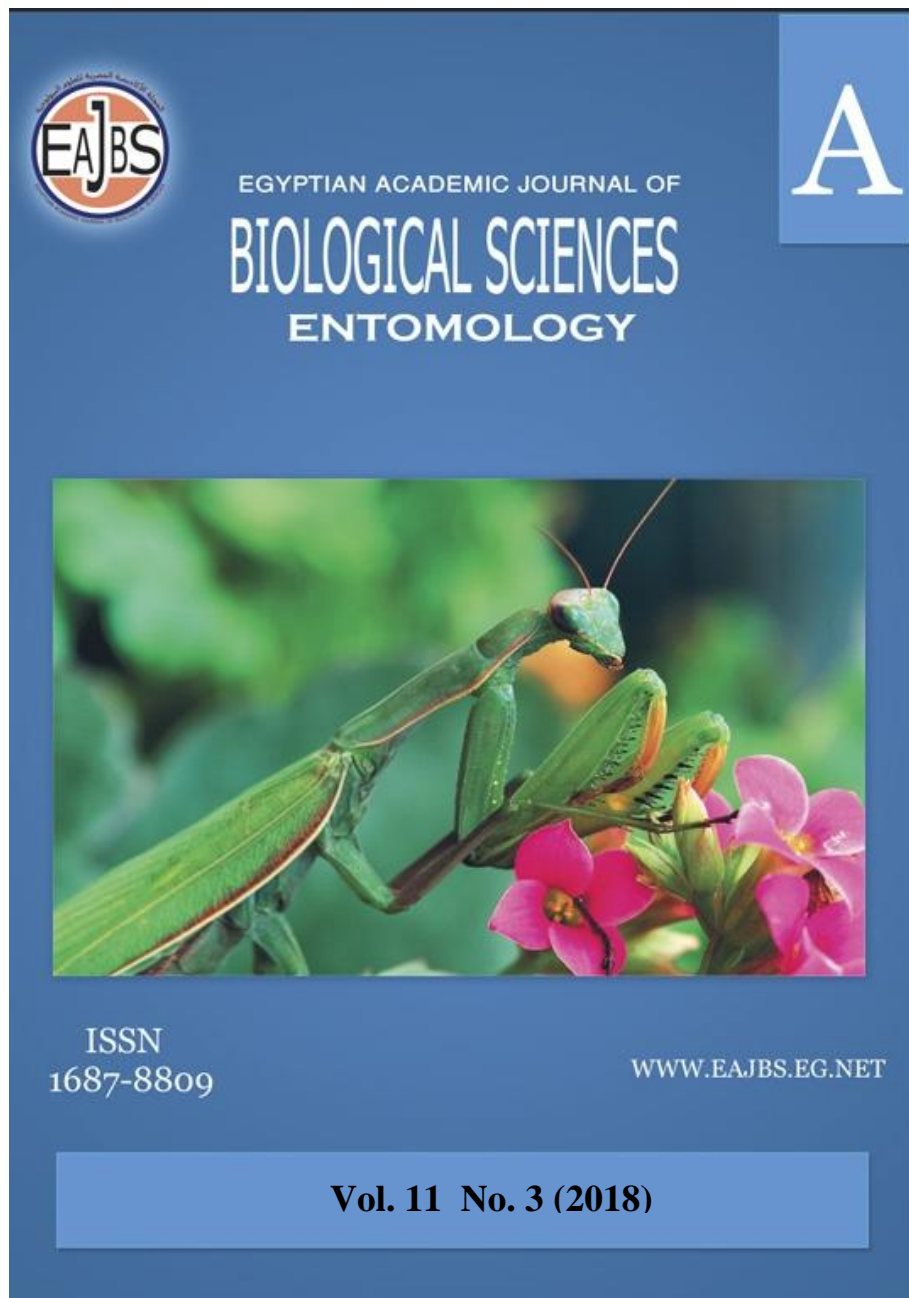


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The Toxic Effect of Certain New Alternative Insecticides against *Bactrocera zonata* under Laboratory Conditions

Elnagar Heba M.; Mohamed H.A.Soliman; Hussein A. El-Naggar and Mansour A.E.Bashar

