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Original Article - Veterinary Medicine

Evaluation of *Nerium oleander* extracts against *Pachycondyla sennaarensis* (Hymenoptera, Formicidae) adults

[*Avaliação de extratos de* Nerium oleander *contra adultos de* Pachycondyla sennaarensis (*Hymenoptera, Formicidae*)]

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ABSTRACT

The Samsum (black) ant, *Pachycondyla sennaarensis* (Formicidae; Hymenoptera) is widely distributed throughout sub-Saharan Africa, and it is also the most common species of the family in southern Arabia. Samsum ant is very invasive and economically damaging and has become a big problem in Saudi Arabia and other distribution countries due to their aggressive nature, severe stings, and harm to agriculture, natural ecosystems, and human health. The physicochemical properties and varied effects against insect pests make plant extracts a potential alternative in the development of pesticides. In this study, the *Nerium oleander* leaves extract toxicity effects against *P. sennaarensis* adults. Exposure of *P. sennaarensis* adult to the oleander leaf extracts produced 96.7% mortality in the insecticidal bioassay, especially at 40% concentration. The mortality % ranged from 3.3-96.7% after 48hrs. The mortality percentage of the Samsum ant adults decreased by increasing exposure periods. The mortality % was highly negatively correlated with exposure times (R= - 0.80 to - 0.94; P = 0001) at 40 to 10% concentrations, respectively. The overall results of the current study suggest that the leaf extract of *N. oleander* may possess potential insecticidal properties, which could potentially be employed in pest management. The GC-MS investigation uncovered that *N. oleander* leaf extracts numerous bioactive compounds associated with plant secondary metabolites with their retention time (RT), and peak area %. We conclude that the *N. oleander* leaf extracts have the potential to be useful in managing insect pests, particularly Samsum ants, but that they must be handled and applied with extreme caution.

Keywords: oleander, bioassay, samsum ant, mortality, insecticidal

RESUMO

A formiga Samsum (preta), Pachycondyla sennaarensis (Formicidae; Hymenoptera), é amplamente distribuída na África subsaariana e é também a espécie mais comum da família no sul da Arábia. A formiga Samsum é muito invasiva e economicamente prejudicial e se tornou um grande problema na Arábia Saudita e em outros países de distribuição devido à sua natureza agressiva, picadas graves e danos à agricultura, aos ecossistemas naturais e à saúde humana. As propriedades físico-químicas e os efeitos variados contra pragas de insetos tornam os extratos de plantas uma alternativa em potencial para o desenvolvimento de pesticidas. Neste estudo, os efeitos de toxicidade do extrato de folhas de oleandro contra adultos de P. sennaarensis. A exposição de adultos de P. sennaarensis aos extratos de folhas de oleandro produziu 96,7% de mortalidade no bioensaio inseticida, especialmente na concentração de 40%. A porcentagem de mortalidade variou de 3,3 a 96,7% após 48 horas. A porcentagem de mortalidade dos adultos da formiga Samsum diminuiu com o aumento dos períodos de exposição. A porcentagem de mortalidade foi altamente correlacionada negativamente com os tempos de exposição (R= -0,80 a -0,94; P=0001) em concentrações de 40 a 10%, respectivamente. Os resultados

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gerais do estudo atual sugerem que o extrato da folha de Nerium oleander pode ter propriedades inseticidas em potencial, que poderiam ser empregadas no controle de pragas. A investigação de GC-MS revelou que a folha de N. oleander extrai vários compostos bioativos associados a metabólitos secundários de plantas com seu tempo de retenção (RT) e área de pico %. Concluímos que os extratos de folhas de N. oleander têm o potencial de serem úteis no manejo de pragas de insetos, especialmente formigas Samsum, mas devem ser manuseados e aplicados com extrema cautela.

Palavras-chave: oleandro, bioensaio, formiga samsum, mortalidade, inseticida

INTRODUCTION

Samsum ant, *Pachycondyla sennaarensis* (Mayr, 1862), is a species of ant belonging to the family Formicidae (Nikbakhtzadeh *et al*., 2009). Samsum ants, also called the Sennaar ants, are a species recognized for their inclination to cause harm to crops and disrupt the equilibrium of natural ecosystems (Al-Khalifa *et al*., 2015). *Pachycondyla* is a diverse and worldwide ant genus recognized for its interesting activities and remarkable species variety. While numerous *Pachycondyla* spp. are found on other continents, *P. sennaarensis* is a well-known example of desert ants on the Arabian Peninsula (Sturm *et al*., 2023).

Samsum ant is very invasive and economically damaging and has become a big problem in Saudi Arabia (Collingwood *et al*., 2004). These ants are notorious for their aggressive nature, severe stings, and harm to agriculture, natural ecosystems, and human health (Nikbakhtzadeh *et al*., 2009). The Samsum ant has been identified as a causative agent in incidents of anaphylaxis worldwide (Klotz *et al*., 2005). The envenomation produced by ants often elicits pain, localized inflammation, itching, and irritation in humans. However, in some cases, it may also give rise to severe allergic responses. These reactions are induced by a varied combination of structurally different chemicals (Hoffman, 1996; Steen *et al*., 2005). The use of chemical pesticides is often employed as a primary approach in managing populations of *P. sennaarensis*. However, it is important to acknowledge that these pesticides might potentially yield negative consequences for non-target species in the surrounding environment (Douglas, 2010; Main *et al*., 2020).

