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RESEARCH ARTICLE

Effect of Phytopesticide Piperidine on Biochemical changes in the adult male insect *Odontopus varicornis* (Heteroptera: Pyrrhocordiae)

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ABSTRACT

Glucose, glycogen, protein, amino acid and lipid are the major components of the body play an important role in the body construction and energy metabolism. The biochemical estimation were made in the tissues of the fat body, testis, seminal vesicle, vas deferens and male accessory reproductive glands (MARGs). The present study was find out the effect of (phytopesticide) Piperidine on *Odontopus varicornis.* The insects were exposed to Piperidine for 48h of duration differ significantly, the sub lethal concentration for Piperidine was found to be about 0.15%for 48 hours LD_{50}. The glycogen, protein, lipid content appears to be decreased in the treated insects when compared to the control insects and glucose and amino acid content appears to be increased in the treated insects when compared to the control insects. The values of mean glucose, glycogen, protein, amino acid and lipid content of the fat body, testis, seminal vesicles, vas deferens and MARGs of control and treated insects are compared for significance of difference for which t – values are calculated. The observed t-values are significant at 0.05 level.

INTRODUCTION

The use of synthetic chemical pesticide on a crop can be very costly for the farmer and can also produce environmental pollution (Embrapa, 1992). Natural products with potential insecticidal activity have been considered as an alternatives to conventional synthetic pesticides used in agriculture. Plant secondary metabolites such as pyrethrins, rotenoids and limonoids have interesting ecological advantages, particularly with respect to their short lifetimes in the environment (Johansen, 1977; Johansen and Mayer, 1990). The widespread application of insecticides in household products and public health programs create a major concern due to their effects on human and environmental health. Certain synthetic pesticides provide a broad range of toxic effects (Isman and Machial, 2006). Insecticides have caused significant negative effects on the non-target organisms; therefore, a toxicity evaluation is important (Celik and Suzek, 2008). Proliferations of research were carried out to produce insecticides from natural product as alternatives to synthetic insecticides in order to reduce their negative health impacts. The use of natural products insecticide is environmentally desirable and economically profitable (Mittal and Subbarao, 2003). As such, the use of plants for insecticidal purpose is becoming more popular due to virtually non-existent adverse effects.

Alkaloids are one of the most important groups of natural products, i.e. compounds that are synthesised by the secondary metabolism of living organisms. These molecules play an important roles in the species that synthesis them but are not essential for life, unlike products of primary metabolism, e.g. nucleosides, amino acids, carbohydrates or lipids (Mann 1994 and Clayden *et al.,* 2001). Although rare in mammals, alkaloids are particularly abundant in higher plants, insects, amphibians and fungi. Within these species, alkaloids are synthesised to act as poisons or anaesthetics for predators, or even as mediators of ecological interactions. In any case, their purpose is to increase the chances of survival. The medicinal plants are an attractive alternative for pest management because they pose low threat to the environment or to human health compared to synthetic insecticides (Moreira *et al.,* 2007). Insect pests are a major constraint on crop production, especially in developing countries. Natural plant extracts play an increasingly prominent role as alternatives to synthetic pesticides due to the increasing concern on health hazards, environmental pollution and negative effects on non target organisms (Sharma *et al.,* 2006).

Many plant derived molecule have shown a promising effect in therapeutics (Lokhande *et al.,* 2007). Among the plants investigated to date, one showing enormous potential is the pepper family otherwise known as Piperaceae (Dodson *et al.,* 2000). *Piper longum, Piper nigrum* and *Piper cubeba* is a flowering vine in the family Piperaceae. *P. longum* (long pepper) is a small shrub large woody root and numerous creeping, jointed stem, thickened at the node. The fruit contain 1% volatile oil, resin, a waxy alkaloid. It is used for several medicinal properties. It has much pharmacological action such as antifungal, anti-inflammatory, antioxidant and anti cancer effect (Atal *et al.,* 1985) and it is known to have insecticidal activity against mosquitoes, bugs, beetles and flies (Miyakado *et al.,* 1989). Carbohydrates together with proteins and lipids are the principle class of organic compounds found in insect as well as other organisms. In most insects, carbohydrates reserves are present as glycogen and trehalose, which can be readily converted into glucose (Islam and Roy, 1981). Carbohydrates provide instant energy for activities such as flight in most of the insects. Trehalose, which is a non-reducing

insects, also function as an important reserve of carbohydrate in them (Keeley 1985 and Siegert, 1995).
Proteins are the complex organic nitrogenous compounds. The building blocks of protein are the amino acids. Amino acids are utilized for the production of hormones and enzymes, the

