JWPR
Journal of World's Poultry Research

© 2015, Scienceline Publication

J. World's Poult. Res. 5(4): 73-83, December 25, 2015
Research Paper
PII: S2322455X1500011-5

Use of Neem (*Azadirachta indica* A. Juss) Oil in The Control of *Musca domestica* L.(Diptera:Muscidae) in Poultry Breeding Farms

Amanda Flávia S Rovida[1*], Karina Midori Endo[1], Andressa Domingos Polli[1], Ligia Maria Cruvelatti Bulla[1], Elton Luiz Scudeler[2], Jéssica Aline Soares Abreu[1] and Hélio Conte[1]

[1]*Department of Biotechnology, Genetic and Cell Biology – State University of Maringá, Maringá, Paraná, CEP, 87020-900, Brazil*
[2]*Laboratory of Insects, Department of Morphology, Bioscience Institute,UNESP, São Paulo, State University, Botucatu, SP, CEP, 18618-970, Brazil*
*Corresponding author's Email: helconte@gmail.com

Received: 12 Nov. 2015
Accepted: 15 Dec. 2015

ABSTRACT

Pest infestation, in general such as flies, is a problem faced by poultry workers in poultry breeding farms. Control of these populations is desirable, due to the damage they cause and, most times, it is done through chemical management, with insecticide application. *Musca domestica* is one of the species with the greatest ability to develop resistance to insecticides, and the digestive system, especially the midgut, is one of the most vulnerable organs to structural alteration, because in it there is production of digestive enzymes and absorption of nutrients. Damage caused by use of synthetic insecticides reactivated studies with bioinsecticides for being one of the most selective options in pest control. Furthermore, they do not cause environmental alterations such as food, soil, water poisoning and they are practically nontoxic to mammals. Among vegetable species with insecticidal activities that have been used a lot, we have neem, subtropical plant that shows low residual power and lower risk of intoxication for mammals and poultry. Thus the objectives that guided this work, was the observation of morphological alteration in *M. domestica* larvae and malformation of pupae after the use of neem, powder and emulsifiable oil at different concentrations (0,5%, 1%, 1,5%) were tested. Following, after the applications, the midgut of flies in larval stage was isolated, processed and analyzed under light microscopy, scanning and transmission electron microscopy. As in most dipterous, the cell types found were columnar cells and regenerative cells, which showed alterations such as: coalescing vacuoles, intercellular spaces, formation of cytoplasmic protrusions, broken microvilli, rough endoplasmic reticulum and dilated mitochondria. The statistical analysis registered for pupa malformation showed significant results in comparison to control, but there was no difference among treatments. The different concentrations of neem oil contributed in altering morphological and ultrastructurally the midgut in *M. domestica* larvae, interrupting its development. These results showed that in poultry breeding farms, neem oil can be a pratical alternative to the control of *M. domestica*.

Key words: Housefly larvae, Alimentary canal, Bioinsecticide, Ultrastructure.

INTRODUCTION

The common housefly, *Musca domestica* L, has made some considerable effects on the poultry industry, as it can spread approximately 100 pathogenic organisms, cause nuisance to workers, and deposit dirt and microorganisms, this results in losses in egg production. A housefly can fly several kilometers, visiting homes, settling on food and excreta, and invade aviaries and other local animal creations and hence the species is considered a serious problem for public health, livestock and poultry (Greenberg, 1973; Förster et al., 2007; Pedroso-de-Paiva, 2007; Acevedo et al., 2009; Bosly, 2013; Zhong et al., 2013; Acharya et al., 2015 and Solà-Ginés et al., 2015).

The systematic application of chemicals provided advances in the development of pest management beginning in the 1960s, but the harm that they caused soon became evident. This harm included environmental imbalances, elimination of beneficial arthropods and contamination of agriculture, food, the environment, soil, water systems, workers and the surrounding population. These factors led to a resurgence of pests and favored the rapid selection of resistant individuals, causing irreparable damage to ecosystem and health, such as poisoning, genetic mutations, cancer and death (Campanhola, 1990; Prabhaker et al., 1998; Menezes, 2005; Veiga et al., 2006 and Khan et al., 2014).

The damages of synthetic insecticides caused a resurgence in studies on botanical insecticides and biopesticides, which have received great attention in recent decades (Viegas Junior, 2003). These compounds are considered better options for insect control because they are more selective for the target insect. Moreover, they did not affect predators and

To cite this paper: Rovida AFS, Endo KM, Polli AD, Bulla LMC, Scudeler EL, Abreu JAS and Conte H. 2015. Use of Neem (*Azadirachta indica* A. Juss) Oil in The Control of *Musca domestica* L.(Diptera:Muscidae) in Poultry Breeding Farms. *J. World's Poult. Res.* 5(4): 73-83.
Journal homepage: http://jwpr.science-line.com/

other beneficial organisms, including humans and also did not cause environmental changes such as contamination of food, soil and water, they are practically nontoxic (Beaty and Marquardt, 1996 and Gallo et al., 2002).

Among the economically viable bioinsecticides, neem (*Azadirachta indica* A. Juss) stands out as it is less polluting and has low residual power and risk of toxicity to mammals and birds (Quintela and Pinheiro, 2004; Rossi and Palacios, 2015). This product generally has been used in Integrated Pest Management (IPM) to control flies. The presence of pests in poultry breeding farms risks biological and chemical damage to eggs. These risks relate to the spread of microorganisms from pests and egg contamination by chemicals applied in pest control (Pedroso-de-Paiva, 2007). Brazilian laying hens rank seventh worldwide in egg production and Brazilian farms produced 730.156 million dozen eggs in the first quarter of 2015, according to data released by the Brazilian Institute of Geography and Statistics (IBGE, 2015). The Brazilian state of Paraná produced 65.159 million dozen eggs in the same period. Paraná had 13.036.000 heads of laying hens in March 2015. In modern times, the rapid treatment of pest infestations is essential. DNA barcoding allows flexibility in the identification of new species and the confirmation of species and makes it possible to identify to a certain taxonomic classification without a skilled taxonomist, which would require more time for this work. The DNA barcode database organizes and maintains all sequences already deposited and is accessible to search worldwide (Stoeckle et al., 2005 and Hebert et al., 2003). The objectives that guided present study were keeping man and the egg, which is our product of consumption, pesticide free by seeking alternatives to control the fly population and consequently reduce contamination of eggs by pathogenic organisms. For this, using light, scanning and transmission electron microscopy, we recorded the cellular changes in *M. domestica* larvae and pupae malformations after the application of different concentrations of neem oil.