Plant Protection Research Institute, Agricultural Research Center, Giza, Egypt

, E.Mail : :drmohamedsoliman351@yahoo.com

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ABSTRACT

Tephritid fruit flies are a group of dangerous insects, attack fruits of fruit trees and certain vegetable fruits in all over the world causing direct and indirect economic injury, from it *Bactrocera zonata* (Saunders) (Diptera: Tephritidae) is known as a most serious pest of tropical and subtropical. The experiments carried out during first February, 2018, *Bactrocera zonata* adults (Saunders) (Diptera: Tephritidae), adults of *B. zonata* were taken from plant protection research institute at Doki , Giza, Egypt, thenceforth transferred to plant protection research, Sharkia branch .The current study aimed to study the toxic effect of certain two marine sponges, *Callyspongia crassa* and *Grayella cyathophora*, bath extracted with ethanol against and used against *Bactrocera zonata* under laboratory condition at branch of plant protection research institute at Sharkia governorate. The results indicated that the *B. zonata* female was more susceptibility to these materials compared with *B. zonata* male . Especially, LC₅₀ of *Callyspongia crassa* recorded 1482.6, 1482.6, 705.8 and 496.6 ppm after 48, 72h., 5 days and 10 days against male , respectively but *Crella cyathophora* extract on *B. zonata* after 48, and 72 h., 5 days and 10 days recorded 2900, 989.1, 989.1, and 429.9 ppm against male , respectively. Also, the results showed that the toxic effect of *Callyspongia crassa* and *Crella cyathophora* extract with ethanol on *B. zoata* female after 48, and 72 h., 5 days , where after 48h, the data revealed that the lowest LC₅₀ was 530 ppm in case *Callyspongia crassa* and *Crella cyathophora* with 95% confidence 2.83 While, the highest LC₅₀ was 989.1 ppm with confidence value 5.45. Moreover, the highest LC₉₀ was 3577.6 ppm at confidence value 5.5 but the lowest LC₉₀ was 1504.9 ppm at confidence value 5.5 After 48h and 10 days , respectively.

INTRODUCTION

Tephritid fruit flies are a group of dangerous insects, attack fruits of fruit trees and certain vegetable fruits in all over the world causing direct and indirect economic injury. Economic injury levels of these groups were studied by (Joomaye et al., 2000; Sarwar, 2006). The damage of genus *Bactrocera* has a wide host range of its species

and the invasive power of some species within the genus (Clarke et al., 2005). Peach fruit fly (PFF), *Bactrocera zonata* (Saunders) (Diptera: Tephritidae) is known as a most serious pest of tropical and subtropical fruits (Fletcher, 1987). It was recorded on more than 50 cultivated and wild plant species, mainly those with fleshy fruits including guavas, mangoes, peach, apricots, figs and citrus (White and Elson-Harris 1992, EPPO, 2005; Ghanim, 2009). It originated in South and South-East Asia (Agarwal et al., 1999), and spread to other parts of the world. In December 1998, *B. zonata* was officially identified and recorded for the first time, on infested guavas collected in Agamy and Sabahia, near Alexandria. In 1999, the first traps were set up and showed high capture rates in Egypt (El-Minshawy et al., 1999). In October 2000, *B. zonata* was detected in North Sinai and different localities in Egypt such as Kalubia (Hashem et al., 2001) and El-Behera ,(Draz et al., 2002). Presence of the pest has now been confirmed from all areas of the Sinai, throughout the Nile Delta region and the entire Nile Valley (Cayol et al., 2002; EPPO, 2002). Distribution and infestation patterns of *B. zonata* in the New Valley Oases were also studied Abdel-Galil (2007). *B. zonata* can be monitored by different kinds of traps (Jackson or Steiner traps, though Jackson traps are preferable) baited with the male lure methyl eugenol (O-methyl eugenol), which attracts male flies at very low concentrations (Qureshi et al., 1992). In 2001, Egypt initiated a project to monitor and control this pest in the Sinai, using the male annihilation technique (MAT). Many authors interested in monitoring and studying population fluctuation of PFF males such as (Ishtiaq et al.1999, Rai et al.2008, Deepa et al. 2009, Dale and Patel , 2010, El-Gendy , 2012, Thakur et al. 2013,Venkatachalam et al. 2014, Sundar et al. 2015 and Darwish et al. 2015) by using of methyl eugenol (ME), while others such as (Sarada et al. 2001, Rajitha and Viraktamath ,2005, Rizk et al. 2014, Darwish et al. 2014 and Nagaraj et al. 2014) concerned monitoring both sexes by food attractant traps (Mcp hail), seasonally or along year on different host plants to detect rearing and abundance period of PFF, best position of traps or study impact of physical environmental factors on trap capture on different host plants. Tephritid fruit flies are serious pests in Egypt , of which *Bactrocera zonata* (Saunders) (Diptera: Tephritidae) . Insecticides used to control this pest but the insecticides cause many problems to environmentalespecially, humans , plants , animals , air, water and soil,Nadeem *et al.*2014 found that trichlorfon was observed susceptible to high resistance level (1.01-fold to 41.13-fold), bifenthrin and malathion were found susceptible to moderate resistance level (1.00-fold to 14.27-fold and 1.00-fold to 20.37-fold), lambda-cyhalothrin and spinosad were showed susceptible to low resistance (1.00-fold to 9.57- fold and 1.20 -fold to 9.95-fold), while effect of methomyl were remained as susceptible to all the tested populations.which required adopting new strategies to overcome resistance in this pest. So that beginning search on the alternative to chemical insecticides such detergent, mineral and plant oils , plant extracts, entomopathogen, natural products, and finally beginning the search on other alternatives, Soliman.1998 , 2004 and Soliman *et al.*2015, One of the pathogens of fruit flies is *Beauveria bassiana* (Balsamo) Vuillemin; the introduction of this entomopathogen into the wild fly population would be beneficial for suppression of fruit fly populations.

