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### Impact of Methoprene Against the Egg Biochemistry of Rice Moth, *Corcyra cephalonica* Staint. (Lepidoptera: Pyralidae)

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**Abstract:** Exposure of first instar larvae of rice moth, *Corcyra cephalonica* to the sublethal concentrations i.e. 4, 8 and 12 ppm of methoprene caused dose-dependent alterations in the biochemical constituents of the eggs (laid within 24 h of mating by different crosses involving one or the other or both sexes emerged from treated culture). Such biochemical changes in eggs might lead to impairment of embryonic and post-embryonic growth and development of this pest, and hence, evolution of a new generation of this lepidopterous pest for the eventual establishment on stored cereals and cereal commodities can be considerably restricted.

**Keywords:** *Corcyra cephalonica*, Methoprene, Egg, Biochemistry, Growth, Development

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### Introduction

The rice moth, *Corcyra cephalonica* Staint. is a notorious pest of stored cereals and cereal commodities in Asia, Africa, North America, Europe and other tropical and sub-tropical regions of the world. Its larval stages cause serious damage to rice, gram, *sorghum*, maize, groundnut, cotton seeds, peanuts, linseeds, and milled products (Ayyar, 1919; Chittenden, 1919; Atwal, 1976; Piltz, 1977).

In recent years, there has been great concern over the toxicity of pesticides on non-target organisms and the environment. The use of conventional organic insecticides to control insect pests have given rise to problems of the proliferation of resistance and accumulation of residues in the environment with adverse ecological effects due to non-biodegradability, biomagnification and

toxicity to non-target organisms (Hoffmann and Lorenz, 1997).

Thus, there is a need of new alternatives to traditional insecticides used in stored product pest management (Mbata and Philips, 2001; Arthur and Philips, 2003). In the search of safer insecticides technologies i.e. more selective modes of action and reduced risks for non-target organisms and the environment, progress has been made with the development of natural and synthetic compounds capable of interfering with process of growth, development and metamorphosis of the target insects (Smet *et al.*, 1989, 1991; Oberlander *et al.*, 1997). In this regard, the insect growth regulators (IGRs) (Fox, 1990), which mimic insect's hormone and regulate the insect population through the disruption of moulting and metamorphosis (Williams, 1956; Oberlander *et al.*, 1997) have captured the interest of stored-product entomologists. IGRs have generally little mammalian toxicity, specific to insects and degrade rapidly in the environment (Kostyukovsky *et al.*, 2000; Ghasemi *et al.*, 2010), therefore, considered as reduced risk insecticides and are often exempted from tolerance requirements of regulatory agencies. They are superior in several aspects to the usual insecticides as, due to its rapid degradation they do not affect beneficial parasites and predators (Fox, 1990) and have a wide spectrum activity on economically important pest species (Menn *et al.*, 1981). Generally the insects do not develop resistance to IGRs (El-Ibrashy, 1970; Moreno *et al.*, 1992; Degheele *et al.*, 1993).

JH acts on so many aspects of insect reproduction that it can just be called a master regulator of the "female reproduction syndrome". In addition to its central role in

oogenesis, JH affects dispersal and flight activity, calling behaviour, post-copulatory changes in female behavior, oviposition behaviour and follicular development (Ahmed *et al.*, 2020).

The objective of the present study is to investigate the influence of methoprene on the internal biochemistry of eggs. This knowledge in turn, is likely to generate new insights into devising ways and means for controlling *Corcyra cephalonica*, by disrupting its embryonic and post-embryonic growth, development, reproductive behaviour and metabolic framework so that evolution of a new generation of this pest for the eventual establishment on stored cereals and cereal products can be considerably restricted.

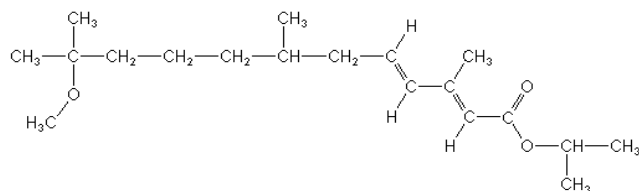
## Materials and Methods

*Corcyra cephalonica* Staint. adults were obtained from already existing laboratory stock culture maintained on normal dietary medium composed of coarsely ground jowar, *Sorghum vulgare* mixed with 5% (w/w) powdered yeast inside large glass containers (150 mm diameter, 200 mm height) at temperature  $26 \pm 1$  C, relative humidity (R.H.)  $93 \pm 5\%$  and a light regime of 12 h light and 12 h darkness. Such a standard culture was maintained throughout the year.

From the above culture, whenever needed, newly emerged males and females were transferred to oviposition glass chambers (35 mm diameter, 200 mm height). Since, *C. cephalonica* individuals do not feed during their adult stage, no food was provided to them during their confinement in these vessels. Eggs laid by the females were collected and then placed in glass chambers (consisting of 250 ml beakers) with the help of zero number camel hair brush for hatching.

Methoprene ( $C_{19}H_{34}O_3$ ); Isopropyl (2E, 4E)- 11- methoxy - 3, 7, 11 trimethyl – 2 - 1, 4 dodecadienoate; a juvenile hormone analogue, P-157N, Lot 18828, was obtained from AccuStandard, New Haven, CT06513, USA and used throughout the investigation.

