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BINARY ACTION OF CHLORPYRIFOS-METHYL AND METHOPRENE ON THE LARVAL BIOCHEMISTRY OF ALMOND MOTH, *EPHESTIA CAUTELLA* WALKER (LEPIDOPTERA:PYRALIDAE)

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ABSTRACT: Sub-lethal concentrations i.e. 1, 2 and 4 ppm mixture of chlorpyrifos-methyl and methoprene in the ratio of 9:1 caused a significantly dose-dependent reduction in the levels of total protein, DNA, RNA and RNA/ DNA ratio and a significantly dose-dependent enhancement in the total free amino acid levels in haemolymph and fat body tissues of the larva of almond moth, *Ephestia cautella* Walker, a serious pest of stored cereals. The highest concentration (4ppm) of this mixture caused abrupt changes in the metabolic framework of the larva that impairs its physiological fitness, which ultimately leads to death, if exposed to 8ppm concentration of this binary mixture. The present study states that methoprene enhances the toxicodynamic property of chlorpyrifos-methyl in a synergistic way.

KEYWORDS: *Ephestia cautella,* haemolymph, fat body, biochemistry, chlorpyrifos-methyl, methoprene

INTRODUCTION

Post-harvest losses and quality deterioration due to storage pests are a major problem throughout the world. The almond moth, *Ephestia cautella* (Walker) is a notorious pest of stored cereals and cereal commodities in India as well as throughout tropical and temperate regions of the world[12,65,51]. Its larval stages cause serious damage to wheat, maize, ground nuts, pulses, peanuts and beans etc[5,56,51,3].

Considerable measures have been adopted in relation to application of organic pesticides since World War II, which brought inestimable benefits to humanity in terms of human lives saved, diminished sufferings, and economic gain[61,45]. but continuous tremendous use of organic pesticides, since last four decades, has polluted the whole environment in addition to causing adverse effect on non- target organisms like parasites, predators and pollinators. They have also posed serious problems like bioaccumulation and development of insecticide resistance. So, there is urgent need of safe and suitable insecticides for the efficient control of almond moth, *Ephestia cautella* in particular and lepidopterous pests in general.

Chlorpyrifos-methyl exposure has shown good result against rice weevil, *Sitophilus oryzae* (L.); granary weevil, *S. granarius* (L.); maize weevil, *S. zeamais* Motschulsky; lesser grain borer, *Rhyzopertha dominica* (Fabricius); *T. confusum* and *T. castaneum*[39]. It was also found to be effective against khapra beetle,

Trogoderma granarium Everts and *T. confusum* in wheat grain[25], *P. interpunctella*[6] and lesser grain borer, *Rhyzopertha dominica*[31] (Fabricius). In mammals chlorpyrifos-methyl is rapidly absorbed and metabolized, the principal metabolite being 3, 5, 6-trichloro-2-pyridol. The present compound and metabolite are excreted primarily in the urine and faeces and are not stored to any extent in the body[42]. It has lower mammalian toxicity, LD_{50} (oral, rat) 3000 mg/kg. Its limited environmental persistence and lack of cross resistance makes chlorpyrifos-methyl a more attractive prospect than DDT for indoor residual spraying[68] (Tomlin, 2000).

Development of insecticide resistance in insects is a great concern and to overcome such problem mixtures of certain insecticides having different mode of action can be used to nullify each others resistance. Application of insect growth regulators mixed with synthetic organic insecticides has shown to be a suitable alternative to suppress the development of such resistance.

Insect growth regulators (IGRs) also called "Third-Generation Pesticides", mimic insect's hormone and regulate the insect population through the disruption of moulting and metamorphosis[73,49]. They have a good margin of safety for most non-target biota, as they display a very low toxicity for human and other mammals, are readily biodegradable (i.e. very low persistence in the environment), highly toxic to target insects, and leave no hazardous residues, making JHAs very useful in food preservation and storage[70]. It has been useful in combinations with other neurotoxic pesticides particularly for control of pest species that have developed resistance to such pesticides.

