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INFLUENCE OF THE ESSENCIAL OILS OF *Origanum majorana* AND *Copaifera officinalis* IN COMPARISON TO FORMULATED PRODUCTS ON ADULT GONADS OF *Neoleucinodes elegantalis* (GUENÉE) (LEPIDOPTERA: CRAMBIDAE)

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Abstract: *Neoleucinodes elegantalis* is one of the key pests of the tomato crop, responsible for causing large losses in the production. Therefore, control measures with new modes of action or target sites have been investigated, such as the use of essential oils. Thus, the present study evaluated the effects of the essential oils of *Copaifera officinalis* L. and *Origanum majorana* L. on the histology and histochemistry of *N. elegantalis* gonads, based on the analysis of morphology, carbohydrates and proteins, compared to commercial formulated products. The essential oils were analyzed by gas chromatography (GC-MS). To carry out the bioassays after emergence, 48h old adults were sprayed with sublethal concentrations of 666.34 and 528.37 mg/L, of *C. officinalis* and *O. majorana* oils, respectively. The formulated insecticides, Decis ® and Azamax ®, in turn, were used in their respective indicated concentrations. After 48h, the testicles and ovarioles were collected for analysis. As a result, it was observed yolk reduction and changes in the concentration of carbohydrates and proteins in the gonads of *N. elegantalis*, and among all treatments *O. majorana* caused greater damage to the gametogenesis. Regarding gas chromatography, E-Caryophyllene and Terpinene were the major components of the essential oils.
Keywords: E-Caryophyllene, Terpinene, bioactive, histophysiology, tomato fruit borer, gametogenesis.

INTRODUCTION

For decades, the use of synthetic insecticides was the most efficient and viable control method for insect pests. However, factors like the development of resistant populations and the damage caused to non-target organisms triggered the search for ecologically viable control alternatives (Desneux *et al.*, 2007; Benelli, 2015a). Therefore, botanical products, such as essential oils, gained prominence. They are defensive substances derived from the secondary metabolism of plants and with high insecticidal properties (Isman, 2020; Pavela *et al.*, 2020).

Essential oils have a wide range of target sites and act on different metabolic pathways. They act by causing neurotoxicity, regulating insect growth, preventing digestive enzymes, inhibiting glutathione-S-transferase, and altering gametogenesis processes (Hummelbrunner and Isman, 2001; Park and Tak, 2016).Essential oils are promising sources of insecticide molecules, overcoming most of the challenges associated with synthetic insecticides: they are biodegradable, have greater selectivity, and are relevant possibilities for use in insecticide rotation programs to minimize the emergence of resistant insects (Pontual *et al.*, 2014; Benelli *et al.*, 2015b; Isman, 2020).

According to the Brazilian Institute of Geography and Statistics, IBGE, the tomato crop in 2020 is estimated at 4.3 million tons, with a planted area of more than 79 million hectares (IBGE, 2019; IBGE, 2020). However, its cultivation and productivity can be compromised due to phytosanitary problems caused by insect pests.The tomato fruit borer, *Neoleucinodes elegantalis* Guenée (Lepidoptera: Crambidae), is one of the most relevant pests for tomato production in South America. Females lay their eggs under the sepals of small green fruits and, after hatching, the caterpillar pierces the fruit and stays until it completes the development, feeding on the mesocarp and endosperm. Therefore, the pest is protected inside the fruit, and can cause damage of up to 90% on the final production (Gravena and Benvenga, 2003; Picanço *et al.*, 2007; Montilla *et al.*, 2013; EPPO, 2015; Silva *et al.*, 2016; Silva *et al.*, 2018).

The biological behavior of housing and

developing inside the fruit makes it difficult to control the pest, reducing the possibilities of viable products for its management. It explains the applications of synthetic insecticides on tomato plants (Badji *et al.*, 2003; Fragoso *et al.*, 2021).In Brazil, about 102 insecticides are registered for the control of *N. elegantalis* in tomato plants (Silva *et al.*, 2018; AGROFIT, 2021). However, although it exists a large number of registered pesticides, crop losses are still annually registered in tomato production. Besides, the use of synthetic insecticides is expensive, potentially harmful to the agroecosystem, and constantly selects resistant insect populations (Omoto, 2000; Benvenga *et al.*, 2010).

The essential oils of *Origanum majorana* L (Lamiaceae) and *Copaifera officinalis* L. (Fabaceae) demonstrate bioactivity on a large number of economically important insect species (Benelli *et al.*, 2017; Demirel and Erdogan, 2017).However, even with the growing number of researches that investigate the insecticidal action of these essential oils on agricultural pests and the importance of *N. elegantalis* for tomato crops, there are no reports of research addressing the effects of these substances on this pest.Research with this objective is extremely important to elucidate the insecticidal potential that essential oils can exert and be used in association with synthetic insecticides to reduce production costs and damage to the environment (Isman, 2008). Another aspect to be analyzed is that small producers also carry out tomato cultivation, and this type of control is economically viable, as it does not require large resources compared to commonly used methods (Picanço *et al.*, 2004).

The reproductive system of insects enables the perpetuation of the species. In holometabolic insects such as lepidopterans, interference in the immature phase can result in changes that affect several parameters of adults, such as longevity, oviposition, and even the formation of their sexual structures (Sâmia *et al.*, 2016; Ataide *et al.*, 2020). Sometimes, the gonads of pest insects have been a target site for the evaluation of bioactive compounds that act on reproduction and help in pest management (Alves *et al.*, 2014; Cruz *et al.*, 2015; Silva *et al.*, 2016).

Therefore, this research aimed to evaluate the effects of essential oils from *C. officinallis* and *O. majorana* on the histology and histochemistry of the gonads of *N. elegantalis* in comparison to the formulated products Decis ® and Azamax ®, indicated for the control of the tomato borer. This parameter was chosen because it interferes with the establishment of the pest in the culture.

MATERIALS AND METHODS

The present research was developed in the Laboratory of Chemical Ecology in the Fundamental Chemistry Department of the Federal University of Pernambuco (DQF-UFPE), in the Laboratory of Histology and Physiology of Insects in the Department of Morphology and Animal Physiology (DMFA), and in the Research Support Center (CENAPESQ) of the Federal Rural University of Pernambuco (UFRPE), Recife, Pernambuco, Brazil.

TOMATO PLANTING

To use tomato fruits for the rearing and the development of experiments, the cultivar Yoshimatsu was planted. The tomato seedlings were produced in polyethylene trays in 128 cells and the transplant was carried out 29 days after sowing, when the plants had four definitive leaves, on average. At this time, they were transplanted to the Horta of the Department of Agronomy, UFRPE, using a spacing of 1.0m × 0.5m, where they received standard cultural treatments, such as pruning, tying, staking, and fertilization.