Nerium oleander has poisonous properties that may be harmful to humans, animals, and insects (Farkhondeh *et al*., 2020). The botanical specimen encompasses a diverse array of cardiac glycosides, such as oleandrin, and others, which possess toxicity against several insect species (Ceci *et al*., 2020; Ayouaz *et al*., 2023). Several studies have investigated the insecticidal properties of *N. oleander* extracts against a wide range of insect species. According to the results of a study, *N. oleander* leaves extracts had substantial insecticidal action against the cotton bollworm, *Helicoverpa armigera*. The results of the study found that the extracts killed the larvae and slowed their growth and development (Sivakumar *et al*., 2022). Additionally, another study looked at the effects of *N. oleander* leaves extracts on the larvicidal *Culex quinquefasciatus* the results were positive (Raveen *et al*., 2014). The Oleander leaves extracts reduced physiological and reproductive fitness in the cotton bollworm, *H. armigera* (Sivakumar *et al*., 2022).

In recent years, there has been an increasing inclination to investigate alternate approaches to pest control that prioritize environmental friendliness and sustainability (Tal, 2018). One such scientific inquiry has focused on the possible use of plant-derived chemicals and extracts as biopesticides (Ferraz *et al*., 2022). The plant species *N. oleander* is extensively spread and is recognized for its poisonous characteristics. It has a diverse range of secondary metabolites, such as alkaloids, cardenolides, and terpenoids, which have been the subject of research due to their possible insecticidal effects (Bandara *et al*., 2010; Sinha and Biswas, 2016; Garima and Amla, 2010). Oleandrin and digitoxigenin, two of its bioactive ingredients, are of special interest owing to their poisonous qualities and possible uses in pest control (Shridhar, 2022). The oleander plant a widespread and plentiful plant in Saudi Arabia, has a long history of usage in traditional medicine and a diverse spectrum of bioactive chemicals, some of which have insecticidal activities against a variety of pests (Zaid *et al*., 2022; Qari *et al*., 2021).

Traditional *P. sennaarensis* management measures, such as chemical insecticides, often cause environmental and health risks. As a result, there is an urgent need for ecologically acceptable and sustainable options for managing these invading ants. So, the present study seeks to evaluate the effect of *N. oleander* extracts against *P. sennaarensis* adults.

MATERIALS AND METHODS

The black ant adults *P. sennaarensis* were gathered from Ad Diriyah governorate in Riyadh, Saudi Arabia. The collected adults were transferred to the Zoology Department of the College of Sciences, King Saud University, in July 2023. The ants were kept in small plastic jars at room temperature (26±2°C; 60-70% RH) for one hour. To prevent escape, the plastic jars were fitted with perforated covers featuring small holes.

Fresh leaves of *N. oleander* were harvested in December 2022 from Modhi Park, located in the Ad Diriyah governorate of Riyadh, Saudi Arabia (24°45'02.2"N 46°34'40.8" E). The plant's identity was verified by a botanist at the herbarium of the Botany Department, College of Sciences, King Saud University, Riyadh, Saudi Arabia. To prepare the collected oleander leaves, they were thoroughly rinsed with distilled water and subsequently air-dried in a shaded area for two weeks. This drying process was further complemented by placing the leaves in a hot air oven set at 25°C for 30 minutes to eliminate any residual moisture. Following this, the dried leaves were transformed into a fine powder using an electric grinder (Senses, MG-503T, Korea). The powdered material was subjected to an ultrasound-assisted extraction process, where it was steeped in 70% methanol for 24 hours. This method was employed to facilitate the extraction of a diverse array of active compounds from the concoction. Additionally, maceration extraction was conducted on 200 grams of the dried oleander leaves powder at a temperature of 4°C, with the mixture being percolated multiple times (2-5 times) to ensure a comprehensive extraction. A vacuum extraction process was carried out at room temperature, following established methods from previous studies (Mu *et al*., 2012; Borjigidai *et al*., 2014; Teng and Choi, 2014). The extracted material was then filtered through two layers of filter paper, collected, and concentrated using a rotary vacuum evaporator (Yamato RE300, Japan) at 50°C under reduced pressure. Before application, the extract's concentration was adjusted by dilution with ethanol to reach a final concentration of 0.5mg/mL. Once the crude extract was obtained, it was lyophilized and stored at -20°C until needed for further use.

The process known as gas mass–chromatography spectrometry (GC–MS) was run out with the assistance of a piece of equipment known as a 7000D Triple Quad GC–MS (Agilent Technologies, Thermo Scientific, Austin, Texas, United States). In Miami, California, in the United States, a Thermo Scientific Trace GC Ultra and an ISQ single quadruple MS were utilized. Both the Agilent 7890A and the Agilent 5975C inert XL EI/CI MSDs come equipped with a solitary quadrupole mass analyzer. The capillary employed was an HP-5MS UI (Ultra Inert) model with the following specifications: a length of 25m, an inner diameter of 0.25mL, a film thickness of 0.25mL, a stationary stage containing 5% phenyl, and low-polar methylpolysiloxane. The split ratio was 50:50, the temperature was 250°C, and the carrier gas was helium. The flow rate was 1 ml/minute, and the temperature was 250°C. After running the GC-MS at 30 degrees Celsius for 5 min at a ratio of 5 counts/min (Co/min), the temperature was raised to 250°C for 40min. The temperature of the transfer line was set to a maximum of 250°C. In the MS conditions, the ionization energy was measured to be 70 eV. We utilized the full scan detection mode, and the mass range we looked at was from 50 to 500 Da. One minute passed during the solvent delay. Methanol was the liquid used to dissolve the sample. To identify the compounds, the Wiley 9 database, the answers, and the libraries were utilized. Name, molecular weight, molecular formula, and peak area were the metrics that were utilized in the analysis of the test substance's constituent parts.

Hep-G2/2.2.15 Human Hepatoblastoma Cell Line were obtained from Sigma-Aldrich Chemie GmbH (Taufkirchen - Germany). The cell lines were cultivated in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% Fetal Bovine Serum (FBS) in a humid environment with 5% CO_2 at 37°℃.

