© JLS 2010 J Life Science, 2(2): 107-115 (2010)
PRINT: ISSN 0975-1270 ONLINE: ISSN 2456-6306 DOI: 10.31901/24566306.2010/02.02.07

# Biochemical Alterations in the Gonads of *Chrotogonus trachypterus* (Blanchard) Treated with Sub-lethal Dose of Monocrotophos

**Princy Shakeet and Saroj Bakshi**

*Eco-Toxicology Laboratory, Entomology Section, Center for Advanced Study, Department of Zoology, University of Rajasthan, Jaipur 302 004, Rajasthan, India*
*E-mail: shakeetprincy@gmail.com, sarojbakshi@gmail.com*

**KEYWORDS** Acid Phosphatase. Alkaline Phosphatase. ATPase. Cholesterol. *Chrotogonus trachypterus*. Monocrotophos. Protein

**ABSTRACT** Effects of sub-lethal doses (176.95 and 75.5 ppm) of monocrotophos on gonads of female and male *Chrotogonus trachypterus* (Blanchard) were studied respectively. The changes in the level of total protein, cholesterol, alkaline phosphatase, acid phosphatase and ATPase at intervals of 12, 24 and 48 h of treatment were evaluated. Monocrotophos led to highly significant (P≤0.001) decrease in protein at 12, 24 and 48 h of treatment in both the sexes. Cholesterol level showed highly significant (P≤0.001) rise after 12 and 24 h of treatment but at 48 h it significantly (P≤0.05) decreased in male while in female a continuous highly significant (P≤0.001) rise was recorded at 12, 24 and 48 h of treatment. Alkaline phosphatase level decreased non-significantly at 12 h but this decrease became highly significant after 24 and 48 h (P≤0.001) in male while in female non-significant decrease was recorded at 12 h, significant (P≤0.01) at 24 h and after 48 h decrease was highly significant (P≤0.001). Acid phosphatase level increased non-significantly at 12 and 24 h but significantly (P≤0.05) increased at 48 h in male while in female non-significant increase was recorded at 12 and 24 h. After 48 h it was more significantly (P≤0.01) observed. ATPase activity increased significantly (P≤0.05) at 12 h, highly significant (P≤0.001) increase at 24 and 48 h in male but in female ATPase level non-significantly decreased at 12 h and highly significant (P≤0.001) increase was observed at 24 and 48 h. The relationship of these changes in biochemical parameters with insecticide has been discussed in this paper.

## INTRODUCTION

The surface grasshopper, *Chrotogonus trachypterus* (Blanchard) (Orthoptera- Acrididae) is a destructive pest of wheat, barley, oil seed crops, maize, jowar, sunhemp, bajra, rice, sorghum, groundnut, pearl millet, cotton, vegetables, indigo, opium, red gram in different parts of the World (Jotwani1975; Latif 1975; Batra 1976; Rai 1976; Singh and Singh 1978). Monocrotophos is the most effective commonly employed insecticides for insect pest management in agriculture. Results of earlier observations established that the monocrotophos affects the gonads of insects. Thereby it is understandable as to how monocrotophos acts to bring about a certain level of sterility in the insects. The effect of monocrotophos (an organophosphorus insecticide) on the biochemical parameters of the insect has not yet been dealt thoroughly. Perhaps the biochemical studies of treated insects may throw more light on the mode of action of this insecticide. These thoughts led to conduct the experiments to discover the effect of monocrotophos on the biochemical parameters of the insect. The present investigation was, thus, conceived to direct the efforts towards the elucidation of the mode of action of this insecticide through a molecular basis for controlling the pest.

## METHODOLOGY

### Rearing of *Chrotogonus trachypterus*

*C. trachypterus* were collected from the fields, low crop ground, bare soil and grass or the waste land. The collected insects were conditioned in the laboratory. Rearing was done at room temperature ranging from 27 to 37°C in summer months and rainy days. During winter the temperature range of 27 to 32°C was maintained in the cages. The rearing was carried out at room humidity 35 to 65 per cent.

