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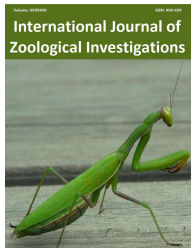

Toxicity of Bioresmethrin on the Developmental Stages and Larval Biochemistry of Rice-Moth, *Corcyra cephalonica* Staint, (Lepidoptera: Pyralidae)

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Abstract: Larvicidal and pupicidal effects of bioresmethrin were investigated on the third instar larvae of rice-moth, *Corcyra cephalonica* (Staint). The observations revealed that 0.0008% dose level of bioresmethrin caused 100% larval mortality. In addition, sublethal doses (0.0001%, 0.0002% and 0.0003%) of bioresmethrin caused a significant dose-dependent reduction in the total protein content, DNA, RNA, RNA/DNA ratio, glycogen and in the activity of alkaline phosphatase and a significant dose-dependent enhancement in the levels of total free amino acids, reducing sugar and in the activity of acid phophatase in haemolymph and fat body tissues of the larva of rice-moth, *C. cephalonica*.

Key words: Bioresmethrin, Toxicity, Ontogeny, *Corcyra cephalonica*, Biochemistry.

Introduction

Stored-product pests cause post harvest losses, estimated from 9% in developed countries to 20% or more in developing countries (Phillips and Throne, 2010). Rice-moth, *Corcyra cephalonica* is a major pest of stored cereals and cereal commodities in India as well as other tropical and subtropical regions of the world. Its larval stages cause serious damage to rice, gram, sorghum, maize, ground nut, cotton seeds, peanuts, linseeds, raisins, nutmeg, chocolates, army biscuits, wheat, coffee, cocoa beans and milled products (Chittenden, 1919; Ayyar, 1919; Atwal, 1976; Piltz, 1977; Allotey, 1991).

Influence of insecticidal agents like organochlorines, organophosphates, a few synthetic pyrethroids and certain plant materials have already been reported against the ontogeny as well as larval biochemistry of this lepidopterous pest (Tiwari and Bhatt, 1987a, 1987b, 1988, 1989, 1992, 1993, 1994a, 1994b, 1994c, 1994d, 1994e, 1996, 1999a, 1999b, 2000a, 2000b; Tiwari and Tripathi, 2001, 2003, 2005, 2006; Pathak and Tiwari, 2010a, 2010b, 2012; Shukla and Tiwari, 2011). Persistent use of synthetic organic insecticides affect immune system of insects, develop resistance (Champ and Dyte, 1976; Zettler, 1982; Zettler and Cuperus, 1990; Yusof and Ho, 1992; White, 1995 and

Ramesh and Birthal, 1997) cause toxicity to non-target organisms (Sighamony et al., 1986), cause residue in food grains (Fishwick, 1988), biomagnification, and of course pollute natural environment due to non-biodegradability, leading to biological imbalance due to the destruction of beneficial species such as parasites and predators of pests beside the destruction of pollinating insects such as honey bees. They cause health hazard to man and other animals (Pichaet and Philongene, 1993). Hence, there is urgent need for a safe but effective and biodegradable pesticide causing no/least toxic effect on non-target organisms. This has created a world-wide interest in the development of alternative strategies, including the search for new type of insectcides, and the re-evolution and use of age-old, traditional botanical pest control agents (Heyde et al., 1983). Different type of plant preparations such as powder, solvent extracts, essential oils and whole plants are being investigated for their insecticidal activity including their action as fumigants, repellents, antifeedants, anti-ovipositions and insect growth regulators (Isman, 2000; Weaver and Subramanyam, 2000; Koul, 2004; Modure, 2004; Erturk, 2004; Negahban and Moharramipour, 2007). The effectiveness of many plant derivatives against stored product insect pest has already been demonstrated (Su, 1977, 1990; Malik and Naqvi, 1984; Delobel and Molonga, 1987; Weaver et al., 1991; Khaire et al., 1992; Hu, 1993; Xie et al., 1995). Bioresmethrin is one of the synthetic pyrethroid which is effective against insects in stored products such as grain, especially where resistance to organophosphate insecticides has built-up. It is slightly toxic for warm blooded animals. Several studies have shown that this active product has a very low toxicity to mammals. Hence, in the present study, for the first time, experiments have been designed and conducted to examine the impact of bioresmethrin (a synthetic pyrethroid), at various doses, on the life-cycle stages as well as on the various biochemical constituents viz. total protein, total free amino acids, nucleic acids, carbohydrates and on the activity of acid and alkaline phosphatases in the haemolymph and fat body tissues of the larva of rice-moth, *C. cephalonica*. Such synthetic pyrethroid induced changes on the ontogeny as well as in the larval haemolymph and fat body biochemistry may be regarded one of the objective criteria permitting an assessment of the effectiveness of synthetic pyrethroid control measures against *C. cephalonica* in particular and lepidopterous pest in general.

