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Insecticidal Action of Pyrethrum Extract on the Carbohydrate and Phosphatase Biochemistry of the Larva of Rice-Moth, *Corcyra cephalonica* Staint. (Lepidoptera : Pyralidae)

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Abstract: Sub-lethal doses (0.0001, 0.0002 and 0.0004%) of pyrethrum extract caused a significantly dose-dependent reduction in glycogen level and alkaline phosphatase activity and a significantly dose dependent enhancement in reducing sugar level and acid phosphatase activity in haemolymph and fat body tissues of the larva of rice-moth, *C. cephalonica*. It was observed that 0.0004% dose level of this extract caused maximum effect on these biochemical parameters. It may be concluded that pyrethrum extract induced alterations in the carbohydrate levels and phosphatase activities in haemolymph and fat body tissues which results into biochemical perturbations leading to death. So, application of pyrethrum extract is of course beneficial for the effective control of rice-moth, *C. cephalonica* in particular and lepidopterous pests in general in eco-friendly way.

Keywords: Pyrethrum extract, *Corcyra cephalonica*, Haemolymph, Fat body, Biochemistry

Introduction

The rice-moth, *Corcyra cephalonica* (Staint.) is a major pest of stored cereals and cereal commodities in Asia, Africa, North America, Europe and other tropical and subtropical regions of the world. This moth was first identified and reported by Stainton (1866), who named it *Melissoblaptes cephalonica.* Later, Ragonot (1885) gave it the generic name *Corcyra.* The only recognized species of this genus is *cephalonica.* Ayyar (1919) made the first record of *Corcyra cephalonica.* Its larval stages cause serious damage to rice, gram, sorghum, maize, groundnut, cotton seeds, peanuts, linseeds, raisins, nutmeg, currants, chocolates, army biscuits and milled products (Chittenden, 1919; Ayyar, 1919; Munro and Thompson, 1929; Richards and Herford, 1930; Noyes, 1930; Herford, 1933; Atwal, 1976; Piltz, 1977).

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. Storage loss of food grains at the level of Government and its agencies such as Food Corporation of India, Central and State Warehousing Corporations and State Civil Supplies Departments/ Corporation have been reduced to the minimum. 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. According to a survey made by the food and agricultural organization of the United Nation in some 29 countries in mid-forties, the estimated total loss of cereals was 25,750,000 tons, out of which 50 per cent loss could be attributed to the insects alone.

Persistent use of synthetic organic insecticides affect immune system of insects, develop resistance (Chand 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 pose problems also such as poisoning in man and other animals (Pichaet and Philongene, 1993). Thus, there is an urgent need to develop safe and suitable alternatives to synthetic organic insecticides for the protection of grain and grain products against insect infestations. Botanical insecticides, compared to synthetic ones may be safer for the environment, are generally less expensive, easily processed and used by farmers and small scale industries (Belmain *et al*., 2001). Since, these insecticides are often active against a limited number of species, are often biodegradable to non-toxic products and are potentially suitable for use in integrated pest management. They could lead to the development of new classes of safer insect control agents (Kim *et al*., 2003).

In the early 1800's pyrethrum flowers were used by Caucasian tribes and in Persia to control body lice. The flowers were first produced commercially in Armenia in 1828. Production started in Dalmatia (Yugoslavia) about 1840 and was centered there until the First World War, in Japan until shortly before the Second World War, and in east Africa since then. More than half of the world's current production comes from Kenya, with important amounts from Tanzania, Rwanda, and Ecuador. Pyrethrum plants were first imported into the United States in about 1860, and several unsuccessful attempts were made over the next 90 years to produce the flowers commercially in this country. Since about 60 years ago the flowers were extracted with Kerosene or similar solvents to give liquid sprays which aremore effective than the powders.

