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Histopathological and morphological alterations in salivary gland of House fly *Musca domestica L.*, induced by oral administration Thiamethoxam

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Abstract: ***The current work the effect of Actara insecticide belongs to chemical family Neonicotinoid. active component of thiamethoxam in three concentrations: 0.750 ppm, 1.5 ppm and 2.25ppm on adult house fly salivary glands . Histopathological and morphological effects revealed important alterations produced by this insecticide in histological and morphology of the adult house fly gland tissue categorized by increasing gland duct lumen diameter. These alterations are possibly related with excretion function of salivary gland might be accountable for removing this insecticide. Results show thiamethoxam is a powerful insecticide that performances histologically in salivary glant tissue, triggering alterations in the glands form, cytoplasm with extreme vacuolation , disruption cell membrane, obvious disorganization tissues cells ,terminating in progressive deteriorating phase with changes in nucleus glandular cell's, such alterations occurred together in its size and form of gland , disintegration of nucleus ,and presence of apoptosis(fragmentation) nucleus, accelerating the process of glandular degeneration ,and interfering with feeding process of house fly particularly when the peak concentration of insecticide was used.***

Keywords: *Musca Domestica, Muscidae, Salivary Glands, Thiamethoxam.*

1. INTRODUCTION

Musca domestica Linnaeus (Diptera: Muscidae) is exist in all over the warm regions in world, it is more adaptable [1].House fly is distributed globally and is a pest in homes, dairies, food processing, barns, poultry houses , and recreation areas. It can develop a generation within two weeks in summer as it has great breeding potential [2]. House flies characterizes a pest of great importance of economic in poultry production, and live stocks, contaminating animals; products and transferring a various pathogens to animals, as well as producing problems by attacking domestic regions nearby livestock units, disturbing life quality of these inhabitants [3]. House fly is the significant veterinary and medical pests that reasons annoyance, putrefied food and performance as a carrier for more than 100 pathogens to animal human [4,5].

It is recognized as a public health pest producing a severe threat to livestock and human by transmission several pathogenic organisms such as cysts of protozoa , parasitic helminthics , enteropathogenic bacteria ,and enterovirus .[6,7, 8, 9].The structure and function salivary glands of insects veterinary and medical importance have been slight studied [10].

Insect salivary glands are the biggest exocrine organs and are essential for feeding. [11]. The structure of salivary glands is complex, than can create materials necessary for nutrient mobilization, and digestive enzymes, which can be used to distinguish nourishing behaviors of insects [12]. Insect salivary glands generally have at least a secretory and restorative region.

The major secretary component is saliva which is chiefly composed of water which is transport from hemolymph across cells of salivary gland and into lumen of the gland [13].Since these glands remove substances from the hemolymp[14, 15, 16],they have ability to absorb poisonous substances that are not removed by the tubules of malpighian. These compounds can accelerate cell death in salivary glands and change their gene expression.

2. METHODS AND MATERIALS

2.1. Rearing Technique

Adult house flies were collected from local area using a sweep net and reared in laboratory at 25±2C° RH 40-50%, photoperiod 12:12 (L:D). The rearing method described by [17]. Flies kept in typical boxes of 40 cm x 30cm x 30 cm. The sides of the boxes had meshes and the base hard board of wood. Frontal side with long sheath cloth for purpose feeding and cleaning [18].

Adult flies were supplied with food consisting of sugar solution 10% and powdered milk. Mixture of milk and wheat flour was prepared at a weight proportion 3:1 and 50gm of this mixture was put into small pots for oviposition site [19].

2.2. Preparation of Insecticide

Thiamethoxam belongs to the Neonicotinoids, with contact, stomach and systemic activity, is used to control sucking insects both on plants and on companion animals. Thiamethoxam acts on the nervous system of the insect.

The compound mimics acetylcholine and binds to the acetylcholine receptor site, which damages the target insect's nervous system causing death. [20]. 0.0125 gm of thiamethoxam ® was dissolved in a volume glucose solution(10% W/V) then the volume completed to 1000 ml by glucose solution(10% W/V) to prepare a stock solution with 25 ppm Thiamethoxam and from stock solution different concentration (0.750 ppm ,1.5 ppm,and 2.25 ppm were prepared.