Methoprene is a long chain hydrocarbon ester considered to have higher potency and better field stability than do naturally occuring juvenile hormone[32]. It is a selective, stable and potent larvicide; an ether and diunsaturated fatty acid ester; and its toxicity to insects is to manifest through interference with metamorphosis, a process without parallel in mammals. It is non-persistent and non-toxic to mammals and presents no long-term hazards to other species at recommended application rates.

The prolongation of larval life presents a distinct disadvantage to using IGRs as a means of controlling stored product insects[64] but binary approach of IGRs with certain reduced risk insecticides may be considered to reduce the prolongation of larval life.

Several studies indicate that methoprene in combination with other reduced risk-insecticides can control stored grain insects, including those populations that are resistant to orgaophosphorous insecticides[22,23,49,15].

The haemolymph is the only extracellular fluid in the insect body and haemocytes suspended in plasma serve principally in phagocytosis[38]. Hormones that regulate larval moulting, growth, longevity, metamorphosis, metabolism and reproductive behavior of insects are secreted

and circulated in the haemolymph[46,33]. The plasma contains about 85% water, is usually slightly acidic and includes inorganic ions, plenty of amino acids, proteins, fats, sugars, organic acids in variable amounts[74,21,29]. It provides a store of water on which the tissue can draw during desiccation and in some cases acts as an appreciable organ of storage for food. It also transports food materials and hormones and exerts a mechanical function in the eversion of protrusible structures.

The fat body, irregular masses or lobes of rounded or polyhedral cells (trophocytes)[52], is a dynamic tissue discharging a variety of important functions like storage of various nutrients, detoxification of foreign chemicals and biosynthesis of circulating metabolites like mammalian liver[35]. It maintains a constant exchange relation with haemolymph[29].

Insecticides have shown to exert massive effect on the haemolymph and fat body biochemistry of insects[48,55,34,4,66,67].

Juvenile hormone analogues (JHAs) have also been reported to influence the biochemistry of proteins[75,47,27,30,71,18,19]; amino acids[18,19] and nucleic acids[24,60,69] in various tissues of insects that leads to metabolic perturbations and consequently biochemical lesion.

Scientific contribution in relation to binary action of synthetic organic insecticides and IGRs influencing haemolymph and fat body biochemistry of the larva of *E. cautella* is completely wanting. Hence, as an objective of such programme, the present work has been designed and conducted to examine into the impact of chlorpyriphos- methyl synergized with methoprene on the haemolymph and fat body biochemistry of the larva of almond moth, *Ephestia cautella* for its effective and safe control.

MATERIALS AND METHOD

The almond moth, *Ephestia cautella* (Walker) (Lepidoptera: Pyralidae) was collected from the go-downs of Central Warehouse Corporation, Nandanagar, Gorakhpur, U.P.; Food Corporation of India, Sardarnagar, Gorakhpur, U.P.; State Warehouse Corporation, Chauri Chaura, Gorakhpur, U.P. and State Warehouse Corporation, Sahjanwa, Gorakhpur, U.P.

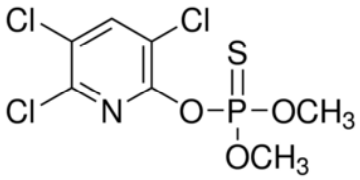
A rich standard culture of this insect was maintained in the laboratory on a normal dietary medium composed of coarsely ground wheat (*Triticum aestivum*) mixed with 5% (w/w) yeast powder and10% (w/w) glucose inside large glass containers (150 mm diameter, 200 mm height) at a temperature of 26 ± 1^0C, relative humidity 93 ± 5% and a light regime of 12 hr light and 12 hr darkness.