Pyrethrum extract contains six closely related insecticidal esters, collectively referred to as the pyrethrins, which differ only in the terminal substituent in the side chains of the acid and alcohol components. The acid is a substituted cyclopropane-carboxylic acid and the alcohol a substituted cyclopentenolone. Three alcohols are involved, pyrethrolone, cinerolone and jasmolone for the pyrethrins, cinerins and jasmolins, respectively. The two acids are chrysanthemic acid for the I series and pyrethric acid for the II series.

The pyrethrins are localized in the secretory ducts of the achenes, where they are

protected from photodecomposition and isolated so they are not toxic to insects feeding on or visiting pyrethrum flowers. The flowers are handpicked when four or five rows of disc florets are open, and each flower contains about 3-4 mg pyrethrins. After drying in the sun or mechanically, the flowers are ground into fine powder, the product is called pyrethrum and extracted with hexane. Evaporating the hexane yields a dark viscous oleoresin concentrate containing about 30% pyrethrins. The concentrate is either diluted with plant or mineral oil to 25% pyrethrins (oleoresin extract) or purified by methanol extraction and charcoal treatment to produce a dewaxed and decolorized refined extract. This purification removes components which earlier gave allergic responses evidenced as dermatitis in humans.

The biological activities of the pyrethrum constituents depend on the structures and stereochemical characteristics of both the acid and alcohol components. Pyrethrins I and II are considerably more potent than the cinerins and jasmolins. The chrysanthimates (I) are generally more potent for kill and the pyrethrates (II) for knockdown. Thus, pyrethrum contains a combination of an excellent knock down agent (Pyrethrin II) and a potent insecticidal component (Pyrethrin I). These compounds act both on the central nervous system causing repetitive discharges, followed by convulsions. Pyrethrins work by creating multiple potentials across the membranes by delaying the closing of the ion channel. They act as contact poisons affecting the insect's nervous system but even though they are a nerve poison, they are not an anti-cholinesterase as are organophosphates and carbamates. Usually pyrethroids contain a synergist which allows the primary insecticide to be more effective by restricting the production of an enzyme the insect uses to detoxify the pyrethrins, one of the most well known is piperonyl butoxide.

Most insects are highly susceptible to low concentrations of pyrethrins. The toxin cause immediate knockdown or paralysis on contact, but insects often metabolize these pyrethrins and recover. Pyrethrins break down quickly, have a short residual, and low mammalian toxicity making them among the safest insecticides in use. Pyrethrum extract is important to control the pest insects in the house hold, in barns and in stored products, and for direct application to man and live stock. They may also be used against a broad range of pests including ants, aphids, roaches, fleas, flies, and ticks. They are available in dusts, sprays, and aerosol "bombs" and may be mixed with synthetic pesticides or other botanicals.

Numerous investigations have shown that botanicals/plant extracts and pyrethroids affect ontogeny as well as the biochemical constituents of various tissues in insects (Shukla, 2011, Shukla and Tiwari 2011a, 2011b, 2011c, 2012; Pathak and Tiwari, 2010a, 2010b, 2012, 2015a, 2015b, 2016, 2017a, 2017b, 2017c; Pathak, 2011). Insecticidal influence of different plant extracts on carbohydrate contents have been explored by Olga *et al.* (2006), Razak and Sivasubramanian (2007) and Vijayaraghavan *et al*. (2010). Alterations in the activity of enzymes concerning those of plant extracts have been reported by Nurulain (1987), Naqvi *et al*. (1991), Josephrajkumar *et al.* (1999), Bream (2003), Akhtar and Islam (2004), Mannan *et al.* (2008), Basiouny *et al.* (2010)

and Zibaee and Badani (2010). As a safe and suitable alternative to synthetic organic compounds, the present work has been designed and carried out to examine the impact of pyrethrum extract at various doses on the glycogen and reducing sugar levels 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, India. 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 (R.H.).

From the above culture whenever needed, newly emerged males and females were transferred to oviposition glass chambers (35 mm diameter, 200 mm height). Eggs laid by the females were collected and then placed in glass chambers (250 ml beakers) for hatching.