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composition of protein in the body as a whole may be greatly modified (Wigglesworth, 1979). In addition to sugars and lipids, insects use true amino acids as they are readily available source of respiratory fuels (Padmanabhan 1992 and Selvisabhanayam, 1995). The fundamental structural unit of protein is the amino acids. Most of the non essential amino acids can be synthesized from simple amino acids. Amino acids, which cannot be synthesized in the animals body, are called essential amino acids and must be supplemented only through diet (Gilmour 1961; Padmanabhan 1992; Selvisabhanayakam, 1995).

Lipids are the chief form in which energy is stored in insects. The ability to synthesize lipids for storage is widespread, but except for specific item as small amounts they are not usually essential constituents of their diet. Insects utilize lipids and can also synthesize from proteins and carbohydrates. However, certain species cannot synthesize certain fatty acids like linolieic and linolenic acids. Hence, these species depend on exogenous source for these fatty acid requirements (Rock Stein, 1978). Lipids are water soluble, low weight, high-energy compounds that can be extracted from cells and tissue by non polar solvents like chloroform, methanol, or benzene, which play vital roles in the life cycle of insect. Insect growth hormones, pheromones and sex attractants are lipoidal in nature (Gillbert, 1967).They are also important constituents of cell membrane (Robbins *et al*., 1971).

It has been established that lipids provide the energy reserve, which can be used during starvation periods or in some insects such as *Schistocera gregaria* during sustained flight activity Chapman, (1982). Kilby, (1963) has suggested that the lipid synthesis take place in the fat body and elsewhere from amino acids, sugars and simple fatty acids. Thus, lipids act as an alternative source of energy. It is evident from the above studies that the information regarding the effect of phytopesticide on biochemical changes in insect tissues (fat body, testis, seminal vesicle, vas deferens and MARGs) are meagre. Hence, an attempt has been made to find out the effect of Piperidine on biochemical changes with special reference to lipid metabolism on *Odontopus varicornis.*

MATERIALS AND METHODS

Insects

The insects collected from the fields and gardens were reared in wooden cage, each measuring about 30 × 22 × 28cm at the laboratory temperature of 29 ± 2°C and relative humidity of 80 ± 5%. The insects were fed daily with soaked cotton seeds (*Bombax ceiba*) as well as with seeds of its higher plant, *Stericulia foetida* and *Gossypium* sp. An additional food of the pieces of chow-chow (*Sechium edule*) was also given to these insects. The insect's cage was cleaned properly, every alternative day, by removing the excreta and other waste materials. The egg laid by them was transferred to another cage, and thus a continuous culture was maintained.

Piperidine

Piperidine was purchaged from Aldrich chemical company (Molecular formula: $C_5H_{11}N$,

Density: 0.8629 g/mol, Molecular wt: 85.15 g/mol, Bolling point: 106ºC) and used as such without purification.

Biochemical studies

Adult male insects were collected from rearing cages and vivisected in insect Ringer solution (Ephrussi and Beadle, 1936). The following biochemical estimations were made in the tissues of fat body, testis and vasa deferens, seminal vesicle and MARGs. The colorimetric method of Kemp and Kits Van Heijninger (1954) was employed for the quantitative estimation of glucose and glycogen. The colorimetric method of Lowery *et al.* (1951) was employed for the quantitative estimation of protein. The colorimetric method of Moore and Stein, (1954) was adopted for the quantitative estimation of total free amino acids and lipid content was estimated by the semi micro determination method of Pande *et al*. (1963).

