvae. The test larvae fed for 24 hours on leaves treated with different concentrations of ammonium fluoride, cobalt sulphate and zinc sulphate where as control larvae were raised on untreated fresh citrus leaves.

Larvae were isolated, weighed and homogenized in the cold distilled water using a glass mortar and pestle. The homogenate were diluted with cold distilled water so as to get 1 % (wt/vol) concentration and used for the assay of triacylglycerol ester hydrolase [8; 15]. The assay system contained 0.25 mL of 5% olive oil dispersed in gum acacia; 1.0 mL of 0.2 M tris-maleate buffer (pH 7.8) and 0.25 ml of 1 % (wt/vol) enzyme solution in a total volume of 1.5 mL. The incubations were carried out in a shaker with a continuous shaking for 25 minutes in glass stoppered vessels at 37^{o} C. The reaction was stopped with 2 mL of Cu-TEA reagent (1N acetic acid: 1M 2, 2', 2" nitrilotriethanol: 6.45% cupric nitrate solution Cu $(NO_3)_2$, (1:9:10, v/v/v/). Exact 10 ml of chloroform was added, the contents were vigorously mixed and the phases were allowed to separate. A 5ml aliquot of the chloroform phase was transferred to a conical centrifuge tube by syringe, 2 mL of distilled water were added without mixing and the tube was centrifuged for a few minutes. After removal of all water, exact 2 mL of the chloroform phase was mixed with 1.0 mL of 0.5 % solution of diphenyl carbazone and diphenylcarbazid (5:95 w/w) in methanol as a colour reagent. The free fatty acids were read at 540 nm absorbance [10]. All enzyme assays were replicated three times. One triacylglycerol ester hydrolase activity unit was expressed as the amount of the enzyme triacylglycerol ester hydrolase capable of catalyzing absorbance change of 0.01 in 60 s at wavelength of 540 nm.

RESULTS

Partial characterization of larval triacylglycerol ester hydrolase of *P. demoleus* (Linnaeus) revealed pH 7.8, incubation time 25 minutes, temperature 37 ^{0}C, day larval enzyme concentration 1 %, substrate concentration 5% and Michaelis-Menten constant 0.1735 $\times 10^{-2}$ mM. The gradual increase in triacylglycerol ester hydrolase activity was observed from 6-day to 9-day, decrease from 9-day to 12-day and maximum activity in 9-day in control, fluoride treated, cobalt treated and zinc treated larvae of *P. demoleus* (Linnaeus). The triacylglycerol ester hydrolase activity of 9-day control larvae is 1.2384, 1.1333 and 1.0625 times as compared to 9-day fluoride, cobalt and zinc respectively treated larvae of *P. demoleus* (Linnaeus). The larval triacylglycerol ester hydrolase of *P. demoleus* (Linnaeus) inhibition noted in the order fluoride > cobalt > zinc with percent value 19.26 %, 11.77 % and 5.89 % respectively. Arithmetic mean and standard deviation were computed to 0.2972, 0.2535, 0.2667, 0.2802 and 0.03698, 0.02538, 0.03006, 0.03350 µ-Equiv. free fatty acids /100mg /25 minutes respectively of 6 to 12-day control, fluoride treated, cobalt treated and zinc treated larval triacylglycerol ester hydrolase activity of *Papilio demoleus* (Linnaeus). Bioefficacy of fluoride, cobalt and zinc on larval triacylglycerol ester hydrolase activity during larval growth of *P. demoleus* (Linnaeus) is presented in figure 1.