Pyrethrum extract 23.3% (a.i.) used throughout the investigation, was obtained from the Sigma - Aldrich (Lot. SZE7135X).

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

Toxicity experiment was designed and performed to find out the sub lethal doses of pyrethrum extract for biochemical estimations (Shukla, 2011; Shukla and Tiwari, 2012).

For biochemical estimations, out of various dose levels of the biopesticides mentioned above, only such doses of pyrethrum extract (0.0001, 0.0002 and 0.0004%) 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 pyrethrum extract as biopesticidal control measures against this lepidopterous pest.

As stated earlier, 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

0.0001, 0.0002 and 0.0004% of pyrethrum extract 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 as follow:

(i) 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.

(ii) 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 immediately mixed with known volume of required solvent to prepare the homogenate for each biochemical estimation.

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 reagents 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. Both the phosphatases (acid and alkaline) catalyze the hydrolysis of p-nitrophenylphosphate into phosphoric acid and p-nitrophenol. Activity of phosphatases is directly proportional to the amount of p-nitrophenol formed. The activities of acid and alkaline phosphatases were expressed as µmoles 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

All the three sub-lethal doses of pyrethrum extract caused a significantly dose-dependent ($P < 0.05$) reduction in the level of glycogen and a significantly dose-dependent ($P < 0.05$) enhancement in the level of reducing sugar in both the tissues of larva (Table 1).

In case of untreated larvae, the glycogen level was 2.489 and 14.875 mg/g in haemolymph and fat body, respectively. Larvae

fed on 0.0004% dose level of pyrethrum extract showed a maximum reduction in the glycogen content in haemolymph (12% of the control) and fat body (23% of the control). Glycogen levels in haemolymph were reduced to 69% (1.717 mg/g), 32% (0.796 mg/g) and 12% (0.299 mg/g) of the control value while these levels, in fat body, were reduced to 62% (9.223mg/g), 38% (5.653mg/g) and 23% (3.421mg/g) of the control value following treatment with 0.0001%, 0.0002% and 0.0004% of the pyrethrum extract, respectively (Table 1) .

The level of reducing sugar, in control larvae, was 2.812 and 1.051 mg/g in haemolymph and fat body, respectively. The maximum enhancement in the level of reducing sugar in haemolymph (194% of the control value) and fat body (218% of the control value) was observed in larvae treated with 0.0004% of pyrethrum extract. Reducing sugar levels, in haemolymph, were enhanced to 140% (3.937 mg/g), 177% (4.977 mg/g) and 194% (5.455 mg/g) of the control value while these levels, in fat body, were increased to 136% (1.429 mg/g), 176% (1.850 mg/g) and 218% (2.291 mg/g) of the control value following treatment with 0.0001%, 0.0002% and 0.0004% of the pyrethrum extract, respectively (Table 1).

Pyrethrum extract caused a significantly dose-dependent ($P < 0.05$) enhancement in the activity of acid phosphatase and a significantly dose-dependent ($P < 0.05$) decrease in the activity of alkaline phosphatase in both the tissues of larva (Table 2).

In the control larvae, the acid phosphatase activity was 0.621 and 2.598 µmoles/30 min/mg protein in haemolymph and fat body, respectively. The maximum enhancement in the acid phosphatase activity in haemolymph (430% of control value) and fat body (339% of control value) was observed in larvae treated with 0.0004% of pyrethrum extract. Acid phosphatase activity in haemolymph was enhanced to 187% (1.161 µmole), 254% (1.577 µmole) and 430% (2.670 µmole) of the control value while its activity in fat body was enhanced to 153% (3.975 µmole), 233% (6.053 µmole) and 329% (8.547 µmole) of the control value following treatment with 0.0001%, 0.0002% and 0.0004% of the pyrethrum extract, respectively (Table 2).

