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Insecticidal Action of *Dryopteris filix*-mas (Linn.) Schott Against Larval Biochemistry of Rice Moth *Corcyra cephalonica* Staint. (Lepidoptera: Pyralidae)

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Abstract: Third instar larvae of rice-moth, *Corcyra cephalonica* were exposed to sub-lethal doses (0.25, 0.50 and 1.00 %) of *Dryopteris filix*-mas root and rhizome's powder in order to evaluate its potential effects on the larval haemolymph and fat body biochemistry of this pest. Results revealed that sublethal doses of this botanical insecticide caused a significantly dose-dependent alteration in the metabolic flux that impairs the physiological fitness of the larva and contributes to the lethal action of this biopesticide.

Keywords: *Corcyra cephalonica*, *Dryopteris filix*-mas, Haemolymph, Fat body,Biochemistry

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Introduction

The control of insect pests is a puzzling problem since many decades. It is estimated that the insects pull down 5 to 6 per cent of the grains of the world's produce. However, 60-70% of the total production are retained by the farmers for their own food, cattle feed, seed etc., and they generally store their grains in traditional storage structures where maximum loss occur that require intensive care of pest management. The damage to the stored products could cause weight loss, detriment in quality and reputation. The loss due to a single larva may be small, only a few milligrams, but with populations measured in millions this would be a remarkable amount.

The protection of stored products by the use of plant materials is a common practice among small scale farmers in tropical and subtropical regions of the world. Repellents, antifeedants and insecticidal substances have been identified in a large variety of plant species, long before the "industrial insecticide revolution" in the 1930's and 1940 when compounds such as nicotine, derris and

pyrethrum were the only effective insecticides (Green *et al.*, 1979). Azadirachtin, a component of *Azadirachta indica* A. Juss, and *Melia azedarach* L. (Meliaceae), is considered as promising alternative to synthetic insecticides (Jotwani and Srivastav, 1981; Ivbijaro, 1983; Jilani, 1983). Studies on natural plant products (botanicals) have revealed that they play a significant role in the control of a variety of stored cereal pests of different orders. They function as ovicidal, larvicidal, antifeedants, repellents, toxicants, protectants, deterrents and insecticidal.

Earlier findings reveal that the rhizome and young shoots (fiddleheads) of the male fern *Dryopteris filix*-mas (Synonyms-*Nephrodium filix*-mas L. Strempel; and *Polypodium filix*-mas L.) have deworming properties that have long been recognized in Europe against tapeworms (Taenia). The ferns are effective in arresting embryonic development in insects. The extracts of pteridophytes have toxic effects on *Spodoptera littura* and *Helicoverpa armigera*. About 1.5 to 2.5% filicin (a polyketide compound) is found in the root and rhizomes of *D. filix*-mas (Chopra *et al.,* 1986). Filicin, which is isolated from the rhizomes of *Dryopteris filix-mas,* is a potential insecticide Mannan *et al.* (2008). Filicinic acid derivatives (well known in *Dryopteris*) possess various biological activities like active antioxidants, antibacterial and antitumor promoting activities and may lead to therapeutic applications of great interest (Widen *et al.*, 2001; Magalhaes *et al.*, 2010).

Numerous investigations have shown that like synthetic insecticides, botanicals/plant extracts and pyrethroids affect the biochemical constituents of various tissues in insects. Insecticidal influence of plant extracts on free amino acids have been explored by Reddy *et al.* (1993) and Vijayaraghavan and Chitra (2002). Such influence of plant extracts on the total protein content have been observed by Gordon and Burford (1984); Subramanyam and Rao (1986); Young and Gordon (1987); Bhagwan *et al.* (1992); Reddy *et al.* (1993); Ramakoteswara Rao *et al.* (1995); Vijayaraghavan and Chitra (2002) and Brisca and Sahayaraj (2009). Similarly changes in carbohydrates levels concerning plant extracts have been reported by Olga *et al.* (2006); Razak and Sivasubramanian, (2007) and Vijayaraghavan *et al.* (2010). Changes concerning nucleic acids biochemistry influenced by plant compounds have been reported by Shakoori *et al.* (1988); Naqvi *et al.* (1991 b) and Tabassum (1994). 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). Plant products/biopesticides induced changes in the biochemical constituents of various tissues of insects may be regarded as one of the objective criteria permitting an assessment of the effectiveness of botanical/ biopesticides control measures against *C.cephalonica* in particular and insect pests population in general.

Persistent use of synthetic organic insecticides affect immune system of insects, develop resistance (Ramesh and Pratap, 1997) and of course pollute our own environment due to non-biodegradability, cause biomagnification and toxicity to non-

target organisms 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 also pose problems such as poisoning in man and other animals (Pichaet and Philongene, 1993). Thus, there is an urgent need to use botanicals/ biopesticides as safer alternatives to synthetic insecticides for the protection of grain and grain products against insect infestations.

Scientific contribution concerning role of natural plant products and synthetic pyrethroids as safer insecticidal agents influencing life-cycle stages as well as biochemistry of rice moth, *C. cephalonica* have been explored by Tiwari and Tripathi (2006), Pathak and Tiwari (2010a, 2012b), Shukla andTiwari (2011a, 2011b, 2011c), Pathak and Tiwari (2012), Shukla and Tiwari (2012, 2018, 2019) and Pathak and Tiwari (2015a, 2015b, 2016, 2017a, 2017b, 2017c). But, the effect of *Dryopteris filix*- mas L. (Family-Filicales) root and rhizome powder on the heamolymph and fat body larval biochemistry of this pest is completely wanting. Hence, as an objective of such programme the present research work has been designed and carried out to examine into the impact of this natural plant product 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.* This knowledge, in turn, is likely to generate new insights into divising ways and means for controlling *C. cephalonica*, by disrupting its 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 adults were collected from Biological Control Station, Gorakhpur, U.P. A rich standard culture of this insect was maintained in the laboratory 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 26 ± 1 C and 93 ± 5% relative humidity.

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) for hatching.