In this experiment, adults house flies were picked up randomly(male and female) from the rearing cage the adult house flies exposed to three concentration of thiamethoxam (0.750ppm,1.5ppm and 2.25 ppm). These concentrations were by preliminary rang-finding tests [21, 22, 23, 24]. Thiamethoxam was used as formulated product Actara 25 WG, from (syngenta) [25]. Prior the experiment, flies were starved two hours. Bioassay experiments were performed on first progeny of adults produced by the field-collected flies [26]. A bait (oral) method was used for all insecticide bioassay in order to evaluate the toxicity of insecticide, ten adult flies 3-days old were used for each dose in the three replication, to be 30 insects in each dose and the control take the same trend while the control groups were provided by sugar solution 10 % [27].

2.3 Dissection

Observations have been made with light microscopic (Olympus, Japan) on salivary gland. The adult of house fly was dorsally fixed on a slide, then body of fly flooded by physiology saline 0.9 %NaCl (0.9 gm NaCl per 100 ml distilled water). Insect's abdomen cut longitudinally by sharp scalpel [28].Salivary glands were picked up by using a spear-shaped head needle firmly and stained by dilute methylene blue (0.01 %).

The Salivary gland were exam by light microscopic (Hamilton BLP 1400, Taiwan), the magnification of 40X. Photographs were taken by computerized microscope-camera (GKB color digital camera, Taiwan).System magnification power 1680X.

3. RESULT

The salivary gland of adult house fly is simple and tubular with mono layer of epithelial cells surrounded by membrane and connective tissue. The fine structure of house fly salivary gland shows epithelial cell. The cell nucleus is large with nucleolus. The glands are composed of glandular cells (epithelial cell) which are convex externally. They are of uniform width throughout their whole length, except the slightly swollen blind termination. These blind ends lie one both sides of the ventral and posterior region of the abdomen, generally embedded in the fat-body (Fig.1a).

Ultra structural examination of untreated salivary gland of house fly (orally administrated with 10% sugar solution) revealed that the cells of normal adult salivary gland control flies regular shape polygonal shape and intact cells, surrounded by cell membrane, which are convex externally. The cell nucleus is large with nucleolus (Fig.1 a).

The treated salivary gland cells of adult house fly with different concentration (0.750 ppm, 1.5ppm, 2.25ppm) of thiamethoxam lost their original form, becoming irregular increasing lumen gland diameter (dilated lumen) and the cells become swollen than those of control group and the posterior end of salivary gland became flattened the adult salivary glands of flies treated with different concentration revealed numerous morphological alternations (Fig.1 b, c and d) compared to control (Fig1 a).

The salivary gland of treated adult house flies with low concentration (0.750 ppm) of thiamethoxam (Fig.2 b). The cells appear to somewhat affected. This effectiveness caused a slight damage of the cells. The first sign is fine granulation of cytoplasm with course of nucleus and cytoplasmic vacuolation in comparison with control (Fig.2 a).

While this affection was clearly observed on salivary gland cells of adult house flies were treated with (1.5 ppm) of thiamethoxam. The nuclei of cells the enlargement and invagination of some nuclear cell envelope, also an increase in vacuole quantity and highly granulation of cytoplasm with the disruption of cell membrane between the cells (Fig.1c).

The salivary gland cells of adult house flies that were treated with (2.25ppm) of thiamethoxam , the treated glands with sever morphological changes, the cell with malformation nuclei (irregular in shape) ,the cytoplasm with highly vacuolation and course granulation and the salivary gland cells characterized by completely decay cells and rupture of cell membranes (Fig.1d).

