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"Laboratory analysis on sublethal impact of synthetic pesticides on the histology of midgut in *Callosobruchus chinensis* Linnaeus".

Corresponding Author: Dr. Shamim Ahmed Malik

Research Centre: Department of Zoology, Progressive Education Society's Modern College of Arts, Science and Commerce Ganeshkhind-16 Pune.

Co-Author: Sayed Zarin Sana

Research Centre: Department of Zoology, University of Kashmir Hazratbal Srinagar-190006

Dr. Shakira Inamdar
(Head and Associate Professor Department of Zoology),
Progressive Education Society's, Modern College of Arts Science and Commerce Ganeshkhind Pune 16.

1. Abstract

A laboratory study was carried out to evaluate the sublethal impact of synthetic pesticide on the histology of midgut in *Callosobruchus chinensis* L. This study reviews the sub-lethal impact of cypermethrin on the histology of midgut in *Callosobruchus chinensis* L. The experimental insect was subjected to a given sublethal dose of the selected pesticide. The exposure did not cause immediate death of the pest, but had delayed and prolonged sublethal impact on its midgut histology. The exposure caused progressive destructives changes in the histology of midgut of the experimental insect. The exposure disrupted the circular and longitudinal muscles in the midgut of the pest. The well-developed columnar epithelium of the midgut was altered. The peritrophic membrane of midgut which is usually attached with gut epithelium got constricted and detached from epithelial lining. It got highly degenerated and clustered at the centre of the gut cavity. The result of this study clearly demonstrate that the synthetic pesticide under investigation displayed significant sublethal impact on the histology of midgut in *Callosobruchus chinensis* L. Further the result of this study reveals that the selected pesticide can be used to control population of the experimental pest provided applied in controlled manner.

Key words: *Callosobruchus chinensis* L., Gut, Histology, Pest, Synthetic pesticides etc.

2. Introduction:

Like other physiological parameters of the insect and other animals, digestive system is one of the important physiological systems in insects. The main role of digestive system in insects is digestion and aabsorption of food. The digestive system in insects helps to extract nutrients and other subsances from the food it consumes (N. C. State University 2009). The food is mostly ingested in the form of macromolecules and other complex substances such as carbohydrates, proteins, fats and nucleic acids. The ingested food must be broken down into simpler, smaller and diffusible molecules before used by body tissues for energy source, growth and development and reproduction. This process of converting complex, non-diffusible food particles into simpler and diffusible molecules is called digestion and is carried out in the digestive system of the insect. The digestive system of an insect is a closed system with a long enclosed coiled tube called alimentary

canal or simply the gut. The alimentary canal runs lengthwise throughout the body. In insects the length of alimentary canal is generally assumed to be correlated with diet. The insects which consume higher protein diet have usually shorter gut (Pradhan 1939). The food is taken into the alimentary through mouth, and then gets processed as it travels toward the anus. The alimentary canal has specific sections for specific roles like storage of food, grinding of food, enzyme production and nutrient aabsorption (Elzinga et *al* 2003). The gut is provided with many sphincters which control movement of food and other substances between different regions of the gut. The alimentary canal is divided into three regions such as stomatodeum- the foregut, mesenteron- the midgut and proctodeum- the hindgut. In addition to the above regions the alimentary canal in insects contains paired salivary glands, which usually reside in the thorax adjacent to the foregut. These glands produce saliva which initiates break down of food in the buccal cavity (Duncan et al, 1939). The fore gut *and* hind gut are epidermal invaginations and are lined with cuticle called intima while as mesenteron lack cuticle. The mesenteron instead is lined with rapidly dividing epithelial cells (Richard et al, 2003). The movement of food down the gut is controlled by muscular *c*ontraction called peristalsis (Gullan et al, 2005).