Dryopteris filix-mas root and rhizome's powder was used throughout this investigation. *Dryopteris filix*-mas (Family-Filicales) plants were collected from adjacent areas in Gorakhpur and its neighbour districts, Uttar Pradesh, India and identified by Professor V. N. Pandey, Department of Botany, D.D.U.Gorakhpur University, Gorakhpur (U.P.) and Dr. H.C. Pandey, Deputy Director, Botanical Survey of India, Dehradun and specimens were deposited in the herbarium.

Fresh collected root and rhizome of *D. filix*-mas was washed with fresh tap water, cut into small pieces, cooked in boiling water for more than one hour to destroy the thiaminase,

dried in sun light for six to seven days, pulverized in a mortar and pestle and then it was ground in an electric grinder. The powder so obtained was used for biochemical investigations.

D. filix-mas root and rhizome powder was directly mixed with the normal food to prepare its different dose levels. For control purposes, the normal food was used.

For biochemical estimations, out of various dose levels of this biopesticide mentioned above only such doses were selected which allowed the larvae to survive and develop (Shukla and Tiwari, 2011 b) but caused considerable effect in the internal biochemistry of the larva that could be easily detected and assessed to prove the effectiveness of *D. filix*-mas root and rhizome as biopesticidal control measures against this lepidopterous pest.

For biochemical estimation, freshly hatched larvae were allowed to feed on a normal dietary medium (kept inside 250 ml beakers) for 15 days. On the 16th day, 25 larvae were transferred to each similar rearing chambers containing dietary medium mixed with 0.25, 0.50 and 1.00% of *D. filix*-mas powder and were allowed to feed for 10 days. 25 larvae were also kept as control with each set of experiment. 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 follow:

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 (12 mm diameter; 55 mm height). For each biochemical estimation, after ascertaining 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 absorbant 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.

The entire programme of biochemical estimation includes the quantitative measurement of total protein, total free amino acid, nucleic acids and carbohydrate levels and activity of acid and alkaline phosphatases in haemolymph and fat body tissues of the larva of rice-moth treated with sublethal doses of *Dryopteris filix*-mas powder as well as control.

The total protein was measured according to the method of Lowry *et al.* (1951) using bovine serum albumin as standard and results were expressed as µg protein/mg wet weight of tissues.

Estimation of total free amino acid was carried out according to the method of Spies (1957) using glycine solution as standard and results were expressed as µg free amino acid/ mg wet weight of tissues.

Deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) levels were estimated according to the method of Schneider (1957). Diphenylamine reagent was used for DNA estimation while orcinol reagent was used for RNA estimation and their values were expressed as µg/mg wet weight of tissues.

Glycogen and reducing sugar were estimated according to the method of Van der Vies (1954) and Folin Wu (1920), respectively. Anthrone reagent was used for glycogen estimation while for glucose estimation, alkaline copper reagent and phosphomolybdic acid reagent were used and their values were expressed as mg/g wet weight of tissues.

Acid and alkaline phosphatase activity in haemolymph and fat body was determined according to the method of Andersch and Szcypinski (1947) as modified by Bergmeyer (1967) using p-nitrophenylphosphate as substrate. The activities of acid and alkaline phosphatases were expressed as µ moles of p-nitrophenol liberated/30 minutes/mg protein.

Results have been expressed as the mean ± SE of six replicates. Significant differences between treatment groups, in order to show dose dependence, were determined by one way analysis of variance ($P < 0.05$ to $P < 0.001$) (Sokal and Rohlf, 1969). Student's t-test was applied to determine the significant differences between the corresponding treated groups and the controls ($P < 0.05$ to $P < 0.001$) (Sokal and Rohlf, 1969).

Results

Alterations caused by sublethal doses of *Dryopteris filix*-mas root and rhizome powder on the levels of total protein and total free amino acids in the haemolymph and fat body tissues of the larva of rice-moth, *C. cephalonica* have been represented in Table 1. *Dryopteris filix*-mas powder caused a significantly dose-dependent ($P< 0.001$) reduction in the level of total protein and a significantly dose-dependent ($P< 0.001$) enhancement in the level of total free amino acids in haemolymph as well as in fat body. The maximum decrease in total protein level in haemolymph (29% of the control value) and fat body (46% of the control value) was observed in larvae treated with 1.00% dose level of *Dryopteris filix*-mas powder. Protein levels, in haemolymph, were reduced to 80% (55.141 µg/mg), 61% (42.044 µg/mg) and 29 % (19.988 µg/mg) of the control value while these levels in fat body, were reduced to 90% (11.483 µg/mg), 61% (7.783 µg/mg) and 46% (5.869 µg/mg) of the control value following treatment with 0.25, 0.50 and 1.00% dose levels of *Dryopteris filix*-mas powder, respectively (Table 1).

Larvae treated with 1.00% dose level of *Dryopteris filix*-mas powder showed a maximum enhancement in the total free amino acid level in haemolymph (161% of the control) and fat body (168% of the control). Total free amino acid levels, in haemolymph, were increased to 103% (91.201 µg/mg), 126% (111.567 µg/mg) and 161% (142.557 µg/mg) of the control while these levels, in fat body, were increased to 135% (15.779 µg/mg), 151% (17.649 µg/mg) and 168% (19.636 µg/mg) of the control following treatment with 0.25, 0.50 and 1.00% of

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 *Dryopteris filix*-mas root and rhizome powder

Per cent *D. filix*-mas dose	Total protein # (µg/ mg, wet wt.)		Total free amino acid # (µg/ mg, wet wt)	
	Haemolymph	Fat body	Haemolymph	Fat body
Control (untreated)	68.926 ± 1.614 (100)	12.759 ± 0.411 (100)	88.545 ± 3.025 (100)	11.688 ± 1.032 (100)
0.25	55.141 ± 1.640 (80)	11.483 ± 0.401 (90)	91.201 ± 3.614 (103)	15.779 ± 0.841 (135)
0.50	42.044 ±1.166 (61)	7.783 ± 0.241 (61)	111.567 ± 3.104 (126)	17.649 ± 0.688 (151)
1.00	19.988 ± 0.342 (29)	5.869 ± 0.125 (46)	142.557 ± 4.110 (161)	19.636 ± 0.826 (168)

Values are expressed as the mean ± SE 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 *D. filix*-mas powder was dose-dependent ($P< 0.001$).

