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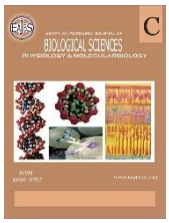
Citation: *Egypt.Acad.J.Biolog.Sci. (C.Physiology and Molecular biology) Vol. 15(2) pp435-442 (2023)*
DOI: 10.21608/EAJBSC.2023.320872

Egypt. Acad. J. Biolog. Sci., 15(2):435-442 (2023)

Egyptian Academic Journal of Biological Sciences
C. Physiology & Molecular Biology
ISSN 2090-0767
www.eajbsc.journals.ekb.eg



Effect of Licorice Root Aqueous Extract, *Glycyrrhiza glabra* (Linnaeus): (Fabaceae) against Peach Fruit Fly, *Bactrocera zonata* (Saunders), (Diptera: Tephritidae) on Three Fruits, Guava, Peach And Apricot At Qulioubia Governorate And Enzymes Effect Of The Treated Adults On Its Behavior Under Laboratory Conditions

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ARTICLE INFO

Article History
Received:6/9/2023
Accepted:11/10/2023
Available:15/10/2023

Keywords:
Bactrocera zonata, *Glycyrrhiza glabra*, root aqueous extract, guava, apricot, peach,AChE & GST enzymes.

ABSTRACT

In order to measure LARE extract efficiency against the adults of *B. zonata* in both of first generation and second generation of the same pest. Data showed that the concentration effect (1: 40) of LARE extract sprayed against adults of *B. zonata* (first generation) on three groups of fruits,Guava, Peach and Apricot were sprayed by hand sprayer under laboratory conditions during the season (2022) successively. The percentages of mortality after 24 h., 48 h. and 72 hours were 73.75%,5% and 21.25% respectively. But in the case of second-generation experiments, laying eggs for females' adults of *B. zonata*. Percentages total numbers of pupae were 100% of distorted adultsexit were 32.56%. Data, illustrated that the percentage of (AChE), (GST) and (SOD) activity decreased significantly when sprayed LARE compared with the control by 22.5%, 39.14% and – 2.98% respectively.

INTRODUCTION

The peach fruit fly, *Bactrocera zonata* (Saunders), (Diptera: Tephritidae) is one of the destructivepests infesting many fruit crops and some vegetables in the world and Egypt causing substantial losses Allwood *et al.* (1999). To reduce the dangerous residues of pesticides used to control this pest on human health and the environment Aktar *et al.* (2009). *G. glabra* plant grows inmost countries in the world and Egypt. Its roots have many medicinal uses as pharmacological effects for human beings, have toxic effects on some pests keeping natural enemies in the environment Fenwick *et al.* (1990). SPONINS was prepared According to the method mentioned by El-Genaidy *et al.* (2021). Saponins are structurally and functionally the largest group of secondary metabolites, profusely produced in plants, and play a very critical role in plant defensemechanisms Moses *et al.* (2014). Due to the growing concern about the potential risks and harmsof chemical pesticides. The impact on non-target organisms stimulates the development and application of environmentally friendly alternative products. It seems necessary to develop alternatives to traditional products and those already widely used in organic farming, for example, copper. Licorice root extract and leaf extract can be effective alternatives for organic and integrated farming, as they contribute to reducing copper used for controlling and resistancemanagement Sophie Hermann *et al.*,*(*2022).

Esterases are the most important enzymes involved in the metabolism of various pesticides in insects. These hydrolases catalyze the hydrolysis and degradation of a wide variety of aliphatic and aromatic esters, organophosphorus compounds, and choline esters. AChE catalyzes the hydrolysis of the acetylcholine neurotransmitter so this enzyme plays a vital role in the cholinergic synapses neurotransmission El-Gendy *et al.,* (2021). Saponin was reported to bind with AChE active site, inhibiting the enzyme, and this causes the accumulation of acetylcholine in the post-synaptic membrane (Sami *et al.*, 2018). This substance accumulation increases stimulations that lead to behavioral changes, asphyxia, and death in insects Silva *et al.,* (2020). Glutathione S-transferase (GST) is an enzyme that plays a significant role in insect metabolism and pesticide resistance. GST widely exists in insects and catalyzes the nucleophilic reaction of glutathione (GSH) with a variety of electrophilic compounds. GST plays an important role in exogenous substance biotransformation, drug metabolism, and protection of the organism against oxidative damage Koirala *et al.,* (2022). This study was concerned with choosing the appropriate concentration of Licorice extract action for applied use in the field. Toidentify the effect of an aqueous extract of licorice on the two stages of the first generation of *B. zonata* adults, the second generation of egg masses and the first larval stages for *B. zonzta*. Simulating the nature of the adult insect stage of *B. zonata* in the laboratory because it was notpossible to monitor this stage under field conditions. Follow-up of the second generation of*B. zonata* inside the fruit and the effect of the extract on the egg masses and larvae stages.Recognizing the insect's preference for the infested fruits during the season of study.

