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EVALUATION OF EFFECT OF PESTICIDES ON AMYLASE ACTIVITY IN WORKER BEES OF *APIS MELLIFERA* L.

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ABSTRACT

The effect of sub lethal concentrations of four pesticides (dimethoate – 30 EC, methyl parathion – 50 EC, malathion – 50 EC and neem oil – 25 EC) on the activity of digestive enzyme Amylase in worker bees of *Apis mellifera* L of different age was studied. The data obtained from treated groups of worker bees revealed that all the pesticides reduced the activity of Amylase in all age groups but maximum reduction was found in foragers (25 days old worker bees). The results suggested that organophosphates (methyl parathion, malathion and dimethoate) had great inhibitory action on amylase activity. Neem oil a biopesticide, showed insignificant inhibitory effect. The order of inhibitory effect of different pesticides on Amylase activity was found as follows -

*Methyl parathion* > *Malathion* > *Dimethoate* > *Neem oil*

5 days old < 15 days old < 25 days

Key words: Pesticides, Amylase, *Apis mellifera*, Organophosphates, Neem oil

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INTRODUCTION

Amylase is one of the most important digestive enzymes for honeybees. This enzyme helps in digestion and conversion of nectar (Carbohydrates) into honey (Reddy, 1979). The honeybees poisoning is a serious adverse effect of pesticides application which leads to a decrease in insect population, reduction of honey yields and other bee products, pesticide residues in food, and to a significant loss of beekeeper's income. As the application of the pesticides increased, it brought great problems of eco-imbalance and caused serious damage to the crop pollinating and eco-friendly insects such as honeybees (Atkins, *et al.* 1978 and 1986; Smirle *et al.* 1984). In Almost cases, the bee poisoning result from pesticides, which are being applied to blooming crop or being allowed to drift onto blooming crops or weeds. Poisoning of honeybees is a serious adverse effect of insecticide application which leads to a decrease in insect population, crop pollination, reduction of honey yields and other bee products, insecticide residues in food, and to a significant loss of beekeeper's income. In bee poisoning, the identification of the responsible toxicant is necessary by both environmental and biological monitoring, to prevent bee poisoning and for the protection of public health. Pesticides applications usually are not recommended for blooming crop but improper

attention and illiteracy of the farmers result use of pesticides on blooming crop, which in tern cause a great lose to apiculture industry either directly or indirectly (Habes D. *et al* 2006). It may result into reduced foraging activity due to repellency (Shires *et al.* 1983, Stoner *et al.* 1984). Sub-lethal doses can also influence other bee behavior patterns i.e., dance rhythm, flight velocity, walking speed, wing beat frequency, etc. (Brandes, 1984). Pesticides can also cause physiological injury to bees when their applications are repeated (Kumar and Gupta, 2010; Kumar and Yadav 2012). In such cases it is not the bee mortality, which is significant, but it may reduce longevity (Smirle *et al.* 1984; Fries and Wibran, 1987; Makenzie and Wiston, 1989). The effect of pesticides on the activity of adenosine triphosphatase, acetyl cholinesterase and digestive enzyme protease was reported by Bai and Reddy (1977a), Reddy (1979) Reddy (1983) in *Apis cerena indica* and Kumar and Gupta, (2007 & 2009) in *Apis mellifera* L., and relative decrease in the activity of the enzyme was used to determine the degree of toxicity of pesticides. Therefore, in the present study the effect of Dimethoate, Methyl parathion, Malathion and Neem oil (Biopesticide) (¼ and ½ of LC<sub>50</sub> at 96 hrs.) on digestive enzyme Amylase activity in different age groups of honeybees *Apis mellifera* L. has been made.