The alkaline phosphatase activitiy in control larvae was 0.480 and 2.618 µmoles/30 min/mg protein in haemolymph and fat body, respectively. The maximum decrease in the alkaline phosphatase activitiy in haemolymph (33% of control) and fat body (39% of control) was observed in larvae treated with 0.0004% of pyrethrum extract. Alkaline phosphatase activitiy in haemolymph was reduced to 76% (0.365 µmole), 53% (0.254 µmole) and 33% (0.158 µmole) of its control value while the activity of this enzyme in fat body was reduced to 80% (2.094 µmole), 60% (1.571 µmole) and 39% (1.021 µmole) of its control value following treatment with 0.0001%, 0.0002% and 0.0004% of the pyrethrum extract, respectively (Table 2).

Discussion

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 pyrethrum extract caused a significantly dose-dependent ($P < 0.05$) decrease in glycogen level and a significantly dose-dependent ($P < 0.05$) enhancement in reducing sugar level in haemolymph as well as fat body tissues of the larva of this pest. A drastic reduction (93.38 %) in carbohydrate content 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,

Table 1: 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 pyrethrum extract

Per cent pyrethrum extract concentration	Glycogen# (mg/g. wet wt.)		Reducing sugar# (mg/g. wet wt.)	
	Hamolymph	Fat body	Haemolymph	Fat body
Control (untreated)	2.489 ± 0.099 (100)	14.875 ± 0.528 (100)	2.812 ± 0.118 (100)	1.051 ± 0.056 (100)
0.0001	1.717 ± 0.084 (69)	9.223 ± 0.521 (62)	3.937 ± 0.266 (140)	1.429 ± 0.114 (136)
0.0002	0.796 ± 0.059 (32)	5.653 ± 0.322 (38)	4.977 ± 0.344 (177)	1.850 ± 0.128 (176)
0.0004	0.299 ± 0.021 (12)	3.421 ± 0.214 (23)	5.455 ± 0.392 (194)	2.291 ± 0.164 (218)

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 pyrethrum extract was dose dependent $P < 0.05$.

Table 2: Changes in acid and alkaline phosphatase activity in haemolymph and fat body of the larva of rice-moth, *C. cephalonica* treated with pyrethrum extract

Per cent pyrethrum extract concentration	Acid phosphatase#		Alkaline phosphatase#	
	Hamolymph	Fat body	Haemolymph	Fat body
Control (untreated)	0.621 ± 0.044 (100)	2.598 ± 0.110 (100)	0.480 ± 0.017 (100)	2.618± 0.107 (100)
0.0001	1.161 ± 0.058 (187)	3.975 ± 0.168 (153)	0.365 ± 0.018 (76)	2.094 ± 0.118 (80)
0.0002	1.577 ± 0.055 (254)	6.053± 0.207 (233)	0.254± 0.012 (53)	1.571 ± 0.127 (60)
0.0004	2.670 ± 0.062 (430)	8.547 ± 0.254 (329)	0.158 ± 0.009 (33)	1.021 ± 0.088 (39)

The activities are given as µmoles of p-nitrophenol liberated per 30 min per mg of protein and expressed as mean ± SE of six replicates.

Values in the parentheses are 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 $P < 0.05$.

Schistocerca gregaria when exposed with *Metarhizium anisopliae* and by Razak and Sivasubramanian (2007) in *Chelomenus sexmaculata* Fabricius and *Chrysoperla carnea* Stephens treated with three botanical oils -- neem, pungam and madhuca. The findings of the present study 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

pyrethrum extract stress. The depletion in glycogen level may also be due to a direct action of pyrethrum extract 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 has been shown in rabbits treated with organophosphorus pesticides (Stitcher *et al*., 1975).

