

INSECTICIDAL ACTIVITY OF SOME PLANT EXTRACTS AGAINST *PECTINOPHORA GOSSYPIELLA* (SAUNDERS) IN RELATION TO THEIR PHENOLIC CONTENTS

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(Manuscript received 8 May 2006)

Abstract

Phenolic contents of some plant extracts (pomegranate peels, acacia pods and leaves of olive, mulberry & guava) were determined as gallic acid equivalents (GAE) by reference to a standard curve ($y = 52.002x - 0.0328$, $r^2 = 0.9963$). The amount of phenolic compounds varied widely in the aforementioned plant materials and ranged between 1.86-196.63 mg GAE/g dw. Among the tested extracts, mulberry extract contained the lowest level (1.86-19.31 mg GAE/g dw) while acacia extract contained relatively the highest one (8.96-139.76 mg GAE/g dw). Moderate phenolic levels were found in pomegranate, guava and olive extracts. Phenolic compounds also varied according to the used solvent. The tested solvents can be arranged according to its efficacy in extracting phenols as follows: acetone > ethanol > ethyl acetate > butanol > methanol.

On the other hand, the insecticidal activity of these plant extracts against *Pectinophora gossypiella* (Saunders) was studied. Data indicated that the tested plant extracts have an insecticidal effect against newly hatched larvae. Tested extracts differed in their efficacy against pink bollworm larvae while, mortality rates increased with the increasing of the used concentration and the period after treatment. Regarding the LC₅₀ values after 2 days of treatment, it could be arranged the efficacy of the tested extracts in a descending order as follows: pomegranate > olive > mulberry > acacia > guava. Data, however, also revealed that the efficacy of the tested plant extracts does not necessarily correlated with exists of high amounts of phenolics.

Key words: *Pectinophora gossypiella*, plant extracts, pomegranate (*Punica granatum*), olive (*Olea europaea*), mulberry (*Morus* sp.), acacia (*Acacia* sp.) and guava (*Psidium guajava*), phenolic compounds.

INTRODUCTION

Cotton is considered a major economic crop in Egypt, it represents the first cash crop for the national income and known as white gold. The pink bollworm (PBW) *Pectinophora gossypiella* (Saunders) is one of the most important insect pests attacking the green cotton bolls in Egypt, which cause great reduction in quantity and quality of the yield. This insect is extremely difficult to control because larvae spend their life sealed and protected deep within the boll.

Insect pest management in cotton has traditionally relied upon synthetic insecticides to maintain insect populations below established economic injury levels. However, insect resistance to insecticides and increasing insecticide costs has made effective and economical insect control difficult. Consequently, novel strategies for controlling these insects need to be developed. The wide-scale commercial use of plant extracts as insecticides began in the 1850s (Isman, 1997). The toxic constituents present in the plant represent the secondary metabolites. Phenolic compounds (PCs) are one of such group of aromatic secondary plant metabolites widely spread throughout the plant kingdom. The term phenolics encompass approximately 8000 naturally occurring compounds, all of which possess one common structural feature, a phenol (an aromatic ring bearing at least one hydroxyl substituent). Current classification divides the broad category of phenolics into polyphenols and simple phenols, based solely on the presence of the number of phenol subunits. The biosynthetic pathways of phenolic compounds in plants are quite well known. PCs have many functions in plants. They act as cell wall support materials and as colorful attractants for birds and insects helping seed dispersal and pollination (Harborne, 1994). Plant phenolics also play important roles in protection against environmental stresses (e.g., herbivory, infection, UV radiation). The role of phenolics in plant defense against herbivores has been a particularly intense area of study and has been the basis of several plant defense theories. The toxicity of many phenolics from simple phenolic acids to complex polyphenols has been attributed to their ability to function as prooxidants (Summers & Felton, 1994).

This work was conducted to determine the amount of phenolic compounds in five selected plants and evaluate the toxicological effects of these botanical extracts against 1st instar larvae of pink bollworm (*Pectinophora gossypiella* Saund.).