In insects the mechanism of digestion and associated organs display great variations due to the variation in consumption of food type. The successful adaptation of an insect to a particular food as its source of nutrients, essential for growth, development, reproduction and population maintenance & requires a unique combination of behavioural, physiological and biochemical processes (Slansky 1982). According to several authors to study the histology of alimentary canal in different groups of beetles is a matter of interest (Mukherji et al, 1973, Talbot 1928, Miller 1961). The gut in beetles is divided into three regions such as foregut, midgut and hindgut. The foregut in beetles is generally short, straight tubular structure which can be divided into anterior short pharynx and a narrow tubular oesophagus. Proventriculus may be present at the point where foregut joins midgut (Gupta et al, 1971). The Proventriculus may act as grinding organ and in many other insects it may form a storage organ called crop. The crop is usually absent or poorly developed in beetle larvae and adults which are polyphagous in nature, where as it is generally present in adult beetles which are monophagous in nature (Crowson 1981). The second and most significant part of the insect gut called midgut is ectodermal in origin and lacks chitinous linings. Midgut in insects is considered as the chief site of digestion because, it secretes most of the digestive enzymes (Wigglesworth, 1972). Midgut in some adult coleopteran is long and coiled tube like structure (Schneider et al 1969). According to a study, it is reported that midgut in beetles shows three types of epithelial cells such as columnar epithelial cells, goblet cells and regenerative cells (Shinoda 1930). These cells are reported to perform different functions in different insects (Lewis 1926). The goblet cells are also reported in the midgut of other insects like Lepidopteron larvae and Trichopteran, Ephemeroptera orders (Chapman 1972). The third and final part of the alimentary canal in insects is hindgut. Anteriorly hind gut begins with ileum and posteriorly terminates exteriorly with the anus. Histologically hind gut is lined by a thin layer of permeable cuticle which prevents loss of useful substances (Maddrell et al, 1980).

Several studies have been carried out from time to time to study the histology of gut in several species of beetles (Kumar et al 1975, Helms 1972). However, some economically significant members of coleopteran have been relatively neglected so for from such studies. One of the economically significant members of coleopteran is *Callosobruchus chinensis* L. - the cowpea beetle. There is very negligible information available about the gut histology of *Callosobruchus chinensis* which is a grave concern for farmers as the beetle is a serious pest on legumes especially cow-pea seeds and green gram. The beetle is a major pest of cowpea and is responsible for maximum damage to the crop on field or under storage conditions. The larval forms of the experimental beetle are voracious feeders and feed on various varieties of legumes especially cowpea, green gram and chickpea. The beetle is known to cause severe damage to legume industry. Infestation of legumes with *Callosobruchus chinensis* L. not only reduces the quantity of legumes but also affects its quality which later results in declining its market value. Thus the experimental beetle is considered one of the detrimental pests of legume industry. Extensive research has been carried out on stored product beetles but the information available on the impact of synthetic pesticides on the mid gut histology of beetles is scanty. Therefore to overcome this issue the efforts were made to examine the sublethal impact of synthetic pesticide on the histology of midgut in *Callosobruchus chinensis* L. to provide necessary information about digestive mechanism which may be a useful tool to control its population.

3. Methodology

3.1 Place of research: The experiment to study the efficacy of synthetic pesticide on the histology of midgut in *Callosobruchus chinensis* L. was conducted in the laboratory of the Department of Zoology, Prof. Ramkrishna More Arts Commerce and Science College Akurdi Pune 411044, M.S. from August 2020 to August 2021.

3.2 Stock Culture of *Callosobruchus chinensis* L.: The *Callosobruchus chinensis* L. colony was raised from different collections of chickpea seeds brought from Dhanakwadi area of Yeola district Nasik Maharashtra during field visits and at times from a local market in Ahmednagar Maharashtra.

3.3 The test chemicals:

The designed synthetic pesticide was procured from a local Agrochemical Pesticide Dealer Sharad Krushi Seva Kendra 22, Vasant Talkies Road Market-yard Ahmednagar-414001(Maharashtra). The solutions of insecticides were prepared and were used for the experimental purpose. The Commercial formulation of Cypermethrin used in this experiment is (Cymbush 25% EC).

