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In Vitro Comparative Study: Toxicity of *Calotropis procera*, *Nerium oleander* Extracts, Methomyl, and their mixtures on *Monacha obstructa*

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ABSTRACT: Ethanolic extracts of fresh Osher, *Calotropis procera* and Daflla, *Nerium oleander* leaves were assessed *in vitro* for their molluscicidal activity against the glassy clover snail, *Monacha obstructa* (Pfeiffer). The evaluation involved both contact and leaf dipping techniques over a 72 hrs. period. Additionally, combinations of each plant extract with methomyl as a chemical pesticide, were tested at a 1:1 ratio (v/v) in order to mitigate the harmful effects of methomyl while maintaining its high molluscicidal activity. Results demonstrated high toxicity of the plant extracts against the target snails, with the contact technique exhibiting greater efficacy than the dipping leaf method for both plant extracts and methomyl. The LC_{25} and LC_{50} values of *C. procera* via contact and leaf dipping methods were found to be (8.24 and 13.86) and (27.09 and 36.02) ppm, respectively. Similarly, sub-lethal concentrations of *N. oleander* achieved via the contact method were (12.55 and 19.84) ppm for LC_{25} and LC_{50}, respectively, while the leaf dipping application yielded LC_{25} and LC_{50} values of (20.83 and 29.25) ppm, respectively. Methomyl exhibited LC_{25} and LC_{50} values of (7.68 and 12.88) ppm via the contact method and (27.13 and 37.89) ppm for the leaf dipping method. Furthermore, mixing methomyl with either *N. oleander* or *C. procera* extracts resulted in varying rates of mortality among the tested snails, with different combinations leading to different mortality rates over specific time intervals.

Key words: Plant extracts; *Monacha obstructa*; *Calotropis procera*; *Nerium oleander*; Methomyl.

INTRODUCTION

Monacha obstructa (Pfeiffer) snails are terrestrial gastropods known as dangerous economic pests due to their wide-ranging attacks on horticultural and agricultural crops, particularly in high-humidity habitats, resulting in poor-quality vegetables and fruits which in turn causes economic losses (Carlsson *et al.*, 2004; Al-Sarar *et al*., 2012; El-Halim *et al*., 2021 and Emara *et al*., 2022). These snails are widely distributed and increased rapidly across Egyptian governorates (El-Halim *et al*., 2021 and Emara *et al*., 2023). While chemical control, particularly with methomyl, is effective against *Monacha obstructa*, it poses environmental hazards and affects non-target organisms (Edwards *et al*., 2009). To address these concerns, there is a need for less hazardous molluscicides with high efficacy, and natural plant extracts offer a promising solution. Plants contain bioactive organic chemicals, particularly secondary metabolites with defensive properties (El-Tantawy *et al*., 2012), including alkaloids, terpenoids, phenols, tannins, and glycosides, which have physiological or behavioral effects on land snails. In developing countries, the use of plant extracts is common due to their cost-effectiveness compared to synthetic pesticides (Abou-Hashem, 2012). Building on these considerations, this study examines the *in vitro* toxicological effects of two plant extracts, Osher, *Calotropis procera* and Daflla, *Nerium oleander*, on *Monacha obstructa*. Furthermore, it conducts a comparative analysis of the toxicity of these plant extracts and methomyl, a traditional chemical pesticide, and evaluates the impact of mixing sub-lethal concentrations of these materials on the mortality periods of tested snails.

MATERIALS AND METHODS

Tested Plants and Chemicals

Osher, *Calotropis procera* leaves were collected from Ismailia city, Ismailia

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Governorate, Egypt. Moreover, leaves of Daflla, *Nerium oleander* were obtained from Sers Ellyan, Menoufia Governorate, Egypt. Methomyl (Copter 90% SP) having a chemical formula; ($C_5H_{10}N_2SO_2$) was achieved from Egypt Chem International for Agrochemicals Company, Cairo, Egypt. The solvents and reagents used in this study were purchased from commercial sources and used as it was without any additional purification.

