Licorice (Glycyrrhiza glabra (Linnaeus, 1753)) roots aqueous extract and some additives against Bactrocera zonata (Saunders, 1841) (Diptera: Tephritidae)

Mervat Abdel-Moneauim Mostafa El-Genaidy1, Mohamed Abd El-Aziz Mohamed Hindy2, Nehad Abdel-Hameed Soliman1

1 Horticultural Insect Research Department (HIRD), Plant Protection Research Institute (PPRI), Agricultural Research Center (ARC), Ministry of Agriculture (MOE), Egypt.
2 Plant Protection Institute (PPRI), Agricultural Research Center (ARC), Ministry of Agriculture (MOE), Egypt.

* Corresponding author: nehadpprie@hotmail.com

Abstract: Peach fruit fly, Bactrocera zonata (Saunders, 1841) is a destructive polyphagous pest threatening the horticultural production in Egypt. Licorice, Glycyrrhiza glabra (Linnaeus, 1753) is a plant growing in Egypt and many other countries and famous for saponins groups that have insecticidal effect against broad spectrum of insect pests. In the present study, the insecticidal effect of licorice roots aqueous extract (LRAE), petroleum oil, KZ light mineral oil 96% (EC), water and an emulsion (1/4 L LRAE + ¾ L petroleum oil + ½ L KZ light oil 96% (EC)) treatments in a ratio 1 L: 29 L water were used in Matabi® sprayer of 20 L capacity against B. zonata pupae in sandy and clay soils. In sandy and clay soils LRAE reduced B. zonata population by 74.44% and 87.55% while petroleum oil, KZ light mineral oil 96% (EC) prevented flies emergence (100% reduction). Water treatment suppressed B. zonata population by 78.61% in sandy soil but caused 100% population reduction in clay soil. The emulsion reduced B. zonata population by 96.94% in sandy soil and 100% in clay soil. The best method for application of the emulsion was to spray as one target spray technique for eight seconds that was sufficient to obtain suitable coverage on soil with spray speed 1.2 km / hour. The persistence of the emulsion that highly reduced B. zonata larval populations was 3.5 and 4.5 days in sandy and clay soils, respectively. The flies emerged from B. zonata pupae treated with the emulsion neither feed nor move naturally. The histological studies showed that these flies suffered changes in the eyes, labellum, muscles and midgut tissues that were different from the emerged control treatment flies.

Key words: pest control, green pesticides, insecticidal effects, spray flow rates, low volume spraying

Introduction

Peach fruit fly, Bactrocera zonata (Saunders, 1841) (Diptera: Tephritidae) is a destructive polyphagous pest infesting all horticultural fruits and some vegetables (Allwood et al. 1999) and was listed as a quarantine pest A2/302 (EPPO, 2020). This pest causes severe damage to the infested fruits when female flies oviposit their eggs inside fruits that hatch into larvae feeding on the fruit flesh causing its destruction, so, they cause high loss in fruit production annually. Larvae pass three larval stages inside the fruit, then the full-grown larvae pop up to the soil for pupation. In about nine days, new flies emerge to attack fruits again. Dependence on the use of pesticides only as a control method became an obstacle in the way of exporting fresh horticultural products due to pesticides residues. In addition, the insecticides residues are dangerous to the sustainable environment and have adverse effects on arthropods fauna, flora, microorganisms and human as well (Aktar et al. 2009). Development of an effective method that can suppress B. zonata population under the economical threshold or possibly eradicate it has become an essential demand. Nowadays, the insecticidal effects of plant extracts alone or with some compatible additives attracted the attention of insect pests control
Researchers, as they are economically approachable, biodegradable and easy to use towards alternative pest management products (Campos et al. 2019). Licorice, *Glycyrrhiza glabra* (Linnaeus, 1753) (Fabaceae) is a plant that grows in Egypt and some other countries in the world. Its roots have insecticidal effect on certain pests keeping natural enemies in the environment (Fenwick et al. 1990). The roots of *Glycyrrhiza* spp. contains triterpenoid saponins (glycyrrhizin, glycyrrhizic acid), which are the major characteristic constituents of licorice (Blumenthal et al. 2000, Shah et al. 2018). In the present study, licorice roots aqueous extract (LRAE) was analyzed spectrophotometrically to confirm the presence of saponins. Saponins extracted from plants are known to be steroidal or triterpenoidal compounds, also phenolic compounds with a diverse range of bioactivities against insect pests (Joel 1978, Herrera 1982, Podolak et al. 2010 & Diaz et al. 2019). Petroleum and mineral oils were so effective against tephritid fruitflies (Nguyen et al. 2007; Daniel 2014). Furthermore, high soil water content level can decrease flies emergence from *B. zonata* pupae (El-Gendy & Abd Allah, 2019). Previous studies have reported that petroleum oils with plant extracts are compatible for combined effect and worked synergistically or additively for insect pest control (Haroon et al. 2011, Loongsai et al. 2012, Shah et al. 2008). In the current study, the authors tested the insecticidal effect of LRAE for the first time in Egypt and may be in the world on *B. zonata* in sandy and clay soil. In addition, we investigated the effect of petroleum oil, light KZ mineral oil (96% EC), distilled water and an emulsion contains LRAE and the other tested materials in different ratios (El-Genaidy et al. 2019) against *B. zonata* pupae in sandy and clay soils under laboratory conditions. The flies emerged from *B. zonata* pupae treated with the emulsion were subjected to histological studies.

