Benha Journal of Applied Sciences (BJAS) **print: ISSN 2356–9751**
Vol. (9) Issue (3) (2024), (59-64) **online: ISSN 2356–976x**
http://bjas.bu.edu.eg

Efficacy of *Eucalyptus citradora* (Hook.) extract against the peach fruit fly pupae, *Bactrocera zonata* (Saund.) (Diptera: Tephritidae)

[1]Radwa. F. Sallam, [2]Naglaa. F.Abdel-Hameid, [1]Souad. A. Shairra, [2] Adel. A. Hafez and [2]Fawzy. F. Shalaby
[1]Biological Control Department, Plant Protection Research Institute, Agricultural Research Center, Giza - Egypt.
[2]Plant Protection Department, Faculty of Agriculture, Benha University, Qalyubia, Egypt.
E-Mail: radwasallam84@gmail.com

Abstract

The peach fruit fly (PFF), *Bactrocera zonata* (Saunders) (Diptera: Tephritidae), is one of the most invasive and economically significant pests in Egypt for a variety of fruits in temperate, tropical, and subtropical regions. The efficacy and the latent effect of *Eucalyptus citradora* (Hook.) extract on the newly formed pupae of *B. zonata* were assessed. Results indicated that the efficacy of *E. citradora* extract increased as the applied concentration increased. The highest concentration of the *E. citradora* extract (2.5%) had a significant effect and caused high mortality among pupae reaching 92% and the LC_{50} = 2.20. The investigations showed inhibition in the feeding ability, delaying sexual maturity and eggs hatching rate. On the other hand, the histological sections showed a change in inner tissues such as the basement membrane of the midgut which was destructed. Also, sections in the female reproductive system showed mal-developing oocyte chambers before sexual maturity, shrank oocytes, and degenerated leaving vacuoles after sexual maturity. Also, cross sections in testis showed mild reduction of spermatogenesis series.

Key words: Bactrocera zonata, Eucalyptus essential oil, midgut, reproductive system, Biological control.

1. Introduction

The genus *Bactrocera spp* cause harm to the infested fruits because of the extensive host ranges of its members and the invasive potential of certain of those species **[6]**. According to **[12]**, the peach fruit fly (PFF), *B. zonata* (Diptera: Tephritidae) has a wide variety of hosts, including fruits and vegetables. It spread quickly in great numbers throughout Egyptian Governorates. This insect lowers fruit quality, which in turn has a negative impact on fruit exportation **[24]**. The indiscriminate application of chemical pesticides led to several health problems, environmental issues, insecticide resistance, and harm to natural enemies **[26]**. As a result, research on safer and more effective substitutes for the classical chemical insecticides is necessary. The natural plant products are inbetween these options.

Numerous aromatic secondary metabolites, including phenols, phenolic acids, quinones, flavones, flavonoids, flavonols, tannins, and coumarins, are produced by plants and have the potential to harm insects **[7]**. Essential oils from different plant species possess ovicidal, larvicidal, and repellent properties against various insect species and are regarded as environmentally compatible pesticides **[14]** and **[5]**. **[3]** indicated that *Eucalyptus* leaf extracts (*Eucalyptus globulus*) are effective against *Bactrocera cucurbitae*. In mature specimens of several pest species, extracts of Northern Pakistani plants, such as *Eucalyptus sideroxylon*, *Isodon rugosus*, *Cinnamomum camphora*, *Calotropis procera*, *Boenninghausenia albiflora*, *Tagetes minuta*, and *Daphne mucronate* discourage oviposition, and cause high mortality rates **[15].**

The present investigation was undertaken to study the toxicity and latent effect of *Eucalyptus citradora* (Hook.) extract against *B. zonata* one day old pupae. Also, the present study aimed at find out histological effects on the midgut, ovaries and testes of the emerged *B. zonata* adults resulted from one day treated old pupae with 2.5 % *E. citradora* extract.

