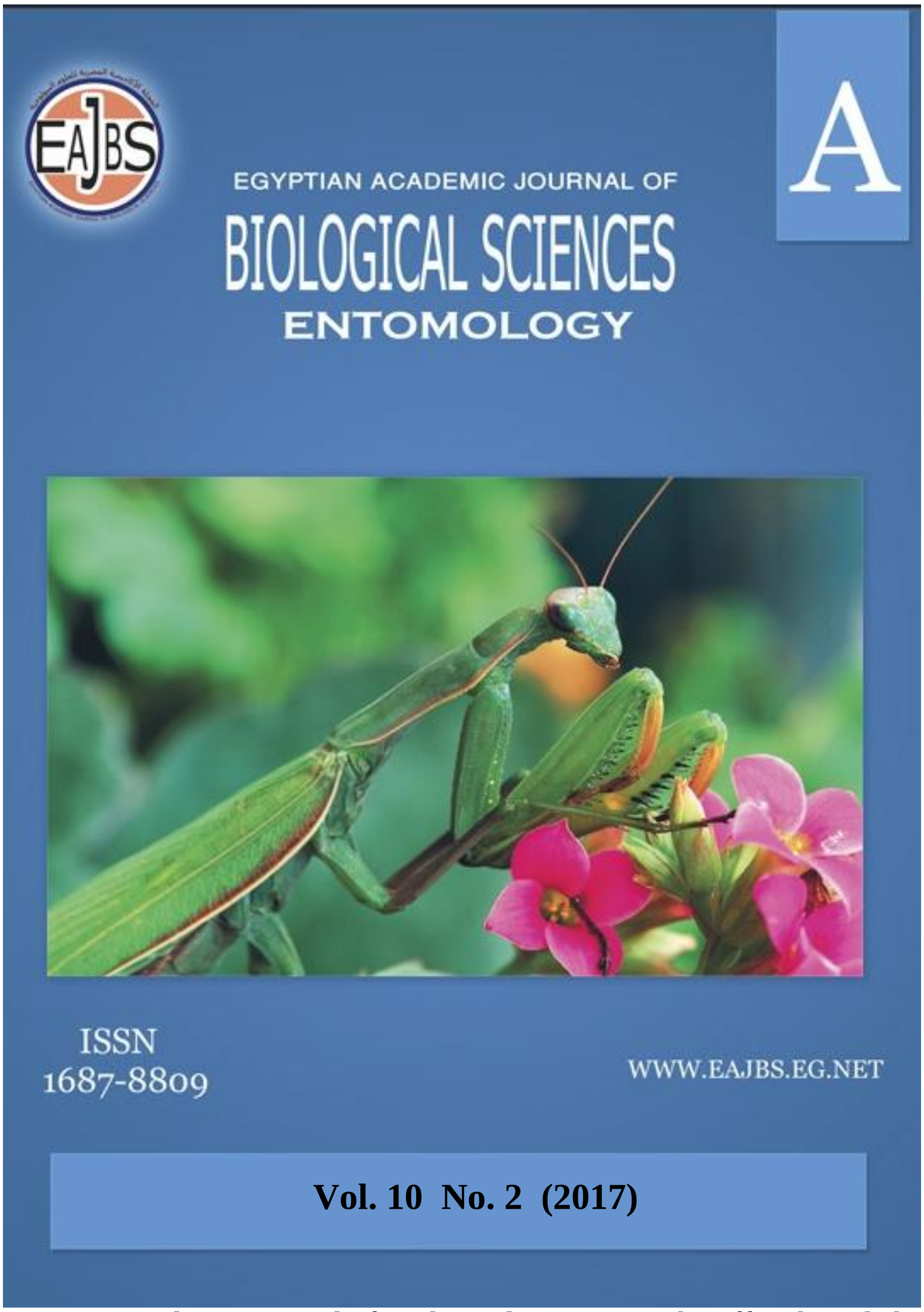

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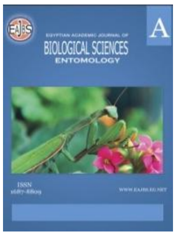

Repellency Effect of Some Biochemical Extracts of Castor Bean Leaf on Two Sap-Sucking Insect Pests