REARING OF *NEOLEUCINODES ELEGANTALIS*

For the beginning of the pest rearing in the laboratory, infested tomato fruits were collected from plantations in the cities Camocim de São Félix (8º21'31" S and 35º45'43" W) and Bezerros-PE (8º14'00" S and 35º47'49" W), in Pernambuco, Brazil.The tomato fruits were taken to the Laboratory of Insect Physiology at UFRPE and were kept in plastic trays covered with paper towels and voil until the caterpillars reached the last larval instar, when they left the fruit, passing to the pupae stage. Subsequently, pupae were placed in wooden and organza screened cages (60 × 60 × 60 cm) until the emergence of the adults, which were fed with a 10% sucrose solution. Unripe tomato fruits of approximately 3 cm in diameter, of the Yoshimatsu variety, were offered for oviposition in plastic containers (500 mL) containing water, daily changed. Tomato leaves were also used as stimuli for oviposition.The eggs were transferred to organic jilo fruits (obtained from commercial establishments) of approximately 7 cm in length. The fruits were kept in plastic trays covered with paper towels for about 15 days, until the caterpillars reached the last larval instar, when they abandoned the fruits and passed the pupal stage on the paper towel. The rearing of *N. elegantalis* was maintained at 26°C ± 2 °C and 70% ± 10% relative humidity, with a 12h period of exposure to light.

OBTAINING ESSENTIAL OILS AND FORMULATED INSECTICIDES

The essential oils of *C. officinalis* and *O. majorana*, used in the experiments, were obtained from Empresa Ferquima Ind. e Com. Ltda. (Vargem Grande Paulista, São Paulo, Brazil). The technical information on these products and their quality parameters (color, purity, odor, density at 20 °C, and refractive index at 20 °C) are described in a technical report on the company website < http://ferquima.com.br >.

The insecticides azadirachtin (Azamax ®) and deltamethrin (Decis ®) were acquired from pesticide distributors in Recife-PE. Azamax ® is a natural insecticide of the tetranortriterpenoid group that acts as both a repellent and an insecticide, by inhibiting feeding and insect growth (AGROFIT, 2021). In turn, Decis ® is a contact and ingestion insecticide of the pyrethroid group (AGROFIT, 2021).

GAS CHROMATOGRAPHY - MASS SPECTROMETRY ANALYSIS (GC-MS) OF ESSENTIAL OILS

Chemical composition analysis of the essential oils was carried out at the Laboratory of Chemical Ecology of the Federal University of Pernambuco. The analyses used gas chromatography coupled to a mass spectrometer in an Agilent 5975C Series quadrupole system (GC-EM) (Agilent Technologies, Palo Alto, USA), equipped with a DB-5 non-polar column (Agilent J&W; 60 m × 0.25 mm i.d., 0.25 μm film thickness). The 1.0 μL solution of known concentration, containing the essential oil diluted in hexane was injected in a 1:20 split, as well as the solution of the C9-C34 hydrocarbon standard mixture.This hexane solution is composed of commercial standards from Sigma-Aldrich ®. The temperature of the GC was held at 60°C for 3 min, and then raised to 240 °C at 2.5°C min-1, which was maintained for 10 min. Helium flow was kept at a constant pressure of 100 kPa.The MS interface was set at 200 °C and the mass spectra were recorded at 70 eV in the EI mode, with a scan rate of 0.5 scans-1 at 20 – 350 m/z. The retention index for each component of the oil was calculated from the retention times of the compounds in the sample in the hydrocarbon standard and the combination of the essential oil with the mixture of this standard, according to the Van den Dool and Kratz equation (1963).The components of essential oils were identified by the similarity of the retention index values and later their identities were confirmed by comparing the respective mass spectra with those available in the GC-MS library: MassFinder 4, NIST08 and Wiley Registry™ 9th Edition and those described by Adams (2009). Finally, the peak areas in the chromatograms were integrated to obtain the total ionic signal and its values were used to determine the respective relative proportions for each compound.

BIOASSAYS

Solubility tests were carried out to identify the best diluents for each oil. The diluents used were acetone, ethyl alcohol and DMSO (Dimethyl Sulfoxide) and the essential oils were *C. officinalis* and *O. majorana*. The diluents were mixed with the oil and distilled water (1 ml of the diluent + 0.1 ml oil + 100 ml of distilled water). Subsequently, the resulting homogeneity of the various solutions was observed. The best diluents for *C. officinalis* oil and *O. majorana* were acetone and DMSO, respectively.

The bioassays were carried out with 48h old adults after emergence. The treatments consisted of the dilution of *C. officinalis* oil in acetone and the dilution of *O. majorana* oil in dimethyl sulfoxide (DMSO), in lethal concentrations (LC_{45}) of 666.34 and 528.37 mg/L, respectively. For the botanical insecticide azadirachtin (AzaMax ® 0.6 mg/L) and pyrethroid insecticide deltamethrin (Decis ® 12.5 mg/L) the respective recommended field dosage to control *N. elegantalis*was taken as a guideline. The adults were immobilized at low temperatures (4°C) and sprayed with the respective concentrations of each product. Each treatment consisted of 40 adults and the control bioassay was treated only with acetone. After the treatments, the insects were kept in cages and fed with a 10% sucrose solution, and 48 hours after the application of the products, the adults were dissected.

HISTOLOGICAL AND HISTOCHEMICAL ANALYSIS OF *N. ELEGANTALIS* ADULT GONADS

Adults undergoing treatments were used 48h after exposure for histological and histochemical analysis of their gonads. They were immobilized at a temperature of 4°C and dissected under a stereomicroscope. Ten ovarioles and ten testicles were collected and fixed in 10% formaldehyde for 24 hours and preserved in 70% alcohol.The ovarioles and testicles were dehydrated in increasing baths of ethyl alcohol (70 - 100%) for 10 minutes each, then they were soaked in alcohol + historesin solution (1:1 v/v) for a period of 24 hours and, finally, included in pure historesin (Leica ®). Sections of 5 μm thick slices were obtained using a Leica ® 2035 semi-automatic microtome. The material obtained was submitted to toluidine blue stain for morphological analysis of the organs, the Periodic Acid Schiff (PAS) stain to detect neutral polysaccharides(Junqueira and Junqueira, 1983), and Xylidine Ponceau staining to detect total proteins (Pearse, 1960).All slides were examined using a Leica ® photomicroscope. The images were captured and digitized by the LAS Leica Image software.

AVERAGE QUANTIFICATION OF NEUTRAL POLYSACCHARIDES AND TOTAL PROTEINS IN ADULTS OF *N. ELEGANTALIS*

The captured images of the adult gonads of *N. elegantalis* were submitted to the GIMP ® 2.8 image editor program (GNU Image Manipulation Program, UNIX platforms) which converts digital images to a grayscale (black and white). This segmentation of colors facilitates the measurement of the number of pixels related to the marking selected in the tissue (Solomon, 2009). For each histochemical analysis, three slides from different individuals were used, with four fields from each analysis being measured, totaling 12 fields per group.The results of carbohydrate and protein contents were submitted to the Kruskal-Wallis non-parametric test at a 5% level of significance using the SAS Institute program.