3.4 Preparation of dosage: To prepare 1000 ppm solution, 1 ml of liquid pesticide was added to 1 litre of distilled water. The prepared diagnostic assay solution was stored at room temperature and later used for the bioassay.

3.5 Treatment of Chickpea seeds with pesticides:

The Chickpea seeds were treated by soaking them in designed sublethal concentration of cypermethrin solution for a period of 30 minutes. After soaking these treated seeds were dried at room temperature for a period of 4-5 hours. Immediately after drying, these seeds were filled in wide mouth plastic jars followed by introduction of beetles into these jars. The jars were labelled and kept in the laboratory at a temperature of 25-27ºC with relative humidity 65-70% and a photoperiod of 14:10h (L.D). The jars were covered with muslin cloth on the top tied with rubber bands. The beetles were exposed to a given sublethal dose for a period of 24 hours. After successful treatment for 24 hours the beetles were removed from jars and were dissected to evaluate the sublethal impact of cypermethrin exposure on their midgut histology.

3.6 Preparation of tissues for histological studies: The adults of *Callosobruchus chinensis* L. collected from the jars treated with designed concentration of selected pesticide were dissected in chilled Insect Ringer solution under stereoscopic dissecting binocular microscope. The alimentary canals were removed and different parts of alimentary canal were fixed in Bouin's fixatives for 20 hours. After fixation the tissues were washed in tap water to remove excess fixative. Immediately after washing, the tissues were gradually dehydrated using ethyl alcohol. The gradual dehydration of tissues was done through a series of ethanol 30%, 50%, 70% and 100% for 30 minutes each. The tissues were cleared in xylene for one hour followed by infiltration and were embedded in paraffin wax at 50ºC. The tissues were sectioned at 5 to 6 µm thickness using microtome. The slides containing sections were stained with haematoxylin and eosin. After staining observations were recorded and microphotography was done.

4. Result and Discussion

Fig.i.Showing histology of midgut of before Cypermethrin treatment *C.chinensis* Linnaeus.

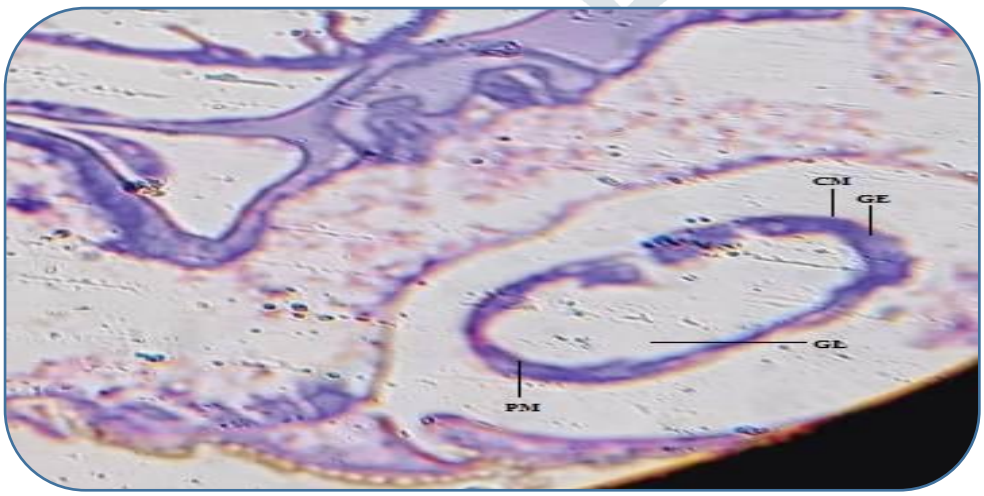
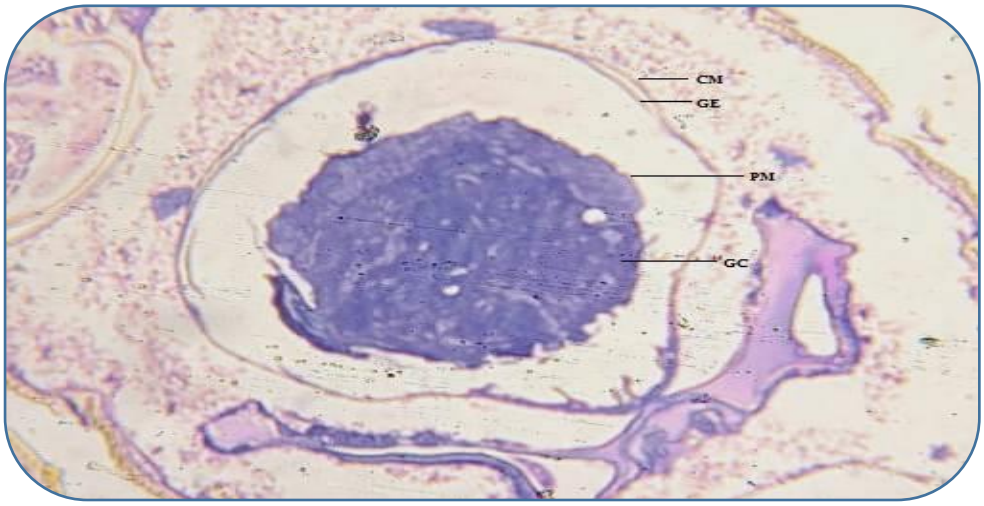