MTT Assay (3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium Bromide, cat#475989-1GM, Sigma-Aldrich, Germany) was utilized to determine the viability of cells as well as the rate at which cells were growing. In a nutshell, aliquots of 120 of the suspended cells ($5x10^4 mL^{-1}$) were supplied to 60 ng/mL of a serial dilution of the *N. oleander* Leaves extract in a 96-well plate. This was done so that the concentration of the *C. procera* Leaves extract could be monitored. After an initial period of incubation lasting three days, twenty microliters of MTT solution were added to each well, and the cells were cultured for an additional two hours after that. Crystals of formazan were dissolved in an isopropanol solution. Using a microplate reader made in the United States by BioTek, the intensity of the color that was produced was measured at 595 nm. The percentage of viable cells was determined using the following formula:

Cell Viability (%) = Mean absorbance [treated cells/untreated cells] × 100

The IC_{50} values (concentration of extract that triggered 50% inhibition) were specified from the dose-response curve of cell viability percent using OriginPro software.

Active tested adults were meticulously sorted using a camel hairbrush and placed into individual small plastic jars. These ants were subsequently distributed into Petri dishes with a diameter of 9cm for testing. Five different concentrations of *N. oleander* leaves extracts in 70% Methanol (5, 10, 20, 30, and 40%) were prepared, each replicated three times. For each concentration, ten individuals were subjected to the bioassay. In the control group, insects were treated solely with distilled water. The treated adults were then confined within Petri dishes with a diameter of 9 cm, maintaining a consistent room temperature of 26±2°C and relative humidity between 60-70%. Within these Petri dishes, a piece of filter paper soaked with 1 mL of the respective extract concentration was placed, following a method outlined by (Hameed *et al*., 2012) with specific modifications. The assessment of mortality percentage was conducted at regular intervals: 12, 24, 36, and 48 hours post-treatment. These recorded mortalities provided valuable data for the evaluation of the insecticidal efficacy of the oleander leaves extracts under various concentrations and exposure times.

Before the analysis, the mortality data were transformed using a log (y + 1) transformation to normalize the data. A completely randomized design (CRD) was used for the bioassay analysis. One-way analysis of variance (ANOVA) SPSS Statistics was used. Duncan's Multiple Range test ($p \leq 0.05$) was used to compare mortality means. All the mortality data are presented as mean ± standard error (SE). Furthermore, a linear correlation was used to explore the relationship between the duration of exposure and mortality percentage.

RESULTS

The GC-MS investigation uncovered in the *N. oleander* extracts leaves methanolic extract several bioactive compounds associated with plant secondary metabolites with their retention time (RT), and peak area %. The components most available and potentially effective are as follows: high be 3-O-Methyl-d-glucose, almost 69.8%, 5-Hydroxymethylfurfural 15.65%. Oleic Acid 5.85%, Linoleic acid 2.97%, and n-Hexadecanoic acid 1.14%. (Table 1 and Fig. 1).

The results of the cytotoxicity tests conducted on the extract at a range of concentrations are shown in Figure 2, where they are provided along with the findings about the extract's cytotoxicity. As part of the experiment, hepatoblastoma cell line Hep-G2 cells were subjected to an extract at a range of doses, including 1, 12.5, 25, 50, 100, 200, 400, and 800 µg/mL. At these doses, the survival rates of the cells were determined to be 99.65, 81.00, 73.41, 63.12, 53.17, 34.99, 17.98, and 5.39% correspondingly. The half-maximal inhibitory concentration (IC_{50}) for Hep-G2 was determined to be 44.2+/-0.007g/mL. It can be demonstrated in Figure 2 that the percentage of cancer cells that managed to survive was lowered when the concentration of the extract was raised.

A range of Oleander extract concentrations (5, 10, 20, 30, and 40%) were selected based on previous studies (Al-Ansi *et al*., 2024). The mortality percentage of *P. sennaarensis* adults to 5, 10, 20, 30, and 40% concentrations of the Methanol extracts of oleander leaves, and control is shown in Figure 3. The results showed a significant effect of most oleander extract

concentrations on adults' mortality (P<0.05). The obtained results of this study showed that all concentrations of oleander leaves extracts have an insecticide effect on *P. sennaarensis* mortality percentage. The results revealed that the mean mortality among tested individuals of *P. sennaarensis* varied depending on exposed time and oleander extract concentrations used in the current study. The results indicated that the mortality percentage ranged from 10% after 12 hours at 5% concentration up to 96.7% after 48 hours at the 40% concentration. The highest average mortality % reached 83.3% at 40% concentration after 12hrs, 66.7% at 30% concentration after 12hrs, 46.7% at 20% concentration after 12hrs, and 40% at 10% concentration after 12hrs (Table. 2). All concentrations showed a significant effect on the black ant mortalities after 12hrs of exposure time. The study results indicated that there are significant effects of mortality percentage between concentrations (P<0.05) (Table 2; Figure 3). The mean mortality % ranged from 40% at 5% concentration and 96.7% at 40% concentration after 48 hours (Table 2). No mortality was observed in control for three intervals of exposed time except after 48 hours. The mortality % ranged from 6.7% at 5 and 40% concentrations and 33.3% at 20% concentration after 24hrs. The mortality percentage ranged from 0.0% at 10% concentration and 20% at 5% concentration after 36hrs.