**Materials and Methods**

**Insect flies**

*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 1:3, respectively; furthermore supplied with a water source. Larvae were reared using artificial larval rearing medium according to Tanaka et al. (1969).

**Preparation of the licorice roots aqueous extraction (LRAE)**

The Licorice roots aqueous extract was prepared under laboratory conditions according to Siam & El-Genaidy (2021) as the following steps: 100 gm of edible parts of Licorice roots were weighted and added with 175 ml of 6% commercial acetic acid and left for fermentation process until 6 hours under room temperature. The concentrated extract was precipitated through filter paper Wattman No. 1 with 500 ml distilled water for eight hours. Another 500 ml of distilled water were added to the filtrate and left for more four hours until the concentrated extract became colorless. The two filtration parts were mixed to obtain one liter of aqueous extract.

**Evaluation of saponins in LRAE**

The crude LRAE solution was subjected to measure saponins by UV-Visible Spectrophotometer model (Spector UV/VIS Dual Beam 8 Auto cell) at wavelength nm 587. Saponins in the crude LRAE were compared to pure saponins powder solution (1 g/L=10 000 ppm). The pure saponins powder and the measurement process belong to “The Scientific Research Center and Measurements”. Faculty of Science, Tanta University, Egypt.
Sterilization of sandy and clay soils

Sandy and Clay soils were sterilized to avoid any contamination factors effect on the pupae beside the effect of our tested materials. Two Aluminum trays (depth 4.0 cm and total capacity 5.0 kg) contained dry sandy soil and dry Clay soil, separately were coated with Aluminum paper and placed inside the oven about 40 minutes under temperature 93.0°C. After sterilization, the trays were left outside the oven until complete cooling and ready to utilization at experimental treatments.

The tested compounds used in the experiments:

1. Licorice roots were obtained from Haraz Co. for food industry, Egypt and used for the aqueous extraction. LRAE concentration rate 1 L: 29 L, compound: water, respectively prepared under laboratory conditions (32°C ± 3°C, RH 70–75%).
2. Petroleum oil was obtained from Kafr El Zayat Pesticides & Chemicals Co., Egypt: aromatic chains and aliphatic hydrocarbons.
3. KZ oil light mineral oil concentration 96% (EC) obtained from Kafr El Zayat Pesticides & Chemicals Co., Egypt: one of the mineral oils consists mainly of aliphatic hydrocarbons, saturated and unsaturated. Common name: Miscible oil, summer oil, KZ oil 96–5% (EC).
4. Distilled (De-ionized) water obtained from Alexandria Detergents & Chemicals Co., Egypt.
5. Emulsion (El-Genaidy et al. 2019) consisted of 1/4 L licorice roots aqueous extraction+1/4 L petroleum oil+1/2 L KZ oil with dilution to 29 L water (contains saponins 3 000 ppm).

Spraying equipment tested on experimental treatments under laboratory condition (common sprayer)

Knapsack sprayer, hand held-lever operated Matabi® sprayer was used during spraying operations of the treatments under laboratory conditions. Techno operational data and calibration of the sprayer were presented in Table 1.

Effect of tested compounds on B. zonata stages

Experimental tests were carried out under PPRI labs conditions (32°C ± 3°C, RH 70–75%). The area of each experimental test was 1.5×3 m² divided into three equal parts. Each part contained five plastic boxes, contained 1/2 kg sandy soil each and arranged in a row (20 cm apart from each other) for the following test experiments. Sensitive papers were used to confirm uniformity of the sprayed materials in the treated soil in boxes (blue color of the papers means spray uniformity in soil) (Fig. 1a, b, c). The same process was repeated for the tests in clay soil.

Effect of tested compounds on different B. zonata pupal ages - first experiment

Twenty B. zonata pupae (4 days old) (Fig. 3a) were distributed randomly at different depths ranged from 1–4 cm inside each sandy

Fig.1. Sensitive paper saturated with droplet sizes after spraying with Matabi® sprayer (a), on sandy (b) and clay (c) soils under laboratory conditions.
Table 1. Techno-operational data and calibration of hand held operated Hydraulic Mitabi sprayer under laboratory conditions.