2. Materials and Methods

Insect culture

The stock culture of *B. zonata* flies used in the present study were reared in cages measured 35 x 30 x 30 cm at the Biological Control Department, Plant Protection Research Institute, Agricultural Research Center Giza - Egypt. Rearing was carried out under greenhouse conditions of 25 ± 2°C. and 54 – 65 % R .H. and the photoperiod ranged between 14/ 8: 16 /10 L: D. on artificial diet. The artificial diet for larvae was prepared according to **[13]** as follows: 1000 gm short wheat, 300 gm sugar, 250 gm yeast, 10 gm sodium benzoate, one ml Conc. HCL (or 20 ml HCL 2N) and 300 ml water.

Adults feeding diet was described by **[18]** as: 2 pieces of Bananas, yolk from 6 Eggs, 4 table spoon honey, 2 table spoons Vit. B. Complex, one table spoon yeast, and 8 table spoons sugar. All these components were mixed in a blinder to make a thick syrup solution to be stored in the refrigerator for further use. For pupation, the third instar larvae were relocated to plastic strainers and placed inside or on large plastic boxes after being later covered with a thin layer of fine sand. After pupation, the sand was sifted to extract pupae which were transmitted to adult cages till emergence according to **[13]** and **[21]**.

Laboratory experiment (inside incubator) using *Eucalyptus citradora* (Hook.) essential oil against one day old pupae of *B. zonata.*

The *E. citradora* essential oil was purchased from the oil extraction unit of the National Research Center (NRC), Dokki, Giza. The *E. citradora* extract concentrations were prepared from the stock solution at 10% concentration. The concentrations 0.5, 1, 1.5, 2, and 2.5% were prepared by dilution with water and adding 2 drops of Tween 80 as emulsifying agent. Freshly formed 150 *B. zonata* pupae (in 5 replicates of 30 pupae / each) were used for each concentration that was no older than one day old and were added to 23gm sandy soil in plastic cans of 5.5 x 4 cm. sprayed with 3ml of *E. citradora* extract emulsion at the above-mentioned concentrations. Five replicates were used for each concentration **[4]**. The pupal mortality was estimated 9 days post-treatment and the LC_{50} was calculated.

Biological effects of *E. citradora* extract against *B. zonata* one day old pupae

Control and treated pupae were placed in separate cages measured (35 x 30 x 30cm) under greenhouse conditions (25±2 °C and 54–65 % R.H. and photoperiod ranged between 14-16: 8 - 10 L: D) and monitored for two generations in terms of emergence time, feeding behavior, sexual maturity period, and egg-hatching rate. For the egg hatching rate, ten replicates were used, and 100 eggs were used for each replicate placed in Petri-dishes by Syringe above a black piece of cloth to make eggs easier to see and counted under light microscope using a brush. Thereafter, eggs were mixed with the previous mentioned larval artificial diet and incubated at 25±2 ºC and 60-70% R.H..

Histopathological studies

For histological studies on mid-gut and ovaries to detect changes and distortions, if any, that occurred on these organs after treatment by *E. citradora* extract concentration (2.5%). The PFF, *B. zonata* adults emerged from treated one-day-old pupae were placed in fixative solution (formalin 10%) for 24 hours in order to process them for general histopathological techniques, which were carried out by standard methods of microtomy. Sections were cut at 3-4 µ thickness using HistoCore Biocut microtome, stained with hematoxylin-eosin (H&E), and examinated under Leica DMLS light microscope **[9]**.

Statistical analysis

Obtained data were analyzed according to **[11]** using Ehabsoft LDP line software to calculate probit analysis **(www.Ehabsoft.com/LDP line)**.

3. Results and Discussion

Toxicity of *E. citradora* extract on *B. zonata* one day old pupae

Data in Table (1) revealed that *E. citradora* extract had high toxicity on the PFF, *B. zonata* freshly formed pupae (LC_{50} =2.20% & LC_{95} = 5.86%).

Table (1) Mortality percentages among freshly formed *B. zonata* pupae treated with different concentrations of *E. citradora* extract 9 days post treatment.

***Eucalyptus* Conc.**	0.5%	1%	1.5%	2%	2.5%	LC_{50} (%)	LC_{95} (%)	Slope
Mortality %	3.33	20	26.66	41.33	92	2.20	5.86	3.86
Control			1.33					

Data clarified that the increasing concentrations of *E. citradora* extract caused an increase in mortality which ranged between 3.33% for treatment with the lowest concentration (0.5%) and 92 % in case of the highest concentration (2.5%). So, the highest concentration caused the highest mortality percentages on the one day-old pupae.