MATERIALS AND METHODS

Insect

Larvae of *M. domestica*, specifically the second instar identified by a body size of approximately 1 mm and the presence of food in its alimentary canal, easily visible due to the transparency of the body were collected at São Carlos Egg Farm in Cruzeiro do Sul, Paraná, Brazil (22°57′59.0″S, 52°11′00.3″W). The larvae were collected from poultry manure collected in 50 mL plastic cups, each containing approximately 5 g of manure and sealed with thin and permeable (voile) tissue, which were placed randomly in the poultry manure. A total of 450 larvae were collected and distributed into 45 batches containing 10 larvae each, which were subsequently divided into 5 groups of 9 batches.

DNA extraction

DNA extraction was performed according to the methods of Waldschmidt et al. (2002) with modifications. Ten larvae were macerated together with liquid Nitrogen in a porcelain crucible. The mash was distributed into microtubes with 700 µL of extraction buffer and incubated for 30min at 65°C. Then, 700 µL phenol-chloroform (1:1) was added and the contents were homogenized and centrifuged at 1080×*g* for 5 min. The supernatant was transferred to another microtube with 700 µL of chloroform and centrifuged at 1080×*g* for 5min. Again, the supernatant was removed, added to 2 volumes of cold ethanol, and incubated for 2–24 hours at -20°C. After this period, the samples were centrifuged at 1480×*g* for 40 min and oven-dried at 37°C for 30 min. The samples were resuspended in 200 µL of elution buffer with RNAse A (100 µg/mL), and incubated for 30 min at 37°C. Then 200 µL chloroform was added and the sample centrifuged at 1080×*g* for 5 min. Next, 2 volumes of ethanol were added in the supernatant incubated for 30 min at -20°C, centrifuged at 1480×*g* for 40 min and oven-dried. Finally, they were resuspended in 50 mL of elution buffer.

Amplification was performed using the TY-J-1460 primers (5′TACAATCTATCGCCTAAACTTCAGCC 3′) and C1-C-1560 (3′TGTTCCTACTATTCCGGCTCA 5′) to a region of the cytochrome oxidase gene of Diptera (Gibson et al., 2011). PCR was performed using a mixture of 5 µL buffer (200 mM Tris-HCl, pH 8.4; 500 mM KCl, once concentrated), 5 µL dNTP (2.5 mM), 3 µL of each primer (10 pmol, Ludwig Biotechnology), 0.4 µL Taq DNA polymerase (5 U/mL), 3.75 µL $MgCl_2$ (50 mM), 27.85 µL Milli-Q sterile water and a 2µL sample of the previously extracted DNA (10–20 ng/µL). The PCR conditions were: initial denaturation at 94°C for 4 min, followed by 25 cycles of denaturation at 94°C for 30 s, annealing at 54°C for 1 min, extension at 72°C for 1 min, and final extension at 72°C for 7 min. PCR was performed in a MG96G Peltier-based model Thermo Cycler.

Species identification

The sample was sequenced from PCR products by Ludwig Biotechnology LTD, using the ABI-PRISM 3100 Genetic Analyzer (Applied Biosystems). The sample was prepared in a 0.5 mL microcentrifuge tube by adding 30–60 ng DNA sample, 4.5 pmol primer TY-J-1460, and the volume made up to 6 µL with Milli-Q water. The sequence obtained was evaluated for quality

using the BioEdit software v7.2.5 by cutting the initial and end regions of the sequencing result, yielding an 80–90 bp fragment. The nucleotide sequence was compared with those deposited in the NCBI database (National Center for Biotechnology Information website) using the BLAST tool, the species being determined based on the best value obtained.

Bioassays

Database sequences with a high percentage of identity with the sequence obtained were rescued and aligned using Clustal W in the MEGA software (version 6.05) along with the sequence of the sample. The phylogenetic tree was constructed with the neighbor-joining method (Saitou and Nei, 1987), using p-distance for nucleotides with the "pair wise gap deletion" option and bootstrap with 10,000 repetitions.

The 45 batches of 10 larvae were divided into five groups (nine batches each): treatment at 0.5%, 1%, or 1.5% neem oil, 1 g neem leaf powder and control (no neem), maintained in plastic trays and treated for 3 days. Each lot also received 1 g of manure daily, simulating the natural deposition of birds.

We used neem oil of 100% purity, 1600 ppm of *Azadirachta indica*, guaranteed by the manufacturer, Natuneem, with record of Protocol Nº 21052.12435/2005-84 in the Ministry of Agriculture, Livestock and Supply, country and certified by the renowned International Organic BSC Öko-Garantie Doc. Nº: NATUR - 9009/09.95/7331-BR. Neem leaf powder was also purchased from this country of the company.

Pupae malformation

Pupal abnormalities were identified from pupae with larvae shapes or partially larval morphology. The distribution of data of malformations in pupae were analyzed using the Shapiro-Wilk test ($W = 0.9102$, $p = 0.01506$) indicating that these data were not normally distributed. Also it was decided to analyze the data using Kruskal-Wallis test ($P<0.05$) to identify whether there were differences between the effects of different neem oil concentrations, when significant differences were found, a post-hoc Dunn's test was applied. All tests were performed using the software R (R Core Team, 2013).

Histological analysis

After dissection as described in the previous section, the midgut regions of 10 larvae were isolated and fixed in aqueous Bouin for 6 hours. After dehydration in an ascending ethanol series (70, 80, 90 and 100%) and diaphanization in xylene, fragments were embedded in paraffin and subjected to histological cuts made on a 6 µm Leica RM 2250 microtome. The sections were stained with hematoxylin and eosin (Junqueira and Junqueira, 1983) and analyzed using a Zeiss Axioskop 2 Plus capture microscope in the Microscopy Center (MC) in the Central Complex of Research Support (COMCAP) at the State University of Maringá (UEM), Brazil.

Scanning Electron Microscopy (SEM)

For scanning electron microscopy, the fragments of 10 midguts were fixed for 48 hours at room temperature about 20-25°C, in 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.3) and then washed in distilled water. They were then post-fixed in 1% osmium tetroxide diluted in distilled water for 30 min at room temperature and dehydrated in a graded series of ethanol (50–100%). After dehydration, samples were subjected to critical point drying and coated with gold dust, then examined in a Fei Company Quanta 200 scanning electron microscope at the Electron Microscopy Center of the Biosciences Institute, UNESP, Botucatu, SP, Brazil.

Transmission Electron Microscopy (TEM)

For studies of transmission electron microscopy, the fragments of 10 midguts were fixed in 2.5% glutaraldehyde and 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.3) for 24 hours at room temperature and post-fixed with 1% osmium tetroxide in the same buffer for 2 hours at room temperature. After washing in distilled water, the material was stained with 0.5% aqueous uranyl acetate for 2 hours at room temperature. The specimens were dehydrated in a graded acetone series (50%, 70%, 90% and 100%) and embedded in Araldite® resin. Semi-thin sections were stained with 1% toluidine blue, whereas ultra-thin sections were stained with uranyl acetate and lead citrate and then examined using a Tecnai Spirit by Fei Company transmission electron microscope in the Electron microscopy center of the Biosciences Institute, UNESP, SP.