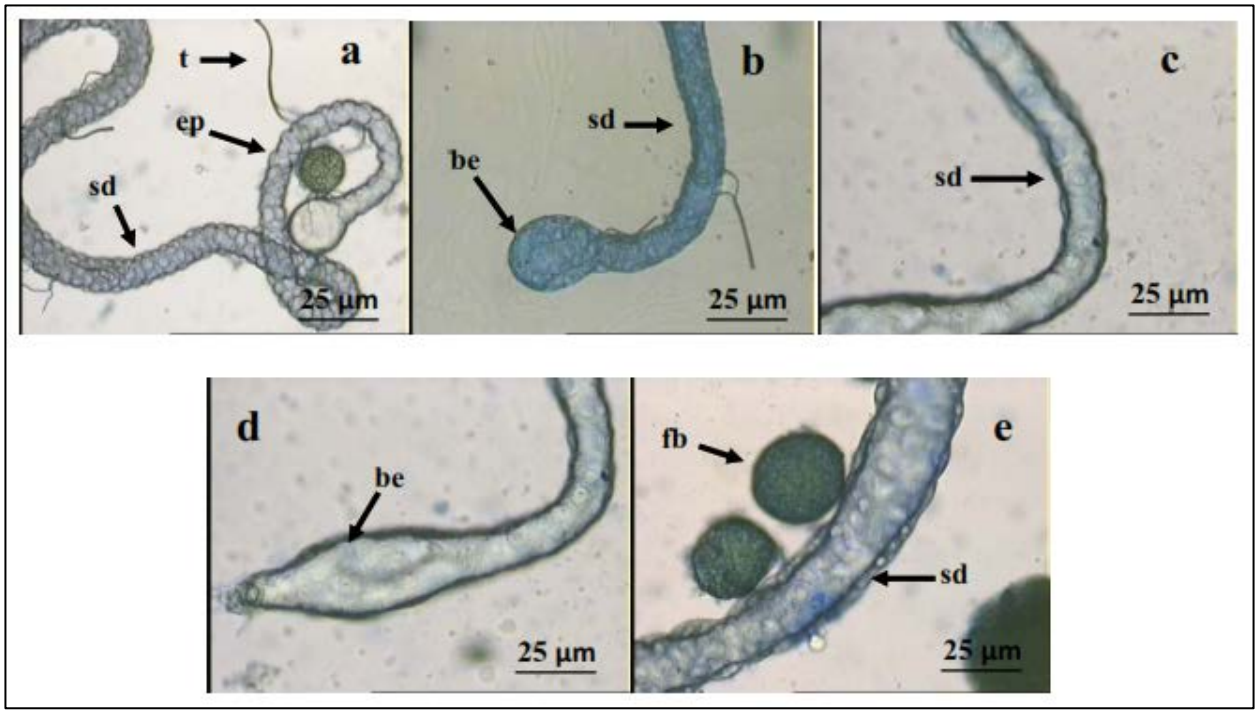

Fig. 1: Salivary gland of adult house fly (*Musca domestica* L) ingested different Concentrations of thiamethoxam(Neonicotinoids).

(a) Morphology apart of control salivary gland duct of house fly the gland is simple and tubular. It has a monolayer of epithelial cells **(ep)** surrounded by basement membrane **(bm)** and connective tissue. They are of uniform width throughout their whole length, except the slightly swollen blind end **(be)** termination.

(b) Apart of control salivary gland duct of house fly provided by sugar solution (10 %) have normal duct shape with intact cells and with circular blind end.

(c) Apart of treated salivary gland duct of house fly treated with 0.750 ppm thiamethoxam, show primary sign of poising the salivary duct, the cells swollen.

(d) Apart of treated salivary gland duct of house fly treated with 1.5 ppm thiamethoxam , show advanced of poising the salivary duct ,the cells more swollen , and the blind end of salivary **(be)** gland change in shape become cylindrical shape.

(e) Apart of treated salivary gland duct of house fly treated with 2.25 ppm of thiamethoxam , show primary more of poising the salivary duct**(sd)**,the cells swollen , and the gland duct become dilated in diameter.

