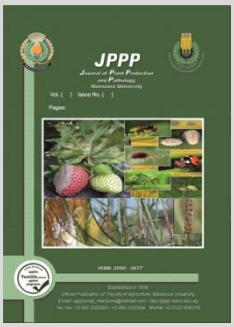
Journal of Plant Protection and Pathology

Journal homepage: www.jppp.mans.edu.eg
Available online at: www.jppp.journals.ekb.eg

Bioactivity of *Moringa oleifera* and *Ruta angustifolia* Oils on *Spodoptera littoralis* (Boisd.) Moths' Vitality

Rehab M. El-Gendy* and Hend M. Sabry

Plant Protection Research Institute, Agriculture Research Center (ARC), Dokki, Giza, Egypt.



ABSTRACT

Spodoptera littoralis, cotton leafworm is a destructive pest for several economic crops. The more use of chemical pesticide the more increase of this pest resistance, that lead to discovering new natural pesticides from plant extracts and oils. The present search is targeted to suggest alternative components from natural sources that more safety to the ecosystem and had the ability to reduce insect prohibition. The experimental results exhibited highly significance effect on adult moths' vitality aspects with the pre-pupal treatments by *Moringa oleifera and Ruta angustifolia* at all tested concentration especially 4% concentration as shorten female moths' longevity and reduced the oviposition period. Obviously, moringa recorded the shortest oviposition period (3.00 days) while ruta oil recorded (5.33 days) compared to control (10.66 days). Significantly reduced fecundity and hatchability and increased the sterility% compared to control. The tested plant oils caused severe ovarian and testicular histological deformation and significant increase in total lipids and total carbohydrate contents also, marked DNA fragmentation in variable levels in ovaries and testes in each oil treatments at 4% concentration. The promising results of both oils on *S. littoralis* reproductive system can prove them be used in the integrated management programs of this pest.

Keywords: *Moringa oleifera*, *Ruta angustifolia*, *Spodoptera littoralis*, histopathology of ovary and testes, DNA fragmentation, total lipid

INTRODUCTION

The cotton leafworm, *S. littorals* (Boisd.) is the most severe destructive pests in Egypt, Africa, Mediterranean Europe and Asia. (Azab *et al.*, 2001). It infests cotton and many vegetable and fruit crops (Ali and Abdallah, 2018). Mostly, control strategies of cotton leafworm based on different applications by chemical insecticides eventually resulted in many of the problems as raising pest resistance and pollution. (El-Seedi *et al.*, 2017; Abd-ElAzeem *et al.* 2019). Recently, there are a great effort to develop alternative safe control strategies with new modes of action.

The sterile insect technique (SIT) was one of the biological insect control methods evaluated in this area which earned a reputation as a part of IPM strategies for the suppression, containment, prevention (Klassen *et al.*, 2005). There are different methods used to carry out SIT, irradiation is popular (Abass *et al.*, 2017; Staten and Walters. 2021), as well genetic methods Alphey, 2007 and Alphey *et al.* 2006) and Magnetic field (Kandil *et al.* 2018 and EL-Shennawy *et al.*, 2019).

At all events, The SIT mostly applied on larvae or pupa and evaluates their effects in the resulting adults, rather than the application of the adult itself. Consequently, it targets the reproductive organ's development and reduces progeny (Klassen *et al.*, 2005). Many plants extracts and some plant essential oils showed the same biological effects; are reported there sterilizing action against various insect by reducing or completely hindering their fecundity and fertility (Papachristos and Stamopoulos, 2004; Nenaah *et al.* 2015; de Araújo *et al.* 2017; Campolo *et al.* 2018). Also, the inhibition in oviposition and reduction in egg hatching caused by white mustard oil against the cotton leafworm were reported by El-Kholy, *et al.* (2014).

Thus, researchers targeted to find out natural sources like Plants allelochemicals that appears to be promising like alkaloids; oils and quinones. Oils are considered the most promising materials because of their broad-spectrum pest control properties and high level of safety for human, animals and fishes, in addition of its minimal impact upon natural pest predator or parasitoid as well as pollinating insects(Mead *et al.*, 2016, and Nollet and Rathore, 2017).

Moringa oleifera (Moringa) and *Ruta angustifolia* (Ruta) are widely cultivated in Egyptian lands, also, both have high contents of oil in seeds and predictable to be effective. Further, the recent investigations cleared an insecticidal activity and repellence activity against many insect, *S. littoralis* and *Anopheles gambiae* of *M. oleifera* (Dimetry *et al.*, 2017). Likewise, Ruta extracts and oils were powerful in controlling various pests (Majdoub *et al.*, 2014; Akkari *et al.*, 2015). Also, showed biological effects against various pests as *Cydia pomonella* Linnaeus (Lepidoptera: Tortricidae) and *Aedes aegypti* Linnaeus (Diptera: Culicidae) (Tabanca *et al.*, 2012).

The purpose of this work was to investigate the effect of *Moringa oleifera (Moringa)* and *Ruta angustifolia* (Ruta) oils on reproductive vitality organs of the cotton leafworm, *S. littoralis* as a part of IPM program of this pest.

MATERIALS AND METHODS

1. Plants material and oils Extraction.

Fixed oils were extracted from *M. oleifera* (kernel) and *R. angustifolia* (seed) that collected from Faculty of Agriculture, Zagazig University. Seeds and kernels were dried at room temperature then crushed into powder. The powders about 200g were soaked separately in 400ml petroleum ether (60/80) for one week then filtered on anhydrous sodium sulphate and finally the solvent was evaporated under vacuum by rotary evaporator. The residual oils were weighted and subjected to bioassays at three concentrations (1, 2 & 4%) of *M. oleifera* and *R. angustifolia* separately in 0.1% tween 80 solution**.**

* **Corresponding author.**
E-mail address: rehabgendy4@gmail.com
DOI: 10.21608/jppp.2021.160690

2. Insect culture source.

The pre-pupa of *S. littoralis* (Boisd.) which used to investigate *M. oleifera* and *R. angustifolia* activities were picked up from susceptible strain occurred in laboratory of the Cotton Pest Research Department, Plant Protection Research Institute, ARC, Egypt.

