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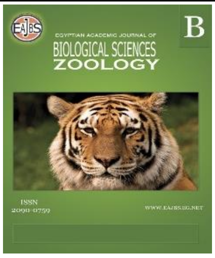

Toxicity of Some Botanical Oils and Ginger Extract against *Monacha cartusiana* Snail

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ABSTRACT

Currently using natural materials for *Monacha cartusiana* (Müller), (Gastropoda, Stylommatophora, Hygromiidae) snail control is deliberated to be the utmost realistic attitude. The objective of this study was to evaluate the effect of six crucial botanical oils. (*Camphora officinarum* Nees (Laurales, Lauraceae), *Citrus limon* Osbeck (Sapindales, Rutaceae), *Mentha perpirata* L. (Lamiales, Lamiaceae), *Nigella sativa* L. (Ranunculales, Ranunculaceae), *Ocimum basilicum* L. (Lamiales, Lamiaceae) and *Syzygium aromaticum* L. (Myrtales, Myrtaceae) were used at concentrations (4, 10, 20, and 40%) while *Zingiber officinale* Roscoe (Zingiberales, Zingiberaceae) extract at (1, 5, 10, and 20%) against *M. cartusiana* snails. The LC_{50} and LC_{90} of each botanical oil and ginger extract were determined and the activity of the binary mixture of LC_{50} extract with LC_{50} of the three most effective oils against snails was estimated under laboratory conditions. All treatments caused a relative mortality of the snail individuals which made it possible to calculate the toxicity index and relative toxicity. Moreover, this study proved that the effect of *C. officinarum*, *N. sativa* and *O. basilicum* oils are higher than that of the other used oils, as they achieved 86.66, 76.66 and 86.66% mortality respectively, at the highest tested concentration after 14 days of treatment. On the other hand, *Z. officinale* extract caused 73.33% mortality at the highest concentration when used individually and the binary mixing of its LC_{50} with LC_{50} of *C. camphora*, *N. sativa* and *O. basilicum* oils separately induced 66.66, 100.00 and 60.00% mortality, respectively. Thus, botanical oils and their mixtures are preferably used in the control of land snails instead of the traditional chemical pesticides.

INTRODUCTION

Land snails represent one of the furthermost severe pests of numerous crops and vegetables, feeding on the leaves, roots, and fruits, leading to a decline in their quality and economic loss. The glassy clover snail, *Monacha cartusiana* (Müller) (Gastropoda, Stylommatophora, Helicidae) is considered the greatest major land snail in Egypt confronting all plants (Ali, 2017). The use of chemical approaches should be avoided in the control of this pest because it causes detrimental effects on non-target organisms, is harmful to many living organisms, and progresses to the pest's resistance. For this reason, natural molluscicides are the best alternative to chemical methods for controlling pests. In this respect, researchers' courtesy has been focused on, observing the molluscicidal activity of different plants (Nikoli *et al*., 2014). Essential oils (Eos) are a unique substitute approach to reducing chemical pesticide usage in pest control. They are brilliant natural botanical products due to their great bioactive potential, constituting a gorgeous source of bioactive amalgams that are environmental yields, tranquil availability, and economic sustainability. Besides, *Peppermint*, *Syzygium aromaticum*, *Citrus limon*, *Nigella sativa*, *Ocimum basilicum* and *Camphora officinarum* have repellent, antifeedant, antioxidant, insecticidal, antibacterial, antifungal, and antimycotoxigenic effects (Popović *et al*., 2018; Mutlu-Ingok *et al*., 2020; Yeddes *et al*., 2022). The plant extracts are also recommended in pest control due to their low toxicity against mammals, low costs and fast biodegradability (Singh *et al*., 2000). The extract of *Zingiber officinale* represents a natural and environmentally safe molluscicide. It has great extermination activity against *Monacha cartusiana* snails at low concentrations (Abd El-Atti *et al*., 2019). On the other hand, the binary mixture of potent botanical agents is more effective than its use individually for the management of the same snail species (Hend, 2018).

This study aimed to investigate the toxic effect of the botanical oils (*C. officinarum*, *C. limon*, *M. perpirata*, *N. sativa*, *O. basilicum* and *S. aromaticum*) and the ethanolic extract of *Z. officinale* individually and as binary mixing with the three most toxic oils separately against *M. cartusiana* snail under the laboratory conditions.