MATERIALS AND METHODS

Rearing of *P. gossypiella*

Newly hatched larvae of *P. gossypiella* were obtained from a colony maintained in the Bollworms Department Laboratory, Plant Protection Research Institute, Ministry of Agriculture, Dokki, Giza for several generations at $27 \pm 1^\circ\text{C}$ and $75 \pm 5\%$ relative humidity (R.H.). Larvae were reared on a modified artificial diet as described previously by Abd El-Hafez *et al.* (1982).

Tested materials

Five selected plant extracts (pomegranate peels *Punica granatum*, olive leaves *Olea europaea*, mulberry leaves *Morus* sp., acacia pods *Acacia* sp. and guava leaves *Psidium guajava*) were tested against the pink bollworm newly hatched larvae.

Preparation of extracts

The phenolic compounds were extracted in the Bollworms Department Laboratory, Plant Protection Research Institute, Ministry of Agriculture, Dokki, Giza from grounded dry plant materials according to the method of Kähkönen *et al.* (1999) with some modifications. Grounded material (500 mg) was extracted with 2 X 10 mL of 80% aqueous solvent (acetone, ethanol, ethyl acetate, butanol and methanol) at room temperature on an orbital shaker set at 200 rpm. Samples were centrifuged for 20 min at 2000 rpm, and combined extracts were taken to dryness. The solid residue was redissolved in water.

Determination of phenolic compounds

The amount of phenolic compounds in the aforementioned extracts was determined according to the Folin-Ciocalteu procedure (Singleton and Rossi, 1965). Samples (200 μ L, three replicates) were introduced into test tubes, 1.0 mL of Folin-Ciocalteu's reagent and 0.8 mL of sodium carbonate (7.5%) were added. The tubes were mixed and allowed to stand for 30 min. Absorption at 765 nm was measured (6505 UV-vis spectrophotometer, JENWAY).

Toxicological studies on the pink bollworm

All bioassay trials were done on the newly hatched larvae of the pink bollworm. Crude plant extracts were homogenately mixed with 100 g of artificial diet to obtain the tested concentrations, i.e., 90, 60, 30 and 10 mL/100 g diet for olive, acacia and mulberry extracts, 30, 20, 10 and 5 mL/100 g diet for pomegranate extract and 180, 90, 60, 30 and 10 mL/100 g diet for guava extract. The treated diet (100 g) of each concentration of the tested material was folded in 10 Petri dishes (9 cm in diameter) and then ten newly hatched larvae of pink bollworm were placed on the surface of the diet using a soft brush. Another group of 10 Petri dishes was prepared containing the same diet but mixed with water (used as control) and an equal number of the maintained insects were placed on their surface. Larvae were allowed to feed on the treated diet for two hours after which any alive larvae were transferred individually to glass vials (2 X 7 cm) containing untreated diet. Vials were plugged with absorbant cotton and incubated at $27 \pm 1^\circ\text{C}$ and $75 \pm 5\%$ R.H. Larval Mortalities were recorded 2 hrs, 2 days and 7 days after treatment.

Statistical analysis

Toxicological data were statistically calculated through a Proban program, software computer program (Jedrychowski, 1991). The variability in response to the tested materials was determined based on LC_{50} and slope in addition to illustrating concentration-mortality regression lines. The toxicity index (T.I.) was calculated using the equation of Sun (1950) as follows:-

$$\text{Toxicity index (T.I.)} = \frac{\text{LC}_{50} \text{ of the material A}}{\text{LC}_{50} \text{ of the material B}} \times 100$$

Where A: is the most effective material

B: is the other tested material

Analysis of variance (ANOVA) was conducted on all data and when statistical differences existed within a data set, Duncan's multiple range test was used to separate the means.