| Spray parameters       | Equipment:          |
|-----------------------|---------------------|
|                       | hand held operated hydraulic Matabi® sprayer |
| No. of units sprayer  | One                 |
| Manufacture           | Matabi – spane      |
| Unit sprayer type     | Hollow cone nozzle with spray Angle 80° |
| Pump type             | Manual Piston Pump  |
| Tank capacity (L)     | 20.0                |
| Pressure pump bar.    | 5.0                 |
| Spraying experimental area (m²) | 4.5 |
| Flow rate (L/min)     | 0.540 (one minute spraying) |
| Flow rate (L/30 sec.) | 0.270 (for half minute spraying) |
| Flow rate (L/8.0 sec.)| 0.073 (for one swath width) |
| Area of experiment (m²) | 6.0                    |
| Sweathe width (m)     | 0.75                |
| Spray height (m)      | 0.70                |
| Spraying type         | Target spray technique in all treatments |
| Working speed (km/h)  | 1.2 at all treatments, i.e. 20.0 meter/min. |

soil box. Licorice root aqueous extract (1L LRAE: 29L water) in Matabi® sprayer of capacity 20 liters as in Table 1 was sprayed on the soil surface. The boxes were placed in screen cages to receive the emerged flies that were examined and the observed malformations were recorded. Dead pupae in each box were counted. Each treatment was replicated three times. A control treatment (without spray) was carried out in parallel. The same procedures were repeated for the test in clay soil. The same steps was repeated to test the petroleum oil, KZ light mineral oil 96% (EC) and the emulsion against B. zonata pupae in sandy and clay soils.

**Effect of spray time of emulsion on pupal mortality - second experiment**

The same steps in the first experiment for testing the effect of the emulsion on B. zonata pupae (4 days old) were proceeded except the spray time under laboratory conditions according to El-Genaidy et al. (2019). Three different spray flow rates were tested. The first was target spray for on swath width for 8.0 sec. at 1.2 km/hour. The second spraying was multiple swath width for 30 sec. third experiment were multiple swath on the same experimental area with another boxes of B. zonata pupae for sandy and clay soils, the multiple spraying for 60 sec. continuously with mixture solution from Matabi® sprayer. The emulsion was sprayed according to Table 1 for 8, 30 and 60 seconds. The treatments were replicated three times and control treatments ran in parallel.

**Persistence of the emulsion in sandy and clay soils - third experiment**

The third experiment was designed after El-Genaidy et al. (2019) to evaluate the persistence of the emulsion under laboratory conditions. Sandy soil in 25 boxes contains 1/2 kg/box were sprayed with the emulsion (one swath for 8 seconds/box). The treated sandy soils were divided into five groups (five boxes/row) to measure the persistence of the emulsion effect on B. zonata fresh full-grown larvae along successive seven days. The first treatment started two hours after soil spraying; twenty B. zonata full-grown larvae were freshly harvested from the rearing medium and placed on the sprayed soils surfaces. Larvae were left to burry themselves naturally to pupate in soil. The same steps were repeated for the rest of groups (1, 3, 5
and 7 days). Control treatments ran in parallel. The treated and control soil boxes were kept in screen cages separately to receive the emerged flies. After ten days from each soil-larval treatment, the soils were inspected for dead larvae and pupae and all malformed individuals were recorded. Also, the emerged flies that seem normal were counted and the same for the control treatments as well. All these steps were proceeded for testing the emulsion persistence in the clay soil.

Effect of the emulsion on the emerged flies - histological studies

The emerged flies which seemed normal were subjected to some histological studies (El-Genaidy et al. 2019) for effect of the emulsion. The studied flies’ parts were the eye, mouthparts (labellum), abdominal muscles and the midgut. The preparation of insect paraffin embedded sections was done according to Kucherenko et al. (2010). The paraffin blocks were cut with 7–10 μm. The sectioned tissues were stained with hematoxylin and eosin (Suvarna et al. 2013).

Statistical analysis

The percentage of pupal and larval mortalities were corrected according to Abbott’s formula (1925). All data were subjected to one-way analysis of variance (ANOVA) for the differences of variance (SAS, 1985). Pearson’s chi squared test was applied for describing the insecticidal effects of the tested compounds against B. zonata pupae and larvae. Pearson’s correlation coefficient was calculated according to (Snedcor & Cochran 1980) to study the relation between time and the emulsion persistent effect on B. zonata population reduction.

Results

Evaluation of saponins in LRAE

Spectrophotometrical analysis chromatographs of the crude licorice roots aqueous extract (LRAE) that were compared to the pure saponins powder solution for calibration presented in Fig. 2a and 2b were almost identical.