From the above culture whenever needed, newly emerged males and females were transferred to oviposition glass chambers (35 mm diameter, 200 mm height). Since, *E. cautella* 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

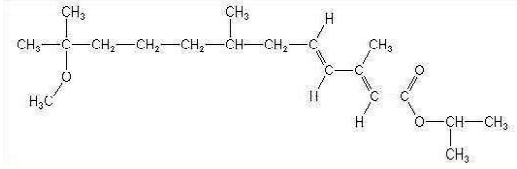
were collected and then placed in glass chambers (consisting of 250 ml beakers) for hatching.

A mixture of two insecticides i.e. chlorpyrifos-methyl (an organophosphate compound) and methoprene (a juvenile hormone analogue), in the ratio of 9:1 were utilized throughout the investigation.

Chlorpyrifos-methyl (0,0-dimethyl 0-(3,5,6-trichloro-2-pyridyl), Molecular formula:$C_7H_7Cl_3NO_3PS$, 98.5% (a.i.) and methoprene (isopropyl (2E, 4E)-11-methoxy-3, 7, 11- trimethyl-2-1,4-dodecadienoate), Molecular formula: $C_{19}H_{34}O_3$ (7.4% cis and 90.4% trans) 97.8% (a.i.) used throughout the investigation were obtained from AccuStandard, Inc.125 Market Street, New Haven, CT 06513 and have the following structural formula:



Chlorpyrifos-methyl



Methoprene

Toxicity results of methoprene[16] (Chandra and Tiwari, 2013), chlorpyrifos-methyl[17] (Chandra and Tiwari,2014b) and their mixture[20] in the ratio of 9:1 (Chandra and Tiwari, 2014d) against the ontogeny of *Ephestia cautella*, at various dose levels have been reported.

For biochemical estimations, out of various concentrations of the mixture of chlorpyrifos-methyl + methoprene in ratio of 9:1, only such concentrations i.e. three from this mixture (1, 2 and 4 ppm) were selected, which allowed the larvae to survive and develop but caused considerable effect in the internal biochemistry of the haemolymph and fat body tissues of the larva that could be easily detected and assessed to prove the effectiveness of this mixture as a chemical control measure 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 13 days. On the 14th day, 25 third instar larvae were transferred to each similar rearing chambers containing dietary medium mixed with 1, 2 and 4 ppm concentrations of the mixture of chlorpyrifos-methyl + methoprene in ratio of 9:1 and were allowed to feed for 10 days. 25 larvae were also kept as control with each set of experiment.

On the completion of 23 days, 10-15 larvae from each set, experimental as well as control, were taken out and their haemolymph and fat body tissues were separately collected (Krishna and Pandey, 1974) for biochemical assay[37].

The total protein level was measured according to the method of Lowry et al. (1951)[44] using bovine serum albumin as standard while total free amino acids were

determined according to the method of Spies (1957)[63] using glycine solution as standard. Deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) levels were estimated according to the method of Schneider (1957)[54] using calf thymus DNA and yeast RNA as standard for DNA and RNA respectively.

Results have been expressed as the mean ± s.e. of six replicates. Significant differences between treatment groups, in order to show dose dependence, were determined by one way analysis of variance (one way ANOVA)[62] (Sokal and Rohlf, 1969).

RESULTS AND DISCUSSION

Sub-lethal concentrations (1, 2 and 4ppm) of the mixture of chlorpyrifos-methyl + methoprene in the ratio of (9:1) caused a significantly dose-dependent ($p < 0.001$) reduction in the levels of total protein in haemolymph as well as in fat body (Table I). In the control larval groups, the total protein content was 54.865 and 67.784 µg/mg in haemolymph and fat body respectively. The maximum decrease in total protein levels in haemolymph (31% of the control value) and fat body (38% of the control value) was observed in larvae treated with 4 ppm concentration of the mixture. Protein levels, in haemolymph, were reduced to 70 (38.649 µg/mg), 46 (25.405 µg/mg) and 31% (17.189 µg/mg) of the control while these levels, in fat body, were reduced to 80 (54.189 µg/mg), 54 (36.487 µg/mg) and 38% (25.892 µg/mg) of the control value following treatment with 1, 2 and 4 ppm concentrations of the mixture respectively (Table 1).