MATERIAL AND METHODS

1. BEE REARING UNDER LABORATORY CONDITIONS FOR BIOCHEMICAL ANALYSIS

The target organism in the present study was Italian honeybee, *Apis mellifera* L. The bees were reared in the laboratory of Zoology Department, Govt. P.G College, Bisalpur, Pilibhit using standard Langstroth cages with wax sheet foundation frame under controlled conditions. The initial bee colonies were obtained from Nearby Apiaries of Tehseel Bisalpur and acclimated for five days in the cages before toxicity tests. The temperature and relative humidity maintained were 20-25°C (± 2°C) and 61-66 R.H. respectively.

Five replicates of control and treated worker bee of different ages were used for biochemical analysis. Worker bees were collected with a cotton cone net from the landing board as they departed for biochemical analyses. For the data based on worker honeybees, the colonies were divided into two groups.

(i) The group of control bee colony (fed on 50% sucrose syrup).

(ii) The treated groups of bee colonies were fed on pesticides mixed (accordingly) 50% sugar syrup.

## 2. BIOCHEMICAL ANALYSIS

Amylase activity was determined by well-known method of Bernfeld, 1955. Amylase hydrolyses the starch resulting production of reducing sugars. The reaction is followed by measuring the increase in the concentration of reducing sugar by using 3: 5 dinitro salicylic acid as a reagent. Alkaline solution of 3: 5 dinitro salicylic acid is reduced to 3 - amino, 5 - nitro salicylic acid by the reducing sugars, produced during the reaction. Reaction is measured at the extinction 540 nm. Specific activity was expressed as  $\mu\text{g}$  of maltose liberated per mg protein per min.

### RESULTS AND DISCUSSION:-

Both concentration levels of pesticides resulted inhibition in the activity of amylase, but concentration level -2 ( $\frac{1}{2}$  of  $\text{LC}_{50}$  at 96 hrs.) proved to be more toxic causing inhibition of enzymes activity to large extent as compared to concentration level-1. The activities of amylase in control groups and pesticides treated groups of worker bees of different age, analyzed during this investigation are presented in table 1.

#### Alterations in amylase activity of worker bees induced by the pesticides at concentration level-1 ( $\frac{1}{4}$ of $\text{LC}_{50}$ at 96 hrs.)

Table 1 (Fig.1) represents an analysis of the variance (ANOVA) of the amylase activity in worker honeybees in response to concentration level-1 and 2. It indicates that the different pesticides had different degree of toxicity. The maximum inhibition of amylase activity was found in 25 days old worker bees. Methyl parathion had maximum inhibitory effect on the activity of amylase in all age groups inhibiting the activity of the enzyme 27.60%\*\*, 33.07%\*\*\* and 39.24%\*\*\* in 5, 15 and 25 days old worker bees respectively.

Malathion was the second most toxic pesticide, It reduced the activity of amylase up to 22.64%\*, 29.5%\*\* and 34.92%\*\*\* in 5, 15 and 25 days old worker bees respectively. Dimethoate reduced the activity of the enzyme to some lesser extent than methyl parathion and malathion. It reduced the activity of amylase up to 17.70%\*, 19.59%\* and 26.43%\*\* in 5, 15 and 25 days old worker bees, respectively. Neem oil had no toxic effect on any group of the worker bees. It caused a very little inhibitory effect on the activity of the enzyme, i.e., 2.85%, 2.89%, and 4.74% in 5, 15 and 25 days old worker bees, respectively.

#### Alterations in amylase activity of worker bees induced by the pesticides at the concentration level-2 ( $\frac{1}{2}$ of $\text{LC}_{50}$ at 96 hrs.)

The changes in amylase activity in worker honeybees of different age groups in response to concentration level-2 ( $\frac{1}{2}$  of  $\text{LC}_{50}$  at 96 hrs.) of pesticides is shown in Table-1 Fig. 1 (ANOVA). Concentration level-2 of pesticides proved to be more toxic to all age groups of bees as compared to concentration level-1. Methyl parathion showed extremely toxic effect inhibiting the amylase activity up to 36.64%\*\*\*, 43.67%\*\*\* and 48.05%\*\*\* in 5, 15 and 25 days old bees respectively. Malathion, the second most toxic pesticide inhibited the activity of amylase up to 27.36%\*\*, 35.37%\*\*\* and 42.01%\*\*\* in 5, 15 and 25 days old worker bees respectively.