RESULTS

In the present study, the significant changes in the content of glycogen, glucose, protein, amino acid and lipid of fat body, testis, seminal vesicle, vas deferens and MARGs have been observed in the insects treated with sub lethal concentration 0.15% for 48 hours of phytopesticide, Piperidine. The glycogen content of the fat body, testis, seminal vesicle, vas deferens and MARGs of control and treated insects are presented in (Table-1 Fig.1). The glycogen content of the fat body, testis, seminal vesicle, vas deferens and MARGs of control insects were found to be 6.06 ± 0.31, 5.09 ± 0.25, 3.22 ± 0.18, 6.18 ± 0.22 and 5.41 ± 0.34 µg/mg, respectively. Likewise, glycogen contents of the fat body, testis, seminal vesicle, vas deferens and MARGs treated insects indicate 5.01 ± 0.20, 3.18 ± 0.07, 1.15 ± 0.33, 4.17 ± 0.05 and 3.09 ± 0.06 µg/mg, respectively. Glycogen content appear to be decreased in the treated insects when compared to the control insects. The values of mean glycogen content of the fat body, testis, seminal vesicle, vas deferens and MARGs of control and treated insects are compared for significance of difference and the calculated t – values are presented in (Table-1). The observed t-values are significant at 0.05 levels.

Table 1.Glycogen content of fat body, testes, seminal vesicle, vas deferens and MARGs of control and phytopesticide, Piperidine treated adult male insects, *Odontopus varicornis*

Tissues	Control (µg/mg)	Treated (µg/mg)	Percentage change over control	t - value
Fat body	6.06±0.31	5.01±0.20	17.32	2.853*
Testis	5.09±0.25	3.18±0.07	37.52	7.346*
Seminal vesicle	3.22±0.18	1.15±0.33	64.56	5.594*
Vas deferens	6.18±0.22	4.17±0.05	32.52	9.136*
MARGs	5.41±0.34	3.09±0.06	42.88	6.823*

Mean ± standard error of six observations
*Significant at 0.05 level.

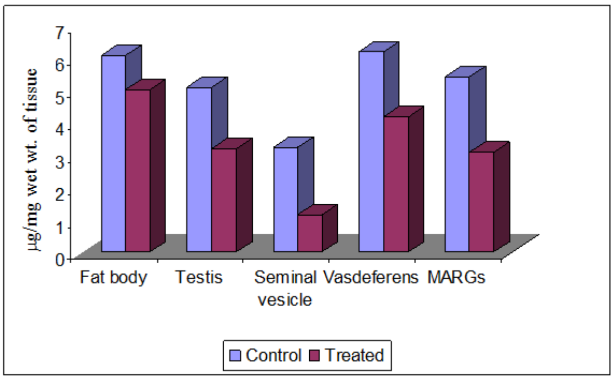

Fig.1. Glycogen content of fat body, testis, seminal vesicle, vas deferens, and MARGs of control and phytopesticide, Piperidine treated adult male insects, *Odontopus varicornis*

The glucose contents of the fat body, testis, seminal vesicle, vas deferens and MARGs of control and treated insect are presented in (Table-1 Fig.1). The glucose contents of the fat body, testis, seminal vesicle, vas deferens and MARGs of control insects are found to be 3.01 ± 0.05, 5.08 ± 0.19, 5.18 ± 0.22, 3.10 ± 0.33 and 5.22 ± 0.18 µg/mg, respectively. Likewise, glucose contents in the fat body, testis, seminal vesicle, vas deferens and MARGs of the treated insects indicate 4.55 ± 0.24, 6.86 ± 0.52, 8.50 ± 0.44, 5.46 ± 0.48 and 6.08 ± 0.77 µg/mg, respectively. Glucose content appears to be increased in the treated insects when compared to the control insects. The values of mean glucose content of the fat body, testis, seminal vesicle, vas deferens and MARGs of control and treated insects are compared for significance of difference and the calculated t – values are presented in (Table-2). The observed t-values are significant at 0.05 level.

Table 2. Glucose content of fat body, testis, seminal vesicle, vas deferens, and MARGs of control and phytopesticide, Piperidine treated adult male insects, *Odontopus varicornis*

Tissues	Control (µg/mg)	Treated (µg/mg)	Percentage change over control	t - value
Fat body	3.01±0.05	4.55±0.24	-51.16	-3.142*
Testis	5.08±0.19	6.86±0.52	-35.43	-3.157*
Seminal vesicle	5.18±0.22	8.50±0.44	-64.09	-6.775*
Vas deferens	3.10±0.33	5.46±0.48	-98.00	-4.338*
MARGs	5.22±0.18	6.08±0.77	-16.47	-1.088*

Mean ± standard error of six observations
* Significant at 0.05 level.

