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Evaluating the insecticidal efficiency of some legumes extracts against *Bactrocera zonata* (Diptera: Tephritidae)

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Abstract:

Insecticidal and biological activity of three legumes; *Lupinus luteus* L., *Glycine max* L. and *Cicer arietinum*; seeds ethanolic extracts against adult and egg stages of peach fruit fly *Bactrocera zonata* (Saunders) (Diptera: Tephritidae) was investigated under laboratory conditions. The phytochemical screening of ethanolic extract of the tested three legumes showed the presence of alkaloids, steroids, flavonoids, terpenoids, tannins and saponins in *G. max* extract and absence of saponins in *C. arietinum* . Also, terpenoids, flavonoids and tannins are absent in *L. luteus* ethanolic extract. Ethanolic extracts of the tested legume seeds achieved variable toxicity against adult and egg stages of *B. zonata*. Ethanolic extracts of *G. max* and *C. arietinum* seeds (LC_{50} = 366.2 and 437.3 mg L^{-1}, respectively) were more toxic against insect adults than *L. luteus* seeds ethanolic extract (LC_{50} = 627.5 mg L^{-1}) after 8 days of exposure. Ethanolic extract of *C. arietinum* achieved the higher ovicidal activity (Egg hatchability reached to 0% at 1000 mg / L) compared to the other extracts. *G. max* and *C. arietinum* seeds ethanolic extracts were the most potent on the adult fecundity with 2.0 and 0.0 egg laid / female at 1000 mg / L, respectively compared to 49.0 egg / female in control. The tested ethanolic extracts of the three tested plant seeds reduced the adult longevity of *B. zonata* and the effect was concentration dependent. Results of the present study suggest that, plant extracts can be an effective tool in integrated pest management programs for the control of fruit flies.

Introduction

The peach fruit fly *Bactrocera zonata* (Saunders) (Diptera: Tephritidae) is a serious pest of fruits in Egypt and many world regions. It attacks a wide range of fruit and vegetable hosts (White and Elson-Harris, 1994). Saafan *et al.* (1993) recorded *B. zonata* in many orchards in several Egyptian Governorates. Peach fruit fly originates in South and South- East Asia (Kapoor, 1993 and Duyck *et al.,* 2004). The presence of this insect reduces the quality of fruits and subsequently negatively affects their exportation (Shehata *et al*., 2008). Damage is caused mainly by the larvae, which feed during the growth and development of the fruit (Stonehouse *et al*., 2005). Therefore, the control of

larvae is difficult using a single control measure (Dhillon *et al*., 2005).

Control methods of this insect are ranged from foliage and soil spraying by the specific insecticide, bait-application, male annihilation techniques, releases of sterilized flies and parasitoids, and cultural controls (Khan *et al.,* 2017). The unwise use of synthetic insecticides resulted in many environmental problems, health problems, development of insecticide resistance and natural enemies toxicity (Victor, 2009). Therefore, there is a need to search about more effective and safe alternative methods to these synthetic insecticides. Between these alternatives are the plant natural products.

Plants synthesize many aromatic secondary metabolites, like phenols, phenolic acids, quinones, flavones, flavonoids, flavonols, tannins and coumarins which can negatively affect insects (Cowan, 1999). These compounds can be used as repellents, feeding deterrents, toxins, defense chemicals and growth regulators. Many are also effective against biting Diptera, especially those volatile components (Gurjar *et al*., 2012). It is documented that, the leaf extracts of eucalyptus *(Eucalyptus globulus)* shows efficacy against *Bactrocera cucurbitae* (Ali *et al*., 2011). In addition, Mahmoud and Shoeib (2008) showed that low concentrations of neem can be applied effectively as sterilant and oviposition deterrent for the peach fruit fly populations. Neem blocks the ovarian development and can be used as safe alternative of insecticides for the control of *a* species (Mahfuza *et al.*, 2007). In the present study, the toxic effects of ethanolic extract of three legume plant seeds; *Lupinus luteus L*. (*L. luteus*), *Glycine max L* (*G. max*) and *Cicer arietinum L* (*C. arietinum*), were tested against *Bactrocera zonata* adult. Effects on the fecundity and fertility of treated adults were also evaluated.