Table 1. GC-Mass analysis to identification of phytochemical components biologically active in methanolic leaves extracts

$t_{R\ (min)}$	Proposed compound	MW	Formula	Peak area%
6.49	3-Methoxycarbonylpyrazole	126	$C_5H_6N_2O_2$	0.94
7.48	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	144	$C_6H_8O_4$	0.86
8.98	5-Hydroxymethylfurfural	126	$C_6H_6O_3$	15.65
13.47	2,5-Dimethoxy-4-ethylamphetamine	223	$C_{13}H_{21}NO_2$	0.815
14.08	Megastigmatrienone	190	$C_{13}H_{18}O$	0.205
14.23	Cyclopenta[1,3]cyclopropa[1,2]cyclohepten-3(3aH)-one, 1,2,3b,6,7,8-hexahydro-6,6-dimethyl-	190	$C_{13}H_{18}O$	0.65
15.56	3-O-Methyl-d-glucose	194	$C_7H_{14}O_6$	69.8
17.88	n-Hexadecanoic acid	256	$C_{16}H_{32}O_2$	1.14
18.03	Mandelic acid, 3,4-dimethoxy-, methyl ester	226	$C_{11}H_{14}O_5$	0.43
19.62	Linoleic acid	280	$C_{18}H_{32}O_2$	2.97
19.67	Oleic Acid	282	$C_{18}H_{34}O_2$	5.85
19.85	Octadecanoic acid	284	$C_{18}H_{36}O_2$	0.55
24.68	Pregn-5-en-20-one, 3-hydroxy-	316	$C_{21}H_{32}O_2$	0.18

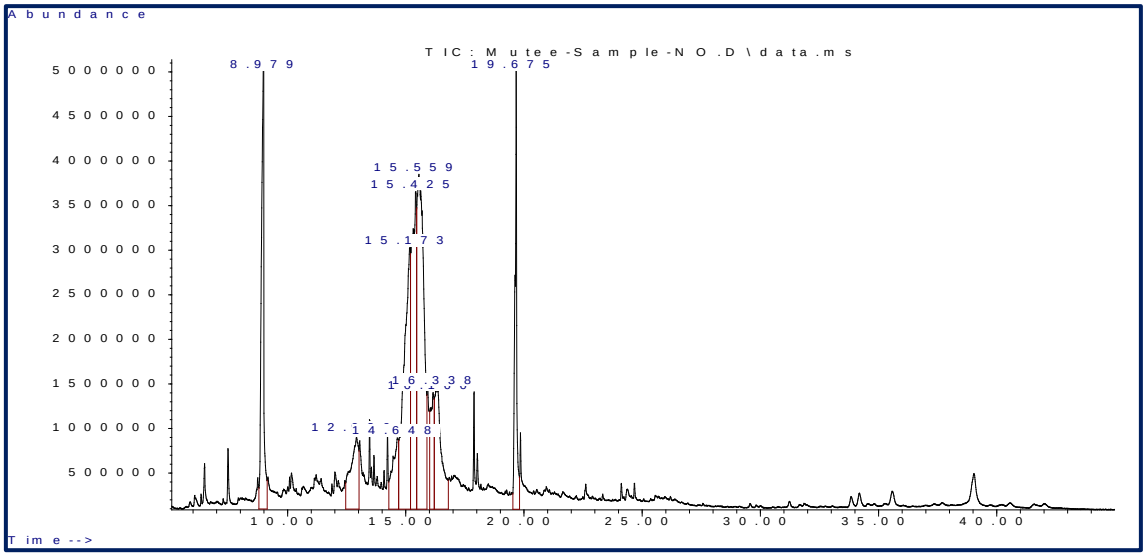

Figure 1. Infrared spectroscopy of *Nerium oleander* extract leaves methanolic.

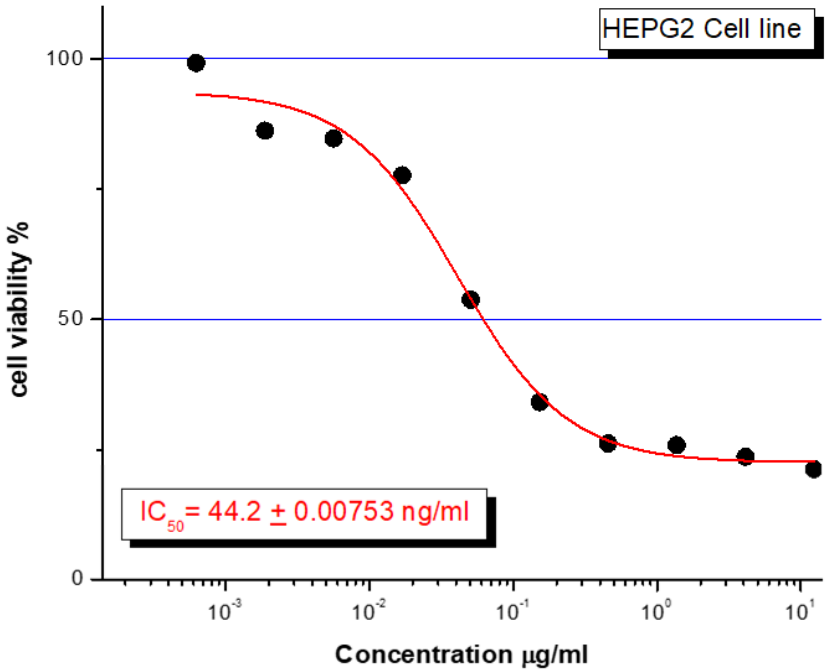

Figure 2. The effect of different doses of *Nerium oleander extract leaves* on the cytotoxicity and subsequent survival of the HT29 cell line. HT-29: Human Colorectal Adenocarcinoma Cell Line.