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ABSTRACT

Two castor bean landraces; Grey Small Seeded Landrace (GSSL) and Red Medium Seeded Landrace (RMSL) were detected during this work using seed external features and molecular analysis by ISSRs technique. Leaves of the GSSL were heavy infested with the castor bean whitefly, *Trialeurodes ricini*, while the RMSL leaves were not infested at all with this whitefly. Main four biochemical groups; phenols, terpenoids, alkaloids, and tannins were analyzed in the two landrace leaves. Analysis results indicated highly significant differences between the two landraces in phenols, terpenoids, and tannins while no differences were detected in alkaloids. Repellency effect of phenols, terpenoids, and tannins extracted from whitefly-resistant castor bean landrace leaf (RMSL) on two sap-sucking insects; *T. ricini* and the striped mealybug, *Ferrisia virgata* was determined. Results indicated that the three extracts have repellency effect on *T. ricini* with PR of 69.3, 58.3, and 17.7% for phenols, terpenoids, and tannins; respectively. Also, terpenoids only showed repellency effect on *F. virgate* with PR 57% and phenols showed low repellency effect. While tannins showed attractiveness effect on *F. virgate*, so it could be used in traps for this mealybug pest. These results suggested that the three biochemical extracts from castor bean had a repellent and/or attractant effect on *T. ricini* and *F. virgata* and could be used as effective and environmentally sustainable bio-insecticides for controlling the two sap-sucking pests.

INTRODUCTION

The castor bean plant, *Ricinus communis* L., is a member of the family Euphorbiaceae. It is widely cultivated and naturalized in tropical and subtropical regions of America and Asia and in many temperate areas of Europe. It naturalizes easily and grows in many areas as a common ruderal plant (Daisie, 2014; Govaerts, 2014; Prota, 2014). It is used as fertilizer or as fuel. The castor oil also has commercial value for making soap, margarine, lubricants, paints, inks, plastics, and linoleum. The crop is also regarded as a useful feedstock for biodiesel production (Okechukwu *et al.*, 2015; Razzazi *et al.*, 2015). There are nearly 250 cultivars of castor (Ovenden *et al.*, 2009). There is a wide variation: vegetative traits: leaf and stem colors, presence of wax on stem (Savy Filho, 2005). Reproductive traits: as color and size of seeds (Popova and Moshkin, 1986). In Egypt, it was cultivated for its oil as long as 6000 years ago and from here it spread through the Mediterranean, the Middle East, Asia, the Far East, and India long time ago (Deacon, 1986). Despite being an important crop, castor bean has never been realized as a commercial crop in Egypt. It is grown on marginalized land without much care and attention. There are three castor bean landraces in Egypt; the small seeded landrace, the medium seeded landrace, and the large seeded landrace, these landraces identified based on seed external features such as size and colour (Algharib and Kotb, 2013).

Castor bean leaves are heavy infested with the castor bean whitefly, *Trialeurodes ricini* (Misra) (Mound and Halsey, 1978; Bink-Moenen, 1983; Vora*et al.*, 1984; Abd-Rabou *et al.*, 2000). Also this whitefly species was recorded on many plant species. Both *T. ricini* nymph and adults cause direct plant injury by sucking sap from lower leaf surfaces and the resulting honeydew deposits lead to the development of sooty moulds. Heavy infestations can produce a large amount of honeydew and sooty moulds can cause a significant reduction in photosynthesis, which reduces plant growth. Seed yield may be reduced in castor due to sooty moulds (Patel *et al.*, 1986). As well as *T. ricini* was not known to be a virus vector until Idriss *et al.* (1997) who reported this whitefly as a vector of tomato yellow leaf curl virus (TYLCV) in Egypt.

The striped mealybug, *Ferrisia virgata* Cock. belongs to Pseudococcidae family and is considered as one of the most highly polyphagous mealybugs known, attacking plant species belonging to some 203 genera in 77 families (García *et al.*, 2016). Many of the host species belong to the Fabaceae and Euphorbiaceae. Among the hosts of economic importance are avocado, banana, betel vine, black pepper, cassava, cashew, cauliflower, citrus, cocoa, coffee, cotton, custard apple, egg-plant, grape-vine, guava, jute, lantana, *Leucaena*, litchi, mango, oil palm, pigeon pea, pineapple, soybean, and tomato. (Kaydan and Gullan, 2012). Also *F. virgata* causes direct plant injury by sucking plant sap in addition to causing indirect plant injury by transmitting *swollen shoot virus* (CSSV) in West Africa, cocoa Trinidad virus (CTV, Diego Martin valley isolate) in Trinidad (Thorold, 1975), and a badnavirus disease of black pepper in India (Bhat *et al.*, 2003).