Dimethoate, third most toxic pesticide inhibited the enzyme activity but less than methyl parathion and malathion. It reduced the activity of amylase up to 23.64%\*\*, 27.37%\*\* and 33.53%\*\*\* in 5, 15 and 25 days old worker bees respectively.

In all cases, the maximum inhibition in the activity of amylase was found for 25 days old worker bees followed by 15 days old and 5 days old worker bees in response to both of the concentration levels. Among all the pesticides, used for this investigation, Neem oil had no significant toxic effect on any age group of the worker bees. It inhibited the enzyme activity up to

3.59%, 4.27% and 5.14% in 5, 15 and 25 days old worker bees respectively.

**TABLE - 1 Alterations in the activity of Amylase in worker honeybees at sub lethal concentrations of pesticides.**

Treatment	Amylase Activity						GROUPS
	Conc. Level-1 ( $\frac{1}{4}$ of $\text{LC}_{50}$ at 96 hrs.)			Conc. Level-2 ( $\frac{1}{2}$ of $\text{LC}_{50}$ at 96 hrs.)			
	5 days old Bees	15 days old Bees	25 days old Bees	5 days old Bees	15 days old Bees	25 days old Bees	
Sugar Syrup (50%)	8.08 $\pm$ 1.39 (---)	8.68 $\pm$ 1.62 (---)	12.26 $\pm$ 2.77 (---)	8.08 $\pm$ 1.39 (---)	8.68 $\pm$ 1.62 (---)	12.26 $\pm$ 2.77 (---)	CONTROL GROUP
Neem oil 25EC	7.85 $\pm$ 0.99 (2.85)	8.43 $\pm$ 1.57 (2.89)	11.68 $\pm$ 1.72 (4.74)	7.79 $\pm$ 0.93 (3.59)	8.31 $\pm$ 1.61 (4.27)	11.63 $\pm$ 1.48 (5.14)	T R E A T E D  G R O U P
Malathion 50EC	6.25 $\pm$ 0.79 (22.64) *	6.12 $\pm$ 1.09 (29.50) **	7.98 $\pm$ 0.91 (34.92) ***	5.87 $\pm$ 0.83 (27.36)* *	5.61 $\pm$ 1.24 (35.37)* **	7.11 $\pm$ 0.72 (42.01)* **	
Dimethoate 30EC	6.65 $\pm$ 0.82 (17.70) *	6.98 $\pm$ 1.05 (19.59) *	9.02 $\pm$ 1.31 (26.43) **	6.17 $\pm$ 0.89 (23.64)* *	6.31 $\pm$ 0.96 (27.37)* *	8.15 $\pm$ 1.29 (33.53)* **	
Methyl Parathion 50EC	5.85 $\pm$ 0.85 (27.60) **	5.81 $\pm$ 0.97 (33.07) ***	7.45 $\pm$ 1.18 (39.24) ***	5.12 $\pm$ 0.59 (36.64)* **	4.89 $\pm$ 0.84 (43.67)* **	6.37 $\pm$ 0.77 (48.05)* **	

Amylase Activity ( $\mu\text{g}$  of maltose liberated/mg protein/min.)

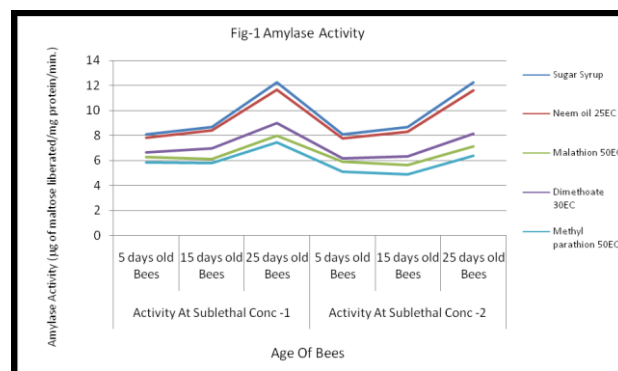
Each value is the mean of five replicates.