Dryopteris filix-mas powder, respectively (Table 1).

All the sublethal doses of *Dryopteris filix*-mas powder caused a significantly dose-dependent ($P< 0.01$) reduction in the DNA and RNA levels in both the tissues of the larva (Table 2). Larvae treated with 1.00% dose level of *Dryopteris filix*-mas powder showed a maximum decrease in the DNA level in haemolymph (69% of the control value) and fat body (59% of the control value). DNA levels, in haemolymph, were reduced to 90% (9.485 µg/mg), 81% (8.536 µg/mg) and 69% (7.272 µg/mg) of the control value while these levels, in fat body, were reduced to 89% (5.792 µg/mg), 73% (4.751 µg/mg) and 59% (3.839 µg/mg) of the control value following the treatment with 0.25, 0.50 and 1.00% of *Dryopteris filix*-mas powder respectively, (Table 2).

The maximum decrease in RNA level in haemolymph, (49% of the control value) and

fat body (46% of the control value) was observed in larve treated with 1.00% of *Dryopteris filix*-mas powder. RNA levels, in haemolymph, were reduced to 85% (13.335 µg/mg), 65% (10.197 µg/mg) and 49% (7.687 µg/mg) of the control value while these levels, in fat body, were reduced to 84% (9.145 µg/mg), 62% (6.749 µg/mg) and 46% (5.008 µg/mg) of the control following treatment with 0.25, 0.50 and 1.00% of *Dryopteris filix*-mas powder, respectively (Table 2).

The RNA/DNA ratio, in control larvae, was 1.489 in haemolymph and 1.673 in fat body. The maximum decrease in this ratio in haemolymph (71% of the control value) and fat body (78% of the control value) was observed in larvae treated with 1.00% of *Dryopteris filix*-mas powder. The RNA/DNA ratios, in haemolymph, were reduced to 94% (1.406 µg/mg), 80% (1.195µg/mg) and 71% (1.057 µg/mg) of the control while these ratios in fat body, were reduced to 94% (1.579 µg/mg), 85% (1.421 µg/mg) and 78% (1.305 µg/mg) of the control value following treatment with 0.25, 0.50 and 1.00% of *Dryopteris filix*-mas powder, respectively (Table 3).

Sublethal doses of *Dryopteris filix*-mas powder caused a significantly dose-dependent ($P < 0.05$) reduction in the level of glycogen and on the other hand, a significantly dose-dependent ($P < 0.05$) enhancement in the level of reducing sugar in both the tissues of the larva (Table 4).

In case of untreated larvae, the glycogen level was 2.477 and 14.881 mg/g in haemolymph and fat body, respectively. The maximum decrease in glycogen level in haemolymph (18% of the control value) and fat body (30% of the control value) was observed in larvae treated with 1.00% dose level of *Dryopteris filix*-mas powder. Glycogen levels, in haemolymph, were reduced to 78% (1.932 mg/g), 40% (0.991 mg/g) and 18% (0.446 mg/g) of the control value while these levels, in fat body, were reduced to 67% (9.970 mg/g), 44% (6.548 mg/g) and 30% (4.464 mg/g) of the control value following treatment with 0.25, 0.50 and 1.00% dose levels of *Dryopteris filix*-mas powder, respectively (Table 4).

The level of reducing sugar, in control larvae, was 2.801 and 1.047 mg/g in haemolymph and fat body, respectively. The dose level of 1.00 % *Dryopteris filix*-mas powder caused a maximum enhancement in the amount of reducing sugar which was 188% in haemolymph and 215% in fat body with respect to their control values. The amounts of reducing sugar, in haemolymph, were increased to 127% (3.557 mg/g), 168% (4.706 mg/g) and 188% (5.266 mg/g) of the control value while these levels, in fat body, were increased to 133% (1.392 mg/g), 169% (1.769 mg/g) and 215% (2.251 mg/g) of the control value following treatment with 0.25, 0.50 and 1.00% of *Dryopteris filix*-mas powder, respectively (Table 4).

Changes in acid and alkaline phosphatase activities in haemolymph and fat body of the larva of *C. cephalonica* treated with sublethal doses of *Dryopteris filix*-mas powder have been expressed as µ moles of p-nitrophenol liberated per 30 min per mg of protein and represented in Table 5. *Dryopteris filix*-mas powder caused a significantly dose-dependent ($P < 0.05$) increase in the activity of acid phosphatase and a significantly dose-

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 *Dryopteris filix*-mas root and rhizome powder

Per cent *D. filix*-mas dose	**DNA # (µg/ mg wet wt)**		**RNA # (µg/ mg wet wt)**	
	Haemolymph	**Fat body**	**Haemolymph**	**Fat body**
Control (Untreated)	10.539 ± 0.714 (100)	6.508 ± 0.466 (100)	15.688 ± 0.688 (100)	10.887 ± 0.399 (100)
0.25	9.485 ± 0.599 (90)	5.792 ± 0.389 (89)	13.335 ± 0.444 (85)	9.145 ± 0.301 (84)
0.50	8.536 ± 0.561 (81)	4.751 ± 0.288 (73)	10.197 ± 0.406 (65)	6.749 ± 0.211 (62)
1.00	7.272 ± 0.442 (69)	3.839 ± 0.229 (59)	7.687 ± 0.321 (49)	5.008 ± 0.312 (46)

Values are expressed as the mean ± SE 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 control
Analysis of variance showed that the response to the *D. filix*-mas powder was dose-dependent (P< 0.01).