RESULTS AND DISCUSSION

Concentration of phenolic compounds in plant extracts

Phenolic compounds contents in extracts of pomegranate peels, acacia pods and olive, mulberry & guava leaves were determined as gallic acid equivalents (GAE) by reference to a standard curve ($y = 52.002x - 0.0328$, $r^2 = 0.9963$). The amount of phenolic compounds varied widely in the aforementioned plant materials and ranged between 1.86-196.63 mg GAE/g dw (Table, 1). Among the tested plants, mulberry extract showed the lowest levels (1.86-19.31 mg GAE/g dw), which are in closed to those achieved by Kähkönen *et al.* (1999). On the contrary, acacia extract contained relatively the highest amounts of phenolics (8.96-139.76 mg GAE/g dw). On the other hand, moderate phenolic levels were found in pomegranate extract (12.06-161.72 mg GAE/g dw). These levels are much higher than those (2-10 mg/g dw) reported by Narr *et al.* (1996) in pomegranate juice. Many investigators reported different levels of the phenolic compounds in plant extracts. In the present study, the phenolic compounds in guava leaves extract (6.35-56.28 mg GAE/g dw) are close to those cited by Jimenez-Escrig *et al.* (2001) and are much lower than those reported by He & Venant (2004). In olive leaves extract, 2.05-21.27 mg GAE/g dw phenolics were determined. These levels are nearly consists with the results of Blekas *et al.* (2002) who found that the phenolic compounds contents in commercially available olives in Greece expressed as caffeic acid, was found to range between 0.18-1.78 mg/g of fresh weight.

Data in Table (1) also reveal that the phenolic compounds contents varied tremendously according to the used solvent. In acacia, the ethanol proved to be the most effective solvent in extraction process (196.63 mg GAE/g dw) followed by acetone (139.76 mg GAE/g dw), ethyl acetate (123.15 mg GAE/g dw) and butanol (95.81 mg GAE/g dw), while methonal (8.96 mg GAE/g dw) had a low efficacy in extracting phenolic compounds. The same trend of these solvents was found in pomegranate and guava except in the case of acetone, which proved to be the most

effective one. In addition, this solvent (acetone) found to be the most effective, not only in the case of pomegranate (161.72 mg GAE/g dw) and guava (56.28 mg GAE/g dw) but also in olive (21.27 mg GAE/g dw) and mulberry (19.31 mg GAE/g dw) also. Regarding of plant material and the used solvent, acetone extract of acacia and pomegranate found to have a high level of phenols comparing to other plant extracts. This may be attributed to tannins, which exist in higher concentrations in acacia (Heil *et al.*, 2002) & pomegranate (Narr *et al.*, 1996) and to the great efficacy of acetone in extracting this type of phenols.

Aqueous methanol had the lowest efficacy in extracting phenols from tested plants (12.06 for pomegranate, 6.35 for guava, 2.05 for olive and 1.86 mg GAE/g dw for mulberry). Otherwise, ethanol, ethyl acetate and butanol had a moderate efficacy. Phenolic compounds contents of pomegranate, guava, olive and mulberry extracted with these solvents were 110.23, 112.85 & 61.93 mg GAE/g dw for pomegranate, 39.78, 27.74 & 22.47 mg GAE/g dw for guava and 20.82, 7.31 & 11.07 mg GAE/g dw for olive, respectively. The present tested plants can be arranged according to its phenolic contents as follows: acacia > pomegranate > guava > olive > mulberry. While the tested solvents can be arranged according to its efficacy in extracting these phenols as follows: acetone > ethanol > ethyl acetate > butanol > methanol.

It is well known that different phenolic compounds have different responses in the Folin-Ciocalteu method. This can be attributed to the great diversity in structures between different types of phenols. The analysis of phenolics begins with extraction. The extraction procedure depends on the type of analyzed plant, the phenolic compound in question, and the analytical procedure to be used. It must be borne in mind that there is no single extraction protocol which can be considered optimally for all types of samples. In the present study, mixture of water and some solvents (recommended for extracting phenols) were used. Based on the efficiency of extraction, acetone was the most effective solvent in extracting phenols. This results are not consists with the previous studies. Bailon and Buelga (2003) mentioned that the most widely used solvent for extracting phenolic substances is methanol and methanol/water mixtures, while acetone, ethyl acetate provide lower yields. While, Brenes *et al.* (2000) mentioned that ethyl acetate is especially suitable for the extraction.