Effect of tested compounds on different B. zonata pupal ages - first experiment

Insecticidal effect of the tested compounds was expressed in B. zonata dead pupae and flies malformations (Fig. 3c, d) percentages in sandy and clay soils (Table 2). All the tested compounds significantly affected B. zonata population in both soil types (F=275.58, df=11, 179, P<0.0001). In sandy soil treatments, LRAE, petroleum oil, KZ light mineral oil 96% (EC), water and the emulsion suppressed B. zonata population significantly (F=10.03, df=4, 74, P<0.0001) and showed high significance with the control treatment (F=270.91, df=5, 89, P<0.0001). Petroleum and KZ oils treatments prevented fly’s emergence (100% reduction) in sandy and clay soil treatments. Water treatments in sandy and clay soils reduced B. zonata population significantly (F=9.38, P<0.001) but showed highly significant difference as compared to control treatment (F=548.36, df=3, 59, P<0.0001). The emulsion treatments caused population reduction in both soil types insignificantly (F=3, 54, P>0.05) and reflected high significant difference with control treatments (F=4 098.22, df=3, 59, P<0.0001). In sandy soil, the emulsion suppressed the pupal population more than water treatment (F=9.94, P<0.001) while in clay soil both of them prevented flies emergence. The highest pupal mortality percentage accompanied the petroleum oil treatment in both soil types while the least appeared with water treatment in sandy soil and KZ mineral oil 96% (EC) in clay soil treatments. The highest flies’ malformations percentages accompanied the KZ mineral oil 96% (EC) pupal treatments reached 60% while the least resulted in the petroleum oil treatment (16%). Head only emerged flies (Fig.3c) differed insignificantly after the tested compounds in sandy soil (F=1.48, df=5, 89, P>0.05) and (F=1.11, df=5, 89, P>0.05) in clay
Fig. 2. Comparison of the chromatographs of the standard pure saponin solution g/L=10,000 ppm/L (a) and the licorice roots aqueous extract LRAE (b) detected at recorded 244–592 nm.

Fig. 3. Malformations recorded due to licorice roots aqueous extract, petroleum oil, KZ light mineral oil 96% (EC), water and emulsion: a) normal B. zonata pupae; b) dead pupae; c) head emerged fly; d) fly emerged with wrinkled wings.

soil. The flies emerged with wrinkled wings (Fig. 3d) revealed significant differences in sandy soil treatments (F=15.33, df=5, 89, P<0.0001) and (F=31.85, df=5, 89, P<0.0001) in clay soil as well. KZ mineral oil treatments appeared linked to the flies emerged with wrinkled wings without significant difference between sandy and clay soils (F=3.25, P>0.05) but reflected significance with control treatments F=536.47, df=3, 59, P<0.0001). Malformations of the emerged flies due to tested compounds are presented in Fig. 3.

Effect of spray flow rates of the emulsion on pupal mortality - second experiment

The emulsion tested spray flow rates (8, 30 and 60 seconds) reduced B. zonata population (dead pupae (Fig. 3b) and malformed
individuals (Fig. 3c, d) in treated sandy soil insignificantly (F=0.408, df=2, 44, P=0.6675) and in clay soil as well (F=2.02, df=2, 44, P=0.1233) (Table 3). In the sandy soil treatments, the dead pupae percentages showed non-significant differences (F=3.40, P=0.0685) and the same was in the clay soils (F=2.72, P=0.1059) at the tested spray flow rates. The dead pupae percentages in treated sandy and clay soils showed non-significant difference (F=2.15, P=0.1849). The examination of the observed malformed individuals resulted in the tested spray flow rates in the sandy soil treatments, reflected significant differences at the flies emerged with head only (F=11.76, P=0.0015) and the emerged flies with wrinkled wings (F=16.73, P=0.0003). The percentages of B. zonata flies emerged with wrinkled wings at the emulsion flow rates 8 seconds showed significant difference with 30 seconds (F=21.21, P<0.001) but were insignificant at 60 seconds spray flow rate. In clay soil treatments, the head only emerged flies showed significant difference (F=6.48, P=0.0123) while the wrinkled wings flies differed insignificantly (F=2.43, P=0.1304).

Perspective of the emulsion in soils - third experiment

Effect of the emulsion sprayed on B. zonata full-grown larvae in sandy and clay soils varied significantly along the seven days of test period (Table 4). There was a significant difference between B. zonata total population reduction percentages (dead larvae or pupae and malformed emerged flies) in sandy and clay soils after two hours of the emulsion spray (F=12.39, P<0.0001) and a high significance when compared to control treatments (F=1765.65, df=3, 59, P<0.0001). The highest population reduction in sandy soil treatments was achieved after two hours of the emulsion spray and the lowest was after five days. The ability of the emulsion to reduce B. zonata population in sandy and clay soils varied significantly after a day of soils spray (F=11.65, P<0.001) while after three days it was not significant (F=0.954, P>0.05). The emulsion reduced population in sandy and clay soils significantly after five days of spray (F=6.88, P<0.001) and after seven days (F=38.85, P<0.001). The larval-pupal mortality percentages showed significant difference in the treated sandy soil along the test period (F=70.28, P<0.0001). The two hours, day and three days treatments affected the larvae insignificantly (F=4.00, P=0.0467). The emerged flies malformations showed significance (F=5.11, P=0.0053) as the highest recorded malformations was 31% after five days treatment and the least was 22% after seven days. A significant decrease in the emulsion effect on larval mortality the appeared after five (F=87.30, P<0.0001) and seven days treatments (F=28.35, P<0.0001). Larval-pupal population reduction percentages reflected an inversely proportional relationship with the emulsion along the test period (r=-0.874). Results showed that reduction percentages of B. zonata treated populations were effective until 3.5 days after treatment in sandy soil. In the clay soil, the highest total reduction of B. zonata population was reached after a day post soil spray with the emulsion and the least was after seven days post treatment (Table 4). The larval mortality percentages differed significantly along the test period (F=131.61, P<0.0001). The highest recorded percentage of B. zonata emerged flies’ malformations (37%) accompanied the two hours treatment and the least (8%) was after seven days treatment reflecting a significance difference (F=48.13, P<0.0001). Effect of the emulsion on B. zonata larvae decreased significantly after five and seven days of treatments and was inversely proportional with time (r=-0.966). The emulsion treatment in the clay soil was effective against B. zonata larval population for 4.5 days soil after spraying.

