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Quinalphos Induced Changes in Amino Acid Contents of Forager Worker bees *Apis mellifera* L.

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ABSTRACT

The effect of sublethal concentrations ¼ and ½ of LC_{50} at 96 hrs of Quinalphos – 25 EC, an organophosphate pesticide was studied on the forager bees of European honeybee *Apis mellifera* L. The results indicated that there were significant changes in total amino acid contents in forager bees at sublethal concentration ½ of LC_{50} at 96 hrs (conc-2) of Quinalphos. No greater changes were observed in forager bees at sublethal concentration ¼ of LC_{50} at 96 (conc-1) hrs of Quinalphos over control bees. Overall Quinalphos proved to be harmful to forager bee of *Apis mellifera* L.

Keywords: Sublethal concentrations, Quinalphos, Forager worker bees, *Apis mellifera* L. Amino acids

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INTRODUCTION

Honeybees are one of the most important insect to human beings and ecosystem. They help to the farmers by pollinating their crops, fruit trees and to the environment by pollinating wild plants [1]. Honeybees are well known for their art of manufacturing the honey, royal jelly, bee wax and propolis. Since historical period, honey is one of the most important natural products of honeybees and has been used as medicine and as an option of hygienic food substitute, especially for new born [2].

A large number of pesticides are being used in crop fields against the different types of pests all over the world. Unfortunately pesticides are not selective to pest species alone. Often non-target insects such as honeybees, which are economically and socially important to human beings, are destroyed in the process of pest control [3]-[4]. Pesticides cause not only morphological but physiological dysfunction also to the bees when their applications are repeated. In such cases it is not the bee mortality, which is significant, but it may reduce longevity [5] and biochemical imbalance. Organophosphates have been reported to be neuroactive poison acting at the synapses of the neurons [6] and have been found very poisonous to honeybees [7].

Amino acids are important metabolites being required at every stage of development in honeybees and others. They play a key role in honeybee metabolism [8]. They are the building blocks of all proteins present in living beings. In foragers, flight muscles play an important role during flight and all the muscles are derived from proteins whose ultimately source are amino acids. The toxic effect of pesticides on the level of ions and amino acids was reported by Reddy (1983)[9] in *Apis cerena indica.* Gupta and Kumar (2010)[10] reported the significant alterations in essential and nonessential amino acids present in forager of *Apis mellifera* L under stress of Malathion, an organophosphate, estimated as dry weight. The present work reports the quantitative changes in the amino acids contents of foragers of *Apis mellifera* L. measured as their dry matter under stress of Quinalphose, an organophosphate.

MATERIAL AND METHODS

Quinalphos (25EC) :

Quinalphos is an organophosphate chemical chiefly used as a pesticide. It is a reddish-brown liquid. The chemical formula is $C_{12}H_{15}N_2O_3PS$, and IUPAC name *O*,*O*-diethyl *O*-quinoxalin-2-yl phosphorothioate, ranked 'moderately hazardous' in World Health Organization's (WHO) acute hazard ranking, use of Quinalphos is either banned or restricted in most nations. Quinalphos, which is classified as a yellow label (highly toxic) pesticide in India, is widely used in the following crops: wheat, mustard, rice, coffee, sugarcane, cotton etc. The sublethal concentrations of Quinalphos were determined by acute toxicity assay tests.

Forager Honeybees

The laboratory experiments were conducted with forager honey bees of *Apis mellifera* L (Hymenoptera: Apidae) in Zoology Laboratory of Govt PG College, Bisalpur, Pilibhit. The adult worker bees were obtained from nearby apiary established by Govt Horticulture Department, Bareilly, UP (India) where honey bee colonies were maintained according to the standard commercial technique in the field. For this kind of risk assessment, forager honey bees are considered the most ecologically relevant when they start performing external tasks [11]. Extensive literature confirms that foragers are those higher than 20 days of age in a typical colony of honey bees [12]. Based on farming records, no obvious diseases were observed on units or colonies, and no hives were treated with pesticides. This was confirmed during the collection of bees. Foraging workers were collected as explained [13]. Briefly, four hives were exposed to smoke twice for 30–60 sec. before collection. Worker honey bees were collected by shaking from the top super or from the front of the hives into a clean and large plastic container. The container was covered with a solid lid, kept in good condition, and transported to the laboratory in 1.5 hrs. The bees were kept in experimental cages (10×7×12 cm) in groups of 100 at 30 ±2 °C with 62 ±5% RH, and fed a 50% (w/v) sucrose solution.