Table-1. Changes in the total protein levels in the haemolymph and fat body of the larva of *E. cautella* treated with the mixture of the chlorpyrifos-methyl + methoprene in the ratio (9:1)

Concentration of chlorpyrifos-methyl + methoprene (9:1)(ppm)	Total protein #(µg/ mg, wet wt.)	
	Haemolymph	Fat body
Control (untreated)	54.865 ± 1.351 (100)	67.784 ± 1.401 (100)
1	38.649 ± 1.285 (70)	54.189 ± 1.034 (80)
2	25.405 ± 1.159 (46)	36.487 ± 0.912 (54)
4	17.189 ± 0.799 (31)	25.892 ± 0.564 (38)

#Values are expressed as the mean ± s.e. of six replicates.

Values in the parentheses indicate the percentage change with control values taken as 100%.

Analysis of variance showed that the response to the mixture of chlorpyrifos-methyl + methoprene (9:1) was dose-dependent $p < 0.001$.

A significantly dose-dependent ($p < 0.001$) enhancement in the level of total free amino acids was recorded in haemolymph and fat body tissues of the larva of *E. cautella* following treatment with sub-lethal concentrations (1, 2 and 4 ppm) of the mixture of chlorpyrifos-methyl + methoprene (9:1) ratio (Table 2).

In the control larval groups, the total free amino acids content was 68.503 and 12.245 µg/mg in haemolymph and fat body respectively. Larvae treated with 4 ppm concentration of the mixture of chlorpyrifos-methyl + methoprene (9:1) ratio showed a maximum enhancement in the total free amino acids level in haemolymph (183% of the control) and fat body (197% of the control). Total free amino acids level, in haemolymph, were increased to 124 (85.273 µg/mg), 151 (103.223 µg/mg) and 183% (125.258 µg/mg) of the control value while these levels, in fat body, were increased to 148 (18.173 µg/mg), 182 (22.271 µg/mg) and 197% (24.145 µg/mg) of the control value following treatment with 1, 2 and 4 ppm concentrations of the mixture respectivel (Table 2).

Table-2. Changes in the total free amino acids level in the haemolymph and fat body of the larva of *E. cautella* treated with the mixture of chlorpyrifos-methyl + methoprene in the ratio (9:1)

Concentration ofchlorpyrifos-methyl + methoprene (9:1) (ppm)	**Total free amino acids #(µg/ mg, wet wt.)**	
	Haemolymph	**Fat body**
Control (untreated)	68.503 ± 1.477 (100)	12.245 ± 0.491 (100)
1	85.273 ± 1.503 (124)	18.173 ± 0.507 (148)
2	103.223 ± 2.975 (151)	22.271 ± 1.541 (182)
4	125.258 ± 1.430 (183)	24.145 ± 0.548 (197)

#Values are expressed as the mean ± s.e. of six replicates.
Values in the parentheses indicate the percentage change with control values taken as 100%.
Analysis of variance showed that the response to the mixture of chlorpyrifos-methyl + methoprene (9:1) was dose-dependent $p < 0.001$.

A significantly dose-dependent ($p < 0.01$). reduction in the levels of DNA was recorded in haemolymph and fat body tissues of the larva following treatment with sub-lethal concentrations (1, 2 and 4 ppm) of the mixture of chlorpyrifos-methyl + methoprene (9:1) ratio (Table 3).