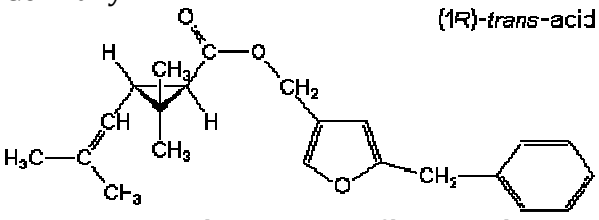
Material and Methods

1. Insects

Culture of rice-moth, *C. cephalonica* was maintained in the laboratory on normal dietary medium composed of coarsely ground jowar (*Sorghum vulgare*) mixed with 5% (w/w) yeast powder inside glass containers (150 mm diameter, 200 mm height) at 26±10C and 93± 5% relative humidity (R.H.).

2. Collection of bioresmethrin

Bioresmethrin (C22H26O3), 97.5% (a.i.), 5-benzyl-3-furylmethyl (1R,3R)-2,2-dimethyl-3-(2-methylprop-1-enyl) cyclopropanecar-boxylate or 5-benzyl-3-furylmethyl(1R)-trans-2,2-dimethyl-3- (2-methylprop-1enyl) cyclopropanecarboxylate, Batch No. SZE8287X, Article/Product No. 31496 was obtained from Sigma-Aldrich Steinheim, Germany.



Structural organization of bioresmethrin

3. Preparation of different dose levels of bioresmethrin in dietary media

For the preparation of different dose levels of bioresmethrin in dietary media, a stock solution of known concentration of bioresmethrin was prepared in required volume of ethanol and then adjusted via serial dilutions to achieve its required concentrations. Required volume of ethanolic concentration of bioresmethrin was thoroughly mixed with the required quantity of normal food (roughly ground jowar mixed with 5 % w/w yeast powder) to get desired dose levels of bioresmethrin. This food was then air dried at room temperature to eliminate completely the excess of organic solvent. For control purposes, the normal food was thoroughly mixed with a required volume of organic solvent similar to that of treated food and then air dried in the same way.

4. Evaluation of toxicity of bioresmethrin against the life-cycle stages of C. cephalonica

To evaluate the toxic effects of various doses of bioresmethrin, freshly hatched larvae of *C. cephalonica* were allowed to feed on a normal dietary medium (kept in 250 ml beakers) for 15 days. On the 16th day, 25 third instar larvae were transferred to each similar rearing chambers containing 50g of dietary medium mixed and treated separately with different known dose levels of bioresmethrin. Experiments were conducted on 8 different concentrations of bioresmethrin (0.0001%, 0.0002%, 0.0003%, 0.0004%, 0.0005%, 0.0006%, 0.0007% and 0.0008%). Twenty five larvae were also kept on a normal dietary medium to be employed as control. On the completion of life-cycle, per cent adult emergence and per cent pupal death was observed and on that basis percent pupation and per cent larval death was calculated. Experiments were replicated five times and the values have been expressed as the mean ± S.D. Straight line regression equation was applied between different concentrations of natural plant products and their corresponding % larval death, % pupation, % pupal death and % adult emergence to observe the significant correlation (Sokal and Rohlf, 1969). Amount of insecticide consumed by larvae were calculated as µg/larva at each dose level of bioresmethrin. LD_{50} values (µg/larva), 95% confidence limits (lower and upper confidence limits) of LD_{50}, slope values, g values and heterogeneity of bioresmethrin was calculated by Polo Plus, Probit and Logit Analysis, Version: 2.0, LeOra Software based on probit analysis (Finney, 1959) (Table 1).

5. Dose selection of bioresmethrin for biochemical assay:

For biochemical estimations, out of various dose levels of bioresmethrin only such doses (0.0001%, 0.0002% and 0.0003%) were selected, which allowed the larvae to survive and develop but caused considerable effect in the internal biochemistry of the larva that could be easily detected and assessed to prove the effectiveness of bioresmethrin control measures against this lepidopterous pest.

6. Larval rearing and their treatment for biochemical estimation:

Freshly hatched larvae were allowed to feed on a normal dietary medium (kept in 250ml beakers) for 15 days. On the 16th day, 25 larvae were transferred to each similar rearing chambers containing dietary medium mixed with 0.0001%, 0.0002% and 0.0003% of bioresmethrin and were allowed to feed for 10 days. 25 larvae were kept as control with each set of experiment.