Chemical structure of methoprene (Isopropyl (2E, 4E)-11-methoxy-3, 7, 11 trimethyl – 2 - 1, 4 dodecadienoate):



Preparation of different concentrations i.e. 2, 4, 8, 12, 16, 20 and 24 ppm of methoprene in dietary media, evaluation of toxic effects of these concentrations at different life stages of *C. cephalonica*, amount of methoprene consumed ( $\mu\text{g}/\text{larva}$ ) at each concentration of methoprene have been reported by Tripathi and Tiwari (2015).

For biochemical estimations, out of various concentrations of the methoprene mentioned above only such concentrations i.e. sub-lethal (4, 8 and 12 ppm) were selected, which allowed the larvae to survive, develop and emerge as adult but caused considerable effect in the internal biochemistry of the eggs that could be easily detected and assessed to prove the effectiveness of hormonal control measures against this lepidopterous pest.

For such purpose, freshly hatched larvae were allowed to feed on a normal dietary medium (kept inside 250 ml beakers) for 4 days. On the fifth day, 25 first instar larvae were transferred to each similar rearing chambers containing dietary medium mixed with 4, 8 and 12 ppm concentrations of

methoprene and were allowed to complete their life-cycle and just after emergence, the males and females were collected and used for mating experiments. For this purpose, four types of crosses were made as following:

1. Normal male x Normal female (serving as control)
2. Treated male x Normal female
3. Normal male x Treated female
4. Treated male x Treated female

(Treated male and treated female refers to the male and female moths emerged from treated food).

These pairs were allowed to mate and lay eggs in the oviposition chambers (35 mm diameter, 200 mm height glass tubes) separately. At each cross of each concentration, six pairs of males and females (each pair in separate mating/oviposition chamber) were kept for experimentation.

The eggs laid within 24 h of mating by the females of all the four crosses (i.e. Normal male x Normal female, Treated male x Normal female, Normal male x Treated female and Treated male x Treated female) were collected separately for their biochemical estimations.

The entire programme of biochemical estimation includes the quantitative measurement of total protein, total free amino acids, nucleic acids and glycogen levels and the activity of acid and alkaline phosphatases of eggs laid within 24 h of mating by the females of all the four crosses (i.e. Normal male x Normal female, Treated male x Normal female, Normal male x Treated female and Treated male x Treated female).

The methods used for the analysis of various biochemical constituents are as follows:

The total protein in eggs was measured according to the method of Lowry *et al.* (1951) using bovine serum albumin as standard. Total free amino acids was determined according to the method of Spies (1957) using glycine solution as standard. Deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) levels in eggs of *C. cephalonica* were estimated according to the method of Schneider (1957). Diphenylamine reagent was used for DNA estimation while orcinol reagent was used for RNA estimation. Glycogen was estimated according to the method of Van der Vies (1954) by using anthrone reagent. Acid and alkaline phosphatase activities in eggs were determined according to the method of Andersch and Szczypinski (1947) as modified by Bergmeyer (1967) using p-nitrophenyl-phosphate as substrate.

Results were expressed as the mean  $\pm$  SE of six replicates. Significant differences between treatment groups, in order to show dose-dependence, were determined by one way analysis of variance ( $P < 0.001$  to  $p < 0.05$ ) by using StatPlus® version 2009. Student's t-test was applied to determine the significant differences between the corresponding treated groups and the controls ( $P < 0.001$  to  $p < 0.05$ ).

## Results

Exposure of sublethal concentrations i.e. 4, 8 and 12 ppm of methoprene caused a significantly dose-dependent reduction in the levels of total protein, DNA, RNA, glycogen and in the activity of alkaline phosphatase and a significantly dose-dependent enhancement in the levels of total free amino acids and in the activity of acid phosphatase in the eggs (laid

within 24 h of mating by different crosses involving one or the other or both sexes emerged from treated culture) of *C. cephalonica* (Tables 1-7).

The total protein content in the eggs obtained from control cross (normal males and normal females), was recorded to be  $1.80 \pm 0.15$   $\mu\text{g/egg}$  (Table 1). The maximum decrease in total protein level in the eggs, collected from the crosses between treated males and normal females, normal males and treated females and treated males and treated females was recorded to be 57, 51 and 36% of the control value, respectively following treatment with 12 ppm concentration of methoprene (Table 1). Total protein levels in eggs, obtained from the crosses between treated males (i.e. emerged from treated cultures) and normal female, were reduced to 79% ( $1.42 \pm 0.08$   $\mu\text{g/egg}$ ), 73% ( $1.32 \pm 0.06$   $\mu\text{g/egg}$ ) and 57% ( $1.02 \pm 0.06$   $\mu\text{g/egg}$ ) of the control value following exposure of 4, 8 and 12 ppm concentrations of methoprene, respectively. The total protein levels in the eggs, collected from the crosses between normal males and treated females (i.e. emerged from treated cultures), were recorded to be 76% ( $1.36 \pm 0.05$   $\mu\text{g/egg}$ ), 66% ( $1.18 \pm 0.04$   $\mu\text{g/egg}$ ) and 51% ( $0.91 \pm 0.04$   $\mu\text{g/egg}$ ) of control value following treatment with 4, 8 and 12 ppm concentrations of methoprene, respectively.