In the control larval groups the deoxyribonucleic acid level was 9.615 and 6.255 µg/mg in the haemolymph and fat body respectively. Larvae treated with 4 ppm

concentration of the mixture of chlorpyrifos-methyl + methoprene (9:1) ratio showed a maximum decrease in the DNA level in haemolymph (59% of the control value) and fat body (39% of the control value). DNA levels, in haemolymph, were reduced to 87 (8.384 µg/mg), 76 (7.342 µg/mg) and 59% (5.710 µg/mg) of the control value while these levels, in fat body, were reduced to 81 (5.090 µg/mg), 66 (4.105 µg/mg) and 39% (2.428 µg/mg) of the control value following treatment with 1, 2 and 4 ppm concentrations of this mixture respectively (Table 3).

Table-3. Changes in the DNA levels in the haemolymph and fat body of the larva of *E. cautella* treated with the mixture of chlorpyrifos-methyl + methoprene in the ratio (9:1)

Concentration of chlorpyrifos-methyl + methoprene (9:1) (ppm)	**DNA #(µg/ mg, wet wt.)**	
	Haemolymph	**Fat body**
Control (untreated)	9.615 ± 0.115 (100)	6.255 ± 0.245 (100)
1	8.384 ± 0.124 (87)	5.090 ± 0.119 (81)
2	7.342 ± 0.260 (76)	4.105 ± 0.126 (66)
4	5.710 ± 0.361 (59)	2.428 ± 0.132 (39)

#Values are expressed as the mean ± s.e. of six replicates.
Values in the parentheses indicate the percentage change with control values taken as 100%.
Analysis of variance showed that the response to the mixture of chlorpyrifos-methyl + methoprene (9:1) was dose-dependent $p < 0.01$.

In the control larval group, the ribonucleic acid content in the haemolymph and fat body was 14.496 and 9.921 µg/mg respectively. Sub-lethal concentrations (1, 2 and 4 ppm) of the mixture of chlorpyrifos-methyl + methoprene in the ratio (9:1) caused a significantly dose-dependent ($p < 0.01$) reduction in the levels of RNA in both the tissues of the larva (Table 4). The maximum decrease in RNA levels in haemolymph, (34% of the control value) and fat body (29% of the control value) was observed in larve treated with 4 ppm concentration of the mixture of chlorpyrifos-methyl + methoprene (9:1) ratio. RNA levels, in haemolymph, were reduced to 75 (10.895 µg/mg), 57 (8.098 µg/mg) and 34% (4.988 µg/mg) of the control value while these levels, in fat body, were reduced to 75 (7.399 µg/mg), 52 (5.109 µg/mg) and 29% (2.913 µg/mg) of the control value following treatment with 1, 2 and 4 ppm concentrations of this mixture respectively (Table 4).

RNA/DNA ratio, in control larvae, was 1.507 in haemolymph and 1.586 in fat body, when treated with sub-lethal concentrations of the mixture of chlorpyrifos-methyl + methoprene (9:1) ratio. The maximum decrease in this ratio in haemolymph (58%

Table-4. Changes in the RNA levels in the haemolymph and fat body of the larva of *E. cautella* treated with the mixture of chlorpyrifos-methyl + methoprene in the ratio (9:1)

Concentration ofchlorpyrifos-methyl + methoprene (9:1) (ppm)	RNA #(µg/ mg, wet wt.)	
	Haemolymph	Fat body
Control (untreated)	14.496 ± 0.276 (100)	9.921 ± 0.215 (100)
1	10.895 ± 0.307 (75)	7.399 ± 0.207 (75)
2	8.098 ± 0.274 (57)	5.109 ± 0.136 (52)
4	4.988 ± 0.1.63 (34)	2.913 ± 0.227 (29)

#Values are expressed as the mean ± s.e. of six replicates.
Values in the parentheses indicate the percentage change with control values taken as 100%.
Analysis of variance showed that the response to the mixture of chlorpyrifos-methyl + methoprene (9:1) was dose-dependent $p < 0.01$.