Fig. ii. Sublethal impact of Cypermethrin on the histology of midgut in *C.chinensis* Linnaeus.



Where

CM= Circular Membrane, GE= Gut Epithelium, PM= Peritrophic Membrane, GL= Gut Lumen, GC= Gut Contents.

The sublethal effect of cypermethrin on the histology of midgut in *Callosobruchus chinensis* L. was determined. The insect was exposed to a given sublethal dose of the selected pesticides. It was observed that cypermethrin at its designed sublethal concentration triggered progressive destructives changes in the histology of midgut of the experimental insect. The exposure disrupted the circular and longitudinal muscles in the midgut of the pest. The well-developed columnar epithelium of the midgut was altered. The peritrophic membrane of midgut which is usually attached with gut epithelium got constricted and detached from epithelial lining. It got highly degenerated and clustered at the centre of the gut cavity. The result of this study clearly demonstrate that cypermethrin at the designed sublethal dose displayed significant sublethal impact on the histology of midgut in *Callosobruchus chinensis* L.The promising result of this study approves the use of cypermethrin to control population of the experimental pest provided applied in controlled manner.

Significance: The knowledge of sub-lethal dose and its toxicity is a significant approach for better assessment of the toxic nature of a particular pesticide. This study may have great implication in predicting the sublethal toxic nature of the given pesticides. Such premonition should help us to take appropriate control measures against the given insect pest. This study is significant as it reviews the sub-lethal effects of synthetic pesticide on vital physiological parameter in *C.chinensis*, in order to update our knowledge on the current dose response status of insect pest and reassess the preference and design of the chemicals for pest control programme. Bioassay can be a significant tool for evaluation and study of diverse agricultural pesticides. This study can help to identify toxicants. Bioassay is an important method to help reassess the preference and design chemicals for effective pest control programme. This study can be useful to evaluate pesticides for their safety to pollinators, predators and other insects.

Recommendation: The synthetic pesticide tested for its sublethal toxicity against the experimental pest successfully declined the population of the insect pest. In this view the use of selected synthetic pesticides for the control of the experimental pest is recommended provided used in appropriate manner.

Ethical Standard: The experiment was conducted in an organised manner under controlled laboratory conditions to avoid any conflict with ethical standard.

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