7. Separation and collection of tissues:

On the completion of 25 days, 10- 15 larvae from each set, experimental as well as

control were taken out. From these groups of larvae, haemolymph and fat body were separately collected and pooled in a manner outlined as thus:

Haemolymph was obtained from these larvae following the procedure of Krishna and Pandey (1974) which involved making of a small puncture by means of a sharp needle at the dorsolateral side of the prothoracic segment and drawing the blood, easily oozing out through this puncture, into a fine glass capillary tube. The haemolymph thus obtained from caterpillars was collected in a previously weighed small glass vial (12mm diameter; 55mm height). For each biochemical estimation, after ascertainning the weight of the haemolymph, a known volume of required solvent was added to prepare the homogenate.

Fat bodies were taken out from these larvae following careful dissections performed on a clean glass slide containing minute quantities of distilled water under a stereoscopic binocular microscope. The water and the flowed out haemolymph surrounding these tissues were then completely drained off with the help of absorbent paper. Later this fat body material was weighed and swiftly mixed with known volume of required solvent to prepare the homogenate for each biochemical estimation.

8. Biochemical estimation:

The entire programme of biochemical estimation includes the quantitative measurement of total protein, total free amino acid, nucleic acids and carbohydrates levels and activity of acid and alkaline phosphatases in haemolymph and fat body of the larva of rice-moth treated with sublethal doses of bioresmethrin as well as control. Total protein level was estimated according to the method of Lowry et al. (1951) using bovine serum albumin as standard. The homogenate (10mg/ml, w/v) was prepared in 10% TCA. Total protein content was expressed as µg protein /mg wet weight of tissue. Total free amino acid level was measured according to the method of Spies (1957) using glycine solution as standard. The homogenate (10 mg/ml, w/v) was prepared in 96% ethanol. The total free amino acid level was expressed as µg /mg wet weight of tissue. The nucleic acids, (DNA and RNA) levels were measured according to the method of Schneider (1957), using diphenylamine reagent and orcinol reagent, respectively. The homogenates (10mg/ml, w/v) were prepared in 5% TCA at 90C. The DNA, RNA levels were expressed as µg/mg wet weight of tissues. Glycogen was measured according to the method of Van der Vies (1954). The homogenate (10 mg/ml, w/v), was prepared in 5% TCA. Anthrone reagent was used for glycogen estimation. Glycogen levels were expressed as mg/g wet weight of tissues. Reducing sugar was estimated according to the method of Folin Wu (1920). Alkaline copper and phosphomolybdic acid reagent was used for reducing sugar estimation. The homogenate (50 mg/g, w/v), was prepared in 4.0 ml of ice cold double distilled water. Reducing sugar levels were expressed as mg/g wet weight of tissues. Acid and alkaline phosphatase activity was measured according to the method of Andersch and Szcypinski (1947) as modified by Bergmeyer (1967) using p-nitrophenyl-phosphate as substrate. Homogenates (2%, w/v) were prepared in ice-cold 0.9% sodium chloride solution. The activities of acid and alkaline phosphatases were expressed as µ moles of p-nitrophenol liberated/30 minutes/mg protein.

Results

Table 2 and Figure 1 represent the toxicodynamic properties of bioresmethrin (a synthetic pyrethroid) on the ontogeny of

C. cephalonica. A significant larval death was obtained with the increase of bioresmethrin concentration in the diet. At 0.0001% dose level of bioresmethrin, larval mortality was 20% while 100% larval death was recorded at 0.0008% dose level of bioresmethrin. As the bioresmethrin concentration increases in the diet a significant reduction in pupation and an insignificant enhancement in pupal death occur. A pupation of 80% was recorded at 0.0001% dose level of bioresmethrin which decreased to 8% at 0.0007% dose level. Under similar conditions, 2.94% pupal death was recorded at 0.0002% dose level of the bioresmethrin while 100% pupal death was recorded at 0.0007% dose level of bioresmethrin. Below 0.0002% dose level of bioresmethrin there was no pupal death. A significant reduction in adult emergence was recorded following exposure of increased dose levels of bioresmethrin. At 0.0001% dose level of bioresmethrin 80% adult emergence was recorded that decreased to 12% at 0.0006% dose level of bioresmethrin.

It deserves mention that the dietary medium (roughly ground *Sorghum vulgare*) was richly mixed with 5% (w/w) yeast powder, hence, in control set there was no larval and pupal mortality resulting into 100% adult emergence. Thus, in the present investigation Abbott's formula need not apply.