Plant secondary metabolites can fulfil important functions in the interaction between plants and their biotic and abiotic environment, providing protection against attack by herbivores and microbes and serving as attractants for pollinators and seed-dispersing agents. These plant secondary metabolites are used to form insecticidal plants which have several effects. When not leading to insect mortality, it may cause repellency, deterrence, deformation in different insect stages, reduce intestinal motility, interfere in the synthesis of ecdysone and chitin (Schmutterer, 1990), growth rate (Nathan *et al.*, 2008), life span, and fecundity (Isikber *et al.*, 2006). Researches confirming insecticidal plants efficiency to control forest pests have been performed (Kanat and Alma, 2004; Sharma *et al.*, 2006; Parel *et al.*, 2014).

This work aims at evaluating the repellency effect of some castor bean biochemicals extracted from whitefly-resistant landrace on castor bean whitefly, *T. ricini*, and the striped mealybug, *F. virgate*.

MATERIAL AND METHODS

Sampling and Counting of *Trialeurodes ricini*:

Two groups of castor bean shrubs with the same vegetation and height were observed at Shebin El-Qanater, Qalyubiya Governorate during 2014 where one shrubs group was heavy infested with the castor bean whitefly, *T. ricini*, while the other shrubs group was not infested at all (Fig. 1). So weekly interval visits to these shrubs were achieved during the period from December 2014 till February 2015 to monitor *T. ricini* infestation. Twenty leaves were picked out from each group of castor bean shrubs and transferred to the Laboratory to count population density of *T. ricini* eggs, nymphs, and adults per leaf inch². When maturated, seeds of these shrubs appeared and some of seeds from each shrubs group were gathered for two purposes; landraces recognition and planting for another monitoring *T. ricini* season 2015. Seeds were planted in early July 2015 at an experimental area of faculty of Agriculture, Ain Shams University, Shoubra, Qalyubiya. *T. ricini* population density was inspected for the second season from November to February, 2016.

Castor Bean Landraces Identification:

For identifying the two castor bean shrub groups two identification ways were applied.

External Features of Each Group Seeds:

Some external morphological features of castor bean seeds such as colour, weight, height, and wide were detected according to Kotb and Algharib, 2013.

Analysis of DNA Using Inter Simple Sequence Repeats (ISSRs) Technique:

Isolation of genomic DNA was done according to Purohit *et al.* 2012. DNA was extracted from 0.3- 0.5 g of germinated seed from each group separately. Trials were done on 12 primers while only 7 were successful at least in one of the two samples (Table 1). Primers showed no bands in both samples were discarded. Thermal cycle used is one cycle initial denaturation (94C for 4 min.); 35 cycles contained the three steps; denaturation (94C/35 sec), annealing (40C/45sec), and extension (72C/2 min.) one cycle for final extension (72C/ 10 min).

Table 1: Seven ISSRs-specific primers amplified polymorphic bands in the two castor bean landraces.

Primer name	Sequence
ISSR-5	5′-AGAGAGAGAGAGAGAGT-3′
ISSR-7	5′-GAGAGAGAGAGAGAGAT-3′
ISSR-8	5′-CTCTCTCTCTCTCTCTG-3′
ISSR-15	5′-ACACACACACACACACGA-3′
RAMP-TAG	5′-TAGAGAGAGAGAGAGAGAG9-3′
RAMP-GAC	5′-GACACACACACACACACAC-3′
LK7	5′-CCACTCTCTCTCTCTCTCT-3

Castor Bean Leaf Bio-Chemicals Analysis:

Main four biochemical groups in castor bean leaves (Total phenols, Tannins, Flavonoids, and Alkaloids) were determined in the each shrubs group according to Harborne, 1983; Dihazi *et al.*, 2003; Bushra *et al.*, 2009 and Helmi & Mohamed, 2016.

