EFFECT OF PESTICIDES ON AMYLASE ACTIVITY IN FORAGERS OF APIS MELLIFERA L. UNDER LABORATORY CONDITIONS

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ABSTRACT

The effect of sub lethal concentrations of three organophosphates (dimethoate – 30 EC, methyl parathion – 50 EC, malathion – 50 EC) and one biopesticide (neem oil – 25 EC) on the activity of digestive enzyme Amylase in foragers of Apismellifera L of different was studied. The experiments were performed in laboratory conditions.The data obtained from treated groups of bees revealed that all the pesticides reduced the Amylase activity. The results suggested that organophosphates (Methyl parathion, Malathion and Dimethoate) had great inhibitory action on amylase activity. Neem oil a biopestcide, showed insignificant inhibitory effect. The maximum inhibition was observed for Methyl parathion followed by, Malathion, Dimethoate, Neem oil.

Keywords:Pesticides, Amylase, Apismellifera, Organophosphates, Biopesticide, Neem oil

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). 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 enzymes and protease was reported by Bai and Reddy (1977a), Reddy, C.C. (1979), Reddy (1983) in Apiscerenaindica and by Kumar and Gupta, 2007 & 2009 in Apismellifera L. The relative decrease in the activity of the enzymes was used to determine the degree of toxicity of pesticides. Among all age groups of bees, only foragers face direct encounter with pesticides, used against pests in crop fields especially at the blooming crops. Therefore, in the present study the effect of Methyl parathion, Dimethoate, Malathion and Neem oil (Biopesticide) (¼ and ½ of LC50 at 96 hrs.) on activity of digestive enzyme Amylase in foragers of Apismellifera L. has been analyzed.

MATERIAL AND METHODS

I. BEE REARING UNDER LABORATORY CONDITIONS FOR BIOCHEMICAL ANALYSIS

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The target organism in the present study was Italian honeybee, *Apismellifera* L. The bees were reared in the laboratory of Zoology Department, Govt. P.G College, Bisalpur, using standard Langstroth cages with wax sheet foundation frame and specialized iron net experimental cages of dimensions 1'×1'×1' under controlled conditions. The initial bee colonies were obtained from Nearby Apiaries of TehseelBisalpur and acclimated for five days in the cages before toxicity tests.The temperature and relative humidity maintained were 20-25oC (± 2^{o}C) and 61-66 R.H. respectively.

Five replicates of control and treated forager bees were used for biochemical analysis. The 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 samples fed on pesticides mixed (accordingly) 50% sugar syrup.

II. 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 asμg of maltose liberated per mg protein per min.

RESULT

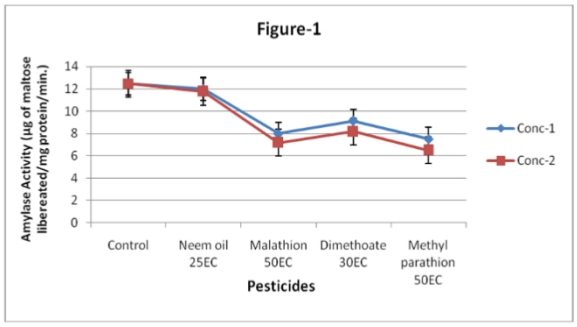
The analysis of variance in amylase activity in control and pesticides treated foragers of *Apismellifera* L. are presented in table 1 (Figure-1). Both sub lethal concentration levels of pesticides resulted inhibition in the activity of amylase, but concentration level –2 [Cons-2 (½ of LC_{50} at 96 hrs.)] proved to be more toxic causing inhibition of enzymes activity to large extent and significant as compared to concentration level-1 [Cons-1 (¼of LC_{50} at 96 hrs.)]..Table 1 (Fig.1) represents an analysis of the variance of the activity of amylase in foragers at conc-1 and 2. It indicates that the different pesticides had different degree of toxicity. Methyl parathion had maximum inhibitory effect on the activity of amylase.

It inhibited the enzyme activity 39.64%*** and 47.75%*** at conc-1 and conc-2 respectively. Malathion was the second most toxic pesticide reducing the activity of amylase up to 35.87%***, 42.38%*** at conc-1 and conc-2 respectively. Dimethoate reduced the activity of amylase up to 26.72%**, 34.43%*** at conc-1 and conc-2 respectively. Results show that Dimethoate reduced the activity of the enzyme to some lesser extent to Malathion. 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 3.77% and 5.70% at conc-1 and conc-2 respectively.

Treatment	Amylase Activity in Foragers	
	Concentration Level-1(¼ of LC_{50} at 96 hrs.)	**Concentration Level-2 (½ of LC_{50} at 96 hrs.)**
Control	12.46 ±2.81 (---)	12.46 ±2.81 (---)
Neem oil 25EC	11.99 ±1.82 (3.77)	11.75±1.58 (5.70)
Malathion 50EC	7.99 ±1.01 (35.87)***	7.18 ±0.89 (42.38)***
Dimethoate 30EC	9.13 ±1.35 (26.72)**	8.17 ±1.24 (34.43)***
Methyl parathion 50EC	7.52 ±1.27 (39.64)***	6.51 ±0.83 (47.75)***

Table 1: Alterations in Amylase activity in foragers of Apismellifera L. at sub lethal concentrations of pesticides.

Amylase Activity (μg of maltose liberated/mg protein/min.)
Each value is the mean of five replicates.
Values are expressed as mean ±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.



DISCUSSION

The inhibitory effect of pesticides on physiology of honeybee is well established **(Bai and Reddy, 1977a; Kumar and Gupta, 2007, 2009 and 2010)**. Laboratory tests suggest that both the sub lethal concentration levels of the pesticides were toxic to the activity of digestive enzyme Amylase but the conc-2 of pesticides produced extremely significant inhibitory action. This study was carried out to evaluate the effect of two different concentration levels (¼ and ½ of LC_{50} at 96 hrs.) of the pesticides on the activity of digestive enzyme Amylase in forager honeybees of different age groups. 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 conc-1 was less toxic as compared to the conc-2. Maximum inhibitory effect was observed for Methyl parathion followed by Malathion, Dimethoate respectively. **Reddy (1979) and Grogan & Hunt(2005)**also reported the inhibitory effects of the different pesticides on Amylase activity in Indian bee *Apiscerenaindica.* The maximum inhibition of amylase activity in forager bees may be due to their direct encounter with pesticides at the time of nectar collection. The exciting results were obtained from Neem oil, a Biopesticide which showed insignificant action on amylase activity and supposed to be bee friendly.

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_{50} at 96 hrs.) of the pesticides on the activity of digestive enzymes amylase in worker honeybees of different age groups. The inhibitory action of pesticides may be due to number reasons. One of them may be due to biotransformation of pesticide molecules which in turn bind up to enzyme not allowing working on substrate.

However, 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 **(Grogan and Hunt, 2005 and Chan *et al*, 2006)**. Anyhow, the decrease in the activity of this enzyme in worker honeybees would cause low production and bad quality of honey i.e. economic loses to the beekeeper as well as decline in bee colony strength and brood rearing activity.

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