Sub-lethal doses of bioresmethrin were enough to alter the biochemical constituents in haemolymph and fat body tissues of larva of rice-moth, *C. cephalonica*. Total protein, nucleic acids (DNA and RNA), RNA/DNA ratio, glycogen contents and activity of alkaline phosphatase were reduced after exposure of bioresmethrin, while total free amino acid, reducing sugar levels and activity of acid phosphatase increased after exposure of bioresmethrin in haemolymph and fat body tissues of larva of rice-moth, *C. cephalonica.*

In the haemolymph and fat body tissues of the *C. cephalonica* exposed to 0.0001%, 0.0002% and 0.0003% of bioresmethrin, total protein level was reduced to 74, 51 and 24% in haemolymph, 84, 53, 38% in fat body, DNA level was reduced to 86, 75 and 67%, in haemolymph, 87, 74 and 63% in fat body, RNA level was reduced to 78, 56 and 37% in haemolymph 80, 62 and 41% in fat body respectively. RNA/DNA ratio level was reduced to 91, 75 and 55% in haemolymph, 91, 83, and 65% in fat body, glycogen level was reduced to 74, 47 and 22% in haemolymph, 65, 43 and 25% and activity of alkaline phosphatase was reduced to 74, 55 and 34% in fat body, total free amino acid content was increased to 112, 132 and 172% in haemolymph, 133, 154 and 174% in fat body, reducing sugar level was increased to 135, 178 and 198% in haemolymph 133, 168 and 210% in fat body and activity of acid phosphatase was increased to 184, 248 and 415% in haemolymph 146, 233 and 329% in fat body (Table 3).

Table 1: LD_{10}, LD_{50} and LD_{90} values, confidence limits (LCL and UCL) of LD_{50}, slope values, g values and heterogeneity of bioresmethrin to the 3rd-5th instar larvae of rice-moth, *C. cephalonica*.

Effective doses (µg / larva)	**Confidence Limits**	**Slope Values**	**g Values**	**Heterogeneity**
LD_{10}= 0.0001 **LD_{50} = 0.0003** **LD_{90} = 0.0009**	**LCL = 0.00025** **UCL = 0.00030**	**2.824 ± 0.158**	**0.015**	**1.20**

LCL = Lower Confidence Limit
UCL = Upper Confidence Limit

Table 2: Toxicity of bioresmethrin against the life-cycle stages of rice-moth, *Corcyra cephalonica*.

Bioresmethrin** concentrations %	Bioresmethrin# consumed (µg/larva)	Percent* larval death	Percent* pupation	Percent* pupal death	Percent* adult emergence	Acute toxicity to the pest
Control	0	0	100	0	100	--
0.0001	0.20	20 ± 2.82	80 ± 2.82	0	80 ± 2.82	Poorly toxic
0.0002	0.38	32 ± 4.00	68 ± 4.00	2.94 ± 2.19	66 ± 2.19	Moderately toxic
0.0003	0.51	41 ± 3.34	59 ± 3.34	5.08 ± 1.78	56 ± 4.00	Moderately severe
0.0004	0.58	60 ± 2.82	40 ± 2.82	7.50 ± 3.34	37 ± 3.34	Moderately severe
0.0005	0.62	72 ± 6.32	28 ± 6.32	17.85 ± 1.78	23 ± 5.21	Severely toxic
0.0006	0.67	83 ± 1.78	17 ± 1.78	29.41 ± 1.78	12 ± 2.82	Severely toxic
0.0007	0.59	92 ± 2.82	8 ± 2.82	100	__	Extremely severe
0.0008	0.48	100	__	__	__	Extremely toxic

**Percent bioresmethrin dose levels in food enriched with 5 % (w/w) yeast powder.

*Values have been expressed as mean ± S.D. of five replicates.

Known weight of treated diet was given to each set of 25 larvae. After the completion of the life cycle, the remaining food was reweighed to calculate the amount of bioresmethrin consumed per larva.
Straight line regression equation was applied between different concentrations of bioresmethrin and their corresponding percent larval death/percent pupation/percent pupal death /percent emergence to observe the significant correlation:

Percent larval death y = 5.622+124833.88x; r = 0.99 P < 0.001

Percent pupation y = 95.75 -130714.28x; r = -0.99 P < 0.001

Percent pupal death y = -16.91+106450.00x; r = 0.77 P insignificant

Percent emergence y = 97.04-145358.09x; r = -0.99 P < 0.001

Table 3: Total protein (TP), total free amino acids (TFAA), nucleic acids (DNA and RNA), RNA/DNA ratio, glycogen (GN), reducing sugar (RS) levels, and activity of acid phosphatase (ACP) and alkaline phosphatase (ALP) in haemolymph and fat body tissues of the larva of rice-moth, *Corcyra cephalonica* following treatment with sublethal doses of bioresmethrin.