The total protein content in the eggs, collected from the crosses between sexes both emerged from treated cultures was observed to be 69% ( $1.25 \pm 0.07$   $\mu\text{g/egg}$ ), 53% ( $0.95 \pm 0.05$   $\mu\text{g/egg}$ ) and 36% ( $0.64 \pm 0.02$   $\mu\text{g/egg}$ ) of the control value following exposure of 4, 8

Table 1: Effects of methoprene on the total protein level ( $\mu\text{g}/\text{egg}$ ) in the eggs of *C. cephalonica*<sup>#</sup>

Methoprene concentration (ppm)	Treated male x Normal female	Normal male x Treated female	Treated male x Treated female
0.00 (control)	1.80 $\pm$ 0.15 (100)	–	–
4	1.42 $\pm$ 0.08 <sup>c</sup> (79)	1.36 $\pm$ 0.05 <sup>c</sup> (76)	1.25 $\pm$ 0.07 <sup>b</sup> (69)
8	1.32 $\pm$ 0.06 <sup>c</sup> (73)	1.18 $\pm$ 0.04 <sup>ba'</sup> (66)	0.95 $\pm$ 0.05 <sup>ab'</sup> (53)
12	1.02 $\pm$ 0.06 <sup>ab'</sup> (57)	0.91 $\pm$ 0.04 <sup>ac'</sup> (51)	0.64 $\pm$ 0.02 <sup>aa'</sup> (36)

<sup>#</sup> Values are expressed as the mean  $\pm$  SE of six replicates; the values in the parentheses indicate the percentage change with control value taken as 100%.

a, b and c indicate significant differences,  $P < 0.001$ ,  $P < 0.01$  and  $P < 0.05$ , respectively compared with control; a', b' and c' indicate significant difference,  $P < 0.001$ ,  $P < 0.01$  and  $P < 0.05$ , respectively from corresponding treated group when t-test was applied.

Analysis of variance showed that the response to the methoprene was dose-dependent  $P < 0.001$ .

and 12 ppm concentrations of methoprene respectively (Table 1).

Sublethal concentrations of methoprene caused a significantly dose-dependent ( $P < 0.001$ ) enhancement in total free amino acids level in the eggs collected from the different crosses of *C. cephalonica* (Table 2). The total free amino acids level in the eggs, obtained from control cross (normal males and normal females) was recorded to be  $0.96 \pm 0.05$   $\mu\text{g}/\text{egg}$ . The maximum increase in total free amino acid levels in the eggs, collected from the crosses between treated males and normal females, normal males and treated females and treated males and treated females was recorded to be 125, 127 and 131% of the

control value, respectively following treatment with 12 ppm concentration of methoprene. Total free amino acid levels in eggs, obtained from the crosses between treated males (i.e. emerged from treated cultures) and normal female, were increased to 106% ( $1.02 \pm 0.07$   $\mu\text{g}/\text{egg}$ ), 115% ( $1.10 \pm 0.07$   $\mu\text{g}/\text{egg}$ ) and 125% ( $1.20 \pm 0.06$   $\mu\text{g}/\text{egg}$ ) of the control value following treatment with 4, 8 and 12 ppm concentrations of methoprene, respectively.

The total free amino acids level in the eggs collected from the crosses between normal males and treated females (i.e. emerged from treated cultures), were recorded to be 113%

Table 2: Effects of methoprene on the total free amino acids level ( $\mu\text{g}/\text{egg}$ ) in the eggs of *C. cephalonica*<sup>#</sup>

Methoprene concentration (ppm)	Treated male x Normal female	Normal male x Treated female	Treated male x Treated female
0.00 (control)	0.96 $\pm$ 0.05 (100)	–	–
4	1.02 $\pm$ 0.07 (106)	1.08 $\pm$ 0.05 (113)	1.11 $\pm$ 0.07 (116)
8	1.10 $\pm$ 0.07 (115)	1.15 $\pm$ 0.06 <sup>c</sup> (120)	1.18 $\pm$ 0.05 <sup>c</sup> (123)
12	1.20 $\pm$ 0.06 <sup>b</sup> (125)	1.22 $\pm$ 0.06 <sup>b</sup> (127)	1.26 $\pm$ 0.07 <sup>b</sup> (131)

<sup>#</sup> Values are expressed as the mean  $\pm$  SE of six replicates; the values in the parentheses indicate the percentage change with control value taken as 100%.

b and c indicate significant difference,  $P < 0.01$  and  $P < 0.05$ , respectively compared with control when t-test was applied.

Analysis of variance showed that the response to the methoprene was dose-dependent  $P < 0.001$ .

(1.08  $\pm$  0.05  $\mu\text{g}/\text{egg}$ ), 120% (1.15  $\pm$  0.06  $\mu\text{g}/\text{egg}$ ) and 127% (1.22  $\pm$  0.06  $\mu\text{g}/\text{egg}$ ) of control value when treated with 4, 8 and 12 ppm concentrations of methoprene, respectively. The total free amino acid contents in the eggs obtained from the crosses between treated males and treated females (i.e. both sexes emerged from treated cultures) were observed to be 116% (1.11  $\pm$  0.07  $\mu\text{g}/\text{egg}$ ), 123% (1.18  $\pm$  0.05  $\mu\text{g}/\text{egg}$ ) and 131% (1.26  $\pm$  0.07  $\mu\text{g}/\text{egg}$ ) of the control value following exposure of 4, 8 and 12 ppm concentrations of methoprene, respectively (Table 2).