RESULTS

CHEMICAL COMPOSITION OF THE ESSENTIAL OILS OF *ORIGANUM MAJORANA* AND *COPAIFERA OFFICINALIS*

The composition of *C. officinalis* oil (Table 1), presented a total of 30 compounds, representing 98.6% of the oil.The main components identified were E-caryophyllene (53.89%), α-humulene (8.73%), and E-α-bergamotene (7.79%). In the *O. majorana* oil, a total of 22 compounds were identified (Table 2), representing 100% of its composition. The major components were terpinen-4-ol (25.97%), e-sabinene hydrate (15.19%) and y-terpinene (13.67%).Most of the compounds in both essential oils belongs to the chemical group of terpenes and are classified as monoterpenes and sesquiterpenes.

HISTOLOGICAL ANALYSIS OF THE GONADS OF *N. ELEGANTALIS*

In control group, the ovarioles of *N. elegantalis* are covered by a connective tissue sheath and contain a region with a lot of yolk. In the ovarioles treated with *C. officinalis*, *O. majorana*, Decis® and Azamax®, the same pattern was observed.However, in the ovarioles of the insects treated with *O. majorana*, there was a reduction in the quantity of yolk (Fig. 1).

The testicles of *N. elegantalis* in control group showed a connective tissue lining with an abundance of cysts with spermThe testicles from the treated adults exhibited the same pattern as that of the control group. However, in the testicles of insects treated with *O. majorana*, empty spaces, not filled with sperm were observed (Fig. 2).

HISTOCHEMICAL ANALYSIS OF THE GONADS OF *N. ELEGANTALIS*

Histochemical analysis detected a positive reaction for Periodic Acid Schiff in the ovarioles of the adults in the control and treatment groups (Fig. 3A-E), showing that all products reduced the levels of neutral polysaccharides. However, Azamax® caused the greatest reduction ($\chi^2 = 36.01$; $P < 0.0001$) (Fig. 3F). In the detection of proteins by Xylidine Ponceau all groups showed positive staining in the ovarioles (Fig. 4A-E), with a reduction in the protein contents in all treatments. The *O. majorana* oil and Azamax® caused the most significant reduction ($\chi^2 = 33.12$; $P < 0.0001$) (Fig. 4F).

In the testicles of adults of *Neoleucinodes elegantalis* there was a positive reaction for PAS (Fig. 5A-E). All treatments reduced carbohydrate contents in these organs. However, those treated with *O. majorana* and Decis® showed the greatest reduction ($\chi^2 = 33.71$; $P < 0.0001$) (Fig. 5F). In the detection of proteins by Xylidine Ponceau, both the control and the treatments groups showed positive staining in the testicles of adults (Fig. 6A-E). The groups treated with *O. majorana*, Decis®, and Azamax® caused a reduction in protein levels of their gonads ($\chi^2 = 19.06$; $P = 0.0008$) (Fig. 6F).

DISCUSSION

Essential oils are made up of bioactive compounds that have different modes of action and different target sites, increasing their insecticidal or insectistatic activity due to the synergistic action between their constituents (Pauliquevis and Favero, 2015; Mossa, 2016). Lipophilicity, variations in the size, in the shapes of molecules, and in the type offunctional group, are characteristics that influence the efficiency of oils (Castilhos *et al.*, 2017). This can alter the physiology of insects resulting, for example, in an interruption of reproductive processes, such as oogenesis, vitellogenesis, the maturation and growth of spermatocytes, as well as biochemical dysfunction and mortality (Lee *et al.*, 2001; Castilhos *et al.*, 2017).

Terpenes are the most abundant constituents of essential oils and can reduce the reproductive capacity of insects (Zuarte and Salgueiro, 2015). All the compounds present in the oils studied in this research belong to this group. Terpinen-4-ol, E-sabinene hydrate, γ-terpinene, α-terpinene, Z-babinene hydrate, and sabinene were the major components of *O. majorana*, and are classified as monoterpenes.Meanwhile, E-caryophyllene, α-humulene, E-α-bergamotene, α-copaene, and δ-cadinene were detected as the major compounds of *C. officinalis*, and are classified as sesquiterpenes.

The terpinen-4-ol and γ-terpinene monoterpenes, identified as major components in the essential oil of *O. majorana*, are widely known for their biocidal activity (Demirel and Erdogan, 2017). According to Abbassy *et al.* (2009) these bioactives exhibited significant insecticidal activity against fourth-instar caterpillars of *Spodoptera costalis* (Boisd.) (Lepidoptera: Noctuidae) and adults of *Aphis fabae* Scopoli (Homoptera: Aphididae), and γ-terpinene was the most toxic. When tested in a binary mixture with the synthetic insecticides Profenofos and Methomyl, both compounds increased the insecticidal action two to three times. The use of a mixture instead of a single product, according to Isman (1997), interferes with the development of resistance by the pest, since it is more difficult to detoxify a complex of substances than just one or a few components. In addition, a mixture can present more than one mode of activity and multiple action sites, acting on physiological and behavioral parameters, demonstrating that essential oils can contribute to possible formulations to control insect pests.

Benelli *et al.* (2017) attributed the toxicity of *C. officinalis* essential oil to the compound α-humulene (LC_{50} = 20.86 μg/ml) when administered by ingestion over *Helicoverpa armigera*. This essential oil is described in the literature as a promising source of toxic molecules to vector insects, such as *Aedes aegypti* and *Anopheles darlingi* (Sharma *et al.*, 2011; El-Sherbini *et al.*, 2014; Prophiro *et al.*, 2012; Trindade *et al.*, 2013), and our results highlight histophysiological disorders on *N. elegantalis*. These findings are similar to those obtained by Silva *et al.* (2016), who reported a smaller amount of yolk, and a reduction in neutral carbohydrates and proteins in gonads of *S. frugiperda* when treated with *Cymbopogon winterianus* (Poaceae). The authors attribute this effect to the citronellal, a majority compound of this oil.

In the ovarioles of insects, the yolk consists of large protein-filled vesicles interspersed with small lipid droplets and glycogen particles. The development of the yolk uses the reserves of proteins, lipids, and carbohydrates that are accumulated in the larval period and stored in the pupae (Telfer, 2009). Therefore, the sexual maturation of lepidopterans depends on the nutrients acquired during the immature stage (Alves *et al.*, 2014). Presumably, the reduction of yolk caused by essential oils and by the formulated products in the ovarioles of *N. elegantalis* was induced by physiological stress conditions promoted by the products. Similar results were verified by Ghazaway *et al.* (2007), where they observed histopathological alterations in the ovarioles of *Heteracris littoralis* Ramb when treated with azadirachtin, showing a reduction of yolk in the oocyte. These results are also comparable to the reduction of yolk in the oocyte of *S. gregararia* treated with the synthetic insecticide Lufenuron (Ghazawai, 2005), demonstrating that the essential oils used in this research are efficient when compared to synthetic insecticides.

