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Evaluation of the Insecticidal Activity of *Nerium oleander* L. against the Red Flour Beetle, *Tribolium castaneum* (Herbst) (Tenebrionidae, Coleoptera)

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Abstract

Background: The red flour beetle, *Tribolium castaneum* (Herbst) (Tenebrionidae, Coleoptera) is a pest of worldwide distribution and can cause destructive damage to stored grains. The physicochemical properties and varied effects against insect pests make plant extracts a potential alternative in the development of pesticides.

Methods: In this study, we assayed the oleander leaf extract toxicity effects against *T. castaneum* adults in the laboratory. We used four concentrations of Oleander methanol leaf extract.

Result: Exposure of *T. castaneum* adult to the oleander leaf extracts produced 100% mortality in the insecticidal bioassay, especially at 40% concentration. The mortality % ranged from 13.3-100% after 48hrs. The mortality percentage of the red flour beetle adults decreased by increasing exposure periods. The mortality % was highly negatively correlated with exposure times (R= -0.97, P =.0001; R= -0.80 and P=.0001) at 40 and 20% concentrations, respectively. The overall results of the current study suggest that the leaf extract of *Nerium oleander* may possess potential insecticidal properties, which could potentially be employed in pest management. The infrared analysis of the oleander leaf extract showed many bioactive components associated with plant secondary metabolites; some of these identified phytochemical compounds have biological activity. Further, the phenol and flavonoid total were estimated. We conclude that the oleander leaf extract has the potential to be useful in managing stored grain insect pests, particularly *T. castaneum*, but that it must be handled and applied with extreme caution.

Key words: Bioassay, Concentrations, Insecticidal, Mortality, Oleander, Stored grain.

Introduction

Grain and stored materials are exposed to great losses during storage as a result of infestations with insect pests, fungi and rodents. Drying the grain to prevent its deterioration due to fungus does not protect the grain from insect infestation. Controlling insect pests and preventing them in various ways is important and necessary, to reduce the losses of grain and stored materials to the least possible extent. Traditional pest control methods using chemicals as protectants or to control stored grain insect pests are not acceptable methods due to issues of insecticide residues in the commodity coupled with the development of insecticide resistance (Padín *et al*., 2002). Using chemical pesticides to control grain and warehouse pests results in many risks, including contamination of food and drinking water and environmental pollution. Owing to above said potential problems of synthetic chemical management, there is a need to look for more sustainable alternative management methods that are eco-friendly (Ačanski *et al*., 2022).

The red flour beetle, *Tribolium castaneum* (Herbst, 1797) (Tenebrionidae; Coleoptera:) is a destructive pest of stored grains and their products worldwide as they feed on the germ of the grain and devour them completely in case of severe infestation (Zettler, 1991; Biswas *et al*., 2016). *T. castaneum* is a polyphagous insect pest, that causes great economical losses by attacking several grains in grain stores, flour mills, poultry feed, cereal products and stored dried foods (Aboelhadid and Youssef, 2021). This insect can contaminate food with its faces and body parts besides direct feeding (Jamilah *et al*., 2021), thus reducing the market value. With its ability to reproduce rapidly and survive under a wide range of conditions, it has gained a notorious reputation as a persistent nuisance pest of stored grain.

An oleander plant (*Nerium oleander* L.) is an evergreen shrub belonging to the dogbane family Apocynaceae. It

