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In vitro Assessment of the Insecticidal Activity of *Nerium oleander* Extract against German Cockroaches (*Blattella germanica*)

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ABSTRACT

Background: German cockroaches are the most common cockroach species in the world. Although German cockroach infestations occur in many human-inhabited spaces, they are most commonly associated with restaurants, food processing facilities, hotels, nursing homes. It's also carried viruses, protozoa and infectious bacteria on the outside and inside of body surfaces and play a role in causing dizziness, asthma and nausea reactions in people. So, *Blattella germanica* is considered as an important medical and economic.

Methods: This study aimed to evaluate the efficacy of *Nerium oleander* extract against cockroaches and inhibition hatching eggs *in vitro* using a dipping method test. Two graduated concentrations of extract (50 and 100 mg/ml) were tested at different periods and changes over time in the viability of cockroaches were registered for (1 and 2 hours) and in relation to Inhibition hatching eggs after 30 days. Distilled water and cypermethrin (0.1%) were used as negative and positive control, respectively. A chemical assay was performed by FT-IR to investigate the presence of several anticipated active chemical compounds in *Nerium oleander* leaf extract.

Result: The analysis of phytochemicals by FT-IR for alcoholic extracts of *Nerium oleander* extracts revealed the presence of 12 effective chemical ingredients such as carbohydrates, alkaloids, flavonoids, glycosides and tannins. The results showed that the extract had high efficacy in killing Cockroaches that were exposed to a high concentration of extract (100 mg/mL), the mortality rate was (90 and 100%), compared to the (cypermethrin 0.1%), the mortality rate was (70 and 100%) during the periods (1 and 2 hr) respectively. While the eggs cockroaches that were exposed to concentration of extract (100 mg/mL) and positive control (cypermethrin 0.1%) the hatching rate was (60 and 60%) after 30 days period respectively. While the negative control (without treated) was the hatching eggs cockroaches rate is (100%) during that period.

Key words: German cockroaches, Insecticide, *Nerium oleander*, Plant extracts.

INTRODUCTION

German cockroaches are invasive pests and are widely distributed in households, hospitals, hotels and restaurants of worldwide, inhabiting in human communities (Tang *et al*., 2019). German cockroach infestations are on the rise and play a role in causing dizziness, asthma and nausea reactions in people (Ladonni, 2001). It's also carry viruses, protozoa and infectious bacteria inside its body and on its external surface, causing health risks in many different environments (Mindykowski *et al*, 2010; Nasirian, 2019). However, *B. germanica* is considered as an important medical and economic pest because it has a shorter generation time and higher fecundity than the other cockroaches, which makes it difficult to control (Scott *et al*., 1990). Synthetic pesticides are effective tools for pest control but have potential negative effects such as pesticide resistance, environmental pollution and human health problems (Sparks and Nauen, 2015). Insecticide resistance has become one of the major interests for the development of new compounds. Cockroaches resistance has been reported to a wide range of chemicals, including pyrethroids, carbamates and organophosphates (Arthur, 1996), especially in the most prevalent cockroaches such as German cockroaches (*Blattella germanica*) (Enayati and Motevali, 2007). The cockroaches' resistance to various pesticides is an important factor in its survival, reproduction and disease transmission (Nasirian, 2010). Therefore, it is necessary to develop a new type of environmentally friendly pesticide. Plant extracts are certainly an ideal candidate due to their low toxicity to mammals, ease of biodegradation and low environmental risk (Rajendran and Sriranjini,