The reduction of sperm in the testicles of *N. elegantalis* adults caused by the essential oils and the formulated products is similar to the reduction in sperm bundles verified by Alves *et al.* (2014) using *Piper hispidinervum* Jacq. oil in *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae). According to Ghazawai *et al.* (2005), high concentrations of azadirachtin prevent spermatogonia from cysts formation and the spermatocytes are unable to make their normal divisions and complete spermatogenesis. On the other hand, at lower concentrations, the spermatocyte formation process continues until the appearance of sperm bundles, but with deformations and degenerations.

The essential oil of *O. majorana* showed greater activity because it increased the reduction of yolk in females and the formation of vacuoles not filled by sperm in males.This fact was due to the abundance of monoterpenes, which have lipophilic properties and may be able to promote changes in the metabolism and physiology of insects (Cox *et al.*, 2000; Inoue *et al.*, 2004). This chemical property could explain the efficiency of *O. majorana* oil in histophysiological changes.

Histological changes in ovarioles and testicles can cause a decrease in fecundity and fertility, promoting a population reduction and keeping it at a level that does not justify the adoption of control measures, reducing the costs of the production (Cruz *et al.*, 2015; Silva *et al.*, 2016). Nutritional status is another factor that directly influences reproductive fitness in holometabolic insects, as sexual maturation depends on nutrients acquired in acceptable qualities and quantities during the immature stage (Alves *et al.*, 2014). Carbohydrates, for example, are required in several metabolic processes; participate in the development of the cuticular and reproductive structures, and the previtellogenic process. Besides, they act as the main energy source for the insect (Arrese *et al.*, 2010; Chapman, 2013).

Furthermore, proteins are also of great importance for the functioning and metabolism of insects. They participate in the structuring of the tegument, in the synthesis of hormones and enzymes, and enable the fat body to synthesize vitellogenin, the main protein that constitutes the yolk and is necessary for the reproductive success of females (Kunkel and Nordin, 1985; Klowden, 2007). The reduction of proteins is probably due to the interference caused by bioactives present in botanical insecticides, acting on the hormones that regulate protein synthesis (Senthilkumar *et al.*, 2009). The reduction of proteins, carbohydrates, yolk, and sperm, observed in *N. elegantalis* infer that these histochemical changes caused by essential oils and formulated products directly and negatively affect its vitellogenesis and spermatogenesis, compromising the reproductive activities (Sharma *et al.*, 2011).

FINAL CONSIDERATIONS

Although it does not express direct lethality in the treated insect, sublethal doses cause secondary effects in the gonads, as observed in this research. These can be of great importance for pest management programs since by interfering with gametogenesisbioactive affects the quantity and quality of the offspring and, consequently, affects the deleterious and economic effects on the final product.

Therefore, the histopathological changes observed in this study demonstrate that botanical molecules can interfere with the reproductive parameters of pest insects. Then, further studies may support the hypothesis that deleterious effects on the gonads of economically important insects may be beneficial for their control.

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Nº	Compound	IR^{L}	IR^{C}	%
1	δ-Elemene	1335	1337	0,45
2	α-Cubebene	1348	1350	0,80
3	α-Ylangene	1373	1371	0,10
4	α-Copaene	1374	1376	5,34
5	β-Elemene	1389	1392	1,51
6	Cyperene	1398	1399	0,36
7	α-Gurjunene	1409	1410	0,05
8	E-Caryophyllene	1417	1421	53,89
9	β-Gurjunene	1431	1429	0,51
10	E-α-Bergamotene	1432	1436	7,79
11	Aromadendrene	1439	1444	0,35
12	<epi-β>Santalene	1445	1448	0,20
13	α-Humulene	1452	1454	8,73
14	allo-Aromadendrene	1458	1461	0,74
15	y-Muurolene	1478	1477	2,43
16	Germacrene D	1480	1482	3,01
17	y-Amorphene	1495	1498	1,86
18	α-Muurolene	1500	1500	0,86
19	β-Bisabolene	1505	1509	2,52
20	y-Cadinene	1513	1515	1,33
21	δ-Cadinene	1522	1524	3,70
22	E-Cadina-1,4-diene	1533	1533	0,24
23	α-Cadinene	1537	1538	0,21
24	Caryolan-8-ol	1571	1571	0,34
25	Caryophyllenoxide	1582	1584	0,35
26	Junenol	1618	1620	0,35
27	<1-epi->Cubenol	1627	1630	0,06
28	Cubenol	1645	1644	0,14
29	α-Muurolol	1644	1648	0,16
30	α-Cadinol	1652	1657	0,22
	Total			**98,60**

IR^{L} Kratz retention indices of literature (Adams, 2009);

IR^{C} Calculated Kratz retention indices;

% Percentage of the compound.

Table 1. Chemical profile of distilled *Copaifera officinalis* essential oil.

Nº	Compound	IR^L	IR^C	%
1	α- Thujene	924	924	0,65
2	α- Pinene	932	930	0,69
3	Sabinene	969	970	6,07
4	Myrcene	988	990	1,20
5	α- Phellandrene	1002	1002	0,38
6	α- Terpinene	1014	1014	8,29
7	o- Cymene	1022	1022	2,64
8	β- Phellandrene	1025	1026	3,85
9	y- Terpinene	1054	1057	13,67
10	Z- Sabinene hydrate	1065	1065	7,05
11	Terpinolene	1086	1087	2,74
12	E- Sabinene hydrate	1097	1096	15,19
13	Linalool	1095	1099	1,17
14	Z-p- Menth-2-en-1-ol	1118	1120	1,04
15	E-p- Menth-2-en-1-ol	1136	1138	0,76
16	Terpinene-4-ol	1174	1176	25,97
17	α- Terpineol	1186	1189	3,04
18	Z-Piperitol	1195	1194	0,50
19	E-Piperitol	1207	1207	0,32
20	Linalool acetate	1254	1256	1,94
21	E- Caryophyllene	1417	1420	2,11
22	Bicyclogermacrene	1500	1497	0,74
	Total			**100,0**

IR^L Kratz retention indices of literature (Adams, 2009);

IR^C Calculated Kratz retention indices;

% Percentage of the compound.

Table 2. Chemical profile of *Origanum majorana* essential oil.