Marine organisms are a rich source of biologically active metabolites. Recently, studies have suggested that some bioactive compounds isolated from marine organisms have been shown to have antibacterial, antiviral, anti-fungal, anti-cancer, antimalarial, antihelmintic, antituberculosis, antiprotozoal, or anti-inflammatory and other pharmacological activities (Somnath and Ghosh, 2010; Mayer et al., 2013). To date, several chemical compounds from marine organisms have been isolated and are under

investigation. In the last decades, researchers of natural products chemistry focused their research on a wide variety of bioactive compounds from marine species. Marine sponges (Porifera) have been still the champion producers with the large diversity of natural components from marine source. They remain the most prolific phylum have been ranked at the top with respect to the discovery of bioactive compounds with potential pharmaceutical applications. (Faulkner, 2000 Molinski et al., 2009; Gordaliza, 2010). It was proved that marine sponges produce an enormous array of antitumor, antiviral, anti-inflammatory, immunosuppressive, antibiotic, and other bioactive molecules that have the potential for therapeutic use (Frota et al., 2012; Hutagalung et al., 2014). The use of marine natural products is an alternative pest control method, which helps to minimize the usage of toxic pesticides and their deleterious effects on insects, livestock, wildlife and on the environment (Fatope et al., 1993). In recent years, researchers are concentrating on marine organisms to study their biological activities; especially, marine sponges (Porifera) which attracted significant attention from various scientific disciplines (Thakur et al., 2008; Hasaballah and El-Naggar, 2017). In this work, the new strategy will be done in biological control of plant pests by using marine origin extracts.

Therefore, the current study aimed to study the toxic effect of certain two marine sponges, *Callyspongia crassa* and *Grayella cyathophora*, bath extracted with ethanol against *Bactrocera zonata* under laboratory condition at the branch of the plant protection research institute at Sharkia governorate.

MATERIALS AND METHODS

The experiments carried out during first February, 2018, *Bactrocera zonata* adults (Saunders) (Diptera: Tephritidae) were taken from the plant protection research institute at Doki , Giza, Egypt thenceforth transferred to plant protection research, Sharkia branch . The *B. zonata* were transferred in the cages 30X30 cm in the laboratory, adult of *B. zonata* feed on sugar solution 10 % on the cotton piece, the cotton pieces spooned in the cage top. The number of treatment are two treatments as marine sponges of *Callyspongia crassa* and *Grayella cyathophora* (Figs.1 & 2) were extracted and analysed by Ibrahim *et al.* (2017), three concentrations per treatment were prepared but control used water only, four reps / concentration . Ten adults per replicate placed in test tube 5 X 7 cm diameter contain cotton piece dipped in concentration at 10 second period, while control the cotton piece dipped ten seconds in distilled water. The test tube was placed on temperature lab. and a photoperiod of 12 h. the adults were examined after 72 h, 5 days, 7 days and ten days from treatments. *B. zonata* adults were classified as dead or alive and then counted. Adults were considered dead when they did not move after treatment to determine as a way of assessing the LC_{50s} and LC_{90s} effects of *Callyspongia crassa* and *Grayella cyathophora* extracted were observed and recorded. extract stock solution (1000 ppm) per type was serially diluted with distilled water to obtain the different extract concentrations as mentioned in Table (1). Each dilution was prepared on the day of the experimental trial.