Pesticide Treatment

The acute toxicity of the pesticide was determined on foraging workers of honey bees *(A. mellifera* L.)

by oral administration through sugar syrup application at controlled laboratory conditions. The worker bees were orally treated with aforesaid pesticide with different concentrations in 50% sugar syrup. Each treatment of each concentration was composed of four replicates of plastic cups of 25 bees each covered with a nylon mesh with 100 honey bees total/treatment (four replicates with 25 bees/cup). The pesticides solution (25 ml) was applied on a cotton bed and then attached to the upper surface of the nylon mesh cover of each cup (four replicates/each concentration) and bees were left to feed for 96 hrs by lapping from the fibers of the cotton wool. The control group of bees was fed on 50% (w/v) sucrose solution. The tests were carried out at 30 ±2 °C with 62 ±5% RH. For Quinalphos (25 EC), the subtethal concentrations were estimated as 0.016% [$^1/_4$ of LC_{50} at 96 hrs (conc-1)] and 0.032% [$^1/_2$ of LC_{50} at 96 hrs (conc-2)] supplied by Gujrat Pesticides Limited, India.

Estimation Of Amino Acids

Samples of 25 forager bees were collected randomly from each of the four replicates of control and treated bee colonies. The individuals were randomly selected for analysis from control and treated colonies. After the removal of gut contents, they were dried to constant weight under vacuum at 40 ^{0}C and their amino acid contents were determined. Duplicate analysis on forager bees for nitrogen were performed by a micro-kjeldal method [14] and amino acids were analysed by a single-column buffer system after acid hydrolysis. The nitrogen analysis enabled the amino acid analyses to be expressed as gram per 16 gram of nitrogen. A Technicon amino acid auto analyzer (114-AAA, Technicon Instruments Limited, U.K.) was used to separate the amino acids with a modified buffer system in single columns [15][16]. 9% cross-linking : average particle size 24μm was used as cationic resin. The colour reagent was 2, 4, 6 - trinitrobenzene sulphonic acid. The pumping rate was adjusted to 0.9 ml/min. Each chromatogram took about 10 hours to complete. Norleucine was used as an internal standard. In no case the standard error of the mean colour factor for any amino acid was greater than ± 0.02. The test of significance was calculated by adopting Fisher's't' test at $p<0.05$*, $p<0.01$** and $p< 0.001$***.

RESULT AND DISCUSSION

The findings of amino acid analysis of Quinalphos treated forager honeybees are presented in table-1 (fig.- 1). An analysis of variance of these results indicated that there were significant alteration in total amino acid contents in forager bees against conc-2 but a few at conc-1 of Quinalphos treated forager over control bees. The amino acids that showed significant changes at conc-1 over control were aspartic acid (-17.33%**), serine (-12.24%*), glutamic acid (-12.12%*), valine (-18.64%*), phenyl alanine (-16.66%*) and arginine (-11.36%*). However, conc-2 of Quinalphos caused greater changes on almost amino acid contents of treated foragers over control bees. The amino acids which showed significant changes at conc-2 were aspartic acid (-37.33%***), threonine (+30.76%**), serine (-34.69%**), glutamic acid (-20.20%**), glycine (-26.92%**), alanine (+36.36%***), valine (-23.72%**), cystine (-15.15%*), methionine (+43.47%***), isoleucine (-20.40%**), leucine (-16.86%*), tyrosine (-14.63%*), phenyl alanine (-38.09***), lysine (-14.00%*), histidine (-22.22%**) and arginine (-20.45%**). The relative amount of various amino acids in the control and Quinalphos treated forager honeybees at conc-2 were significantly different from the control bees and showed drastic decrease in the level of amino acids estimated as dry weight of bees. The data presented in table 1 vividly indicate that the treatment of Quinalphos to the forager bees at conc-1 was not so much harmful to the bees.