### Mode of Application

*Chrotogonus trachypterus* were treated by dipping method because of the smaller size and hopping behaviour of the insect. About 5 ml solution of a sub-lethal concentration of the

monocrotophos was taken in a small crucible, cleared and sterilized. A grasshopper held dorsally at the thoracic region by forceps was just dipped in the insecticide contained in the crucible. It was then kept in a wooden cage measuring 15 x 15 x 183 fitted with mesh windows on the sides and a glass door in the front under normal condition for experiments.

**Determination of Sub-lethal Dose of Monocrotophos Against *Chrotogonus trachypterus***

The $LC_{50}$ value of monocrotophos against female and male *C. trachypterus* were 353.9 and 151.0 ppm, respectively (Shakeet and Bakshi 2009). In continuation of above results the sub-lethal dose of monocrotophos on female and male *C. trachypterus* were 175.95 and 75.5 ppm, respectively when applied by the dipping method. The effects were observed after 12, 24 and 48 h intervals and compared with control group which received double distilled water (DDW).

## BIO-CHEMICAL ESTIMATION

### 1. Estimation of Protein

Total protein was estimated by the Lowry et al. (1951) procedure. Protein homogenate was prepared in glass distilled water and precipitated with trichloroacetic acid. When folin reagent was added to a copper treated protein, a bluish green colored complex resulted, the intensity of which accounts for the amount of protein present and was measured in ultraviolet-visible spectrophotometer with filter number 5. The absorbance was read at 640 nm against blank. The total amount (UV-VIS) of NaOH added was sufficient to neutralize the acid of the folin reagent. The folin reagent was destroyed on contact with alkali. This neutralization was necessary in order to allow the sodium carbonate to buffer the mixture near pH 10, because the maximum stability and intensity of the color results if the reduction takes place at pH 10. It is therefore, essential to mix this reagent thoroughly with copper protein complex immediately after addition. Serum-bovine albumen was used as a standard for calculations. The activity was expressed in mg/g of the wet tissue.

### 2. Estimation of Cholesterol

Total cholesterol was determined by the method described by Zlatkis et al. (1953). Cholesterol has unsaturated bonds and phenanthrene ring structure. After macerating and homogenizing the tissues in glacial acetic acid, it was kept and centrifuged. The supernatant was treated with a color reagent ferric chloride in concentric sulphuric acid. The reaction after half an hour, resulted in pinkish to colored complex, the intensity of which accounts for the amount of cholesterol present and was read on ultraviolet-visible spectrophotometer with filter number 4. The absorbance was read at 540 nm against blank. The activity was expressed in mg/g of wet tissue.

### 3. Estimation of Alkaline Phosphatase

Alkaline phosphatase was estimated by the method after Fiske and Subba-row (1925) for the determination of phosphate liberated with modification including the incubation procedure of Bodansky (1932, 1933), using alkaline buffer. The protein of the tissue homogenate was precipited with trichloroacetic acid. The filterate was then treated with acid molybdate solution. This resulted in the formulation of phosphomolybdic acid which was then reduced by adding 1, 2, 4-aminonaphthosulfonic acid reagent to produce a blue colour whose intensity was proportional to the amount of phosphate liberated. The color intensity of unknown and standard samples was read on ultraviolet-visible spectrophotometer with filter number 5 which was set to zero density with blank. The absorbance was read at 660 nm. The phosphatase activity is the difference between the inorganic phosphate content of the incubated and control samples and is expressed in terms of Bodansky unit corresponding to the liberation of inorganic phosphorus from the tissue in mg $^{pi}$/g/h.

### 4. Estimation of Acid Phosphatase

Acid phosphatase activity was also estimated by the method after Fiske and Subba-row (1925) for the determination of phosphatase libarated with modifications to include the conditions suggested by Shinowara et al. (1942). The procedure of acid phosphatase estimation was similar to that of alkaline phosphatase except that the buffered acid phosphatase substrate at an optimum pH 5.0 of Shinowara.