MATERIALS AND METHODS

Botanical Oils:

The botanical oils C*amphora officinarum* Nees (Laurales, Lauraceae), *Citrus limon* Osbeck (Sapindales, Rutaceae), *Mentha perpirata* L. (Lamiales, Lamiaceae), *Nigella sativa* L. (Ranunculales, Ranunculaceae), *Ocimum basilicum* L. (Lamiales, Lamiaceae) and *Syzygium aromaticum* L. (Myrtales, Myrtaceae)) were selected for use in this study, as they were purchased from Al-Tasnem Company, Cairo, Egypt.

No.	**Scientific name**	**Trade name**	**Active compound**	**Reference**
1	*Camphora officinarum* Nees	Camphor oil	Linalool	Lee *et al*. (2022)
2	*Citrus limon* Osbeck	Lemon	Linalool	Hojjati and Barzegar (2017)
3	*Mentha perpirata* L.	Mint	Menthol	Lawrence (2006)
4	*Nigella sativa* L.	Black seed	Thymoquinone	Fatima *et al*. (2021)
5	*Ocimum basilicum* L.	Basil	Linalool	Reza *et al*. (2022)
6	*Syzygium aromaticum* Perry	Clove oil	Eugenol	Chaieb *et al*. (2007)

Plant Extract:

A fine powder of *Zingiber officinale* Roscoe (Zingiberales, Zingiberaceae) (250 g) (**Photo 1**) was deeply macerated in 70% ethanol for 7 days. At the end of the drenching period, the extract was filtered and concerted. Ethanol was evaporated, using a rotary evaporator (IKA-WERK, RV10, China) at 60^{o}C, and then oven-dried to concentrate at 45^{o}C (Bahrin *et al*., 2018).

***Scientific name**: *Zingiber officinale* Roscoe
Trade name: Ginger
Active compound: The main active constituent is gingerol (Mao *et al*., 2019).

Photo 1: Fine powder of *Zingiber officinale* plant.

Tested Snail:

Adult individuals of *M. cartusiana* snail were collected from infested clover fields at Kafr El-Ashraf village, Zagazig district, Sharkia Governorate, Egypt. Snails were transported to the laboratory and retained in a glass container (50 × 30 × 30 cm) containing humid clay soil. Snails were nurtured daily with fresh Lettuce leaves for two weeks for adaptation before conducting any experiment.

Toxic Effect of Botanical Agents Against Snails:

The molluscicidal influence of six botanical oils of C. *officinarum*, *C. limon*, *M. perpirata*, *N. sativa*, *O. basilicum* and *S. aromaticum*, and *Z. officinale* extract against snails was investigated separately using baits technique under laboratory conditions. The used concentrations of each oil 4, 10, 20 and 40% were prepared by adding the amount of each tested oil required to obtain the wanted concentration to 5 parts of sugar cane syrup mixed with 91, 85, 75 and 55 parts of wheat bran. While, the concentrations of *Z. officinale* extract 1, 5, 10 and 20% were prepared by adding the amount of extract to get the required concentration to 5 parts of the sugar cane syrup and combined with 94, 90, 85 and 75 parts of wheat bran. Three replicates for each tested concentration of each plant agent were used in plastic boxes containing the baits that were prepared and ten adult snails/ box. The other three control boxes were prepared in the same manner but without any treatment. All boxes were covered with muslin cloth fixed with rubber bands to prevent snails from escaping (Hilmy and Hegab, 2010). The mortality percentages of snails were recorded after 1, 3, 7, 14 and 21 days of the treatment. On the 7th day of the experiment, the lethal concentrations LC_{50} and LC_{90} of each plant agent were calculated according to Finney (1971). The toxicity index and relative toxicity were also estimated according to Sun (1950).

$$\text{Toxicity Index} = \frac{LC_{50}\,\text{of the most effective compound}}{LC_{50}\text{ of the other compound}} \times 100$$

$$\text{Relative toxicity } = \frac{LC_{50}\,\text{of the most effective compound}}{LC_{50}\text{ of the other compound}}$$

Efficiency of Botanical Mixtures Against Snails:

The molluscicidal effect of the binary mixture of *Z. officinale* extract (LC_{50}) with (LC_{50}) of the three most toxic oils separately was explored against snails. Three replicates were prepared for each mixture; each replicate containing ten adult snails and bait whose method of preparation was previously explained and three other replicates were prepared in the same way but without treatment as a control. All boxes were examined after 1, 3, 7, 14 and 21 days to calculate the mortality percentages of snails.