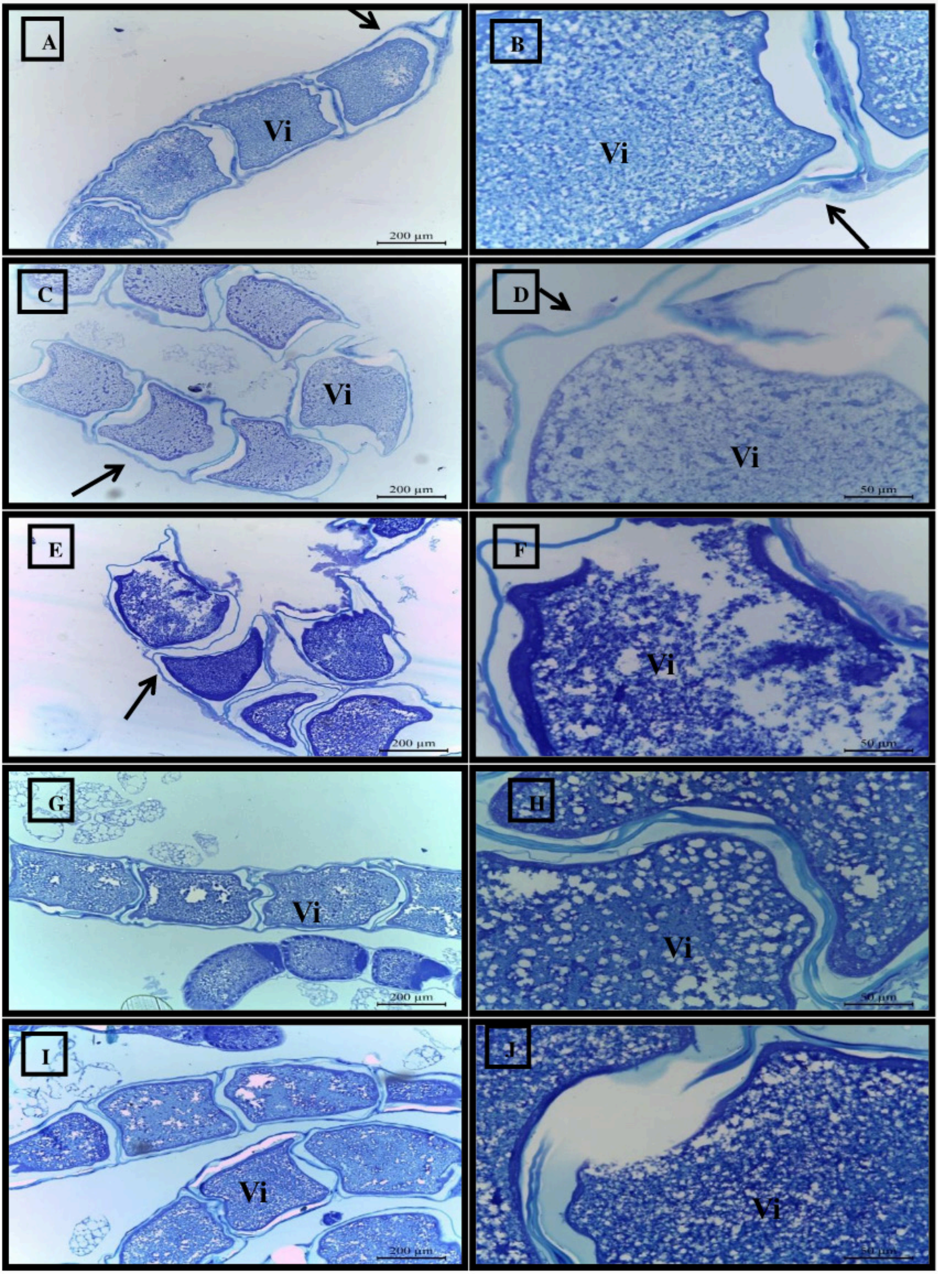

Figure 1. Longitudinal sections of ovarioles of *Neoleucinodes elegantalis* adults sprayed with the CL_{45} of the essential oils of *Copaifera officinalis* and *Origanum majorana*, and with the commercial concentration of the formulated products Decis® and Azamax®.A and B – Control; C and D – *Copaifera officinalis*; E and F – *Origanum majorana*; G and H – Decis®; I and J – Azamax®. Coloration with Toluidine Blue.Yolk (Vi);s heath of connective tissue (long arrow).