RESULTS

Confirmation of species by barcode

According to sequencing and phylogenetic analyses, the analyzed sample showed 99% identity with accession numbers KM200723.1, EU154477.1 and AF104616.1. In the phylogenetic tree, Sample 1 (larvae) was located in a clade with these three hits identifying it as *M. domestica* (Table 1, Figure 1a and figure 1b).

Larval action

Treatments containing neem oil were different from control ($\chi2 = 15.9742$, $df = 4$, $P = 0.0030$), with

significant differences when treatments (0.5%, P= 0.0085; 1.0%, p = 0.0025; 1.5%, p = 0.0004) and neem powder (P = 0.0002) were compared to the control (Table 2 and figure 3). These results indicated that when fly larvae are subjected to neem oil treatment, they showed defects at the pupal stage. However, when comparing the generated defects between the different concentrations of neem oil and neem powder, the difference was not significant (P=0.13).

Table 1. List of accession numbers and their degree of identity with sample Md-01

Sample	Species	Accession Number	Identity
Sample Md-01	*Musca domestica*	KM200723.1	99%
	Musca domestica	EU154477.1	99%
	Musca domestica	AF104616.1	99%

Table 2. Overview of number of malformed pupae in the following treatments: control; 0.5%, 1% and 1.5% neem oil and neem powder

Repetitions	Number of pupae with malformations				
	Control (60 larvae)	Neem oil 0.5% (60 larvae)	Neem oil 1.0% (60 larvae)	Neem oil 1.5% (60 larvae)	Neem Powder (60 larvae)
Number of malformed pupae	0	15	17	20	21

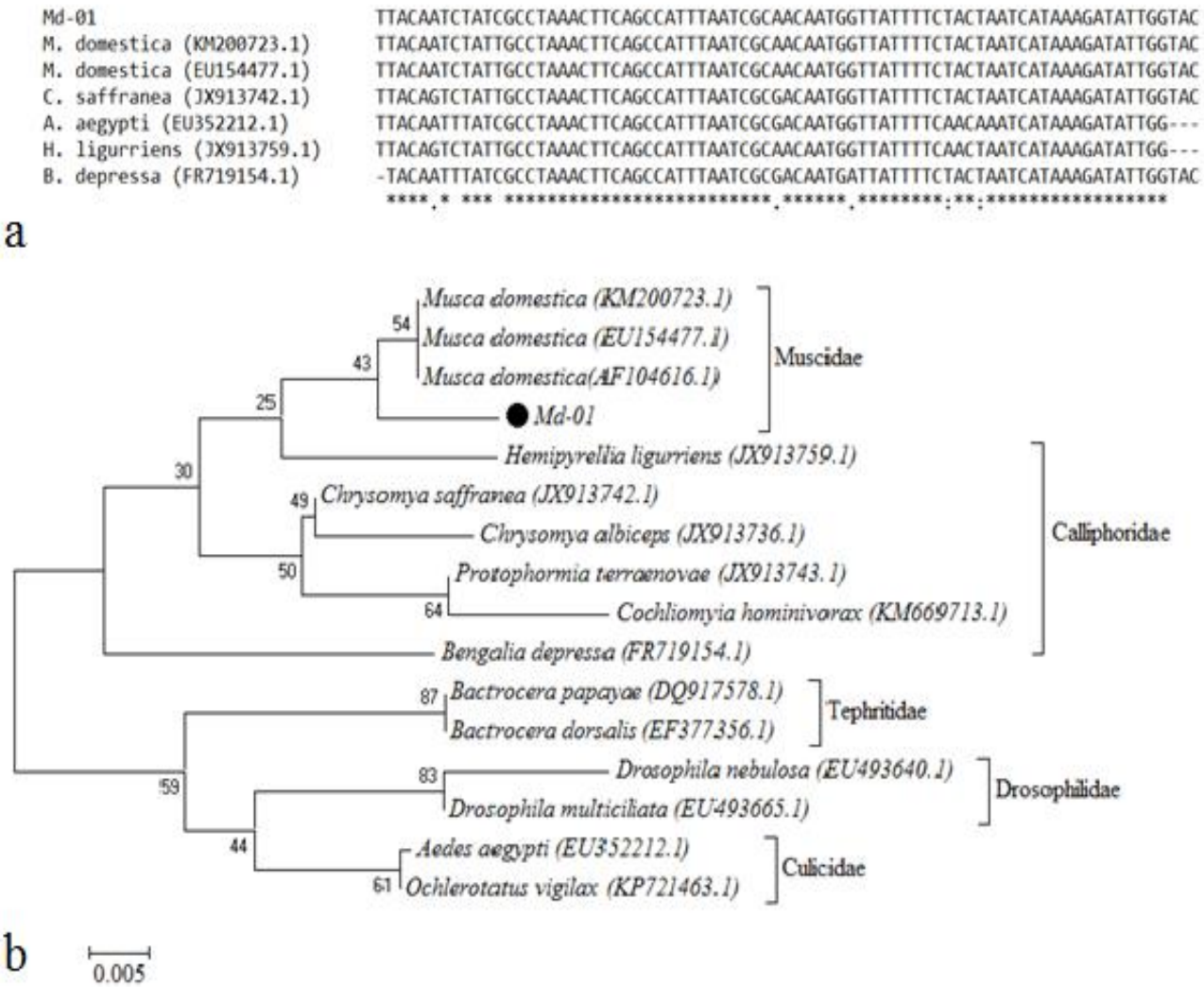

Figure 1. (**a**) Obtained with the ClustalW alignment program with other similar sequences retrieved from GenBank database (NCBI); (**b**) Dendrogram resulting from alignment of sample Md-01 with species that showed high degrees of identity.