Statistical Analysis:

Data means obtained from the toxicological studies were analyzed by F test using Costat Program software Version 6.311, Costat (2005).

RESULTS AND DISCUSSION

Toxic Experiments:

Toxic Influence of Botanical Oils Against Snails:

As indicated in **Table 1.** the snails' mortality increased with increasing the exposure time (1, 3, 7, 14 and 21 days) and concentrations (4, 10, 20 and 40%) of the botanical oils (*C. officinarum*, *C. limon*, *M. perpirata*, *N. sativa*, *O. basilicum* and *S. aromaticum*). It seems clear that the three oils; *C. officinarum*, *N. sativa* and *O. basilicum* achieved the highest mortality rates of snails, which were 86.66, 76.66 and 86.66% at a concentration of

40% after 21 days of the exposure. Moreover, there is a highly significant difference between the means of snail mortality at different concentrations of the tested oils.

Table 1. Efficacy of botanical oils against *M. cartusiana* snail.

Tested oils	Conc. (%)	Mean of mortality % after indicated days				
		1	3	7	14	21
C. officinarum	**4**	20.00 ^{b}	23.33 ^{de}	23.33 ^{fg}	23.33 ^{gh}	23.33 ^{gh}
	10	20.00 ^{b}	20.00 ^{e}	23.33 ^{fg}	23.33 ^{gh}	40.00 ^{ef}
	20	43.33 ^{a}	60.00 ^{a}	60.00 ^{bc}	83.33 ^{a}	83.33 ^{a}
	40	46.66 ^{a}	60.00 ^{a}	83.33 ^{a}	86.66 ^{a}	86.66 ^{a}
C. limon	**4**	0.00 ^{c}	0.00 ^{f}	0.00 ^{h}	0.00 ^{i}	0.00 ^{i}
	10	0.00 ^{c}	0.00 ^{f}	20.00 ^{fg}	26.66 ^{gh}	26.66 ^{gh}
	20	0.00 ^{c}	0.00 ^{f}	43.33 ^{cde}	43.33 ^{ef}	43.33 ^{ef}
	40	0.00 ^{c}	0.00 ^{f}	60.00 ^{bc}	66.66 ^{bc}	66.66 ^{bc}
M. perpirata	**4**	0.00 ^{c}	20.00 ^{e}	20.00 ^{fg}	23.33 ^{gh}	40.00 ^{ef}
	10	0.00 ^{c}	20.00 ^{e}	33.33 ^{def}	33.33 ^{fg}	40.00 ^{ef}
	20	20.00 ^{b}	43.33 ^{abc}	43.33 ^{cde}	43.33 ^{ef}	46.66 ^{e}
	40	46.66 ^{a}	46.66 ^{ab}	60.00 ^{bc}	60.00 ^{cd}	60.00 ^{cd}
N. sativa	**4**	0.00 ^{c}	0.00 ^{f}	10.00 ^{gh}	16.66 ^{h}	16.66 ^{h}
	10	0.00 ^{c}	0.00 ^{f}	30.00 ^{ef}	33.33 ^{fg}	33.33 ^{fg}
	20	43.33 ^{a}	43.33 ^{abc}	50.00 ^{cd}	50.00 ^{de}	50.00 ^{de}
	40	46.66 ^{a}	46.66 ^{ab}	73.33 ^{ab}	76.66 ^{ab}	76.66 ^{ab}
O. basilicum	**4**	20.00 ^{b}	20.00 ^{e}	23.33 ^{fg}	23.33 ^{gh}	23.33 ^{gh}
	10	23.33 ^{b}	40.00 ^{bcd}	60.00 ^{bc}	60.00 ^{cd}	60.00 ^{cd}
	20	40.00 ^{a}	46.66 ^{ab}	60.00 ^{bc}	63.33 ^{c}	63.33 ^{c}
	40	53.33 ^{a}	60.00 ^{a}	80.00 ^{a}	86.66 ^{a}	86.66 ^{a}
S. aromaticum	**4**	0.00 ^{c}	0.00 ^{f}	0.00 ^{h}	0.00 ^{i}	0.00 ^{i}
	10	0.00 ^{c}	20.00 ^{e}	20.00 ^{fg}	33.33 ^{fg}	40.00 ^{ef}
	20	0.00 ^{c}	20.00 ^{e}	43.33 ^{cde}	43.33 ^{ef}	60.00 ^{cd}
	40	0.00 ^{c}	26.66 ^{cde}	70.00 ^{ab}	74.66 ^{ab}	76.66 ^{ab}
Control		0.00 ^{c}	0.00 ^{f}	0.00 ^{h}	0.00 ^{i}	0.00 ^{i}
P		.0000 ***	.0000 ***	.0000 ***	.0000 ***	.0000 ***
$LSD_{0.05}$		1.36	1.71	1.88	1.32	1.21