The oleander extract at 5% concentration caused mild ant mortality, where at 12 hours, 10% of ants died. This effect slightly decreased to 6.7% at 24 hours but increased to 20% after 36 hours. By 48 hours, mortality returned to the baseline at 3.33%. The extract exhibited moderate effectiveness, especially at the 36-hour mark (Table 2). At 10% concentration, the extract had a more significant impact, causing 40% mortality at 12 hours and 30% mortality at 24 hours. However, the extract lost its effectiveness entirely after 36 and 48 hours, resulting in no

mortality. The 20% concentration of oleander extract caused substantial initial mortality, 46.7% at 12 hours and 33.3% at 24 hours. The effectiveness waned, leading to 3.33% mortality at 36 hours and no mortality at 48 hours. Overall, the oleander extracts at this concentration caused 83.3% mortality. At a 30% concentration, the extract was highly effective initially and gave 66.67% mortality at 12 hours. However, its effectiveness decreased significantly, resulting in only 10% mortality at 36 hours and no mortality after 48 hours. In general, this concentration caused 90% mortality. The highest concentration (40%) led to significant initial mortality, causing 83.33% mortality after 12 hours. The extract retained some effectiveness, causing 3.33% mortality at 48 hours (Table 2).

In the current study, the effectiveness of extract concentrations was increased with increasing exposure time (Figure. 3). 40% concentration revealed the highest insecticidal activity with 96.7% mortality after 48hrs. The 10, 20, and 30% concentrations also demonstrated insecticidal activity, with 70, 838.3, and 90% individual mortality after 48hrs. The 5% concentration showed the lowest insect mortality 40% after 48hrs. Overall, no significant differences (P=0.05) were observed between all concentrations after 36, and 48hrs. The obtained results showed significant differences between the mortality mean of 40% concentration after 12 hours and 10% concentration after 24hrs. . Also, there were significant differences between 5, 30, and 40% concentrations and at 10, and 20% concentrations after 24hrs, respectively. Moreover, significant differences were observed between concentrations after 12hrs, especially at 40% and other concentrations (Table 2).

The oleander extract exhibits concentration-dependent effects, with higher concentrations leading to higher initial mortalities. The effectiveness of the extract diminishes over time, indicating a short-lived impact on *P. sennaarensis*. There appears to be a concentration threshold (around 30%) beyond which the effectiveness significantly drops after the initial hours. These results suggest that oleander extract while showing promise as an insecticidal agent against these ants, has limitations in terms of sustained effectiveness over longer durations. Further studies are warranted to explore the reasons behind the diminishing effectiveness and to optimize the concentration and application methods for practical pest management strategies.

Table 2. Means of mortality % (M ± SE) of oleander extracts at various exposure periods (12, 24, 36, and 48hrs and total mean) against *Pachycondyla sennaarensis*

Concentration (%)	Mortality (%)				Total mean
	12hrs	24hrs	36hrs	48hrs	
Control	0± 0^{c}	0± 0^{c}	0± 0^{c}	3.3±0.3^{c}	3.3±0.1^{c}
5%	10±0.5^{c}	6.7±0.5^{c}	20±1.6b^{c}	3.3±0.3^{c}	40±0.5^{b}
10%	40±0.9^{b}	30±2.1^{b}	0± 0^{c}	0± 0^{c}	70±0.8^{ab}
20%	46.7±1.1^{b}	33.3±2.0^{b}	3.3±0.3^{c}	0± 0^{c}	83.3±0.8^{ab}
30%	66.7±1.0^{b}	13.3±0.7^{c}	10±0.5^{c}	0± 0^{c}	90±0.8^{a}
40%	83.3±1.4^{a}	6.7±0.5^{c}	3.3±0.3^{c}	3.3±0.3^{c}	96.7±1.1^{a}

The means with different lowercase letters in columns or rows are significantly different (p<0.05). Those with the same lowercase letters are not significantly different (p>0.05) based on Duncan's Multiple Range Test following ANOVA. Each value is expressed as mean ± standard error.

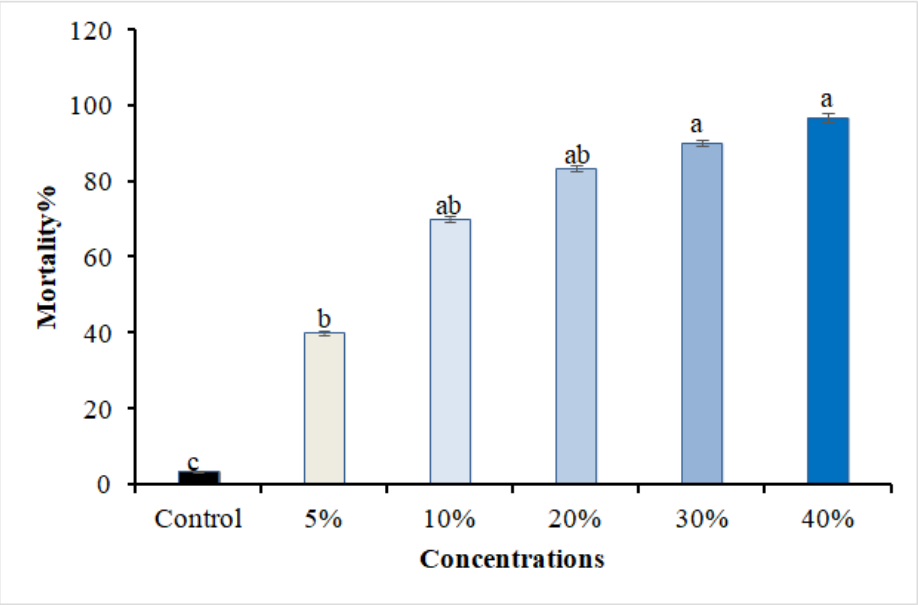

Figure 3. Total mean (M±SE) of mortality % of *Pachycondyla sennaarensis* adults at various concentrations. Values with different lowercase letters are significantly different (p<0.05). Those with the same lowercase letters are not significantly different (p<0.05) based on Duncan's Multiple Range Test (p<0.05).