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2008).Many medicinal plant extracts are reported to have insecticidal properties against public health pests, such as mosquitoes (Kamaraj *et al*., 2010), houseflies (Chauhan *et al*., 2015) and German cockroaches (*B. germanica*) (Liu *et al*., 2015). *Nerium oleander* is an ornamental evergreen shrub or small tree native to the Mediterranean region and Southeast Asia (Lans, 2007). All parts of the oleander plant are toxic to humans and animals and repel some insects (Bagari *et al*., 2013). *N. oleander* has been used as a medicinal plant for the treatment of a variety of ailments. Traditional medicine uses oleander to treat epilepsy and cancerous tumors (Abbas *et al*., 2012). *Nerium* is employed in the practice of traditional medicine. The flower makes a green dye that is beneficial for treating skin conditions. Moreover, this dye has the power to treat wounds and lessen skin inflammation (Lans, 2007). The juice prepared from the stem bark of *Nerium oleander* is used as an earache remedy in the local traditional medical systems in the Kancheepuram region of Tamil Nadu, India (Nanyingi *et al*., 2008). It has also been found to have various medicinal activities such as antidiabetic (El Sawi *et al*., 2010), immunomodulatory (Mwafy and Yassin, 2011) and cardiac tonic (Al-Farwachi, 2007). Since *Nerium oleander* extract has been shown to have several medicinal properties. The present study aimed to evaluate the insecticidal activity of *Nerium oleander* extract against German cockroaches.

MATERIALS AND METHODS

Preparation of extracts

The *Nerium oleander* leaves were collected from Botanical Gardens in Riyadh city, Saudi Arabia. The leaves (1000 g) were air-dried at 40°C, ground into a powder, after which extracted powder totaling 500 g from the leaves was extracted with 70% ethanol as follows: 100 g of dry powder was added to 400 ml of 70% ethanol and mixed gently for 1 h using a magnetic stirrer. The obtained solution was left at room temperature for 24 h, then stirred again and filtered. The solvent was then evaporated on a rotary evaporator (Inter world highway, LLC). After obtaining the crude extract, it was lyophilized and kept at a temperature of -20 degrees Celsius until usage (Chen *et al*., 2006).

Infrared spectroscopy

After the completion of the processing steps, a minute portion of the material was homogenized by mixing it with an excessive quantity of potassium bromide powder (1: 99 wt %). After that, the material went through a coarse crushing process before being loaded into a pellet-forming die. The infrared spectrum was analyzed using an optical spectrometer from Thermo Scientific called the NICOLET 6700 Fourier-transform infrared spectroscopy (FT-IR). This allowed for the prediction of the most probable constituent classes. The greatest number of waves absorbed is denoted by the expression “a number of waves” (cm^{-1}). Spectra were recorded at 25°C, with a resolution of 4 cm and the range of the spectrum was from 4000 cm^{-1} to 400.

Collection Adult cockroaches and egg capsules

Adult cockroaches and egg capsules were collected from the kitchens of workers’ apartments located in the city of Riyadh. They were placed in plastic tubes with holes that allowed air to enter the tube. After the collection process, they were transferred to the Parasitology Laboratory in the Department of Zoology, King Saud University.

Preparation of concentrations of *Nerium oleander* extract

The dry extract was diluted in distilled water to the concentrations coveted for biological assays (50 and 100 mg/ml) for the tested plant. The concentrations were used to test the insecticidal effect activity *Nerium oleander* extract against cockroaches and the eggs capsule cockroaches. Distilled water and cypermethrin (0.1%) were used as negative and positive control. The positive control, 0.1% cypermethrin was diluted in water according to the manufacturer’s recommendation (1:1000) before being utilized for the further experiment (Heukelbach *et al*., 2006).

Assay of insecticidal activity of *Nerium oleander* extract of adult cockroaches

In vitro testing commenced within 1 hr of cockroaches collection. Ten adult cockroaches active in three replicates were placed in a petri dish and 3 mL of each concentration was added directly to three repeat Petri dishes. 3 ml of distilled water and 0.1% cypermethrin 60 EC were used as negative and positive controls. The insects were maintained at room temperature. Cockroaches dead were determined in each petri dish at 1 hr and 2 hr periods from treatment. Adult cockroaches were considered dead if their appendages did not move when prodded with a brush.

Assay of insecticidal activity *Nerium oleander* extract of eggs capsule cockroaches

In vitro testing commenced within 1 hr of eggs capsule cockroaches collection. Five eggs capsule in two replicates were placed in a petri dish and 1 mL of concentration 100 mg/ml of Nerium oleander extract was added directly to two repeat. Five eggs capsule in Petri dishe negative control (without treated) and other Petri dishe contant 0.1% cypermethrin 60 EC was used as positive control. The egg capsules were maintained at 25±1°C and 60% RH. The hatching of the eggs was determined in each petri dish one month after they were collected from female cockroaches and treatment.