3. Pre-pupal application technique.

Pre-pupa of *S. littoralis* were dipped in various concentrations along with 0.1% tween 80 solution as control for 10 sec. then put in jar lined with filter paper in incubator at 25 ± 2°C and 65 ± 5% RH. and left to develop into pupa then adults. Three replicates (10 pre-pupa/ replicates) were considered. The resulted adults were investigated to calculate adult longevity, fecundity, egg hatching as percentage (hatchability %) as well as the Percent of sterility was calculated according to Toppozada *et al.* (1966)

Sterility % = 100- ((a×b/A×B)×100) where

a = No. of eggs/female in treatment.
b = percent hatchability in treatment.
A = No. of eggs/female in control.
B = percent hatchability in control.

4. Histological study.

The ovaries and testes of control adult moths and those developed from and treated pre-pupa at 4% conc. were dissected in Ringer's saline solution. Bouin`s fluid was used to fix the reproductive organs. After that dehydrated in series of ethanol. Then cleared in xylene and embedded in paraffin wax. Serial sections, 5µm thick, were cut, stained with hematoxylin and eosin, then cleared and mounted in DPX and photographed using the light microscope.

5. Preparation of samples and biochemical assay.

One gram from ovaries and testes resulted from 10 adults resulted from treated pre-pupa of *M. oleifera* and *R. angustifolia* oils at 4% conc. and those of normal control were collected after 24 hrs. of emergence for biochemical assays. Samples for protein assay were homogenized in distilled water (50mg/ml) using chilled glass Teflon homogenizer. Homogenates were centrifuged at 5000 rpm for 20 minutes at 5 ºC in a refrigerated centrifuge. The deposits were discarded and the supernatants were subjected to determination of total soluble protein that carried out as described by Gornall *et al.* (1949). Total lipids were estimated in tissue according to the method of Zollner and Kirsch (1962) using kits from Diamond Diagnostics. Also, total carbohydrate were estimated in tissue according to the method of Van Handel (1985).

6. DNA fragmentation analysis of reproductive organs samples.

DNA damage determined by DNA fragmentation assay according to Bortner, *et al.* (1995) summarized as the following:

Fifty micrograms of separated reproductive organs resulted from treated pre-pupa at 4% conc. Of moringa and ruta fixed oils and control were put in 1.5 ml microfuge tube. Extraction buffer was added to 0.2-0.3 ml mark in tube. Tissues were crushed in buffer until it dispersed, and then extraction buffer was added till 0.5 ml mark. 50.0 µl of proteinase-K solution (10.0 mg/ml) was added then the tubes were closed and inverted to mix. Tubes were incubated at 50 °C for 12 hour to 3 days with occasional vigorous mixing. Tubes were removed from incubator and DNA was extracted with a mixture of 0.7-0.8 ml phenol, chloroform and isoamylalcohol (25.0: 24.0: 1.0) and vortex samples 2.0-5.0 sec. Samples were centrifuged at 12000 rpm for 3.0-5.0 min after that 400-500 µl aqueous layer for each sample was removed carefully into new tube and 40.0-50.0 µl of 3.0 M sodium acetate (pH= 5.3) was added to each tube. Pure ethanol (100%) was added till mark 1.5 ml. Tubes were inverted to mix and precipitate DNA then let to set at -20 °C overnight and, centrifuged at 12000 rpm for 20.0 min. The supernatant and any fluid were removed and 50.0 µl of tries EDTA buffer was added overnight till complete dissolving. Samples were run on electrophoresis using 1.2% agarose gel and run the gel at a low voltage which improves resolution of DNA fragments (50.0 volt), gel was stained using ethidium bromide. Samples were analyzed using image analyses software.

The analysis of agarose gel image was determined using Gel Analyzer Soft Ware (version 19.1) by GelAnalyzer.com. http://www.gelanalyzer.com/?i=1

7. Statistical analysis

The biological and biochemical results were subjected to (ANOVA) variance analysis in addition to the Tukey-HSD significant difference to estimate significance differences between samples by (Costat, 2005) version 6.311.

RESULTS AND DISCUSSION

Biological studies

The obtained data in Table (1) showed adult moths' vitality aspects which affected with highly significant differences. the early effects of the treatments by *M. oleifera* and *R. angustifolia* resulted in shorten female moths' longevity to 5.66 and 9.00 days respectively at the highest tested concentration of 4% compared to control female longevity of 13.00 days. Even so, males were more sensitive, recorded 6.83 and 6.16 days respectively compared to the control of 11.33 days. The concentration was directly effective. Consequently, the oviposition periods were reduced with all treatments but moringa oil was more effective recordings the shortest oviposition period (3.00 days) at concentration of 4% while ruta oil recorded (5.33 days) at same concentration compared to control (10.66 days)

Table 1. Effects of *Moringa oleifera* and *Ruta angustifolia* oils on S. *littoralis* moths vitality .