Values are expressed as mean  $\pm$  Standard Error.

Values are significant - \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ .

(Fisher's 't' test)

Values in parenthesis are %age inhibition of enzyme activity over control bees.



The inhibitory effect of pesticides on physiology of honeybee is well established (Bai and Reddy, 1977a; Kumar and Gupta, 2009 and 2010). Laboratory tests suggest that both the concentration levels of the pesticides were toxic to the bees in reference to the activity of digestive enzyme Amylase but the concentration level-2 of pesticides produced extremely significant inhibitory effect. This study was carried out to evaluate the effect of two different concentration levels ( $\frac{1}{4}$  and  $\frac{1}{2}$  of  $\text{LC}_{50}$  at 96 hrs.) of the pesticides on the activity of digestive enzyme Amylase in worker honeybees of different age groups.

Two aspects were considered during this study.

1. Age dependent changes in activity of digestive enzymes in control group of worker bees.
2. Age dependent inhibitory action of pesticides on activity of digestive enzyme in worker bees.

In reference to 1<sup>st</sup> aspect, we made study on the activity variations of the enzyme in control bees. The control group of bees revealed that 5 days old bees (brood chamber bees) had least and 25 days old worker bees (foragers) had maximum activity of the enzyme. The results also expressed that the enzymatic activities increase very rapidly after the age of 15 days (Fig. 1).

In reference to 2<sup>nd</sup> aspect, the results obtained from pesticide treated group revealed that all the pesticides inhibited the activity of the enzyme. Organophosphates proved to be more toxic as compared to neem oil to be non-toxic. The concentration level-1 was less toxic as compared to the concentration level-2 (½ of LC<sub>50</sub> at 96 hours). For concentration level-2 and maximum inhibitory effect was observed in 25 days old and least in 5 days old bees. Reddy (1979) and Grogan & Hunt (2005) also reported the inhibitory effects of the different pesticides on Amylase activity in Indian bee *Apis cerena indica*. The maximum inhibition of Amylase activity in 25 days old bees may be due to their direct encounter with pesticides at the time of nectar collection.

Although these laboratory tests suggest that both the concentration levels of the pesticides were highly toxic to the bees in reference to the activity of digestive enzymes amylase but the concentration level-2 of pesticides produced extremely significant inhibitory effect on the activity of the enzymes. This study was carried out to evaluate the effect of two different lethal concentrations (¼ and ½ of LC<sub>50</sub> at 96 hrs.) of the pesticides on the activity of digestive enzymes amylase in worker honeybees of different age groups. This study is quite important because any factor causing change in the normal activity of Amylase, directly effects to bee health, honey production and its quality and pollination capability of bees (Badiou *et al* 2008). This enzyme helps in the conversion of nectar and pollen into honey and is very essential for the proper digestion of carbohydrate contents of bee meal (D.E. Grogan and Hunt, J.H., 2005 and Chan *et al*, 2006). Anyhow, the decrease in the activity of this enzyme in worker honeybees would cause low production and unhygienic bee products *i.e.* economic loses to the beekeeper as well as decline in bee colony strength and brood rearing activity.

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#### References

- Atkins, E. L.; Kellum, D.; Atkins, K. W. 1978. Integrated pest management strategies for protecting honeybees from pesticides. *Am. Bee J.* 118: 542-543, 547-548.
- Atkins, E.L. and Kellum, D. 1986. Comparative morphogenic and toxicity studies on the effects of the pesticides on honeybee broods. *J. Apic. Res.* 25 (4): 242-255.
- Badiou A., Meled M., Belzunces L.P., 2008. Honeybee *Apis mellifera* acetylcholinesterase- A biomarker to detect delmethrin exposure. *Ecotoxicol. Environ. Saf.* 69: 246-253.
- Bai, A. R. K. and Reddy, C. C. (1977a). Inhibition of acetylcholinesterase as a criterion to determine the degree of pesticidal poisoning in *Apis cerena indica*. *J. Apic. Res.*, 16: 112-114.