Table 3: Alterations in the RNA/DNA ratio in haemolymph and fat body of the larva of rice-moth, *C. cephalonica* treated with *Dryopteris filix*-mas root and rhizome powder

Per cent *D. filix*-mas dose	**RNA/DNA ratio**	
	Haemolymph	**Fat body**
Control (untreated)	1.489 (100)	1.673 (100)
0.25	1.406 (94)	1.579 (94)
0.50	1.195 (80)	1.421 (85)
1.00	1.057 (71)	1.305 (78)

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

Table 4: Changes in the levels of glycogen and reducing sugar in the haemolymph and fat body of the larva of rice-moth, *C. cephalonica* treated with *Dryopteris filix*-mas root and rhizome powder

Per cent *D. filix*-mas dose	**Glycogen# (mg/g wet wt)**		**Reducing sugar# (mg/g wet wt)**	
	Haemolymph	**Fat body**	**Haemolymph**	**Fat body**
Control (untreated)	2.477 ± 0.112 (100)	14.881 ± 0.416 (100)	2.801 ± 0.147 (100)	1.047 ± 0.052 (100)
0.25	1.932 ± 0.094 (78)	9.970 ± 0.314 (67)	3.557 ± 0.168 (127)	1.392 ± 0.068 (133)
0.50	0.991± 0.018 (40)	6.548 ± 0.224 (44)	4.706 ± 0.170 (168)	1.769± 0.058 (169)
1.00	0.446 ± 0.013 (18)	4.464 ± 0.213 (30)	5.266 ± 0.164 (188)	2.251 ± 0.099 (215)

Values are expressed as the mean ± SE 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 *D. filix*-mas powder was dose-dependent (P< 0.05).

dependent (P < 0.05) decrease in the activity of alkaline phosphatase in both the tissues of the larva.

In contol larvae, the acid phosphatase activity was 0.622 and 2.599 µ moles/30 min/mg protein in haemolymph and fat body, respectively. The maximum enhancement in acid phosphatase activity in haemolymph (423% of the control value) and fat body (326% of the control value) was observed in larvae treated with 1.00% dose level of *Dryopteris filix*- mas powder. Acid phosphatase activity, in haemolymph, was increased to 180% (1.120 µmole), 250% (1.555 µmole) and 423% (2.631 µmole) of the control value while the activity of this enzyme, in fat body, was enhanced to 148% (3.846 µmol), 223% (5.796 µmole) and 326% (8.473 µmole) of the control value following treatment with 0.25, 0.50 and 1.00% dose level of *Dryopteris filix*-mas powder, respectively (Table 5).

In the control larvae, the alkaline phosphatase activity was 0.468 and 2.615

µmoles/ 30min/mg protein in haemolymph and fat body, respectively. The maximum decrease in alkaline phosphatase activitiy in haemolymph (37% of the control value) and fat body (45% of the control value) was observed in larvae treated with 1.00% dose

Table 5: Changes in acid and alkaline phosphatase activity in haemolymph and fat body of the larva of rice-moth, *C. cephalonica* treated with *Dryopteris filix*-mas root and rhizome powder

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Per cent	**Acid phosphatase#**		**Alkaline phosphatase#**	
***D. filix*-mas dose**	**Hamolymph**	**Fat body**	**Haemolymph**	**Fat body**
Control (untreated)	0.622 ± 0.041 (100)	2.599 ± 0.114 (100)	0.481 ± 0.027 (100)	2.615 ± 0.133 (100)
0.25	1.120 ± 0.062 (180)	3.846 ± 0.157 (148)	0.380 ± 0.010 (79)	2.301 ± 0.108 (88)
0.50	1.555± 0.074 (250)	5.796 ± 0.217 (223)	0.279 ± 0.011 (58)	1.830 ± 0.128 (70)
1.00	2.631 ± 0.080 (423)	8.473 ± 0.228 (326)	0.178 ± 0.014 (37)	1.177 ± 0.076 (45)

The activities are given as µ moles of p-nitrophenol liberated per 30 min per mg of protein and expressed as mean ± SE of six replicates.
Student's t-test showed significant differences (P< 0.05 to P< 0.001) between the corresponding treated groups and the controls.
Values in the parentheses are the percentage change, with control values taken as100%.
Analysis of variance showed that the response to the *D. filix*-mas was dose-dependent (P < 0.05).

level of *Dryopteris filix*-mas powder. Alkaline phosphatase activity in haemolymph, was reduced to 79% (0.380 µmole), 58% (0.279 µmole) and 37 % (0.178 µmole) of the control value while its activity, in fat body, was reduced to 88 % (2.301 µmole), 70% (1.830 µmole) and 45%.(1.177 µmole) of the control value following treatment with 0.25, 0.50 and 1.00% dose level of *Dryopteris filix*-mas powder, respectively (Table 5).

Discussion

Available evidences reveal that ferns are effective in arresting embryonic development in insects and filicin isolated from root and rhizome of *D. filix*-mas is a potential

insecticide which has been reported to be toxic against *Spodoptera littura* and *Helicoverpa armigera* (Mannan *et al.*, 2008). Plant extracts interfere with the normal embryonic development by suppressing hormonal and biochemical process. Such physiological interference was observed by Ofuya *et al.* (1992), Chiranjeevi and Sudharkar (1996) and Jayakumar *et al.* (2003). Since, the ferns arrest the embryonic development of insects (Mannan *et al.*, 2008), thus, it may be probably considered that active compounds present in *D. filix*-mas attack the insect's endocrine system affecting the levels of juvenile hormone secreted from corpora allata and ecdysone (moulting hormone) secreted from prothoracic gland causing the insect unable to moult. *D. filix*-mas induced such hormonal imbalance in addition to biochemical perturbations in *C. cephalonica* results into its ultimate 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. In the present investigation, all the three sublethal doses of *D. filix*-mas root and rhizome's powder 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 botanical insecticides 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 in agreement with findings of above workers.

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, all the three sublethal doses of *D. filix*-mas root and rhizome's powder 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 this biopesticide induced 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*. Since, *D. filix*-mas extract 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 whereas 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.*,1981; Tiwari and Bhatt, 1987). Literature 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 far from adequate.

In the present investigation, all the three sublethal doses of *D. filix*-mas root and rhizome's powder 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. Botanicals (neem compounds) and pyrethroid have been shown to inhibit the nucleic acid level in *Musca domestica* L. (Naqvi *et al.*, 1991a). Similar findings have also been observed in case of pulse beetle, *Callosobruchus analis* L. following treatment with neem compounds NfC (Neutral fraction C- which is a crude extract of whole neem seed) and NC (Nimocilin, Azadirachtin) as reported by Tabassum (1994). 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.) as reported by Shakoori *et al.* (1988).