RESULTS AND DISCUSSION

The analysis of *Nerium oleander* leaf extracts using FT-IR explained main bands at 3383.10 cm^{-1}, N-H stretching, 2934.35 cm^{-1}, C-H stretching, 2124.09cm^{-1}, N=C=N stretching, 1633.45 cm^{-1}, C=C stretching, 1515.92 cm^{-1},

N-O stretching, 1423.03 cm^{-1}, O-H bending, 1273.79 cm^{-1}, C-O stretching, 1117.70 cm^{-1}, C-O stretching, 1048.87 cm^{-1}, CO-O-CO stretching, 926.30 cm^{-1}, bending, 828.28 cm^{-1}, C=C bending and 717.43 cm^{-1}, C=C bending (Fig 1 and Table 1).

The effects of the *N. oleander* extract on cockroaches and hatching of eggs cockroaches were studied *in vitro* at therapeutic doses of 50 and 100 mg/mL and compared it to a negative control (distilled water) and control positive cypermethrin 0.1%. Results showed that thorough Cockroaches examination after 1 and 2 hr of concentrations were given to test the cockroaches killer effects and see if it was still alive or dead. The Cockroaches exposed to a low concentration of extracts (50 mg/mL) had lower mortality rates during the periods 1 and 2 hr respectively (Fig 3 and 4), Compared to the positive control and concentration of 100 mg/mL extract.

While the Cockroaches that were exposed to a high concentration of extract (100 mg/mL), the mortality rate was (90 and 100%) during the periods (1 and 2 hr), respectively (Fig 2B, 3 and 4).

Compared to the positive control, the mortality rate was (70 and 100%) during the periods (1 and 2 hr) respectively (Fig 2.C, 3 and 4). While the negative control was not exposed to drugs only water, the death rate is almost zero during the periods (1 and 2 hr) (Fig 2A, 3 and 4). Through these results, it was found that the concentration of (100 mg/mL) from *Nerium oleander* extract are very similar to the positive control (cypermethrin 0.1%) and that the concentration of (50 mg/mL) is also considered effective in killing cockroaches when compared to the negative control (Fig 5).

30 days after exposure, concentrations of 100 mg/ml of *Nerium oleander* extract and cypermethrin 0.1% were