### 5. Estimation of Adenosine Triphosphatase (ATPase)

For quantitative analysis of the activity of ATPase, the method given by Sickevitz and Potter

(1953) was followed. Tissues were homogenized in sucrose. Disodium salt of ATP was used as substrate. The activity was measured in term of inorganic phosphorus liberated from the tissue as for the acid and alkaline phosphatases. The absorbance was read at 640 nm.

## RESULTS

The observations of the experiments conducted on the effect of monocrotophos on biochemical parameters in the gonads of *C. trachypterus* were assessed.

### 1. Protein

*Male:* A highly significant (P≤0.001) decrease was recorded in protein level of treated male insects after 12, 24 and 48 h of treatment (Table 1, Fig. 1).

*Female:* Protein level observation in females recorded similar as in males (Table 1, Fig. 1).

### 2. Cholesterol

*Male:* There was a highly significant (P≤0.001) rise in cholesterol level after 12 and 24 h of the treatment which was followed by significant (P≤0.05) decrease (Table 1, Fig 2).

*Female:* A continuous highly significant (P≤0.001) rise was recorded in the cholesterol level in females treated with sub-lethal doses after 12, 24 and 48 h of the treatment (Table 1, Fig. 2).

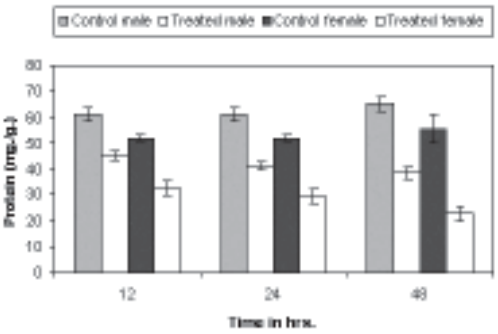


**Fig. 1. Effect of sublethal dose of monocrotophos on the protein level of the gonads of *Chrotogonus trachypterus*.**

### 3. Alkaline Phosphatase

*Male:* A non-significant decrease in alkaline phosphatase activity was recorded 12 hrs after treatment. This decrease became highly significant after 24 and 48 h (P≤0.001) of the treatment (Table 1, Fig. 3).

*Female:* Alkaline phosphatase level decreased non-significantly in females at 12 h after the

**Table 1: Biochemical alterations in gonads of *Chrotogonus trachypterus* in control and treated groups**

| *Parameters* | *Sex* | *Group* | *Time in hrs. after treatment* | | |
|---|---|---|---|---|---|
| | | | *12 hrs.* | *24 hrs.* | *48 hrs.* |
| Protein | Male | Control | 61.28±2.4344 | 61.28±2.4344 | 65.31±2.7274 |
| (mg./g.) | | Treated | 45.14±1.8599*** | 40.96±1.5813*** | 38.30±2.4705*** |
| | Female | Control | 51.92±1.6572 | 51.92±1.6572 | 55.58±5.5895 |
| | | Treated | 32.60±0.7292*** | 29.28±1.1699*** | 22.68±0.7808*** |
| Cholesterol | Male | Control | 6.77±0.4951 | 6.87±0.4570 | 7.55±0.7055 |
| (mg./g.) | | Treated | 11.91±0.5299*** | 12.57±0.2971*** | 10.04±0.3650* |
| | Female | Control | 5.62±0.0877 | 6.13±0.6204 | 6.27±0.5678 |
| | | Treated | 11.07±0.4262*** | 9.43±0.0892*** | 10.65±0.4117*** |
| Alkaline phosphatase | Male | Control | 3.25±0.4706 | 3.07±0.2234 | 3.17±0.2584 |
| ($mg^{pi}$/gm./hrs.) | | Treated | 2.43±0.3221$^{NS}$ | 1.77±0.0799*** | 1.64±0.1273*** |
| | Female | Control | 3.51±0.4480 | 3.35±0.3473 | 3.19±0.2916 |
| | | Treated | 2.62±0.3317$^{NS}$ | 1.90±0.0845** | 1.51±0.2093*** |
| Acid phosphatase | Male | Control | 0.726±0.1688 | 0.788±0.0634 | 0.748±0.0655 |
| ($mg^{pi}$/gm./hrs.) | | Treated | 0.789±0.0979$^{NS}$ | 0.880±0.0717$^{NS}$ | 1.21±0.1464* |
| | Female | Control | 0.671±0.0603 | 0.756±0.0890 | 0.843±0.0310 |
| | | Treated | 0.844±0.1442$^{NS}$ | 0.811±0.0673$^{NS}$ | 1.82±0.2444** |
| ATPase | Male | Control | 31.46±1.1036 | 32.67±1.2654 | 33.37±0.7018 |
| ($mg^{pi}$/gm./hrs.) | | Treated | 52.83±2.8924* | 70.87±1.3238*** | 131.67±2.0404*** |
| | Female | Control | 35.41±2.1124 | 33.73±0.7018 | 32.67±1.2654 |
| | | Treated | 30.06±1.4678$^{NS}$ | 89.17±3.8727*** | 130.53±1.4720*** |

