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Repellency and toxicity of *Citrullus colocynthis* and *Commiphora myrrh*a essential oils against *Periplaneta americana* populations collected from four localities in Egypt

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Abstract
Periplaneta americana L. is an insect of medical important found within the human houses and hospitals that cause serious health problems. Experiments were carried in the laboratory to evaluate contact toxicity and repellent activity of essential oils (Eos) of *Citrullus colocynthis* (colocynthis oil) and *Commiphora myrrh*a (myrrh oil) against laboratory strain (Lab) of *P. americana* and four populations collected from Cairo, Giza, Qalyubia and Beni suef in Egypt. Toxicity experiments by topical application methods revealed that myrrh oil proved to be more effective followed by colocynthis oil. However, there were no significant differences in the tested populations regarding colocynthis and marrh oils toxicity as the confidence intervals for the LD_{50} overlapped. Resistance ratio was very small of all populations against myrrh oil and colocynthis oil. Repellent activity of colocynthis oil and myrrh oil against adult cockroaches were determined by area preference method after 24, 48 and 72 hr of exposure. The essential oils of colocynthis oil and myrrh oil acted as a repellent against the Lab, Giza, and Qalyubia populations of *P. americana* adults at 24h, 48 and 72 h after insect exposure. Colocynthis oil showed moderate repellent activity against the tested populations at all assessment times. In addition, myrrh oil had the strongest repellency of *P. americana* collected from Beni suef with mean percentage of repellency (90%) after 72 h post treatment. It is concluded that myrrh oil and colocynthis oil exhibits both insecticidal and repellent activity against *P. americana* and can potentially be used to management cockroaches in different localities in Egypt.

Keywords: *Periplaneta americana*, *Citrullus colocynthis*, *Commiphora myrrh*a, susceptibility, repellency

1. Introduction
Periplaneta americana L. (Blattodea: Blattidae) is an obnoxious and filthy domestic pest found in tropical countries around the world. In Egypt, *P. americana* is among the medically important insects found within the human hospitals that causes disease transmission [1]. Cockroaches can be mechanical vectors of gastrointestinal diseases [2, 3]. Cockroaches disseminate infections with the fecal-oral route [4, 5]. Additionally, cockroaches damaged stored products and household goods [6, 7] and contaminate food on contact [8-10]. Therefore, Cockroaches must be controlled for reasons of disease prevention and public health [11].
As described earlier, chemical insecticides have been the cornerstone of controlling *P. americana*. However, it has been limited by several factors: the development of natural resistance by *P. americana* and the negative impact on human health [12]. Thus, the demand for botanical insecticides is growing [13]. Botanical insecticides are inexpensive, safe and pose less risk to the environment. Currently, many studies have proven the insecticidal and repellent activity of some plant species against cockroaches [14, 15].
Recently, increased attention has been paid for using *Citrullus colocynthis* as a natural insecticide against many insect pests [16]. *C. colocynthis* is an annual herb found in the sandy lands of tropical Africa and in the Mediterranean region [17]. Also, *Commiphora myrrha* is native to Northeastern Africa, especially Somalia. *C. myrrha* extract possessed antibacterial and antifungal [18], acaricidal [19] and insecticidal activities [20] Myrrh is one of the oldest known medicines and was widely used by the ancient Egyptians [21].
The present study attempted to evaluate the insecticidal activity and repellent efficacy of colocynthis oil and myrrh oil against laboratory and field populations of adults of *P. americana* in different localities in Egypt.

2. Materials and Methods
2.1 Insect collection
Adults of *P. americana* were collected from sewerage manholes in four different localities in Egypt: Cairo, Giza, Qalyubia and Beni suef. Susceptible population of *P. americana* was obtained from the Medical Insect Research Center, Dokki, Giza. The different populations of *P. americana* were kept in the laboratory at 27°C, 75% relative humidity and in darkness.

2.2 Essential oils
The tested essential oils (Eos) selected for susceptibility studies are listed in table 1. Eos were purchased from Harraz Company (Agricultural Seeds, Spices, and Medicinal plants Co.) Cairo, Egypt. The selection of the tested oils was based on the fact that these Eos are effective against other insects.

