International Journal of Agricultural Technology 2013 Vol. 9(3): 553-563
Available online http://www.ijat-aatsea.com
ISSN 2630-0192 (Online)

Insecticidal and histopathological effects of botanical formulations against *Helicoverpa armigera* (Hub.) (Lepidoptera: Noctuidae)

Soosaimanickam Maria Packiam[*], Kathirvelu Baskar, and Savarimuthu Ignacimuthu

Entomology Research Institute, Loyola College, Chennai, 600 034, Tamil Nadu, India.

Soosaimanickam Maria Packiam, Kathirvelu Baskar and Savarimuthu Ignacimuthu (2013). Insecticidal and histopathological effects of botanical formulations against *Helicoverpa armigera* (Hub.) (Lepidoptera: Noctuidae). International Journal of Agricultural Technology 9(3):553-563.

Abstract Different novel botanical formulations were evaluated for their insecticidal activity against the fourth instar larvae of Helicoverpa armigera Hubner. Among them, formulation C (PONNEEM) showed maximum insecticidal activity against H. armigera. In histopathological analysis midgut region of H. armigera was severely damaged by the PONNEEM when compared to control. The effective PONNEEM did not cause any damage to the kidney or liver of male albino wistar rats (Rattus norvegicus). The insecticidal activity of PONNEEM against H. armigera was concentration dependent. PONNEEM could be used in pest management programmes.

Key words: *Helicoverpa armigera*, PONNEEM, Insecticidal, Histopathological, Wister rat

Introduction

Indiscriminate use of chemical pesticides to control pests has led to the developement of resistance in sects; it also affects nontarget organisms. Water resources are contaminated by agricultural chemicals. Hence an eco-frindly alternate method is needed. Plant based products have been used to control the different pests by the farmers at least for two millennia (Thacker, 2002). Since plant materials are rich in phytochemicals (Georges *et al*., 2008), the extracts of plant products and secondary metabolite have been used to control insect pests of various orders (including various insects from Lepidoptera) (Baskar *et al*., 2009; Baskar and Ignacimuthu, 2002; Muthu *et al*., 2012). *H. armigera* is a polyphagous lepidopteran insect pest which is widely distributed in Asia, Africa, Australia, Europe and other countries. This cotton bollworm causes

[*] **Corresponding author:** S. Maria Packiam; **e-mail:** mariaento@hotmail.com ; suribaskar@hotmail.com

great economic losses to cotton crops. This insect pest has developed resistance to most of the chemical pesticides (Kranthi *et al*., 2000). In India 158 million US dollars were lost between 1996 -1997 and about 54% of the total chemical insecticides were spent to control *H. armigera* (Jalali *et al*., 2004). The present study aims to investigate the insecticidal activity of different botanical formulations against *H. armigera.*

Materials and methods

Preparation of oil formulations

Different oils such as neem and pongam were taken at specified ratio in a stainless steel vessel with a stirrer and were stirred at 120 rpm for 10 minutes. Then 8% emulsifier + 1% stabilizer were added to the oils and again it was stirred at 120 rpm for 10 minutes. At last 0.123% Azadirachtin + 2% isopropyl alcohol were added and again it was mixed thoroughly by using a stirrer at 120 rpm for 10 minutes (Packiam and Ignacimuthu, 2012).

Insect Culture

H. armigera larvae were collected from bhendi field at Mangadu, Kancheepuram district. The collected larvae were reared individually in a plastic container (vials) and regularly fed with bhendi till the larvae attained the pupal stage under laboratory conditions (28±2°C and 80±5% RH). Sterilized soil was provided for pupation. After pupation, the pupae were collected from the soil and placed inside the cage. Cotton swabs soaked with 10% honey solution mixed with few drops of multivitamin were provided for adult feeding to increase the rate of fecundity. Black colour muslin cloth was placed inside the oviposition cage for egg laying. The eggs were collected from the cloth and allowed to hatch. After hatching the newly emerged larvae were fed with artificial diet in separate vials. The fourth instar larvae of *H. armigera* was used for the present study.