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grows well in warm subtropical regions, originated in Southwest Asia and is widely cultivated (Farooqui and Tyagi 2018). The plant contains numerous toxic compounds and it is one of the most poisonous plants (Goktas *et al*., 2007). The most toxic compounds are oleandrin and neriine, which are cardiac glycosides and are present in all parts of the plant (Goktas *et al*., 2007), in addition to many other unknown or unstudied compounds that may have significant effects. Recent studies showed the pharmaceutical properties of the oleander extract as having antiviral activity against SARS-CoV-2 (Plante *et al*., 2021). Researchers worldwide reported that the insecticidal compounds derived from plants may delay insect resistance (Regnault-Roger *et al*., 2012), safety for natural enemies, have a wide range of activities and compatible with bio-control organisms for IPM and are eco-friendly (Pavela, 2016; Souto *et al*., 2021). Several studies found that extracts derived from the oleander plant have potential insecticidal properties, making them a promising candidate for controlling stored insect pests such as *T. castaneum* (Hameed *et al*., 2012; Sagheer *et al*., 2014; Al-Ghannoum and Karso, 2015). From time immemorial many plant extracts have been used for management of *T. castaneum*. These plant extracts include *viz*., *Tamarindus indica*, *Azadirachta indica*, *Cucumis sativus*, *Eucalypts sp*., *Switenia mahagoni Psidium guajava* (Mostafa *et al*., 2012); *Ambrosia tenuifolia*, *Baccharis trimera*, *Brassica campestris*, *Jacaranda mimosifolia*, *Matricaria chamomilla*, *Schinusmolle* var. *areira*, *Solanum sisymbriifolium*, *Tagetes minuta* and *Viola arvensis* (Padín *et al*., 2013); *Datura stramonium* and *Zingiber officinale* (Ali *et al*., 2020); *Syzygiumaromaticum* and *Cymbopogon schoenanthus* (Aboelhadid and Youssef, 2021); *Artemisia annua* (Deb and Kumar, 2021).

It has been demonstrated several times that sole reliance on synthetic chemicals for the management of *T. castaneum*can could lead to develop resistance to some insecticides commonly used to control this species such as phosphine (Gautam and Opit, 2015) and other classes of insecticides (Rösner*et al*., 2020). So, it has become imperative to find any an alternate method of management of stored grain insects. Therefore, this study aimed to study the potential of oleander extracts as an insecticidal activity against the red flour beetle, *T. castaneum*.

MATERIALS AND METHODS

Plant preparation and extraction

The *N. oleander* fresh leaves were collected from Ad Diriyah governorate, Modhi Park, (24°45′02.2″N 46°34′40.8″E), Riyadh, Saudi Arabia, in December 2022. The Oleander plant was identified and authenticated by a botanist at the herbarium of the Botany Department, College of Sciences, King Saud University, Riyadh, Saudi Arabia. The collected *N. oleander* leaves were washed thoroughly in distilled water and air dried for two weeks in the shadow area to remove the moisture contents, the leaves were kept in a hot air oven at 25°C for 30 minutes. Then, the dried leaves were pulverized using an electric grinder (Senses, MG-503T, Korea). The powder was steeped in 70% methanol (ultrasound-assisted extraction) for 24 hours to extract a wide variety of active compounds from the decoction. Maceration extraction was performed on the dried powder of oleander leaves (200 g) at 4°C and then total extraction was achieved by percolating the mixture 2-5 times. Based on previous research (Chen *et al*., 2006; Mu *et al*., 2012; Borjigidai *et al*., 2014; Teng *et al*., 2014), the powder underwent a vacuum extraction process at room temperature. Following extraction, the extract was separated using two layers of filter paper, gathered and a concentrated rotating vacuum evaporator (Yamato RE300, Japan), at 50°C and a lowered pressure. Before usage, the extract's concentration was diluted with ethanol to a final concentration of 0.5 mg/mL. After obtaining the crude extract, it was lyophilized and kept at -20°C until usage.

Chemical analysis of plant extract

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 (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.

Total phenolic contents

The Ainsworth method was utilized to estimate the total phenolic content (TPC) of the oleander leaf extract (Ainsworth *et al*., 2007). The mixed-up volume of 100 µL the oleander extract with the Folin–Ciocalteu reagent and 300 µL of a solution of sodium carbonate (20%). After that, the sample was kept at room temperature and incubated in the dark for 30 minutes, recorded the wavelength was 765 nm, utilizing a UV-Visible spectrophotometer (SHIMADZU, UV-1800). The total phenolic content of the samples was calculated by using the linear equation ($y = 0.0021x + 0.0021$ with $R^2 = 0.9995$). This equation was derived from a standard curve that was built by utilizing Gallic acid in a range of values ranging (25-400 µg/mL). The total phenolic content was expressed as mg/g DW.