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/ synthetic pyrethroids have been reported to influence the activities of acid and alkaline phosphatases in insects (Naqvi *et al*.,1991; Josephrajkumar *et al*., 1999; Shukla, 2011; Pathak, 2012; Pathak and Tiwari, 2015a, 2016, 2017b, 2017c). In the present study, sub-lethal doses of pyrethrum extract caused a significantly dose-dependent enhancement in acid phosphatase activity and a significantly dose-dependent reduction in alkaline phosphatase activity in both the tissues of the larva. Similar results have also been observed in paddy borer insect *Oxycarenus lugubris* when exposed to neem compounds - RB-a, RB-A and Magosan-O™ (Nurulain, 1987); *Helicoverpa armigera* treated with plumbagin and azadirachtin (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). However, these studies provide no relevant explanation regarding the mode of action of plant extracts/biopesticides about the activity of phosphatase. Natural plant products / synthetic pyrethroids like *Dryopteris filix*-mas root and rhizome extracts, their powders (Shukla, 2011), Bioresmethrin (Pathak, 2011; Pathak and Tiwari, 2015a), neem products (Pathak, 2011), neem stem bark powder (Pathak and Tiwari, 2016), neem seed ethanol extract (Pathak and Tiwari, 2017b) and neem seed acetone extract (Pathak and Tiwari, 2017c) exerted a promising effect altering the phosphatase activities in larval haemolymph and fat bodies tissues of *C. cephalonica.* It deserves mention that in the present investigation pyrethrum extract caused a fast deviation in the activity of acid phosphatase in haemolymph in comparision to fat body tissues. An enhancement in acid phosphatase activity with reduction in alkaline phosphatase activity during *in vivo* treatment of pyrethrum extract may be due to the interaction of several reactions occurring simultaneously, causing direct or indirect stress on these phosphatase activities in haemolymph and fat body tissues of the larva of *C. cephalonica*.

Conclusion

From the present investigation, it may be concluded that pyrethrum extract induced alterations in the carbohydrate levels and phosphatase activities in haemolymph and fat body tissues and impairs the metabolic framework of larva that results into biochemical perturbations leading to death.

So, application of pyrethrum extract is of course beneficial for the effective control of rice-moth, *C. cephalonica* in particular and lepidopterous pests in general in ecofriendly way.

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References

Abdul Razak T and Sivasubramanian P. (2007) Effects of three botanical oils on carbohydrate content in *Cheilomenes sexmaculata* Fabricus and *Chrysoperia carnea* Stephans. Asian J. Biochem. 2: 124-129.

Abou-Donia MB. (1978) Increased acid phosphatase activity in hens following an oral dose of Leptophos. Toxicology Letters 2: 199-203.

Akhtar Y and Islam MB. (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 MA and Szcypinski AJ. (1947) Use of p-nitrophenylphosphate as the substrate in the determination of serum acid phosphatase. Am. J. Clin. Pathol. 17: 571-574.

Atwal AS. (1976) Agricultural pests of India and South-East Asia*,* pp 502, Kalyani Publishers, Delhi.

Ayyar TVR. (1919) Some insects recently noticed as injurious in South India. Report Proc. 3rd Entomol. Meet. Pusa 1: 314-328.

Basiouny AL, Sh Hamadah Kh and Tanani AM. (2010) Efficacy of wild plant *Fagonia bruguieri* (Zygophyllaceae) on acid and alkaline phosphatase activities in the desert locust *Schistocerca gregaria* (Orthoptera:Acrididae). Egypt. Acad. J. Biolog. Sci. 2:1-10.

Becker NH and Barron KD. (1961) The cytochemistry of anoxic and anoxic ischemic encephalopathy in rats. Am. J. Pathol. 38: 161-175.

Belmain SR, Neal GE, Ray DE and Golop P. (2001) Insecticidal and vertebrate toxicity associated with ethno botanicals used as post-harvest proctants in Ghana. Food Chemical Toxicology 39: 287-291

Bream AS. (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.

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.

Dalela RC, Bhatnagar MC and Verma SR. (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 JR. (1970) *In vivo* glucose metabolism in the normal and poisoned cockroach, *Periplaneta americana*. J. Insect Physiol. 16: 2137- 2156.