Results in Table (1) and Fig. (1) clarified, also, that the LC_{50} of *E. citradora* oil extract after 9 days post-treatment on the 1st day old pupae of the PFF, *B. zonata* was 2.20 %. The current results are similar to those recorded by **[19]** who tested *Moringa oleifera* oil, lemon peel oil and *M. oleifera* leaf extract on *B. zonata* pupae. The authors` data indicated that pupal mortality percent increased gradually with increased of the applied concentration. On the other hand, these results differed from those obtained by **[4]** who tested some plant extracts against one day old pupae of *B. zonata* and results revealed that *Eucalyptus* oil had the highest toxicity against *B. zonata* pupae with low LC_{50} value of 38.88 ml/L The insecticidal constituents of many plant extracts and essential oils were described as monoterpenoids due to their high volatility, they have fumigant activity that might be of importance for controlling stored-product insects **[17]; [22]** and **[2]**. In the present study, the *E. citradora* extract showed insecticidal activity against the pupae and adults resulted from treated *B. zonata* pupae. The toxic effects of the main components in the essential oil of 15 *Eucalyptus* species were reported by **[10]** to be 1,8-cineole, followed by spathulenol. The high toxicity of linalool, linalyl acetate and 1.8-cineole was reported against the rice weevil *Sitophilus oryzae* and *Rhyzopertha dominica* **[23]**.

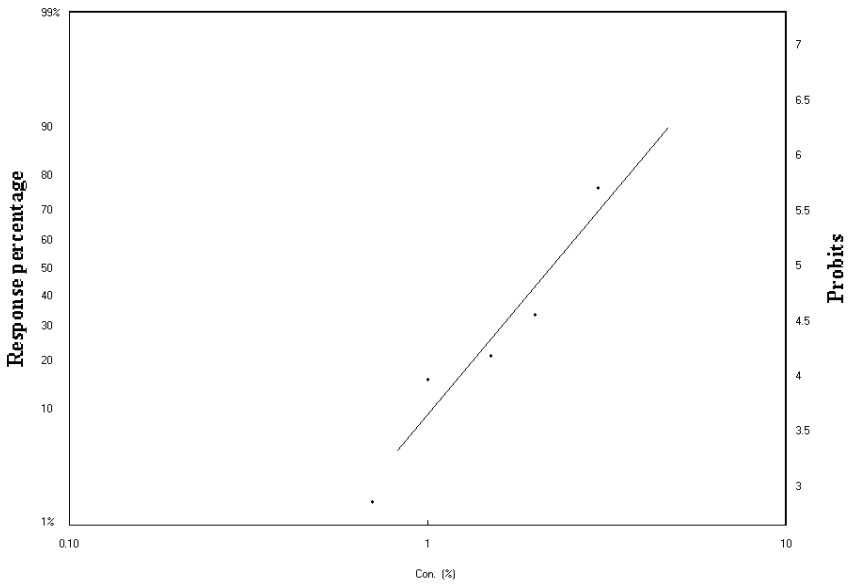

Fig (1) Concentration/mortality line of *E. citradora* extract as surface contact against *B. zonata* one-day-old pupae .

Biological aspects as affected by *E. citradora* extract on *B. zonata* one day old pupae

Results showed that the *E. citradora* extract (2.5%) had a biological action on adults which emerged from one-day-old pupae (treated pupae). These effects were observed in some aspects of adults' behavior or life (Table 2), those may be noted as follows:

***B. zonata* pupal period**

Comparing the control insect adults with those emerged from pupae treated with *E. citradora* extract, it was found that the untreated (control) insects showed nine days as pupal period, while the treated insects emerged one to three days later with an average of 11 days in the first generation. But, in the second generation, the treated adults emerged nine days post-pupation, being the same as that of the control pupae. Statistical analysis showed highly significant differences, as P-value = 0.0004 and the LSD = 0.86. These results are similar to that recorded by **[27]** who tested an aqueous extract of *Centrum parqui* L. ground leaves (which contain saponins) on *Ceratitis capitata* larvae and found a substantial reduction in the emergence rate of flies. **[20]** showed that the tested concentrations of Novaluron, significantly, reduced *B. zonata* adults′ emergence. Also, **[19]** studied the effect of different concentrations of *Moringa oleifera* oil, lemon peel oil and *M. oleifera* leaf extract on some biological aspects such as adults emergence of the peach fly, *B. zonata*. Data indicated that adult emergence decreased with increase of concentrations until reached to very low emergence rate.