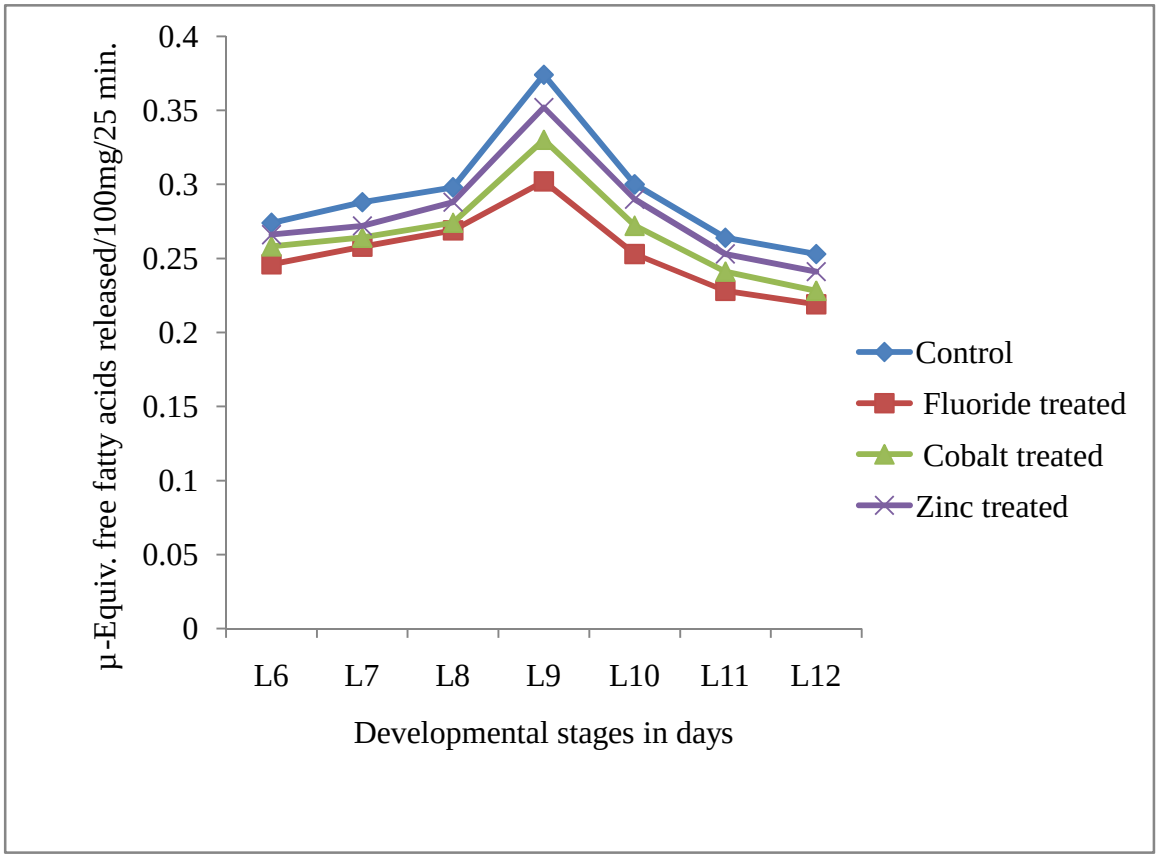

Figure 1: Bioefficacy of fluoride, cobalt and zinc on larval triacylglycerol ester hydrolase activity of *P. demoleus* (Linnaeus).

DISCUSSION

The lipase activity was inhibited by fluoride ion, Co^{2+} and Zn^{2+} (Rao and Subrahmanyam, 19). Lipase enzyme exhibits dual optima at pH 4.9 and 7.0 and is inhibited by fluoride ion [3]. LC_{50} of zinc sulphate for damselfly for 24 hour is 32 mg/L [20]. LC_{50} of zinc sulphate for midge, *Chironomous tentans* at third instar larvae for 48 hrs is 8.2 mg/ L [12]. Zinc is an essential trace element for all living organisms. Zinc has its primary effect on zinc-dependent enzymes that regulate RNA and DNA. Zinc interacts with many chemicals to produce altered patterns of accumulation, metabolism and toxicity; some interactions are beneficial to the oraganism and others are not depending on the organism, its nutritional status and other variables. Knowledge of these interactions is essential to the understanding of zinc toxicokinetics [4]. The azadirachtin treated larvae of rice leaf folder; *Cnaphalocrosis medinalis* had low lipase activity in the midgut [22]. Due to wide spectrum usage of synthetic pesticides and appearing of environmental risks such as pest resurgence, pesticide resistance and poisonous effects on non-target organisms such as human and biocontrol agents, integrated pest management programs have been focused on the using digestive enzyme inhibitors. Digestive enzyme inhibitors occur naturally in many food plants and are particularly abundant in cereals like rice and legumes [25]. Triacylglycerol ester hydrolase have key roles in insect lipid mobilization and are also fundamental to many physiological processes under pinning insect reproduction, development, defence from pathogens and oxidative stress and pheromones signalling [9]. Zinc is an essential electron transfer in many enzymatic reactions. However, its prolonged and excessive intake may lead to toxic effect such as carcinogenesis and mutagenesis. 96 hours LC_{50} of zinc sulphate

value for guppies was found to be 30.8 mg/L [6]. The fat body plays major roles in the life of insects. It is a dynamic tissue involved in multiple metabolic functions. One of these functions is to store and release energy in response to the energy demands of the insect. Insects store energy reserves in the form of glycogen and triglycerides in the adipocytes, the main fat body cell. Insect adipocytes can store a great amount of lipid reserves as cytoplasmic lipid droplets. Lipid metabolism is essential for growth and reproduction and provides energy needed during extended nonfeeding periods [1]. The reduction in insect food consumption by 20-hydroxyecdysone resulting in larval fat body lipoysis of *B. mori* [24]. Low lipase activity noted in the sunn pest, *Eurygaster intergriceps* Puton after treatment of *Artemisia annua* L. (Asteracea) extract [25]. The effects of the broad-specificity lipase inhibitor, tetrahydrolipstain, on the growth, development and survival of the larvae of *Epiphyas postvittana* (Walker) [14]. Gradually reduction of lipase activity noted by increasing concentrations of phenylmethylsulfony fluoride (PMSF), triethylenetetramine hexaacetic acid (TTHA) and ethylenediamine tetraacetic acid (EDTA) in larval gut of *Naranga aenescens* Moore (Lepidoptera: Noctuidae) [26].