Morphological and ultra-structural characterization

Larvae of *M. domestica* have an alimentary canal divided into 3 regions: anterior (stomodeum), middle (midgut) and posterior (proctodeum). The midgut is characterized by a tube of uniform caliper consisting of contoured columnar cells occupying a large part of the abdomen, with the ability to stretch during feeding. The transition between stomodeum and midgut is marked by the presence of the cardia valve, which penetrates the esophagus and in the anterior region are four gastric ceca, arranged symmetrically around the duct, composed of columnar cells. Along the midgut are nests of regenerative cells, sparsely distributed in the basal region, that are small and bulging with circular or oval shapes and characterized by strongly basophilic cytoplasm (Figure 2a, figure 2d and figure 2e). In the columnar cells of the midgut, there was an increase in the number of vacuoles, swelling and apical disruptions, irregularities in cellular morphology and a decrease in the number and size of microvilli (Figure 2c and figure 2d, figure 3c, figure 4d and figure 4e) with increase in the concentration of applied neem. Larvae treated with 1.5% neem oil exhibited a large number of vacuoles in their midguts as observed using light microscopy and also exhibited cell lysis. These results were not observed by light microscopy analysis of larvae treated with 0.5% or 1.0% neem oil (Figure 2a and figure 2e). Scanning electron microscopy revealed profuse microvilli in the columnar cells in the control group. Changes were observed in these cells in the treated groups. Examples of changes observed with the application of 1.5% neem oil included bulging, juxtaposition, flattening and breaking in the apical region. Changes in the organization, shape and number of microvilli, increase or modification of protrusions and the presence of debris in the apical epithelium (Figure 3a and figure 3e). Using transmission electron microscopy, we observed regenerative cells characterized by a basal labyrinth, glycogen stores, lipid droplets and secretory vesicles, rough endoplasmic reticulum, digestive and autophagic vacuoles, mitochondria, multivesicular bodies, microvilli, and defined contours in the nuclei (Figure 4a and figure 4f). In *M. domestica* larvae treated with 0.5% neem we identified spherites in the epithelium of the midgut cells with dilatations expanded in the basal labyrinth; in larvae treated with the 1% concentration we found reductions in the number and length of microvilli and the presence of large amounts of lipid droplets, which were also observed in the 1.5% and powder treatments. Both showed a large number of mitochondria, but the ringed form was most evident in larvae treated with 1.5% neem oil (Figure 4c-f).

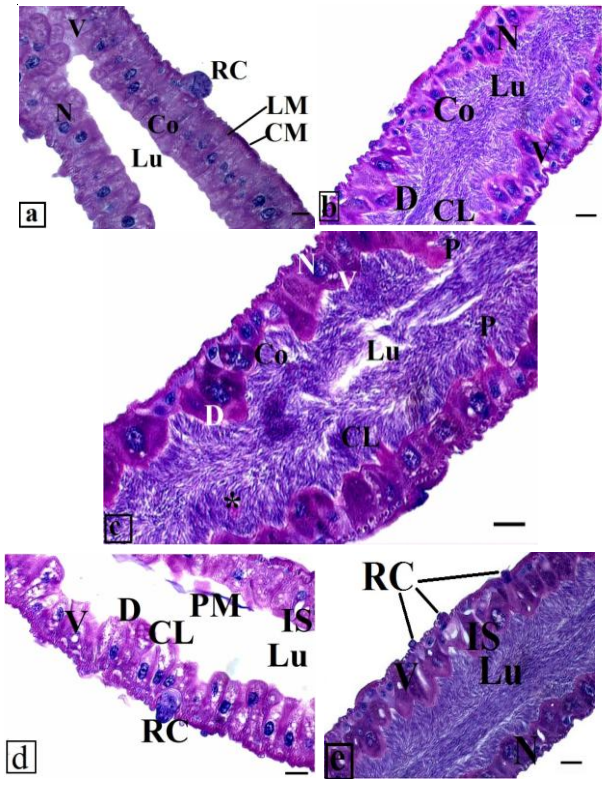

Figure 2. Photomicrographs of the midgut of *Musca domestica* larva, longitudinal sections (hematoxylin-eosin) (**a**) Larva control group. Columnar cells (Co); Regenerative Cells (RC); Nucleus (N); Lumen (Lu); Vacuoles (V); Circular Muscle fiber bundles (CM); Bundles of Longitudinal Muscle fibers (LM); bar = 20 µm; (**b**) Larvae treated with 0.5% neem. Columnar cells (Co); Dilated (D); Cell Lysis (CL) in the apical region of the cell; Intercellular Space (IS); Vacuoles (V); bar = 50 µm (**c**) Larva treated with 1% neem. Cell Lysis presence (CL); Protrusions (P); Possible epithelium fragment (*); slightly vacuolated (V); bar = 50 µm (**d**) Larva treated with 1.5% neem. Cell highly vacuolated (V); with cell expansion (D) and irregular shapes; Cell Lysis (CL); Peritrophic Membrane (PM); bar = 50 µm (**e**) Larva treated with neem powder; Cell highly vacuolated (V); with cell expansion (D) and irregular shapes; bar = 50 µm.