Behavioral resistance.

By observing the feeding behavior of both control and adults of *B. zonata* resulting from treated pupae, it was noticed that the insects from treated pupae have a very weak feeding activity and need to renew feeding at very long intervals for both first and second generations.

***B. zonata* adults sexual maturity period**

Data in Table (2) revealed that there was a delay in sexual maturity and the beginning of laying of eggs (pre oviposition period) estimated at 25 days in the first generation and 35 days in the second generation. Sexual maturity of control insects which take 15 days was compared with treated insects. Statistical analysis showed highly significant differences with P-value = 0.0001 and the LSD = 0.

***B. zonata* eggs hatching rate**

Results recorded in Table (2) indicated that the egg-hatching percentage varied in both the first and second generations. The mean hatching percentage was 20% in the first generation and decreased in the subsequent generation to 15% on average. However, in control treatments, the mean of egg-hatching percentage was 62.7%. Statistical analysis showed highly significant differences at P-value = 0.0001 and the LSD = 13.02.

Table (2) Changes in biological aspects occurred in *B. zonata* adults resulted from newly formed pupae treated with *E. citradora* extract 2.5%.

Biological aspects	Mean (days)	P-value	LSD
Pupal period G1	11		
Control	9	0.0004	0.86
Sexual maturity G1	25		
Sexual maturity G2	35	0.0001	0.00
Control	15		
Eggs′ hatching G1	**Mean percent %**		
	20%		
Eggs′ hatching G2	15%	0.0001	13.02
Control	62.7%		

The previous results are similar to those recorded by **[8]** who reported that derivatives of parthenin (1), obtained from wild feverfew, *Parthenium hysterophorus* act as anti-feedants, repellents, and egg-laying deterrents. **[3]** found that *Eucalyptus* leaf extracts (*Eucalyptus globulus*) are effective against *B. cucurbitae* which minimize population numbers. Also, **[15]** tested some plant extracts including *Eucalyptus sideroxylon*, and reported that these extracts caused resistance, discourage oviposition, and caused, also, high mortality percentage.

Histopathological effects of *E. citradora* extract on the PFF, *B. zonata* midgut, female ovaries, and male testis post emergence.

1- **Effect on midgut**: Transverse sections of the midgut from the control and treated adults (adults emerged from one-day-old pupae treated with *E. citradora* extract 2.5%) were observed under light microscopy (Plate; 1 A & B). In controls, the midgut epithelium appeared formed by large similar cells with central nuclei, and small cells, the regenerative cells, regrouped as crypts. The regenerative cells are located at base of the epithelium. The lumen contained some food surrounded by the peritrophic membrane. By the high concentration (2.5%) treatment; the peritrophic membrane seen less distended (Plate, 1 B) compared to control and the epithelial tissues were destroyed. Cross sections of the midgut, cleared the destruction of epithelial cells and autophagic vacuoles in the lumen (black arrow).

2- **Effect of *E. citradora* extract on ovary 27th day after sexual maturity**: Sections of control ovary showed the nutritive (nurse) cells within the ovariole (red arrow) (Plate, 1 C) and clumped shrunk oocyte, semi absorbed or degenerated leaving vacuoles in treated insects (Plate, 1 D).

3- **Effect of *E. citradora* extract on testis 27th day after sexual maturity:** Testis of control insect show intact follicular cells with spermatogonia, spermatide and spermatocyte cells. The testis are full of sperm bundles (blue arrow), spermatids attached to sperm bundles and free sperms (Plate, 1 E) but, mild reduction of spermatogenesis series (black arrow) could be verified in treated insects (Plate, 1 F).