Sublethal concentrations of methoprene caused a significantly dose-dependent ( $P < 0.01$ ) reduction in DNA levels in the eggs collected from the different crosses of *C. cephalonica* (Table 3). The DNA levels in the

eggs, obtained from control cross (normal males and normal females), was recorded to be  $4.25 \pm 0.11 \mu\text{g}/\text{egg}$ . The maximum decrease in DNA level in the eggs, collected from the crosses between treated males and normal females, normal males and treated females and treated males and treated females was recorded to be 63, 54 and 40% of the control value respectively following exposure of 12 ppm concentration of methoprene. The DNA levels in eggs, obtained from the crosses between treated males (i.e. emerged from treated cultures) and normal females, were decreased to 89% ( $3.80 \pm 0.10 \mu\text{g}/\text{egg}$ ), 69% ( $2.92 \pm 0.08 \mu\text{g}/\text{egg}$ ) and 63% ( $2.69 \pm 0.08 \mu\text{g}/\text{egg}$ ) of the control value following treatment with 4, 8 and 12 ppm concentrations of methoprene, respectively. The DNA levels in the eggs, collected from the crosses between normal males and treated

Table 3: Effects of methoprene on the DNA level ( $\mu\text{g}/\text{egg}$ ) in the eggs of *C. cephalonica*<sup>#</sup>

Methoprene concentration (ppm)	Treated male x Normal female	Normal male x Treated female	Treated male x Treated female
0.00 (control)	4.25 $\pm$ 0.11 (100)	–	–
4	3.80 $\pm$ 0.10 <sup>c</sup> (89)	3.31 $\pm$ 0.08 <sup>a</sup> (78)	2.72 $\pm$ 0.06 <sup>a</sup> (64)
8	2.92 $\pm$ 0.08 <sup>aa'</sup> (69)	2.87 $\pm$ 0.06 <sup>ab'</sup> (68)	2.59 $\pm$ 0.05 <sup>a</sup> (61)
12	2.69 $\pm$ 0.08 <sup>a</sup> (63)	2.29 $\pm$ 0.04 <sup>aa'</sup> (54)	1.70 $\pm$ 0.03 <sup>aa'</sup> (40)

<sup>#</sup> Values are expressed as the mean  $\pm$  SE of six replicates; the values in the parentheses indicate the percentage change with control value taken as 100%.

a, b and c indicate significant differences,  $P < 0.001$ ,  $P < 0.01$  and  $P < 0.05$ , respectively compared with control; a' and b' indicate significant difference,  $P < 0.001$  and  $P < 0.01$ , respectively from corresponding treated group when t-test was applied.

Analysis of variance showed that the response to the methoprene was dose-dependent  $P < 0.001$ .

females (i.e. emerged from treated cultures), were recorded to be 78% (3.31  $\pm$  0.08  $\mu\text{g}/\text{egg}$ ), 68% (2.87  $\pm$  0.06  $\mu\text{g}/\text{egg}$ ) and 54% (2.29  $\pm$  0.04  $\mu\text{g}/\text{egg}$ ) of control value when treated with 4, 8 and 12 ppm concentrations of methoprene, respectively. The DNA contents in the eggs obtained from the crosses between treated males and treated females (i.e. both sexes emerged from treated cultures), were found to be 64% (2.72  $\pm$  0.06  $\mu\text{g}/\text{egg}$ ), 61% (2.59  $\pm$  0.05  $\mu\text{g}/\text{egg}$ ) and 40% (1.70  $\pm$  0.03  $\mu\text{g}/\text{egg}$ ) of the control value following exposure of 4, 8 and 12 ppm concentrations of methoprene, respectively (Table 3).

Sublethal concentrations of methoprene caused dose-dependent ( $P < 0.01$ ) reduction in RNA levels in the eggs, collected from the

different crosses of *C. cephalonica* (Table 4). The RNA levels in the eggs obtained from control cross (normal males and normal females) was recorded to be 0.91  $\pm$  0.05  $\mu\text{g}/\text{egg}$ . The maximum decrease in RNA level in the eggs collected from the crosses between treated males and normal females, normal males and treated females and treated males and treated females, was recorded to be 60, 54 and 45% of the control value, respectively following treatment with 12 ppm concentration of methoprene. The RNA levels in eggs obtained from the crosses between treated males (i.e. emerged from treated cultures) and normal females, were decreased to 91% (0.83  $\pm$  0.06  $\mu\text{g}/\text{egg}$ ), 76% (0.69  $\pm$  0.05  $\mu\text{g}/\text{egg}$ ) and 60% (0.55  $\pm$  0.04  $\mu\text{g}/\text{egg}$ ) of the control value following treatment with 4, 8

and 12 ppm of methoprene, respectively. The RNA levels in the eggs, collected from the crosses between normal males and treated

females (i.e. emerged from treated cultures), were recorded to be 85% ( $0.77 \pm 0.06$   $\mu\text{g/egg}$ ), 69% ( $0.63 \pm 0.04$   $\mu\text{g/egg}$ ) and 54%

Table 4: Effects of methoprene on the RNA level ( $\mu\text{g/egg}$ ) in the eggs of *C. cephalonica*<sup>#</sup>

Methoprene concentration (ppm)	Treated male x Normal female	Normal male x Treated female	Treated male x Treated female
0.00 (control)	$0.91 \pm 0.05$ (100)	–	–
4	$0.83 \pm 0.06$ (91)	$0.77 \pm 0.06$ (85)	$0.70 \pm 0.05^c$ (76)
8	$0.69 \pm 0.05^c$ (76)	$0.63 \pm 0.04^b$ (69)	$0.54 \pm 0.04^{ac'}$ (59)
12	$0.55 \pm 0.04^a$ (60)	$0.49 \pm 0.02^{ac'}$ (54)	$0.41 \pm 0.03^{ac'}$ (45)

<sup>#</sup> Values are expressed as the mean  $\pm$  SE of six replicates; the values in the parentheses indicate the percentage change with control value taken as 100%.

a, b and c indicate significant differences,  $P < 0.001$ ,  $P < 0.01$  and  $P < 0.05$ , respectively compared with control; a' and c' indicate significant difference,  $P < 0.001$  and  $P < 0.05$ , respectively from corresponding treated group when t-test was applied.