Histological studies of B. zonata flies treated as pupae with the emulsion

The seemed normal B. zonata flies emerged
from treated pupae with the emulsion were different from those emerged from the control treatments. The flies were neither able to feed nor to move naturally. Most of these flies died before sexual maturation.

Eyes

The untreated flies showed normal arrangement of the ommatidia (Fig. 4a) while the treated flies reflected degeneration and necrosis of ommatidia units that contain the photoreceptors cone cells and pigment cells (Fig. 4b) showing a distortions of the treated flies’ eyes are clearly presented.

Mouthparts (labellum)

The untreated flies showed normal view of pseudotracheal structure (yellow arrows) with clear chitinacious structure (green arrow) and food (pink arrow) (Fig. 4c) while the treated flies reflected absence of pseudotracheal tubes and necrosis of the labellum cells (Fig. 4d).

Abdominal muscles and gut

The untreated flies showed normal abdominal muscles striation as each fiber consists of a number of parallel fibrillae occupying the whole of the cross section of the fiber (Fig. 4e). The treatment with the emulsion resulted in abdominal muscles lost their normal turgid appearance. Fissures in the muscular band appeared and severe damage to the sarcolemma membrane. All muscle fibers were degenerated and fragmented into separate small parts and large vacuoles were seen occupying the most of the muscle fibers (Fig. 4f). The emulsion affected the gut of the treated flies. The treated flies mid gut showing atrophied enterocyte (yellow arrow), degeneration necrosis of enterocytes and adjacent cells with sloughing in many parts (green arrow), degeneration and necrosis of the muscular layer of gastric mucosa (x400) (Fig. 4g). Epithelium of midgut treated with the emulsion was detached from the basal lamina. Nucleus shape was altered with destruction of chromatin material. Deterioration and necrosis in Malpighian tubules tissue structure is showing the loss of its function that is responsible for absorption of waste products in the flies’ hemolymph (Fig. 4h).
Discussion