Tissues		Control	Sub-lethal dose level (%)		
			0.0001	0.0002	0.0003
TP	HLP	68.941 ± 2.630 (100)	50.996 ± 2.614 (74)	35.146 ± (51) 1.806(74)	16.539± 0.844(24)
	FB	13.021 ± 0.616 (100)	10.938 ± 0.484 (84)	6.901 ± 0.294 (53)	4.948 ± 0.284 (38)
TFA	HLP	88.320 ± 3.838 (100)	98.919 ± 3.884(112)	116.582 ±4.088(132)	151.910 ± 4.729(172)
	FB	11.524 ± 0.762(100)	15.327 ± 1.026(133)	17.474 ± 1.221(154)	20.052 ± 1.164(174)
DNA	HLP	10.562 ± 0.682(100)	9.083± 0.473 (86)	7.922 ± 0.462(75)	7.076 ± 0.392 (67)
	FB	6.402 ± 0.436(100)	5.601 ± 0.388(87)	4.771 ± 0.306(74)	4.031 ± 0.318(63)
RNA	HLP	15.892 ± 0.866(100)	12.396 ± 0.644(78)	8.899 ± 0.572(56)	5.880 ± 0.482(37)
	FB	10.990 ± 0.504(100)	8.792 ± 0.566(80)	6.814 ± 0.414(62)	4.506 ± 0.299(41)
RNA/ DNA ratio	HLP	1.505(100)	1.365(91)	1.123(75)	0.831(55)
	FB	1.717(100)	1.569(91)	1.428(83)	1.117(65)
GN	HLP	2.298 ± 0.076(100)	1.700 ± 0.068(74)	1.080 ± 0.058(47)	0.505 ± 0.036(22)
	FB	14.228 ± 0.815(100)	9.248 ± 0.438(65)	6.118 ± 0.043(43)	3.577 ± 0.041(25)
RS	HLP	2.701 ± 0.088(100)	3.646 ± 0.106(135)	4.807 ± 0.211(178)	5.348 ± 0.204(198)
	FB	1.061 ± 0.068(100)	1.411 ± 0.062(133)	1.782 ± 0.081(168)	2.228 ±0.094(210)
ACP	HLP	0.572 ± 0.044(100)	1.052 ± 0.065(184)	1.418 ± 0.060(248)	2.373 ± 0.116(415)
	FB	2.502 ± 0.176(100)	3.653 ± 0.147(146)	5.829± 0.176(233)	8.231 ± 0.199(329)
ALP	HLP	0.458 ± 0.026(100)	0.338 ± 0.024(74)	0.252± 0.019(55)	0.156 ± 0.012(34)
	FB	2.468 ± 0.136(100)	2.171 ± 0.176 (88)	1.728 ± 0.164(70)	1.110 ± 0.091(45)

*Significant (P<0.001), (P<0.01) and (P<0.05) when Student's t test was applied between treated and control groups. Values are means ± SE of six replicates. Values given in the parentheses are percent values with control taken as 100%. Values are expressed as: µg/mg for TP, TFA, DNA and RNA, RNA/DNA ratio; mg/g for GN and RS; and µ moles/mg for ACP and ALP.

Discussion

The present investigation showed that different dose levels of bioresmethrin exerted a depressive effect on the life-cycle stages of *Corcyra cephalonica*. The toxicity of bioresmethrin increases significantly with the increase in its concentration on each developmental stage i.e. larva, pupa and adult (Table 2, Fig. 1). On the basis of % larval death, % pupation, % pupal death and % adult emergence at different dose levels of bioresmethrin, it is possible to categorize the relative effectiveness of their dose levels (Fitzpatrick and Dowell, 1981). Bioresmethrin at 0.0008% dose level may be considered to be extremely toxic to the pest as no pupation occurred at this dose level indicating 100% larval mortality. At dose level 0.0007% of bioresmethrin very poor pupation took place but all the pupae get perished and hence, there was no emergence of any single adult. This dose level may be regarded as extremely severe to the pest. At dose levels 0.0006% and

0.0005% of bioresmethrin the average emergence was 12 ± 2.82 and 23 ± 5.21%, respectively. These dose levels may be regarded to be severely toxic to the pest. A moderately severe toxicity is accounted to the dose levels of 0.0004% and 0.0003% of bioresmethrin as the average emergence at these dose levels was 37 ± 3.34 and 56 ± 4.00%, respectively. At 0.0002% dose level of bioresmethrin the average emergence was 66 ± 2.19%. This dose level may be regarded as moderately toxic to the pest. At dose level 0.0001% of bioresmethrin the average emergence was 80 ± 2.82%. This dose level may be considered as poorly toxic to the pest.

It deserves mention that, in the present investigation, at the highest dose level of bioresmethrin (0.0008%), which caused 100% larval mortality, the larval food consumption was observed to be very poor. This observation indicates that at this highest dose level the toxicity is considered to be mostly due to contact toxicity rather than oral/stomach toxicity. It further deserves mention that at 0.0007% dose level of bioresmethrin, though, the larval mortality was very high, yet some of the larvae pupate but all of them get perished and hence no adult emergence occur. Pupation at this dose level, ofcourse, justify that larval feeding (still poor) occurs at this dose level. Hence, it may be concluded that larval/pupal toxicity at this dose level may be due to a joint action i.e. contact as well as oral/stomach toxicity of the bioresmethrin. It may also be concluded that active compounds present in bioresmethrin are apolar enough to penetrate the exterior cuticle and also water soluble enough to be carried by the haemolymph throughout the interior of the insect to its site of action. Similar explanation for the toxicity of insecticides has been reported in case of *Calendra granaria* (Armstrong et al., 1951).