Evaluating of Repellency Effect:

The experiment was designed to determine the repellency of three different castor plants extracts; Phenols, Terpenoids, and Tannins on *Trialeurodes ricini* adults and *Ferresia vergata* nymphs.

***Trialeurodes ricini* Adults:**

Four expanded uninfested leaves of susceptible castor bean landrace were placed individually in petri plates. Three of them were sprayed with the tested plant biochemical extracts (one leaf /one biochemical extract) and the other one was sprayed with distilled water (control). The petri plates were placed in a woody cage covered with fine netting material. About 100 immobilized adults especially newly emerged were placed between the four petri plates.

***Ferrisia virgata* Nymphs:**

Forty small fresh uninfected guava leaf pieces of leaves (~1cm^2) were used. Each ten pieces were sprayed with one of the castor plant extracts, while the other ten pieces were sprayed with water. Each ten pieces of guava leaves that were sprayed with one of each castor plant extract were placed near the edge of 10-cm diameter Petri dish while ten of guava leaves pieces which were sprayed with water placed on the other edge of the same dish. *Feresia vergata* nymphs were collected from guava trees before the experiment and were starved to 6 hrs. Nymphs were placed in the center between the two groups of guava leaf pieces. Each dish was covered and maintained at room temperature.

Each plant extract was replicated 5 times. Number of *T. ricini* adults and *F. virgata* nymphs attracted to each group of leaf pieces was recorded after 2, 8, 12, 16, 20, and 24 hours after treatment..

The data were converted to express percentage repulsion (PR %) and classified as mentioned before. The data were converted to express percentage repulsion (PR) by the formula of Talukder and Howse (1994) and Ali (1999). PR % = (N-C) /C X 100 Where: N = the number of insects present in the control half. C = half the number of total insects present. Positive values (+) expressed repellency and negative values (-) attractancy. Mean values were classified according to the following scale:

0	>0.01 to < 0.1
1	0.1 to 20
2	20.1 to 40
3	40.1 to 60
4	60.1 to 80
5	80.1 to 100

RESULTS AND DISCUSSION

Susceptibility of Castor Bean Plants to *Trialeurodesricini* Infestation:

Results obtained in Tables 2 and 3 indicated the mean numbers of *T. ricini* population density on castor bean throughout two seasons, 2014/2015 at Shebin El-Qanater and 2016 season at Shoubra, Qalyubiya Governorate. From these results, two castor bean landraces according to *T. ricini* infestation could be clearly determined whereas heavy infested landrace and uninfested landrace at all. Also results indicated that population density decreased gradually from mid-January to reach the lowest population density in mid-February in the both studied seasons.

Table 2: Weekly mean numbers of *Trialeurodes ricini* population density on two castor plant cultivars during 2014/2015 at Shebin El-Qanater, Qalyubiya Governorate.

Inspection Dates	Landrace A				Landrace B			
	Eggs	Nymphs	Adults	Total	Eggs	Nymphs	Adults	Total
1 Dec.	141.6	218.2	1	360.8	0	0	0	0
8 Dec.	192.8	390.8	13.2	596.8	0	0	0	0
15 Dec.	125.9	287.6	27.2	440.7	0	0	0	0
22 Dec.	229.2	235.4	71.6	536.2	1	0	0	1
29 Dec.	114.8	269.2	36.8	420.8	0	0	0	0
6 Jan.	245.1	280.5	21.6	547.2	0	0	0	0
12 Jan.	250.2	329.2	46.8	626.2	0	0	0	0
19 Jan.	164.2	127.6	7.2	299	0	0	0	0
26 Jan.	115.2	47	0.8	163	0	0	0	0
2 Feb.	33.4	39.8	0.1	73.3	0	0	0	0
9 Feb.	22.1	2.9	0	25	0	0	0	0
16 Feb.	11.6	2.4	0	14	0	0	0	0
Total	2340.1	2704.8	292.6	5337.5	1	0	0	1
Mean	156.0	180.32	19.51	355.83	0.08	0	0	0.08

Table 3: Weekly mean numbers of *Trialeurodes ricini* population density on two castor plant cultivars during 2016 at Shoubra, Qalyubiya Governorate.