Table.1. Concentration preparation from *Callyspongia crassa* and *Grayella cyathophora* crude extracts and dilutions with water .

Name of Extract	Solvents	stock solution	Concentrations		
<i>Callyspongia crassa</i>	Ethanol	1000	400	200	100
<i>Grayella cyathophora</i>	Ethanol	1000	400	200	100
Control	Water	-	-	-	-

Data analysis:

Extract concentration - mortality curves for all bioassays were estimated using Probit analysis in the SPSS software (version 11.0.1) (SPSS Inc., 2010). Extract concentrations, and their 95 % confidence limits, required to kill 50 and 90 percentage (LC50 and LC 90) of the cowpea aphids (nymph) were estimated using the Probit regression. We developed a probit model that used the number of dead aphids as a response variable, the total number of aphids subjected to an extract concentration as a total observations variable, the type of solvent used in leaf extraction as a factor, coded (CE, chloroform extracts) for chloroform, (EE, ethanol extracts) for ethanol, and (WE, water extracts) for water. We used Extracts concentrations, then a single analysis was therefore executed for all solvent extracts, with the assumption of similar or common probit regression slopes checked with the test of parallelism. Pearson's Chi-square test was used to determine the fit of the statistical model (Finney, 1971). Data from all bioassays were corrected using Abbott's formula (Abbott, 1925).



Fig.1. *Callyspongia crassa* (Keller, 1889)

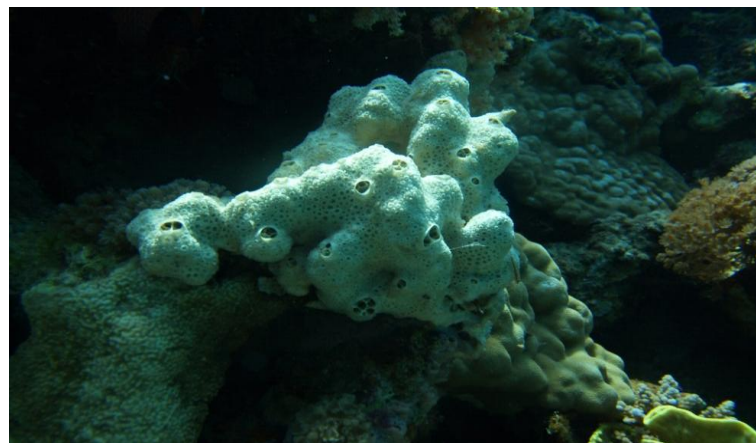


Fig. 2. *Crella* (Grayella) *cyathophora* (Carter, 1869)

RESULTS AND DISCUSSION

Toxic effects of the *Callyspongia crassa* and *Grayella cyathophora* crude extracts against *Bacterocera zonata* male under laboratory conditions.

Problems with the use of pesticides led to the search for alternatives to insecticides, some of these alternatives are marine substances extracted from