Folin O and Wu H. (1920) A system of blood analysis: Supplement I. A simplified and improved method for determination of sugar. J. Biol. Chem. 41: 367-374.

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

Herford GVB. (1933) The more important pests of cacao, tobacco and dried fruit. Bull. Imp. Inst. 31: 39-55.

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

Josephrajkumar A, Subrahmanyam B and Srinivasan (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.

Kim S, Roh JY, Kim DH, Lee HS and Ahn YJ. (2003) Insecticidal activities of aromatic plant extracts and essential oils against *Sitophilous oryzae* and

Callosobruchus chinensis. J. Stord. Prod. Res. 39: 293-303.

Krishna SS and Pandey GC. (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.

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

Munro JW and Thompson WS. (1929) Report on insect infestation of Cacao. Emp. Marketing Bull. 24.

Naqvi SNH, Akhtar K and Azami MA. (1991) 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 SNH, Nurulain SM and Tabassum R. (1991b) Effect of neem fraction and pyrethroid on the nucleic acid of *Musca domestica* L. Proc. Ist Nat. Biochem. Symp. pp. 21.

Noyes WM. (1930) Moth pests in cocoa and confectionery. Bull. Ent. Res. 21: 77-121.

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.

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 CS. (2011) Studies on the effect of certain natural plant products and syntheticpyrethroids on the chemistry of haemolymph and fat body of the larva of rice-moth, *Corcyra cephalonica* Staint. (Lepidoptera: pyralidae). Ph.D. Thesis, Dept. of Zoology, DDU Gorakhpur Univ. Gorakhpur, U.P., India.

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

Pathak CS 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 CS and Tiwari SK. (2015a) 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 CS and Tiwari SK. (2015b) 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 CS and Tiwari SK. (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 CS and Tiwari SK. (2017a) 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 CS and Tiwari SK. (2017b) Influence of neem seed ethanol extract against the larval biochemistry of rice moth, *Corcyra cephalonica* Staint. (Lepidoptera: Pyralidae) Intern. J. Zool. Invest. 3: 155-174.

Pathak CS and Tiwari SK (2017c) Influence of neem seed acetone extract on the larval biochemistry of rice moth, C*orcyra 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 W and Bernard Philogene JR. (1993) A natural path to pesticides. IDRC Reports (Canadian Collaboration for development). 21: 760.

Pilo B, Ansari MV and Shah RV. (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.

Piltz H. (1977) *Corcyra cephalonica* (Staint.) In: J. Kranz, (H. Schmutterer and W. Koch. (eds.). Disease pests and weeds tropical crops. 439-440 pp. Verlag Paul Parey, Berlin and Hamburg.

Ragonot E L. (1885) Revision of the British species of Phycitidae and Galleriidae. Entomological monthly Mag. 22: 17-32.

Chand Ramesh and Pratap SB. (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.

Richards OW and Herford GVP. (1930) Insects found associated with Cacao, spices and dried fruits in London warehouses. Ann. App. Boil. 17: 367-395.

Shukla S. (2011) Studies on the effect of certain natural plant products 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.

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

Shukla S and Tiwari SK. (2011b) 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 SK. (2011c) 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 SK. (2012) The influence of pyrethrum extract on the developmental stages of the rice-moth, *Corcyra cephalonica* Stainton (Lepidoptera: Pyralidae). Egyptian J. Biol. 14: 57-6.

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

Sokal RR, and Rohlf FJ. (1969) Introduction to Biostatistics. W.H. Freemann and Co. San Franscisco.

Stainton HT. (1866) Description of a new species of family Galleriidae. Entomological monthly Mag. 2:172-173.

Stitcher DL, Harris LW and Garry VF. (1975) Fed. Proc. 34: 737: Cited by, Singhal RL. (1979)“Cyclic AMP in environmental toxicology”. Trends Pharmacological Sciences, December, 100-102.

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

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

Vijayaraghavan 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.

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

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