Treatment	Conc.	Longevity (day)		Oviposition period (day)	Fecundity (egg/female)	Hatchability %	Sterility %
		female	Male				
Moringa oil	1%	8.66^{bc}	9.5^{abc}	5.33^{bc}	1288.66^{b}	71.14^{ab}	47.57^{f}
	2%	8.00^{bc}	7.75^{cd}	4.00^{bc}	666.66^{cd}	53.29^{bc}	79.87^{c}
	4%	5.66^{c}	6.83^{d}	3.00^{c}	235.83^{d}	57.02^{bc}	92.85^{a}
Ruta oil	1%	8.16^{bc}	8.00^{bcd}	6.66^{b}	1021.83^{bc}	73.45^{ab}	57.51^{e}
	2%	8.16^{bc}	10.26^{ab}	6.66^{b}	829.99^{bc}	59.72^{bc}	71.95^{d}
	4%	9.00^{b}	6.16^{d}	5.33^{bc}	543.25^{cd}	37.81^{c}	88.39^{b}
Control		13.00^{a}	11.33^{a}	10.66^{a}	1830^{a}	96.42^{a}	0.00^{g}
LSD		3.13	2.39	2.70	531.08	33.31	0.52
P		0.0096**	0.0037 **	0.0009 ***	0.0004 ***	0.0502 ns	0.0000 ***

Data expressed as (Means), and statistical treatments were performed using ANOVA for differences comparing means with Tukey's HSD test, P < 0.05.Same letters means non-significant effect while different letters means significant effect (* = highly significant, ** = significant and ns =non-significant)**

The results in Table (1) indicated that concentrations 1, 2 and 4% of both oils significantly reduced fecundity (eggs/female) and fertility (hatchability %) and increased the sterility % compared to those of control. Moringa oil gave better results than Ruta oil at all concentrations that reached 92.855% and 88.39% sterility percentages respectively, a gradual decrease was detected in reproductive parameters led to an increase in sterility% when increasing both oils concentration.

The effect may be due to physiological disturbance as hormones secretions or decrease of the vital constituents in adults but the inhibition of egg hatchability may due to disturbance in embryonic development Mogahed *et al*. (1997). Rather, the sterility may due to damages to reproductive organs tissues. This results in the same trend of those obtained by Abass *et al.*, (2017) when used gamma radiation on cotton leafworm pupa, also El-Kholy, *et al*. (2014) reported a same effects when used mustard oil and some plant extracts.

Histopathological studies

The histopathological studies were carried out for ovaries and testes of both normal adult moths and those developed after treatment of pre-pupa of *S. littoralis* with 4% conc of *M. oleifera* and *R. angustifolia* fixed oils. Longitudinal section in the ovaries of control group of adult female moths revealed that the follicular epithelial sheath was regular in shape and continued in many positions. Oocyte cytoplasm is dramatically homogenate and the developing oocyte in particular stage of growth surrounded by follicular cells Fig. (1a).In female ovaries developed after treatments of last instar larva with *M. oleifera* the follicular oocytes appeared with altered structure moreover great separation between oocyte and follicular cells breakage of follicular epithelium with wavy shape. Fig. (1 b,c&d).While in case of *R. angustifolia* treatments there was marked increase in number of fissures and vacuoles in yolk body and chorion is deformed. A marked separation of inner epithelial membrane of follicular cells furthermore, deformation in the shape oocyte and warping of egg chorion Fig. (1 e&f).

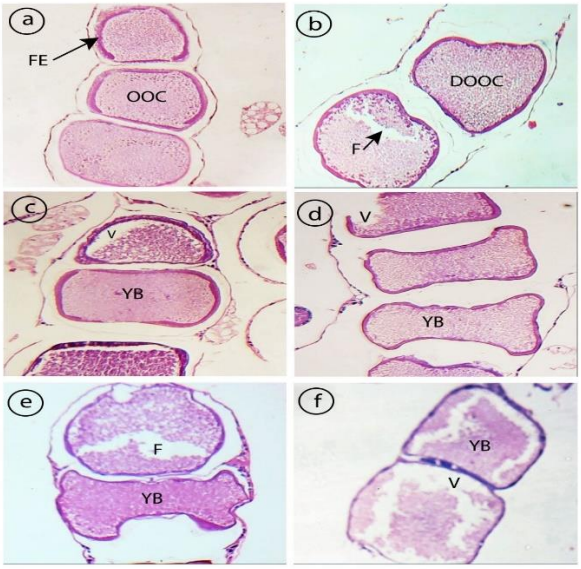


Figure 1. Photomicrograph showing longitudinal section in ovariole of female moth of *S. littoralis* developed from (a): normal pre-pupae; (b-d) pre-pupae treated with 4% of *M. oleifera*; (e-f) developed from pre-pupae treated with 4% of *R. angustifolia* (Dooc) deformed oocyte (F) fissure (FE) follicular epithelium (ooc) oocyte (V)vacuole (YB) yolk particle

Transverse sections in the ovaries of control females moths showed normal oocyte during oogenesis where the follicular epithelium accommodates itself to increase the volume of the oocyte till it reaches about more than one half of the chamber. The nurse cells are getting smaller gradually and oocyte occupies most of the egg chamber Fig. (2a). Different profiles of oocyte degeneration chorion formation failure noticed with *M. oleifera* treatment Fig. (2 b&c). Another sing of oocyte deformation obviously seen in *R. angustifolia* treatments, deteriorated oocyte with high vacuolization in the follicular oocyte in addition to chorine ruptured and yolk body divided into two halves follicular epithelial cells appear of lost their boundaries structure great separation among cytoplasm of oocyte is recorded fig. (2d).