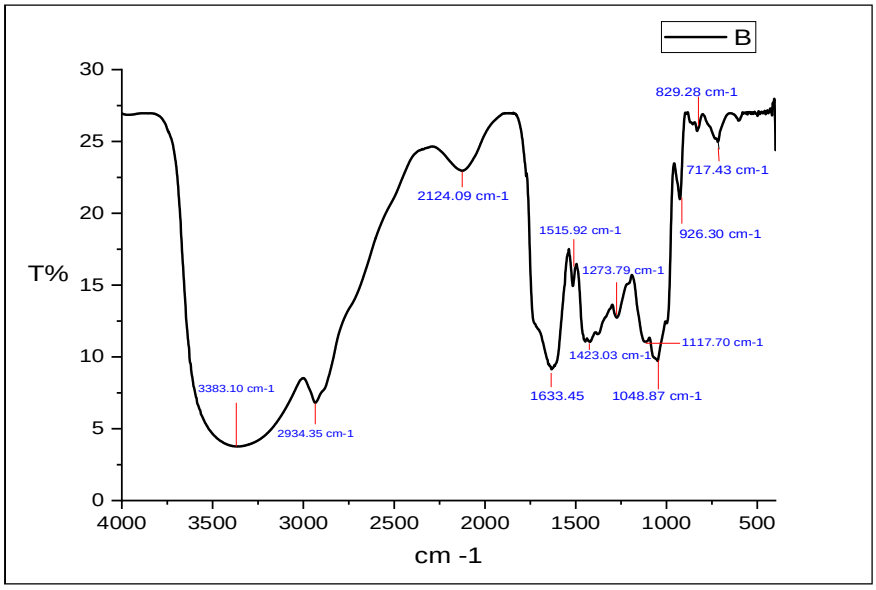


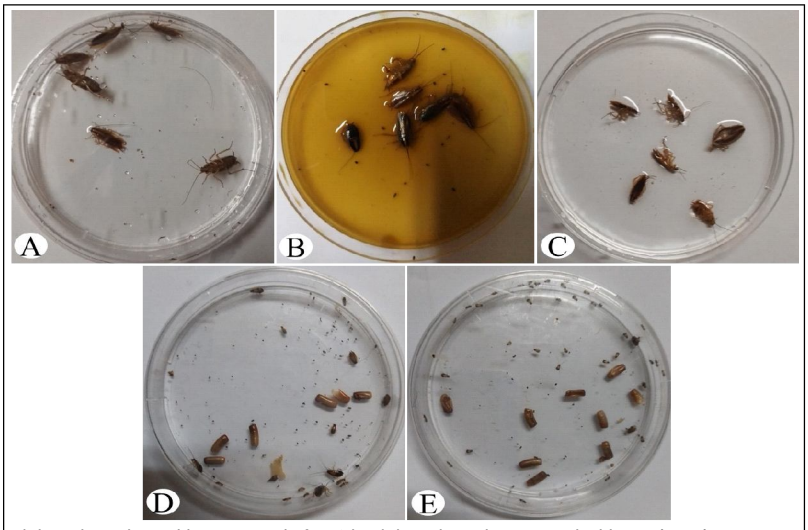
Fig 1: FT-IR chromatogram of *Nerium oleander* leaf extracts in methanolic medium showing the functional characteristic of the active chemical compounds.

Table 1: Analyze NOLE to identify potential active chemical compounds using FT-IR.

Absorption (cm^{-1})	Appearance	Transmittance (%)	Group	Compound class
3383.10	Medium	4	N-H stretching	Aliphatic primary amine
2934.35	Medium	7	C-H stretching	Alkane
2124.09	Strong	23	N=C=N stretching	Carbodiimide
1633.45	Medium	9	C=C stretching	Alkene
1515.92	Strong	15	N-O stretching	Nitro compound
1423.03	Medium	11	O-H bending	Alcohol
1273.79	Strong	12	C-O stretching	Alkyl aryl ether
1117.70	Strong	10	C-O stretching	Secondary alcohol
1048.87	strong, broad	9	CO-O-CO stretching	Anhydride
926.30	Strong	21	C=C bending	Alkene
829.28	Medium	26	C=C bending	Trisubstituted
717.43	Strong	25	C=C bending	Disubstituted (cis)

more effective against hatching of eggs cockroaches than the negative control was not exposed to drugs, the not hatching of eggs cockroaches rate is almost zero during that period in negative control. While the eggs of cockroaches that were exposed to concentration of extract (100 mg/mL) (Fig 2D) and control positive cypermethrin 0.1% the hatching rate was (60 and 60%) after 30 days from exposed (Fig 6). While the negative control was the hatching rate is (100%) during that period (Fig 2E and 6).

The results obtained from this study are consistent with El-Sayed and El-Bassiony, (2016) that diethyl ether extract of *N. oleander* leaves is potential control against to *Culex pipiens*. Also, aqueous extract of *Nerium oleander* flowers were reported for larvicidal activity against the filarial vector, *Culex quinquefasciatus* (Raveen *et al*., 2014). The aqueous *Nerium oleander* extract exhibited larval toxicity against the first to fourth instar larvae and pupae of *Anopheles stephensi* (Roni *et al*., 2013). These results are in line with obtained Rathi and Al-Zubaidi, (2011) that *N. oleander* has toxic effects on the development of larval and pupal stages for *Bemisiatabaci* species. Morsi *et al*. (2022) found the effect of *Nerium oleander* leaf extract on the schistosomiasis



(A) cockroaches without treated after 2 hr. (B) cockroaches treated with *N. oleander* extract (100 mg/ml) after 2 hr. (C) cockroaches treated with cypermethrin 0.1% after 2 hr. (D) eggs capsule cockroaches treated with *N. oleander* extract (100 mg/ml) after 30 days. (E) eggs capsule cockroaches without treated after 30 days.

Fig 2: The effect of *Nerium oleander* extract on cockroaches and the eggs capsule cockroaches.