Each value represent - Mean±S.E. Significance level – Control vs. Treated NS = Non significant
*P<0.05 = Significant **P<0.01 = More significant ***P<0.001 = Highly significant

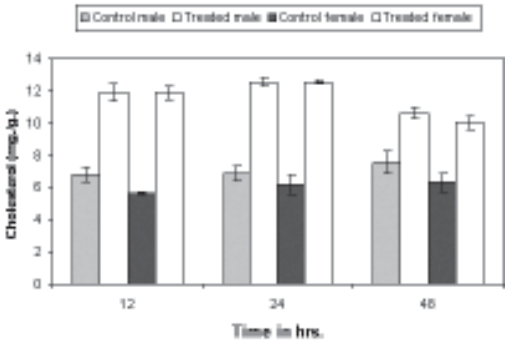


**Fig. 2. Effect of sublethal dose of monocrotophos on the cholesterol level of the gonads of *Chrotogonus trachypterus*.**

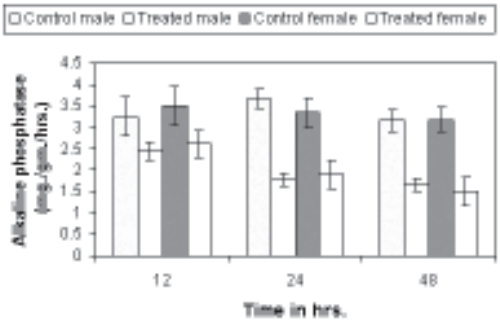


**Fig. 3. Effect of sublethal dose of monocrotophos on the alkaline phosphatase level of the gonads of *Chrotogonus trachypterus*.**

treatment. After 24 h of the treatment the decrease was more significant (P≤0.01) which continued as duration increased till 48 h and became highly significant (P≤0.001). (Table 1, Fig. 3).

### 4. Acid Phosphatase

*Male:* Acid phosphatase activity showed a non-significant increase at 12 and 24 h of the treatment but at 48 h acid phosphatase level increased significantly (P≤0.05) (Table 1, Fig 4).

*Female:* The increase of acid phosphatase was not significant at 12 and 24 h. Thereafter, the increase was more significant (P≤0.01) at 48 h of treatment (Table 1, Fig. 4).

### 5. ATPase

*Male:* ATPase level significantly (P≤0.05) increased after 12 h which was followed by a highly significant (P≤0.001) increase after 24 and 48 h of treatment (Table 1, Fig. 5).

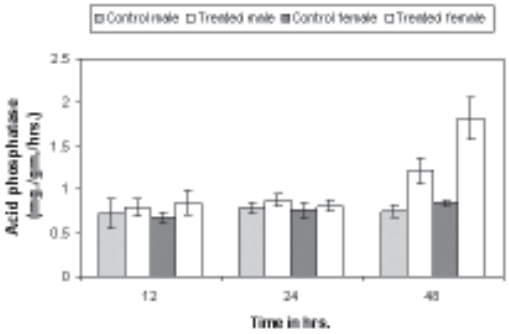


**Fig. 4. Effect of sublethal dose of monocrotophos on the acid phosphatase level of the gonads of *Chrotogonus trachypterus*.**

*Female:* No significant decrease was recorded after 12 h of treatment in the ATPase activity of females treated with sub-lethal dose. This was followed by highly significant (P≤0.001) increase after 24 and 48 h treatment (Table 1, Fig. 5).