- Bernfeld, P. 1955. *Methods in Enzymology*. Volume-I. Eds. (S. P. Colowick and N.O. Kaplan) Academic Press, New York p. 149.
- Brandes, K. P. (1984). Study of the digestive enzyme in the three species of the major carp of India. *J. Biol. Sci.* 11: 63-74.
- Chan Q.W.T., Howes C.G., Foster L.J., 2006. Quantitative comparison of caste differences in honeybee hemolymph. *Mol. Cell. Proteomics* 5: 2252-2262.
- D. E. Grogan and Hunt, J. H. (2005). Effects of soybean trypsin inhibitor on hypopharyngeal gland protein content, total midgut protease activity and survival of the honey bee (*Apis mellifera* L.). *Cellular and molecular Life Sci.* 5: 1347-13 48.
- Fries, I. and Wibran, K. (1987). Effect on honeybee colonies following application of pyrethroids, cypermethrin and PP321 in flowering oil seed rape. *Amer. Bee J.* 127 (4): 266-269.
- Habes D., Morakchi S., Aribi N., Farine J.-P., Soltani N., 2006. Boric acid toxicity to the German cockroach, *Blattella germanica*: Alteration in midgut structure, and acetylcholinesterase and glutathione S-transferase activity. *Pestic. Biochem. Physiol.* 84: 17-24.
- Kumar Sushil and Gupta A.K. (2009). Pesticides induced changes in activity of digestive enzyme protease in worker honeybees, *Apis mellifera* L. *J. Flora and fauna* Vol. 15(1) 108-114.
- Kumar Sushil and Gupta A.K. 2007. Age dependent Study of the impact of certain field pesticides on Acetylcholinesterase activity of *Apis mellifera* . *J. Ento. Res.* 31(2) 115-119.
- Kumar Sushil and Gupta A.K.2010. Effect of Malathion on Amino Acid contents of Worker Honeybees of *Apis mellifera* L. *J. Entomological Res.* 34(2) 310-314.
- Kumar Sushil and Yadav Himshikha 2012. Effect of Malathion on Amino Acid contents of Worker Honeybees of *Apis mellifera* L. *Proceedings National Conference* 28-29 Jan. 2012 PP 149-153
- Mackenzie, K.E. and Winston, M. L. (1989). Effects of sub lethal exposure to Diazinon on longevity and temporal division of labor in the honeybees. *J. Econ. Ento.* 82(1): 75-82.
- Reddy, C. C. (1979). Effects of insecticides on the activities of digestive Amylase and protease of Indian honeybee *Apis cerena indica*. *Indian Bee J.* 1: 9-11.
- Reddy, C. C. (1983). Effect of pesticides on the level of ions and amino acids in the haemolymph of worker bees. *Indian Bee J.* 45(4): 105-7.
- Shires, S. W.; Murray, A; Deray, D. and Blanc, J. Le. (1983). A field study on the effects of a new insecticide Fastac on honeybees. 10<sup>th</sup> intern. Cong. of plant prot. Brighton, England 2: 135-140.
- Smirle, M. J; Mark, L.W and Kenneth, L.W. (1984). Development of a sensitive bioassay for evaluating sub lethal pesticides effect on honeybees. *J. Econ. Ent.* 77(1): 63-67.
- Stoner, A.; Wilson, W. T. and Moffett, J.O. (1984). Effects of long term feeding of low doses of Fenvalerate in sugar syrup on honeybees in standardized field colonies. *J. Georgia Ento Soc.* 19 (4): 490-498.

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