MATERIALS AND METHODS

Bactrocera zonata flies used in this experiment were reared in PPRI, Giza, Egypt. Adult flies kept in a controlled environment (Temperature 27±2°C, 70±10% R.H., 12:12 L:D photophase) in cages (80cm, 50cm, 40cm). The flies were fed on enzymatic protein hydrolysate and sugar at a ratio of 1:3, respectively; furthermore supplied with a water source. Larvae were reared using an artificial larval rearing medium according to Tanaka *et al*. (1969). Preparation of licorice root aqueous extraction (LRAE), fresh root parts of licorice roots, *Glycyrrhiza glabra* Linn. (Family: Fabaceae) was collected from private nursery (Libidy Nursery), Berqash, Giza Governorate, and was taxonomically identified in Horticultural Research Center, Botanic Department, ARC. Egypt. One kilogram of dry mature roots was prepared as aqueous extraction (LRAE) according to the method of El-Genaidy *et al.,* (2021).

Experiment Design:

The experiment was carried out in laboratories of the Plant Protection Research Institute under room conditions Licorice, G. glabra root aqueous extract was prepared with four different concentrations as follows: -1:30 (with 3 ml of aqueous extract: 90 ml distal water), 1:40 (with 3 ml of aqueous extract: 120 ml distal water), 1:50 (with 3 ml of aqueous extract: 150 ml distal water), Pure concentration without dilution, and distilled water was used as a control successively.

A preliminary experiment was applied to find the appropriate concentration that does not cause damage to the plant parts (leaves, fruits). Twelve trees from 3 guava, apricot, peach and their control were sprayed with different concentrations**.** From each fruit trees were divided into four groups; each group consisted of three trees. Trees were sprayed at the stage of fruit hgtsd ripening exudation at a rate of three trees from each concentration, the treated trees inspected three healthy fruits were picked from each treated variety under studying were sprayed by hand laboratory sprayer with different concentrations of Licorice extract 1:30, 1: 40, 1:50 pure extraction and distal water as control successively. After one hour of praying three treated fruits in each replicate

with different concentrations of extract and control were dried on treated fruits every concentration was put in a cage containing 50 males and 50 females at sexual maturity adults with treated three fruits Guava, Peach, Apricot and control treatments. Also, each cage added artificial food which consists of enzymatic protein, hydrolysate and sugar at a ratio of 1:3, respectively and was supplied with a water source inside each cage. Cages were checked regularly every 12 hours and recorded every remark and note we took the dead adults of *B. zonzta*. After 12 hours from the treatment observations were recorded. The treated adults weresluggish moving non-ability to fly with their abdomen distention. After 24 hours 50% of treated adults of *B. zonata* died. At 36 hours 70% died. After 48 hours 80% died. But at 72 hours all treated adults in the cages have died as shown in Figures (1 & 2).

Fig. 1: Spray the fruits with different concentrations of the extract before placing them inthe cage and exposure insects to fruits treated with the extract at all concentrations.

Fig. 2: The first-generation enzymes were analyzed after exposure to the treated fruits.

Fruits Incubation:

Every single fruit was incubated in incubator boxes to know the number of larvae that completedits life cycle and converted to the next stage (pupae) we did that to know the ability of the extract to penetrate the fruit surface &its effect upon the mass of eggs in one prick**.**

Fig. 3: The second generation was larvae inside the fruit that could not jump or pupate. *B. zonata* larvae and pupae incubated from the treated fruits**.**

After hatching eggs to larvae was recorded the mortality percentages and the morphological changes, and these larvae failed to complete the stage into pupae. (All morphological symptoms were photographed) as shown in Figure (3).