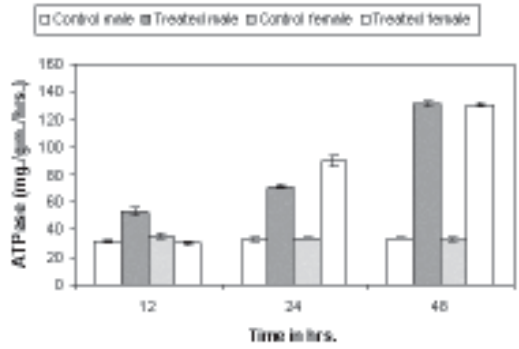


**Fig. 5. Effect of sublethal dose of monocrotophos on the ATPase level of the gonads of *Chrotogonus trachypterus*.**

## DISCUSSION

Tests with monocrotophos found potentially effective controlling insecticide by screening experiments were performed for the quantitative estimation of biochemical parameters *viz*. protein, cholesterol, alkaline phosphatase, acid phosphatase and ATPase in the gonads of *C. trachypterus*.

### Protein

*C. trachypterus* treated with sub-lethal dose

of monocrotophos exhibited hypoproteinemia or the decreased protein level in both the sexes being more pronounced in females than in males. The decrease recorded was highly significant for both the sexes till 48 h when the experiment was terminated. It shows utilization of protein in energy production. The decline in protein level with increase in transaminase activities suggests the mobilization of amino acids during insecticide stress to meet the energy demands. The fluctuation in the protein level in the insects treated with monocrotophos are in accordance to Gadallah et al. (1970, 1971a and 1972b) who reported a 6-fold increase in the protein synthesis during maturation of ovaries, an increase in the ribosomal protein in the eggs during embryogenesis and an accumulation of haemolymph protein and ovarian protein in the adult *Musca domestica* L. treated with P, P'-bis (1-aziridimyl)-N (3methoxypropyl) phosphinothionic acid. Painter et al. (1972) also reported an accumulation of haemolymph protein in apholate treated houseflies when sexually sterile have similar accumulation of some haemolymph proteins determined by acrylamide gel electrophoresis pattern. On the contrary, Masher and Macha (1968) and Macha (1969) observed the protein contents of the fat body to be strongly suppressed in the lime bug, *Pyrrhocoris apterus* (L.) treated with 6-azauridine, a nucleic acid metabolite. Turner and Maheshwary (1969) recorded a decrease in the specific activity of the ovarian protein content by apholate in the susceptible strain of *Aedes aegypti* (L.). Mitlin and Wiggal (1971) reported an inhibited protein biosynthesis in the weevil, *Anthonomus grandis* Boheman, fed with busulfan. Gadallah et al. (1971b) by sucrose density gradient (SDG) separation method demonstrated that in the thiotepa sterilized housefly eggs, normal formation of heavier aggregates of protein synthesis did not occur. Young and Lovell (1971), Pawar and Ramakrishnan (1977) and Mazzone (1985) reported reduction of protein in Lepidopteran species such as *Mamestra brassicae* (L.), *Trichoplusia ni* (Hubner) and *Lymantria dispar* (L.) infected with NPV. Subba (1985) studied the effects of organophosphates (quinalphos and monocrotophos) and a pyrethroid (sumicidin) on protein metabolism in the haemolymph of *Periplanata americana* (L.). Sneh and Quan-chuafhan (1994) and Salama et al. (1999) investigated mode of action of Bt. in *Chilo agamenon* (Bles.) and *Ostrinia nubilalis* (Hubner) in the laboratory tests and found marked decrease in total protein in B.t. treated larvae. Dhiman and Kumkum (2006) observed low level of total protein on gonads of *Leptocoris augur* Fabr. parasitized by *Hexamermis vishwakarma* Dhiman. Etebari (2006) showed that many insecticides decreased protein amount of an insect's body. Etebari et al. (2007a) reported that Grasseri is one of the most important diseases of silkworm with significant yield loss, which is caused by nuclear polyhedrosis virus (NPV). When its total proteins were measured the result showed decreased level of protein. Etebari et al. (2007b) reported significant decrease in protein contents in haemolymph of silkworm larvae due to pyriproxyfen residue. Renuga and Sahayaroj (2009) found significantly reduced total head protein after 24, 48, and 72 h treated of *Ageratum conyzoides* L. and *Artemisia vulgaris* (L.) in third and fourth instar larvae of *Spodoptera litura* Fabr. Hussain et al. (2009) reported depletion of total protein contents in *Tribolium castaneum* (Herbst) treated with spinosad.