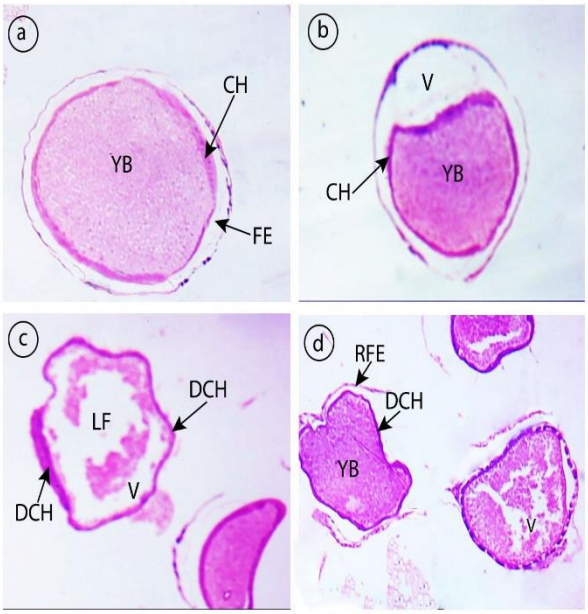


Figure 2. Photomicrograph showing transverse section in ovariole of female moth of *S. littoralis* developed from (a): normal pre-pupae; (b&c) developed from pre-pupae treated with 4% of *M. oleifera* (d) developed from pre-pupae treated with 4% of *R. angustifolia*. (CH) chorion (DCH) deformed chorion (F) fissure (FE) follicular epithelium (LF) large fissure (ooc) oocyte (RFE) ruptured follicular epithelium (V) vacuole (YB) yolk particle.

Examination of longitudinal sections in testis of control moths reveals that each oval testis lined by a testicular membrane which enclose testis follicles, within each follicle the developing sperm are in different stages of maturation divided in cysts, Fig. (3a). spermatogonia were occurred near the periphery while primary and secondary spermatocytes are inside, spermatogonia are spherical cells their round nuclei have dark stained in contrary, spermatocytes larger than spermatogonia with less intensity stainable nuclei fig. (3b). Abundance in Sperm bundles which disperses in the interstial tissue filling most of the central area they within one bundle, sperms are aligned, roughly parallel to one another and oriented in the same direction fig. (3c).

In the testis of male moths developed from *M. oleifera* treatment separation between epithelial cells of testicular follicles and presence of large mass of protoplasm in the follicular edge fig. (3d) disintegrations and damage of testicular germ cells, spermatogonia, spermatocytes, spermatids and spermatozoa and vacuolation inside testes were observed in fig. (3e) also

degeneration of spermatocytes and inhibition in the formation of sperm bundles were noticed accompanied by low number of spermatid and sperm bundle in testes compared by density of control number fig. (3f). In case of *R. angustifolia* treatment the testicular wall epithelial cells were dimensioned leaving many spaces away from tests component, death of germ cells leave liquefied testicular contents and widest vacuolated area fig. (3g) Cytoplasmic vacuolations of spermatogonia and absence of sperms and spermatids fig. (3h) delay in the development and the amount of spermatids, sperm bundles were absorbed disintegrations of spermatocytes were observed fig. (3k).

Histopathological abnormalities were appeared in the ovaries and testis of adult moths developed from treated pre-pupae may have collective signs of diminished activity and cellular degeneration. The two fixed oils have similar mode and site of action. In conclusion, tested plant oils caused severe ovarian and testicular histological deformation and this is may be attributed to the interference of the oils with the endocrine system and this harmony with Su and Mulla (1999) stated that, ecdysteroid is one hormone regulating vitellogenesis, and azadirachtin can modify hemolymph ecdysteroid by inhibiting the release of PTTH and allatotropins from the brain-corpus cardiacum complex, adverse effects on ovarian development, fecundity, and fertility. Fixed oils may be debilitate the normal osmotic properties of plasma membrane of the old oocytes. This lead to dehydration and subsequently appearance of vacuoles within the oocytes warping of egg chorion and shrinkage or degeneration of the yolk similar observations were noticed by Ahmed *et al.* (2015) The ovarian follicles of treated F1 females with both Dimilin® and Virtu® exhibited high potency and efficacy of the two IGRs on developed ova of *S. littoralis*. Abdelgaleil and El-Sabrout (2018) revealed undifferentiation ovarioles of adults developed from 4th instar larvae that fed on leaf discs treated with *A. monosperma* and *C. macrocarpa* essential oil. Sabry *et al.* (2017) in ovaries and tests of adults derived from treated larvae with LC_{50} methoxyfenozide treatment may be affecting the plasma membrane osmotic properties, leading to dehydration and appearance of vacuoles within the oocytes ovaries while testes revealed clear reduction in cellular content and bundles of spermatocytes and spermatogonia were severely reduced in number. The tested oils succeed to cause spermatocyte depletion and this play an important role reducing gamete maturing rate and this in finding with Amaldoss (1989) spermatocytes develop into spermatids and sperm bundles are formed as a result of maturation of the spermatids, which completes spermatogenesis. Hazza *et al.* (2009) the treatment with different concentrations of *Nerium oleander* caused histological changes in the ovaries including vacuolation and shrinkage of oocyte tissue, clumped of chromatin material and thickness of epithelial cells at some areas. Also vacuolation of the testes and absorption of sperm bundles also represent the damage of germ cells was most prominent in these organs. Bakr *et al.* (2010) in adult males developed from treated one day old of the 5th nymphal instar of *Schistocerca gregaria* with LC_{50} of Consult. The testicular follicles showed damage in zones of reduction and necrosis appeared in many spermatids and spermatozoa. While those treated with Lufox LC_{50} showed severe degeneration and necrosis of the most spermatogenic stages. Hatem *et al.* (2011) detected morphological anomalies in the testicular follicles of male reproductive system of *S. littoralis* when treated newly moulted third instar with sublethal concentrations (1.38 mg/ml) of Azadirachtin, and showed some follicles with minor wall thickness and disorganization of spermatocytes.