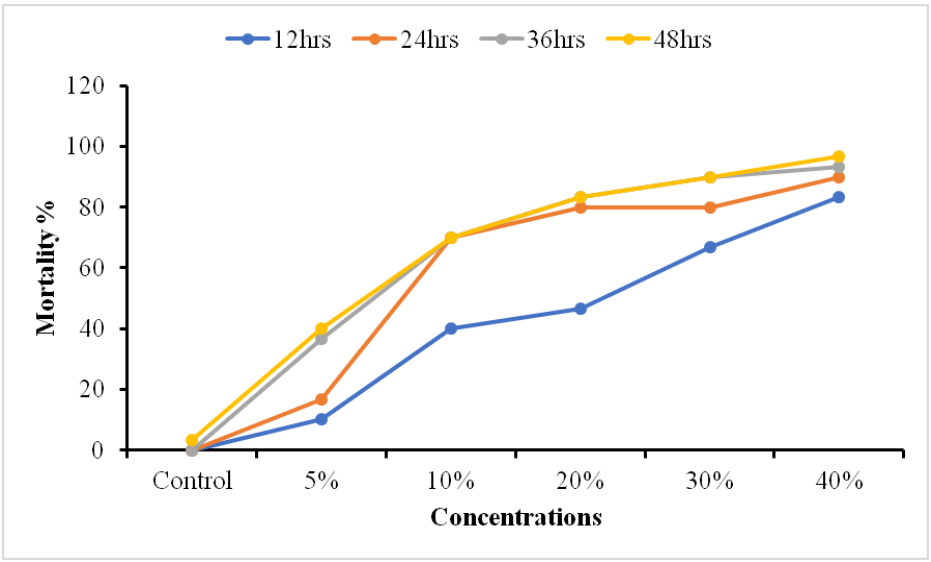

Figure 4. Mortality % of *Pachycondyla sennaarensis* assayed with leaves oleander extracts at various exposure periods (12, 24, 36, and 48hrs).

The mortality percentage of black ant adults decreased with increasing exposure periods (Figure 5). The mortality % was highly negatively correlated with exposure times (R= −0.94; R=-0.93; -0.90; -0.80 and p=0001) at 10, 20, 30, and 40% respectively (Figures 5; 6; 7; and 8). Whereas the correlation coefficient at 5% was weak negatively (R= −0.10, and P=0.0001). The overall results of the current study suggest that the leaves extracts of *N. oleander* may possess potential insecticidal properties, which could potentially be employed in pest management.

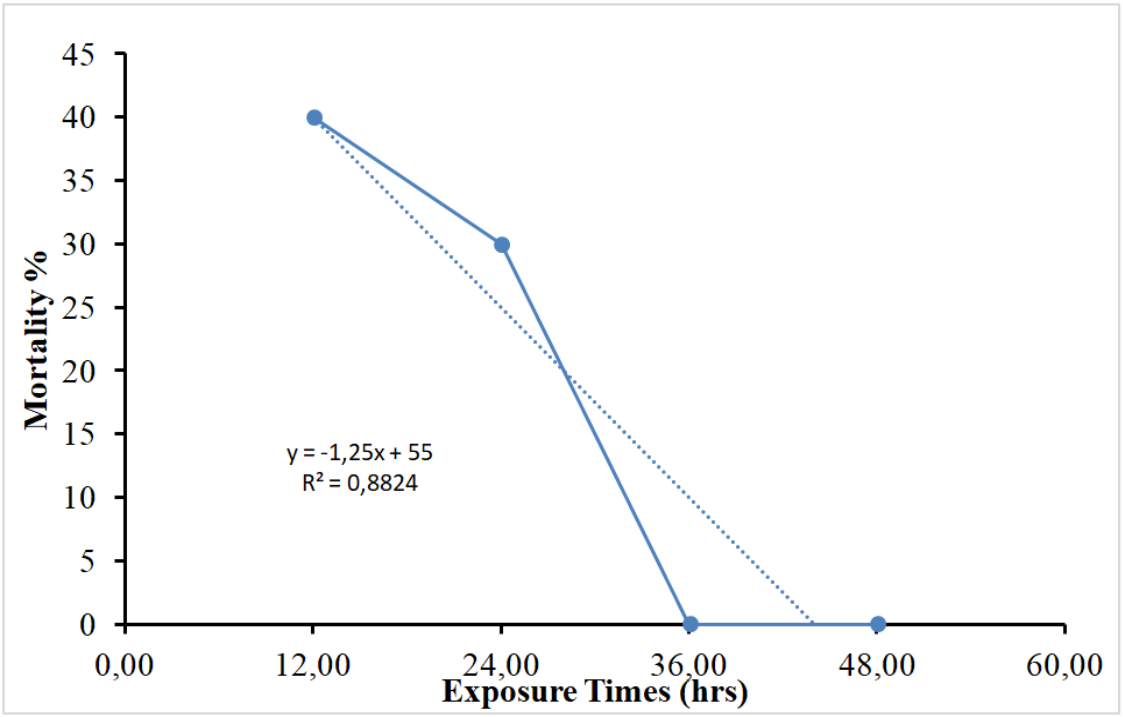

Figure 5. Mean mortality % of *Pachycondyla sennaarensis* adults at 10% concentration for various exposure times (hrs).