Analysis of variance showed that the response to the methoprene was dose-dependent  $P < 0.01$ .

( $0.49 \pm 0.02$   $\mu\text{g/egg}$ ) of control value when treated with 4, 8 and 12 ppm of methoprene, respectively. The RNA content in the eggs, obtained from the crosses between treated males and treated females (i.e. both sexes emerged from treated cultures), were found to be 76% ( $0.70 \pm 0.05$   $\mu\text{g/egg}$ ), 59% ( $0.54 \pm 0.04$   $\mu\text{g/egg}$ ) and 45% ( $0.41 \pm 0.03$   $\mu\text{g/egg}$ ) of the control value following exposure of 4, 8 and 12 ppm concentrations of methoprene, respectively (Table 4).

Sublethal concentrations of methoprene caused dose-dependent ( $P < 0.05$ ) reduction in glycogen levels in the eggs collected from the different crosses of *C. cephalonica* (Table 5). The glycogen levels in the eggs obtained from control cross (normal males and normal females) was recorded to be  $0.94 \pm 0.08$

$\mu\text{g/egg}$ . The maximum decrease in glycogen levels in the eggs collected from the crosses between treated males and normal females, normal males and treated females and treated males and treated females was recorded to be 63, 53 and 42% of the control value, respectively following exposure of 12 ppm concentration of methoprene. It was observed that the glycogen level in eggs, collected from the crosses between treated males (i.e. emerged from treated culture) and normal females, were decreased to 80% ( $0.75 \pm 0.04$   $\mu\text{g/egg}$ ), 72% ( $0.68 \pm 0.05$   $\mu\text{g/egg}$ ) and 63% ( $0.59 \pm 0.03$   $\mu\text{g/egg}$ ) of the control value following treatment with 4, 8 and 12 ppm concentrations of methoprene, respectively. The glycogen level in the eggs collected from the crosses between normal males and treated females (i.e. emerged from treated culture)



were recorded to be 75% ( $0.70 \pm 0.04$   $\mu\text{g/egg}$ ), 67% ( $0.63 \pm 0.03$   $\mu\text{g/egg}$ ) and 53% ( $0.50 \pm 0.02$   $\mu\text{g/egg}$ ) of control value when treated with 4, 8 and 12 ppm concentrations of methoprene, respectively. The glycogen content in the eggs obtained from the crosses between treated males and treated females (i.e. both sexes emerged from treated culture) were found to be 69% ( $0.65 \pm 0.05$   $\mu\text{g/egg}$ ), 55% ( $0.52 \pm 0.04$   $\mu\text{g/egg}$ ) and 42% ( $0.39 \pm$

$0.03$   $\mu\text{g/egg}$ ) of the control value following exposure of 4, 8 and 12 ppm methoprene concentrations, respectively (Table 5).

Sublethal concentrations of methoprene caused a significantly dose-dependent ( $P < 0.05$ ) enhancement in the activity of acid phosphatase in the eggs collected from the different crosses of *C. cephalonica* (Table 6). The acid phosphatase activity in the eggs obtained from control cross (normal males

Table 5: Effects of methoprene on the glycogen level ( $\mu\text{g/egg}$ ) in the eggs of *C. cephalonica*<sup>#</sup>

Methoprene concentration (ppm)	Treated male x Normal female	Normal male x Treated female	Treated male x Treated female
0.00 (control)	$0.94 \pm 0.08$ (100)	–	–
4	$0.75 \pm 0.04$ (80)	$0.70 \pm 0.04^c$ (75)	$0.65 \pm 0.05^c$ (69)
8	$0.68 \pm 0.05^c$ (72)	$0.63 \pm 0.03^b$ (67)	$0.52 \pm 0.04^a$ (55)
12	$0.59 \pm 0.03^b$ (63)	$0.50 \pm 0.02^{ab'}$ (53)	$0.39 \pm 0.03^{ac'}$ (42)

<sup>#</sup> Values are expressed as the mean  $\pm$  SE of six replicates; the values in the parentheses indicate the percentage change with control value taken as 100%.

a, b and c indicate significant differences,  $P < 0.001$ ,  $P < 0.01$  and  $P < 0.05$ , respectively compared with control; b' and c' indicate significant difference,  $P < 0.01$  and  $P < 0.05$ , respectively from corresponding treated group when t-test was applied.

Analysis of variance showed that the response to the methoprene was dose-dependent  $P < 0.05$ .

Table 6: Effects of methoprene on the activity of acid phosphatase in the eggs of *C. cephalonica*<sup>#</sup>

Methoprene concentration (ppm)	Treated male x Normal female	Normal male x Treated female	Treated male x Treated female
0.00 (control)	$14.80 \pm 1.12$ (100)	–	–
4	$19.72 \pm 1.16^c$ (133)	$22.41 \pm 1.18^a$ (151)	$24.02 \pm 1.33^a$ (162)
8	$23.22 \pm 1.44^a$ (157)	$25.66 \pm 2.04^a$ (173)	$27.75 \pm 1.64^a$ (188)
12	$26.55 \pm 1.57^a$ (179)	$28.71 \pm 2.16^a$ (194)	$30.32 \pm 1.84^a$ (205)

<sup>#</sup> The activities are given as  $\mu$  moles substrate hydrolyzed /30 minutes / mg protein and expressed as mean  $\pm$  SE of six replicates; the values in the parentheses are the percentage change with control value taken as 100%.

a, b and c indicate significant differences,  $P < 0.001$ ,  $P < 0.01$  and  $P < 0.05$ , respectively compared with control when t-test was applied.