The reduction in the DNA and RNA levels, in the present study, may be due to interference of active ingredients present in *D. filix*-mas root and rhizome powder 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.). Similar explanation for decrease in DNA and RNA contents have also been reported in fenpropathrin exposed larvae of *Tribolium castaneum* (Herbst.) (Shakoori *et al.*, 1988) and in rice moth, *Corcyra cephalonica* exposed to neem stem bark powder (Pathak and Tiwari, 2016), neem seeds acetone extract (Pathak and Tiwari, 2017a), neem seeds ethanol extract (Pathak and Tiwari, 2017b) and pyrethrum extract (Tiwari, 2019).

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 demonstrates the reduction in the total protein level in both the tissues of the larva following treatment with *D. filix*-mas powder. 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 *D. filix*-mas powder's active ingredients influencing the amino acids 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).

Data obtained on the carbohydrate level indicate that *D. filix*-mas powder 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* Burm. and *Vitex negundo* Linn. extracts poisoned larvae of cabbage leaf webber, *Crocidolomia binotalis* Zeller (Vijayaraghavan *et al.*, 2010). They 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* Fabricius and *Chrysoperla carnea* Stephens. 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, 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 *D. filix*-mas stress.

The depletion in glycogen level may also be due to a direct action of active compounds of *D. filix*-mas powder on oxidative phosphorylation as observed in case of *Periplaneta americana* following treatment with lindane (Ela *et al.*, 1970).

Acid phosphatase plays a significant role in catabolism, pathological necrosis, autolysis and phagocytosis (De Duve, 1959; Becker and Barron, 1961; 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).

Plant extracts/biopesticides and synthetic pyrethroids influence the activities of acid and alkaline phosphatases as reported by Naqvi *et al.* (1991); Josephrajkumar *et al.* (1999); Akhtar and Islam (2004); Mannan *et al.* (2008); Pathak and Tiwari (2015, 2016, 2017b, 2017c) and Tiwari (2018).

Data presented on phosphatase activity clearly demonstrate that all the three sublethal doses of *D. filix*-mas root and rhizome's powder 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 of *C. cephalonica*. Similar observations have been recorded in haemolymph and fat body tissues of the larva of this pest exposed to neem stem bark

powder (Pathak and Tiwari, 2016), neem seeds ethanol extract (2017 b) and neem seeds acetone extract (Pathak and Tiwari, 2017 c). Such results have also been observed by neem compounds (RB-a, RB-A and Magosan -O™) exposed insect *Oxycarenus lugubris* (Nurulain, 1987). Although many studies have been reported pertaining to effects of plant extracts including growth retardation and arrest of ovarian as well as embryonic development but their exact mode of action has not yet has been elucidated (Akhtar and Islam, 2004; Mannan *et al.*, 2008). 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 studies 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.

D. filix-mas powder 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), *Fragonia 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 on 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 *D. filix-mas* powder 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*.

The entire finding of the present investigation i.e. *D. filix*-mas powder influenced reduction in the levels of total protein, DNA, RNA, RNA/ DNA ratio, glycogen and inhibition of alkaline phosphatase activity as well as enhancement in the levels of total free amino acids, reducing sugars and in the activity of acid phosphatase perturbs the biochemical framework of the larva resulting into death. Of course, application of this biopesticide would be able to reduce the population build up of *C. cephalonica* in particular and lepidopterous pests in general, in ecofriendly way.

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References

Abou-Donia M B. (1978) Increased acid phosphatase activity in hens following an oral dose of Leptophos. Toxicol. Lett. 2: 199-203.

Akhtar Y and Islam M B. (2004) Comparative growth of inhibitory and atifeedant effects of plant extract and pure allelochemicals on some phytophagus insect species. J. Applied Entomol. 128: 32-38.

Andersch M A and Szcypinski A J. (1967) Am. J. Clin. Pathol. 17: 571, In: Methods in Enzymatic Analysis. Bergmeyer, U.H. (ed.), Academic Press, New York.

Beadle L and Shaw J. (1950) The retention of salt and the regulation of the NPN fraction in the blood of the aquatic larva, *Sialia lutaria.* J. Exp. Biol. 127: 96-109.

Becker N H and Barron K D. (1961) The cytochemistry of anoxic and anoxic ischemic encephalopathy in rats. Am. J. Pathol. 38: 161-175.

Bhagawan C N, Reddy K D and Sukumar K. (1992) Annona-induced growth anomalies and protein depletion in red cotton bud *Dysdercus koenigii*. Indian J. Exp. Biol. **30**: 908-912.

Bhunya S P and Das N. (1976) Effect of a syntetic insecticide, dimethoate, on spermatocytic chromosomes of a short-horned grasshopper, *Poecilocerus pictus.* Sci. Cult. 42: 571.

Bishop G H, Briggs A P and Ronzoni E. (1926) Body fluids of honey bee larva. J. Biol. Chem. 66: 77-88.

Brachet J. (1955) In: The Nucleic Acids. Vol. II, Academic Press, New York.

Brisca Renuga F and Sahayaraj K. (2009) Influence of botanicals in total head protein of *Spodoptera litura (*Fab.). J. of Biopest. 2: 52-55.

Bream A S. (2003) Effect of azadirachtin on phosphatases and transaminases activities in pupae of the red palm weevil, *Rhyncophorus ferrugineus* (Coleoptera: curculionidae). Proc. Int. Conf. Date Palm. 16-19 Sep.

Buck J B. (1953) The internal environment in regulation and metamorphosis. In: Insect Physiology K. Roeder(ed.). Wiley, New York, 191-217.

Chefurka W. (1959) Glucose metabolism in insects. In: Biochemistry of Insects (Symposium XII of the IV Int. Congress Biochem. Levenbook, L. (ed) Pergamon Press.

Chefurka W. (1964) Intermediary metabolism of carbohydrates in insects. In: The physiology of Insects. Rockstein, M. (ed.) 2: 11. Academic Press, New York.