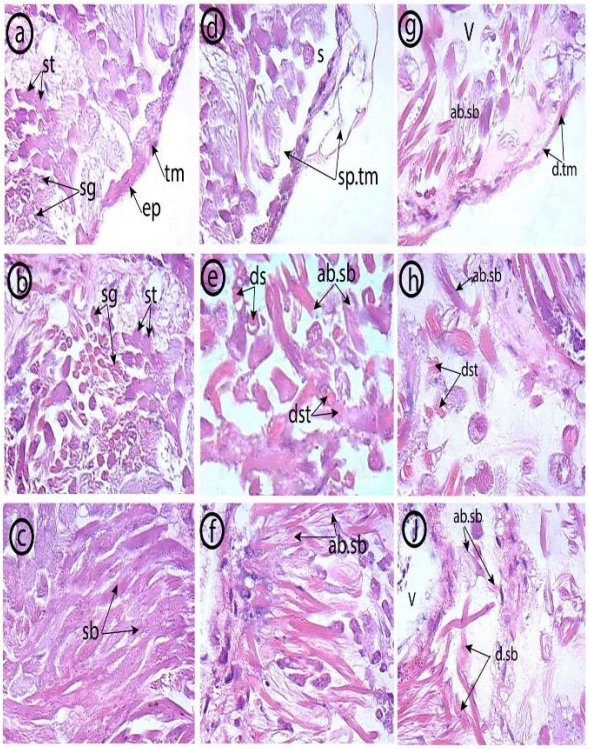


Figure 3. Photomicrograph showing longitudinal section in testes of male moth of *S. littoralis* developed from (a,b&c): normal pre-pupae; (d,e&f) developed from pre-pupae treated with 4% of *M. oleifera* (g,h&k) developed from pre-pupae treated with 4% of *R. angustifolia*. (ab.sb) absorbed sperm bundles (ab.st) absorbed spermatids (dsg) degenerated spermatogonia (dst) degenerated spermatid (DTm) disintegrated testicular membrane (ep) testicular epithelium (s) separation (sb) sperm bundles (sp.tm) separated testicular membrane (sg) spermatogonia (st) spermatids (tm) testicular membrane (v) vacuole.

Biochemical studies

In general, biochemical assays revealed a non - significant variation in total protein and significant increase in total lipids and total carbohydrate contents in each oil treatments in both ovaries and testes.

Data in fig. (4A) cleared that total protein, lipids and carbohydrates in ovaries were increased in all treatments of the two oils under investigation on testes compared to control insuring that moringa oil was more effective than ruta oil. Qualifications of tested oils on testes were illustrated in fig. (4B) the ruta oil was more effective in raising protein, lipids and carbohydrates contents than moringa oil.

Indeed, one of the evolutionary advances of insects, which responsible for their extraordinary success in life are the development of chorionated eggs with a large quantity and fertilize eggs by the production of healthy sperms. Hence, the reproductive organs of female and male insects require a massive maintenance of nutrients and energy resources (Smykal, and Raikhel, 2015). In the same trend (Sabry *et al.* 2017) demonstrated that protein and lipids are known as nutritional indices in insects. Proteins are the fundamental

components in both hormones and enzymes. Lipids are major constituent in synthesizing the phospholipids which are very important components in many hormones, cell structures as membrane and provide the insect with its energy requirements (Das and Medda, 1988) reported the enhancement in protein contents in the ovary of *Bombyx mori* treated with cyanocobalamin. Likewise, (Ge *et al*., 2009) found that some insecticides treatments raising the protein contents in the ovary of *Nilaparvata lugens*. On contrary, results obtained by (Khebbeb *et al.*, 2008) showed that tebufenozide treatment caused a significant decrease on carbohydrate, lipids and protein in both ovaries and testes of Mediterranean flour moth, *Ephestia kuehniella*. Upon these data, we can say that the disturbance or fluctuation in the nutritional values can cause damages even was increase or decrease.

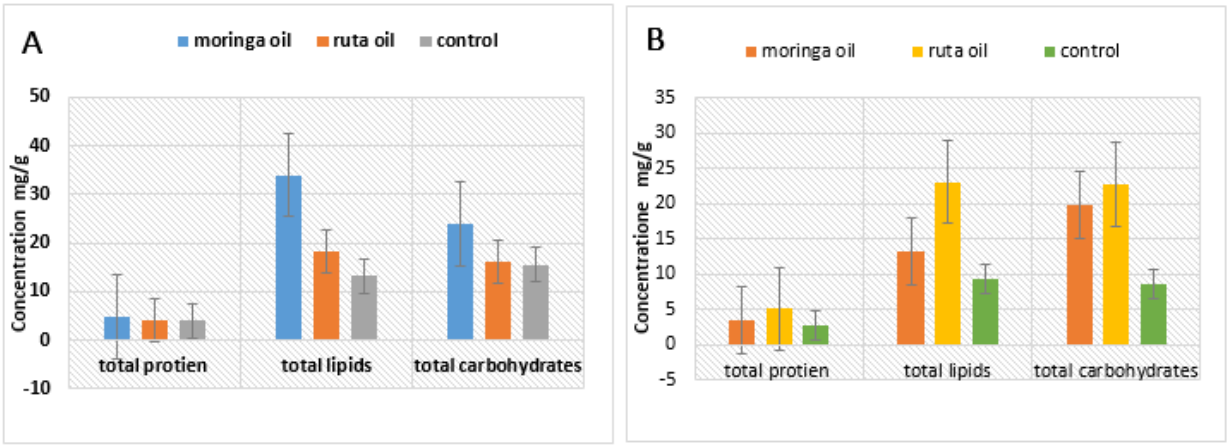


Fig. 4. Biochemical aspects of *S. littoralis* moths' reproductive organs (A) Ovaries (B) Testes.