Insecticidal activity of the tested plant extracts

Data indicated that tested extracts differed in their efficacy against pink bollworm larvae. The illustrated concentration-mortality regression lines (Fig. 1) confirmed positive relationship ($r = 0.3428 \pm 0.1171$) between the applied concentration of plant

extracts, period after treatment and the mortality percentages. Mortality continued at different rates among larvae of *P. gossypiella* according to the extract used and its concentration (Fig. 2). It could be noted that the whole percent of mortality was occurred within the first 2 days following treatment with the pomegranate and olive extracts. On the other hand, higher concentrations of olive extract caused an acute effect where latent effect was observed in case of lower ones. For example, most larvae died (91.69%) within the first two hours following treatment with the highest concentration (90 mL/100g diet) of olive extract but only 4.23% of larvae died within the same period when treated with the lowest concentration (10 mL/100g diet). In addition, mortality progressively increased to reach five folds (20.43%) after two days in case of treatment with the lowest concentration opposite to few percent of increase (1.87%) in case of treatment with the highest one. As for mulberry, acacia and guava extracts, corrected mortality percentages increased after two days of treatment and continued among pink bollworm larvae until the seventh days of treatment with the three aforementioned extracts. Regarding to mulberry extract treatment, about 50% of the recorded mortality occurred within the first two hours at all concentrations tested, except the lowest one. In the case of acacia and guava extracts, most of mortality occurred within the first two days following treatment. The present data indicated that the tested plant extracts showed insecticidal effect against newly hatched larvae of *P. gossypiella*.

Based on the LC_{50} values, olive was the most potent extract after two hours of treatment (LC_{50} reached 33.59 mL/100g diet with toxicity index (TI)= 100). On the other hand, pomegranate extract (48.69 mL/100g) came second to olive in its efficiency against pink bollworm, followed by mulberry extract (114.35 mL/100g diet). On the contrary, acacia (820.7 mL/100g diet) and guava (882.85 mL/100g diet) were the least effective extracts (Table 2). Two days after treatments, pomegranate became the most effective extract, (TI= 100) as it had the lowest LC_{50} value, (17.80 mL/100g diet). While, acacia and guava extracts had the highest LC_{50} values (183.96 and 197.85 mL/100g diet, respectively). On the other hand olive (LC_{50} value= 27.31 mL/100g diet) and mulberry (62.20 mL/100g diet) extracts had a moderate effect against pink bollworm larvae. Seven days following treatment, the efficacy of the tested extracts follow the same trend as previously observed after 2 days of treatment. In addition, LC_{50} of pomegranate and olive extract did not change while, acacia and guava still had the least effect (toxicity indices = 11.24 and 13.48, respectively). As for mulberry extract, LC_{50} value reached 56.69 mL/100g diet.

The present data indicated that five tested plant extracts showed insecticidal effect against newly hatched larvae of *P. gossypiella*. Regarding to the LC₅₀ values after 2 days, it could be arranged the toxicity of the tested extracts in a descending order as follows: pomegranate > olive > mulberry > acacia > guava. The efficacy varies according to the differences in chemical structure and mode of action. Similar results were observed against different insects. In this respect, Sharaby (1989) found that the LC₅₀ values of guava leaves powder admixed with rice grains against *Sitophilus oryzae* and *S. granarius* were 2.551 and 2.28 g leaves/100 g rice grains, respectively.

Data in Table (3) reveal that efficacy of the tested plant extracts does not necessarily correlated with exists of high amounts of phenolics. Although phenolic compounds content can sometimes influence insect feeding, the presence and concentration of specific phenolic, constituents can be more important. The relationship between concentration and toxicity demonstrated in diet may not be as strong with indigenous plant phenolics. This assuming that the various complexed forms are not equally available to the insect than total concentrations of some phenolic compounds may not reflect the effective dose to the insect. Thus, higher indigenous concentrations of phenolics may not always results in increased toxicity. In the present results, pomegranate and acacia extracts had a high concentration of phenolic compounds comparing to other extracts. This can explain by existence of high levels of tannins in both plants (Narr *et al.*, 1996 and Heil *et al.*, 2002). The pomegranate extract found to be more effective than acacia extract, this can be attributed to the kind of tannins. Tannins belong to two major groups, the condensed tannins and hydrolysable tannins (Harborne, 1994). The latter can be metabolized by insects, while condensed tannins probably cannot, thus show markedly antifeedant activity (Hurrel and Finot, 1982).

In general, further studies are needed to develop the aforementioned plant extracts to be used as insecticides. The advantage of that is decreasing the chemical insecticide amounts which used against crop pests and would be helpful to decrease the environmental pollution as well as the total cost of pest control. Moreover, it would have to keep in mind that each crude extract has more than one component and if the most active one is isolated, the effect would be magnified. However, the integrated pest mangment (IPM) has been programmed to diminish as low as possible the application of pesticides, so it would be useful to use these natural extracts in IPM program.