**Cholesterol**

An initial increase observed after 12 and 24 h followed by a decrease approaching the control value at 48 h after the treatment was recorded in the cholesterol level of males treated with monocrotophos. The increase being continuous in treated female after 12, 24 and 48 h. The effect being more pronounced in females than males. The results of observations showed that the level of cholesterol in the whole tissue was immediately affected by the monocrotophos exhibiting a sudden rise in the cholesterol level in both the sexes. The initial rise may be the result of imbalance caused in the metabolism due to immediate effect of monocrotophos in the insect body. Thereafter, it was observed that a decrease resulted in the treated males which tended to approach the control values. This type of recovery in the cholesterol level approaching the control values observed in males showed that with the passage of time, the effect of the monocrotophos on the cholesterol diminishes and therefore an attempt to recovery was made. However, it is expected that the changes in the cholesterol level in the tissue may contribute to the dis-functioning of hormonal balance due to the disturbed metabolism thus, affecting reproduction.

El-Ibrashy and Boctor (1970) observed diminish cholesterol level in fat when they study the effect of allatectomy upon lipid metabolism of the female moth *Spodoptera littoralis* (Boisduval). Saleem et al. (2005) found that cholesterol content was most sensitive when they observed effects of sublethal doses of cypermethrin on the sixth instar larvae of *T. castaneum*. Dhiman and Kumkum (2006) found high cholesterol level on gonads of *L. augur* parasitized by *H. vishwakarma.* Etebari et al. (2007) reported effect of pyriproxyfen residue on silkworm larvae in haemolymph showed that cholesterol decreased significantly in 24 h after while after 120 h this level increased in some doses.

**Alkaline Phosphatase and Acid Phosphatase**

A continuous decrease was observed in the activity of the enzyme alkaline phosphatase in both the sexes treated with monocrotophos. In acid phosphatase, initially no significant effect was recorded in both the treated sexes at 12 and 24 h treatment with monocrotophos. At 48 h treatment significant increase was observed in males and still more significant increase was observed in females. These finding are in accordance to Mendoza (1964) and Mendoza and Peters (1968), who also reported a decrease in the activity of alkaline phosphatase detected histochemically in the gonads of southern corn rootworm in both males and females after the administration of apholate. Similar observations have also been recorded by Campion and Lewis (1971) in the testes of *Diparopsis castanea* (Hmps.) after tepa treatment of male moths. Kugler et al. (1956) reported the absence of alkaline phosphatase in the ovary of cockroach. Saxena and Aditya (1971, 1974) reported the absence of both alkaline and acid phosphatase in the testes and the ovaries of even untreated *Poecilocerus pictus* Fabr. They explained that either the phosphatase are absent or the enzymes are in too low concentrations to be detected quantitatively by the histochemical techniques employed. On the contrary, Turner and Maheshwary (1969) using biochemical assay technique reported high alkaline phosphatase activity in the developing ovaries of *A. aegypti*. According to Turner (1968), phosphatase are generally found in the cytoplasm of growing cells in which protein synthesis is taking place although they are important participants in the metabolism of lipids, carbohydrates, nucleic acids and nucleotides. The term alkaline phosphatase includes several enzymes which have similar although not identical properties.