The pyrethroids (bioresmethrin) share similar mode of action, resembling to that of DDT and are considered as axonic poisons but their stimulating effect is much more pronounced than that of DDT. They apparently work by keeping open the sodium channels in neuronal membranes and affect both the peripheral and central nervous system of the insect. They initially stimulate nerve cells to produce repetitive discharges and eventually cause paralysis. Such effects are caused by their action on the sodium channel, a tiny hole through which sodium ions are permitted to enter the axon to cause excitation resulting into hyperactivity, tremors, convulsions, paralysis and finally death of the insect (George and Whitacre, 2004). Thus, it may be concluded that bioresmethrin, in the present investigation, disturbs the permeability of sodium across the nerve membrane which ultimately results into hyperactivity, tremors, convulsions, paralysis and death.

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 (Lee et al., 1981). 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 (Shigematsu, 1960), which, in later instars are sequestered from the blood into the fat body. Regarding their synthesis Simmon and Mitchell (1962) have suggested that in *Drosophila* amino acids are first incorporated into peptides and later enter into proteins (Weinmann, 1964).

In the present investigation, all the three sublethal doses of bioresmethrin caused a dose dependent ($P < 0.001$) reduction in the level of total protein in both the tissues of the larva. Earlier investigations have revealed that synthetic insecticides, botanical insecticides (natural plant products/biopesticides) and synthetic pyrethroids influence the biochemistry of insect pests. Bhagawan et al. (1992) have reported that application of *Annona squamosa* seed extracts caused a significant reduction in protein content in the nymphs of *Dysdercus koenigii*. In a similar way, *Polyscias quilfolei* extracts (Rajendra and Gopalan, 1982) and azadirachtin (Subramanyam and Rao, 1986) have also been reported to cause significant alteration in protein contents in certain other insects. The present results are also in agreement with findings of above investigators. Synthetic insecticides have also been shown to decrease the total protein level in insects by inhibiting amino acid incorporation into protein causing adverse effect on protein biosynthesis (Agosin et al., 1965; Molchanov et al., 1980; Lee et al., 1981). Organochlorine and organophosphate insecticides induced reduction in total protein level and associated enhancement in total free amino acid level have already been reported in haemolymph and fat body tissues of the larva of this pest (Tiwari and Bhatt, 1987b; 1989; 1996) and in gonadal tissues of this adult moth (Tiwari and Bhatt, 1994e).

Protein synthesis in the insect fat body is also inharently regulated by endocrine secretions. β-ecdysone stimulated the protein synthesis (*in vitro*) in the larval fat body of *Calliphora stygia* (Neufeld, Thompson and Horn, 1968) and α- ecdysone stimulated the same in oak worm, *Antheraea pernyi* (Sahota and Mansingh, 1970). Similarly, juvenile hormone enhanced the rate of protein synthesis in larval fat body of the milkweed bug *Oncopeltus fasciatus* (Bassi and Feir, 1971). Thus, it may be concluded that these plant insecticides might affect the endocrine secretion in addition to amino acid incorporation into protein, resulting into poor protein biosynthesis which ultimately leads to reduced level of protein in haemolymph as well as in fat body.

One of the most characteristic features of insect haemolymph is the high level of free amino acids (Buck, 1953; Florkin, 1959; Gilmour, 1961, 1965; Wyatt, 1961; Clements, 1963; Chen, 1966; Florkin and Jeuniaux, 1974) whereas insect fat body is an active site for the intermediary metabolism of these amino acids (Kilby, 1963; Chen, 1966). The high concentration of free amino acid is believed to play an important role in osmoregulation (Bishop et al., 1926; Beadle and Shaw, 1950) buffering of the blood to some extent, energy production for flight and cocoon construction (Wyatt, 1961) with the predominant function of serving as units for protein synthesis (Buck, 1953) and taking part in other metabolic activities.