Table 1: list of the essential oils used in this study.

Scientific name	Family name	English name	Used part
Citrullus colocynthis (L.) (Schrad.)	Cucurbitaceae	colocynthis	Seeds oil
Commiphora myrrha (Nee) Engl.	Burseraceae	Myrrh	Bark oil

2.3 Topical application method
Susceptibility assays were carried out according to the method described by [22]. Ten adults (5 males and 5 females) of each collected populations in 4 replicates in 10 cm diameter Petri-dishes have been used for susceptibility evaluation. For each Eo, serial dilutions were prepared using acetone to obtain five concentrations. Serial dilutions for each of colocynthis oil and myrrh oil were 1.0, 2.0, 3.0, 4.0 and 5.0 µl/ml. By using micro applicator, 1.0 µl of each essential oil concentration was topically applied to the sternum of the first abdominal segment. Control group received 1.0 µl acetone only. The treated cockroaches were provided with bread and moistened cotton plug as food source. All experimental beakers were kept in laboratory condition mentioned above. Mortality was recorded after 24-hr exposure to Eo.

2.4 Repellency test
The repellency of Eos against cockroaches was determined by area preference method according to [23]. On one half of the filter paper 2 ml of tested essential oils were applied (treated area) and 2 ml of water were applied on the other half (control area). Food and drink for the cockroaches was placed at both sides of the treated and control area. Ten adults of the *P. americana*, were placed in the center point of filter paper. The repellency was observed after 24, 48 and 72 hours under laboratory conditions. Each experiment was repeated six times.

2.5 Data analysis
Data were subjected to probit analysis to determine LD_{50} for colocynthis oil and myrrh oils [24]. The mortality of treated populations was corrected according to Abbott's formula [25]. The Resistant ratio was calculated as the ratio between LD_{50} of the field population and LD_{50} of the laboratory population of *P. americana*. The repellency against the cockroaches was calculated for each test [26] following this formula: the percent repellency: PR (%) = [(C-T) / (C+T)] ×100. Analysis of variance (ANOVA) and Tukey's post hoc tests were used to compare the mean percentage of repellencies between essential oils according to [27]. Descriptive statistics and LD_{50} values were estimated using SPSS (Version 20) statistical software.

3. Results
3.1 Topical application method
Topically applied essential oils caused the death of adults of *P. americana* collected from different areas in Egypt (Table 2). Marrh oil was more toxic to insect populations collected from Cairo (2.02µl/insect), Qalyubia (2.56µl/insect) and Giza (LD_{50}=2.65 µl/insect) than to those from Beni suef (LD_{50} = 3.07µl/insect). Treatment with colocynthis was found to have least effect on the tested populations collected from Giza (LD_{50} = 4.24µl/insect) and Qalyubia (LD_{50} = 4.61µl/insect). There were no significant differences in the tested populations regarding colocynthis and marrh oils toxicity as the confidence intervals for the LD_{50} overlapped. The Lab strain of *P. americana* showed more susceptibility to Eos. LD_{50} of colocynthis oil and myrrh oil against the Lab strain of *P. americana* were 2.3 and 2.1 µl/insect, respectively. In all treatments, the resistant ratio was very small in comparison to the Lab strain (Table 2), indicating that *P. americana* collected from Cairo, Giza, Qalyubia and Beni suef did not developed resistant to tested Eo. Slopes recorded show that the least steepness of the lines was for colocynthis oil against Qalyubia population (slope= 2.02) and the highest steepness was for myrrh oil against Cairo population (slope = 4.17). This indicated that the toxicity is concentration dependent for both tested oils.

Table 2: Susceptibility of *Periplaneta americana* adults from different populations to colocynthis oil and myrrh oil after 24h of topical application.