Management of fruit flies is challenging because full-grown larvae leave infested fruits and drop to the soil to pupate; consequently, both larvae and pupae are protected from surface-applied insecticides (Heve et al. 2017). Green pesticides are mostly derived from plant origin and are used as organic pesticides. Licorice, *Glycyrrhiza glabra* (Linnaeus, 1753), roots contain phenolics, tannins, flavonoids but the major ingredient are saponins (glycyrrhizin). Saponins have insecticidal activities against broad range of pest insects (De Geyter et al. 2007). Licorice roots aqueous extract (3000 ppm saponins) reduced *B. zonata* pupal population in sandy soil (74.44%) but acted more actively in clay soil (87.55%). This difference in population reduction between the sandy and clay soils may be due to the distribution of LRAE in them, as the soil particles of sand are larger and loose while the clay soil particles are tiny and somewhat compact. The same reasons may explain the difference of population reduction on using water treatment in sandy soil (78.61%) and in clay soil (100%). Zapata et al. (2006) tested the aqueous extract of ground leaves of *Cestrum parqui* (L'Hér., 1788) (Solanales: Solanaceae) that contains saponins on *Ceratitis capitata* (Wiedemann, 1824) larvae and reported a significant decrease of flies’ emergence. Pelah et al. (2002) found that saponins extracted from soapbark tree, *Quillaria saponaria* (Molina, 1782) (Fabales: Quillajaceae) have larvicidal activity against the mosquitos’ species, *Aedes aegypti* (Linnaeus, 1762) (Diptera: Culicidae), and *Culex pipiens* (Linnaeus, 1758) (Diptera: Culicidae); 100% mortality was obtained by using and amount of 1 000 mg/L (1 000 000 ppm/L) during five days. In the present study, LRAE in a concentration 3 000 ppm/L reduced *B. zonata* pupal population by 87.55% in clay soil i.e. saponins act differently according to plant origin sources. Petroleum and KZ 96% (EC) light mineral oils reduced *B. zonata* pupal population (100%) in sandy and clay soils treatments. Petroleum oils were used in cherry fruit fly management to kill pupae in soil but these compounds may show some unacceptable effects as negative effects on trees’ roots that lead to trees death because of large amount usage of oils in soil (Weismann 1934). Mineral oils create a thin layer on the insects’ treated stage surface where it stops the gas exchanges followed by death (Micks & Berlin 1970, Helmy et al. 2012). The emulsion contained LRAE, petroleum oil, KZ light mineral oil 96% (EC) and all were added to water reduced *B. zonata* population in sandy soil (96.94%) and in clay soil (100%) more than LRAE alone. The aim of mixing the emulsion ingredients is to obtain higher efficacy as the petroleum and mineral oils increase the LRAE distribution horizontally and vertically in soil then quickly dissipate by evaporation leaving little residues harmless to plant roots. From the same perspective, Thaochan & Ngampongsai (2018) studied the effect of mixing petroleum oil and seed kernels, *Azadirachta excelsa* (Jacobs, 1961) (Sapindales: Meliaceae) extract against melon fruit fly, *Zeugodacus cucurbitae* (Coquillette, 1899) (Diptera: Tephritidae) and found that it increased larval and pupal mortality more than acted singly in laboratory and greenhouse experiments. In the same way, aqueous saponins extract can more easily adhere than just water to the target pest. Adding of material as petroleum or mineral oils to the aqueous extract can increase the kinematic viscosity coefficient that is beneficial to the effective adhesion of the mixed material to the target pest and crops during spraying and is positively correlated to the utilization rate of pesticides (Gil & Sinfort 2005). Our results agree with that as the emulsion rate used as one side target for eight seconds showed non-significant difference with the multisided spray for 30 and 60 seconds. Malformations of the emerged flies accompanied all the applied treatments. These malformations indicate that physiological disturbance occurred during
B. zonata development. Focusing on saponins effect, it presents an excellent model of insecticidal effect and multitude physiological effects (Chaieb 2010). Khater (2020) studied the effect of azadirachtin (neem leaves extract) on B. zonata larvae and pupae and reported flies' morphological abnormalities represented in incomplete flies emergence from pupae and crumbled or wrinkled wings. Azadirachtin contains saponins molecules that inhibit enzymes activity of treated red flour beetle, Tribolium castaneum (Herbst, 1797) (Coleoptera: Tenebrionidae) (Sami & Shakoori 2014). Khater (2020) studied the insecticidal activity of Azadirachtin 15% (EC) on the lesser pumpkin fly, Dacus ciliatus (Loew, 1862) (Diptera: Tephritidae) larvae and pupae and recorded malformed emerged flies with wrinkled wings and deformed female's ovipositor. These findings may explain the similar malformation recorded in the present study. The morphological abnormalities of azadirachtin insecticide may be a result of inhibition of the release of prothoracicotropic hormones and allatotropins (Mordue & Blackwell 1993, Williams & Mansingh 1996). Sharma (1992) investigated the growth-inhibiting properties of azadirachtin by topical application on the development of rice meal moth, Corcyra cephalonia (Stainton, 1866) (Lepidoptera: Pyralidae). Larval development was inhibited and at higher doses, lead to disturbance of both larval-pupal and pupal-adult moulting was interpreted as interference with the morphogenetic hormone. Khatter (2011) demonstrated the latent effects of azadirachtin on the confused flour beetle, Tribolium confusum (Du Val, 1863) (Coleoptera: Tenebrionidae) progenies as manifested by the reduced growth and development of immature and mature stages. Malformation accompanied the use of petroleum and mineral oils. Greenberg and Amos (1996) found low growth rate of yellow mealworm, Tenebrio molitor (Linnaeus, 1758) (Coleoptera: Tenebrionidae) larvae under hypoxia (10%) that resulted in 20% survivors. Hallman (2004) used pupal hypoxia on oriental fruit moth, Grapholita molesta (Busck, 1916) (Lepidoptera: Tortricidae) before irradiation treatment and found partially abnormalities in emerged moths. Petroleum and mineral oils form a thin layer on treated B. zonata preventing oxygen supply and gas exchange causing long period of hypoxia. In the present work, the histological studies of the flies emerged from emulsion treated pupae tissues are different from the untreated ones (Fig. 4).