Total flavonoid contents

Using the method applied by Ordonez *et al*. (2006) to determine the total flavonoid content (TFC) in plant materials. Methanol extract 0.5 mL was mixed with a water solution containing 2% AlCl3 having the same volume. The wavelength was measured at 420 nm at 25°C after 2 hrs. The TFC was calculated using a calibration curve that was constructed using different concentrations (50-0400 g/mL) of quercetin standard and the following equation (y = 0.0172x + 0.0507 with R^2 = 0.995). The curve was generated using the data from the previous step. The calculated TFC has been represented as quercetin (mg/g DW).

Insects collection and rearing

Adults of *T. castaneum* were collected from poultry feed mill stores in Riyadh, Saudi Arabia. The red flour beetle individuals were cultured in the Zoology Department, College of Sciences, King Saud University, from June to July 2023 on poultry feed in small plastic jars at room temperature (26±2°C; 60-70% RH). *T. castaneum* colonies were kept in jars containing poultry feed and the plastic jars were covered with perforated cover with small holes to prevent the individuals escape. The red flour beetle was identified following the taxonomic characteristics, such as the antennae being distinctly club-like with a three-segmented club (Bousquet, 1990) and returned to the identified specimens deposited in the King Saud University Museum of Arthropods (KSMA), Plant Protection Department, College of Agriculture and Food Sciences, King Saud University, Riyadh, Saudi Arabia.

Adults bioassay

The active adults were separated using a camel hairbrush in small plastic jars, distributed in Petri dishes of 9 cm diameter and tested. Four concentrations of Methanol 70% *N. oleander* leaves extracts (5, 10, 20 and 40%) were prepared with three replicates and ten individuals were assayed for each replicate. The control was treated with distilled water only. Treated adults were confined into Petri dishes (9 cm diameter) at room temperature (26±2°C; 60-70% RH). The tested adults were placed in a Petri dish containing filter paper soaked with 1 mL of extract for each concentration according to Hameed *et al*., (2012) method with some modifications. Mortality percentage was recorded at 12, 24, 36 and 48 after treatments.

Statistical analysis

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) SAS 9.2 software (SAS Institute, 2008) was used. Duncan's Multiple Range test (P≤0.05) was used to compare mortality means. All the mortality data are presented as mean ± standard error (SE). A linear correlation was used to analyze the relationship between exposed time and mortality percentage.

RESULTS AND DISCUSSION

Chemical analysis of plant extract

The FT-IR analysis and qualitative phytochemical investigation of alcoholic extracts of the leaves of *N. oleander* showed the presence of 12 compounds of active chemical constituents such as carbohydrates, alkaloids, flavonoids, glycosides and tannins (Fig 1 and Table 1), which are mainly responsible for insecticidal activity. The total phenol and total flavonoid contents of oleander leaf extract are shown in Fig 2. The analysis of *N. 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, 1515.92cm^{-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).