Crude Extracts Technique

Fresh green plant leaves were gathered from a public garden within the study area, subsequently washed with tap water, and left to dry in the shade at room temperature for approximately 10 days. Following drying, the leaves were finely ground into a fine powder using an electric blender mill. The extraction process, adapted from the methods described by Freedman *et al.* (1979), involved immersing the ground leaves in 70% ethanol. Each batch of 50 g of dried plant powder was combined with 500 ml of ethanol in brown-colored bottles fitted with light stoppers, and left to macerate for 7 days. An electric shaker was employed for two hours daily to agitate the bottles. Subsequently, the plant extracts were filtered off over anhydrous calcium chloride. A rotary evaporator was then utilized to remove the solvent, leaving behind semi-solid crude plant extracts. These extracts were weighed, transferred into glass vials, and stored in a freezer until required.

Tested Snails

During February, 2024, healthy adult individuals of the glassy clover snail, *Monacha obstructa*, were manually collected from untreated infested fields at Sers Ellyan Agricultural Research Station in Menoufia Governorate, Egypt. Following collection, the snails were transported to the laboratory using muslin sacks. To ensure acclimation, the collected snails were housed in plastic aerated cages filled with moist, optimal soil for two weeks prior to the commencement of the studies. During this acclimation period, the snails were provided with fresh green lettuce leaves for nourishment. Only adult *Monacha obstructa* snails of approximately equal size were selected for inclusion in the treatments.

Toxicity Tests

Contact Method

To determine the median lethal concentration of the compounds under investigation, the contact method was employed, following the protocol outlined by (Mourad, 2014). Stock solutions of the chemicals were prepared using a mixture of distilled water and dimethylformamide (DMF) in a ratio of 3:1 by volume. Four different concentrations of each compound; (10, 20, 30, and 40 ppm) were prepared, and a parallel control test was included. Each treatment comprised three replicates, with each replicate containing ten snails, and the experiments were conducted for 72 hrs.

Leaf Dipping Method

For the leaf dipping technique, four concentrations of the tested materials; (20, 30, 40, and 50 ppm) were prepared and applied. Fresh lettuce leaves with dimensions; (10×15 cm) were dipped in each concentration for three minutes and then left to dry under laboratory conditions, following the method outlined by (Ghamry, 1994). Subsequently, the treated leaves were placed inside plastic boxes dimensioned; (24×10×12 cm) filled with optimal soil and a piece of filter paper to absorb moisture. Each treatment comprised of three replicates, each replicate involved ten healthy adult *Monacha obstructa* snails. The snails were provided with treated leaves for a duration of 72 hrs., followed by untreated leaves for four successive days. Control snails were fed untreated leaves moistened with water.

Joint Action Tests

This experiment aimed to assess the effects of combination processes on the mortality period of the tested snails, where, individuals of *Monacha* *obstructa* were exposed to LC_{25} of plant extracts mixtures, both alone and in combination with methomyl at a ratio of 1:1 (v/v), using contact technique (Kandil *et al.*, 2014).