Eyes, labellum, midgut and abdominal muscles were deteriorated and damaged (Fig. 4b, d, f, g, h). Khater (2020) treated D. ciliatus larvae and pupae with Azadirachtin 15% (EC) and found muscular fragmentations of the muscular layers, appearance of vacuoles between cuticle and epidermis. The flies emerged after pupal emulsion treatment showed sever damage in midgut tissue. Khater (2020) reported the effect of Azadirachtin 15% (EC) on D. ciliatus midgut tissues and showed agreement with our results. She found severe destruction in midgut muscular cells and detachment of the basement membrane and muscle layer and the brush border of the Malpighian tubules were coagulated and detached from epithelial cells. The midgut is responsible for the digestion and absorption of flies’ food. In the present study, the damage of midgut tissues due to the emulsion treatment may explain why the emerged flies neither feed nor move normally. Chaieb et al. (2009) showed structural modifications at the fat body of Spodoptera littoralis (Bois, 1833) (Lepidoptera: Noctuidae) as well as on the foregut and the gastric caeca of the desert locust, Schistocerca gregaria (Forskål, 1775) (Orthoptera: Acrididae) that were due to the cytotoxicity effect of Cestrum parqui saponins. Similar effects are obtained by treatment of Culex pipiens mosquito larvae by C. parqui saponins. The microscopic observations of treated insect tissue cuts show smaller sized cells of the fat body appear darker due to the loss of their contents probably caused by the modification of their membrane permeability,
and even with the disorganization of their molecular architecture (Chaeib et al. 2007). Their results indicate that tea saponins can cause physiological and morphological damage to midgut epithelial cells. Cui et al. (2019) tested total saponins of ethanolic extract of tea oil camellia, Camellia oleifera (Abel, 1818) (Ericales: Theaceae) seeds on the tea geometrid moth, Ectropis obliqua (Prout, 1915) (Lepidoptera: Geometridae) larvae by contact toxicity and found that the cells exhibited vacuolization and vesicle release for energy detoxification. Excessive toxicity of tea saponins leads to smaller microvilli in the midgut and to cell death. The mode of action of saponins seems in relation to the property of its molecules to be interacted either with structural cholesterol (membrane) or with metabolic cholesterol (food). Saponins affect the water balance of the treated pest by destroying the waxy layer on the epidermis surface allowing penetration into the body and finally resulting in death due to water loss (Balabanidu et al. 2018).

Conclusion

Licorice, Glycyrrhiza glabra (Linnaeus, 1753) roots aqueous extract (LRAE) has an insecticidal potential against Bactrocera zonata (Saunders, 1841) larvae and pupae in sandy and clay soils. Spectrophotometer analysis confirmed the presence of saponins in the crude LRAE in a concentration 10000 ppm. The emulsion used as 1 L: 29 L water in Matabi® sprayer containing saponins 3000 ppm is enough to reduce B. zonata larval and pupal population in soil easily. In order to increase the efficacy of LRAE, petroleum oil and KZ light mineral oil 96% (EC), were added (1/4 L LRAE+1/4 L petroleum oil+1/2 L KZ oil) to form the emulsion. It could be recommended that the emulsion could be sprayed as a target spray technique sufficient to obtain suitable spray coverage on different sandy and clay soils with spray speed of 1.2 km/hour.

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Table 2. Effect of LRAE and additives on *B. zonata* pupae (4 days old) in sandy and clay soils under laboratory conditions.

| Tested compounds | Sandy soil | Clay soil |
|------------------|------------|-----------|
|                  | Mean % pupal mortality ± SE | Mean % of pupal-flies malformations ± SE | Total % mortality ± SE | Mean % pupal mortality ± SE | Mean % of pupal-flies malformations ± SE | Total % mortality ± SE |
|                  | Head | Wrinkled wings | Seem normal flies | Head | Wrinkled wings | Seem normal flies |
| LRAE             | 58.19±1.24 | 9.10±0.78 | 23.90±1.81 | 8.41±1.41 | 74.44±0.63 | 61.22±1.03 | 7.65±0.68 | 14.35±0.78 | 12.05±0.81 | 87.55±0.80 |
| P                | 83.64±0.74 | 3.05±0.40 | 12.91±0.20 | 0.00±0.00 | 100.00±0.00 | 86.75±0.98 | 0.00±0.00 | 12.99±1.01 | 0.00±0.00 | 100.00±0.00 |
| KZ               | 38.72±1.48 | 1.25±0.20 | 58.75±0.43 | 0.00±0.00 | 100.00±0.00 | 34.74±0.74 | 1.12±0.20 | 61.88±0.81 | 0.00±0.00 | 100.00±0.00 |
| Water            | 38.13±0.95 | 3.86±0.80 | 35.14±1.14 | 20.99±0.93 | 78.61±0.58 | 69.48±0.71 | 8.45±0.75 | 24.55±1.08 | 0.00±0.00 | 100.00±0.00 |
| Emulsion         | 70.35±1.16 | 4.98±0.45 | 21.02±1.57 | 2.06±0.25 | 96.94±0.24 | 77.61±0.25 | 5.98±0.50 | 16.02±0.73 | 0.00±0.00 | 100.00±0.00 |
| C                | 2.33±0.24  | 0.00±0.00 | 0.00±0.00 | 97.67±0.50 | 2.33±0.01 | 2.67±0.003 | 0.00±0.00 | 0.00±0.00 | 97.33±0.08 | 2.67±0.003 |

The treatment labels are LRAE=licorice roots aqueous extract (3000ppm saponins), P=petroleum oil, KZ=light mineral 96% (EC) and C=control, LRAE, P and KZ were used in 1L: 29 water in Matabi® sprayer of capacity 30 L. The emulsion contained 1/4L LRAE+ 1/4L P+1/2 KZ: 29 L water in Matabi® sprayer 30 L capacity.