The lethal concentrations of LC_{50} and LC_{90} of botanical oils were also determined as shown in **Table 2.** *O. basilicum* oil has the highest toxicity against snails, so it recorded the lowest values of LC_{50} and LC_{90}, which were 11.17 and 69.73%, respectively. The toxicity indexes of *C. officinaruma*, *N. sativa*, *S. aromaticum*, *M. perpirata* and *C. limon* were 68.82, 55.55, 45.93, 40.06 and 39.17%, respectively when compared with the most effective oil, *O. basilicum* which had 100% toxicity index. On the other hand, the relative toxicity of *O. basilicum*, *C. officinarum*, *N. sativa*, *S. aromaticum* and *M. perpirata* were 2.55, 1.76, 1.42, 1.17 and 1.02 folds sequentially compared with the least effective oil, *C. limon*. These results were in accordance with Lahlou (2004) who reported that botanical oils were successful natural molluscicides due to the high bioactive potential of their constituents which represent a rich source of compounds that are biodegradable to non-toxic products and economic viability. In addition, the toxic influence of natural products against animals is generally due to their ability to induce programmed cell death in intracellular tissues (EL-Shafey *et al*., 2022). The clove oil, *S. aromaticum* has a significant molluscicidal activity against the giant African snail, *Achatina fulica* (Parvate and Thayil, 2017). As reported (Abdel-Rhman, 2020) this oil was also the most effective against *Monacha* snails; as it caused 90% mortality of snails at the concentration of 26% after only one day of the treatment. It was followed by *N. sativa* and *Brassica alba* oils which recorded 80 and 70% mortality sequentially at the same time of exposure and the same concentration. So, *S. aromaticum* recorded the lowest value of LC_{50} which was 2.81% after 96 hrs of treatment followed by *N. sativa* and *B. alba* which recorded 7.31 and 11.73%, respectively. The toxicity indexes of *N. sativa* and *B. alba* oils were 38.44 and 23.95% sequentially when compared with the most effective oil, *S. aromaticum* which had a 100% toxicity index. On the other hand, the relative toxicity of *S. aromaticum* and *N. sativa* oils were 4.17 and 1.60 folds, respectively compared to the lowest effective oil, *B. alba*. Similarly, Ismail and Abdel Kader (2011) indicated that the essential oil, *S. aromaticum* achieved 39.6, 57.2 and 62.4% mortality of *Monacha cartusiana* snails at the concentrations of 1, 2 and 4%, respectively after 21 days of the treatment by baits technique. The potent effect of *S. aromaticum* oil may be attributed to the presence of eugenol as its main component (Kumar and Singh, 2006) which consists of large members of phenolic compounds (Chaieb *et al*., 2007).

Table 2. Determination of lethal concentrations of tested botanical oils.