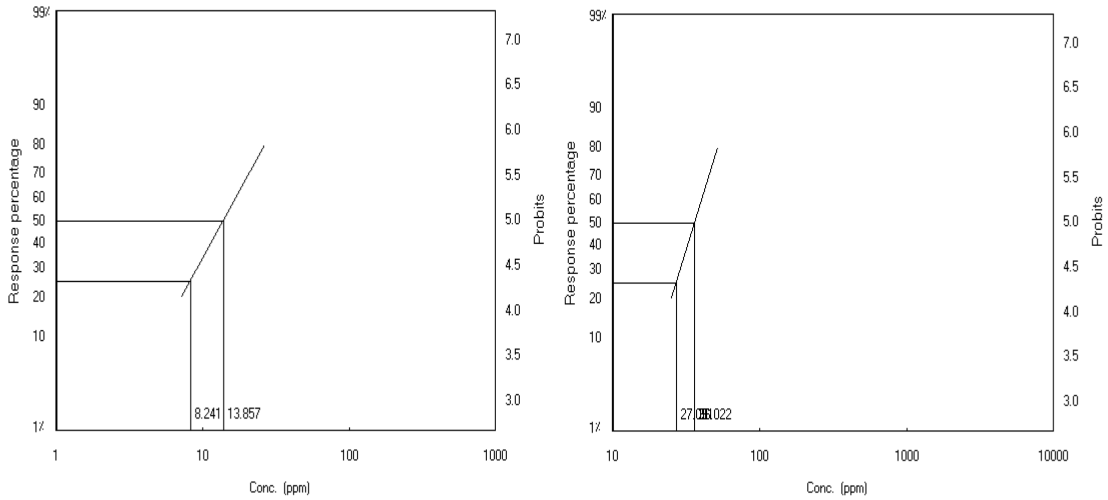
Statistical Analysis

Experiment protocols followed (Finney, 1971) to assess the sub-lethal concentrations of plant extracts in combination with methomyl, along with determination of their slope and fiducial limits. Outcome data were presented as (Mean ± S.E.). Subsequently, variance analysis (ANOVA) was conducted. Significance testing utilized the LSD method, comparing means at a probability of 0.05, as outlined by (Steel *et al.*, 1981).

RESULTS AND DISCUSSION

Toxicological Influence of *Calotropis Procera*

The results of laboratory treatments of *Monacha obstructa* with Osher, *Calotropis procera* are presented in Table (1) and shown in Fig. (1). The obtained data indicate that *C. procera* exerts a significant toxicological influence against the target snails, whether through contact or leaf dipping techniques. Specifically, the LC_{25} and LC_{50} of *C. procera* via the contact method are 8.24 and 13.86 ppm, respectively, with a slope ± S.E. = 2.99 ± 0.323. Furthermore, when applied through the leaf dipping technique, *C. procera* exhibits an LC_{25} of 27.09 ppm and an LC_{50} of 36.02 ppm with a slope ± S.E. = 5.45 ± 0.535 (Table 1).



Contact Assay **Leaf Dipping Assay**

Fig. 1. LC_{25} and LC_{50} of *Calotropis procera* against *Monacha obstructa.*

Tab1e 1. Toxicity of *Calotropis procera* against *M. obstructa* using contact and leaf dipping methods

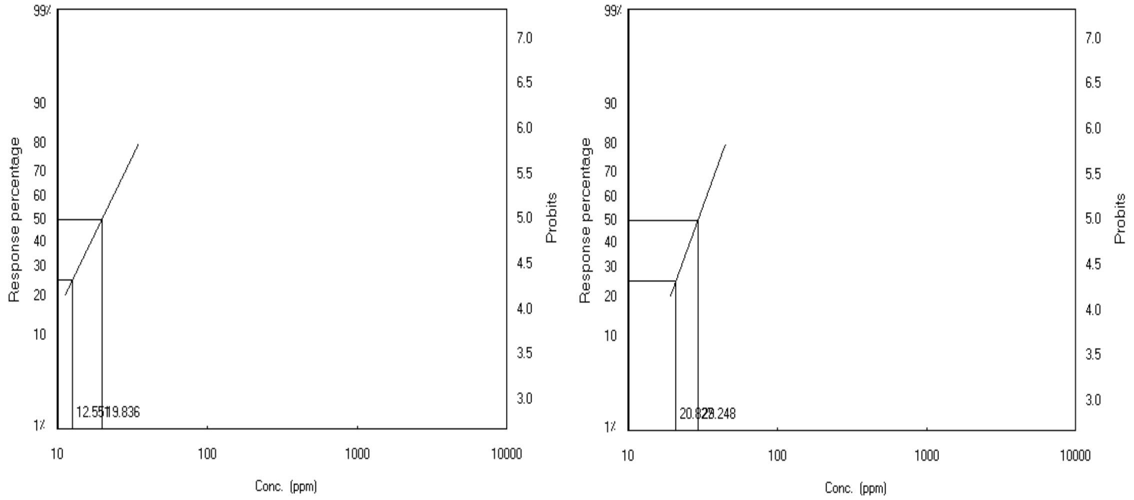
Technique	Concentrations (ppm)	Corrected Mortality (%)	LC_{25} (ppm)	LC_{50} (ppm)	Slope ± S.E.
Contact	10	33.33	8.24	13.86	2.99 ± 0.323
	20	66.67			
	30	88.89			
	40	88.89			
Leaf Dipping	20	11.11	27.09	36.02	5.45 ± 0.535
	30	33.33			
	40	44.45			
	50	88.89			