Analysis of variance showed that the response to the methoprene was dose-dependent  $P < 0.05$ .

and normal females), was recorded to be  $14.80 \pm 1.12$   $\mu$  moles substrate hydrolyzed/30 min/mg protein. The maximum increase in acid phosphatase activity in the eggs collected from the crosses between treated males and normal females, normal males and treated females and treated males and treated females was recorded to be 179, 194 and 205% of the control value, respectively following exposure of 12 ppm concentration of methoprene. The acid phosphatase activities in the eggs, obtained from the crosses between treated males (i.e. emerged from treated culture) and normal females, were increased to 133% ( $19.72 \pm 1.16$ ), 157% ( $23.22 \pm 1.44$ ) and 179% ( $26.55 \pm 1.57$ )  $\mu$  moles substrate hydrolyzed /30 min/mg protein of the control value following treatment with 4, 8 and 12 ppm concentrations of methoprene, respectively. The acid phosphatase activities in the eggs collected from the crosses between normal males and treated females (i.e. emerged from treated culture), were recorded to be 151% ( $22.41 \pm 1.18$ ), 173% ( $25.66 \pm 2.04$ ) and 194% ( $28.71 \pm 2.16$ )  $\mu$  moles substrate hydrolyzed/30 min/mg protein of control value when treated with 4, 8 and 12 ppm concentrations of methoprene, respectively. The acid phosphatase activities in the eggs obtained from the crosses between treated males and treated females (i.e. both sexes emerged from treated culture) were found to be 162% ( $24.02 \pm 1.33$ ), 188% ( $27.75 \pm 1.64$ ) and 205% ( $30.32 \pm 1.84$ )  $\mu$ moles substrate hydrolyzed /30 min/mg protein of the control value following exposure of 4, 8 and 12 ppm concentrations of methoprene, respectively (Table 6).

Sublethal concentrations of methoprene caused a significantly dose-dependent ( $P < 0.05$ ) reduction in the activity of alkaline phosphatase in the eggs collected from the different crosses of *C. cephalonica* (Table 7). The alkaline phosphatase activity in the eggs obtained from control cross (normal males and normal females), was recorded to be  $39.52 \pm 2.14$   $\mu$  moles substrate hydrolyzed /30 min/mg protein. The maximum decrease in alkaline phosphatase activity in the eggs collected from the crosses between treated males and normal females, normal males and treated females and treated males and treated females was recorded to be 66, 59 and 40% of the control value, respectively following exposure of 12 ppm concentration of methoprene. The alkaline phosphatase activities in the eggs obtained from the crosses between treated males (i.e. emerged from treated culture) and normal females, were decreased to 84% ( $33.15 \pm 1.81$ ), 72% ( $28.24 \pm 1.64$ ) and 66% ( $26.11 \pm 1.42$ )  $\mu$  moles substrate hydrolyzed/30 min/mg protein of the control value following treatment with 4, 8 and 12 ppm concentrations of methoprene, respectively. The activities of alkaline phosphatase in the eggs collected from the crosses between normal males and treated females (i.e. emerged from treated culture), were recorded to be 76% ( $30.11 \pm 1.66$ ), 66% ( $26.16 \pm 1.72$ ) and 59% ( $23.44 \pm 1.38$ )  $\mu$  moles substrate hydrolyzed /30 min/mg protein of the control value when treated with 4, 8 and 12 ppm concentrations of methoprene, respectively. The alkaline phosphatase activities in the eggs obtained from the crosses between treated

males and treated females (i.e. both sexes emerged from treated culture), were found to

be 65% ( $25.86 \pm 1.62$ ), 57% ( $22.52 \pm 1.11$ ) and 40% ( $15.75 \pm 0.77$ )  $\mu$  moles substrate

Table 7: Effects of methoprene on the activity of alkaline phosphatase in the eggs of *C. cephalonica*<sup>#</sup>

Methoprene concentration (ppm)	Treated male x Normal female	Normal male x Treated female	Treated male x Treated female
0.00 (control)	39.52 $\pm$ 2.14 (100)	–	–
4	33.15 $\pm$ 1.81 <sup>c</sup> (84)	30.11 $\pm$ 1.66 <sup>b</sup> (76)	25.86 $\pm$ 1.62 <sup>a</sup> (65)
8	28.24 $\pm$ 1.64 <sup>bd'</sup> (72)	26.16 $\pm$ 1.72 <sup>ac'</sup> (66)	22.52 $\pm$ 1.11 <sup>a</sup> (57)
12	26.11 $\pm$ 1.42 <sup>a</sup> (66)	23.44 $\pm$ 1.38 <sup>a</sup> (59)	15.75 $\pm$ 0.77 <sup>aa'</sup> (40)

<sup>#</sup> The activities are given as  $\mu$  moles substrate hydrolyzed /30 minutes / mg protein and expressed as mean  $\pm$  SE of six replicates; the values in the parentheses are the percentage change with control value taken as 100%.

a, b and c indicate significant differences,  $P < 0.001$ ,  $P < 0.01$  and  $P < 0.05$ , respectively compared with control; a' and c' indicate significant difference,  $P < 0.001$  and  $P < 0.05$ , respectively from corresponding treated group when t-test was applied.