Tested oils	LC_{50} (%)	LC_{90} (%)	Slop	Toxicity index	Relative toxicity
C. officinarum	16.23	80.27	1.85 ± 0.19	68.82	1.76
C. limon	28.52	145.49	1.81 ± 0.32	39.17	1
M. perpirata	27.88	429.05	1.08 ± 0.18	40.06	1.02
N. sativa	20.11	104.33	1.79 ± 0.20	55.55	1.42
O. basilicum	11.17	69.73	1.61 ± 0.14	100	2.55
S. aromaticum	24.32	88.87	2.28 ± 0.32	45.93	1.17

Molluscicidal Potency of *Zingiber officinale* Extract Against Snails:

The influence of *Z. officinale* extract against snails increased with the increasing of concentrations and exposure period as shown in Table 3. The highest mortality rates were 73.33 and 60% at the highest concentrations 20 and 10%, sequentially after only 7 days of treatment and these rates remained constant until the end of the experiment. While it achieved 53.33 and 26.66% mortality of snails at its other concentrations 5 and 1%, respectively after 14 days of treatment. The obtained data also showed highly significant differences between the means of mortality percentages at all tested concentrations of the extract and all the exposure periods. The LC_{50} and LC_{90} were 5.78 and 95.90%, respectively (**Table 4).** These findings were in harmony with Abd El-Atti *et al*. (2019) who revealed that the ethanolic extract of *Z. officinale* caused 66.7 and 90% mortality of *M. cartusiana* snails at the concentrations 20 and 40% sequentially after 28 days of exposure. Moreover, the mortality rate of snails increased with increasing the extract concentrations and also with increasing the time elapsed. It causes cell injuries in the digestive gland of snails and also causes toxically hepatitis (Farkas *et al*., 2004). Likewise, Tahir *et al*. (2015) showed that the treatment with *Z. officinale* extract promoted apoptosis. The presence of alkaloids, tannins, saponins, flavonoids and terpenoids represents the main reason for the molluscicidal potency of this extract (Sharma *et al*., 2016).

Table 3. Efficacy of *Zingiber officinale* extract against *M. cartusiana* snail.

Tested Conc. (%)	Mean of mortality % after indicated days				
	1	3	7	14	21
1	0.00 ^{c}	0.00 ^{c}	26.66 ^{bc}	26.66 ^{bc}	26.66 ^{bc}
5	10.00 ^{bc}	33.33 ^{b}	50.00 ^{ab}	53.33 ^{ab}	53.33 ^{ab}
10	23.33 ^{ab}	60.00 ^{a}	60.00 ^{a}	60.00 ^{a}	60.00 ^{a}
20	40.00 ^{a}	46.66 ^{ab}	73.33 ^{a}	73.33 ^{a}	73.33 ^{a}
Control	0.00 ^{c}	0.00 ^{c}	0.00 ^{c}	0.00 ^{c}	0.00 ^{c}
P	.0000 **	.0000 ***	.0000 **	.0000 ***	.0000 ***
$LSD_{0.05}$	1.69	2.10	2.77	2.69	2.69

Table 4. Determination of lethal concentrations of *Zingiber officinale* extract**.**

LC_{50} (%)	LC_{90} (%)	Slop
5.78	95.90	1.05 ± 0.14

Efficiency of Botanical Mixtures Against Snails:

The impact of mixing the LC_{50} of each of *C. camphora, N. sativa* and *O. basilicum* with the LC_{50} of *Z. officinale* extract against snails was demonstrated in Table 5. The mixing of *N. sativa* oil with *Z. officinale* extract was the most effective mixture against snails where it caused 100% mortality after only one day of treatment. It was followed by the binary mixing of the extract with each of *C. officinarum* and *O. basilicum* oils, which achieved 66.66 and 60% mortality of snails on the third day of the experiment, respectively. Our results showed also a highly significant difference between the means of mortality percentages achieved by the tested binary mixtures.

Table 5. Toxic activity of the binary mixtures of botanical oils (LC_{50}) with *Zingiber officinale* extract (LC_{50}) against *M. cartusiana* snail

Botanical mixtures	Mean of mortality % after indicated days				
	1	3	7	14	21
C. officinarum + Z. officinale	66.66 ^{b}	66.66 ^{b}	66.66 ^{b}	66.66 ^{b}	66.66 ^{b}
N. sativa + Z. officinale	100.00 ^{a}	100.00 ^{a}	100.00 ^{a}	100.00 ^{a}	100.00 ^{a}
O. basilicum + Z. officinale	43.33 ^{c}	60.00 ^{b}	60.00 ^{b}	60.00 ^{b}	60.00 ^{b}
Control	0.00 ^{d}	0.00 ^{c}	0.00 ^{c}	0.00 ^{c}	0.00 ^{c}
P	.0000 ***	.0000 ***	.0000 ***	.0000 ***	.0000 ***
$LSD_{0.05}$	0.76	1.08	1.08	1.08	1.08