Statistical Analysis:

Abbott's formula (1925) was used to record the percentage mortality of adults and larvae of peach fruit fly of B. zonata. Data were subjected to a one-way analysis of variance for the differences of variance (spss, ver.20) and GraphPad prism.

Enzyme analysis tests of treated adults of B. zonata by (LARE), AchE (acetylcholinesterase) activity was measured according to the method described by Simpson *et al.* (1964), using acetylcholine bromide (AchBr) as substrate. The reaction mixture contained 200 µl enzyme solution, 0.5 ml 0.067 M phosphate buffer (pH7) and 0.5 ml AchBr (3 mm). The test tubes were incubated at 37 ºc for exactly 30 min. 1 ml of alkaline hydroxylamine (equal volume of 2 M hydroxylamine chloride and 3.5 M NaOH) was added to the test tubes. Then 0.5 ml of Hcl (1 partof conc. Hcl and 2 parts of ΔH2O) were added. The mixture was shaken vigorously and allowed to stand for 2 min. 0.5 ml of ferric chloride solution (0.9 M Fecl3 in 0.1M Hcl) was added and mixedwell. The decrease in AchBr resulting from hydrolysis by AchE was read at 515 nm.

Principle:

The Bio diagnostic Glutathione S-Transferase Assay Kit measures total GST activity (cytosolic and microsomal) by measuring the conjugation of1- Chloro- 2, 4-dinitrobenzene (CDNB) with reduced glutathione. The conjugation was accompanied by an increase in absorbance at 340 nm. The rate of increase was directly proportional to the GST activity in the sample.

Sample Preparation:

1. Homogenize the tissue in 5 – 10 ml cold buffer (i.e., 100 mm potassium phosphate, pH7.0, containing 2 mm EDTA) per gram of tissue.
2. Centrifuge at 4,000 rpm for 15 minutes at 4 °C. 3. Remove the supernatant for assay and store on ice.

If not as said on the same day, freeze the sample at 80°C. The sample will be stable for atleast one month.

Reagents: They were Phosphate buffer, pH 7.4, Glutathione Reduced (GSH) (Powder), Chloro,2,4-dinitrobenzen (CDNB) and Trichloroacetic acid.

Procedure:

Add 1.0 ml of Buffer (Phosphate buffer) then 0.05 ml Sample and 0.1 ml of GSH (Glutathione Reduced) Incubate at 37ºC for 5 min. then add: 0.1 ml of CDNB (Chloro, 2,4-dinitrobenzen) Mix well. Incubate at 37ºC for exactly 5 min. 5 terminate the reaction by adding: 0.1 ml of TCA (Trichloroacetic acid). Mix well, centrifuge at 3000 r. p. m. for 5 min measure the absorbance of sample (A sample) against the blank at 340 nm. GST Activity: Tissue (U /g tissue) = A (sample) x 2.812/ g. tissue used

RESULTS AND DISCUSSION

GST is involved widely in insects' defense against insecticides. Insect resistance is determined bythe activities of detoxifying enzymes and decreased target sensitivity to chemical pesticides. The changes usually involve increased detoxification enzyme activities. Increased activity of detoxifying enzymes in insects represents a response to intoxication with insecticides or xenobiotics. In this work, GST activity was elevated significantly in the licorice group. These results are in line with a prior study reported that Ectropis obliqua larvae treated with 30% tea saponin showed a significant increase in GST activity during the initial period which suggests that this enzyme may act to detoxify tea saponins Zeng *et al.,* (2018).

Because AChE is an enzyme that is vital in neuromuscular and neural communication in insects, its inhibition is an important target for insecticides (Dassanayake *et al.,* 2021). In this study, AChE activity showed a significant decrease in licorice group and this could be explained by the presence of 40% of saponins in licorice extract. These

results agree with a previous study reported that AChE is a possible target of licorice, which was implicated in the termination of pulse propagation transmission in several cholinergic routes in the central and peripheral nervous systems by rapidly hydrolyzing the neurotransmitter acetylcholine (Chen *et al.*, 2019). From this study, licorice can be used as an AChE inhibitor in the peach fruit fly.