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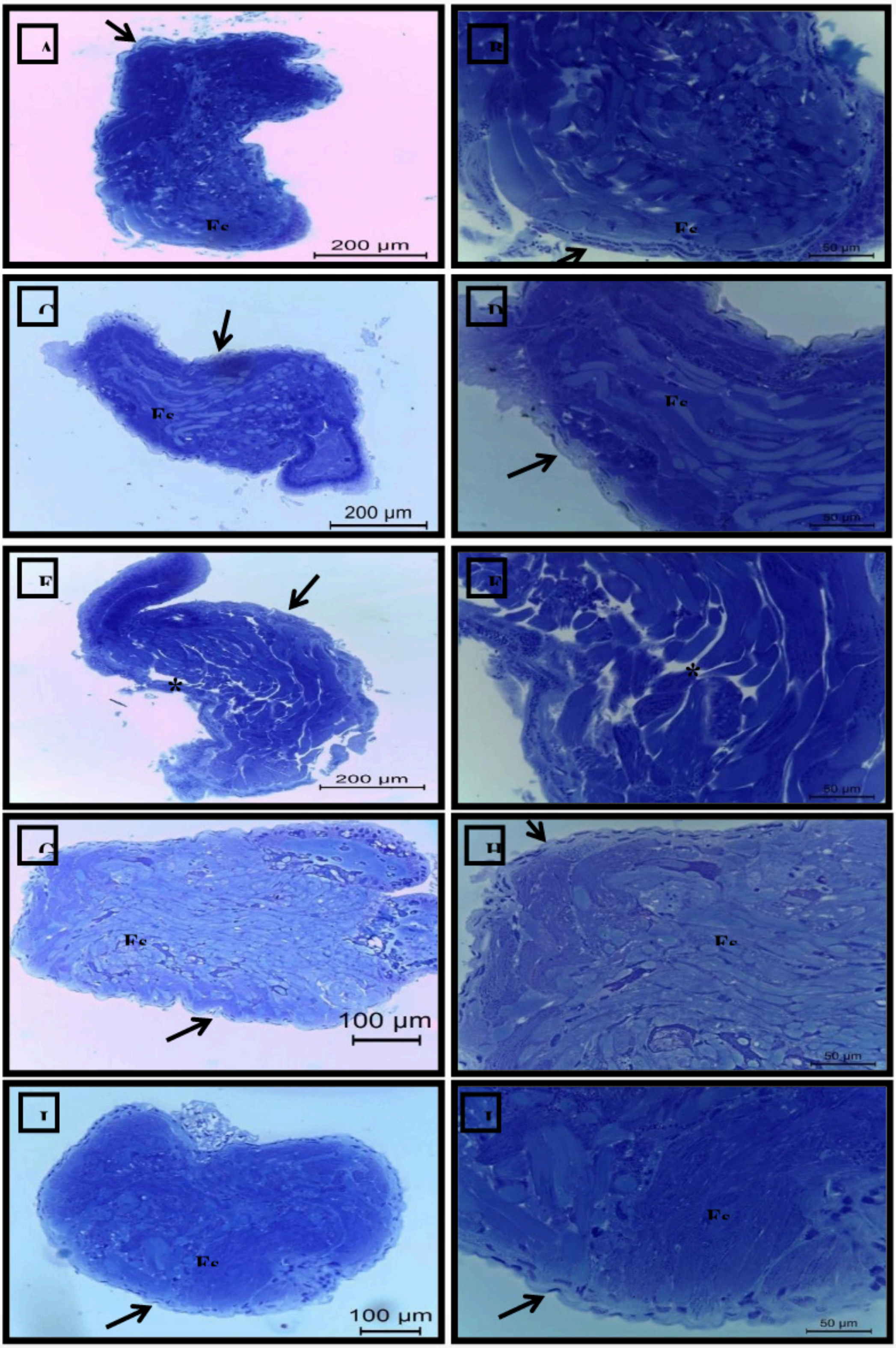
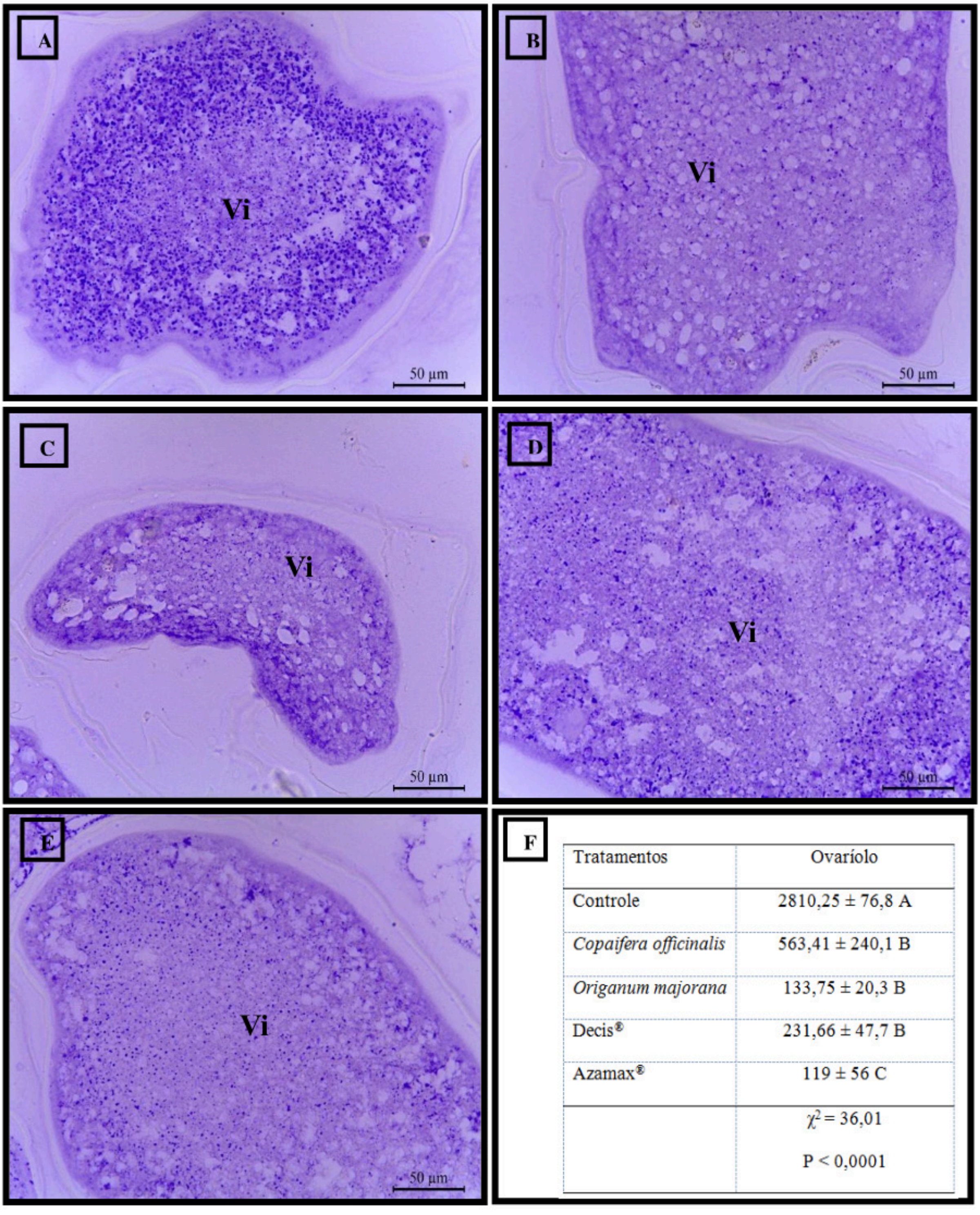
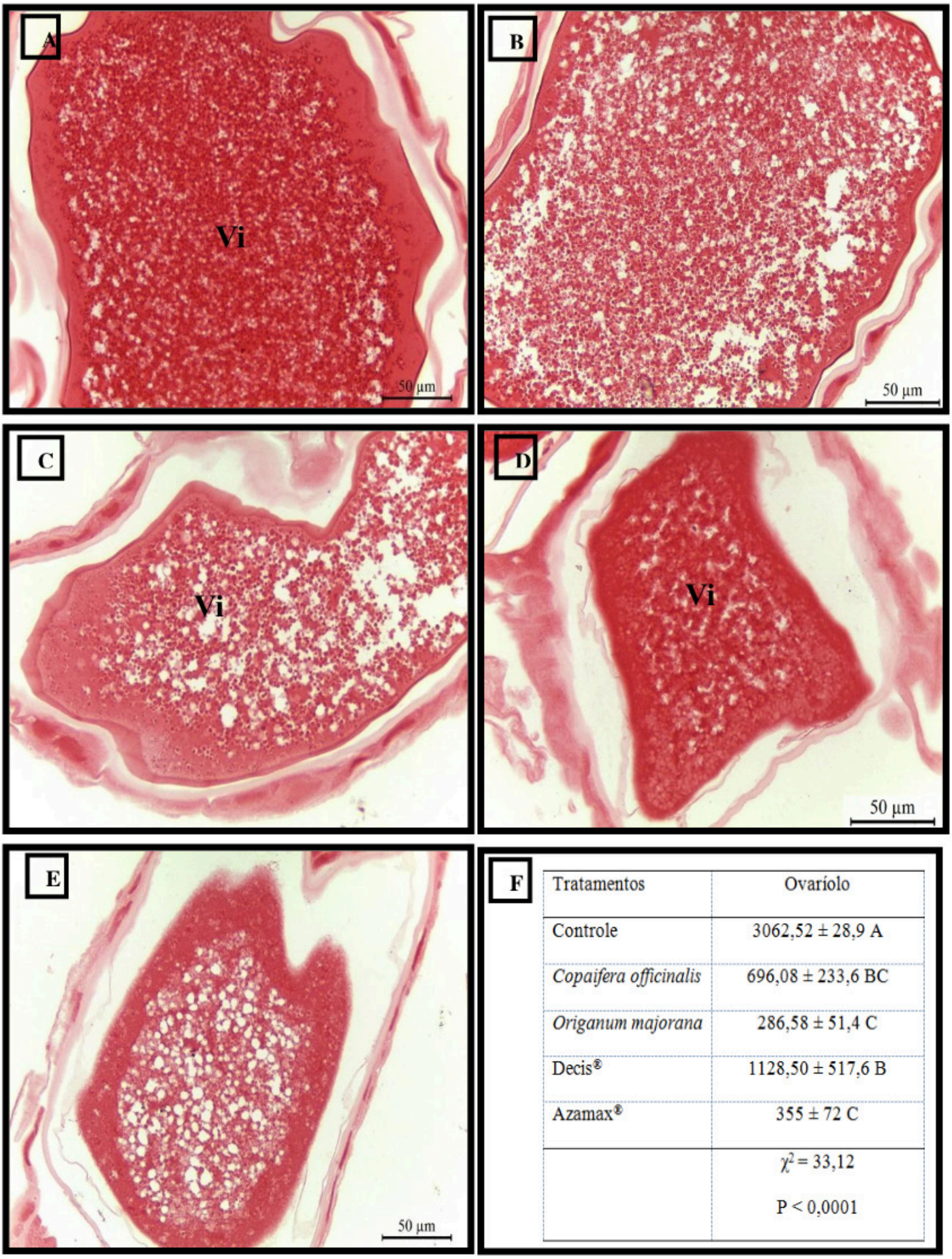