In the present study, it is evident that the contact method is more effective than the dipping leaf method. This finding aligns with the observations of Abdel-Kader *et al*. (2007), who investigated the efficacy of *C. procera* extract against two land snails, *Monacha cartusiana* and *Theba pisana*. The results indicated that the application of certain plant water extracts through spraying techniques was more efficient against land snails compared to incorporating the extracts into lettuce leaves as poisonous food or using the ground plant parts directly. Similar conclusions were also reported by El-Hwashy *et al*. (1996), who investigated the toxicity effects of six plant extracts on *Eobania vermiculata* snails in Egypt, including; Cauliflower (*Brassica oleracea*), Atma (*Pergularia tomentosa*), Khilla (*Ammi visnaga*), Radish (*Raphanus sativus*), Osher (*Calotropis procera*), and Datura (*Datura stramonium*). The preliminary screening of their molluscicidal activity revealed that three extracts, namely; Cauliflower, Atma, and Osher, exhibited high potency against snails when extracted with ethanol and tested using the residue film technique. Among these, the Atma extract demonstrated the highest activity, followed by Osher, while Cauliflower exhibited the least activity. These results are also in agreement with the findings of Hattan, (2004) that Osher plant had molluscicidal obvious effect against the land snail *Thepa pisana*. Hattan, (2004) mentioned that the areal parts of Osher plant include the following active ingredients; saponin, tannins, triterpenes, alkaloids, cardiac glycosides, and flavonoids.

Molluscicidal activity of *Nerium oleander* against *Monacha obstructa*

The toxic impact data of *Nerium oleander* extract on *Monacha obstructa* species after 72 hrs. of treatments are presented in Table (2) and displayed in Fig. (2). The results demonstrate the elevated toxicity of the tested plant extract against the target pests. Additionally, the toxic effect induced by the contact technique is more efficient than the leaf dipping method (Table 2). Sub-lethal concentrations achieved by applying the contact method are 12.55 and 19.84 ppm for LC_{25} and LC_{50}, respectively, with a slope ± S.E. of 3.39 ± 0.332. In contrast, the leaf dipping application established LC_{25} and LC_{50} values of 20.83 and 29.25 ppm, respectively, with a slope ± S.E. of 4.57 ± 0.484.

The obtained data are in agreement with Supriatno *et al.,* (2023) who stated that *N. oleander*, a toxic plant, has been identified as a potential solution for controlling significant agricultural pests. The effect of Oleander leaf extract on golden snail mortality is attributed to the presence of naturally produced toxic substances in plants. Oleander leaf extract significantly affects the mortality of golden snails due to the active compounds it contains (glycosides) such as oleandrin and neriin, which belong to the alkaloid group. Oleander leaf extract acts as an appetite suppressant (antifeedant) for golden snails. Additionally, Oleander extract exhibits toxicity that inhibits respiration, affects the nervous system, and serves as a repellent (Salim *et al.*, 2020). Strychnin, another toxic substance found in Oleander plants, contributes to its effectiveness. Furthermore, Oleander leaves contain polyphenolic compounds (up to 61%) and cinnamic acid, which function as antioxidants. These findings are consistent with Ahmed *et al*. (2023), who found promising results in their leaf phytochemical screening, which included alkaloids, terpenoids, saponins, glycosides, carbohydrates, and tannins. The toxicity of *N. oleander* targets pests with reproductive, digestive, or nervous systems (Zaid *et al.*, 2022).



Contact Assay **Leaf Dipping Assay**

Fig. 2. Laboratory LC_{25} and LC_{50} of *Nerium oleander* against *Monacha obstructa*

Tab1e 2. Toxicity of *Nerium oleander* against *M. obstructa* using contact and leaf dipping methods