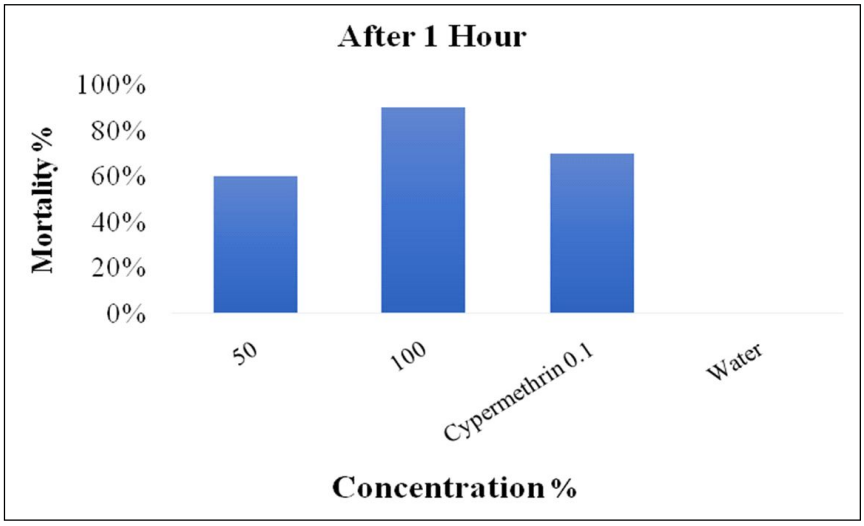


Fig 3: The influences of various Concentrations of leaf extracts (50, 100 mg/ml) of *Nerium oleander* with positive and negative controls cypermethrin 0.1% and water on the mortality rate of cockroaches after 1 hr.

parasite. confirmed Siddiqui *et al*. (2012) the extract of *N. oleander* leaves contains many effective compounds, including flavonoid glycosides, oleanderocinoic acid, a cardenolide, oleandigoside, neridiginoside and odoroside-H and these compounds have CNS depressant effects and growth inhibitory and cytotoxic activities, leading to paralysis and killing cockroaches. Also, the cockroaches work to swallow the extract, which in turn works to cause cockroaches poisoning, which results in the death of the cockroaches and effect hatching of eggs cockroaches. This is consistent with the mention in Bandara *et al*. (2010) that *Nerium oleander* is a plant that can be fatal if ingested. All parts of the plant are toxic and contain various cardiac glycosides. Ingestion of *oleander* causes nausea, vomiting, abdominal pain, diarrhea, cardiac arrhythmias and hyperkalemia in animals. In a study that proved the presence of a group of effective compounds in the *Nerium oleander* extract, such as alkaloids, phenols, flavonoids, tannins and actins (Huang *et al*., 2009), as for flavonoids, they act to reduce sugars, causing an imbalance in carbohydrate metabolism and re-ducing the amount of energy units (ATP) supplied in the Organisms vital activity (Hamad, 2021).

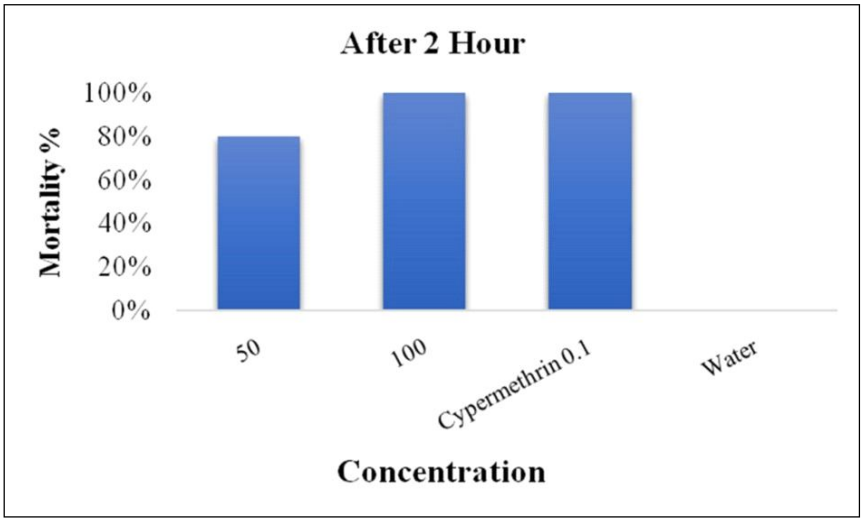


Fig 4: The influences of various concentrations of leaf extracts (50, 100 mg/ml) of *Nerium oleander* with positive and negative controls cypermethrin 0.1% and water on the mortality rate of cockroaches after 2 hr.