Figure 2. Longitudinal sections of testicles of *Neoleucinodes elegantalis* adults sprayed with the CL_{45} of the essential oils of *Copaifera officinalis* and *Origanum majorana*, and with the commercial concentration of the formulated products Decis® and Azamax®.A and B – Control; C and D – *Copaifera officinalis*; E and F – *Origanummajorana*; G and H – Decis®; I and J – Azamax®. Coloration with Toluidine Blue. Connective tissue (long arrows); sperm (Sp); spaces not filled by sperm (*).



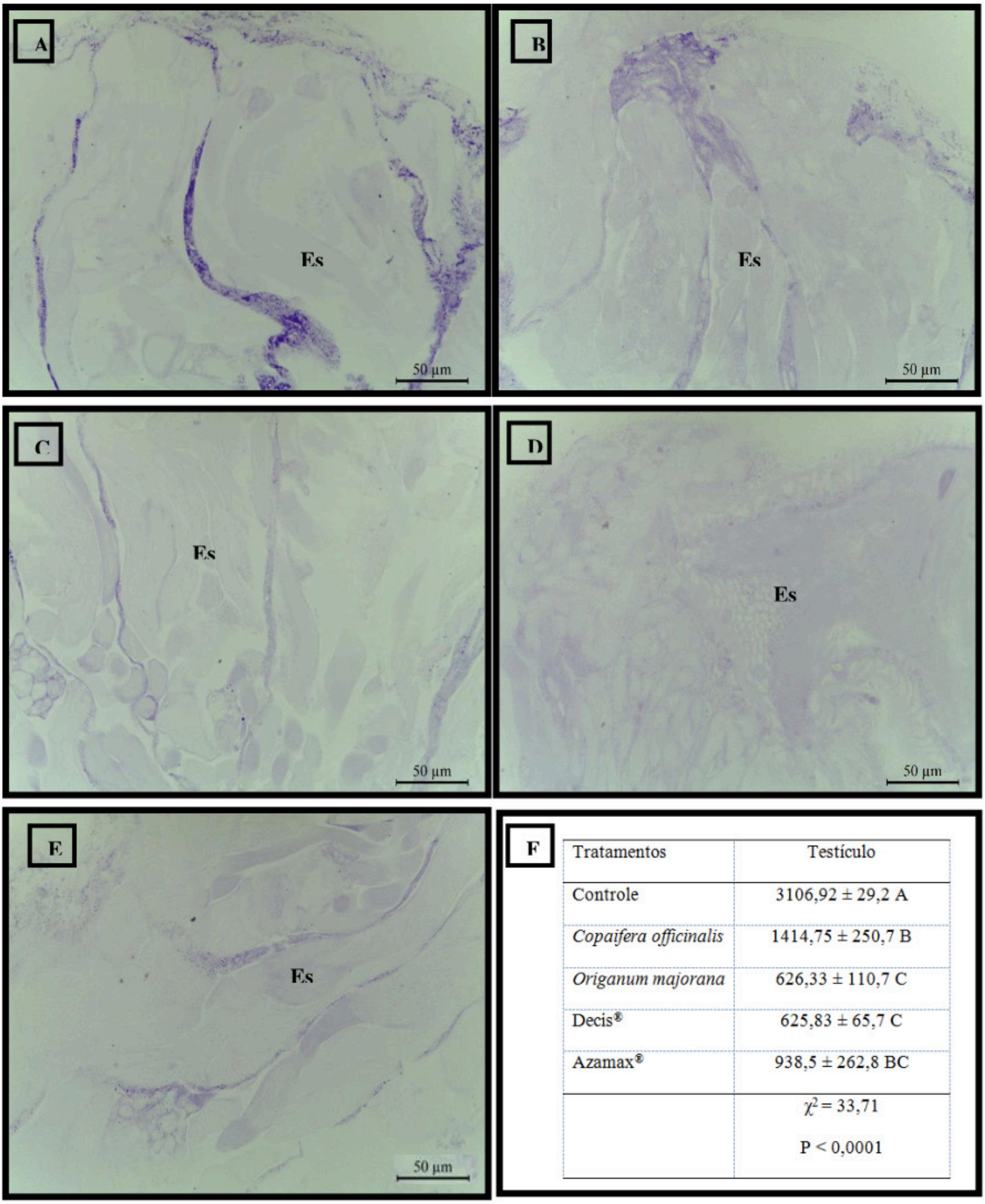
Tratamentos	Ovaríolo
Controle	2810,25 ± 76,8 A
Copaifera officinalis	563,41 ± 240,1 B
Origanum majorana	133,75 ± 20,3 B
Decis®	231,66 ± 47,7 B
Azamax®	119 ± 56 C
	$\chi^2 = 36{,}01$ P < 0,0001

Figure 3. Histochemistry of ovarioles of *Neoleucinodes elegantalis* adults sprayed with the CL_{45} of the essential oils of *Copaifera officinalis* and *Origanum majorana*, and with the commercial concentration of the formulated products Decis® and Azamax®. A– Control; B – *Copaifera officinalis*; C – *Origanummajorana*; D – Decis®; E – Azamax®; F - Number of pixels (mean ± SEM) for neutral polysaccharides. Means followed by the same letter do not differ by Kruskal-Wallis test at 5% significance level. Coloration with Periodic Acid of Schiff (P.A.S.).Yolk (Yo).