First Generation of Adults of *B. zonata:*

-Adult insects of the first generation were attracted to the fruits sprayed with the extract , especially Guava fruits. They left the available nutrition in the cage (sugar and protein).

-Swelling in the abdominal area.

-Abstain from feeding completely (antifeeding effect).

-Slow movement and inability to fly.

-Onset of death after 24 hours, about 60%, and complete death 100% after 72 hours.

Table 1: Percentage mortality of adults of *B. zonata* (first-generation) with four concentrations of Licorice root solution on three groups of fruits Guava, Peach and Apricotby hand sprayer under laboratory conditions during season (2022).

Concentration (LARE)	24 hour. %	48 hour. %	72 hour. %	96 hour. %
1:30	68.5	9	22.5	0
1:40	73.75	5	21.25	0
1:50	77.78	4.94	17.28	0
Pur.	69.07	17.53	13.4	0
control	1	1	3	95

- Control treatment was distilled water only.
- Results were corrected by using Abbot equation (1925).

Shown from statistical analysis Influencing variables were introduced from different concentrations, and it was found that these variables combined affect the death of insects, as they explain about 84.4% of the changes that occurred in that treatment.

The result of the analysis showed that there was a strong significant inverse relationship between the concentrations and the death of the insect, as the change in the concentration by one unit affects the insect by about 0.109 units, and there was a significant inverse relationshipbetween the death rate of insects and the concentration rate of the extract, as a decrease in the concentration by one unit leads to an effect on the Insect death by about 46.8 units.

For The Second Generation*:*

- Delaying hatching and the growth of larval stages in the treated fruits by about 2 to 4 days from normal growth of control treatment.
- The systolic movement of the caterpillar was slow with its presence in the state of larvalage, despite the passage of about 14 days after laying eggs, with the availability of suitable temperatures for growth, and this was shown in examining the incubated fruits.
- The presence of a number of prickles and pupal formation in guava fruits 2.6 grams of protein per 100 gm incubated was greater than that of peach fruits, including 2.18 grams of protein per 100 grams and apricots 1.4 gm protein. This might be due to the protein content in guava fruits being higher than peaches and apricots Wikipedia (2023).

Table 2: Laying eggs for adult *B. zonzta* (second generation) on three treated fruits Guava, Peach and Apricot with Licorice extract with conc. 1: 40 by hand sprayer under laboratory conditions for seasons (2022).

The crop	% Total number of pupae *B.zonata*	% Distorted adults exit	% Failure adults to come out
Guava	100	32.56	67.44
Peaches	45.7	94.92	5.08
Apricot	22.5	82.76	17.24

- All adult incites that resulted from pupae were distorted antifeedant, without the ability to aviation and were dead after 72 hours.
- Control fruits that pricked all their larvae completed their natural life cycle.

The variables were introduced for the characteristics of the second generation, and it was found that these variables do not affect the incidence of prickles (egg masses), as they explain only about 8.7% of the characteristics of the second generation in that treatment.

It was also found that the relationship was not significant between the characteristics of the second generation of the insect, as all insects of the second generation were unable to complete the life cycle, but it was noted that the percentage of prickles in peaches represents about 45.7%, and apricots were about 22.5% of the percentage of prickles in guava fruits.

Acetylcholinesterase (AChE) activity decreased significantly in licorice group ($p < 0.01$) whencompared with the control group. On the other hand, GST activity showed a significant increase in licorice group ($p < 0.001$) in comparison with the control group. However, there was a non-significant change in SOD activity in licorice and control groups.

Table 3: Enzyme analysis (AChE), GST and SOD of first-generation adult insects treatedwith LARE concentration (1:40).