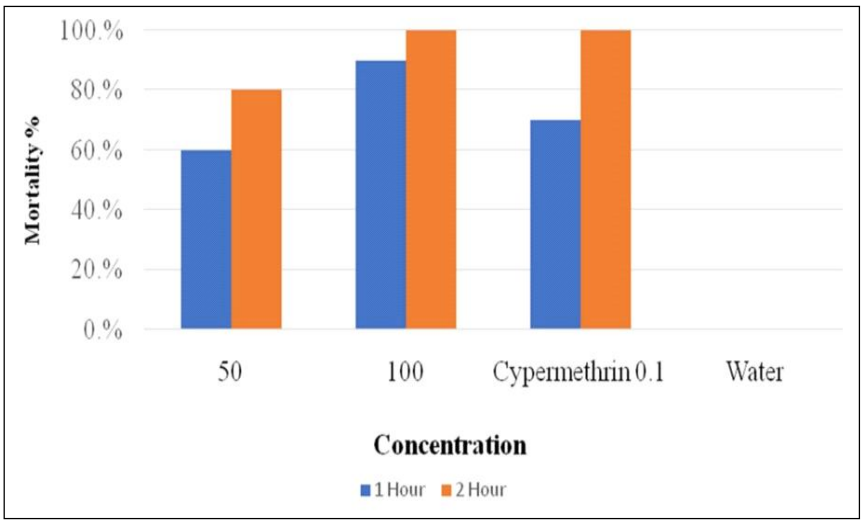


Fig 5: The difference between the general influences of various concentrations of leaf extracts (50, 100 mg/ml) of *Nerium oleander* with positive and negative controls cypermethrin 0.1% and water on the mortality rate of cockroaches of 1 to 2 hr.

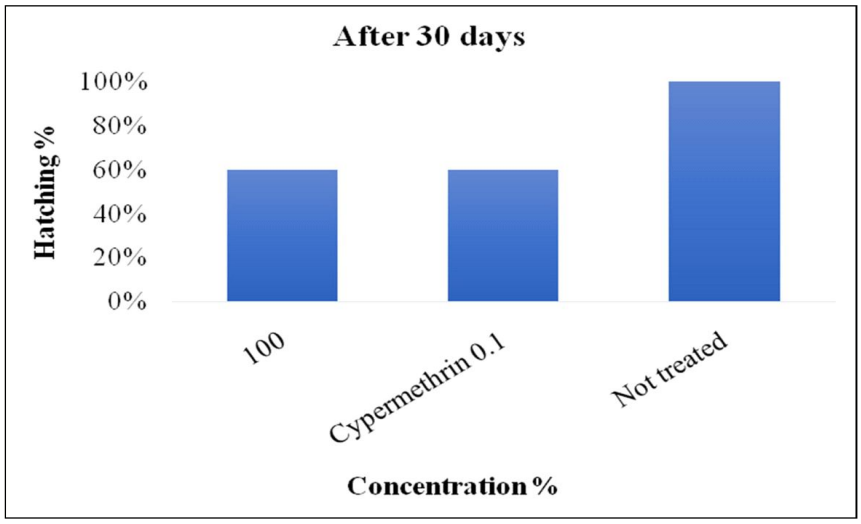


Fig 6: The influences of concentration of leaf extracts (100 mg/ml) of *Nerium oleander* with positive and negative controls on the hatching eggs capsule cockroaches rate after 30 day.

CONCLUSION

Our study indicates that *Nerium oleander* leaf extract had strong Inhibition activity hatching of eggs cockroaches and killer it greatly comparable to the effect of cypermethrin 0.1% at higher concentrations. Also, further studies are needed to isolate the pharmacologically active compounds responsible for these activities.

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Data availability statement

All the datasets generated or analyzed during this study are included in this published article.

Conflict of interest statement

The author(s) declare that they have no conflict of interest regarding the content of this article.

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