Treatment	Insect group	LD_{50} (µl/insect)	Confdience limit		χ^2	Slope	Resistant ratio
			Lower	Upper			
Colocynthis oil	Lab.	2.16	0.63	3.78	12.64	3.55	-
	Cairo	2.30	1.00	3.74	12.04	3.97	1.06
	Giza	4.24	3.59	5.47	0.545	2.81	1.96
	Qalyubia	4.61	3.65	7.09	1.85	2.02	2.13
	Beni suef	3.25	2.79	3.90	1.57	2.82	1.50
Myrrh oil	Lab.	1.90	0.05	3.55	12.77	3.00	-
	Cairo	2.02	0.59	3.40	4.6	4.17	1.06
	Giza	2.65	2.23	3.13	3.00	2.67	1.39
	Qalyubia	2.56	2.20	2.93	3.29	3.28	1.34
	Beni suef	3.07	2.72	3.47	1.94	3.93	1.61

The LD_{50} values were considered significantly different ($P < 0.05$) if the 95% confidence intervals did not overlap.

Resistant ratio = LD_{50} of tested oil against field population/LD_{50} of tested oil against Lab population.

3.2 Repellency test

The area preference test on filter paper revealed that colocynthis oil exhibited repellency against the five populations of *P. americana* during the entire assessment period (Figure 1). It was the least repellant at 24 h (F=1.503., df =24, P>0.05) and 48 h (F=0.153, df=29, P>0.05) but its repellency to best at 72 h (F=0.96, df=29, P<0.05). While, the repellency of myrrh oil was significantly higher than the control at 48h and 72h, However, it was not significant different from the control after 24 h of exposure (Figure 2). Myrrh oil showed repellency at 24 h (F= 4.51, df =29, P>0.05), at 48 h (F=1.64, df= 29, P<0.05) and at 72 h (F=1.57, df=29, P<0.05).

The essential oils of colocynthis oil and myrrh oil acted as a strong repellent against the Lab, Giza, and Qalyubia populations of *P. americana* adults at 24h, 48 and 72 h after insect exposure (Figure 3 A and B). In addition, myrrh oil had the strongest repellency of *P. americana* collected from Beni suef with mean percentage of repellency (90%) after 72 h post treatment. Finally, colocynthis oil and myrrh oils were good repellent at 48 and 72 h. Furthermore, myrrh oil was more repellent than colocynthis oil against cockroaches showing percentage of repellency between 10 and 90% repellency and (10 and 63 % repellency), respectively (Figure 3 A and B).

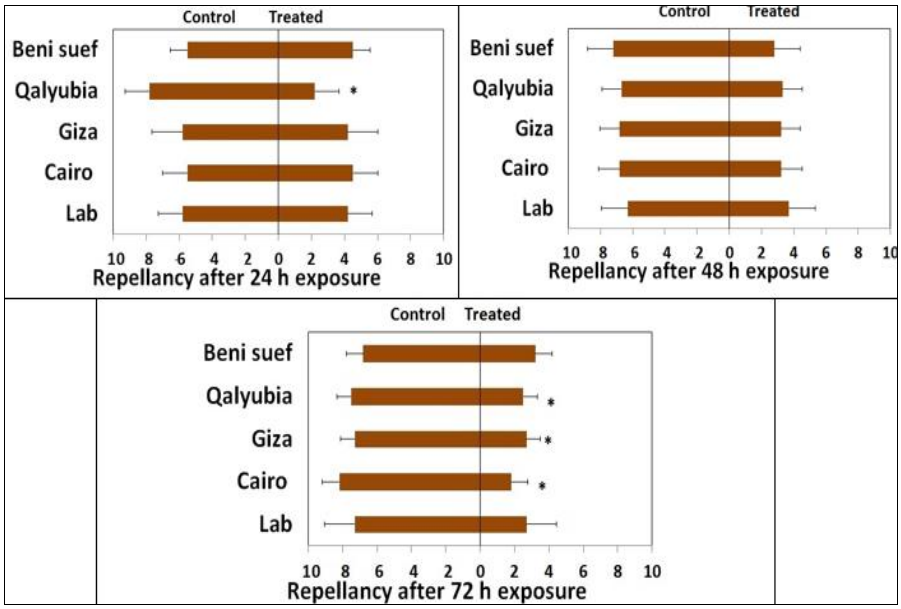

Fig 1: Repellency effect of colocynthis oil on *Periplaneta americana* adults from different localities as experimented by using the area preference test at 24, 48 and 72 h of exposure. The mean numbers of adults in the treated and control were analyzed by paired t-test at significance level of P < 0.05.