Enzymes activity	Control group	licorice group(1S)
AChE (ug AchBr/min/g tissue)	640.28 ± 29.77	419.8311 ± 11.16 ($p < 0.01$)
GST (U/ g tissue)	183.34 ± 5.84	255.10 ± 1.81 ($p < 0.001$)
SOD (U/ g tissue)	27916.86 ± 757.62	27354.75 ± 372.81 N.S

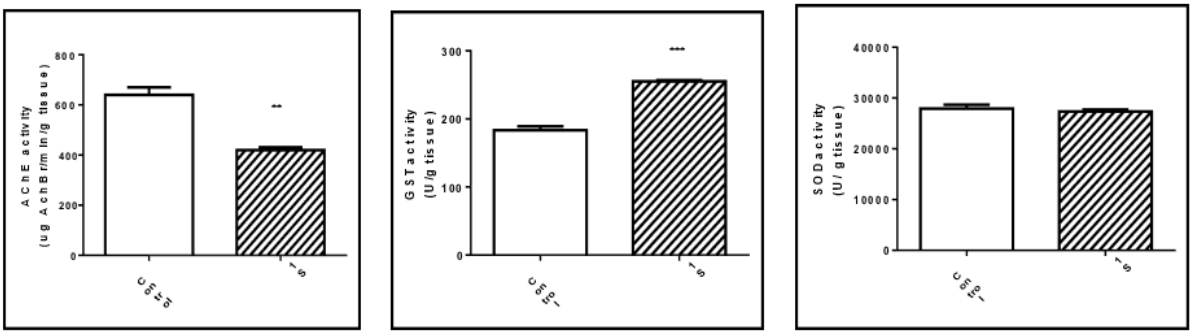

Fig.4: It shows the relationship between enzymes (AChE), GST and SOD in control andtreated adults**.**

Acknowledgements

The authors would like to express their deep gratitude to Dr. Marian N Gerges Department of Chemistry, Faculty of Science, Tanta University to explain the chemical effect of LARE extract on the biological activity of the adult insect *B. zonata*.

REFERENCES

Abbott's formula (1925) was used to record the mortality of adult and larvae of peach fruit fly of B. zonata. Data were subjected to one-way analysis of variance for the differences of variance (spss, ver.20). and Graphpad prism

Aktar Md. Wasim, Dwaipayan Sengupta and Ashim Chowdhury(2009): *Impact of pesticides use in agriculture: their benefits and hazards. Interdisciplinary Toxicology* , 2009 Mar; 2(1): 1–12.

Allwood,A.J. ; Chinajariyawong,A. ; Krits aneepaiboon, S. ; Drew, R. A. I. ; Hamacek, E. L. ; Hancock, D. L. ; Hengsawad, C. ; Jipanin, J. C. ; Jirasurat, M. ; Krong, C. K. ; Leong, C. T.S.; and Vijaysegaran, S. (1999): *Host plant records for fruit flies (Diptera: Tephritidae) in Southeast Asia. Raffles Bulletin of Zoology*, Vol.47 No.Supplement 7 pp.1-92 ref.26

Chen M, Zhu J, Kang J, Lai X, Gao Y, Gan H, Yang F. (2019) Exploration in the Mechanism of Action of Licorice by Network Pharmacology. *Molecules*,15;24(16):2959. doi: 10.3390/molecules24162959. PMID: 31443210; PMCID:PMC6720938.

Dassanayake MK, Chong CH, Khoo TJ, Figiel A, Szumny A, Choo CM. (2021): Synergistic Field Crop Pest Management Properties of Plant-Derived Essential Oils in Combination with Synthetic Pesticides and Bioactive Molecules: A Review. *Foods*.;10(9):2016. Published. doi:10.3390/foods 10092016.

El-Gendy, I.R., El-Banobi, M.I. & Villanueva-Jimenez, J.A. (2021): Bio-pesticides alternative diazinon to control peach fruit fly, *Bactrocera zonata* (Saunders) (Diptera: Tephritidae). *Egyptian Journal of Biological Pest Control*,31, 49 https://doi.org/10.1186/s41938-021-00398-2

Fenwick G.R., J. Lutomski, C. Nieman (1990): *Liquorice, Glycyrrhiza glabra L.—Composition, uses and analysis*. *Food Chemistry*, Volume 38, Issue 2, Pages 119-143

Koirala B K S, Moural T, Zhu F. (2022): Functional and Structural Diversity of Insect Glutathione S-transferases in Xenobiotic Adaptation. *International Journal of Biological Sciences*, 11;18(15):5713- 5723. doi: 10.7150/ijbs.77141. PMID: 36263171; PMCID: PMC9576527.