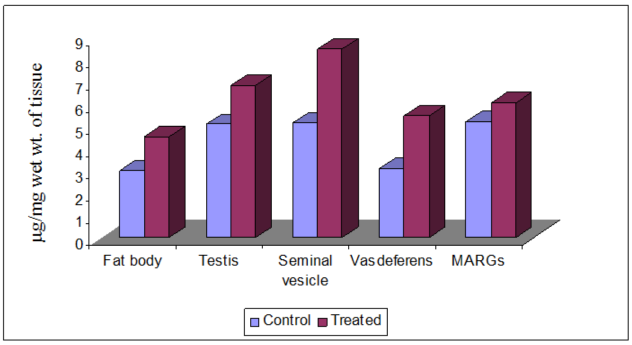

Fig.2. Glucose content of fat body, testis, seminal vesicle, vas deferens and MARGs of control and phytopesticide, Piperidine treated adult male insects, *Odontopus varicornis*

The protein contents of the fat body, testis, seminal vesicle, vas deferens and MARGs of control and treated insects are presented in (Table-3 and Fig. 3). The protein contents of the fat body, testis, seminal vesicle, vas deferens and MARGs of control insects are found to be 55.22 ± 1.77, 28.30 ± 1.08, 50.18 ± 2.19, 54.70 ± 2.80 and 85.77 ± 2.79 µg/mg, respectively. Likewise the protein contents of the fat body, testis, seminal vesicle, vas deferens and MARGs of treated insects are found to be 44.79 ± 1.98, 18.70 ± 1.06, 36.18 ± 1.96, 44.80 ± 2.04 and 72.18 ± 2.50µg/mg, respectively. The protein content appears to be decreased in the treated insects when compared to the control insects. The values of mean protein contents of the fat body, testis, seminal vesicle, vas deferens and MARGs of control and treated insects are compared for significance of difference and the calculated t – values are presented in (Table-3). The observed t – values are significant at 0.05 level.

Table 3. Protein content of fat body, testis, seminal vesicle, vas deferens, and MARGs of control and phytopesticide, Piperidine treated adult male insects, *Odontopus varicornis*

Tissues	Control (µg/mg)	Treated (µg/mg)	Percentage change over control	t - value
Fat body	52.22±1.77	44.79±1.98	18.88	3.935*
Testis	28.30±1.08	18.70±1.06	33.92	6.357*
Seminal vesicle	50.18±2.19	36.18±1.96	27.89	4.778*
Vas deferens	54.70±2.80	44.80±2.04	17.91	2.832*
MARGs	85.77±2.79	72.18±2.50	15.84	3.633*

Mean ± standard error of six observations
*Significant at 0.05 level.

The amino acid content in the fat body, testis, seminal vesicle, vas deferens and MARGs of control and treated insects are presented in (Table-4 and Fig. 4). The amino acid content of the fat body, testis, seminal vesicle, vas deferens and MARGs of control insects found to be 86.44 ± 2.16, 51.06 ± 2.06, 70.19 ±2.55, 68.73 ± 3.19 and 100.01 ± 3.19 µg/mg, respectively. Likewise, amino acid content of the fat body, testis, seminal vesicle, vas deferens and MARGs of treated insects are found to be 109.40 ± 3.66, 81.33 ± 2.71, 88.65 ± 2.50, 83.79 ± 3.19 and 122.30 ± 3.51 µg/mg, respectively. Amino acid

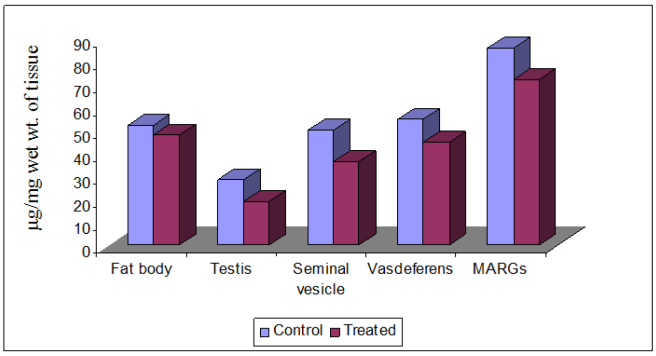

Fig.3. Protein content of fat body, testis, seminal vesicle, vas deferens and MARGs of control and phytopesticide, Piperidine treated adult male insects, *Odontopus varicornis*

content appears to be increased in the treated insects when compared to the control insects. The values of mean amino acid contents of the fat body, testis, seminal vesicle, vas deferens and MARGs of control and treated insects are compared for significance of difference and the calculated t – values are presented in (Table-3). The observed t – values are significant at 0.05 level.