Materials and methods

1.Tested insect: *B. zonata* pupae were obtained from the Department of Pests Horticultural Crops, Plant Protection Institute Research, Agricultural Research Center. Emerged adults were introduced to cages of 30 cm- 30 cm - 30 cm size. Cages were enclosed with mesh screens and have cloth sleeves at front and back sides for the introduction of the food (hydrolyzed yeast and sugar of ratio 1:3) (Khan *et al*., 2016) and oviposition trays (El-Minshawy *et al.,* 1999). The insect larvae were reared under the laboratory conditions (25 °C ± 2; 65% ± 5 RH.) on a semi-artificial diet (Wheat bran 500 gm, molasses 250 gm, dried yeast 150 gm, citric acid 4 gm and sodium benzoates 6 gm in 1liter water) according to Awad (1993).

2.Tested plants:

Seeds of three legumes; *L. luteus*, *G. max* and *C. arietinum*, ethanolic extracts were used to study its potential biocidal activity against the tested insect *B.zonata*. Dried legume seeds were purchased from local markets, Alexandria Governorate. The plants were identified by Department of Horticultural Crops,Agricultural Research Center, Alexandria.

3.Plant extraction:

The legumes seeds were pulverized into fine powder using a grinding mill. The extraction of the investigated plants was carried out according to the method of (Mbatchou *et al.,* 2011). Powder of each of *L. luteus* , *G.. max* and *C* . *arietinum* seeds (200g) were soaked in (700 ml) of ethanol 98% for two weeks with intermittent shaking. The extracts were separately evaporated to dryness at room temperature to obtain

the crude extracts (ethanol extracts). This procedure was repeated 10 times. The resulting crude extracts were stored in glass vials pack closed at (2-4°C) until used for phytochemical and bioassay assessment experiments.

4. Qualitative phytochemical screenings:

The extracts obtained from ethanol extract, were subjected to preliminary phytochemical analysis tests to identify the main chemical groups such as alkaloids, steroids, flavonoids, saponins and tannins according to **Mbatchou *et al*. (2011).**

4.1.Alkaloids test (Wagner's **test):**

One ml of each extract was treated with drops of Wagner's reagent (Dissolve 2 g of iodine and 6 g of potassium iodide in 100 ml distilled water) and observed for the formation of reddish brown precipitate.

4.2. Flavonoids test (Willistatter test):

To an ethanol solution of each extract, a piece of magnesium ribbon was added, followed by dropwise addition of concentrated HCl. Colors ranging from orange to red indicated flavonoids.

4.3. Terpenoids and steroids test (Liebermann Buchart test):

A small quantity of each extract was dissolved in trichloromethane, and a minimum volume of concentrated sulphuric acid added to its content. A blue or green color or a mixture of these two shades was taken as positive test for terpenoids compounds and the formation of dark pink or red color taken as positive test for Steroids compounds.

4.4. Tannins test:

Each plant extract (0.2 ml) was re-extracted with ethanol. The solution obtained was later treated with 5% ferric chloride. A blue-black or blue-green appearance was taken as positive test for tannins.

4.5. Saponins test:

A small portion of each extract was added to 2 ml of distilled water and boiled for 3:5 minutes. The resultant mixture was filtered, allowed to cool with the filtrate shaken vigorously. Honey comb froth higher than the aqueous layer was taken as strongly positive for saponins.

5.Insecticidal activity of legumes extract:

The bioassay experiments were carried out using the extract of the investigated plants to test its potential biocidal activity against *B. zonata*. Series of concentrations of each botanical extract from the three legumes were prepared in ethanol and tested against *B. zonata* adults. Tween-twenty was used as an emulsifier. Twenty adults of *B. zonata* (Males and Females) 10 days old gravid flies (Rehman *et al*., 2009a) put in plastic container containing food (hydrolyzed yeast and sugar of ratio 1:3) and water. (Sultana *et al*., 2013). The tested extract was put as a layer on the banana peel in the plastic container. Emulsifier and ethanol solution without extract was added to a piece of banana peel and kept as untreated control. Each treatment in addition to control was replicated three time. The plastic container covered with muslin cloth and tight with rubber bands and kept under laboratory conditions. The adult mortality and number of laid eggs /female was recorded after 2, 4, 6 and 8 days of treatment. The eggs removed and counted from banana peel by pin and placed in a petri dish having wet black cloth to calculate egg hatchability%. Mortality percentages were calculated and subjected to probit analysis according to Finney (1971). The concentration which cause 50% mortality (LC_{50}) and the time required for 50% mortality (LT_{50}), confidence limits and slope ± SE were calculated.