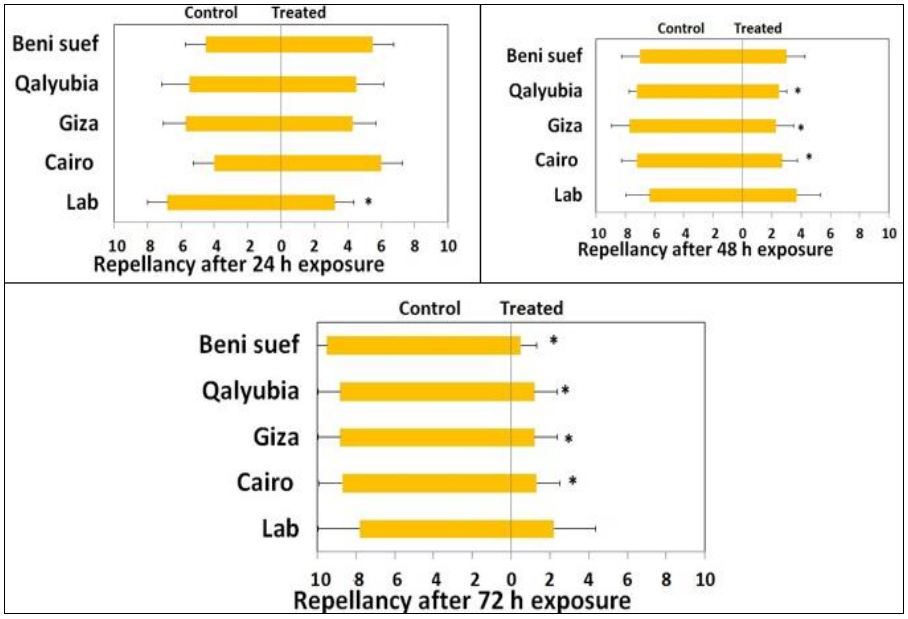

Fig 2: Repellency effect of myrrh oil on *Periplaneta americana* adults from different localities as experimented by using the area preference test at 24, 48 and 72 h of exposure. The mean numbers of adults in the treated and control were analyzed by paired t-test at significance level of P < 0.05.

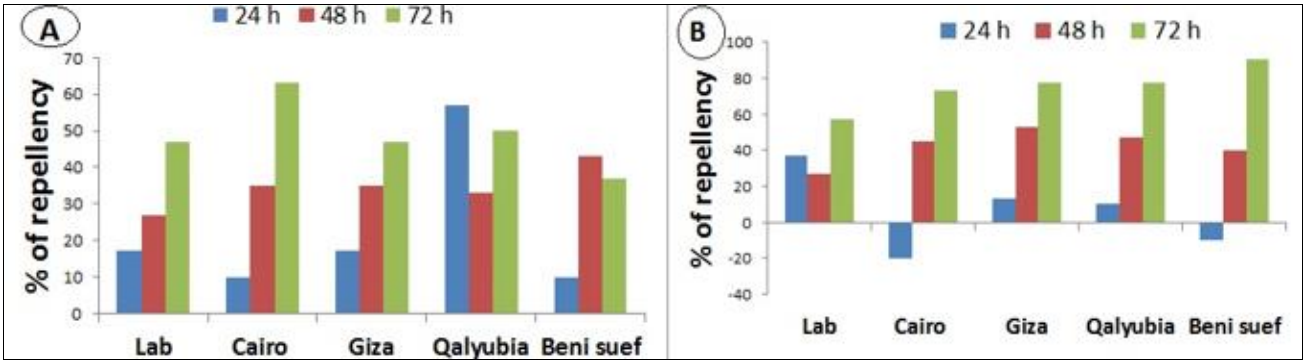

Fig 3: Percentage repellency of colocynthis oil (A) and myrrh oil (B) against different populations of *Periplaneta americana* at 24h, 48h and 72 of exposure.