Table 4. Amino acid content of fat body, testis, seminal vesicle, vas deferens and MARGs of control and phytopesticide, Piperidine treated adult male insects, *Odontopus varicornis*

Tissues	Control (µg/mg)	Treated (µg/mg)	Percentage change over control	t - value
Fat body	86.44±2.16	109.40±3.66	-26.56	-5.415*
Testis	51.06±2.06	81.33±2.71	-59.28	-8.902*
Seminal vesicle	70.19±2.55	88.65±2.50	-26.30	-5.170*
Vas deferens	68.73±3.19	83.79±3.19	-21.90	-3.339*
MARGs	100.07±3.19	122.30±3.51	-22.28	-4.702*

Mean ± standard error of six observations
*Significant at 0.05 level.

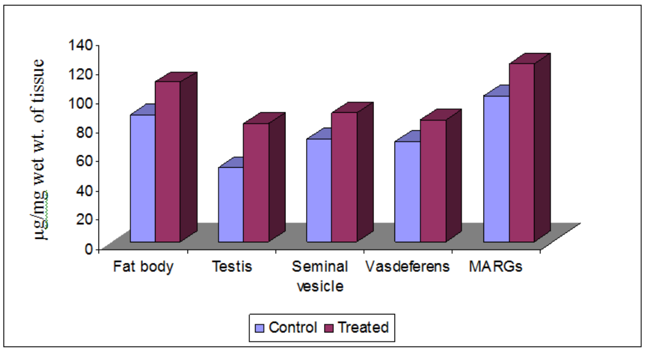

Fig.4. Amino acid content of fat body, testis, seminal vesicle, vas deferens and MARGs of control and phytopesticide, Piperidine treated adult male insects, *Odontopus varicornis*

The lipid contents of the fat body, testis, seminal vesicle, vas deferens and MARGs of control and treated insects are presented in (Table-5 and Fig.5). The lipid contents of the fat body, testis, seminal vesicle, vas deferens and MARGs found to be 12.66 ± 1.71, 10.80 ± 1.16, 9.30 ± 0.80, 8.14 ± 0.55 and 9.14 ± 0.61 µg/mg, respectively. Likewise, lipid content of the fat body, testis, seminal vesicle, vas deferens and MARGs of treated insects are found to be 9.85 ± 0.66, 8.77 ± 0.59, 7.55 ± 0.69, 7.06 ± 0.77 and 7.14 ± 0.54 µg/mg, respectively. Lipid content appears to be decreased treated insects when compared to the control insects. The values of mean lipid contents of the fat body, testis, seminal vesicle, vas deferens and MARGs control and treated insects are compared for significance of difference and the calculated t-values are presented in (Table-5). The observed t–values are significant at 0.05 levels.

Table 5. Lipid content of fat body, testis, seminal vesicle, vas deferens and MARGsof control and phytopesticide, Piperidine treated adult male insects, *Odontopus varicornis*

Tissues	Control (μg/mg)	Treated (μg/mg)	Percentage change over control	t - value
Fat body	12.66±1.71	9.85±0.66	22.19	1.535*
Testis	10.80±1.16	8.77±0.59	18.29	1.560*
Seminal vesicle	9.30±0.80	7.55±0.69	18.81	1.666*
Vas deferens	8.14±0.55	7.06±0.77	13.26	1.148*
MARGs	9.14±0.61	7.14±0.54	21.88	2.469*

Mean ± standard error of six observations
* Significant at 0.05 level.

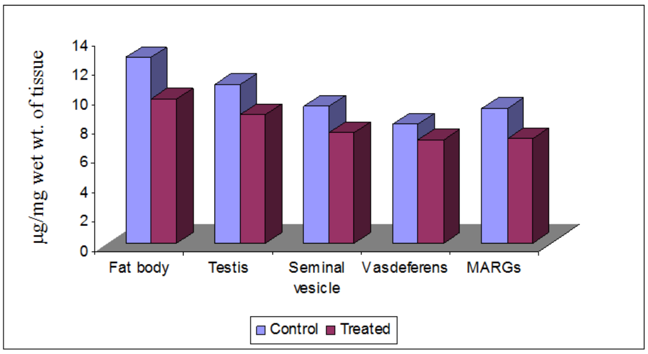

Fig.5. Lipid content of fat body, testis, seminal vesicle, vas deferens and MARGs of control and phytopesticide, Piperidine treated adult male insects, *Odontopus varicornis*