(sd) =salivary duct; (be) =blind end; (fb) =fat body; (t) =trachiole; (ep) =epithelial cell; (cm) =cell membrane. Bars =25 µm

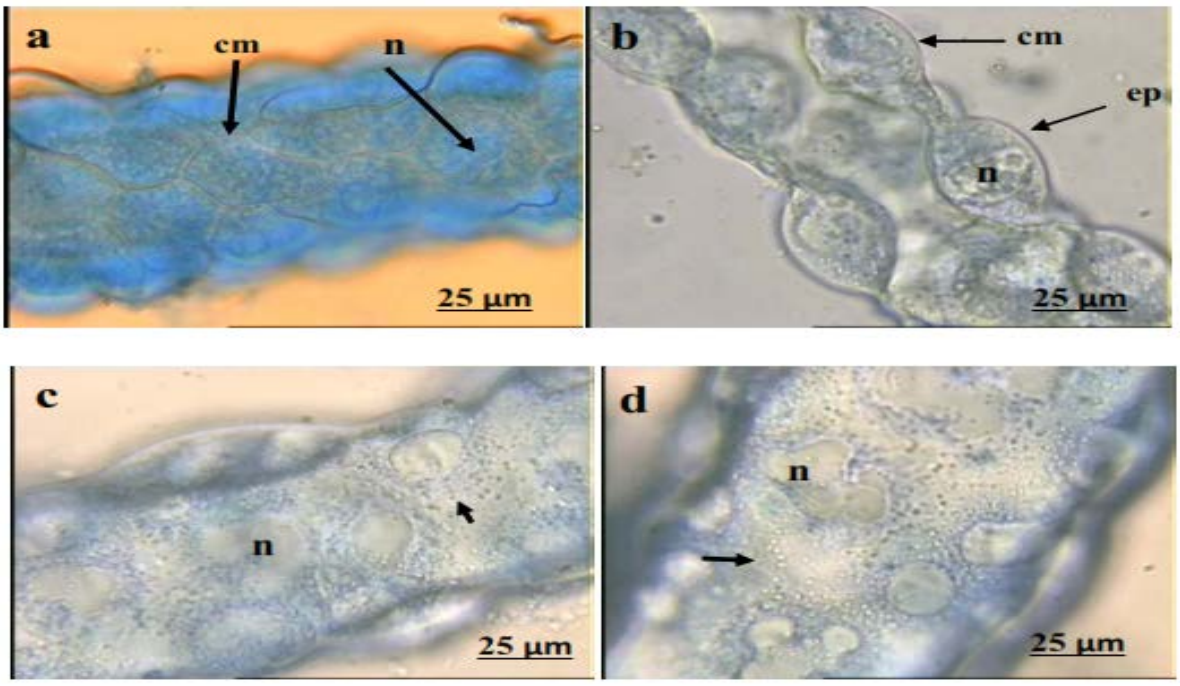

Fig.2. Salivary gland of adult house fly (*Musca domestica* L) ingested different concentrations of thiamethoxam (Neonicotinoids).

(a) Salivary gland duct of house fly provided by sugar solution (10 %) have normal intact cell (polygonal) surrounded by cell membrane with large nucleus and nucleolus.

(b) Salivary gland duct of house fly treated with 0.750 ppm of thiamethoxam(Neonicotinoids), some primary signs of poising appeared on the cells. The cytoplasm of the cells with fine granulation (arrow ahead) ,with small vacuoles.The nuclei changed in size and shape (enlargement of nucleus).The nuclei are containing debris(n), and the cell changes in shape became oval shape.

(c) Salivary gland duct of house fly treated with 1.5 ppm of thiamethoxam(Neonicotinoids), While this affection was clearly observed on salivary gland cells of adult house flies were treated with (1.5 ppm) of thiamethoxam. The nuclei of cells the enlargement and invagination of some nuclear cell envelope, also an increase in vacuole quantity and highly granulation of cytoplasm (arrow ahead) with the disruption of cell membrane between the cells.

(d) Salivary gland duct of house fly treated with 2.25 ppm of thiamethoxam(Neonicotinoids),The salivary gland cells of adult house flies that were treated with (2.25 ppm) of thiamethoxam , the salivary gland with sever morphological changes, the cell with malformation nuclei (irregular in shape) ,the cytoplasm with highly vacuolation(arrow ahead) and course granulation and the salivary gland cells characterized by completely decay cells and rupture of cell membranes.