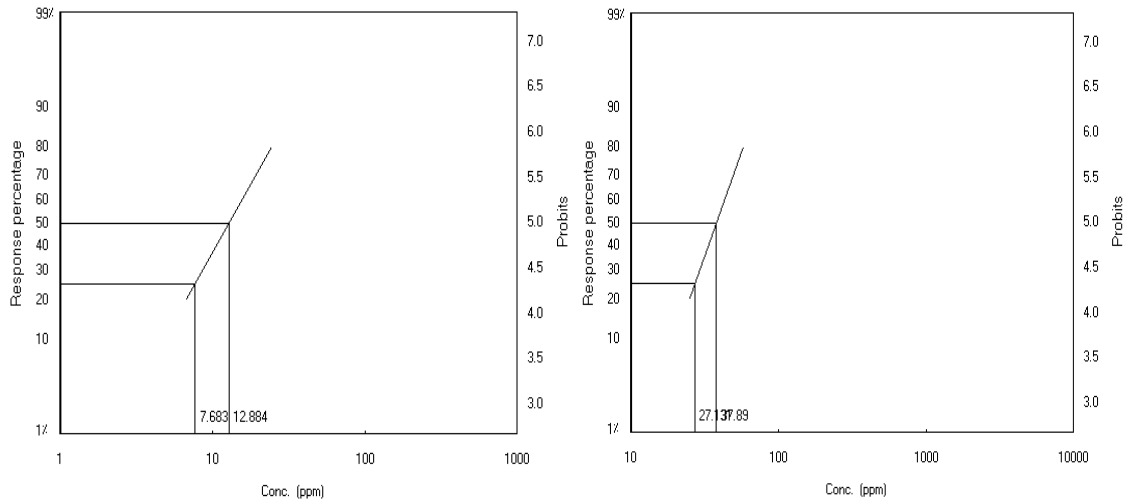
Technique	Concentrations (ppm)	Corrected Mortality (%)	LC_{25} (ppm)	LC_{50} (ppm)	Slope ± S.E.
Contact	10	22.22	12.55	19.84	3.39 ± 0.332
	20	33.33			
	30	77.78			
	40	88.89			
Leaf Dipping	20	22.22	20.83	29.25	4.57 ± 0.484
	30	55.56			
	40	66.67			
	50	88.89			

Toxic Impact of Methomyl against *Monacha obstructa*

The molluscicidal activity of methomyl on *Monacha obstructa* species was assessed after 72 hrs. of application using both contact and leaf dipping methods. Results are provided in Fig. (3) and Table (3). The chemical pesticide exhibits a high toxicity against target snails. Upon contact technique, methomyl displays LC_{25} and LC_{50} of 7.68 and 12.88 ppm, respectively, with a slope ± S.E. of 3.00 ± 0.329. However, dipping leaf method affords LC_{25} and LC_{50} of 27.13 and 37.89 ppm, respectively, with a slope ± S.E. of 4.65 ± 0.510. These findings demonstrate that methomyl is highly effective in controlling *Monacha obstructa* species. Notably, the contact method yielded significantly lower LC_{25} and LC_{50} values compared to the leaf dipping method.

The findings presented in this study align with those reported by El-Okda *et al*. (1989), who investigated the toxicity of various pesticides; (methomyl, aldicarb, oxyamyl, methiocarb, and metaldehyde) against a variety of land snail species; (*Helix sp., E .vermiculata, T. pisana, Rumina* sp.*, Cochlicella* sp.*, Helicella* sp.*, Limax* sp.,and *Oxychilus* sp.) using bait technique. They found that methomyl, oxamyl and aldicarb revealed the highest toxicity. However, metaldehyde and methiocarb showed lower toxicity. Ghamry *et al.* (1994) evaluated fourteen insecticides against two land snail species; *M. contiana* and *E. vermiculata*. Results from bait tests indicated the following effective pesticides for killing snails under laboratory conditions; methomyl, dithiocarb, carbaryl, chlorpyrifos and dimethoate. These insecticides exhibited effectiveness after 12 days of application, with similar results observed under field conditions. El-Okda *et al.* (1989) also evaluated the efficacy of formulated local 0.5% pesticides against various land molluscs. The tested pesticides included aldicarb, oxamyl, methiocarb, Lannate, and metaldehyde. The results showed that aldicarb, oxamyl, and Lannate exhibited the highest toxicity against a wide range of snail and slug species. In contrast, methiocarb and metaldehyde demonstrated lower toxicity levels. Gabr *et al*. (2006) studied the effectiveness of certain pesticides against adult stages of three land snail species; (*H. vestalis*, *M. contiana*, and *E. vermiculata*) under laboratory conditions using three testing methods; direct spray, dipping, and poisonous bait technique. They found that the toxicity of the tested compounds varied according to the method of application. These studies collectively provide insights into the efficacy of different pesticides especially, methomyl against various land snail species and highlight the importance of considering laboratory conditions in pesticide evaluation.