Table 1. Concentration of phenolic compounds in some plant extracts (mg GAE/g dw)

plant	Solvent					Mean	LSD (5%)
	Acetone	Ethanol	Ethyl Acetate	Butanol	Methanol		
Acacia	139.76 ^{ab} ± 5.50 (136.42-146.11)	196.63 ^{ab} ± 9.05 (190.40-207.02)	123.15 ^c ± 5.86 (116.69-128.11)	95.81 ^{cd} ± 4.01 (92.12-100.08)	8.96 ^{cd} ± 2.36 (6.23-10.39)	112.87 ^a ± 63.88 (6.23-207.02)	10.55
Pomegranate	161.72 ^{ab} ± 0.68 (161.04-162.40)	110.23 ^{bc} ± 3.32 (107.38-113.87)	112.85 ^{bc} ± 3.85 (110.46-117.29)	61.93 ^{cd} ± 3.57 (59.53-66.03)	12.06 ^{cd} ± 1.01 (11.04-13.05)	91.76 ^b ± 52.68 (11.04-162.40)	5.15
Guava	56.28 ^{cd} ± 1.31 (55.45-57.79)	39.78 ^{cd} ± 0.95 (39.13-40.87)	27.74 ^c ± 2.33 (25.10-29.52)	22.47 ^{cd} ± 1.89 (20.33-23.92)	6.35 ^{cd} ± 1.21 (5.06-7.46)	30.52 ^c ± 17.41 (5.06-57.79)	2.94
Olive	21.27 ^{cd} ± 0.20 (21.11-21.50)	20.82 ^{cd} ± 0.34 (20.43-21.07)	7.31 ^{cd} ± 0.25 (7.13-7.60)	11.07 ^{cd} ± 0.93 (10.01-11.74)	2.05 ^{cd} ± 0.24 (1.90-2.32)	12.51 ^d ± 7.82 (1.90-21.50)	0.87
Mulberry	19.31 ^{cd} ± 0.49 (19.01-19.87)	16.43 ^{cd} ± 1.83 (14.31-17.57)	1.95 ^{cd} ± 0.14 (1.79-2.03)	7.38 ^{cd} ± 0.03 (7.35-7.42)	1.86 ^{cd} ± 0.06 (1.82-1.92)	93.86 ^b ± 75.57 (1.79-19.87)	1.55
Mean	79.67 ^{ab} ± 62.05 (19.01-0.02)	76.78 ^b ± 71.25 (14.31-207.02)	54.60 ^c ± 54.50 (1.79-128.11)	39.73 ^p ± 35.33 (7.35-100.08)	6.26 ^e ± 4.23 (1.825-13.05)	51.41 ± 56.79 (1.79-207.02)	
LSD (5%)	4.65	8.03	6.01	4.69	2.32		2.21

Means within columns and rows with same letter(s) are not significantly different from each other at 5% level of probability.
Capital letter row
Small letter column

Table 2. LC₅₀ and slope values (mL/100g diet) of some plant extracts tested against the newly hatched larvae of pink bollworm, *P. gossypiella* after 2 hr, 2 days and 7 days following treatment.

Treatment	LC ₅₀ Confidence limits (95%)	Slope ± S.E.	Toxicity index (T.I.)
2 hours after treatment			
Pomegranate	48.69 (32.61-112.51)	1.22± 0.25	68.99
Olive	33.59 (4.83-76.12)	3.28± 0.91	100
Mulberry	114.53 (60.81-80.32)	1.67± 0.47	29.33
Acacia	820.7 (263.75-5.15E+4)	0.81± 0.24	4.09
Guava	882.85 (422.96-5548.7)	1.43± 0.32	3.81
2 days after treatment			
Pomegranate	17.80 (7.86-366.87)	2.14 ± 0.46	100
Olive	27.31 (23.89-30.85)	2.86 ± 0.24	65.18
Mulberry	62.20 (31.23-736.52)	2.42 ± 0.50	28.62
Acacia	183.96 (94.26-1.44E+3)	0.66 ± 0.19	9.68
Guava	197.85 (137.63-358.15)	1.04± 0.16	9.00
7 days after treatment			
Pomegranate	17.80 (7.86-366.87)	2.14± 0.46	100
Olive	27.31 (23.89-30.85)	2.86± 0.24	65.18
Mulberry	56.69 (51.08-63.19)	3.36± 0.35	31.40
Acacia	158.31 (94.56-517.23)	0.87± 0.20	11.24
Guava	132.03 (111.54-164.16)	2.03± 0.22	13.48