Table-5. Alterations in the RNA/DNA ratio in haemolymph and fat body of the larva of *E. cautella* treated with the mixture of chlorpyrifos-methyl + methoprene in the ratio (9:1)

Concentration ofchlorpyrifos-methyl + methoprene (ppm)	RNA/DNA ratio	
	Haemolymph	Fat body
Control (untreated)	1.507 (100)	1.586 (100)
1	1.299 (86)	1.454 (92)
2	1.103 (73)	1.245 (79)
4	0.813 (58)	1.190 (75)

The values in the parentheses indicate the percentage change with control value taken as 100%.

of the control value) and fat body (75% of the control value) was observed in larvae treated with 4 ppm concentrationofthis mixture. The RNA/DNA ratios, in haemolymph, were reduced to 86 (1.299), 73 (1.103) and 58% (0.813) of the control value while these ratios in fat body, were reduced to 92 (1.454), 79 (1.245) and 75% (1.190) of the control value following treatment with 1, 2 and 4 ppm concentrations of this mixture respectively (Table 5).

The present investigation reveals some of the so far unexplored information regarding potential of a synergistic mixture of chlorpyrifos-methyl (an organophosphorus insecticide) and methoprene (a juvenile hormone analogue) in the ratio of 9:1 on the total protein, total free amino acids, DNA and RNA levels

in the haemolymph and fat body tissues of the larva of almond moth, *Ephestia cautella*, pertaining to a specific age group. The findings are discussed here in the light of the influence of this synergistic mixture (in the ratio of 9:1) as insecticidal agents on the basic extrinsic as well as intrinsic cellular mechanisms such as transport, synthesis, degradation and storage in relation to the aforesaid biochemical constituents to come to some such conclusions which may in future help in devising ways and means for the effective control of this lepidopterous pest.

Proteins are among the most complex of all known chemical compounds and also the most characteristic of living organism. 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[41]. Protein synthesized in the early instars of the larval fat body (the main site of protein synthesis of blood protein) are subsequently released into the surrounding blood[57], which, in later instars are sequestered from the blood into the fat body. Regarding their synthesis, it was observed that in fruit fly, *Drosophila* amino acids[59] are first incorporated into peptides and later enter into proteins[72]. Higher concentrations of insecticides inhibit amino acid incorporation into protein causing adverse effect on protein biosynthesis[1].

In the present investigation, all the three sub-lethal concentrations of the mixture of chlorpyrifos-methyl and methoprene (9:1) ratio caused a significantly dose-dependent ($p < 0.001$) reduction in the level of total protein in both the tissues of the larva (Table 1). Similar to this observation, application of sub-lethal doses of organophosphorous insecticides reduced the protein content in the haemolymph of gypsy-moth, *Porthetria dispar*[48] caterpillars, and BHC (a chlorinated insectide) reduced the protein content in haemolymph of German cockroach, *Blattella germanica*[41]. On the contrary, lower DDT concentrations increased the protein content of *Triatoma infestans* by enhancing the amino acid incorporation into protein while higher DDT concentrations inhibited the amino acid incorporation into protein causing adverse effect on protein biosynthesis[1]. Application of a mixture of chlorpyrifos and camphor extract significantly decreased the total protein level by 13.5% in the larva of cotton leafworm, *Spodoptera littoralis* while this level was reduced to 31 and 26% following the exposure of camphor extract and chlorpyrifos repectively[50,28]. In addition, exposure of chlorosan (chloropyrifos 24% + cypermethrin 5%), engeo (thiamethoxam 14.1% + lambda-cyclithrin 10.6%), cygron (flufenoxuron 3% + alpha-cypermethrin7%) and feroban (chloropyrifos 47.5% + lufenuron 2.5%) caused significant reduction in total protein level in the larva of *S. littoralis*[26]. Our present findings are in accordance with the above results as reported in case of *S. littoralis*[26,28,50]. It appears that reduction in protein level in

haemolymph and fat body, in the present study, may be due to the insecticidal (chlorpyrifos-methyl+ methoprin) inhibition of amino acid incorporation into protein, as reported in case of *Triatoma infestans*[1] in relation to higher DDT concentration.