6. Statistical analysis:

Data were subjected to one-way ANOVA test (Duncan's Multiple Comparison Range Tests at 0.05% level). The means were separated through Tukey's HSD (Honest Significant Difference) test at a significance level of 0.05 probability. Percentage repellency (PR) data was calculated by applying this Schneider-Orelli's formula (Püntener, 1981).

$$\text{Corrected \%} = \frac{\text{(Mortality \% in treated plot - Mortality \% in control plot)}}{\text{100 - Mortality \% in control plot}} \times 100$$

Results and discussion

1. Phytochemical screening:

The phytochemical screening of ethanolic extracts showed the presence of different types of active constituents (Table, 1). Alkaloids, steroids, flavonoids, terpenoids, tannins and saponins were present in the *G. max L* ethanolic extract. Alkaloids, steroids and saponins were identified in *L. luteus* extracts. *C. arietinum* ethanolic extract contains alkaloids, steroids, flavonoids, terpenoids and tannins. The variability in active compounds in each extract is expected to affect their biological activities. Results of the present study is in accordance with many previous studies. Phytochemical analysis indicated the presence of flavonoids, alkaloids, steroids, and tannins in *L. luteus, C. arietinum* and *G. max* ethanolic extracts (Maknickiene *et al*., 2013; Mamta *et al*., 2013 and Kumaran and Citarasu 2015). The variability in active compounds and their concentrations in the extracts are expected to affect in the biological activities against pest (Elena *et al*., 2016).

Table (1): Phytochemical screening of various extracts of tested plants.

Experimental Plants	Phytochemical Constituents					
	Alkaloids	**Steroids**	**Terpenoids**	**Flavonoids**	**Tannins**	**Saponins**
Lupinus luteus	+	+	-	-	-	+
Glycine max	+	+	+	+	+	+
Cicer arietinum	+	+	+	+	+	-

2. Toxicity of tested plant extracts against *Bactrocera zonata* adults:

Toxicity of ethanolic extracts of three legume plants *L. luteus*, *G. max* and *C. arietinum* seeds against the *B. zonata* adults after 6 and 8 days of treatment are presented in Table (2). Ethanolic extracts of *G. max* and *C. arietinum* seeds (LC_{50} = 366.2 and 433.9 mg L^{-1}, respectively) were more toxic than *L. luteus* seeds ethanolic extract (LC_{50} = 627.5 mg L^{-1}) after 8 days of exposure. According to confidence limits, the toxicity of ethanolic extracts of both *G. max* and *C. arietinum* seeds against *B. zonata* adults after 8 days of exposure was comparable. Toxicity of the three tested plant extracts against *B. zonata* adults is time dependent (Table, 2). According to the LT_{50} values of the tested extracts, *G. max* and *C. arietinum* act more fast against *B. zonata* adults with LT_{50} values 4.5 and 5.2 days compared to *L. luteus* extract with LT_{50} value 15.5 days at 500 mg / L (Table, 3). Botanical insecticides are good alternatives to chemical insecticides and approved to be effective in insect control (Rehman *et al.*, 2009b).

The over use of chemical pesticides causes many environmental and health problems. Therefore, It is recorded that, *Lupinus* species are a source of alkaloids, which provide the plants with protection from adverse weather conditions, microorganisms, fungi, insects, and herbivores (Rybiński *et al.*, 2018). The ethanolic extract of marine algae *Callyspongia crassa* and *Grayella cyathophora* achieved considerable toxicity against *B. zonata*

under laboratory conditions (Elnagar *et al*., 2018). Acetone and water extracts of *Acacia auriculiformis* A. Cunn. bark significantly prolonged the larval period and total developmental period, decreased percentage pupation, percentage emergence, oviposition and egg hatching of *Bactrocera cucurbitae* (Coquillett) in a dose dependent manner (Kaur *et al*., 2010). Siddiqi *et al*. (2011) reported that the acetone extract of *Curcuma longa* achieved high mortality percentages to *B. zonata* and caused high inhibition of pupa formation.

Table (2): Insecticidal effect of different concentrations of extracts on adult of *Bactrocera zonata.*

Extracts		Exposure period			
		LC_{50}	Confidence limits	Slope±SD	Chi^2 Tabulated
Lupinus luteus	6days	712.5	670.8-755.3	7.3±0.7	3.8
	8days	627.5	592.9– 659.3	7.5±0.6	3.8
Cicer arietinum	6days	483.8	410.4-536.4	4.4±0.7	3.8
	8days	433.9	373.1-473.6	7.6±1.3	3.8
Glycine max	6days	442.6	328.3-514.9	3.4±0.7	3.8
	8days	366.2	254.4-436.2	4.3±0.9	3.8

Table (3): LT_{50} values for adult of *Bactrocera zonata* exposed to *plants* extract at concentration of 500 mg / L.