Table 3. Effect of the emulsion spray flow rates on *B. zonata* population reduction in sandy and clay soils under laboratory conditions.

| Time of spray | Sandy soil | Clay soil |
|---------------|------------|-----------|
|               | Mean % pupal mortality ± SE | Mean % emerged flies malformations ± SE | Seem normal flies ± SE | Total population reduction ± SE | Mean % pupal mortality ± SE | Mean % emerged flies malformations ± SE | Seem normal flies ± SE | Total population reduction ± SE |χ² | P |
|               | Head | Wrinkled wings | | | Head | Wrinkled wings | | | |
| 8 sec.        | 12.25±0.92 | 1.02±0.11 | 33.73±0.51 | 34.63±1.10 | 80.61±1.70 | 30.61±0.62 | 2.12±0.13 | 30.27±1.04 | 16.69±1.08 | 79.59±1.17 | 85.97 | 0.000 |
| 30 sec.       | 14.27±0.20 | 14.24±0.19 | 08.16±0.38 | 44.47±0.27 | 81.63±0.33 | 30.91±0.20 | 20.54±0.11 | 5.10±0.38 | 23.07±0.33 | 79.62±0.29 | 39.12 | 0.000 |
| 60 sec.       | 26.53±0.77 | 2.98±0.21 | 44.98±0.76 | 7.14±0.51 | 82.73±0.56 | 14.36±0.78 | 7.11±0.36 | 35.71±1.71 | 23.74±2.16 | 80.92±2.90 | 62.98 | 0.000 |
| C             | 1.77±011   | 0.00±0.00 | 0.00±0.00 | 98.23±0.08 | 1.77±0.11 | 0.00±0.00 | 0.00±0.00 | 98.67±0.01 | 1.33±0.15 | 87.55±0.80 | 85.97 | 0.000 |

The emulsion contained 1/4L LRAE+ 1/4L P+1/2 KZ: 29 L water in Matabi® sprayer 30 L capacity. Spray flow rate 8 seconds: Target spray 0.073L/ swathe width at 1.2 km/hour. Spray flow rate 30 seconds: Multiple swaths width 0.270 L for 30 seconds. Spray flow rate 60 seconds: Multiple spray 0.540L continuously for 60 seconds. C=control treatment.
| Time after soil treatment | Sandy soil | Clay soil |
|---------------------------|------------|-----------|
|                           | Mean % larval-pupal mortality ± SE | Mean % emerged flies malformations ± SE | Seem normal flies ± SE | Total population reduction ± SE | Mean % larval-pupal mortality ± SE | Mean % emerged flies malformations ± SE | Seem normal flies ± SE | Total population reduction ± SE | $\chi^2$ | $P$ |
| 2 hrs                     | 43.41±0.50 | 2.51±0.25 | 22.61±0.51 | 8.54±0.32 | 90.95±0.50 | 39.76±0.31 | 5.86±0.39 | 30.91±0.74 | 22.88±0.68 | 76.91±0.32 | 21.26 | 0.000 |
| 1 day                     | 37.40±0.24 | 2.32±0.40 | 35.71±0.21 | 34.84±0.31 | 64.82±0.24 | 49.80±0.66 | 4.96±0.58 | 24.89±0.35 | 18.88±0.92 | 80.93±0.36 | 38.76 | 0.000 |
| 3 days                    | 34.37±0.15 | 0.00±0.00 | 17.63±0.21 | 33.83±0.22 | 63.82±0.25 | 37.75±0.25 | 9.87±0.33 | 19.88±0.30 | 32.90±0.78 | 66.88±0.79 | 30.12 | 0.000 |
| 5 days                    | 10.19±0.18 | 0.00±0.00 | 30.70±0.37 | 57.47±0.53 | 41.71±0.18 | 18.67±0.77 | 8.86±0.20 | 6.90±0.44 | 64.95±0.45 | 34.76±0.37 | 11.70 | 0.009 |
| 7 days                    | 24.29±0.31 | 0.00±0.00 | 21.69±0.25 | 52.41±0.55 | 46.73±0.31 | 6.63±0.13 | 0.00±0.00 | 8.05±0.09 | 85.02±0.47 | 14.68±0.24 | 8.65 | 0.034 |
| C                         | 2.76±0.11  | 0.00±0.00 | 0.00±0.00  | 97.24±0.08 | 2.76±0.11  | 1.34±0.15 | 0.00±0.00 | 0.00±0.00 | 98.67±0.01 | 1.34±0.15  |          |       |

Correlation factor “$r$”

For Sandy soil: $r = -0.874 ± 0.281$

Lower confidence interval = -0.992
Upper confidence interval = 0.037
$P = 0.0529$

For Clay soil: $r = -0.996±0.149$

Lower confidence interval = -0.998
Upper confidence interval = -0.567
$P = 0.0075$