Naqvi et al. (1992) studied the effect of some chemosterilants on the enzymes of *A. aegypti* larvae and their colorimetric and histochemical findings indicate that alkaline phosphatase is inhibited by shikonin, hema, tepa and shikonin angelate. Similar inhibition of phosphatase by pesticides was reported by Naqvi et al. (1969a, b), Rashid et al. (1973) and Gearing (1973). Naqvi et al. (1976) reported some inhibition of this enzyme in *M. domestica* by tepa and thiourea (neopesticide). Shafi et al. (1986) and Naqvi et al. (1989) also reported inhibition of alkaline phophatase by dimilin. Ahmed et al. (2004) demonstrated that activity of acid and alkaline phosphatase in cypermethrin treated beetles was significantly increased after 24 hrs exposure as compared to control beetles which had non-significant difference with bifenthrin treated beetles. Nathan et al. (2005) reported decrease activity of alkaline and acid phosphatase when *S. litura* larvae were fed a diet of castor leaves treated with azadiractin and nucleopolyhedrosis virus in bioassay. Saleem et al. (2005a) observed sub-lethal effect of permethrin and malathion on acid and alkaline phosphatase activity and they found that they led to significant changes. Saleem et al. (2005b) observed effects of sub-lethal doses of cypermethrin on the sixth-instar larvae of *T. castaneum* and found that the activity of alkaline and acid phosphatase was raised significantly. Matindoost (2006) showed that Bm NPV had caused a considerable decrease in the activity of alkaline phosphatase in silkworm embryo (Bm-EK1). Etebari et al. (2007b) observed alkaline phophatase activity significantly decrease in silkworm larvae due to pyriproxyfen residue. Hussain (2009) reported that spinosad caused depletion in alkaline phophatase activity while increase in acid phosphatase activity in treated *T. castaneum*.

**ATPase (Adenosine Triphosphatase)**

The activity of the energy enzyme ATPase showed highly significant increase in both the sexes treated with monocrotophos. The finding in the present investigation reveal that enough energy is consumed during metabolism due to

the effect of the monocrotophos since an increase in the activity of ATPase. Agrell (1952) carried out studies on the phosphorus metabolism of the fly *Calliphora erthrocephala* (Meig.) and reported that probably there would be interconversion in the ATPase activity i.e., when ATPase activity increased amount of ATPase decreased and vice-versa. In the absence of sufficient experimental evidences, it may be interpreted that the amount of phosphorus decrease in the body due to consumption of energy which is evident from the increase of ATPase activity. The rate of metabolism slowed down and thus led to physiological abnormalities like infertility of the insect.

Brovko et al. (1978) also observed reduced ATPase activity when they administred immobilized firefly luciferase. Babu et al. (1996) showed that the ATPase activity in *Helicoverpa armigera* (Hubner) was significantly decreased because of toxic effect of chemicals. Nathan et al. (2004) found significant reduction in ATPase activities in rice leaf folder larvae *Cnaphalocrocis medinalis* (Guenée) when fed a diet of rice leaves treated with botanical insecticides and bacterial toxins. Nathan et al. (2005) reported decreased ATPase activities when *S. litura* larvae were fed on a diet of castor leaves treated with azadirachtin and nucleopolyhedrosis virus in bioassay. Zibaee et al. (2008) reported decreased activity levels of ATPase that had significant effect on metabolism of nutrients in *Chilo suppressalis* (Walker) treated with diazinon.

## CONCLUSION

From the results described above, in general it is concluded that the following biochemical activity as the protein level continued to decrease, cholesterol increased, alkaline phosphatase decreased, acid phosphatase increased and ATPase increased so affecting the insect reproductive physiology through the process of gametogenesis in gonads as well as controlling fertility by inhibiting production of sperm and ova by utilization of monocrotophos in pest management.

## ACKNOWLEDGMENT

The authors are grateful to Dr. R.S. Bedwal, Professor and Head, Department of Zoology, University of Rajasthan, Jaipur for providing necessary laboratory facilities.

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