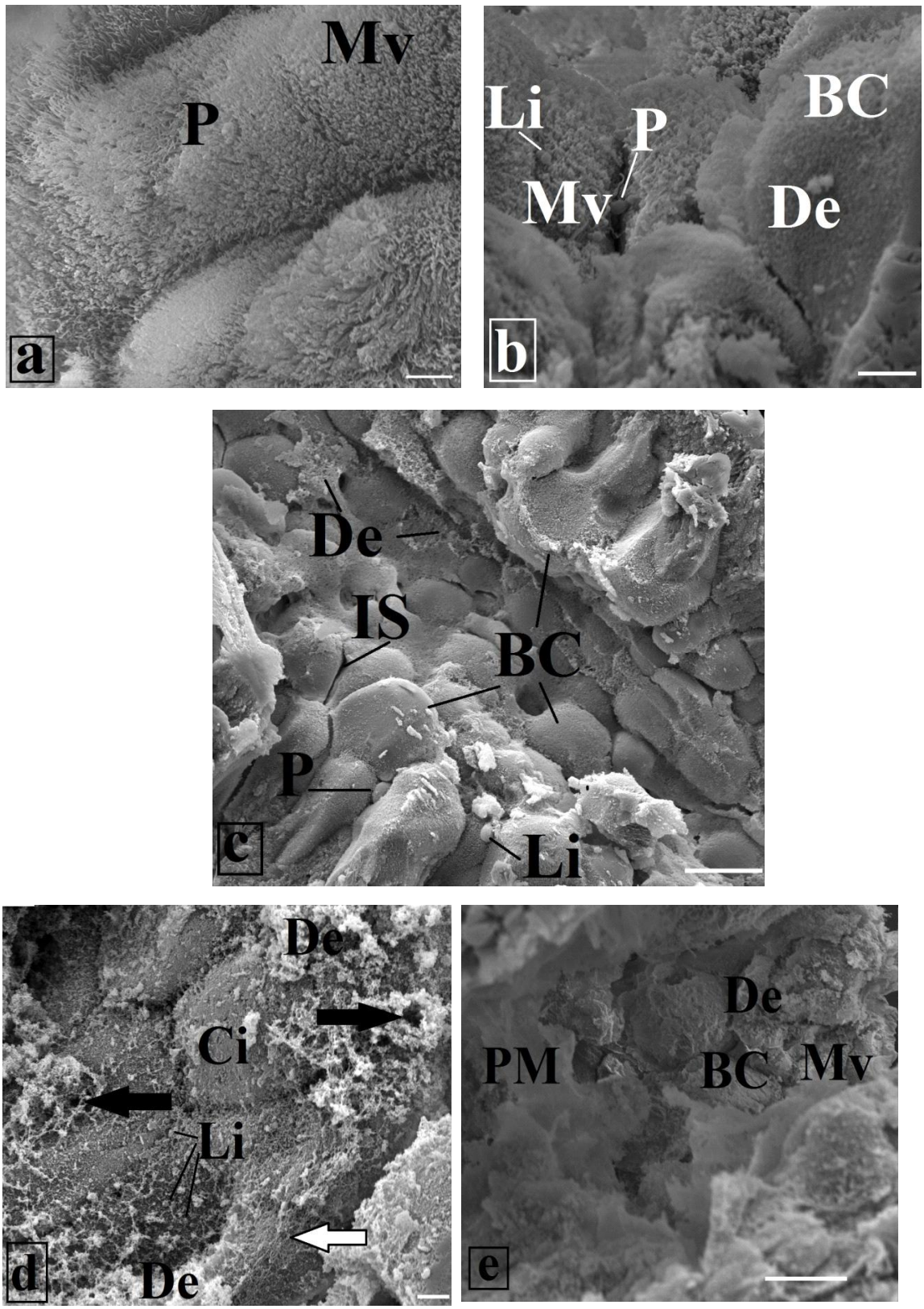

Figure 3. Electron-scanning micrographs of the midguts of *Musca domestica* larvae (control and treatment groups). (**a**) Larval midgut control. Columnar cells with abundant Microvilli (Mv), uniform and organized, smooth Protrusion (P); bar = 5 µm; (**b**) Larvae treated with 0.5% neem. Slightly Bulged Columnar cells (BC); Presence of potential lipid droplets (Li); Microvilli with irregular pattern (Mv); small Debris (De); bar = 5 µm; (**c**) Larvae treated with 1% neem. Variation in the number and size of protrusions (P), Debris (De), lipid droplets (Li); presence of Intercellular Spaces (IS); bar = 25 µm; (**d**) Larvae treated with 1.5% neem. Large presence of Debris (De); Columnar, juxtaposed, flat cells (Ci) with mobile possible disruptions (black arrow); Scattered and disorganized microvilli (white arrow) associated with Debris (De); Lipid droplets (Li); bar = 5 µm; (**e**) Larvae treated with neem powder. Disruption in the apical membrane (*); Peritrophic membrane, Bulged Columnar cells (BC); Disorganized and irregular microvilli; Large presence of debris (De); bar = 25 µm.

To cite this paper: Rovida AFS, Endo KM, Polli AD, Bulla LMC, Scudeler EL, Abreu JAS and Conte H. 2015. Use of Neem (*Azadirachta indica* A. Juss) Oil in The Control of *Musca domestica* L.(Diptera:Muscidae) in Poultry Breeding Farms. *J. World's Poult. Res.* 5(4): 73-83.
Journal homepage: http://jwpr.science-line.com/