Extracts	LT_{50}	Confidence limits	Slope±SD	Chi^2 Tabulated	C
Glycine max	4.5	4 - 5	2.7±0.2	9.5	0.8
Cicer arietinum	5.2	3.8-6.6	3.2±0.3	9.5	0.8
Lupinus luteus	15.5	11.4-20.4	3.5±0.3	9.5	0.8

2. **Ovicidal activity and effects on some biological aspects of tested plant extracts against *Bactrocera zonata*:**

Effects of the three legume plants *L. luteus* , *G. max* and *C. arietinum* seeds ethanolic extracts on the *B. zonata* egg hatchability are presented in Table (4). It is clear that, *C. arietinum* seeds ethanolic extract had the higher activity where the egg hatchability reached to 0% at concentration of 1000 mg / L compared to 88.5% in control. The egg hatchability reached to 69.8 and 58.4% when the *B. zonata* eggs where treated by *L. luteus* L. or *G. max* L. at 1000 mg / L compared to 88.5%in control. The ovicidal activity of the tested three plant ethanolic extracts is concentration dependent. Similar type of response in insects caused by plant extracts has also been reported from other laboratories. Sharaby (1988) reported pronounced reduction in egg production and egg viability when *Phthorimaea operculella* were exposed to the vapours arising from paper treated with 220 µl of *Citrus sinensis*.

The number of laid eggs / female was highly affected when adults of *B. zonata* where exposed to the ethanolic extracts of the three tested plant seeds (Table, 4). *Glycine max* L and *C. arietinum* seeds ethanolic extracts were the most potent with 2.0 and 0.0 egg laid / female at 1000 mg / L, respectively compared to 49.0 egg / female in control. The tested ethanolic extracts of the three tested plant seeds reduced the adult longevity of *B. zonata* and the effect was concentration dependent (Table, 4). The adult longevity reached to 6.7days when adults was exposed to *C. arietinum* seeds ethanolic extract at 1000 mg / L compared to 59.3 days in control.

Table (4): Effects of the tested plants extracts on egg hatchability, number of egg/female and longevity (days) for *Bactrocera zonata*.

Treatments	Concentration (mg/L)	Egg hatchability(%) ±SD	Number of egg/female ±SD	Time of exposure(days) ±SD
Control	**0.0**	88.5±9.1[a]	49.0±8.1[a]	59.3±1.2[a]
Lupinus luteus	**500**	76.5±8.2[b]	12.3±0.4 [b]	32.0±3.5[b]
	750	71.2±5.7[b]	11.25±1.3[b]	12.7±1.2[c]
	1000	69.8±1.6[bc]	5.0±3.0[cd]	9.3±2.3[de]
Glycine max	**500**	75.0±8.3[b]	5.5±3.5[c]	11.3±1.2[cd]
	750	65.5±1.7[cd]	2.0±0[cd]	8.0±0[ef]
	1000	58.4±8.3[d]	2.0±1.0[cd]	7.3±1.2[ef]
Cicer arietinum	**500**	33.3±0[e]	1.1±0.2[cd]	11.3±1.2[cd]
	750	33.3±0[e]	1.0±0[cd]	7.3±1.2[ef]
	1000	0.0[f]	0.0[d]	6.7±2.3[f]

Means with the same letters in the same column are not significantly different according to L.S.D. test at 0.05 level of probability.

Insecticidal properties of any plant extracts depend on the active constitutes. Saponins have strong detrimental effects on insects, causing mortality, growth retardation and decreased fecundity (De Geyter *et al.,* 2007). In addition, lower food intake of insects fed on saponin-containing plant extracts was recorded (Taylor *et al.,* 2004 and Golawska *et al.,* 2006). According to Ishaaya (1986) saponins slow down the passage of food through the insect gut. Perhaps they reduce the digestibility of the food by inhibiting the secretion of digestive enzymes (Proteases and amylases) (Golawska *et al.,* 2006). Shimada (2006) reported tannins to form irreversible complexes with proline rich protein resulting in the inhibition of cell protein synthesis. Steroidal compounds are of importance and interest due to their relationship with various anabolic hormones including sex hormones (Okwu, 2001). Hence, the presence of these compounds in the tested plant extracts corroborates the insecticidal activities observed.

The systemic insecticides are not the preferred choice for fruit flies control in fruit crops. Furthermore, the insecticides having contact action remained insufficient to give successful control of fruit flies, unless targeting the fruit fly adults in abandoned areas and vegetation. This behavior of flies suggests that, such control strategies may be useful as an integrated pest management (IPM) approach. Therefore, plant extract formulations affecting the oviposition have an added advantage over synthetic insecticides and can be a tool in integrated pest management programs for the control of fruit flies (Khattak *et al*., 2006).

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