DISCUSSION

Glycogen is an important nutrient reserve in animal tissue and it is used as an immediate energy source when required by any animals. Therefore, glycogen is an essential component of the normal metabolism (Thunberg and Manchester, 1972). Gilmour, (1961) has reported that the pesticide R-20458 when administered to the larvae of *Spodoptera litura,* the carbohydrate content was decreased. Dimethoate application also caused decrease in the glycogen content in *Odontopus varicornis* (Jayakumar, 1988). Prakash *et al.* (1990) have reported that the quantity of carbohydrate in the ovary and fat body was decreased in the endosulfan treated *Poecilocerus pictus*). However, the increased glycogen level has been observed in the fat body as well as haemolymph of *Laccotrephes ruber* when exposed to monocrotophos Ravichandran, (1996) and mercuric chloride Palanisamy, (1997). Similarly, Tiruvasagam, (1994) and Ramanathan, (1995) have reported a decreased quantity of glycogen and increased quantity of glucose in *Aspongopus janus* and *Periplaneta americana* when these insects exposed to nimbecidine and *Pongamia glabra* leaf extract, respectively. Glucose, a reliable and immediate source of energy is available in all types of tissues and it can be mobilized from glycogen stores at the time of energy requirement and oxidized to co_2 and water and release energy in the form of ATP molecules (Boell, 1965).

As protein are the most important organic constituents of animal tissues, they play an important role in energy production. Normally, tissue proteins in terrestrial insects under toxic stress are known to play a vital role in the activation of compensatory mechanism (Wigglesworth, 1972 and Downer, 1982). Jayakumar, (1988) has reported that the protein content was decreased in the tissues of testes, accessory reproductive glands and fat body of *Odontopus varicornis* when treated with dimethoate. Pazhanichamy, (1997) has reported a sudden depletion of protein content and increased amino acid content in *Laccotrephes ruber* when exposed to heavy metal mercury. Vijayaprabha, (1990) has also reported that the protein content was decreased and amino acid content was increased in the brain, fat body and haemolymph of *Catacanthus incarnatus* when exposed to the sub lethal concentration of dimethoate. Amino acids are the important components of protein and serve as a precursor for many biological compounds. In addition to the dietary proteins, the animal body also synthesizes its own protein from the available free amino acids obtained as a result of proteolysis. Jayakumar, (1988) has reported decreased protein content and an increased amino acid level in *Odontopus varicornis* when treated with dimethoate. It is thought that the high level of amino acids formed by proteolysis in the tissues are transported into the haemolymph and then to the metabolic pathway by the pyruvate, which directly enter in to the TCA cycle in the form of keto acids to provide extra energy during the stress period.

Lipids play an important role in maintaining integrity of cell structure and functions. Normally all animals depend upon the lipid content to overcome the physiological stress caused by toxicants or environmental contamination (Prakash *et al*., 1990 and Jamil and Hussian, 1992). Sastry and Siddiqui, (1984) have reported some biochemical and histochemical changes in the fresh water teleost *Channa punctatus* exposed to sublethal concentrations of quinolphos. Fluctuation of lipid content in different species of insects treated with different toxicants has been reported by several investigators. Copuzzo and Lancaster, (1981) have shown a significant decrease of lipid content in the fat body of *Homarus americanus* when exposed to toxicants. The same trend has been observed in carbohydrate, protein and lipids by *Aspongopus janus* when treated with nimbeciline *(*Thiruvasagam, 1994*), Periplaneta americana* when treated with *Pongamia glabra* leaf extract *(*Ramanathan, 1995*)* and *Laccotrephes ruber* when treated with monocrotophos (Ravichandran, 1996), *Gryllotalpa africana* when treated with endosulfan (Sumathi, 2001), *Laccotrephes ruber* when treated with zinc (Ramesh Kumar, 2004), *Sphaerodema rusticum* when treated with mercury (Rajathi, 2004), *Odontopus varicornis* when treated with pygidial secretion (Lousia, 2010) and *Mylabris indica* when treated with Vijay neem (Vivekananathan, 2011).

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