Mervat Abdel-Moneauim Mostafa El-Genaidy, Mohamed Abd El-Aziz Mohamed Hindy, Nehad Abdel-Hameed Soliman (2021): *Licorice (Glycyrrhiza glabra (Linnaeus, 1753)) roots aqueous extract and some additives against Bactrocera zonata (Saunders, 1841) (Diptera: Tephritidae). Polish Journal of Entomology*, 90(2): 70–85

Moses, T., Papadopoulou, K. K., & Osbourn, A. (2014): *Metabolic and functional diversity of saponins, biosynthetic intermediates and semisynthetic derivatives*. *Critical Reviews in Biochemistry and Molecular Biology*, 49, 439-462.

Sami, A.J., Bilal, S., Khalid, M., Nazir, M.T., Shakoori, A., (2018): A comparative study of inhibitory properties of saponins (derived from Azadirachta indica) for acetylcholinesterase of Tribolium castaneum and Apis mellifera. *Pakistan Journal of Zoology*, 50, 725–733.

Silva, L. S. da, Porfiro, C. A., Silva, F. G., Rodrigues, A. R. dos S., & Pereira, P.

S. (2020): Acetylcholinesterase and α-Amylase inhibitors from Mouriri elliptica Martius leaf extract. *Bioscience Journal,* 36(2). https: //doi.org/10.14393/BJ-v36n2a2020-42714

Sophie Hermann,1 Marc Orlik,2 Petra Boevink,3 Elke Stein,4 Andrea Scherf,2 Ina Kleeberg,1 Annegret Schmitt,2 and Adam Schikora5 (2022*): Biocontrol of Plant Diseases Using Glycyrrhiza glabra Leaf Extract* . *Plant Disease,* Vol. 106 No. 12 p:3133-3144.

Wikipedia (2023): (https://ar. wikipedia. org/wiki).

Zeng C, Wu L, Zhao Y, Yun Y, Peng Y. (2018): Tea saponin reduces the damage of *Ectropis obliqua* to tea crops, and exerts reduced effects on the spiders *Ebrechtella tricuspidata* and *Evarcha albaria compared to chemical insecticides*. PeerJ. 19;6:e4534. doi: 10.7717/peerj.4534. PMID: 29576988; PMCID: PMC5863709.

ARABIC SUMMARY

تأثير المستخلص المائي لجذور عرق السوس *Glycyrrhiza glabra* (Linnaeus) (Fabaceae) ضد ذبابة فاكهة الخوخ *Bactrocera zonata* (Saunders),(Diptera: Tephritidae) على ثلاث ثمار الجوافة والخوخ والمشمش بمحافظة القليوبية وتأثير أنزيمات سلوك الشخص البالغ المعالج تحت ظروف المختبر

مرفت عبد المنعم الجنيدي[1] و محمد عبد العزيز هندى[2]

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من أجل قياس كفاءة مستخلص LARE ضد البالغين من B. zonata في كل من الجيل الأول والجيل الثاني من نفس الآفة. أظهرت البيانات أن تأثير التركيز (1:40) لمستخلص LARE الذي تم رشه ضد بالغات B. zonata (الجيل الأول) على ثلاث مجموعات من ثمار الجوافة والخوخ والمشمش تم رشها بالرش اليدوي تحت الظروف المختبرية خلال الموسم (2022) على التوالي. . النسب المئوية للوفيات بعد 24 ساعة، 48 ساعة. و 72 ساعة كانت 73.75%، 5% و 21.25% على التوالي. ولكن في حالة تجربة الجيل الثاني، يتم وضع البيض للإناث البالغة من نوع B. zonata. وكانت النسب الكلية لأعداد العذارى 100% من خروج البالغين المشوهين 32.56%. أوضحت البيانات أن نسبة نشاط (AChE) و (GST) و (SOD) انخفضت معنوياً عند رش LARE مقارنة مع المقارنة بـ - 22.5%، 39.14% و- 2.98% على التوالي.

الكلمات المفتاحية: ***Bactrocera zonata*** ، AChE **و** GST **و DOS** انزيم ، مستخلص جذور العرقسوس المائى ، الجوافة والخوخ والمشمش