Most of the protein in flying insects is due to presence of the flight muscles, of which $^1/_{3^{rd}}$ part is mitochondrial protein [17]. Thus, an analysis of the amino acids produced from adult insects by acid hydrolysis is supposed to be fairly uniform (amino acids level in control bees). Variations in the amino acid composition of insects have been attributed either to the effect of flight metabolism [18] or to dietary absorption into the haemolymph [19] or under the effect of pesticides causing decrease or increase acid hydrolysis of proteins (proteolysis) [9] and in worker honeybees the relative activity of glands producing larval food may also be implicated [10] and it makes a possibility that in honeybees all three factors are involved in the variations observed (Tables-1).

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Table 1: Amino acid contents (g/16gN) in Control and Quinalphos (25EC) treated foragers of *Apis mellifera* L.

S. No.	Amino Acids	Control	Quinalphos (25EC) Treated Foragerbees	
			Conc-1 (1/4 of LC_{50} at 96 hrs)	Conc-2 (1/2 of LC_{50} at 96 hrs)
1	Aspartic acid	7.5 ± 1.53 (---)	6.2 ± 0.71* (-17.33)	4.7 ± 0.65*** (-37.33)
2	Threonine	3.9 ± 0.28 (---)	4.4 ± 0.21 (+12.82)	5.1 ± 0.49** (+30.76)
3	Serine	4.9 ± 0.51 (---)	4.3 ± 0.71* (-12.24)	3.2 ± 0.96** (-34.69)
4	Glutamic acid	9.9 ± 1.20 (---)	8.7 ± 0.61* (-12.12)	7.9 ± 0.37** (-20.20)
5	Glycine	7.8 ± 1.01 (---)	8.0 ± 0.48 (+2.56)	5.7 ± 0.29** (-26.92)
6	Alanine	7.7 ± 1.90 (---)	7.3 ± 0.53 (-5.19)	10.5 ± 1.04*** (+36.36)
7	Valine	5.9 ± 0.98 (---)	4.8 ± 0.78* (-18.64)	4.5 ± 0.34** (-23.72)
8	Cystine	3.3 ± 0.25 (---)	3.1± 0.17 (-6.06)	2.8 ± 0.05* (-15.15)
9	Methionine	2.3 ± 0.07 (---)	2.5 ± 0.31 (+8.69)	3.3 ± 0.64*** (+43.47)
10	Isoleucine	4.9 ± 0.67 (---)	4.5 ± 0.72 (-8.16)	3.9 ± 0.72** (-20.40)
11	Leucine	8.3 ± 1.33 (---)	7.9 ± 0.89 (-4.82)	6.9 ± 0.55** (-16.86)
12	Tyrosine	4.1 ± 0.18 (---)	4.2 ± 0.37 (+2.43)	3.5 ± 0.31* (-14.63)
13	Phenyl alanine	4.2 ± 0.71 (---)	3.5 ± 0.55* (-16.66)	2.6 ± 0.09*** (-38.09)
14	Lysine	5.0 ± 0.81 (---)	4.8 ± 0.34 (-4.00)	4.3 ± 0.3.9** (-14.00)
15	Histidine	2.7 ± 0.13 (---)	2.5± 0.29 (-7.40)	2.1± 0.08*** (-22.22)
16	Arginine	4.4 ± 0.72 (---)	3.9 ± 0.87* (-11.36)	3.5 ± 0.23* (-20.45)

Each value is the mean of four replicates ± S. E
Values are significant at *P<0.05, **P<0.01, ***P<0.001
Values in parentheses are percent decrease or increase over control