Histopathological results are similar to those recorded by **[1]** whose findings indicated that monoterpenes delayed the formation of female ovarioles in *B. zonata* adults when three oxygenated monoterpenes were assayed against adults. Histopathological alterations were noted, including unfilled egg chambers, germarium construction, empty spaces between ovarioles, and failure of oocyte development. Also, **[16]** treated the early 3rd instar larvae and pupae of *Dacus ciliates* (Diptera: Tephritidae) with Azadrachtin formulation and his results showed that treatment induced severe effect on the midgut where destruction of muscular cells and detachment of the basement memberane. Different results were reported by **[25],** who tested Licorice roots aqueous extract against full-grown larvae and different times after pupation of *Ceratitis capitata* Wied. by dipping treatment. Histological studies showed that the mouth parts and neutrophil foregut neutrophil areas were severely affected, but female ovaries and male testes showed normal view of tissues.

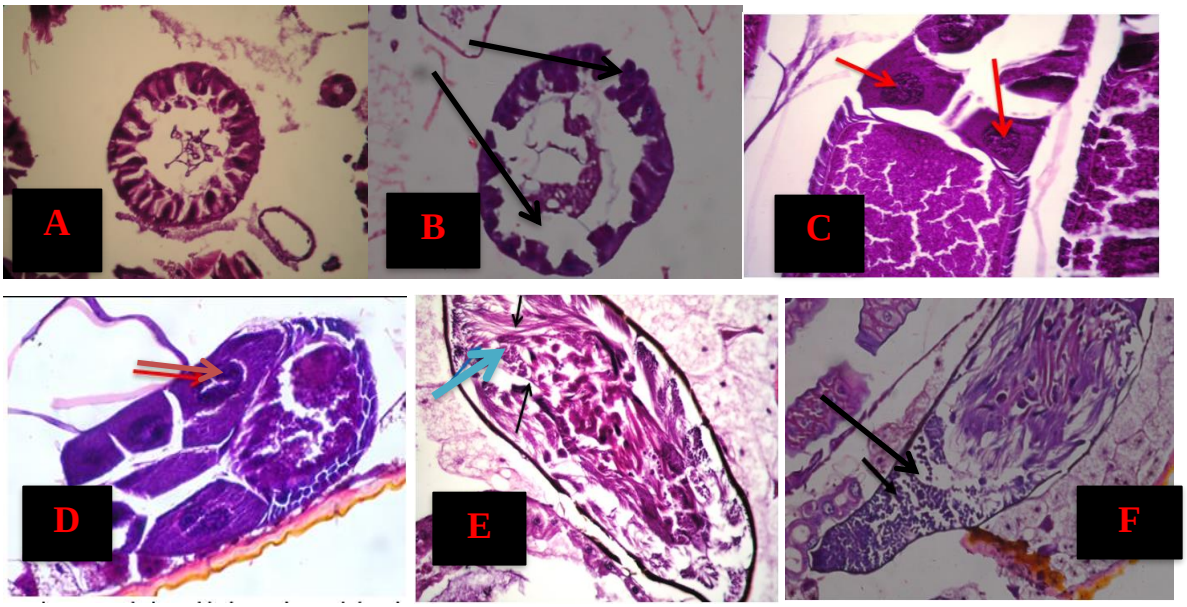

Plate (1). Cross sections in both midgut and reproductive system of *B. zonata*: A) Normal midgut of control insect. B) abnormal midgut of adults resulted from treated pupae. C) Normal ovary of the control insect showing a normal ovariole with mature oocyte. D) Abnormal ovary of the treated insects showing smaller immature ovariole with ruptured follicular epithelium, spaces and degenerated oocyte. E) Normal testis of the control insects showing a normal acini of the testicular follicle. F) Transverse section of the testis of treated insect showing some abnormal testicular acini with necrotic sperms and spaces.

4. Conclusion

E. citradora extract treatments had a toxicity and histological effect against *B. zonata* one day old pupae. Results revealed that mortality percentage increased with increasing the applied concentrations. On the other hand, investigations showed changes in biological aspects and there were histological alterations in midgut, ovary and testes tissues. Therefore, according to the previous results, it could be suggest compination of *E. citradora* extract with Integrated pest control programmes.

