
POTENTIAL OF PYRETHRUM EXTRACT AGAINST LARVAL BIOCHEMISTRY OF RICE-MOTH, *CORCYRA CEPHALONICA* STAIN. (LEPIDOPTERA: PYRALIDAE)

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ABSTRACT: Sub lethal doses of pyrethrum extract caused a significantly dose dependent reduction in the levels of total protein, DNA, RNA, RNA/DNA ratio and a significantly dose dependent enhancement in total free amino acids in haemolymph and fat body tissues of the larva of rice-moth, *C. cephalonica*. 0.0004% dose level of this extract caused maximum effect on these biochemical parameters.

KEYWORDS: Pyrethrum extract, *C. cephalonica*, Haemolymph, Fat body, Biochemistry

INTRODUCTION

The rice-moth, *Corcyra cephalonica* (Staint.) is a notorious pest of stored cereals and cereal commodities in India as well as in other tropical and subtropical regions of the world. Its larval stages cause severe loss to rice, sorghum, currants, gram, milled products, cocoa beans, peanuts, cottonseed, linseed, raisins, chocolates, army biscuits and nutmeg^{3,38,2}. Sufficient knowledge exists on the nutritional and reproductive physiology of this lepidopterous pest^{20,8}, in addition to effects of insecticidal agents such as organochlorines, organophosphates, a few synthetic pyrethroids and botanical insecticides and a few IGRs on the development and larval biochemistry of *C. cephalonica*^{52,53,54,36,33,30,31,32,34,35,48}.

The use of synthetic insecticides pose problems such as poisoning in man and other animals³⁷, pest resistance to pesticides¹², and the risk of contamination causing injury to non-target organisms and pollution to our own environment, thus

disturbing the ecosystem. Hence there is an urgent need to develop safe alternatives to conventional insecticides for the protection of grain and grain products against insect infestations.

Pyrethrum is obtained from flowers of *Chrysanthemum cinerariifolium* harvested shortly after blooming, and either dried or powdered, or the oils within the flowers extracted with solvents. Pyrethrum extract is important in the control of household pests, in barns, stored products, and for direct application to man and livestock. It is generally considered to be the safest insecticide, and hence it has been selected as one of the safer substitutes to control *Corcyra cephalonica* in particular and lepidopterous pests in general.

MATERIALS & METHODS

A standard culture of *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 ± 10 C and $93 \pm 5\%$ relative humidity.

Pyrethrum extract 23.3% (active ingredient) was purchased from Sigma-Aldrich, Steinheim, Germany (Lot. SZE7135X).

Preparation of different dose levels of pyrethrum extract in dietary media, evaluation of toxicity of different dose levels of pyrethrum extract, larval rearing and their treatment for biochemical estimation, separation and collection of haemolymph and fat body tissues has been done⁴⁶. The entire programme of biochemical estimation includes the quantitative measurement of total protein, total free amino acid and nucleic acids in haemolymph and fat body of the larva of rice-moth treated with sublethal doses of pyrethrum extract as well as control.

The total protein was measured according to the method of Lowry et al²⁴. using bovine serum albumin as standard while total free amino acid was estimated according to the method of Spies⁴⁹ using glycine solution as standard.

Deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) levels were estimated according to the method of Schneider⁴³. Diphenylamine reagent was used for DNA estimation while orcinol reagent was used for RNA estimation.

RESULTS AND DISCUSSION

Changes induced by sublethal doses of pyrethrum extract on the levels of total protein and total free amino acids in haemolymph and fat body of the larva of *Corcyra cephalonica* have been represented in Table 1. Pyrethrum extract caused a dose dependent reduction in the level of total protein and an associated enhancement in the total free amino acid level in both the tissues of the larva (Table 1).

In control larval groups, the total protein content in the haemolymph and fat body was 68.879 and 12.886 μ g/mg respectively. The maximum decrease in total protein level in haemolymph (23% of the control value) and fat body (40% of the control value) was observed in larvae treated with 0.0004% dose level of pyrethrum extract. Total protein levels, in haemolymph, were reduced to 74% (50.970 μ g/mg), 55% (37.883 μ g/mg) and 23% (15.842 μ g/mg) of the control value while these levels in fat body were reduced to 88% (11.340 μ g/mg), 57% (7.216 μ g/mg) and 40% (5.154 μ g/mg) of the control value following treatment with 0.0001%, 0.0002% and 0.0004% dose levels of pyrethrum extract respectively (Table 1).