Analysis of variance showed that the response to the methoprene was dose-dependent  $P < 0.05$ .

hydrolyzed/30 min/mg protein of the control value following exposure of 4, 8 and 12 ppm concentrations of methoprene, respectively (Table 7).

## Discussion

The present investigation shows that when first instar larvae of *C. cephalonica* were exposed to sublethal concentrations i.e. 4, 8 and 12 ppm of methoprene, certain biochemical constituents of eggs (laid within 24 h of mating by different crosses involving one or the other or both sexes emerged from treated culture.) were observed to exhibit significant quantitative variations. Earlier investigations revealed that sublethal concentrations i.e. 4, 8 and 12 ppm of methoprene caused a significantly dose-dependent reduction in the levels of total protein, DNA, RNA, glycogen and in the

activity of alkaline phosphatase and a significantly dose-dependent enhancement in the levels of total free amino acids and in the activity of acid phosphatase in the gonadal tissues i.e. testis and ovaries of *C. cephalonica* (Tripathi, 2015).

Proteins are among the most complex of all known chemical compounds and also the most characteristic of living organisms. They serve as an important internal environmental factor for the metabolism, especially having a close relation with fat body, metamorphic hormone, trehalose and sex hormone during development and metamorphosis (Lee *et al.*, 1981).

Methoprene suppresses egg production in insects by several mechanisms. In males JHAs disrupt spermatogenesis (Dumser and Davey, 1974) and functioning of accessory glands in

some insect species and the degree to which the target tissue is affected differs with the species. In females, JHAs affect the development of oviducts (Koeppe *et al.*, 1985), follicular growth (Koeppe *et al.*, 1980), oocyte maturation (Koeppe *et al.*, 1985) and functioning of accessory glands (Bodenstein and Sprague, 1959; Koeppe *et al.*, 1985). JHAs treatment produced severe disorders in the ovaries of *Tenebrio molitor* including cell death in the germarium, resorption of oocytes in the previtellarium and vitellarium, formation of compound egg chambers and undue proliferation of follicular cells sometimes resulting in malformation of the whole ovaries (Metwally *et al.*, 1972). These ovarian defects appear to be consequent with the changes in the course of cell proliferation, and JH compounds seemed to interfere with the cell division and differentiation (Metwally *et al.*, 1972).

Earlier findings suggest that in pre-adult females of *Sitophilus oryzae*, hydroprene and R-20458 treatment prevented successful completion of vitellogenesis, formation of the germinal vesicle and karyosphere, and also blocked retraction of the oolemma and as a result the ovarioles remained atrophied and consequently no new generation was produced (Gupta and Mkhize, 1983). Diflubenzuron reduced oviposition in *Tenebrio molitor*, as a result of inhibition of ovarian DNA synthesis (Soltani-Mazouni and Soltani, 1994) and also due to interference with vitellogenesis via biochemical processes (Oberlander *et al.*, 1975; Soltani *et al.*, 1984; Soltani-Mazouni and Soltani, 1994). Fenoxycarb at 0.1-10 ppm concentration caused abnormal development of the male gonads in *Ephestia kuehniella* (Moreno, 1992)

resulting into reduced fecundity of adults. Larval diet of triflumuron disrupted the testicular follicle in male *Tribolium castaneum* of both susceptible and resistant strains (Parween, 1996). Spermatids and sperm bundles in the treated males appeared to be abnormally large, and spermatogonia were few in number. When these treated males were allowed to mate with untreated females, egg viability was reduced and the effect was found to be dose-dependent. However, the male reproductive system of *Tribolium castaneum* was less affected than the female reproductive system (Parween, 1996). At higher doses of IGR treatment sterility may be permanent but at marginal doses the females may lay viable eggs at the later part of the oviposition period (Adan *et al.*, 1994; Parween, 1996).

JHAs have been observed to disturb the growth and maturation of the gonads in both sexes (Metwally *et al.*, 1972; Soltani-Mazouni and Soltani, 1994, 1995; Parween, 1996) resulting in production of non-viable eggs.

In the present investigation, methoprene caused a significantly dose-dependent reduction in the level of total protein and a significantly dose-dependent enhancement in the levels of total free amino acids in the eggs, laid within 24 h of mating by different crosses involving one or the other or both sexes emerged from treated culture. It may be supposed that methoprene induced reduction in the levels of total protein in the ovaries of *C. cephalonica* (Tripathi, 2015), may be plausibly on account of poor synthesis of proteins and/or inhibition of amino acid incorporation into proteins in ovaries, which might be the possible reasons for the decreased level of

total protein content in the eggs of the rice moth, *C. cephalonica*.

The high concentration of free amino acids is believed to play an important role with the predominant function of serving as units for protein synthesis (Buck, 1953) and taking part in other metabolic activities.

The enhancement in the total free amino acid levels in the eggs laid within 24 h of mating by different crosses involving one or the other or both sexes emerged from the treated culture, in the present investigation, may be due to methoprene induced inhibition of protein synthesis and/or inhibition of amino acid incorporation into protein in the ovaries (Tripathi, 2015) and ultimately into eggs.