Chefurka W. (1965) Some comparative aspects of metabolism of carbohydrates in insects. Ann. Rev. Entomol. 10: 345-382.

Chen P S. (1966) Amino acid and protein metabolism in insect development. In: Advances in Insect Physiology. Beament J.W.L., J.E. Treherne and V.B. Wigglesworth (eds). 3: 53-132. Academic Press, New York.

Chiranjeevi C and Sudhakar T R. (1996) Effect of indigenous plant materials on the fecundity, adult emergence and development of pulse beetle, *Callosobruchus chinensis* (L.) in black gram. J. Research- APAU. 24: 57-61.

Chopra R N, Nayar S L and Chopra I C. (1986) Glossary of Indian Medicinal Plants (Including the Supplement). Council Scintific Industrial Research, New Delhi.

Clements A M. (1963) The Physiology of Mosquitoes. Pergamon Press, Oxoford.

Dalela R C, Bhatnagar M C and Verma S R. (1978) Histochemical studies on the effect of rogor and thiodon on the activity of acid phosphatase in liver, muscles and Kidney of *Chana gachus*. Indian J. Exp. Biol. 16: 1099-1102.

De Duve C. (1959) Lysosomes, a new group of cytplasmic particles. In: Subcellular particles (Hayashi, T., ed.) Symposium of the Society of General Physiologists at the Maine Biological Laboratory, Woods Hole, Mas, June 9-11, 1958, Ronald Press, New York, 128-159.

Ela R, Chefurka W and Robinson J R. (1970) *In vivo* glucose metabolism in the normal and poisoned cockroach, *Periplaneta americana.* J. Insect Physiol. 16: 2137- 2156.

Florkin M. (1959) The free amino acids of insect haemolymph. Proc. VIth Int. Cong. Biochem. 12: 63-73.

Florkin M and Jeuniaux C. (1974) Haemolymph: Composition. In: Rockstein, M. (ed.). The physiology of insects. 5: 255-307, Academic Press, New York.

Folin O and Wu H. (1920*) J. Biol. Chem.* 41: 367, Cited by B.L. Oser (ed.): Hawks Physiological Chemistry. New Delhi, Tata Mc Graw Hill, 1979.

Friedman S. (1970) In: Chemical Zoology. Florkin, M. and B.T. Scheer (eds.), 5(A): pp 167-197. Academic Press, New York.

Galdher N R, Fawade M M, Halde U K and Pawar S S. (1978) Acid and alkaline phosphatase activities during pesticide intoxication. Indian J. Biophysics. 15 : 78.

Gilmour D. (1961) The biochemistry of insects. Academic Press, New York. 235-390.

Gilmour D. (1965) The metabolism of Insects. Oliver Boyd, Edinburg. 115-147.

Glees P. (1967) A morphological and neurological analysis of neurotoxicity illustrated by tricresyl phosphate in toxication in the chick. In : Neurotoxicity of drugs, Proceedings of the European Society for the study of Drug Toxicity (Davey, D.G., ed), Excerpta Medica, Amsterdam, **8**: 136-148.

Gordon R and Burford, I. R. (1984) Effects of methoprene, a juvenile hormone analogue, on the larval and pupal stages of the yellow fever mosquito, *Aedes aegypti*. J. Insect. Physiol. 30: 279-286.

Green M B, Hartley G S and West T F. (1979) Chemicals for Crop protection and Pest Control. Pergamon Press, Oxford.

Ibrahim A M, Higazi M G and Demian E S. (1974) Histochemical localization of alkaline phosphatase activity in the alimantry tract of the snail, *Marisa cornuarietis* (L.). Zool. Soc. Egypt. Bull. 26: 94-105.

Ivbijaro M F.. (1983) Toxicity of neem seed, *Azadirachta indica* A. Juss., to *Sitophilus oryzae* (L.) in stored maize. Prot. Ecol. 5: 353-357.

Jayakumar M, Elumalai K, Jeyasankar A, Raja N and Ignachimuthu S. (2003) Efficacy of crude extracts of *Hyptis suaveolens* and *Melochia chorcorifolia* on pulse beetle, *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae). Proc. Biol. Control Insect Pests, pp. 219- 223.

Jilani G. (1983) Laboratory studies on several plant materials as insect repellents for plant protection of cereal grains. J. Econ. Ent. 76: 19-23.

Josephrajkumar A, Subrahmanyam B and Srinivasan S. (1999) Plumbagin and azadirachtin deplete haemolymph ecdysteroid levels and alter the activity profiles of two lysosomal enzymes in the fat body of *Helicoverpa armigera* (Lepidoptera: noctuidae). Eur. J. Entomol. 96: 347-353.

Jotwani M G and Srivastava K P. (1981) Neem–insecticide of the future. I- As protectant against stored grain pests. Pesticides 15: 19-23.

Kilby B A. (1963) The biochemistry of the insect fat body. In: Advances in Insect Physiology, (Beament JWL, Treherne JE and Wigglesworth VB, (eds.)., 1: 111-174. Academic Press, London and New York.

Krishna S S and Pandey G C. (1974) *In Vivo* studies on the fate of certain carbohydrates in the alimentary canal and haemolymph of the larva of *Earias fabia* Stoll (Lepidoptera: Noctudiae). New Entomologist 23: 1-7.

Lang C A, Lau H Y and Jefferson D J. (1965) Protein and nucleic acid changes during growth and aging in the mosquito. Biochem. J. 95: 372-7.

Lee J J, Shim J C and Lee K R. (1981) The effect of insecticides on haemolymph in the German cockroach, *Blattela germanica* L. Korean J. Entomol. Vol. XI: 25-32.

Lowry O H, Rosenbrough N J, Farr A L and Randall R J. (1951) Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193: 265-275.

Magalhae L G, Kapadia G J, da Silva Tonuci L R, Caixeta S C, Parreira N A, Rodrigues V and Da Silva Filho A A. (2010) *In vitro* schistosomicidal effects of some phloroglucinol derivatives from *Dryopteris* species against *Schistosoma mansoni* adult worms. Parasitology Research 106: 395-401.