Insecticidal activity

Fresh cotton leaf discs of 3 cm in diameter were punched using cork borer and dipped with 5, 10, and 15 µl/L of diffent formulations. Treated leaf discs were placed inside the petridish having wet filter paper to avoid early drying of the treated leaf disc. Leaf discs treated with nimbicidine served as reference control; leaf discs treated with water served as negative control. In each petridish single prestarved 4^{th} instar larva of *H. armigera* was released

individually. For each treatment 20 larvae were introduced. Five replicates were done. The number of dead larvae was recorded after 24 h up to pupation. Percentage of larval mortality was calculated and corrected using Abbott's formula (Abbott, 1925).

$$\text{Abbott's per cent corrected mortality} = \frac{\text{\% Mortality in treated} - \text{\% Mortality in control}}{\text{100 - \% mortality in control}} \times 100$$

Histopathological analysis in the midgut of H. armigera

Histopathological analysis was carried out in the midgut region of *H. armigera* for PONNEEM which was the most effective formulation. The alimentary canals of treated (5 µl/L) and control larvae of *H. armigera* were dissected out under insect ringer solution. The separated pieces of midgut were fixed in Bouin's fluid. The tissues were processed for dehydration after 24 hours. The standard histological techniques with the use of ascending grades of alcohol were followed. The tissues were stained in 70% alcoholic eosin to facilitate the orientation of tissues. The tissues were dehydrated in absolute alcohol and acetone, cleared in benzene and embedded in paraffin wax (58-60°C). Sections were cut at 6 µm thicknesses, deparaffinised and stained with Heidenhain's haematoxylin and counterstained with eosin. The slides were observed under microscope.

Toxicity analysis in male albino wistar rats

Male albino wistar rats weighing 100 to 120 gm were used for the present study. The rats were provided with standard pellet feed (manufactured by Hindustan Liver Limited, Bombay, India). Food and water were given ad libitum.

Histopathological Studies in rat

Six animals were used for control and treatment. PONNEEM was administered for 15 days at a dose of 20 µl/L. The control group received vehicle (water) throughout the experimental period. The change in body weight was recorded weekly, and simultaneously mortality was also noted. The treated

and control animals were sacrificed for histopathological analysis. Kidney and liver were dissected, rinsed in physiological saline and fixed in 10 % formalin.

Tissues thus fixed were washed in double distilled water, dehydrated in alcohol series and cleaned in xylene. The cleaned tissues were infiltrated with molten paraffin at 58-60 °C. Serial sections were taken at 10 µm using rotary microtome and stained using haematoxylin-eosin (Merck). The sections were observed under light microscope with a magnification of 25 x. Histological observation and photographs were taken with a light microscope (Yesilada *et al*., 1997).

Statistical analysis

The antifeedant and growth inhibitory activities were subjected to analysis of variance (ANOVA). Significant differences between treatments were determined by DMRT ($P< 0.05$).

Results

Insecticidal activity

The percentage mortality of *H. armigera* larvae in different concentrations of different oil formulations are presented in Table 1. In all the formulations, the larval mortality was proportionately increased with increasing concentrations. Nimbicidine was used as reference control for comparison. Insecticidal activity of 22.93 and 18.63% were noticed in individual treatment of neem and pungam oil respectively against *H. armigera* at 5µl/L. Formulations A and B showed 24.53 and 25.40% insecticidal activities against *H. armigera* 5µl/L. Maximum insecticidal activity was recorded in PONNEEM (58.16%) at 15 µl/L concentration compared to all other formulations and control. It was statistically significant from other treatments at all the treated concentrations.