Callyspongia crassa and *Grayella cyathophora* against *Bacterocera zonata* under laboratory conditions. The data in Tables 2 and 3 show that the female more susceptible to these materials compared with male. Especially, table 2. the data illustrate value LC₅₀, LC₉₀, confidence, Chi square, slope and standard error. In the same table, the results show that LC₅₀ of *Callyspongia crassa* recorded 1482.6, 1482.6, 705.8 and 496.6 ppm after 48, 72h., 5 days and 10 days against male, respectively and *Grayella cyathophora* extracts from ethanol solvent on *B. zonata* after 48, and 72 h., 5 days and 10 days recorded 2900, 989.1, 989.1, and 429.9 ppm, respectively. On the other hand, LC₉₀ to *Callyspongia crassa* and *Grayella cyathophora* against *Bacterocera zonata* male recorded (18996.2, 21985), (18996.2, 3577.6), (5098.3, 3577.6) and (2357, 1183.6), respectively. The toxic effect of *Callyspongia crassa* and *Grayella cyathophora* were increasing gradually by time beginning from 48 hours to 10 days. Confidence at 95% in case *Callyspongia crassa* and *Grayella cyathophora* after 48, 72 hours, 5 days and 10 days recorded (3.23, 4.57), (3.23, 5.46), (3.46, 5.46) and (3.87, 5.28%), respectively. Whereas slope values ranged between 1.16 to 1.49 and 1.45 to 2.91 in case *Callyspongia crassa* and *Grayella cyathophora*.

Table 2: LC₅₀s and LC₉₀s together with their 95% confidence limits of the *Callyspongia crassa* and *Grayella cyathophora* crude extracts against *Bacterocera zonata* male under laboratory conditions.

Treatments	LC ₅₀ ppm	LC ₉₀ ppm	95 % confidence	Chi. Square	Slope	S.D
After 48 hour of application						
<i>Callyspongia crassa</i>	1482.6	18996.2	3.23	0.013	1.16	1.06
<i>Grayella cyathophora</i>	2900	21985	4.57	0.66	1.45	1.58
After 72 hour of application						
<i>Callyspongia crassa</i>	1482.6	18996.2	3.23	0.013	1.16	1.06
<i>Grayella cyathophora</i>	989.1	3577.6	5.46	0.34	2.29	1.61
After 5 days of application						
<i>Callyspongia crassa</i>	705.8	5098.3	3.46	0.213	1.49	1.0
<i>Grayella cyathophora</i>	989.1	3577.6	5.46	0.34	2.29	1.61
After 10 days of application						
<i>Callyspongia crassa</i>	496.9	2357.6	3.87	0.059	1.89	1.01
<i>Grayella cyathophora</i>	429.9	1183.6	5.28	1.11	2.91	1.2

Data in Table (3) showed the toxic effect of *Callyspongia crassa* and *Grayella cyathophora* extract with ethanol on *B. zoata* female after 48, and 72 h., 5 days, where after 48h, the data revealed that the lowest LC₅₀ was 530 ppm in case *Callyspongia crassa* and *Grayella cyathophora* with 95% confidence 2.83. While, the highest LC₅₀ was 989.1 ppm with confidence value 5.45. Moreover, the highest LC₉₀ was 3577.6 ppm at confidence value 5.5 but the lowest LC₉₀ was 1504.9 ppm at confidence value 5.5. After 48h and 10 days, respectively. From the data in table 3 *Callyspongia crassa* and *Grayella cyathophora* extracts have the same efficacy on *B. zonata* female. Chi-square value calculated less than chi-square tabulated. These results were agreement Richmond *et al.* (1983), Shehata *et al.* (2008), Elnagar M. Heba (2011), Rizk *et al.* (2014) and Soliman *et al.* (2015).

Table 3: LC₅₀s and LC₉₀s together with their 95% confidence limits of the *Callispongia crassa* and *Grayella cyathophora* crude extracts against *Bactrocera zonata* female under laboratory conditions.

Treatments	LC ₅₀ ppm	LC ₉₀ ppm	95 % confidence	Chi. Square	Slope	S.D
After 48 hour of application						
<i>Callispongia crassa</i>	989.1	3577.6	5.5	0.335	2.295	1.61
<i>Grayella cyathophora</i>	989.1	3577.6	5.5	0.335	2.295	1.61
After 72 hour of application						
<i>Callispongia crassa</i>	989.1	3577.6	5.5	0.335	2.295	1.6
<i>Grayella cyathophora</i>	701.85	2519.5	4.9	0.895	2.31	1.31
After 5 days of application						
<i>Callispongia crassa</i>	701.85	2519.5	4.9	0.895	2.31	1.31
<i>Grayella cyathophora</i>	701.85	2519.5	4.9	0.895	2.31	1.31
After 10 days of application						
<i>Callispongia crassa</i>	530.94	1504.9	5.5	0.610	2.83	1.34
<i>Grayella cyathophora</i>	530.94	1504.9	5.5	0.610	2.83	1.34

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