Data expressed as mg/g. body weight (M ± SE), and statistical treatments were performed using ANOVA for differences comparing means with Tukey's HSD test, $P < 0.05$.

DNA fragmentation assay

The precise effect of tested oils on ovaries and testes was evaluated in resulted moths by measuring the level of genomic DNA fragmentation through detecting the DNA laddering on agarose gel electrophoresis compared to organs of untreated control. Fig (5). Imaging revealed that treatment with morimga oil caused marked DNA fragmentation in variable levels in ovaries and testes. Testes found to be more sensitive were proved to increase the DNA fragmentation appeared as smear along the lane while fragmentation was lesser in ovaries which appeared like a low level of laddering

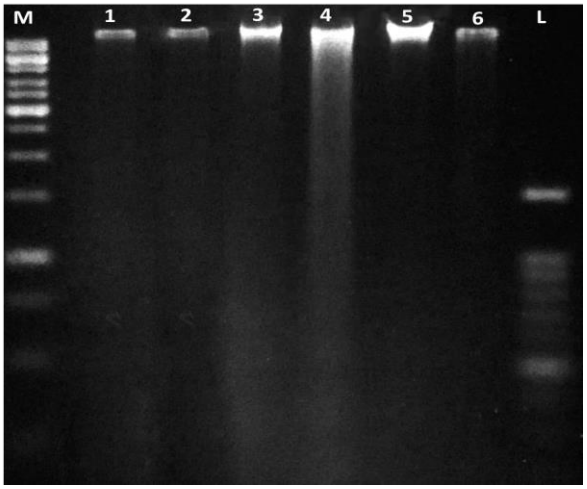


Fig. 5. The electrophoretic pattern of small DNA fragments of ovary and test on 1.5% agarose gel electrophoresis.

Lanes (1 and 2); Control samples of ovary and testes respectively.
Lanes (3 and 4) expressed the moringa treated ovary and testes respectively.
Lanes (5 to 6) expressed the ruta treated ovary and testes respectively.
Lane L; DNA stander ladder 1000bp
Lane M; DNA stander ladder 10000 bp

Regarding to ruta oil,the DNA fragantation or laddering was barely noticeable in ovary sample with slightly fregmanted was detected in testes sample .

The DNA damage was examined in testes tissues by Heikal *et al.* (2014) in Methomyl treated rats. Methomyl expressed more bands of the damaged DNA compared with the control. Also, deltamethrin (Galal *et al*., 2014) recorded a DNA fragmentation in brain cell of rates. About the insects ovarian effects (Sauman and Berry, 2002) detected fragmentation in genomic DNA isolated from developing follicles of *Manduca sexta* ovary by Cytochalasin-D treatment.

REFERENCES

Abass, A. A., Salem, H. M., Abd El Hamid, N. A., Gabarty, A., & Embaby, D. M. (2017). Effects of gamma irradiation on the biological activity of the cotton leafworm, *Spodoptera littoralis* (Boisd.). J Nucl Tech Appl Sci, 5(1), 19-26.

Abd-ElAzeem, E. M., El-Medany, W. A. Z., & Sabry, H. M. (2019). Biological activities of spores and metabolites of some fungal isolates on certain aspects of the spiny bollworms *Earias insulana* (Boisd.)(Lepidoptera: Noctuidae). *Egyptian Journal of Biological Pest Control*, *29*(1), 1-7.

Abdelgaleil, S. A., & El-Sabrout, A. M. (2018). Anti-nutritional, antifeedant, growth-disrupting and insecticidal effects of four plant essential oils on *Spodoptera littoralis* (Lepidoptera: Noctuidae). *Journal of Crop Protection*, *7*(2), 135-150.

Akkari, H., Ezzine, O., Dhahri, S., B'chir, F., Rekik, M., Hajaji, S.,... & Gharbi, M. (2015). Chemical composition, insecticidal and in vitro anthelmintic activities of *Ruta chalepensis* (Rutaceae) essential oil. *Industrial Crops and Products*, *74*, 745-751.

Ali, A.M.H and. Abdallah E.M (2018).Gut microbiota of cotton leafworm *Spodoptera littoralis*: A brief Review. An International J., 10(2018. 92-95

Alphey, L., Baker, P., Condon, G.C., Condon, K.C., Dafa'alla, T.H., Fu, G., Jin, L., Labbe, G., Morrison, N.M., Nimmo, D.D. and O'Connell, S., (2006) . Genetic technologies to enhance the sterile insect technique (SIT). Fruit Flies of Economic Importance: From Basic to Applied Knowledge Proceedings of the 7th International Symposium on Fruit Flies of Economic Importance 10-15 September 2006, Salvador, Brazilpp. 319-3 *(1)*.

Alphey, L.S., (2007). Engineering insects for the sterile insect technique. In *Area-Wide Control of Insect Pests* Springer, Dordrecht. pp. 51-60.

Amaldoss, G. (1989). Persistent occurrence of spermatogenesis in the adult testes of *Spodoptera litura* (Fabricius)(Lepidoptera: Noctuidae). *Proceedings: Animal Sciences*, *98*(6), 405-418.

Azab, S. G., Sadek, M. M., & Crailsheim, K. (2001). Protein metabolism in larvae of the cotton leafworm *Spodoptera littoralis* (Lepidoptera: Noctuidae) and its response to three mycotoxins. *Environmental entomology*, *30*(5), 817-823.

Bakr, R. F., Mohammed, M. I., El-Gammal, A. E. M., & Mahdy, N. M. (2010). Histopathological change in the testis of the desert locust *Schistocerca gregaria* (Forskal) induced by the IGR Consult and Lufox. *Egyptian Academic Journal of Biological Sciences, D. Histology & Histochemistry*, *1*(1), 23-28.