In the present investigation, bioresmethrin caused a dose dependent ($P < 0.001$) enhancement in the level of total free amino acids in both the tissues of the larva. Reddy et al. (1993) have reported that active compounds extracted from seed of *Annona squamosa* has enhanced the amino acid content in *Dysdercus koenigii* possibly due to depletion of protein and/or inhibition of amino acid incorporation into protein. Similarly, Vijayaraghavan and Chitra (2002) have reported botanical insecticides induced alterations in the free amino acid contents of *Spodoptera litura*. Contrary to the present finding, synthetic insecticide malathion caused a dose depedent reduction in free amino acid level in haemolymph of *Dysdercus koenigii* (Singh, 1982) and

Blattella germanica (Mansingh, 1965). Similarly, in the haemolymph of DDT poisoned cockroaches the amino acid concentrations varied inversely with increase in toxicity (Corrigan and Kearns, 1963). Agosin et al. (1965) have reported that higher DDT concentrations inhibited amino acid incorporation into protein causing adverse effect on protein biosynthesis. Since, *Azadirachta indica* seed's ethanol and acetone extracts, its stem bark and leaf powders and bioresmethrin, in the present study, decreased the protein level in the haemolymph and the fat body of the larva of this moth as stated earlier, it may be concluded that a rise in the total free amino acid level in both the tissues is plausibly on account of protein depletion and/or inhibition of amino acid incorporations into protein.

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 (Brachet, 1955; Lang et al., 1965).

Insecticides have shown to alter the nucleic acid levels (Bhunya and Das, 1976; Tayyaba et al., 1976; Tiwari and Bhatt, 1987). Literatures concerning botanical insecticides and pyrethroid induced changes in the nucleic acid levels with special reference to insects (Shakoori et al., 1988; Naqvi et al., 1991b; Tabassum, 1994) are adequate.

In the present investigation, sublethal doses of bioresmethrin caused a dose dependent ($P < 0.01$) reduction in the levels of DNA and RNA and a significant reduction in RNA / DNA ratio, in both the tissues of the larva of this pest. Synthetic insecticides have been reported to be a strong inhibitor of DNA and RNA (Bhunya and Das, 1976; Tayyab et al., 1976) and protein synthesis (Harvey and Sharma, 1978). Pesticides induced DNA damage has also been reported in human cell culture (Ahmad et al., 1977). Botanicals (neem compounds) and pyrethroid have been shown to inhibit the nucleic acid level in *Musca domestica* (Naqvi et al., 1991). Similar findings have also been observed in pulse beetle, *Callosobruchus analis* following treatment with neem compounds NfC (Neutral fraction C- which is a crude extract of whole neem seed) and NC (Nimolicine, Azadirachtin) as reported by Tabassum (1994). But, sublethal and lethal doses of fenpropathrin (a pyrethroid) did not change the RNA and DNA levels in the larvae of *Tribolium castaneum* as reported by Shakoori et al. (1988).

In the present study, the reduction in the DNA and RNA levels, may be due to interference of bioresmethrin with the synthesis site of nucleic acids. Similar explanation for decrease in DNA and RNA contents has also been suggested in fenpropathrin exposed larvae of *Tribolium castaneum* (Shakoori et al., 1988). 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. The present study also demonstrate the reduction in the total protein level in both the tissues of the larva following treatment with bioresmethrin. 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 level is due to the involvement of bioresmethrin in influencing the transport of amino acids as well as their incorporation into the polypeptide chain. The enhancement in total free amino acid level further supports the above presumption.

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, 1964, 1965; Kilby, 1963; Wyatt, 1967; Friedman, 1970).

Results obtained on the carbohydrate level indicate that bioresmethrin caused a dose dependent ($P < 0.05$) decrease in glycogen level and a dose dependent ($P < 0.05$) enhancement in reducing sugar level in haemolymph as well as in fat body of the larva of this pest. A drastic reduction (93.38 %) in the amount of carbohydrates has been reported in *Lippia nodiflora* and *Vitex negundo* extracts poisoned larvae of cabbage leaf webber, *Crocidolomia binotalis* (Vijayaraghavan et al., 2010). They have suggested that under stress conditions, more sugar might be metabolized to meet out the energy expenses. This could be the reason for carbohydrate level depletion in treated insects. Similar results were obtained by Seyoum et al. (2002) in desert locust and by Razak and Sivasubramanian (2007) in *Chelomenus sexmaculata* and *Chrysoperla carnea*. Joshi et al. (1976) have reported a significant reduction in glycogen levels in insect, *Hieroglyphus nigrorepletus* following exposure to dieldrin. Lindane depleted glycogen levels in *Periplaneta americana* (Orr and Downer, 1982) and in desert locust, *Schistocerca gregaria* (Samaranayaka, 1974, 1978) due to insecticide induced release of hyperglycaemic factors from corpus cardiacum. The present findings are in conformity with Vijayaraghavan et al. (2010) and Razak and Sivasubramanian (2007).

A significant decrease in glycogen reserves with a significant enhancement in reducing sugar content observed in this investigation may be ascribed to the decreased activity of glycogen synthetase and/or increased glycogenolysis, perhaps resulting from the enhanced activity of glycogen phosphorylase to encounter bioresmethrin stress.