Inspection Dates	Landrace A				Landrace B			
	Eggs	Nymphs	Adults	Total	Eggs	Nymphs	Adults	Total
15 Nov.	1243	350.0	1.2	1594.2	0	0	0	0
22 Nov.	46.8	352.0	4.6	403.4	0	0	0	0
29 Nov.	274.7	135.0	2.0	411.7	0	0	0	0
6 Dec.	106.4	680.8	3.0	790.2	0	0	0	0
13 Dec.	7.0	156.6	14.6	178.2	0	0	0	0
20 Dec.	22.8	164.4	12.6	199.8	0	0	0	0
27 Dec.	48.2	167.0	7.0	222.2	0	0	0	0
2 Jan.	117.4	147.8	3.0	268.2	0	0	0	0
9 Jan.	51.7	72.0	4.4	128.1	0	0	0	0
16 Jan.	44.9	68.7	1.2	114.8	0	0	0	0
23 Jan.	40.0	33.2	0.2	73.4	0	0	0	0
30 Jan.	39.5	24.9	0	64.4	0	0	0	0
6 Feb.	21.2	15.0	0	36.2	0	0	0	0
13 Feb.	9.7	4.6	0	14.3	0	0	0	0
20 Feb.	5.6	1.1	0	66.7	0	0	0	0
Total	2078.9	2373.1	53.8	4505.8	0	0	0	0
Mean	138.6	158.21	3.6	300.41	0	0	0	0

Castor Bean Landraces Identification:

External Features of Seeds:

Two types of seeds were detected according to some castor bean seed external features (Fig. 2), whereas seeds yielded from the susceptible plants were gray in colour while seeds were yielded from the resistant ones were red in colour. The gray seeds were less than the red seeds in weight, length, and width whereas ranges were 0.18: 0.2 g in weight, 0.82: 90 cm in length, and 0.27: 0.30 cm while the red seeds ranges were 0.4 : 0.48 g, 1.2: 1.3 cm, and 0.57: 0.60 cm for seeds weight, length, and width; respectively.

Molecular Identification Using Inter Simple Sequence Repeats (ISSRs) Technique:

Results of molecular analysis of the two castor bean landraces using 7 primers of ISSRs (Table 4 and Fig. 3) indicated that 60 fragments were generated (13 fragments for the susceptible landrace and 47 fragments for the resistant one) there are 96.7% polymorphism between the two landraces whereas two fragments only were monomorphic (3.3%). RumpTA6 primer produced the maximum number of fragments (12 fragments), while RumpGAG primer produced the minimum number of fragments (5 fragments)

Table 4: Results of molecular analysis of the two castor bean landraces using seven ISSR primers.

Primer	Total Bands	Monomorphic	Polymorphic	% Polymorphism
ISSR5	7	0	7	100
ISSR7	7	0	7	100
ISSR11	10	0	10	100
ISSR15	10	0	10	100
LK7	9	0	9	100
RumpTA6	12	1	11	90.91
RumpGAG	5	1	4	90.91
Total	60	2	58	96. 7

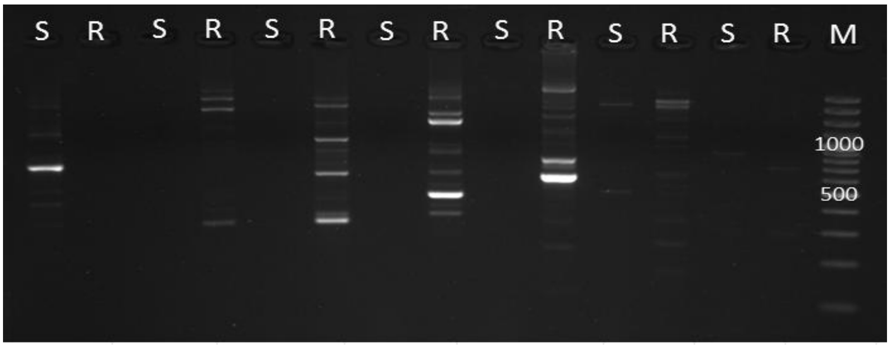

Fig. 3: DNA fragments generated by seven ISSR primers in two landraces of castor bean in Egypt. M: Marker R: Resistant landrace S: Susceptible landrace.