The total free amino acid content, in the control larvae, was 88.580 and 11.729 μ g/mg in haemolymph and fat body respectively. The maximum enhancement in the total free amino acid level in haemolymph (171% of the control value) and fat body (177% of the control value) was observed in larvae treated with 0.0004%

dose level of pyrethrum extract. Total free amino acid levels, in haemolymph, were increased to 109% (96.552µg/mg), 131% (116.040µg/mg) and 171% (151.471µg/mg) of the control value while these levels, in fat body, were increased to 144% (16.890µg/

mg), 161% (18.883µg/mg) and 177% (20.760µg/mg) of the control value following treatment with 0.0001 %, 0.0002 % and 0.0004 % of pyrethrum extract respectively (Table 1).

Table 1. Changes in the total protein and total free amino acid levels in the haemolymph and fat body of the larva of rice-moth, *C. cephalonica* treated with Pyrethrum extract

Percent pyrethrum extract concentration	Total protein# (mg/g. wet wt.)		Total free amino acid# (µg/ mg, wet wt.)	
	Hamolymph	Fat body	Haemolymph	Fat body
Control (untreated)	68.879 ± 2.110 (100)	12.886± 0.564 (100)	88.580 ± 3.440 (100)	11.729 ± 1.106 (100)
0.0001	50.970 ± 1.994 (74)	11.340 ± 0.676 (88)	96.552 ± 3.016 (109)	16.890 ± 0.710 (144)
0.0002	37.883 ± 1.806 (55)	7.216 ± 0.414 (57)	116.040 ± 4.201 (131)	18.883 ± 0.742 (161)
0.0004	15.842 ± 0.742 (23)	5.154 ± 0.226 (40)	151.471 ± 4.016 (171)	20.760 ± 1.004 (177)

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%.

Student's t-test showed significant differences (P < 0.05 to P < 0.001) between the corresponding treated groups and the controls.

Analysis of variance showed that the response to the pyrethrum extract was dose dependent P < 0.001.

A dose dependent reduction in the levels of deoxyribonucleic acid and ribonucleic acid was recorded in haemolymph and fat body tissues of the larva following treatment with pyrethrum extract (Table 2).

In the control larval groups, the deoxyribonucleic acid level was 10.531 and 6.572µg/mg in haemolymph and fat body respectively. The 0.0004% dose level of pyrethrum extract caused a maximum

reduction in the level of DNA which was 51% in haemolymph and 41% in fat body with respect to their control values. DNA levels, in haemolymph, were reduced to 80% (8.425µg/mg), 61% (6.424µg/mg), and 51% (5.371µg/mg) of the control value while these levels, in fat body, were reduced to 78% (5.126µg/mg), 62% (4.075µg/mg) and 41% (2.695µg/mg) of the control value following treatment with 0.0001%, 0.0002% and 0.0004% dose level of pyrethrum

extract respectively. It is also revealed that sublethal doses of pyrethrum extract caused a sharp reduction in DNA levels of fat body in comparison to those of haemolymph (Table 2).

The ribonucleic acid level, in control larvae, was 15.814 and 10.989 $\mu\text{g}/\text{mg}$ in haemolymph and fat body respectively. Larvae treated with 0.0004% dose level of pyrethrum extract showed a maximum reduction (34% in haemolymph

and 32% in fat body of their control values) in the level of RNA. RNA levels, in haemolymph, were reduced to 79% (12.493 $\mu\text{g}/\text{mg}$), 55% (8.698 $\mu\text{g}/\text{mg}$) and 34% (5.377 $\mu\text{g}/\text{mg}$) of the control value while these levels, in fat body, were decreased to 75% (8.242 $\mu\text{g}/\text{mg}$), 54% (5.934 $\mu\text{g}/\text{mg}$) and 32% (3.516 $\mu\text{g}/\text{mg}$) of the control value following treatment with 0.0001%, 0.0002% and 0.0004% dose levels of pyrethrum extract respectively (Table 2).