The depletion in glycogen level may also be due to a direct action of active compounds of bioresmethrin on oxidative phosphorylation as observed in case of *Periplaneta americana* following treatment with lindane (Ela et al., 1970). The observed enhancement in reducing sugar level may be due to gluconeogenesis and/or decreased sugar utilization as noticed in rabbits treated with organophosphorus pesticides like Soman (Stitcher et al., 1975).

Acid phosphatase plays a significant role in catabolism, pathological necrosis, autolysis and phagocytosis (De Duve, 1959; Becker and Barron, 1961 and Abou Donia, 1978). It also helps in energy liberating processes (Dalela et al., 1978). Alkaline phosphatase has been reported to be involved in the transport of metabolites across the membranes (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).

Synthetic insecticides and plant extracts / biopesticides have been reported to influence the activities of acid and alkaline phosphatase (Glees, 1967; Ntiforo and Stein, 1970; Abou Donia, 1978; Galdhar et al., 1978; Mukhopadhyay and Dehadrai, 1980; Shivanandappa and Krishnakumari, 1981; Tiwari and Bhatt, 1987; Naqvi et al., 1991; Josephrajkumar et al., 1999; Akhtar and Islam, 2004; Mannan et al., 2008).

In this study phosphatase activity clearly demonstrate that sublethal doses of bioresmethrin caused a dose dependent ($P < 0.05$) enhancement in acid phosphatase activity and a dose dependent ($P < 0.05$) reduction in alkaline phosphatase activity in both the tissues of the larva. BHC and

malathion induced enhancement in acid phosphatase activity and reduction in alkaline phosphatase activity in haemolymph and fat body of the larva of *C. cephalonica* have already been observed by Tiwari and Bhatt (1987). Similar observation has been reported in ovary of Zebra fish, *Brachydanio rerio* exposed to malathion (Ansari and Kumar, 1987). On the contrary, malathion reduced the acid phosphatase activity in gill as well as in liver and enhanced the alkaline phosphatase activity in intestine of the cat fish *Clarias batrachus* (Mukhopadhyay and Dehadrai, 1980). However, studies pertaining to mode of action of synthetic insecticides on the phosphatase activity in relation to vertebrates have been explored in detail by various workers (Glees, 1967; Ntiforo and Stein, 1970; Abou Donia, 1978; Galdhar et al., 1978; Mukhopadhyay and Dehadrai, 1980; Shivanandappa and Krishnakumari, 1981). Glees (1967) reported increased acid phosphatase activity in nerves and neuroglia of hens following treatment with TOCP possibly due to labilization of lysosomal membranes. This explanation was further supported by Abou Donia (1978) who observed enhancement in the activity of plasma acid phosphatase in hens exposed to leptophos and is also in accordance with the suggestions of Ntiforo and Stein (1970) who showed interaction of anticholinesterase pesticide (malathion) with the structural components of lysosomal membrane altering its permeability. Similar reasons may be assigned for the enhancement in the activity of acid phosphatase in the present study.

Bioresmethrin induced decrease in the activity of alkaline phosphatase, in the present investigation, resembles to that of plumbagin and azadirachtin exposed *Helicoverpa armigera* (Josephrajkumar et al., 1999), NfD exposed *Sitophilus oryzae* (Naqvi et al., 1991), *Fagonia bruguieri* induced *Schistocerca gregaria* (Basiouny et al., 2010) and *Artemisia annua* exposed *Eurygaster intefriceps* (Zibaee and Badani, 2010). But these studies have no relevant explanation regarding the mode of action of plant extracts/biopesticides about the activity of alkaline phosphatase. The reduced activity of alkaline phosphatase in the present investigation also resembles to that of rats exposed to Disulfaton (Galdhar et al., 1978) and BHC (Shivanandappa and Krishnakumari, 1981). The decrease in the activity of this enzyme may be due to fall in pH following rupture of cell membranes as suggested by Mukhopadhyay and Dehadrai (1980) in case of cat fish, *Clarias batrachus* under malathion stress. A reduction in alkaline phosphatase activity during *in vivo* treatment of bioresmethrin may be due to the interaction of several reactions occurring simultaneously, causing direct or indirect stress on the alkaline phosphatase activity in haemolymph and fat body of the larva of *C. cephalonica*.

From the present investigation it may be concluded that 0.0008% dose level of bioresmethrin that caused 100% larval mortality may be utilized for the efficient control of rice-moth, *C. cephalonica* in particular and stored cereal pest population in general.

The entire finding of the present work viz. decreased protein level, increased amino acid titre, and enhanced reducing sugar level may not be only due to reduction in DNA, RNA levels and RNA / DNA ratio but also due to other cellular enzymatic activities such as enhanced acid phosphatase activity and inhibition of alkaline phosphatase activity, and decreased glycogen level which finally leads into metabolic perturbation and consequently biochemical lesion in the bioresmethrin exposed larvae resulting into death.

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