In the present study, partial characterization of larval triacylglycerol ester hydrolase of *P. demoleus* (Linnaeus) at pH 7.8, incubation time 25 minutes, temperature 37 ^{0}C, enzyme concentration 1 %, substrate concentration 5% and Michaelis-Menten constant 0.180×10^{-2} mM indicates triacylglycerol ester hydrolase maximally active at an alkaline pH and high affinity with the enzyme. The inhibitory effect of pyriproxyfen, tubufennozide and lufenuron on lipid content of fat body and haemolymph from nymph of desert locust, *Scistocerca gregaria* **[7]**.

In the present study, decrease in triacylglycerol ester hydrolase activity in fluoride, cobalt and zinc treated larvae as compared to control larvae of *P. demoleus* (Linnaeus) indicates the inhibitory action of fluoride, cobalt and zinc on larval triacylglycerol ester hydrolase. One-way analysis of variance (ANOVA) of triacylglycerol ester hydrolase activity in fluoride treated and control larvae Snedecor's F statistic $F>6.659$ and $p<0.024$ indicates independent samples and different mean values among the larvae of *P. demoleus* (Linnaeus). The $F>1$ and $p<0.05$ also indicates true hypothesis with significant differences with triacylglycerol ester hydrolase activity in fluoride treated and control larvae of *P. demoleus* (Linnaeus). The triacylglycerol ester hydrolase activity of 9-day control larvae 1.2384, 1.1333 and 1.0625 times as compared to 9-day fluoride, cobalt and zinc respectively treated larvae of *P. demoleus* (Linnaeus) suggests enzyme inhibition in the order fluoride > cobalt > zinc with percent value 19.26 %, 11.77 % and 5.89 % respectively. Before considering inhibitors as a control approach, the enzymatic properties of triacylglycerol ester hydrolase must be described. As it was mentioned, a few studies deals with the digestive triacylglycerol ester hydrolase of the insects and the current was could be one of the most complete studies by considering the partial characterization and using some inhibitors. It was suggestd to develop this work by using plant origin inhibitors to reach a winning control of *P. demoleus* (Linnaeus) in the near future.