These outcomes were supported by Oparaeke *et al*. (2005) who confirmed that mixing two or more plant materials is more effective than the use of one plant material. The binary mixing of *Acalypha indica* and *Cedrus deodara* oil was more influential against *Lymnaea acuminata* snail than the individual treatment (Rao and Singh, 2001). The binary combination of *Euphorbia tirucalli* extract with *Codiaeum variegatum* against the same snail species increased the toxic impact by 9.51 times to the single treatments (Chauhan and Singh, 2011). In the same line, Hend (2018) stated that the binary combination of *Citrus limon* juice with the juice of *Mentha spicata* has the highest molluscicidal influence against *M. cartusiana* snails than using each juice alone, as it was recorded 80% mortality of snails after only three days of the laboratory experiment. Similarly, the binary mixing of these botanical juices is more effective than the use of each one alone. The toxic activity of the botanical mixtures varied according to the combined species and dosages (Taguiling, 2015). In addition, the mixing of plant materials was effective due to the synergistic impact of the phytochemical components of the single plant material (Guruswamy *et al*., 2017). The combination of these components increases or prolongs the toxic influence of these botanical mixtures (Oparaeke *et al*., 2005).

CONCLUSION

Due to the distinctive potency of tested botanical agents in this study, they could be used as potent molluscicides for controlling this snail species. As environmentally friendly and easily biodegradable agents, they can be used as the perfect strategy for controlling land snails instead of using harmful traditional chemical pesticides.

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ARABIC SAMMARY

سميه بعض الزيوت النباتيه ومستخلص الزنجبيل ضد قوقع موناكا كارتوسيانا

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يعتبر قوقع البرسيم الزجاجي موناكا كارتوسيانا هو من أكثر القواقع إنتشارا وبالتالي أكثرها ضررا وتلفا للمحاصيل الزراعيه في جمهوريه مصر العربيه. أستهدفت هذه الدراسه إختبار التأثير السام لسته زيوت نباتيه وهي زيت الكافور- الليمون – النعناع – حبه البركه – الريحان وأيضا القرنفل ضد الأفراد البالغه لقوقع موناكا كارتوسيانا عند التركيزات 4 و10 و20 و40٪ لكل زيت. تم أيضا دراسه التأثير القاتل لمستخلص الزنجبيل الإيثانولي ضد أفراد نفس نوع القوقع عند التركيزات 1 و5 و10 و20٪ تحت الظروف المعمليه. تم تحديد التركيز نصف مميت والتركيز الذي يسبب 90٪ موت للقواقع من كل زيت مختبر ولمستخلص الزنجبيل أيضا ثم تم خلط التركيز نصف مميت لأكثر ثلاثه زيوت نباتيه سميه للقواقع كلا على حدي مع التركيز نصف مميت لمستخلص الزنجبيل. أوضحت النتائج أن زيت الريحان والكافور وحبه البركه هم الأكثر سميه لأفراد قوقع موناكا كارتوسيانا حيث حققوا أقل قيم للتركيز النصف مميت وهي 11،17 و16،23 و20،11٪ على التوالي. بينما سجل مستخلص الزنجبيل تركيز نصف مميت يعادل 5،78٪ فقط. أظهرت النتائج أيضا أن للخلط الثنائي ما بين التركيز النصف مميت لزيت حبه البركه مع التركيز النصف مميت لمستخلص الزنجبيل أعلى تأثير على القواقع حيث سجل 100٪ موت لأفراد القوقع بعد يوم فقط من المعامله يليه الدمج الثنائي لنفس المستخلص عند نفس التركيز مع التركيز النصف مميت لزيت الكافور الذي حقق 66،66٪ موت للقواقع بعد نفس الفتره من التجربه بينما سجل الخلط ما بين مستخلص الزنجبيل و زيت الريحان 60 ٪ موت لأفراد القوقع بعد ثلاثه أيام من المعامله و ظل هذا المعدل ثابتا حتى نهايه التجربه و هكذا أشارت النتائج بوضوح إلى أن سميه المخاليط الثنائيه للزيوت النباتيه مع مستخلص الزنجبيل تفوق سميه كلا منهما عند إستخدامهما ضد القواقع بشكل فردي.