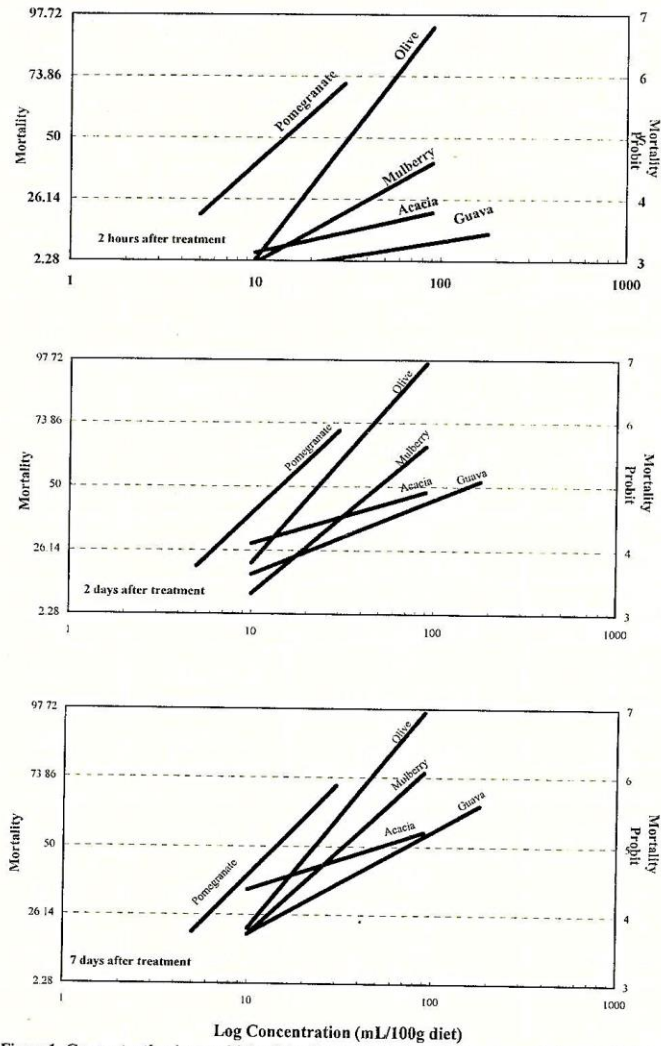


Figure 1. Concentration log probit toxicity lines of some plant extracts against newly hatched larvae of *P. gossypiella* after 2hr, 2days and 7days following treatment