Acknowledgement

I am gratefull to all of my supervisors Emeritus **Prof. Fawzy, F. Shalaby;** Emeritus **Prof. Adel, A. Hafez; Prof. Naglaa, F. Abdel-Hameid and Prof. Souad, A. Shairra** who supported me during my study. Each of the members of my research has provided me extensive personal and professional guidance and taught me a great deal about scientific research. Also, I want to extend my thanks to **Prof. Mohamed Abou-Setta** for his help in performing the statistical analysis**.**

References

[1] Abdelgaleil, S.A.M.; Al-Eryan, M.A.; El-Minshawy, A.M.; Gadelhak, G.G. and Rabab, R.A. (2019). Toxicity, development and histological effects of monoterpenes on peach fruit fly, *Bactrocera zonata* (Diptera: Tephritidae). J. Crop. Prot., 8(3):339-349.

[2] Ahn, Y.J.; Lee, S.B.; Lee, H.S. and Kim, G.H. (1998). Insecticidal and acaricidal activity of caravacrol and β-thujaplicine derived from *Thujopsis dolabrata* var. *hondai* sawdust. Journal of Chemical Ecology, 24:81–90.

[3] Ali, H.; Ahmad, S. ; Hassan, G. ; Amin, A.; Hussain, Z. and Naeem, H. (2011). Bioefficacy of different plant extracts against melon fruit fly in bitter gourd. Pakistan J. Weed Sci. Res., 17(2):143-149.

[4] Ali, M.A. (2018). Toxicity of certain plant oils on pupal stage of the peach fruit fly, *B. zonata* (Saunders) (Tephritidae: Diptera). Adv. Plants Agric. Res. 8 (6) : 372 – 374.

[5] Cetin, H.; Erler, F. and Yanikoglu, A. (2004). Larvicidal activity of a botanical natural product, AkseBio2, against *Culex pipiens*. Fitoterapia, 75:724–728.

[6] Clarke, A.; Armstrong, K.; Charmichael, A.; Milne, J.; Raghu, S.; Roderick, G. and Yeates, D. (2005). Invasive phytophagous pest arising through recent tropical evolutionary radiation the *Bactrocera dorsalis* complex of fruit flies. Annu. Rev. of Entomol., 50: 293-319.

[7] Cowan, M.M. (1999). Plant products as antimicrobial agents. Clin. Microbiol. Rev., 12 : 564- 582.

[8] Datta, S. and Saxena, D.B., (2001). Pesticidal properties of Parthenin (*Parthenium hysterophorus* L.) and related compounds. Pest Manage. Sci., 57: 95-101.

[9] Drury, A.R. and Wallington, E.A. (1980). Carleton's histological techniques, 5th Ed., Oxford University Press, New York.

[10] Elaissi, A.; Rouis, Z.; Mabrouk, S.; Salah, K.B.; Aouni, M. and Khouja, M.L., *et al*. (2012). Correlation between chemical composition and antibacterial activity of essential oils from fifteen *Eucalyptus* species growing in the Korbous and Jbel Abderrahman Arboreta (North East Tunisia). Molecules, 17:3044–57. Search in Google Scholar PubMed Central.

[11] Finney, D.J. (1971). Probit analyses. Cambridge University Press, New York USA.

[12] Hashem, A.G; Shehata, M.N.; Abdel-Hafeez, T.A.; Ibrahim, S.A. and El-Kashef, K.K.H. (2007). Occurence and distribution of *Bactrocera zonata* (Saund.) in North Sinai. Egypt J. Appl. Sci., 22(10B): 682-692.

[13] Hosni, M.E.; El-Husseini, M.M.; El-Heneidy, A.H. and Atallah, F.A. (2011). Biological aspects of the peach fruit fly, *Bactrocera zonata* (Saund.) (Diptera: Tephritidae) and its parasitoid species, *Aganaspis daci* Weld. (Hymenoptera: Eucoilidae). Egypt. J. Biol. Pest Control, 21(2): 137-142.