The collected data verify that the molluscicidal activities of the two plant extracts, along with methomyl, against *Monacha obstructa* species, intensified with increasing concentrations applied. A direct correlation was almost observed between the concentrations of the individual extracts and snails' mortality (Tables 1-3). This observation aligns with the findings of (Chauhan and Sigh, (2011), and El-Din *et al*. 2011), where increase in the concentration of plant extracts led to high mortality rates of various pest snails. Taguiling, (2015) similarly reported a positive correlation between the concentration of plant extracts; (*Sandoricum vidalii*, *Harpulia arborea*, and *Parkia sp.*) and the mortality of *P. canaliculata*. Additionally, a positive correlation between the concentrations of Neem seed crude extract and the mortality of *P. canaliculata* was observed (Massaguni *et al.*, 2015).



Contact Assay **Leaf Dipping Assay**

Fig. 3. Laboratory LC_{25} and LC_{50} of Methomyl against *Monacha obstructa*

Tab1e 3. Toxicity of Methomyl against *M. obstructa* using contact and leaf dipping methods

Technique	Concentrations (ppm)	Corrected Mortality (%)	LC_{25} (ppm)	LC_{50} (ppm)	Slope ± S.E.
Contact	10	33.33	7.68	12.88	3.00 ± 0.329
	20	77.78			
	30	88.89			
	40	88.89			
Leaf Dipping	20	11.11	27.13	37.89	4.65 ± 0.510
	30	33.33			
	40	44.45			
	50	77.78			

Combination between Plant Extracts and Methomyl

Combining stock solutions of two plant extracts at a 1:1 (v/v) ratio dilutes their individual concentrations. As a result, achieving 90% mortality of snails would likely necessitate higher concentrations. However, if the plant extracts demonstrate synergy despite this dilution, the concentration required for 90% mortality may be reduced. The present study discusses the susceptibility of *Monacha obstructa* snails to methomyl compared to two plant extracts, *N. oleander and C. Procera*. It explores the mortality rates of the snails after treatment with methomyl alone and in combination with the two investigated plant extracts over a period of 72 hrs. While the study demonstrates that methomyl is effective in causing mortality among the snails, it also acknowledges the hazardous nature of this chemical pesticide and its potential impact on non-target organisms. To mitigate these risks, the study proposes mixing methomyl with natural plant extracts to reduce its harmful effects while maintaining its molluscicidal activity. The results show that mixing methomyl with either *N. oleander* or *C. Procera* extracts led to varying rates of mortality among the snails, with different combinations resulting in different mortality rates over specific time intervals (Table 4). Where, the mixture of (methomyl + *N. oleander*) recorded 25.34% mortality after only 5 hrs. of treatment compared to 6 hrs. for (methomyl + *C. Procera*) combination and 10 hrs. for (*N. oleander* + *C. Procera*) mixture (Table 4). While the mixtures of (methomyl + *N. oleander*), (methomyl + *C. Procera*) and (*N. oleander* + *C. Procera*) led to 50.56% mortality after 10, 8 and 11 hrs. of treatments, respectively. However, 77.67% mortality of treated snails was achieved after 11, 12 and 36 hrs. of applications with (methomyl + *N. oleander*), (methomyl + *C. Procera*) and (*N. oleander* + *C. Procera*) combinations, respectively (Table 4).