Bortner, C. D., Oldenburg, N. B., & Cidlowski, J. A. (1995). The role of DNA fragmentation in apoptosis. *Trends in cell biology*, *5*(1), 21-26.

Campolo, O., Giunti, G., Russo, A., Palmeri, V., & Zappalà, L. (2018). Essential oils in stored product insect pest control. *Journal of Food Quality*, 1, *2018*.

Costat Statistical Software, 2005. Microcomputer program analysis version, 6.311. Co Hort Software, Monterey, California, USA

de Araújo, A. M. N., Faroni, L. R. D. A., de Oliveira, J. V., Navarro, D. M. D. A. F., Breda, M. O., & de França, S. M. (2017). Lethal and sublethal responses of *Sitophilus zeamais* populations to essential oils. *Journal of Pest Science*, *90*(2), 589-600.

Das, S., & Medda, A. K. (1988). Effect of cyanocobalamin on protein and nucleic acid contents of ovary of silkworm, *Bombyx mori* L., during larval, pupal and adult stages of development. *International Journal of Tropical Insect Science*, 9(5), 641-646.

Dimetry, N. Z., Metwally, H. M., Abdou, W. L., & Abdel-Hakim, E. A. (2017). Acceptability and anti-feedant effect of the drumstick" *Moringa oleifera* leaves towards the cotton leafworm" *Spodoptera littoralis* (Boisd) under laboratory conditions. *Research Journal of Pharmaceutical Biological and Chemical Sciences*, *8*(4), 1006-1014.

Ahmed, K. S., El-Bermawy, S. M., Ismael, E. H., Abdel-Gawwad, R. M., & Saleh, T. A. (2015). Histopathological effects of Diflubenzuron and Chromafenozide (IGRs) on egg development in the cotton leafworm *Spodoptera littoralis* (Boisd) (Lepidoptera, Noctuidae). *Journal of Bioscience and Applied Research*, *1*(4), 147-153.

El-Kholy, R. M. A., El-Bamby, M. M. M., El-Tawil, M. F., & Abouamer, W. L. (2014). Effect of three plant extracts on some biological aspects of cotton leafworm, *Spodoptera littoralis* (Boisd.). *Middle East Journal of Applied Sciences*, *4*(2), 243-251.

El-Seedi, H. R., Azeem, M., Khalil, N. S., Sakr, H. H., Khalifa, S. A., Awang, K., ... & Borg-Karlson, A. K. (2017). Essential oils of aromatic Egyptian plants repel nymphs of the tick *Ixodes ricinus* (Acari: Ixodidae). *Experimental and Applied Acarology*, 73(1), 139-157.

EL-Shennawy, R. M., Husseen, A. M., & Kandil, M. A. (2019). Latent Effects on Adults' Emergence of *Pectinophora gossypiella* (Saund.) and *Earias insulana* (BOISD.), Resulted from Magnetized Pupae. *Journal of Plant Protection and Pathology*, 10(1), 77-80.

Galal, M. K., Khalaf, A. A. A., Ogaly, H. A., & Ibrahim, M. A. (2014). Vitamin E attenuates neurotoxicity induced by deltamethrin in rats. *BMC complementary and alternative medicine*, *14*(1), 1-7.

Ge, L. Q., Hu, J. H., Wu, J. C., Yang, G. Q., & Gu, H. (2009). Insecticide-induced changes in protein, RNA, and DNA contents in ovary and fat body of female *Nilaparvata lugens* (Hemiptera: Delphacidae). *Journal of economic entomology*, *102*(4), 1506-1514.

Gornall, A. G., Bardawill, C. J., & David, M. M. (1949). Determination of serum proteins by means of the biuret reaction. *Journal of biological chemistry*, *177*(2), 751-766.

Hatem, A. E. S., Aldebis, H. K., & Osuna, E. V. (2011). Effects of the *Spodoptera littoralis* granulovirus on the development and reproduction of cotton leafworm S. littoralis. *Biological Control*, *59*(2), 192-199.

Hazaa, M. A. M., Alm El-Din, M. M. S., & El-Akhdar, E. A. H. (2009). The Histological and Histochemical Changes in the Gonads of the Cotton Leaf Worm *Spodoptera littoralis* (Boisd.). *Isotope Rad. Res*, *41*(4 suppl 2), 1465-1484.

Heikal, T. M., Mossa, A. H., & Khalil, W. K. (2014). Protective effects of vitamin C against methomyl-induced injures on the testicular antioxidant status and apoptosis-related gene expression in rat. *J Environ Anal Toxicol*, *5*(2), 255-262.

Kandil, M. A., el-shenawy, R. M., & Amer, r. A. (2018). Impact of magnetic fields and temperatures on biological, life table, morphological and biochemical parameters in *Arias insulana* (boisd.). *Egyptian Journal of Agricultural Research*, *96*(3), 967-985.

Khebbeb, M. E. H., Gaouaoui, R., & Bendjeddou, F. (2008). Tebufenozide effects on the reproductive potentials of the Mediterranean flour moth, *Ephestia kuehniella. African Journal of Biotechnology*, *7*(8).

Klassen, W., Curtis, C. F., Dyck, V. A., Hendrichs, J., & Robinson, A. S. (2005). History of the sterile insect technique. *Sterile Insect Technique: Principles and Practice in Area-Wide Integrated Pest Management. Dordrecht, The Netherlandfs: Springer*, 1-34.

Majdoub, O., Dhen, N., Souguir, S., Haouas, D., Baouandi, M., Laarif, A., & Chaieb, I. (2014). Chemical composition of *Ruta chalepensis* essential oils and their insecticidal activity against *Tribolium castaneum*. *Tunisian Journal of Plant Protection*, *9*(1), 83-90.