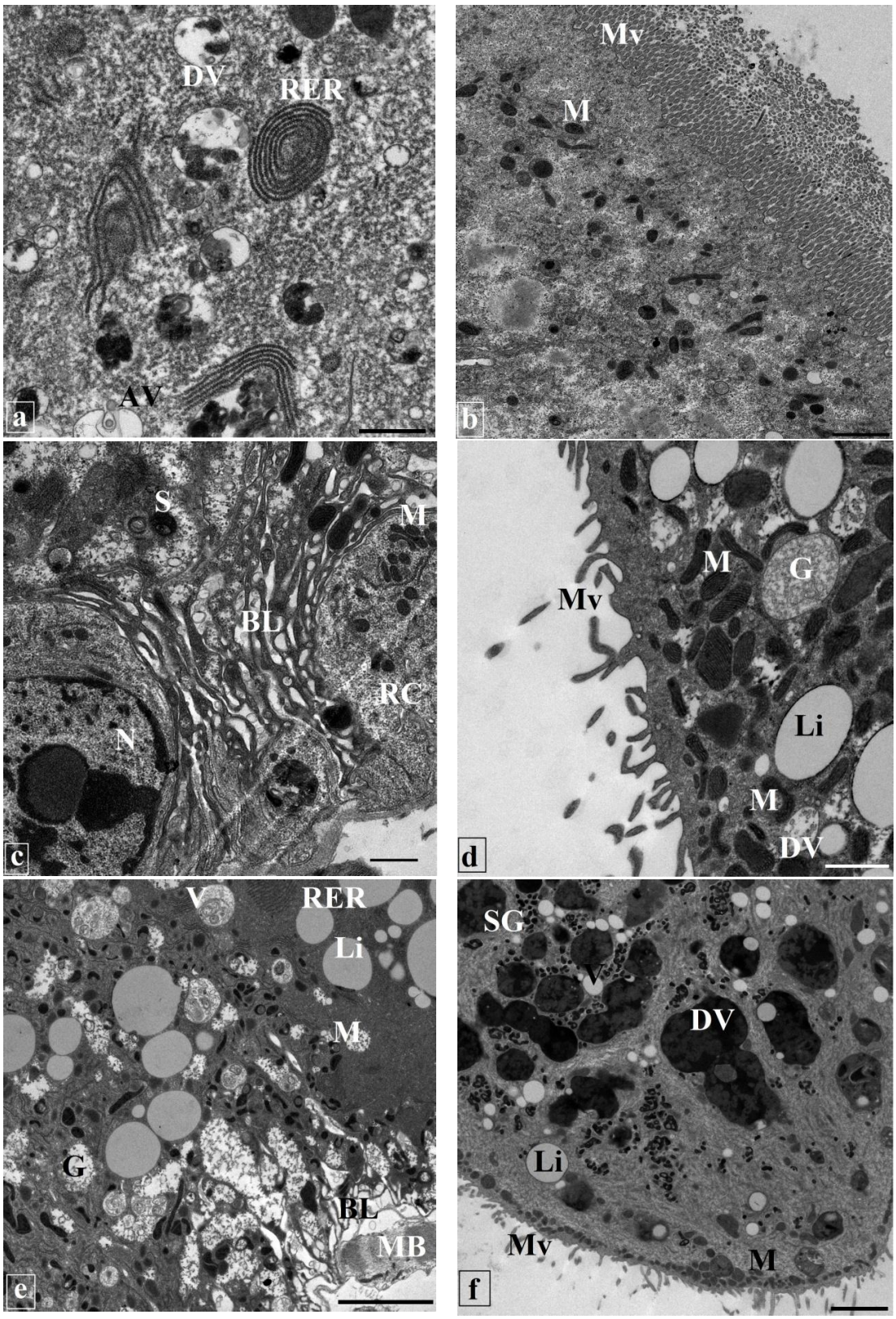

Figure 4. Transmission electron micrographs of the longitudinal sections of the midgut of *Musca domestica* larvae (control and treatment groups). (**a**) Larval midgut control. Presence of Digestive Vacuoles (DV); Autophagic Vacuoles (AV); Lattice rough endoplasmic reticulum; bar = 1 µm (**b** and **c**) Larvae treated with 0.5% neem. Microvilli (Mv); Mitochondria (M); Dilated Basal Labyrinth (BL); Regenerative Cell (RC); Nucleus (N); Spherites (S); bar = 2 µm; (**d**) Larvae treated with 1% neem. Sparse and short Microvilli (Mv); Large lipid droplets (Li); Glycogen store (G); Mitochondria (M); Digestive Vacuole (DV); bar = 5 µm; (**e**) Larvae treated with 1.5% neem. Many Lipid droplets (Li); Vacuole (V); Glycogen store (G) Muscle Bundles in the basal region (MB); bar = 1 µm; (**f**) Larvae treated with neem powder. Large number of digestive vacuoles (DV), Mitochondria in the apical region (M); Sparse and short Microvilli (Mv); Secretory Granules (SG). Lipid droplets (Li); bar = 2µm.

DISCUSSION

From the sequencing that identified the biological material with 99% identity to *M. domestica*, it was possible to construct a phylogenetic tree, as the extraction of sample 1 was in the same clade as other three accessions corresponding to *M. domestica*, which was the object of study in present work. According to Scott et al. (2014), the availability of the domestic fly genome should accelerate the pace of research on this important vector of human and animal diseases. The dilatations of the basal labyrinth observed in the midgut epithelium cells in *M. domestica* treated with concentrations of 0.5% neem oil may be related to detoxification processes, since low concentrations of neem oil fail to prevent molting. In this case, we see the elongation of the insect's life cycle and even those who survive the action of neem tend to harm the population, since they cannot afford to feed, develop and reproduce normally (Mordue (Luntz) and Nisbet, 2000; Martinez and Van Emden, 2001). The incorporation of a 10% emulsion of neem in *M. domestica* diet for just one day maintained until pupation showed no significant results compared to the control, suggesting that the effects vary according to the stage, species and the exposure mode (Fernandes et al., 2010). In *M. domestica*, Ginarte (2003) observed larvicidal efficiency with a neem concentration of 0.2%. Scudeler and Santos (2013) found that 0.5%, 1.0% and 2.0% neem concentrations were larvicidal for *Ceraeochrysa claveri*. Deleito and Borja (2008) tested the effect of neem concentrations ranging from 0.2% to 0.6% on *Lucilia cuprina*, *Chrysomya megacephala*, *Cochliomyia hominivorax* and *M. domestica*, all with positive results. These results were consistent with those obtained in our experiments. Vieira and Lello (2001) identified the epithelial columnar and regenerative cells as the major cells in *Dermatobia hominis* midguts, similar to what we find in *M. domestica*. Intakhan et al. (2014) also reported the presence of endocrine cells in *Ochlerotatus togoi*. In *Cyclorrhapha* larvae, Terra and Ferreira (1994) described oxyntic cells that are thought to pump protons into the ventricular lumen, but these cells were not seen in larvae of *M. domestica*. The columnar cells, also described as digestive cells, in larvae of *M. domestica* showed morphological characteristics of absorptive cells including cytoplasmic granules, microvilli on the apical area, and invaginations on the basal labyrinth. Columnar cells are the most frequently observed cells in the epithelium, characterized by basophilic cytoplasm and acidophilus fluted edge (Levy et al., 2004; Okuda et al., 2007; Fialho et al., 2009; Sousa et al., 2009 and Sousa and Conte, 2013). Based on the morphological comparisons of epithelial cells, especially of the columnar cells in the midgut of *M. domestica* larvae, we verified structural changes associated with the concentration of neem oil used. Morphological changes have also been described in studies on neem and other vegetable oils and derivatives (Nasiruddin and Mordue (Luntz), 1993; Nogueira et al., 1997; Rey et al., 1999; Arruda et al., 2003; Jing et al., 2005; Ndione et al., 2007; Correia et al., 2009; Roel et al., 2010; Lü et al., 2010; Almehmadi, 2011; Qi et al., 2011; Scudeler and Santos, 2013 and Scudeler et al., 2014). According to Cheville (1994, 2009) after progressive injury and dilatations, columnar cells are disrupted and became irregular or are lost altogether. The presence of intercellular spaces as well as the detachment of the basement membranes of these cells (Ndione et al., 2007; Correia et al., 2009 and Scudeler and Santos, 2013) were also observed after a few treatments with neem oil applied to *M. domestica*. The dilation of endoplasmic reticulum is one of the first signs of injury in most cells, which can subsequently end in the fragmentation of membrane vesicles and resulting in vacuolation of the cytoplasm (Endo and Nishiitsutsuji-Uwo, 1980; Nasiruddin and Mordue (Luntz), 1993; Cheville, 1994, 2009; Jing et al., 2005 and Qi et al., 2011). The increase in reticulum membranes in *M. domestica* may be a signal of an attempt to detoxification the cell in response to treatment with neem. Mitochondria shapes, often similar to possible autophagosomes, after covering cytosolic material were observed in larvae of *M. domestica*, similar to that described in *C. claveri* (Scudeler and Santos, 2013). Glycogen stores and lipid droplets were also observed in the midgut, indicating that the absorption and metabolism of carbohydrates and lipids takes place here (Levy et al., 2004; Fialho et al., 2009; Scudeler and Santos, 2013 and Scudeler et al., 2014).

CONCLUSION

The increase in lipid droplets can be interpreted as being related to the neem oil treatments. The numerous morphological changes found in the midgut of the larvae of *M. domestica* after treatment with various concentrations of neem oil probably resulted in malformations of pupae and it allows us to conclude that the neem oil can be a viable alternative to use in the control of *M. domestica*.

Acknowledgements

We thank of the Centro de Microscopia Eletrônica (CME) at the Universidade Estadual Paulista (UNESP), Botucatu, SP and Centro de Microscopia (CMI) of Complexo de Centrais de Apoio à Pesquisa (COMCAP) at the Universidade Estadual de Maringá (UEM), Maringá, PR., Laboratory of Microbial

Biotechnology coordinator by Dr. João Alencar Pamphile, in processing material used and assistance in handling the equipment.

REFERENCES

Acevedo GR, Zapater M and Toloza AC (2009). Insecticide resistance of house fly, *Musca domestica* (L.) from Argentina. Parasitology research, 105(2): 489-493.