4. Discussion

The problems released after the repeated use of residual insecticides for controlling cockroaches have bring out the need for the development of new types of selective cockroach control alternative. Yoon *et al*. [14] reported that plant essential oils have been suggested as potential alternatives to cockroaches chemical control agents. The present study showed that the colocynthis and myrrh oils have adultcidal activities against *P. americana* as well as repellent agents.

The current study also indicated the high efficacy of colocynthis oil and myrrh oil against all tested field populations, indicating an absence of resistance in all tested populations of *P. americana* in comparison to Lab population. The little or lack of plant essential oils use for cockroaches control in different areas in Egypt might be responsible for the high susceptibility of *P. americana*, which is a good indication for the further potential use of this botanical insecticide in *P. americana* management programs. Furthermore, botanical insecticides composed of mixtures of compounds as opposed to conventional insecticides usually containing a single active ingredient. So, it can affect both behavioral and physiological processes and the chance of pests developing resistance to such substances is very low [28]. The present results are in agreement with Asid *et al*. [29]. They found that the laboratory strain of *Musca domestica* was more sensitive to *C. colocynthis* extract than field strain after treatment with two concentrations (10% and 50%) using feeding and dipping bioassay. Furthermore, they recommended using the extract of *C. colocynthis* with concentration 50% against the larval instars of *M. domestica* under field conditions.

Previously, the insecticidal activity of colocynithin and hydrated colocynithin isolated from alcoholic extract of *C. colocynthis* pulp against *P. americana* was noticed in Egypt [30]. Also, *C. colocynthis* extract reduced the reproduction and the life cycle of *Aphis craccivora* [31], adultcidal activity against *Callosobruchus maculatus* [32] and inhibited the percentage of egg hatching of *Phthorimaea operculella* by 29.0% at 1.0% conc [33]. Furthermore, extract of *C. colocynthis* showed larvicidal, ovicidal and repellent activities against *Culex quinquefasciatus* [34] and *Anopheles arabiensis* [35]. The LC_{50} values of the ethanolic extracts of *C. colocynthis* fruit pulp and seeds were 50.11, 30.90 ppm respectively, in *A. arabiensis* and 25.12 and 39.81 ppm aganist *C. quinquefasciatus* [36]. The LC_{50} value for *C. colocynthis* plant on adult *Chrotogonus trachypterus* was calculated 18.58 mg/ml [37]. *C. colocynthis* extract at 10% killed 50% of *Tribolium castaneum* after 72 h exposure [38]. The active constituents of *C. colocynthis* fruits could be effective natural pesticides for managing adults of *Sitophilus oryzae* and *Sitophilus zeamais* [39].

Essential oil of *C. colocynthis* had potential repellent activity against larvae of *Dermestes maculatus* [40]. Methanolic and hexanic extract at 50% (w/v) of *C. colocynthis* showed repellency against *T. castaneum* [41]. *C. colocynthis* extract exhibited strong repellent activity against *Callosobruchus maculatus* [42].

Myrrh oil treatments resulted in more than 90% mortality against the Q-biotype females of *Bemisia tabaci* by using the vapour phase mortality bioassay 24 h post-treatment [20]. *C. myrrha* was a good repellent of *Megalurothrips sjostedti* [43]. Extract from gum of *C. myrrha* showed repellency against *S. zeamais* after 1 h and 3 h of exposure [44].

Essential oils are considered to be a potential source of insecticides due to their ability to inhibit acetylcholine esterase activity in *P. americana* [45]and *Blatella germanica* [46]. EOs act also via the octopaminergic system by increasing octopamine level and calcium in nervous cells of *B. germanica* [47]. Moreover, some EO components tested on *P. americana* compete with octopamine in binding to its tyramine receptors [48]. Alterations in the nervous system functions as the mechanism of action of Eos make them interesting candidates for bio-insecticides [49].

5. Conclusion

It can be concluded from the results that colocynthis oil and myrrh oil can be used to control adults of *P. americana* in Cairo, Giza, Qalyubia and Beni suef in Egypt. These plant materials have shown good efficacy as adulticides, fumigant and repellent against all four population of *P. americana*. Colocynthis oil and myrrh oil can be used in an eco-friendly manner to reduce the number of cockroache populations.

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