Table 1. Per cent insecticidal activity of different oil formulations against 4th instar larvae of *H. armigera* at different concentrations

Treatments	Concentration tested (µl/L)		
	5	10	15
Formulation A (Pungam+Neem oil-3:7)	16.36±2.66^{d}	19.63±3.09^{c}	24.53±5.43^{d}
Formulation B (Pungam+Neem oil-7:3)	8.96±3.24b^{c}	16.43±3.13^{c}	25.40±3.31^{d}
Formulation C (PONNEEM) (Pungam oil + Neem oil – 1:1)	40.96±4.66^{e}	46.66±5.61^{d}	58.16±3.70^{e}
Formulation D (Pungam oil)	6.56±3.72^{b}	9.80±3.56^{b}	18.00±3.46^{b}
Formulation E (Neem oil)	11.46±1.76^{c}	17.20±3.34^{c}	22.93±3.44^{cd}
Formulation F (Nimbicidine)	15.60±3.57^{d}	18.00±5.41^{c}	19.63±3.09^{bc}
Formulation G (Emulsifier control)	0^{a}	0^{a}	0^{a}

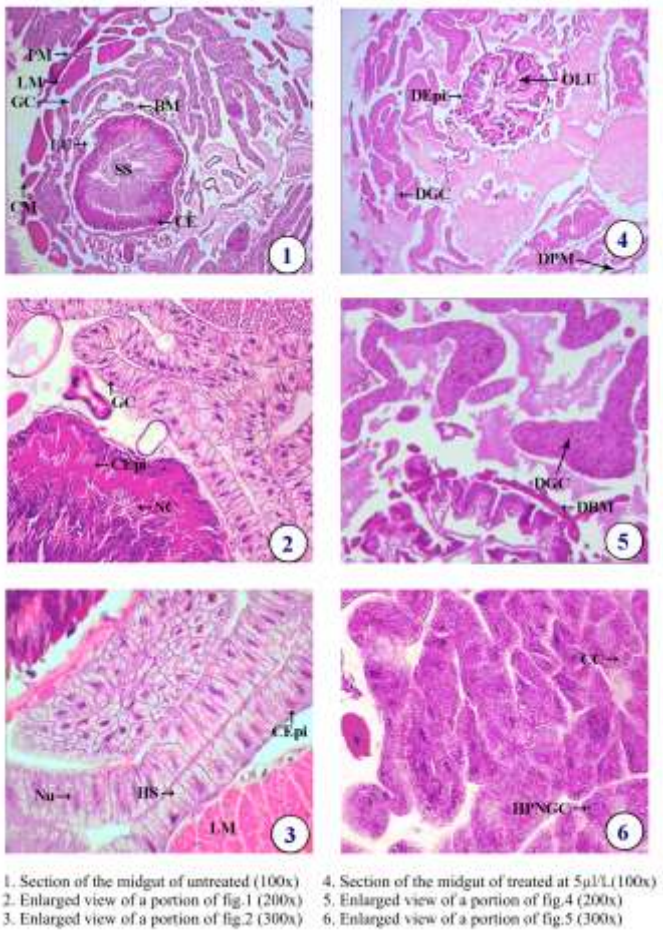
Values are mean of five replications. Means ± SD followed by same letter(s) in a column are not significantly different (P=0.05) by DMRT.

Histopathological Study on the untreated larvae of H. armigera

The control midgut of 4th instar larvae of *H. armigera* showed certain histopathological architecture (Figure 1: 1,2,3). The outer peritoneal membrane below this midgut consisted of longitudinal and circular muscle layers. Interior of this midgut was the centrally located lumen which covered this basal membrane. Below this basal membrane tall columnar epithel cell was present and it was secretory in nature. The lumen consisted of more amounts of secretory substances. Between the peritoneal membrane and basal membrane the space was filled with numerous couplet cells. The cells were longitudinal and columnar. All these cells are called goblet cells which are secretory in nature. The mode of secretion was found to be holocrine. Lumen was surrounded by columnar epithelial cells which are secretory in nature. The mode of secretion was found to be apocrine. These cells were meant for secretion of different enzymes for digestion. In between the columnar epithelial cells, numerous small cells called nidi cells meant for regeneration were present.

Five µl/L PONNEEM treated 4th instar larvae of *H. armigera* showed spectacular changes in the midgut region such as the occurrence of disintegration of peritoneal membrane with dilution of circular and longitudinal

muscles suggesting that the midgut was not in a position to push the food product into the hindgut. The lumen of the midgut treated insect contained structures with disorganized basal membrane and the occurrence of obliterated columnar epithelial cells, suggesting that these cells were affecting the synthesis of various enzymes by these insects. In addition goblet cells were highly pycnotic and necrotic at this concentration (Figure 1: 4, 5, 6).