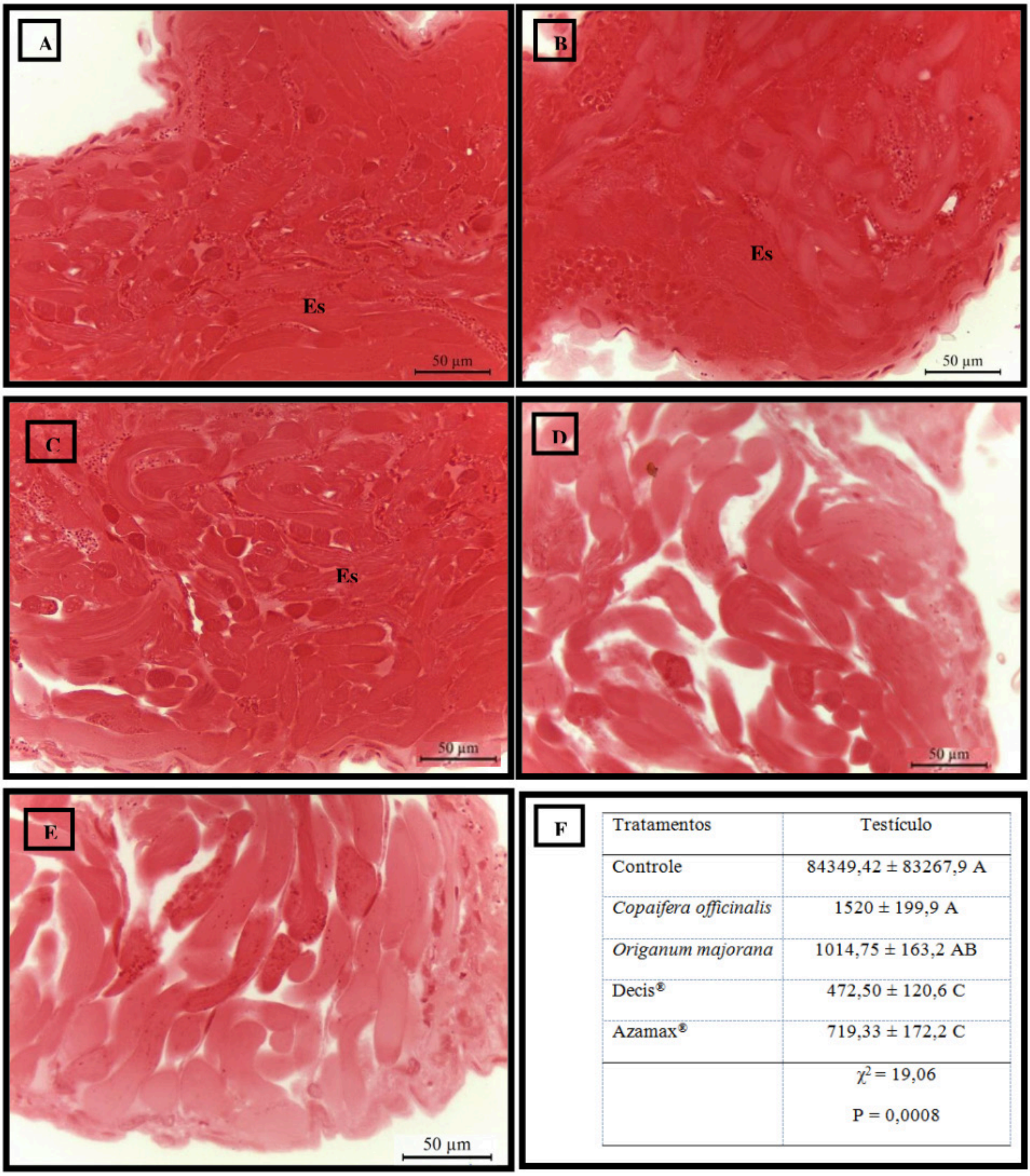
Tratamentos	Ovaríolo
Controle	3062,52 ± 28,9 A
Copaifera officinalis	696,08 ± 233,6 BC
Origanum majorana	286,58 ± 51,4 C
Decis®	1128,50 ± 517,6 B
Azamax®	355 ± 72 C
	$\chi^2 = 33{,}12$ P < 0,0001

Figure 4. Histochemistry of ovarioles of *Neoleucinodes elegantalis* adults sprayed with the CL_{45} of the essential oils of *Copaifera officinalis* and *Origanum majorana*, and with the commercial concentration of the formulated products Decis® and Azamax®. A– Control; B – *Copaifera officinalis*; C – *Originummajorana*; D – Decis®; E – Azamax®; F - Number of pixels (mean ± SEM) for total proteins. Means followed by the same letter do not differ by Kruskal-Wallis test at 5% significance level. Coloration withXylidine Ponceau. Yolk (Yo).



Tratamentos	Testículo
Controle	3106,92 ± 29,2 A
Copaifera officinalis	1414,75 ± 250,7 B
Origanum majorana	626,33 ± 110,7 C
Decis®	625,83 ± 65,7 C
Azamax®	938,5 ± 262,8 BC
	$\chi^2 = 33{,}71$ P < 0,0001

Figure 5. Histochemistry of testicles of *Neoleucinodes elegantalis* adults sprayed with the CL_{45} of the essential oils of *Copaifera officinalis* and *Origanum majorana*, and with the commercial concentration of the formulated products Decis® and Azamax®. A– Control; B – *Copaifera officinalis*; C – *Origanum majorana*; D – Decis®; E – Azamax®; F - Number of pixels (mean ± SEM) for neutral polysaccharides. Means followed by the same letter do not differ by Kruskal-Wallis test at 5% significance level. Coloration with Periodic Acid of Schiff (P.A.S.).Sperm (Sp).



Tratamentos	Testículo
Controle	84349,42 ± 83267,9 A
Copaifera officinalis	1520 ± 199,9 A
Origanum majorana	1014,75 ± 163,2 AB
Decis®	472,50 ± 120,6 C
Azamax®	719,33 ± 172,2 C
	$\chi^2 = 19,06$
	P = 0,0008

Figure 6. Histochemistry of testicles of *Neoleucinodes elegantalis* adults sprayed with the CL_{45} of the essential oils of *Copaifera officinalis* and *Origanum majorana*, and with the commercial concentration of the formulated products Decis® and Azamax®. A– Control; B – *Copaifera officinalis*; C – *Origanummajorana*; D – Decis®; E – Azamax®; F - Number of pixels (mean ± SEM) for total proteins. Means followed by the same letter do not differ by Kruskal-Wallis test at 5% significance level. Coloration withXylidinePonceau.Sperm (Sp).