From the abovementioned results, it could be concluded that there are two landraces in this work according to external features, these two landraces are gray small seeded landrace (GSSL) and red medium seeded landrace (RMSL)**,** this identification is based on the work of Algharib and Kotb, 2013 who detected four castor bean landraces in Egypt based on external morphological features in addition to oil yield percentage. Also, molecular analysis using ISSRs technique confirmed the highly variability between the two landraces, as well as the two landraces showed highly differences in susceptibility to *T. ricini* infestation whereas GSSL showed highly susceptibility while RMSL found to be uninfested with *T. ricini* at all.

Main Four Biochemical Groups in the Two Castor Landraces:

Main four biochemical groups; phenols, tannins, alkaloids, and terpenoids were analyzed in both GSSL and RMSL to detect which of them may play a role in resistance against *T. ricini* so it may be used as a repellent of sap sucking insects. Statistical analysis indicated highly significant differences between the two landraces in three biochemical groups; phenols, tannins, and terpenoids while insignificant differences detected between the two landraces in alkaloids. The resistant landrace (RMSL) contains phenols, tannins, and terpenoids higher than the susceptible landrace. (Table 5).

Table 5: Concentrations of main four biochemical groups (mg/100mg) extracted from the two castor bean landraces; GSSL and RMSL.

	Phenols (mg/100mg)		Tannins (mg/100mg)		Alkaloids (mg/100mg)		Terpenoids (mg/100mg)	
	RMSL	GSSL	RMSL	GSSL	RMSL	GSSL	RMSL	GSSL
	3.278	0.1848	10.6	1.02	68	60.3	31.5	14.2
	3.4408	0.198	10.4	1	65	60.1	34.65	13.3
	3.4188	0.1364	11.4	1.06	62	60.2	34.65	12.9
Mean	3.38±0.1	0.17±0.03	10.8±0.5	1.03±0.03	65±3	60.2±0.1	33.6±1.8	13.5±0.7
P≤0.05	0.0002		0.0004		0.2		0.002	

Repellency Effect:

Results of repellency effect of three biochemical extracts; phenols, terpenoids, and tannins extracted from RMSL leaf on both castor bean whitefly, *T. ricini* adults and striped mealybug, *F. virgate* nymphs were obtained in Table 6. While alkaloids extract was not tested because there were no differences between the two landraces. The repellency effect of the three biochemical extracts on *T. ricini* indicated that all the three biochemical extract have repellent effect on *T. ricini* adults whereas the phenol extract was the one with the strongest repellent effect on *T. ricini* adults (class

4) with PR of 69.3% the maximum PR was 86% recorded after 16 hours from spraying, followed by the terpenoids extract (class 3) with PR of 58.3% the maximum PR was 74% recorded after 12 hours from spraying, while the tannins extract recorded the lowest repellent effect (class 1) with PR of 17.7% the maximum PR was 20% recorded twice after 12 and 20 hours of spraying.

Table 6: Repellency of extracts of three castor bean biochemical groups on *T. ricini* adults and *F. vergata* nymphs.

Insects	Biochemical group	Repellency (%)¹ at:						Mean Repellency (%)
		2h	8h	12h	16h	20h	24h²	
T. ricini	Phenols	72	76	80	86	60	42	69.3
	Terpenoids	68	72	74	66	42	28	58.3
	Tannins	16	18	20	14	20	18	17.7
F. vergata	Phenols	44	28	16	0	-4	-8	-
	Terpenoids	64	78	80	60	40	20	57
	Tannins	8-	-50	-80	-72	-80	-80	-

¹Percentage of repellency ²hours after treatment

The percentage of repellency observed during the 6 recorded times of the test showed a defined behavior either between each time in the case of both phenols and terpenoids whereas the PR% was gradually increased until exact time (16 h) for phenols extract, (12 h) for terpenoids extract, then gradually decreased. While tannins extract did not show a defined behavior either between each time.

The repellency effect of the three biochemical extracts on the striped mealybug, *F. virgata* nymphs showed that the terpenoids extract has the highest repellency effect to *F. virgata*till the end of the experiment recorded class 3 with PR of 57%, the terpenoids extract recorded the maximum percentage repulsion (PR) after 12h (80%) after that PR gradually decreased till the end of experiment. Also, the phenols extract showed a repellency effect but its efficacy was lower than the terpenoids extract where its percentage repulsion (PR) ranged from the third to first class with 44, 28, and 16% during 2, 8, and 12h; respectively after that its effect disappeared. On the other hand, the castor plants extract tannins recorded highly attractiveness efficacy, where its percentage repulsion (PR) were negative and ranged between -8% at the beginning of the experiment to -80% at the end of the experiment so it could be used as an attractiveness substance in this mealybug traps.