1. Section of the midgut of untreated (100x)
2. Enlarged view of a portion of fig.1 (200x)
3. Enlarged view of a portion of fig.2 (300x)
4. Section of the midgut of treated at 5µl/L(100x)
5. Enlarged view of a portion of fig.4 (200x)
6. Enlarged view of a portion of fig.5 (300x)

Fig. 1. Histopathological Analysis of PONNEEM in the midgut of *H. armigera*

Toxicological and Histopathological Analysis in rat

Control kidney of rat exhibited normal histological architecture such as the occurrence of proximal and digital convoluted tubules of kidney. The control kidney showed the presence of Bowman's capsule and distinct endothelial cells. PONNEEM treated kidney at 20µl/L showed some

histopathological changes such as the elongation of proximal convoluted tubules. Otherwise there were no other changes (Figure 2:1 and 2). Similarly, histology of liver, control rats exhibited the occurrence of hepatocytes. The nuclei were stained darkly with distinct nucleoli. The presence of vacuoles was comparatively less with tight sinusotes. The liver of treated rats showed some changes in the hepatocytes which were less intensively stained with hepatoxylin and eosin. The nuclei and nucleoli of all the hepatocytes were distinct (Figure 2: 3 and 4). Comparatively PONNEEM treated liver of rat exhibited only very few changes compared to the control rat. This suggested that PONNEEM did not bring about any derangement in the tissues of rat.

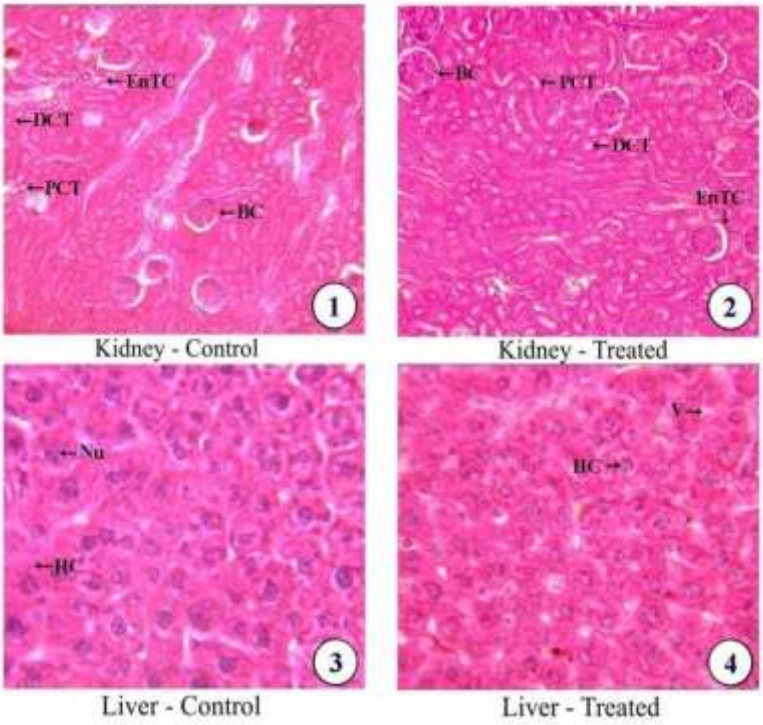


Fig. 2. Histopathological effect of PONNEEM in rat at 20 µl/L

Discussion

Insecticidal activity

Screening of plant extracts for deleterious effects on insects is one of the approaches used in the search for novel botanical insecticides (Isman *et al.*, 2001; Muthu *et al*., 2013; Baskar and Ignacimuthu, 2013). Secondary plant compounds act as insecticides. These compounds also deter or repel an insect

from feeding (Lajide *et al*., 1996). High larval mortality normally indicates potential insecticidal activity. In the present study, irrespective of concentrations, the insecticidal activity varied significantly for all the oil formulations. At lower concentration (5µl/L) of PONNEEM, 40.96% of insecticidal activity was observed against *H. armigera*. At 15 µl/L it exhibited significant insecticidal activity (58.16%) against *H. armigera* when compared to all other botanical formulations. It is possible that the compounds present in PONNEEM arrest the various metabolic activities of the larvae during the development. The larvae failed to moult and finally died. Several workers have already reported insecticidal activity of many plants and their compounds against different groups of insects (Leatemia and Isman, 2004; Baskar *et al*., 2010; 2011; Baskar and Ignacimuthu, 2012b).