(ep) =epithilaial cell; (cm) =cell membrane; (n) =nucleus Bars =25 µm.

4. DISCUSSION

The current work shows the thiamethoxam induced cytological and morphological alterations occurs in salivary gland duct of the adult house fly. The changes observed in treated of house fly by (0.750 ppm.)thiamethoxam of were categorized by alteration in shape and size of salivary duct and chiefly an increase of duct lumen compared to control group .

The damage caused by thiamethoxam in gland duct developed more extensive as the concentration of thiamethoxam increased, culminating in the glands of flies cured with thiamethoxam of (2.25 ppm). This results in agreement with [29] who stated that morphological alterations detected in type 1 acini, as well as in their cells, propose that these organs might be participated in the removing of toxic substances present in circulation hemolymph of tick that needs to eliminate from the gland. These, in treated adult house flies the increase lumen diameter of salivary gland suggest a role of gland for eliminating poisonous compounds from hemolymph. Even at low concentration, thiamethoxam was capable to cause glandular tissue damages cooperating organ metabolism. The current study revealed that the salivary gland cells in individuals subject to (0.750 ppm) of thiamethoxam morphologically altered; change in of cell structure and enlargement of the nucleus, and cytoplasm vacuolation compared to the control group.

This results in agreement with [30, 31]. Who reported that when the salivary gland of female ticks treated with concentration of 206 ppm of permethrin were detected the first signs of glandular tissue disintegration, such as the existence of vacuolation in cytoplasm and more ever, in nucleus of acinar cell, alterations in form of cell which become irregular in the shape, size increasing, and disposition of chromatin were also detected. In medium concentration of thiamethoxam (1.5 ppm) the nuclei of treated adult salivary gland cells showed significant changes in their shape, and some of them fragmented, besides this some nuclei present hypertrophy .This data suggest an advanced in the degenerative processes exposed to higher concentration of thiamethaxam in relation to the other treatment groups. This results is in agreement with [32] who stated

when the females of ticks treated with 1033 ppm, the cortical area cells lost their integrity in structure ,observation cytoplasmic contraction, existence of vacuoles between the nerves cells(vacuolation),loss of form (out line) and difficult to determination cellular boundaries, in addition decreasing cell size , this is in agreement with [33,34,35,36] whose stated that when the cortical region cells of ticks *Rhipicicephalus sanguineus*(Acari :Ixodidae) trated by 1031 ppm permethrin existing nuclei with excessive alterations in shape(irregular in outline) ,with fragmented blebs, decreased in size and condensation of chromatin network. Throughout cell death by apoptosis, breakdown of nucleus is one of the first changes detected, categorized by the compression and sidelining of chromatin, and bleb appearance, finally followed by the breakdown of the nucleus.

As the thiamethoxan insecticide increase (2.25ppm), changes in then salivary gland cells of treated adult house flies developed more sever demonstrating that this concentration would cause more important destruction to treated adult salivary gland cells. The doses triggering glandular cell disintegration progress, resultant in formation amorphous mass consist of cells remains which no longer be recognized, also the existence of numerous apoptotic bodies and several fragmented nuclei with picnotic feature, and rupture of gland cell membrane.

These results in agreement with similar results were found by [37] which suggested that in individuals of ticks subject to 2060 ppm of permithrin, only afew acini cells recognized, with others ones categorized as intermediate, subsequently they lost their histological and morphological characteristic owing to disintegration. In this situation, characteristics of salivary gland tissue like a loss of acini cells form; loss integrity acini cells membrane ; vacuolation of cytoplasm; existence of a number secretory granules in cells and nuclear alterations in size and shape, would be previous measures that terminate in acini interruption with the subsequent formation and discharge of apoptotic bodies.

5. CONCLUSION

The present work has established that thiamethoxam belongs to the (Neonicotinoids), besides its neurotoxic action, which damage the target insect's nervous system causing death [20], directly performances on the

physiology of the adult house flies glands stimulating early disintegration of the tissue. This insecticide acts by intrusive and preventing the feeding process even when administrated at minimal concentrations.

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