Mead, H. M., El-Shafiey, S. N., & Sabry, H. M. (2016). Chemical constituents and ovicidal effects of mahlab, *Prunus mahaleb* L. kernels oil on cotton leafworm, *Spodoptera littoralis* (Boisd.) eggs. *Journal of Plant Protection Research*.

Mogahed, M.I., I. Sharaby, Aziza and M. El-Gengaihi, Souad, (1997). Utilization of Egyptian plants as natural pesticides against *Spodoptera littoralis* (Boisd.). *Bulletin of the Entomological Society of Egypt, Economic Series*, 24: 107-114.

Nenaah, G. E., Ibrahim, S. I., & Al-Assiuty, B. A. (2015). Chemical composition, insecticidal activity and persistence of three Asteraceae essential oils and their nanoemulsions against Callosobruchus maculatus (F.). *Journal of Stored Products Research*, *61*, 9-16.

Nollet, L. M., & Rathore, H. S. (Eds.). (2017). *Green pesticides handbook: Essential oils for pest control*. CRC Press.

Papachristos, D. P., & Stamopoulos, D. C. (2004). Fumigant toxicity of three essential oils on the eggs of *Acanthoscelides obtectus* (Say) (Coleoptera: Bruchidae). *Journal of Stored Products Research*, *40*(5), 517-525.

Sabry, H. M., Mead, H. M., & Khater, K. S. (2017). Inhibitory Effects of Methoxyfenozide on Reproductive Organs of Cotton Leafworm, *Spodoptera Littoralis* (Boisd.). *Journal of Plant Protection and Pathology*, *8* (5), 219-227.

Sauman, I., & Berry, S. J. (2002). Cytochalasin-D treatment triggers premature apoptosis of insect ovarian follicle and nurse cells. *International Journal of Developmental Biology*, *37*(3), 441-450.

Staten, R. T., & Walters, M. L. (2020). Technology used by field managers for pink bollworm eradication with its successful outcome in the United States and Mexico. *Area-Wide Integrated Pest Management: Development and Field Application, J. Hendrichs, R. Pereira, MJB Vreysen, Eds.(CRC Press, Boca Raton, FL, 2020)*, 51-92.

Smykal, V., & Raikhel, A. S. (2015). Nutritional control of insect reproduction. *Current opinion in insect science*, *11*, 31-38.

Su, M., & Mulla, M. S. (1999). Activity and biological effects of neem products against arthropods of medical and veterinary importance. *J. Am. Mosq. Control Assoc*, *15*, 133-152.

Tabanca, N., Demirci, B., Kiyan, H. T., Ali, A., Bernier, U. R., Wedge, D. E., ... & Başer, K. H. C. (2012). Repellent and larvicidal activity of *Ruta graveolens* essential oil and its major individual constituents against *Aedes aegypti*. *Planta Medica*, *78*(05), P_90.

Toppozada, A., Abdallah, S., & Eldefrawi, M. E. (1966). Chemosterilization of larvae and adults of the Egyptian cotton leafworm, *Prodenia litura*, by apholate, metepa, and tepa. *Journal of Economic Entomology*, *59*(5), 1125-1128.

Van Handel, E. M. I. L. E. (1985). Rapid determination of glycogen and sugars in mosquitoes. *J. Am. Mosq. Control Assoc*, *1*(3), 299-304.

Zollner, N., & Kirsch, K. (1962). Microdetermination of lipids by the sulphophosphovanillin reaction. *Z. Ges. Exp. Med*, *135*(545.561).

النشاط الحيوي لزيتي المورينجا والسذاب على حيوية فراشة دودة ورق القطن

ريحاب محمود الجندي* وهند محمد صبري

معهد بحوث وقايه النباتات. مركز البحوث الزراعيه. دقي. جيزة. مصر

دودة ورق القطن هي آفة مدمرة للعديد من المحاصيل الاقتصادية. وكلما زاد استخدام المبيدات الكيماوية زادت مقاومة هذه الآفات مما أدى إلى اكتشاف مبيدات طبيعية جديدة من المستخلصات النباتية والزيوت. يهدف البحث الحالي إلى اقتراح مكونات بديلة من المصادر الطبيعية والتي تكون أكثر أمانًا للنظام البيئي ولديها القدرة على الحد من حظر الحشرات. أظهرت النتائج التجريبية تأثيرًا عالي المعنوية على جوانب حيوية الفراشات البالغة الناتجه من معامله طورما قبل العذراء بواسطة زيت المورينجا و السذاب في جميع التركيزات المختبرة وخاصة التركيز 4٪ حيث يقلل من فترة عمر الإناث ويقلل من فترة وضع البيض. من الواضح أن المورينجا سجلت أقصر فترة وضع للبيض (3.00 أيام) بينما سجلت زيت السذاب (5.33 أيام) بتركيز 4٪ مقارنة بمجموعة التحكم (10.66 يوم). انخفاض كبير في الخصوبة وقابلية الفقس وزيادة نسبة العقم مقارنة بمجموعة الكنترول . تسببت الزيوت النباتية المختبرة في حدوث تشوه نسيجي حاد في المبيض والخصية وزيادة معنوية في الدهون الكلية ومحتويات الكربوهيدرات الكلية أيضًا ، وتميزت تفتيت الحمض النووي بمستويات متغيرة في المبايض والخصيتين في كل علاج زيتي بتركيز 4٪. يمكن أن تؤكد النتائج الجيدة للزيتين على التأثير الشديد لحيوية الجهاز التناسلي لفراشه دودة ورق القطن أنها يمكن أن تستخدم في برامج الإدارة المتكاملة لهذه الآفة.