Table 2. Changes in the DNA and RNA levels in the haemolymph and fat body of the larva of rice-moth, *C. cephalonica* treated with Pyrethrum extract

Percent pyrethrum extract concentration	DNA# (mg/g. wet wt.)		RNA# ($\mu\text{g}/\text{mg}$, wet wt.)	
	Haemolymph	Fat body	Haemolymph	Fat body
Control (untreated)	10.531 \pm 0.441 (100)	6.572 \pm 0.411 (100)	15.814 \pm 0.612 (100)	10.989 \pm 0.401 (100)
0.0001	8.425 \pm 0.467 (80)	5.126 \pm 0.369 (78)	12.493 \pm 0.565 (79)	8.242 \pm 0.364 (75)
0.0002	6.424 \pm 0.411 (61)	4.075 \pm 0.255 (62)	8.698 \pm 0.511 (55)	5.934 \pm 0.312 (54)
0.0004	5.371 \pm 0.402 (51)	2.695 \pm 0.211 (41)	5.377 \pm 0.301 (34)	3.516 \pm 0.214 (32)

Values are expressed as the mean \pm s.e. of six replicates.

Values in the parentheses indicate the percentage change, with control values taken as 100%. Student's t-test showed significant differences ($P < 0.05$ to $P < 0.001$) between the corresponding treated groups and the controls.

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

Sub lethal doses of pyrethrum extract caused a dose dependent reduction in RNA/DNA ratio in both the tissues of the larva. In control larvae, the RNA/DNA ratio, which is an index of the protein synthetic capacity per cell, was 1.502 and

1.658 in haemolymph and fat body respectively. The maximum decrease in this ratio in haemolymph (67% of the control value) and fat body (79% of the control value) was observed in larvae treated with 0.0004% dose level of pyrethrum extract.

RNA/DNA ratios, in haemolymph, were reduced to 99% (1.483µg/mg), 90% (1.354 µg/mg) and 67% (1.001µg/mg) of the control value while these values, in fat body, were reduced to 97% (1.608µg/mg), 88%

(1.456µg/mg) and 79%(1.305µg/mg) of the control value following treatment with 0.0001%, 0.0002% and 0.0004% dose levels of pyrethrum extract respectively (Table 3).

Table 3. Alterations in the RNA/DNA ratio in haemolymph and fat body of the larva of rice-moth, *C. cephalonica* treated with Pyrethrum extract

Percent pyrethrum extract concentration	RNA / DNA ratio (mg/g. wet wt.)	
	Hamolymph	Fat body
Control (untreated)	1.502 (100)	1.658 (100)
0.0001	1.483 (99)	1.608 (97)
0.0002	1.354 (90)	1.456 (88)
0.0004	1.001 (67)	1.305 (79)

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

The present investigation, reveals Pyrethrum extract (a biopesticides) induced changes in the levels of total protein, total free amino acid, DNA, RNA, and RNA/DNA ratio in the haemolymph and fat body tissues of the larva, pertaining to a specific age group, of the rice-moth, *Corcyra cephalonica*. The findings are discussed here in the light of the insecticidal influence of pyrethrum extract 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

dividing ways and means for the effective control of this lepidopterous pest.

Studies, regarding mode of action of botanicals, have revealed that active ingredients present in the insecticidal plants inhibit some of the enzyme systems and subsequent mortality^{13,21,29,40}. Active ingredients of leaves of *Calotropis* and *Ipomea* have been reported to inhibit some of the enzyme systems and subsequent mortality^{21,29}. Active ingredients of *Ocimum* had also been known to act as a reversible competitive inhibitor of acetyl cholinesterase⁴⁰ like azadirachtin, abundantly present in the neem seeds that

have been shown to inhibit the release of prothoracicotropic hormones and allatropins⁴, thereby affecting metamorphosis in insect⁴² is well documented.