Table 4. Mortality % of *M. obstructa* treated with (plant extracts + methomyl) mixtures

Corrected Mortality (%)	Mortality Time Intervals (hr.)		
	(Methomyl + *N. oleander*)	(Methomyl + *C. Procera*)	(*N. oleander* + *C. Procera*)
25.34	5	6	10
50.56	10	8	11
77.67	11	12	36

The outcome results are in agreement with the research findings reported by Taguiling, (2015) who reported that the combination of extracts of three species; (*S. vidalii fruit* and *barks of H. arborea* and *Parkia sp*.) at a ratio of 1:1:1 (w/w) was the most effective against golden apple snails under laboratory and field trials. Furthermore, the binary combination of (*S. vidalii* + *Parkia sp*.) and (H. *arborea* + *Parkia sp*.) recorded a mean mortality time of 8.11 min and 10.11 min, respectively (Taguiling, 2015). A similar synergistic effect was reported with the binary combination of ferulic acid and azadirachtin against *Fasciola larva* in the snail *Lymnaea acuminata*, which was 64.28 times more effective than a single treatment with ferulic acid (Sunita *et al*., 2013). Rao and Singh, (2001) reported that the synergistic action in binary and tri-herbal combinations of A. indica and Cedrus deodara oil (1:7 ratio) and A. indica, piperonyl butoxide, and C. deodara at a ratio of 1:5:7 were found to be more toxic to *L. acuminata* than single treatment. Similarly, the binary (1:1) and tri-herbal (1:1:1) combinations of *Euphorbia pulcherima* latex powder, botulin, and taraxerol decreased significantly the LC_{50} dosages against snail *L. acuminata* (Yadav and Singh, 2008).

Conclusion

This study highlights the effectiveness of the two investigated plant extracts, *C. procera and N. oleander* in combination with methomyl, as well as their binary combinations, against *Monacha obstructa*. Specifically, the research showcased a synergistic molluscicidal effect by selectively combining crude extracts of *N. oleander*, *C. procera*, and methomyl, effectively controlling *Monacha obstructa* species under laboratory conditions. Overall, the study suggests that incorporating methomyl with these plant extracts holds promise in amplifying its toxicity against *Monacha obstructa* snails while potentially mitigating its adverse environmental impacts. Further research is warranted to elucidate the underlying mechanisms and optimize their efficacy in agricultural settings.

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دراسة معملية مقارنة: تقييم سُمية مُستخلصات العُشار والدفلة مع الميثوميل على قوقع البرسيم الزجاجي

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الملخص العربي

في هذه الدراسة، تم تقييم النشاط القاتل للرخويات لمُستخلصات العُشار والدفلة المُستخلصة بالإيثانول من أوراق النباتات في المختبر باستخدام طريقتيّ المُلامسة وغمر الأوراق على مدار ٧٢ ساعة ضد قوقع البرسيم الزجاجي. بالإضافة إلى ذلك، تم إختبار سُمية المستخلصات النباتية مع الميثوميل، وهو مبيد حشري كيميائي، بنسبة ١:١ (حجم/حجم) للتخفيف من آثار الميثوميل الضارة مع الحفاظ على نشاطه العالي في قتل الرخويات. أظهرت النتائج سُمية عالية للمستخلصات النباتية ضد القواقع المُستهدفة، حيث أظهرت تقنية المُلامسة فعالية أكبر من طريقة غمس الأوراق لكل من المستخلصات النباتية والميثوميل. وقد وُجد أن قيم LC_{25} و LC_{50} لمُستخلص العُشار بطريقتيّ المُلامسة وغمر الأوراق هي (٨,٢٤ و١٣,٨٦) جزء في المليون و (٢٧,٠٩ و٣٦,٠٢) جزء في المليون على التوالي. وبالمثل، كانت التركيزات شبه المميتة لنبات الدفلة التي تم الحصول عليها بطريقة المُلامسة (١٢,٥٥ و١٩,٨٤ جزء في المليون) لـ LC_{25} و LC_{50} على التوالي، في حين أن تطبيق طريقة غمر الأوراق أعطى قيم LC_{25} و LC_{50} البالغة (٢٠,٨٣ و٢٩,٢٥ جزء في المليون) على التوالي. كما أظهر الميثوميل قيم LC_{25} و LC_{50} البالغة (٧,٦٨ و ١٢,٨٨ جزء في المليون) بطريقة المُلامسة و (٢٧,١٣ و ٣٧,٨٩ جزء في المليون) بطريقة غمر الأوراق. علاوة على ذلك، أدى خلط الميثوميل مع مُستخلصات الدفلة أو العُشار إلى معدلات متفاوتة للوفيات بين القواقع التي تم إختبارها مع مجموعات مختلفة تؤدي إلى معدلات وفيات مختلفة على فترات زمنية مُحددة.