Mannan Mannar M, Maridas M and Victor B. (2008) A review on potential uses of ferns. Ethanobotanicals Leaflets 12: 281-285.

Mukhopadhyay P K and Dehadrai P V. (1980) Studies on air breathing catfish *Clarias batrachus* (Linn.) under sublethal malathion exposure. Indian J. Experimental Biol. 18: 400-404.

Naqvi S N H, Akhtar K and Azami M A. (1991 a) Toxicity of NfD against *Sitophilus oryzae* L. exposed to impregnated filter paper and its effects on phosphatases and protein metabolites. Acta Biologica. Crac. Zoolgia Ser., X X X III: 49-58.

Naqvi S N H, Nurulain S M and Tabassum R. (1991 b) Effect of neem fraction and pyrethroid on the nucleic acid of *Musca domestica* L. Proc. Ist Nat. Biochem. Symp. pp. 21.

Ntiforo C and Stein M. (1967) Labilization of lysosomes as an aspect of the biochemical toxicology of anticholinesterase pesticides. Biochem. J. 102: 44.

Nurulain SM. (1987) Toxicity and effect of neem fraction and malathion on the enzymes of proteins of *Oxycarenus lugubris* (Heteroptera: Lygaeidae). Thesis, Dept. of Zool., Univ. of Karachi.

Ofuya T I, Okoye B C and Olola A S. (1992) Efficacy of crude extract from seeds of *Monodora myristics* (Gaem.) Dunal as surface protectant against

Callosobruchus maculates (F) attacking legume seeds in storage. J. Herbs SpicesMedicinal Plants 11: 47-69.

Olga S, Uckan F and Ergin E. (2006) Effects of cypermethrin on total body weight, glycogen, protein and lipid contents of *Pimpla turronellae* (L.) (Hymenoptera: Ichneumonidae). Belgian J. Zool. 136: 53-58.

Pathak C S and Tiwari S K. (2010 a) Toxicity of neem seed (*Azadirachta indica* A. Juss, Meliaceae) extract against the immature stages of rice-moth, *Corcyra cephalonica* (Staint.) (Lepidoptera: Pyralidae). J. Appl. Biosci. 36: 173-177

Pathak C S and Tiwari S K. (2010 b) Toxicological effects of neem *Azadirachta indica* A. Juss leaf powder against the ontogeny of *Corcyra cephalonica* (Staint.) (Lepidoptera: Pyralidae). J. Biopest. 3: 617-621

Pathak C S and Tiwari SK. (2012) Insecticidal action of neem seed (*Azadirachta indica* A. Juss) acetone extract against the life-cycle stages of rice-moth, *Corcyra cephalonica* (Staint.) (Lepidoptera: Pyralidae). World J. Agric. Sci. 8: 529-536.

Pathak C S and Tiwari SK. (2015 a) Toxicity of Bioresmethrin on the developmental stages and larval biochemistry of rice-moth, *Corcyra cephalonica* Staint, (Lepidoptera: Pyralidae). Intern J. Zool. Invest. 1: 55-71

Pathak C S and Tiwari SK. (2015 b) Toxicity of neem stem bark powder against the ontogeny of rice moth, *Corcyra cephalonica* Staint, (Lepidoptera: Pyralidae). Intern J. Zool. Invest. 1: 187-191

Pathak C S and Tiwari S K (2016) Potential of neem stem bark powder against the larval biochemistry of rice moth, *Corcyra cephalonica* Staint.(Lepidoptera: Pyralidae). Intern J. Zool. Invest. 2: 304-323

Pathak C S and Tiwari S K. (2017 a) Potential of neem seed's acetone extracts on the haemolymph and fat body biochemistry of rice moth, *Corcyra cephalonica Staint.* (Lepidoptera: Pyralidae). J. Adv. Zool. 38:164-177

Pathak C S and Tiwari SK. (2017 b) Influence of neem seed ethanol extract against the larva biochemistry of rice moth, *Corcyra cephalonica* Staint.(Lepidoptera: Pyralidae) Intern J. Zool. Invest. 3: 155-174

Pathak C S and Tiwari SK. (2017c) Influence of neem seed acetone extract on the larval biochemistry of rice moth, *Corcyra cephalonica* Staint.(Lepidoptera: Pyralidae). J. Appl. Biosci. 43: 90-96

Pavlikova D and Repas S. (1975) Comparative histochemical studies of changes in spermatogenesis and intertubular tissue at male sterility. Biologia Bratisl. 30: 889-895.

Pichaet Wiriyachitra and Bernard Philogene J R. (1993) A natural path to pesticides. IDRC Reports (Canadian Collaboration for development), 21(2).

Pilo B Ansari M V and Shah R V. (1972) Studies on wound healing and repair in pigeon liver: III Histochemical studies on acid and alkaline phosphatase during the process. J. Anim. Morphol. Physiol. 19: 205-212.

Rajendran B and Gopalan M. (1982) Note on biochemical changes induced by *Polyscias guilfoy* Bailey (Araliaceae) in *Dysdercus cingulatus* Fabricius (Hemiptera: Pyrrhocoridae). Indian J. Agric. Sci. 52: 46-48.

Ramesh Chand and Pratap S Birthal (1997) Pesticide use in Indian agricultural in relation to growth in area of production and technological change. Ind. J. of Agri. Econ. 52: 488-498.

Ramakoteswara Rao S, Chitra K C and Kameswara Rao P. (1995) Studies on the effects of certain plant extracts on the protein metabolism of *Spodoptera litura* (Fabr). Indian J. Entomol. 57: 406-408.

Razak AT and Sivasubramaniam P. (2007) Effect of three botanical oils on carbohydrate content in *Cheilomenes sexmaculata* Fabricius and *Chrysoperla carnea* Stephans. Asian J. Biochem. 2:124-129.

Reddy K D, Bhagawan C N and Sukumar K. (1993) Depletion of amino acids and protein in the nymphal haemolymph of the red cotton bug, *Dysdercus koenigii* (Fab.) (Hemiptera: Pyrrhocoridae) treated with *Annona squamosa* extract. Phytophaga 5: 45-50.