Acharya N, Seliga RA, Rajotte EG, Jenkins NE and Thomas MB (2015). Persistence and efficacy of a Beauveria bassiana biopesticide against the house fly, *Musca domestica*, on typical structural substrates of poultry houses. Biocontrol Science and Technology, 25(6): 697-715.

Almehmadi RM (2011). Larvicidal, histopathological and ultra-structure studies of *Atricharia chamomella* extracts against the rift valley fever mosquito *Culex quinquefasciatus* (Culicidae:Diptera). Journal of Entomology, 8(1):63-72.

Arruda W, Oliveira GMC and Silva IG (2003). Toxicidade do extrato etanólico de *Magonia pubescens* sobre larvas de *Aedes aegypti*. Revista da Sociedade Brasileira de Medicina Tropical, 36(1): 17-25.

Beaty BJ and Marquardt WC (1996). The biology of disease vectors. University Press of Colorado.

Bosly AH (2013). Evaluation of insecticidal activities of *Mentha piperita* and *Lavandula angustifolia* essential oils against house fly, *Musca domestica* L.(Diptera: Muscidae). Journal of Entomology and Nematology, 5(4): 50-54.

Campanhola C (1990). Resistência de insetos a inseticidas: importância, características e manejo. EMBRAPA-CNPDA.

Cheville NF (1994). Ultrastructural pathology: an introduction to interpretation. Ames: Iowa State University Press.

Cheville NF (2009). Ultrastructural pathology: the comparative cellular basis of disease. 2. ed. Ames: Willey-Blackwell, Pp: 973.

Correia AA, Wanderley-Teixeira V, Teixeira AAC, Oliveira JV and Torres JB (2009). Morphology of the alimentary canal of *Spodoptera frugiperda* (JE Smith) larvae (Lepidoptera: Noctuidae) fed on neem-treated. Neotropical Entomology, 38(1): 83-91.

Deleito CSR and Borja GEM (2008). Nim (*Azadirachta indica*): uma alternativa no controle de moscas na pecuária. Pesquisa Veterinária Brasileira, 28(6): 293-298.

Endo Y and Nishiitsutsuji-Uwo J (1980). Mode of action of *Bacillus thuringiensis* δ endotoxin: histopathological changes in the silkworm midgut. Journal of Invertebrate Pathology, 36: 90-103.

Fernandes JI, Ribeiro FDA, Correia TR, Coumendouros K, Verocai GG, Fazio Junior PI, Silva DD and Scott FB (2010). Larvicidal efficacy of an emulsion containg 10% neem (*Azadirachta indica*) oil on the control of *Musca domestica* (LINNAEUS, 1758). Revista Brasileira De Medicina Veterinaria, 32: 25-30.

Fialho MCQ, Zanuncio JC, Neves CA, Ramalho FS and Serrão JE (2009). Ultrastructure of the digestive cells in the midgut of the predator *Brontocoris tabidus* (Heteroptera: Pentatomidae) after different feeding periods on prey and plants. Annals of the Entomological Society of America, 102(1): 119-127.

Förster M, Klimpel S, Mehlhorn H, Sievert K, Messler S and Pfeffer K (2007). Pilot study on synanthropic flies (eg *Musca*, *Sarcophaga*, *Calliphora*, *Fannia*, *Lucilia*, *Stomoxys*) as vectors of pathogenic microorganisms. Parasitology research, 101(1): 243-246.

Gallo D, Nakano O, Neto SS, Carvalho RPL, Batista GC, Berti Filho JRP, Parra RA, Zucchi SB, Alves JD, Vendramim LC and Marchini JRS (2002). Entomologia agrícola. Piracicaba: FEALQ, p 920.

Gibson JF, Kelso S, Jackson MD, Kits JH, Miranda GF and Skevington JH (2011). Diptera-specific polymerase chain reaction amplification primers of use in molecular phylogenetic research. Annals of the entomological society of America, 104(5): 976-997.

Ginarte CMA (2003). Efeitos de extratos de plantas e inseticidas de segunda e terceira gerações em populações de *M. domestica*. Efeitos de extratos de plantas e inseticidas de segunda e terceira gerações em populações de *M. domestica.* Tese de Doutorado, Unicamp, São Paulo. 136p.

Greenberg B (1973). Flies and diseases. Biology and disease transmission Vol. II. New Jersey: Princeton Univ. Press, USA.

Hebert PDN, Cywinska A, Ball SL and Dewaard JR (2003). Biological identifications through DNA *barcode*s. The Royal Society, 270: 313-321.

IBGE. Instituto Brasileiro de Geografia e Estatística (2015). Indicadores IBGE. Estatística da Produção Pecuária. 47 p. Junho.

Intakhan N, Jariyapan N, Chanmol W, Sor-Suwan S, Phattanawiboon B, Saeung A, Choochote W and Bates PA (2014). Midgut ultrastructure of fourth instar *Ochlerotatus togoi* (Diptera: Culicidae). Southeast Asian Journal of Tropical Medicine and Public Health, 45(6): 1301-1315.

Jing L, Fang Y, Ying X, Wenxing H, Meng X, Syed MN and Fang C (2005). Toxic impact of ingested jatropherol-I on selected enzymatic activities and the ultrastructure of midgut cells in silkworm, *Bombyx mori* L. Journal of Applied Entomology, 129(2): 98-104.

Junqueira LCU and Junqueira LMMS (1983). Técnicas básicas de citologia e histologia. São Paulo: Santos.

Khan HAA, Akram W and Shad SA (2013). Genetics, resistência cruzada e mecanismo de resistência a espinosad numa estirpe de campo de *Musca domestica L.* (Diptera: Muscidae). Acta Tropica, 130: 148-154.

Levy SM, Falleiros AMF, Gregório EA, Arrebola NR and Toledo LA (2004). The larval midgut of *Anticarsia gemmatalis* (HÜBNER) (Lepidoptera:Noctuidae): light and electron microscopy studies of the epithelial cells. Brazilian Journal Biology, 64(3B): 633-638.

Lü M, Wu W and Liu H (2010). Effects of fraxinellone on the midgut ultrastructural change of *Mythimna separata* Walker. Pesticide Biochemistry and Physiology, 98: 263-268.

Martinez SS and Van Endem HF (2001). Growth disruption, abnormalities and mortality of *Spodoptera littoralis* caused by azadirachtin. Neotropical Entomology, Londrina, 30(1):113-125.

Menezes ELA (2005). Inseticidas botânicos: seus princípios ativos, modo de ação e uso agrícola. Rio de Janeiro: Embrapa Agrobiologia, Pp.58.