From the fore-mentioned results, it could be concluded that both phenols and terpenoids extract have highly repellency effect while the tannins extract showed low repellency effect on *T. ricini* and showed highly attractiveness to *F. virgata*. These results were in agreement with these obtained by several authors who worked on the repellency and toxicity effects of different plant extracts on hemipterans as Emilie *et al.*, 2015 who recorded repellent, irritant, and toxic effects of essential oils on the behaviour of *Bemisia tabaci.* Wubie *et al.*, 2014 who demonstrated repellent and insecticidal activity of *Mentha piperita* extract against *Brevicoryne brassicae*. Many authors mentioned that the repellency and toxicity of phenols in different plant extracts were highly effective against different herbivores (Goławska, 2006; Goławska *et al.*, 2008; Bhonwong *et al.*, 2009 and Haas *et al.*, 2016). While many authors considered tannins acts as feeding deterrents against many insects, so tannins may play role in controlling these insects (Bernays, 1981; Sharma & Agarwal 1983 and Barbehenn *et al.*, 2011).

This current study is represented basic work, consequently it should be used to help select wild plants with repellent properties and these plants could be extracted and detecting the active biochemical compounds responsible for repellency act on the insects and develop environmental-friendly insecticides.

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Fig. 1: Photos show the two castor bean landraces leaf in relation to infestation with the castor bean whitefly, *T. ricini.* A, landrace leaf with no infestation at all B, landrace leaf with heavy infestation.

A **B**

Fig. 2: Castor bean seeds: A, seeds of susceptible landrace B, seeds of resistant landrace to *T. ricini* infestation.

ARABIC SUMMERY

التأثير الطارد لبعض المستخلصات البيوكيميائية من اوراق الخروع على اثنين من الآفات الحشرية الماصة للعصارة النباتية

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تم تحديد اثنين من سلالات الخروع البرية؛ السلالة ذات البذور الصغيرة الرمادية والسلالة ذات البذور المتوسطة الحمراء وذلك باستخدام الصفات الظاهرية وتحليل الحامض النووى دنا باستخدام تقنية التكرارات البسيطة البينية ISSRs. أظهرت نتائج الفحص الدورى للاصابة بذبابة الخروع البيضاء ان اوراق السلالة ذات البذور الصغيرة الرمادية تصاب بشدة بهذه الافة فى حين ان اوراق السلالة ذات البذور المتوسطة الحمراء كانت خالية من الاصابة كلياً. تم تحليل الفينولات، التربينويدات، القلويدات و التانينات فى اوراق كل من السلالتين واظهرت النتائج احتواء سلالة الخروع المقاومة على محتوى عالى من الفينولات، التربينويدات و التانينات فى حين لم تجد فروق بين السلالتين فى محتوى القلويدات. تم تقييم الاثر الطارد لكل من مستخلصات الفينولات، التربينويدات و التانينات المستخلصة من اوراق السلالة المقاومة على كل من الحشرات الكاملة لذبابة الخروع البيضاء و حوريات البق الدقيقى المخطط، وأظهرت النتائج وجود تأثير طارد للثلاث مستخلصات على الحشرات الكاملة لذبابة الخروع البيضاء بمتوسط نسب مئوية ٦٩,٣%، ٥٨,٣% و١٧,٧% لكل من الفينولات، التربينويدات و التانينات، على التوالى. فى حين اظهرت التربينويدات تأثيراً طاردا على حوريات البق الدقيقى المخطط بمتوسط نسبة مئوية ٥٧% فى حين اظهرت الفينولات تأثير طارد منخفض، اما التاتينات فأظهرت تأثيراً جاذباً لحوريات البق الدقيقى المخطط مما يمكن استخدام التاتينات فى المصائد الجاذبة لهذه الافة. يمكن ان نستخلص من النتائج المتحصل عليها امكانية استخدام المستخلصات الكيميائية المستخلصة من اوراق السلالة البرية للخروع كمبيدات طبيعية صديقة للبيئة فى برامج السيطرة على هاتين الافاتين.