Histopathological analysis in the midgut of H. armigera

Insecticides affect the normal functions of specific cells and make the survival of insect very difficult. Studies on the effects of various insecticides on the gut have been undertaken by many workers in insects of different orders such as Orthoptera (Singh, 1990) and Lepidoptera (Woke, 1940). These studies have revealed that administration of chemical pesticides such as dimethoate, endosulfan, dieldrin, sumithion, carbaryl, lead arsenate, and endrin has produced marked changes that include reduction in cell size, disintegration of intima, cytoplasmic vacuolization, chromatin clumping and nuclear condensation of *S. litura*.

The present study showed the toxic effect of PONNEEM in the midgut region of *H. armigera.* Significant changes in the midgut of *H. armigera* included cellular shrinkage, necrosis, disorganisation of peritrophic membrane and epithelium, loss of secretary products, cytoplasmic vacuolization, nuclear pycnosis (conglomeratin of nuclei) and irregular nuclear arrangement. Similar results were recorded by several scientists. Azadirachtin was also found to produce certain histopathological changes in tissues of insect body (Rembold, 1991; Annadurai and Rembold, 1993). Cottee (1984) has recorded changes like necrosis of cells, vacuolization of cytoplasm, reduction in the size of the nuclei and regeneration of cells in *Schistocerca gregaria* and *Locusta migratoria* when treated with botanical pesticides. PONNEEM exhibited good bioinsectical activity and higher damage on the midgut region of *H. armigera* because of the presence of many active biomolecules.

Toxicological and Histopathological Analysis in rat

Kidney plays a critical role in the elimination of most of the toxicants and it is considered to be the major target organ for toxicity (Lu, 1985). Kidney of control rat exhibited normal histological arichecture such as the occurrence of proximal and digital convoluted tubules. PONNEEM treated kidney (20 ppm) showed some histopathological changes such as the elongation of proximal convoluted tubules. All the rest of the structures were observed to be on par with control. The liver is the largest gland of the body and the major site for detoxification of toxicants including heavy metals, which are carried by the portal blood. The hepatocytes have numerous functions. Liver is involved in carbohydrate, protein and lipid metabolism and also plays an important role in the metabolism of toxic substances. In addition, the liver is engaged in secretory activities both exocrine and endocrine. The liver stands directly in the pathway of blood vessels, which conveys the absorbed substance from the digestive tract and enables the toxicant to be metabolized by it (Verma *et al*., 1975). Due to its capacity to degrade toxic substance, most of the biotransformations of toxic substances are carried out in the liver. Histology of liver of control rats exhibited the occurrence of hepatocytes. The nuclei were stained darkly with distinct nucleoli. The presence of vacuoles was comparatively less with tight sinusotes. The liver of treated rats showed no changes in the hepatocytes. Comparatively PONNEEM treated liver of rat exhibited only very few changes compared to the control rat. This suggested that the toxic agent like PONNEEM did not cause any derangement in the tissues of rat. Toxicity study revealed that PONNEEM was non-toxic to rat.

In conclusion, the novel botanical formulation PONNEEM exhibited good control of *H. armigera*. It also affected effect at the midgut region of *H. armigera.* Since this novel botanical formulation is non-toxic to the non-target organisms, environmentally safe, and easily available, it can be used in Pest Management programmes.

Acknowledgement

The authors thank Entomology Research Institute, Loyola College, Chennai, India for financial support (Grant no: ERI/MP/01/2006) and for providing the facilities to carry out the research.

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(Received 4 May 2013; accepted 30 April 2013)