Schneider WC. (1957) Determination of Nucleic Acids in tissues by Pentose Analysis. In: Methods in Enzymology. Colowick, S.P. and N.O. Kaplan (eds), Academic Press, New York, pp 680-684.

Shakoori A R, Fayyaz M and Saleem M A. (1988) Biochemical changes induced by fenpropathrin in the 6th instar larvae of *Tribolium castaneum* (Herbst.) (Coleoptera: Tenebrionidae). J. Stored Prod. Res. 24: 215-220.

Shukla S and Tiwari S K. (2011 a) Toxicological effects of *Dryopteris filix*-mas against the ontogeny of rice-moth, *Corcyra cephalonica* (Staint.) (Lepidoptera: pyralidae. World Appl. Sci. J. 12: 16-20

Shukla S and Tiwari S K. (2011 b) Toxicity of *Dryopteris filix*-mas powder against the ontogeny of rice-moth,

Corcyra cephalonica (Staint.) (Lepidoptera: pyralidae). Asian. J. Exp. Sci. 25: 73-79

Shukla S and Tiwari S K. (2011 c) Insecticidal activity of *Dryopteris filix*-mas(Linn) Schott ethanolic extract against *Corcyra cephalonica* Staint. (Lepidoptera: pyralidae). J. Biopest. 4: 138-143.

Shukla S and Tiwari S K. (2012) The influence of pyrethrum extract on the developmental stages of the rice-moth, *Corcyra cephalonica* Stainton (Lepidoptera: Pyralidae), Egyptian J. Biol. 14: 57-62.

Seyoum E, Bateman R P and Charnley A K. (2002) The effect of *Metarhizium anisopliae* var acridum on haemolymph energy reserves and flight capability in the desert locust, *Schistocerca gregaria*, J. Applied Entomol. 126: 119-124.

Shigematsu H. (1960) Protein metabolism in the fat body of the silk worm, *Bombyx mori* L. Bull. Seric. Exp. Sta. Japan 16: 141-70.

Shivanandappa T and Krishna Kumari M K. (1981) Histochemical and biochemical changes in rats fed dietary BHC. Indian J. Exp. Biol. 19: 1163-1168.

Sokal R R and Rohl FJF. (1969) Introduction to Biostatistics. W.H. Freemann Co. San Franscisco.

Spies JR. (1957) Colorimetric procedures for amino acids. In: Methods in Enzymology. Colowick, S.P. and N.O. Kaplan (eds.), Academic Press, New York, pp: 464-471.

Subramanyam B E and Rao P J. (1986) Azadirachtin effects on *Schistocerca gregaria* Forskal during ovarian development. Curr. Sci. 55: 534-538.

Sumner A T. (1965) The cytology and histochemistry of the digestive gland cells of *Helix.* Q. J. Microsc. Sci. 106: 173-192.

Tabassum R. (1994) Toxicity and residual effect of some neem compound (Nimocinol etc.) in comparision with IGR (Dimilin) against stored grain pests. Ph. D. Thesis, Dept. of Zoology, University of Karachi, Pakistan.

Tayyaba K, Hasan M, Islam F and Khan N H. (1981) Organophosphate pesticide Metasystox-induced regional alterations in brain nucleic acids metabolism. Indian J. Exp. Biol. 19: 688-690.

Tiwari S K. (1987) Studies on the effect of certain insecticides on the chemistry of haemolymph and fat body of the larva of rice-moth, *Corcyra cephalonica* Stainton. (Lepidoptera: Pyralidae). Ph.D. Thesis, Dept. of Zoology, Gorakhpur Univ. Gorakhpur, U.P., India.

Tiwari S K. and Tripathi C P M. (2006) Tetramethrin induced toxicity against the ontogeny of rice-moth, *Corcyra cephalonica* (Staint.) (Lepidoptera: Pyralidae). Uttar Pradesh J. Zool. 26: 227-229.

Tiwari S K. (2018) Insecticidal action of pyrethrum extract on the carbohydrate and phosphatase biochemistry of the larva of rice-moth, *Corcyra cephalonica* Staint. (Lepidoptera : Pyralidae). Intern. J. Zool. Invest. 4: 196-207

Tiwari S K. (2019) Potential of pyrethrum extract against larval biochemistry of rice-moth, *Corcyra cephalonica* Staint. (Lepidoptera : Pyralidae). J. Adv. Zool. (in press).

Van der Vies J. (1954) Two methods for the determination of glycogen in liver. Biochem. J. 59: 410-416.

Vijayaraghavan C and Chitra K C. (2002) Total protein and free amino acid content of *Spodoptera litura* (Fabr.) due to botanicals and conventional insecticides. Indian J. of Entomol. 64: 92-95.

Vijayaraghava C, Sivakumar C, Zadda Kavitha and Sivasubramanian P. (2010) Effect of plant extracts on biochemical components of cabbage leaf webber, *Crocidolomia zeller. J. Biopest.* 3: 275-277.

Vorbrodt A. (1959) The role of phophatase in interacellular metabolism. Postepy, Hig Med. Dosw. 13: 200-206.

Widen C J, Fraser-Jenkins C R, Reichstein T and Sarvela J. (2001) A survey of phenolic compounds in *Dryopteris* and Related fern genera. III. Phloroglucinol derivatives in subgenera *Erythrovaria* and *Nephrocystis* and related genera (Pteridophyta: Dryopteridaceae). Ann. Botanici Fennici. 38: 99-138.

Wyatt G R. (1961) The biochemistry of insect haemolymph. Ann. Rev. Ent. 6: 72-102.

Wyatt G R. (1967) The biochemistry of sugars and polysaccharides in insects. Adv. Insect. Physiol. 4: 287-360.

Young T L and Gordon R. (1987) Effect of the juvenoid methoprene on the hemolymph composition of the cabbage maggot*Delia radicum* (Diptera: Anthomyiidae). Experientia 43: 902-903.

Zibaee A and Badani A R. (2010) A study of toxicity of a medicinal plant, *Artemisia annua* L. (Asteracea) extract to the sunn pest, *Eurygaster integriceps* Puton (Hemiptera: Scutelleridae). J. Plant Proct. Res. 50: 79-85.