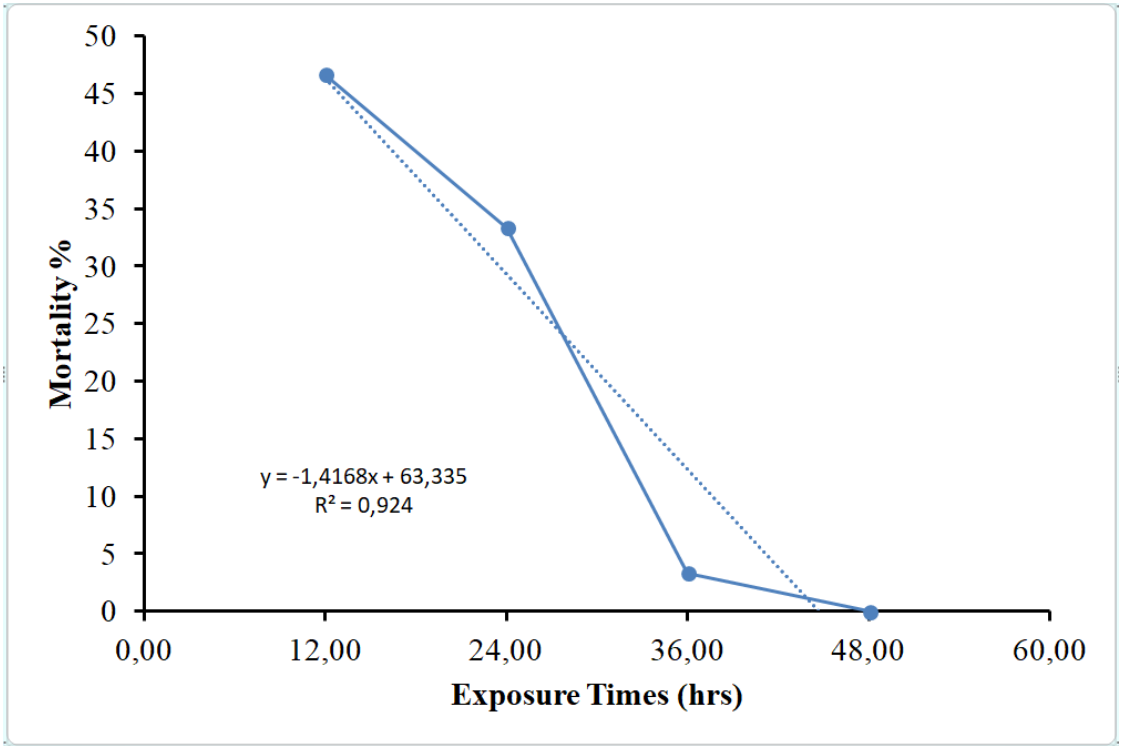

Figure 6. Mean mortality % of *Pachycondyla sennaarensis* adults at 20% concentration for various exposure times (hrs).

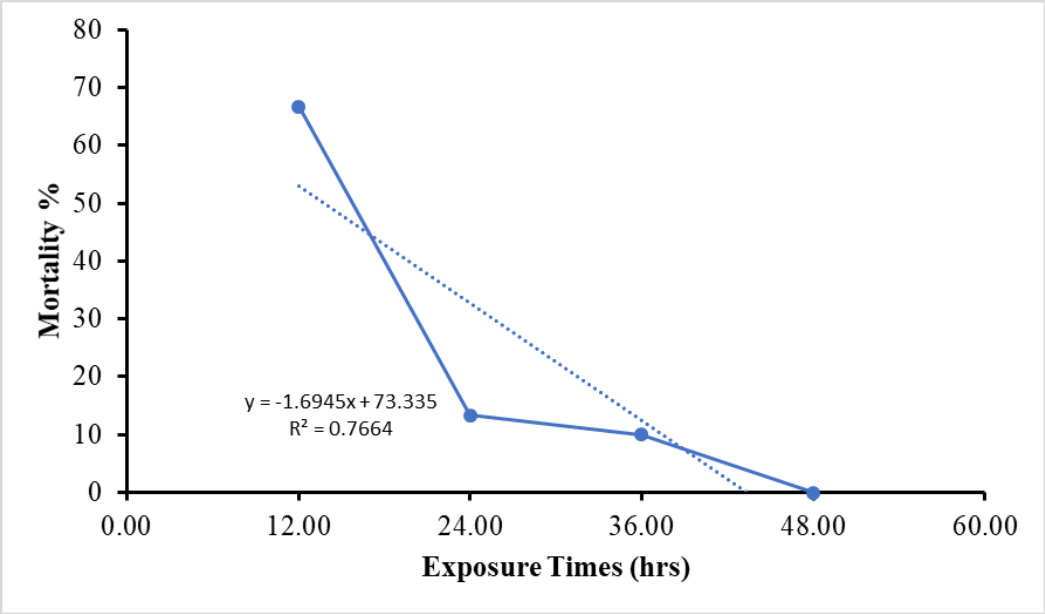

Figure 7. Mean mortality % of *Pachycondyla sennaarensis* adults at 30% concentration for various exposure times (hrs).