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REFERENCES

1. Arrese, E. L., and Soulages, J. L., 2010, Insect fat body: energy, metabolism and regulation. *Annu. Rev*. *Entomol.,* 55: 207-225.
2. Atwal, A. S., and Dhaliwal, G. S., 2003, In: Agricultural Pests of South Asia and Their Management. Fourth edition. Published by Mrs. Usha Raj Kumar for Kalyani Publishers, New Delhi. Printed at Xpress Grafics, Delhi. 17-286.
3. Downer, R. G. H., and Steele, J. E., 1973, Haemolymph lipase activity in the american cockroach, *Periplaneta americana.* Pergamon press, Printed in Great Britain. *J. Insect physiol*., 19: 523-532.
4. Eister, R., 1993, Zinc hazardsto fish, wildlifeand invertebrates: a synoptic review. Published by U.S. Dept. of the Interior Fish and Wildlife Service, Laurel Maryland. 26:1-35.
5. Gillot, C., 1982, In: Entomolotgy. Published by Plenum publishing corporation, New York. Printed by United States of America. 38-663.
6. Gul, A., Yilmaz, M., and Isilak, Z., 2009, Acute toxicity of zinc sulphate ($ZnSO_4.H_2O$) to Guppies, *Poecilia reticulata* P. *Journal of Science*, 22(2): 59-65.
7. Hamadah, K. S., Ghoneim, K. S., and Tanani, M. A,, 2012, Effect of certain insect growth regulators on the lipid content of some tissues of the desert locust, *Scistocerca gregaria*. *African Journal of Biochemistry Research*. 6(9):121-128.
8. Hayase, K., and Tapple, A. L.,1970, Specificity and other properties of lysosomal lipase of rat liver. *J. Biol. Chem*., 245: 169-175.
9. Horne, I., Haritos, V.S., and Oakeshott, J. G., 2009, Comparative and functional genomics of lipases in holometabolous insects. *Insect Biochem Mol. Biol*., 39 (8): 547-567.
10. Itaya, K., 1977, A more sensitive and stable colorimetric determination of free fatty acids in blood. *J. Lipid Res*., 18: 663-665.
11. Jabde, P.V., 2005, In: Text book of Applied Zoology. Published by Discovery Publishing House. Printed at Tarun Offset Printers, Maujpur, Delhi. 290, 421.
12. Khangarot, B. S., and Ray, P. K., 1989, Sensitivity of midge larvae of *Chironomous tentans* Fabricius (Diptera: Chironomidae) to heavy metals. *Bulletin of Environmental Contamination and Toxicology*. 42: 325-330.
13. Khosravi, R., Sendi, J. J., Ghadamyari, M., and Yezdani, E., 2011, Effect of Sweet wormwood *Artemisia annua* crude leaf extract on some biological and physiological characteristics of the lesser mulberry pyralid, *Glyphodes pyloalis*. *J. Insect Science,* 11: 1-13.
14. Markwic, N.P., Poulton, J., McGhie, T. K., Wohlers, M.W., and Christeller, J.T. 2011,The effects of the broad-specificity lipase inhibitor, tetrahydro lipstain, on the growth, development and survival of the larvae of *Epiphyas postvittana* (Walker) (Tortricide, Lepidoptera). *Journal of Insect Physiology,* 57 (12):16-1650.
15. Patil, S.S., Bhandari, C. K., and Sawant, V. A., 1983, Studies on triaclyglycerol ester hydrolase from bat adipose tissue. *J. Biosci*., 5(1): 35-42.
16. Pawar, A S.; Gejage, R. M., and Dubal, R. S., 2013, Studies on larval gut lipolytic activity in *Helicoverpa armigera* (Hubner). *J. Cell Tissue Research*. 13(2): 3697-3699.
17. Pol, J. J., and Sawant, V. A., 1995, Lipase activity during metamorphosis of *Chrysomia rufifacies*. *Entomon*, 20(2): 51-53.
18. Ravindranathan, K. R., 2003, In: Economic Zoology. Published by A. S. Saini for dominant publishers and distributors New Delhi. Printed by Orient Offset, New Delhi.192-194. ISBN 81-7888-130-6.

19. Rao, R. H., and Subrahmanyam, D., 1969, Studies on the phospholipase A in larvae of *Culex pipiens fatigans. Journal of Insect Physiology,* 15(1): 149-159.
20. Rehwoldt, R., Lasko, L., Shaw, C., and Wirshowski, E., 1973, The acute toxicity of some heavy metal ions toward benthic organisms. *Bull. Envoiron. Contan. Toxicol.,*8: 91-96.
21. Schafer, M., Fischer, C., Melodau, S., Seebald, E., Oelmuller, R., and Baldwin, I. T., 2011, Lipase activity in insect oral secretions mediates defense responses in *Arabidopsis thaliana*. *Plant Physiology*, 156: 1520-1534.
22. Senthil-Nathan, S., 2006, Effect of *Melia azedarach* on nutritional physiology and enzyme activities of the rice leaffolder, *Cnaphalocrosis medinalis* (Guenee) (Lepidoptera: Pyralidae). *Pesticide Biochemistry and Physiology*, 84: 98-108.
23. Tierno de Figueroa, J. M., Trenzado, C. E., Lopez – Rodriguez, M. J., and Sanz, A., 2011, Digestive enzyme activity of two stonefly species (Insecta: Plecoptera) and their feeding habits. *Comparative Biochemistry and Physiology,* Part A 160: 426-430.
24. Wang, S., Liu, S., Liu, H., Wang, J. Zhou, S., Jiang, R.J., Bendena, W.G., and Li, S., 2010, 20-hydroxyecdysone reduces insect food consumption resulting in fat body lipolysis during molting and pupation. *J. Mol. Cell Biol.,* 2(3): 128-138.
25. Zibaee, A., and Bandani, A. R., 2010, A study on the toxicity of medicinal plant, *Artemisia annua* L. (Asteracea) extract to the sunn pest, *Eurygaster intergriceps* Puton (Hemiptera: Scutelleridae). *Journal of Plant Protection Research,* 50(1): 79-85.
26. Zibaee, A., and Fazeli-Dinan, M., 2012, Purification and characterization of a digestive lipase in *Naranga aenescens* Moore (Lepidoptera: Noctuidae). *SOAJ of Entomological Studies.*1: 38-54.
27. Zibaee, A., Bandani, A. R., and Ramzi, S., 2008, Lipase and invertase activities in midgut and salivary glands of *Chilo suppressalis* (Walker) (Lepidoptera, Pyralidae), rice striped stem borer. *Invertebrate Survival Journal,* 5: 180-189.