Active compounds of Pyrethrum extract have a quick knock down effect against insects. They act on tiny channels through which sodium is pumped to cause excitation of neurons. They prevent sodium channels from closing resulting in continual nerve impulse transmission, tremors, and eventually death. Thus, it may be concluded that pyrethrum extract, in the present investigation, disturbs the permeability of sodium across the nerve membrane causing body convulsions which ultimately leads to 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 metamorphosis²³. 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 blood⁴⁵, which, in later instars are sequestered from the blood into the fat body. Regarding their synthesis⁴⁷ in *Drosophila* amino acids are first incorporated into peptides and later enter into proteins⁵⁶.

In the present investigation, all the

three sublethal doses of Pyrethrum extract (Table 1) caused a significant dose dependent ($P < 0.001$) reduction in the level of total protein in both the tissues of the larva. Earlier investigations have revealed that botanical insecticides (natural plant products / biopesticides) influence the biochemistry of insect pests. Application of *Annona squamosa* seed extracts caused a significant reduction in protein content in the nymphs of *Dysdercus koenigii*⁷. In a similar way, azadirachtin⁵⁰ has 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 workers. 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^{25,23,1}.

Protein synthesis in the insect fat body is also inherently regulated by endocrine secretions. $\hat{\alpha}$ -ecdysone stimulated the protein synthesis (in vitro) in the larval fat body of *Calliphora stygia*²⁸ and $\acute{\alpha}$ -ecdysone stimulated the same in oak worm *Antheraea pernyi*⁴¹. Similarly, juvenile hormone enhanced the rate of protein synthesis in larval fat body of the milkweed bug *Oncopeltus fasciatus*⁵. 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^{11,17,18,57,15,14,16} whereas insect fat body is an active site for the intermediary metabolism of these amino acids^{19,14}. The high concentration of free amino acid is believed to play an important role in osmoregulation^{9,6} buffering of the blood to some extent, energy production for flight and cocoon construction⁵⁷ with the predominant function of serving as units for protein synthesis¹¹ and taking part in other metabolic activities.

In the present investigation, all the three sublethal doses of pyrethrum extract (Table 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. 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 this biopesticide induced depletion of protein and / or inhibition of amino acid incorporation into protein³⁹. Similarly, botanical insecticides have been reported to induce alterations in the free amino acid contents of *Spodoptera litura*⁵⁵. Thus, in the present investigation, it may be concluded that a rise in the total free amino acid level in both the tissues of the larva is plausibly on account of protein depletion and / or inhibition of amino acid incorporations in to 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^{10,22}. Literatures concerning botanical insecticides and pyrethroid induced changes in the nucleic acid levels with special reference to insects^{27,51} are far from adequate.

In the present investigation, all the three sublethal doses of pyrethrum extract (Table 2) caused a significantly dose dependent ($P < 0.01$) reduction in the levels of DNA and RNA and a significant reduction in RNA / DNA ratio (Table 3) in both the tissues of the larva of this pest. Botanicals (neem compounds) and pyrethroid have been shown to inhibit the nucleic acid level in *Musca domestica* L.²⁶. Similar findings have also been observed in case of pulse beetle, *Callosobruchus analis*⁵¹ L. following treatment with neem compounds NfC (Neutral fraction C- which is a crude extract of whole neem seed) and NC (Nimocilin, Azadirachtin). But, sublethal and lethal doses of fenpropathrin (a pyrethroid) did not change much of the RNA and DNA levels in the larvae of *Tribolium castaneum* (Herbst.)⁴⁴.

The reduction in the DNA and RNA levels, in the present study, may be due to interference of pyrethrum extract with the synthesis site of nucleic acids. This finding is in accordance with Tabassum, (1994) who reported similar possibility of the decreased DNA and RNA due to the action of neem compounds NfC and NC (Nimocilin,

Azadirachtin) on nucleic acids synthesis in the exposed pulse beetle, *Callosobruchus analis* (L.)⁵¹. Similar explanation for decrease in DNA and RNA contents have also been reported in fenpropathrin exposed larvae of *Tribolium castaneum* (Herbst.)⁴⁴. 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 larva following treatment with pyrethrum extract. 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 pyrethrum extract 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.

On the basis of overall findings, in the present investigation, it may be concluded that pyrethrum extract disrupts the metabolic framework of the larva hence, evolution of a new generation of this pest for the eventual establishment on stored cereals and cereal products can be considerably restricted.

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