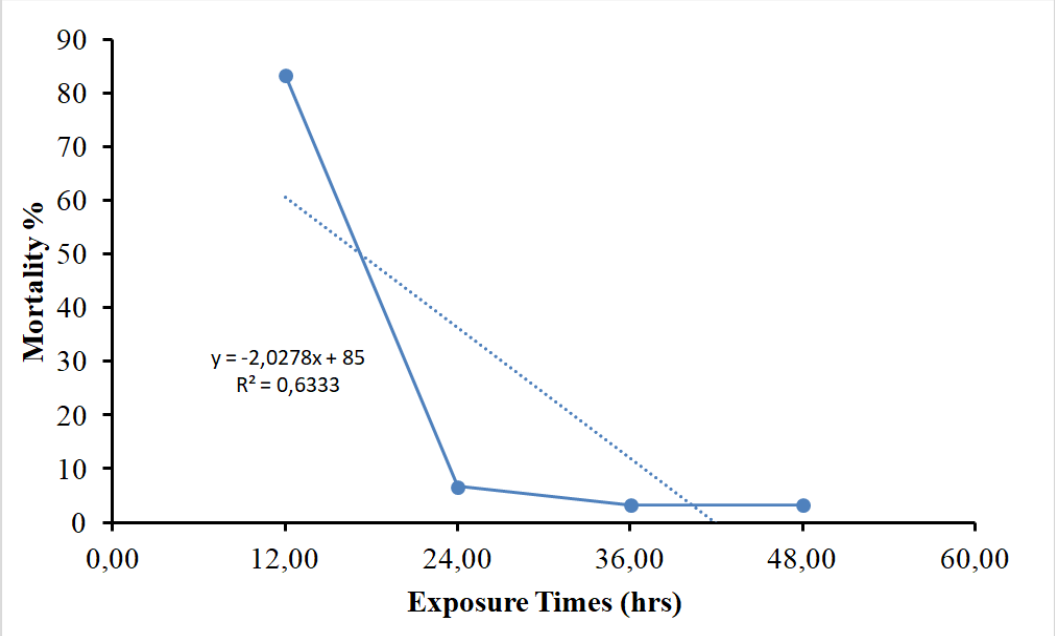

Figure 8. Mean mortality % of *Pachycondyla sennaarensis* adults at 40% concentration for various exposure times (hrs).

DISCUSSION

The study provided investigates the potential insecticidal properties of *N. oleander* extracts against the Samsum ant (*P. sennaarensis*), which is a highly invasive and economically harmful species in Saudi Arabia and other distribution regions. The research is significant because of the ants' aggressive behavior, their influence on agriculture, natural ecosystems, and human health, as well as the possible hazards linked with chemical pesticides that have historically been used to control them.

A study of the phytochemicals in the *N. oleander* extract found that it had several bioactive compounds. These compounds, such as 3-O-Methyl-d-glucose, 5-Hydroxymethylfurfural, and oleic acid, could be responsible for the insecticidal properties observed in this study. These results are consistent with study results on groundnut shell extracts (Arumugam *et al*., 2022) and include alkaloids, cardenolides, and terpenoids, which have been studied due to their possible insecticidal effects (Bandara *et al*., 2010; Sinha and Biswas, 2016; Garima and Amla, 2010).

The study revealed that the application of oleander leaves extracts had a substantial effect on the death rate of Samsum ant adults, especially when used at higher concentrations. The results demonstrated a concentration-dependent effect of oleander extract on *P. sennaarensis*. These findings suggest that extracts from *N. oleander* possess insecticidal capabilities. The results align with other studies that have shown the insecticidal effects of *N. oleander* extracts on different insect species, such as on viviparous females of *Chaitophorus leucomelas* (Zaid *et al*., 2022), cotton bollworm *H. armigera* (Sivakumar *et al*., 2022), *Aedes aegypti* and *C. quinquefasciatus* (Hari and Mathew, 2018). These findings suggest that the oleander extracts may serve as a viable natural substitute for chemical pesticides in managing the Samsum ant populations.

The study also discovered that the mortality rate of adult Samsum ants dropped with increased exposure times. Higher concentrations generally lead to higher mortality rates, indicating the potency of the extract as an insecticidal agent. The efficacy of the *N. oleander* extract diminishes over time. While higher concentrations cause rapid mortality (12 and 24 hrs), the effect wanes by 36 and 48 hrs, indicating a shorter duration of effectiveness. Maybe due to the *N. oleander* extract kills insects more efficiently in the early hours following application, as seen by the negative association with exposure periods. It is critical to consider treatment time for optimal pest control. The results align with other studies such as the assessment of toxins to get rid of the Samsum ants (Mashaly *et al*., 2014) and (Ali and Ali, 2020), and against several insect species (Ceci *et al*., 2020; Ayouaz *et al*., 2023).

The results of the experiment revealed that the *N. oleander* extract has a concentration-dependent impact on Samsum ant mortality. Higher extract concentrations resulted in more deaths. However, the extract's potency faded with time, suggesting a transient effect. The greatest concentration (40%) had the largest initial mortality (83.33%), but its efficacy declined with time, and it had no mortality after 48 hours, these results are consistent with a study (Ali and Ali, 2019). However, its effectiveness diminishes relatively quickly, suggesting that frequent reapplication might be necessary for sustained pest control.

The investigation used an oleander leaves extract to see how harmful it was on Hep-G2 cells. The findings show that the extract had a dose-dependent cytotoxic impact. This suggests that the extract may have potential uses in cancer research. The findings indicate that oleander extracts have effects on Samsum ants. More study is required to enhance concentration and application procedures for effective pest control measures. Furthermore, the safety and environmental effects of employing oleander extracts as pesticides should be evaluated before they are widely used in pest management.

CONCLUSION

The study concludes that *N. oleander* leaf extract have the potential for effective control of *P. sennaarensis* adults. Optimum concentrations, particularly in the range of 10-40% *N. oleander* extract, resulted in significant mortality, surpassing the 50% mortality. This indicates the enhanced efficacy of the plant extract within these concentrations. The research suggests that employing oleander leaves extracts at concentrations ranging from 10% to 40%, coupled with extended exposure periods, offers a viable and eco-friendly alternative to synthetic insecticides for controlling the black ant insect population. This approach not only underscores the potential of natural compounds for pest management but also emphasizes the importance of optimizing concentration and exposure time for maximum effectiveness. Implementing the findings of this study could lead to the development of sustainable pest control strategies, aligning with the growing global need for environmentally conscious practices in agriculture and pest management.

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