[14] Isman, M.B. (2000). Plant essential oils for pest and disease management. Crop Protection, 19:603–608.

[15] Khan, S.; Shah, M.M.; Ahmed, R. and Haq, I. (2016). The insecticidal potential of botanical extracts for management of Peach fruit fly, *Bactrocera zonata* Saunders, 1842 (Diptera: Tephritidae). Türk. Entomol. Derg., 40(4): 445–453.

~~[15]~~[16] Khater, K.S. (2020). Histopathological effects of Azadirachtin on *Dacus ciliates* Loew (Diptera: Tephritidae). Egypt Acad. J. Biolog. Sci., 12(1): 91-104.

~~[16]~~[17] Konstantopoulou, L.L; Vassilopoulou, L.; Mavragani-Tsipidou, P. and Scouras, Z.G.(1992). Insecticidal effects of essential oils. A study of the effects of essential oils extracted from eleven Greek aromatic plants on *Drosophila auraria*. Experientia, 48:616–619.

~~[17]~~[18] Masood, K.K.; Mohammad, F.S. and Ghulam, J. (2006). Effect of different extracts of harmal (*Peganum harmala* l.), rhizomes of Kuth (*Saussurea lappa* c. b. Clarke) and blachar (*Valariana officianalis* l.) on the settling and growth of Peach Fruit Fly, (*Bactrocera zonata* Saunders). Pak. Entomol., (28)1.

~~[18]~~[19] Morsi, G.M.A.; Gazia, E.F.; Farag, S.R.M. and Selem, G.S.H. (2020). Impact certain plant extract on toxicity, biochemical effect and some biological measurements of peach fly, *Bactrocera zonata* (Saunders). Arab Univ. J. Agric. Sci., 28(1):303-314.

~~[19]~~[20] Negm, A. A. K. H. (2014). Effect of some insect growth regulators on vitellogenesis for controlling the peach fruit fly, *Bactrocera zonata* (Saunders) (Diptera: Tephritidae). Ph.D. Thesis, Fac. of Sci., Ain Shams Univ., Cairo, Egypt. 285 pp.

~~[20]~~[21] Rashad, Marwa, M.; El-Heneidy, A.H.; Djelouah, K.; Hassan, N. Shairra, Souad, A. (2015). On the pathogenicity of entomopathogens to the peach fruit fly, *Bacterocera zonata* (Saunders) (Diptera:Tephritidae). Egypt. J. Biol. Pest Control, 25(3): 649-654.

~~[21]~~[22] Regnault-Roger, C. and Hamrouni, A. (1995). Fumigant toxic activity reproductive inhibition induced by monoterpenes on *Acanthoscelides obtectus* (Say), a bruchid of kidney bean. Journal of Stored Products Research, 31:291–299.

~~[22]~~[23] Rozman, V.; Kalinovic, I. and Korunic, Z. (2007). Toxicity of naturally occurring compounds of Lamiaceae and Lauraceae to three stored-product insects. Journal of Stored Products Research, 43:349–355.

~~[23]~~[24] Shehata, N. F.; Younes, M. W. F. and Mahmoud, Y. A. (2008). Biological studies on the peach fruit fly, *Bactrocera zonata* (Saunders) in Egypt. J. Appl. Sci. Res., 4: 1103–1106.

~~[24]~~[25] Soliman, N.A and El-Genaidy, M.A.M. (2021). Toxicological and histological effects of Licorice *Glycyrrhiza glabra* L., roots aqueous extract on Mediterranean fruit fly, *Ceratitis capitata* Wied. (Diptera: Tephritidae) under laboratory conditions. J. Plant Prot. & Pathol., 12(8): 553-562.

~~[25]~~[26] Victor, M. G. S. (2009). Monitoring and pest control of fruit flies in Thailand: New knowledge for integrated pest management. Examensarbete, 2009: 15, Institutionen for Ekologi, Uppsala. 42 pp.

~~[26]~~[27] Zapata, N.; Budia, F.; Viñuela, E. and Medina, P. (2006). Insecticidal effects of various concentrations of selected extractions of *Cestrum parqui* on adults and immature *Ceratitis capitata*. Journal of Economic Entomology, 99(2): 359–365.