Mordue (Luntz) AJ and Nisbet AJ (2000). Azadirachtin from the Neem Tree *Azadirachta indica*: its action against insects. Anais da Sociedade Entomologica do Brasil, 29(4): 615-632.

Nasiruddin M and Mordue (Luntz) AJ (1993). Effect of azadirachtin on the midgut histology of the locusts *Schistocerca gregaria* and *Locusta migratoria*. Tissue and Cell, 25(6): 875-884.

Ndione RD, Faye O, Ndiaye M, Dieye A and Afoutou JM (2007). Toxic effects of neem products (*Azadirachta indica* A. Juss) on *Aedes aegypti* Linnaeus 1762 larvae. African Journal of Biotechnology, 6(24):2846-2854.

Nogueira NFS, Gonzales M, Garcia EM and Souza W (1997). Effect of Azadirachtin A on the fine structure of the midgut of *Rhodnius prolixus*. Journal of Invertebrate Pathology, 69: 58-63.

Okuda K, Almeida F, Mortara RA, Krieger H, Marinotti O and Bijovsky AT (2007). Cell death and regeneration in the midgut of the mosquito *Culex quinquefasciatus*. Journal of Insect Physiology, 53:1307-1315.

Pedroso-De-Paiva D (2007). Controle integrado de moscas em avicultura intensiva de postura – controle mecânico. Concórdia: Embrapa Suíno e Aves.

Prabhaker N, Toscano NC and Henneberry TJ (1998). Evaluation of insecticide rotations and mixtures as resistance management strategies for *Bemisia argentifolii* (Homoptera: Aleyrodidae). Journal of Economic Entomology, 91(4): 820-826.

Qi Z, Shi B, Hu Z, Zhang Y and Wu W (2011). Ultrastructural effects of Celangulin V on midgut cells of the oriental armyworm, *Mythimna separata* walker (Lepidoptera: Noctuidae). Ecotoxicology Environmental Safety, 74(3): 39-44.

Quintela ED and Pinheiro PV (2004). Efeitos de extratos botânicos sobre a oviposição de *Bemisia tabaci* (Genn.) biótipo B (Hemiptera: Aleyrodidae) em feijoeiro (*Phaseolus vulgaris* L.). Embrapa Arroz e Feijão. Comunicado Técnico.

R CORE TEAM R (2013). A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Rey D, Pautou MP and Meyran JC (1999). Histopathological effects of tannic acid on the midgut epithelium of some aquatic dipteral larvae. Journal of Invertebrate Pathology, 73:173-181.

Roel AR, Dourado DM, Matias R, Porto KRA, Bednaski AV and Costa RB (2010). The effect of sub-lethal doses of *Azadirachta indica* (Meliaceae) oil on the midgut of *Spodoptera frugiperda* (Lepidoptera: Noctuidae). Revista Brasileira de Entomologia, 54(3):505-510.

Rossi YE and Palacios SM (2015). Toxicidade inseticida do óleo de *Eucalyptus cinerea* essencial e 1, 8-cineole contra *Musca domestica* e usos possíveis de acordo com a resposta metabólica de moscas. Cultivo de plantas industriais e os produtos, 63:133-137.

Saitou N and Nei M (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. Molecular biology and evolution, 4(4): 406-425.

Scott JG, Warren WC, Beukeboom LW, Bopp D, Clark AG, Giers S, Hediger M, Jones AK, Kasai S, Leichter CA, Li M, Meisel RP, Minx P, Murphy TD, Nelson DR, Reid WR, Rinkevich FD, Robertson HM, Sackton TB, Sattelle DB, Thibaud-Nissen F, Tomlinson C, Zande LV, Walden KK, Wilson RK and Liu N (2014). Genome of the house fly, *Musca domestica* L., a global vector of diseases with adaptations to a septic environment. Genome biology, 15(10): 466.

Scudeler EL and Santos DC (2013). Effects of neem oil (*Azadirachta indica* A. Juss) on midgut cells of predatory larvae *Ceraeochrysa claveri* (Navás, 1911)(Neuroptera: Chrysopidae). Micron, 44: 125-132.

Scudeler EL, Padovani CR and Santos DC (2014). Effects of neem oil (*Azadirachta indica* A. Juss) on the replacement of the midgut epithelium in the lacewing *Ceraeochrysa claveri* during larval-pupal metamorphosis. Acta Histochemica, 116:771-780.

Solà-Ginés M, González-López Jj, Cameron-Veas K, Piedra-Carrasco N, Cerdà-Cuéllar M and Migura-Garcia L (2015). MOSCAS DOMÉSTICAS (*Musca domestica*) como vetores para estendido *Spectrum-β-lactamase-Escherichia coli* produtora de frangos de corte em espanhol Farms. Applied and Environmental Microbiology, 81(11): 3604-3611.

Sousa G and Conte H (2013). Midgut morphophysiology in *Sitophilus zeamais* Motschulsky, 1855 (Coleoptera: Curculionidae). Micron, 51: 1-8.

Sousa MEC, Wanderley-Teixeira V, Teixeira AAC, Siqueira AA, Santos FAB and Alves LC (2009). Ultrastructure of the *Alabama argillacea* (Hübner) (Lepidoptera: Noctuidae) midgut. Micron, 40: 743-749.

Stoeckle M, Waggoner PE and Ausubel JH (2005). Barcoding life, illustrated. Goals, rationale, results.

Terra WR and Ferreira C (1994). Insect digestive enzymes: properties, compartmentalization and function. Comparative Biochemistry and Physiology Part B: Comparative Biochemistry, 109(1): 1-62.

Veiga MM, Silva DM, Veiga LBE and De Castro Faria MV (2006). Análise da contaminação dos sistemas hídricos por agrotóxicos numa pequena comunidade rural do Sudeste do Brasil. Cadernos Saúde Pública, 22(11): 2391-2399.

Viegas Júnior C (2003). Terpenos com atividade inseticida: uma alternativa para o controle químico de insetos. Quim. Nova, 26(3): 390-400.

Vieira AM and Lello E (2001). Desenvolvimento pós-embrionário do intestino anterior de *Dermatobia hominis* (Linnaeus Jr.)(Diptera, Cuterebridae). Revista Brasileira de Zoologia, 18(1): 81-90.

Waldschmidt AM, Marco-Junior P, Barros EG and Campos LAO (2002). Genetic analysis of *Melipona quadrifasciata* LEP.(Hymenoptera: Apidae, Meliponinae) with RAPD markers. Brazilian Journal of Biology, 62(4B), pp: 923-928.

Zhong M, Wang X, Wen J, Cai J, Wu C and Aly SM (2013). Selection of reference genes for quantitative gene expression studies in the house fly (*Musca domestica* L.) using reverse transcription quantitative real-time PCR. Acta biochimica et biophysica Sinica, 45(12): 1069-1073.