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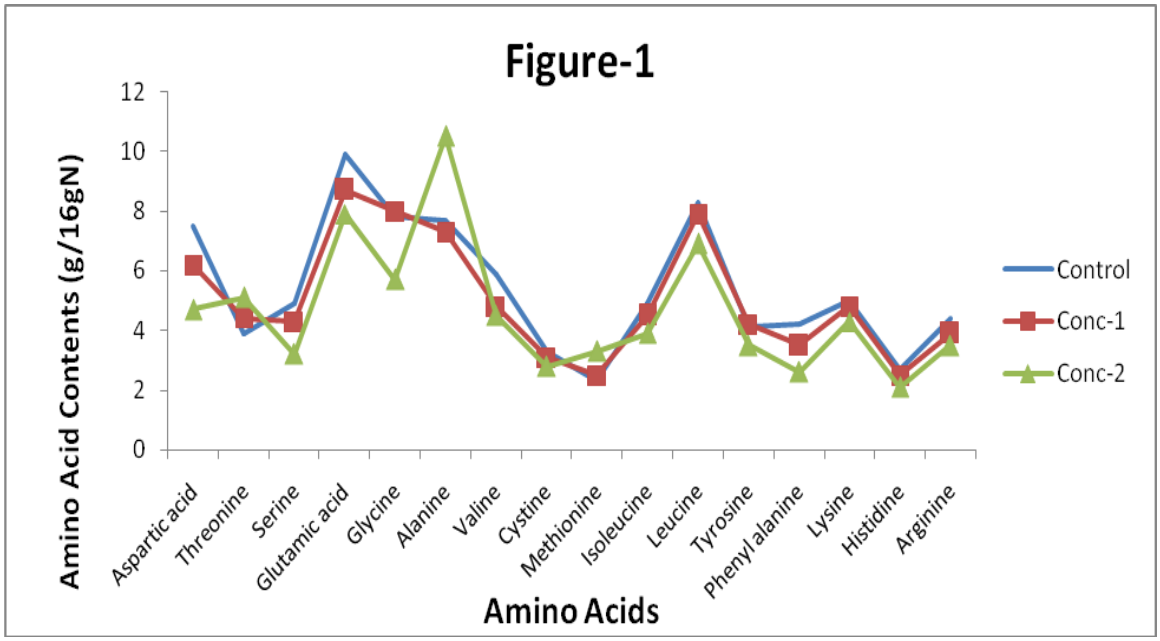

Fig. 1: Amino acid contents in control and Quinalphos treated (at conc-1 and conc-2) forager bees.

CONCLUSION

The quantitative changes in the contents of different amino acids under stress of pesticide suggested that pesticide affected the normal selective regulation of amino acids in honeybees. This study justifies that Quinalphos used in present investigation had almost negative effect on amino acids contents of foragers except for a few, which showed positive changes. The increase in level of a few amino acids indicated either greater proteolysis or greater catabolization of proteins, and their decrease might show further degradation [20]. The pesticidal effects may manifest in the inhibition or over activity of the enzymes specifically and selectively. Although free flying bee and laboratory reared control bee also exhibited a little variation in amino acid contents but it was quite insignificant [21]. The deviation in amino acids contents in forager bees due to intake of Quinalphos might cause adverse effect on their flight performances during foraging. All the cumulative effects of pesticide caused deficiency in efficacy of bee services done by them for their own colony but for nature also. The honeybees are one of most environment friendly insects whose visits to plants provides livelihood of some people but a great job is done for biodiversity sustainability. The results clearly indicate that we need great precautions while using pesticides like Quinalphos over those crops to which honeybees and other like insects have regular visits for nectar collection especially during blooming stage.

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REFERENCES

[1] Free, J. B., 1993. Insect pollination of crops. 2nd edition, Academic press, London, U. K Pp 121-129.