One of the most characteristic features of insect haemolymph is the high level of free amino acids[72,21,29] whereas insect fat body is an active site for the intermediary metabolism of these amino acids[35,21]. The high concentration of free amino acid is believed to play an important role in osmoregulation[11,8], buffering of the blood to some extent, energy production for flight and cocoon construction[74] , with the predominant function of serving as units for protein synthesis[14] and taking part in other metabolic activities.

In the present study, the sub-lethal concentrations of chlorpyrifos-methyl + methoprene (9:1) caused a significantly dose-dependent ($p < 0.001$) enhancement in the level of total free amino acids in both the tissues of the larva of this pest (Table 2). Similar result has also been reported in case of *T. castaneum* larvae following exposure of binary mixture of permethrin (200 ppm) and malathion (20 ppm)[55]. Since chlorpyrifos-methyl+ methoprene, in the present study, decreased the protein level in haemolymph and fat body of the larva of almond moth as stated above, it may be concluded that a rise total free amino acid level in both the tissues is plausibly on account of protein depletion and/or inhibition of amino acid incorporation into proteins.

RNA content can be considered an index of the capacity of organism for protein synthesis where as DNA content provides an estimate of cell number. The RNA/DNA ratio is, therefore, a measure of protein synthetic capacity per cell[13,40].

Insecticides have shown to alter the nucleic acid levels in various tissues of insects[9,36,10,58]. Literatures concerning insecticidal induced changes in the nucleic acid levels with special reference to insects[7,43] are far from adequate.

In the present investigation binary mixture of chlorpyrifos-methyl and methoprene caused a dose-dependent($P < 0.01$) reduction in the levels of DNA and RNA (Table 3 and 4) and a significant reduction in RNA/DNA ratio (Table 5) in both the tissues of the larva of this pest. Similarly, DDT and dieldrin have been shown to reduce the nucleic acids as well as protein content in rats[9,36] but DDT stimulated RNA synthesis in adult houseflies, *Musca domestica*[7] and in nymphs of *Triatoma infecstans*[43]. Organophosphorus compounds have been reported to be a strong inhibitor of nucleic acids[10]. They interfere with the synthesis site of nucleic acids as reported in the fish *Tilapia mossambica*[53] exposed to DDVP. Similar explanation for decrease in DNA and RNA contents have also been reported in BHC fed rats[58]. Pesticides induced DNA damage have also been reported in human cell culture[2]. The reduction in the DNA and RNA levels, in the present study, may be due to

interference of this binary mixture (Chlorpyrifos-methyl + methoprene in the ratio of 9:1) with the synthesis site of nucleic acids. As stated earlier , the two parameters- RNA content and RNA/DNA ratio, show a significant correlation with protein content. Thus, the protein content depends on its synthesis in which RNA plays a vital role. Data in the present study also demonstrate the reduction in the total protein level in both the tissues of the larvae following treatment with this binary mixture. Therefore, it may be presumed that the synthesis of protein is inhibited due to inhibition of RNA. It may also be presumed that reduction in protein levels is due to interference of this binary mixture (Chlorpyrifos-methyl and methoprene in the ratio of 9:1) with the transport of amino acids incorporation into the polypeptide chain. The enhancement in total free amino acid level further supports the above presumption.

The entire findings of the present investigation i.e. decreased protein level, increased amino acid titre and reduced DNA, RNA and RNA/DNA ratio leads to metabolic perturbation and consequently biochemical lesion in the chlorpyrifos-methyl + methoprene exposed larvae resulting into death. Application of a little amount of methoprene to the chlorpyrifos-methyl explores dual significance i.e. it enhances the activity of chlorpyrifos-methyl as synergist and reduces resistance in *E. caulella*, due to their different mode of action.

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