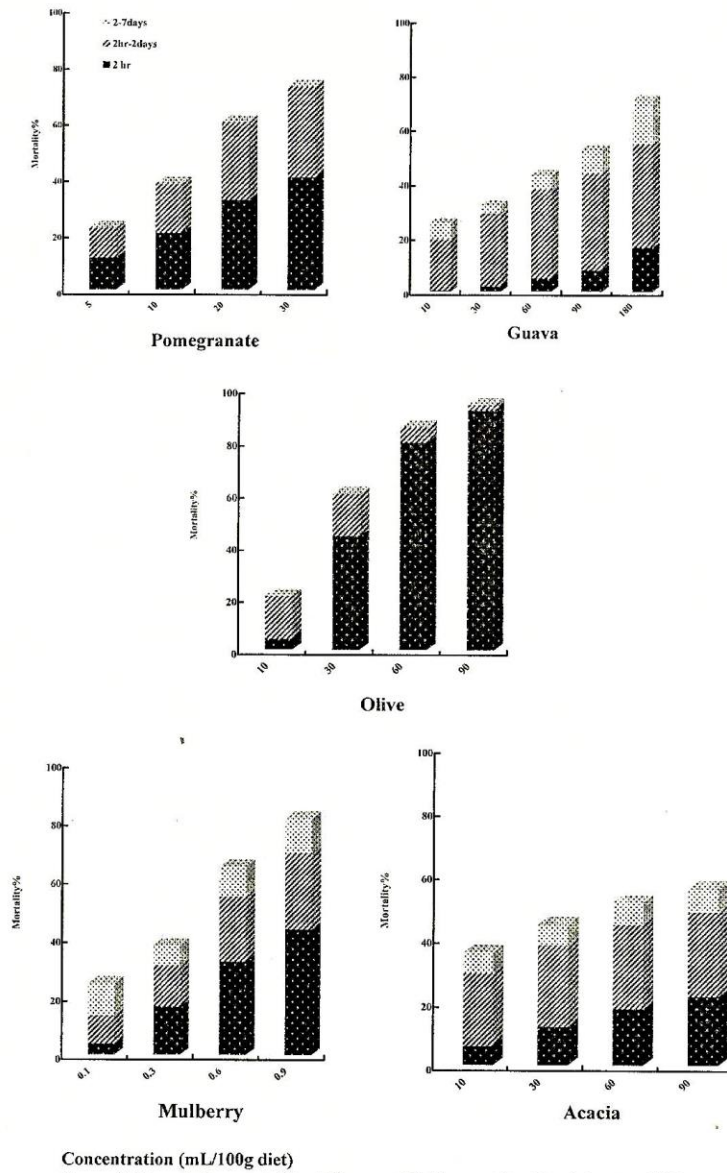


Table 3. Correlation between phenolic compounds (PCs) concentration in tested plant acetone extracts and their efficacy against newly hatched larvae of pink bollworm, *P. gossypiella*

Plant extract	PCs concentration (mg GAE/g dw)	LC ₅₀ (mL/100g diet) after		
		2 hours	2 days	7 days
Acacia	139.76	820.7	114.53	158.31
Pomegranate	161.72	48.69	17.80	17.80
Guava	56.28	882.85	197.85	132.03
Olive	21.27	33.59	27.31	27.31
Mulberry	19.31	114.53	27.31	56.69
Correlation (r) ± S.E.		0.205 ± 0.565	0.111 ± 0.574	0.169 ± 0.569
		ns	ns	ns

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الكفاءة الإيادية لبعض المستخلصات النباتية ضد دودة اللوز القرنفلية وعلاقتها بالمحتوى الكلي من الفينولات

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تم تقدير المحتوى الكلي من الفينولات في بعض المستخلصات النباتية (قشور الرمان، ثمار القرص وأوراق كل من الزيتون والتوت والجوافة) وقد وجد أن هذا المحتوى يختلف باختلاف النبات محل الدراسة حيث سجل مستخلص نبات التوت أقل محتوى من الفينولات (١٩,٣١-١,٨٦ mg GAE/g dw)، في حين كان مستخلص نبات القرص هو أعلى المستخلصات في إحتوائه على الفينولات (١٣٩,٧٦-٨,٩٦ mg GAE/g dw)، بينما احتوت مستخلصات كل من الرمان والجوافة والزيتون على نسبة متوسطة من الفينولات. كذلك اختلفت المذيبات المستخدمة فيما بينها من حيث كفاءتها في استخلاص الفينولات ويمكن ترتيبها من حيث الكفاءة كالتالي: الأسيتون < الإيثانول < الإيثانول أسيتات < البيوتانول < الميثانول

تم دراسة كفاءة هذه المستخلصات النباتية كمبيدات حشرية ضد دودة اللوز القرنفلية، وأظهرت النتائج المتحصل عليها وجود كفاءة إيادية لهذه المستخلصات ضد القفس الحديث لدودة اللوز القرنفلية واختلفت المستخلصات في مستوى تأثيرها ولوحظ أن نسبة الموت تتزايد بزيادة التركيز المستخدم والفترة بعد المعاملة، وقد أمكن ترتيب المستخلصات وفقاً لسميتها كالتالي: قشور الرمان < أوراق الزيتون < أوراق التوت < ثمار القرص < أوراق الجوافة. كما أظهرت النتائج عدم وجود علاقة بين سمية المستخلصات ومحتواها من الفينولات.