DNA content provides an estimate of cell number whereas RNA content can be considered as an index of the capacity of organism for protein synthesis (Brachet, 1955; Lang *et al.*, 1965).

In the present investigation, methoprene exposure caused a significantly dose-dependent reduction in the levels of DNA and RNA in the eggs, laid within 24 h of mating by different crosses involving one or the other or both sexes emerged from the treated culture. Similar result was also reported in the eggs of *Spodoptera litura* exposed to an IGR, chlorfluazuron (Perveen, 2012). IGR-diflubenzuron inhibited ovarian DNA synthesis resulting into reduced oviposition in *Tenebrio molitor* (Soltani-Mazouni and Soltani, 1994) and also due to interference with vitellogenesis via biochemical processes (Oberlander *et al.*, 1975; Soltani *et al.*, 1984; Soltani-Mazouni and Soltani, 1994). It may be concluded that methoprene inhibits ovarian

DNA synthesis that resulted into reduced level of DNA and RNA in gonads of this pest (Tripathi, 2015) and consequently in the eggs in the present investigation.

Carbohydrates are one of the most essential biochemical constituents of insect tissues, many of which support optimum growth, development, reproductive activity and survival of individual species (Chefurka, 1959; Kilby, 1963; Wyatt, 1967; Friedman, 1970).

Glycogen can be synthesized also in the ovary itself, from haemolymph trehalose as major source (Yamashita and Hasegawa, 1985). Engles (1966) and Engles and Bier (1967) have reported that by applying  $H^3$  histidine at various stages of vitellogenesis in honey bee, *Apis mellifica* and *Musca domestica* L., the carbohydrate incorporation occurred primarily during later stages of oocyte maturation. They are of the opinion that the glycogen synthetase, essential for glycogen synthesis, does exist in inactive form in the earlier stages of oocyte maturation. With the help of radioautography, Ramamurty (1968) has also noticed almost similar findings and proposed that the nurse cells and follicle cells of ovaries of scorpion fly, *Panorpa communis* do not supply glycogen to the oocyte. But follicle cells have been reported to be the source of glycogen in the ovaries of *Bombus* (Palm, 1948) and honey bee (Bier, 1954). Engles and Drescher (1964) have observed that the labelled carbohydrate appeared in the oocyte within three minutes of application of  $H^3$  glucose, in case of *Apis mellifica*. This rapid uptake of glucose further suggests that it is the oocyte itself which is involved in carbohydrate synthesis. Blood glucose has also been reported to be utilized in the

glycogen synthesis by the oocytes (Bonhag, 1956; Ramamurty, 1968).

Methoprene, in the present investigation, caused a significantly dose-dependent reduction in glycogen content in the eggs laid within 24 h of mating by different crosses involving one or the other or both sexes emerged from treated culture. It may be concluded that, in the present investigation, methoprene affects the synthesis of glycogen in the oocytes by hampering the activity of glycogen synthetase and/or by blocking the passage of raw materials for glycogen synthesis to the oocytes which results in the reduced levels of glycogen in the eggs. Similar opinion has also been suggested by Engles and Bier (1967) for the reduction of glycogen level by radioisotopic studies in *Apis mellifica* and *Musca domestica* L.

Acid phosphatase plays a significant role in catabolism, pathological necrosis, autolysis and phagocytosis (Abou Donia, 1978; Aruna *et al.*, 1979). It has also been reported to have proteolytic action (Moczon, 1976) and helps in energy liberating processes (Dalela *et al.*, 1978). Alkaline phosphatase is involved in the transport of metabolites across the cell membranes (Danielli, 1952; Vorbrodt, 1959), synthesis of certain enzymes (Sumner, 1965), protein synthesis (Pilo *et al.*, 1972), secretory activity (Ibrahim *et al.*, 1974) and spermatogenesis (Pavlikova and Repas, 1975), hydrolysis of phosphomonoesters under the alkaline condition (Miao, 2002). Both the enzymes (acid and alkaline phosphatases) have been shown to be associated with insect development, especially in relation to nutrition and egg maturation (Tsumuki and Kanehisa, 1984).

In the present study, it was observed that sublethal concentrations of methoprene caused a significantly dose-dependent enhancement in the activity of acid phosphatase and a significantly dose-dependent reduction in the activity of alkaline phosphatase in the eggs laid within 24 h of mating by different crosses involving one or the other or both sexes emerged from the treated culture of *C. cephalonica*.

JHA induces cell proliferation of the gonads that results into significant enhancement in the activity of acid phosphatase in the testes and ovaries of insects as reported in case *Gryllotalpa gryllotalpa* (Mandal, 1982), milkweed bug, *Oncopeltus fasciatus* (Beel and Feir, 1977) and *Chrysocoris stoll*i (Saha *et al.*, 1985). Similar results have also been observed in testes, ovaries and eggs of *C. cephalonica* following exposure of fenoxycarb (Singh, 2015) and methoprene (Tripathi, 2015).

It may be assumed that in this study enhancement in the activity of acid phosphatase and reduction in the activity of alkaline phosphatase in the eggs may be due to methoprene induced cell proliferation in the ovaries of this insect.

## Conclusion

On the basis of the overall findings it may be concluded that methoprene exposure, in the present investigation, disrupts the egg biochemistry of *C. cephalonica*, which might lead to impairment of embryonic and post-embryonic growth and development of this pest, and hence, evolution of a new generation of this lepidopterous pest for the eventual establishment on stored cereals and cereal commodities can be considerably restricted.

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