[2] Adlakha, RL., Dhaliwal, HS., 1979a. Insect pollination of seed cauliflower, *Brassica oleracea* with particular reference to the role of honeybees. *Indian Bee J*. 41 (1-2): 13-16.

[3] Johanson, C.A., 1979. Honeybee poisoning by chemicals: signs contributing factors, current problems and preventions. Bee world 63 (3), Pp 109-127.

[4] Atkins, E. L., MacDonald, RL., and Greywood; Hale, E. A., 1975. Repellent additives to reduce pesticide hazards to honeybees; Field tests. J. Environ. Ent. 4, pp 207-210.

[5] Mackenzie KE., 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), Pp 75-82.
[6] Srivastava, KP., 1993. Mode of action of insecticides. A Text Book of Applied Entomology, pages 77-98.
[7] Kumar, S., Gupta A. K., 2007. Age dependent study of the impacts of certain field pesticides on Acetyl cholinesterase activity in worker bees of Italian honeybee *Apis mellifera* L. J. Ento Res. 31(2), Pp 115-119.
[8] Duchateau, G., Florkin, M., Sarlet, H., 1952. Sur les acides amines libres ou combines sous form non protinique du plasma sanguine de differentes insects. Arch. Int. Physiol. Biochem. 60, pp 539-540.
[9] Reddy, CC., 1983. Effects of pesticides on the level of ions and amino acids in the haemolymph of worker honeybees. Indian bee J. 45(4), Pp 105-107.
[10] Kumar S., Gupta A.K., 2010. Effect of Malathion on Amino Acid contents of Worker Honeybees of *Apis mellifera* L. J. Ento. Res. 34(2), pp 310-314.
[11] Picard-Nizou, AL., Pham-Delègue, MH., Kerguelen, V., Douault, P., Marilleau, R., Olsen L., Grison R., Toppan A., Masson, C., 1995. Foraging behaviour of honey bees (*Apis mellifera* L.) on transgenic oilseed rape (*Brassica napus* L.*var. oleifera*). Transgenic Res. 4: Pp170-176.
[12] Winston, M.L., 1987. The biology of the honey bee. Harvard University Press, Cambridge. Pp133-141.
[13] Iwasa, T., Motoyama, N., Ambrose, J.T., Michael, R., 2004. Mechanism for the differential toxicity of neonicotinoid insecticides in the honey bee, *Apis mellifera* L. *Crop Prot.* 23, 371–378.
[14] Yuen, S.H., and Pollard, A.G., 1953. Determination of nitrogen in soil and plant materials; use of boric acid in micro-kjeldal method. J. Sci. Fd. Agric. 4, 490-496.
[15] Thomson, AR., Miles, BJ., 1964. Ion exchange chromatography of amino acids: improvement in single column system. Nature, Lond. 203, pp 483-484.
[16] Waring, J.J., and Bolton, W., 1967. 2,4,6-trinitrobenzene sulphonic acid as a colour reagent for amino acids analyses. Proc. 5th colloquium in amino acid analysis. *Technichon Mongor.* 2, pp 30-34.
[17] Bartelink, AKM., De Kort, P.A.D., 1973. Synthesis of mitochondrial protein in the flight muscles of the Colorado beetle. Biochem. J. 136, pp 795-802.
[18] Bursell, E., 1963. Aspects of the metabolism of amino acids in the testsefly. J. of Insect Physiol. 9, pp 439-452.
[19] Auclair, J. L., 1959. The influence of dietary amino acids on the blood amino acids of the German cockroach. J. Insect Physiol. 3, pp 127-131.
[20] Lass A., Crailsheim K., 1996. Influence of age and caging upon protein metabolism, hypopharyngeal glands and trophallactic behaviour in the honey bee (*Apis mellifera*. L) Ins Soc 43, pp 347-358.
[21] Gupta, A. K., Kumar, S., 2003. Evaluation of Amino Acid Contents of Adult Worker Bee and Brood of Italian Honeybee Apis mellifera L. Nat. Con. 15 (2), pp 214-223.