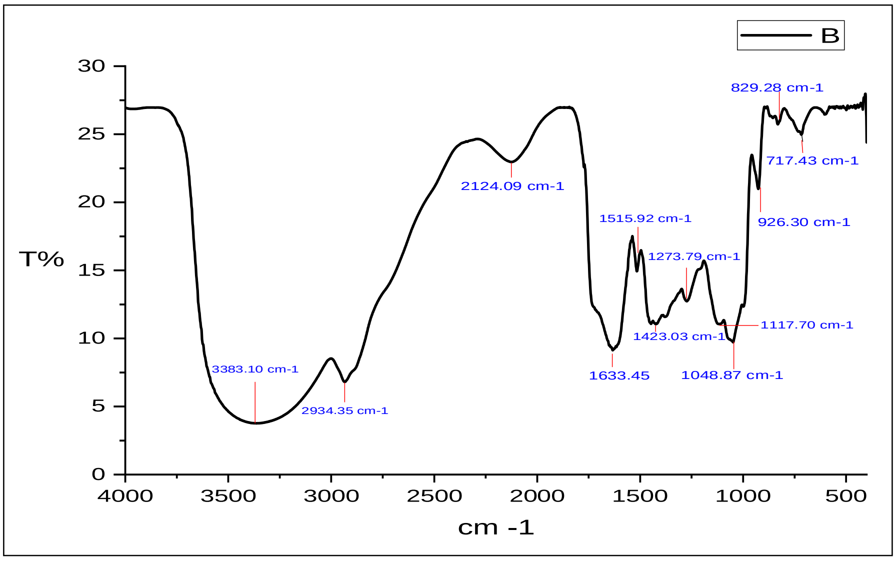


Fig 1: FT-IR chromatogram of *Nerium oleander* leaf extracts in methanolic medium showing the functional characteristic of the active chemical compounds.

Adult bioassay

For many decades, chemical pesticides were the main method of controlling store grain insect pests. However, the world is moving to reduce the use of synthetic pesticides to 50% by 2030 to reduce the risks of the most dangerous chemical insecticides (Hamel *et al*., 2020). Therefore, researchers have been motivated to look for alternative methods to manage these pests such as the use of plant extracts many of which have proved the effectiveness. The oleander toxicity and contents of various plant parts were studied (Farkhondeh *et al*., 2020). Laboratory studies were conducted using oleander leaf extract and the extract activities against the black poplar leaf aphid *Chaitophorus leucomelas* were reported (Zaid *et al*., 2022); they found that the mortality rate reached 100% after 4 days at a concentration of 5.15 g/m^2. A range of Oleander extract concentrations (5, 10, 20 and 40%) were selected based on previous studies and evaluated on the red flour beetle. The mortality percentage of *T. castaneum* adults to 5, 10, 20 and 40% of the Methanol extracts of oleander leaves and control is shown in Fig 3. The results showed a significant effect of most oleander extract concentrations on adult mortality ($P<0.05$). This study's results showed that all concentrations of oleander leaf extracts have an insecticide effect on *T. castaneum* mortality percentage. The results revealed that the mean mortality among tested individuals of *T. castaneum* varied depending on exposed time and oleander extract concentrations used in the current study. Oleander leaf extracts were assayed against *T. castaneum* at 4 concentrations (25-40%), found that the mortality % was 16.7% in dry powder and 70% in the alcohol extract at 40% concentration (Al-Ghannoum and Karso, 2015), these results are compatible with our results, where with increasing extract concentration the mortality increased.

The results indicated that the mortality percentage ranged from 10% after 24 hrs at 5% concentration up to 100% after 48 hrs at the 40% concentration. The highest average mortality % reached 40% at 40% concentration after

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)

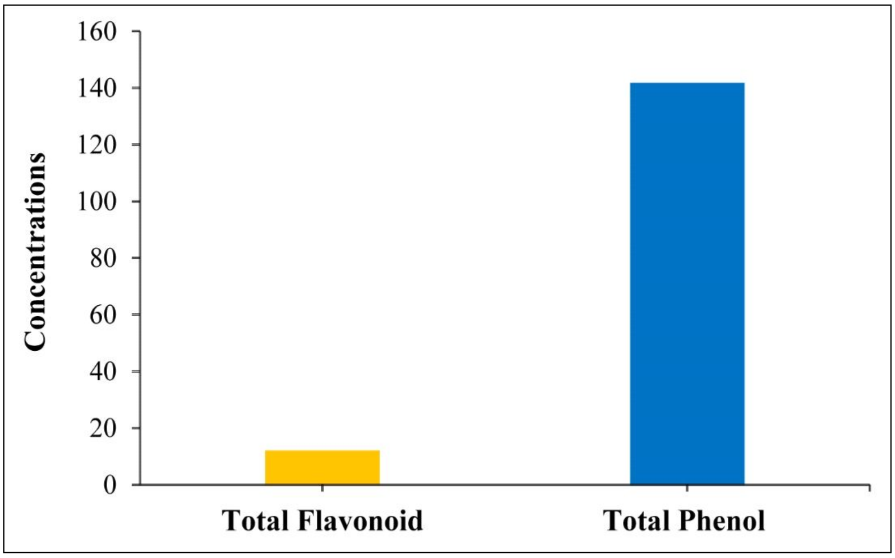
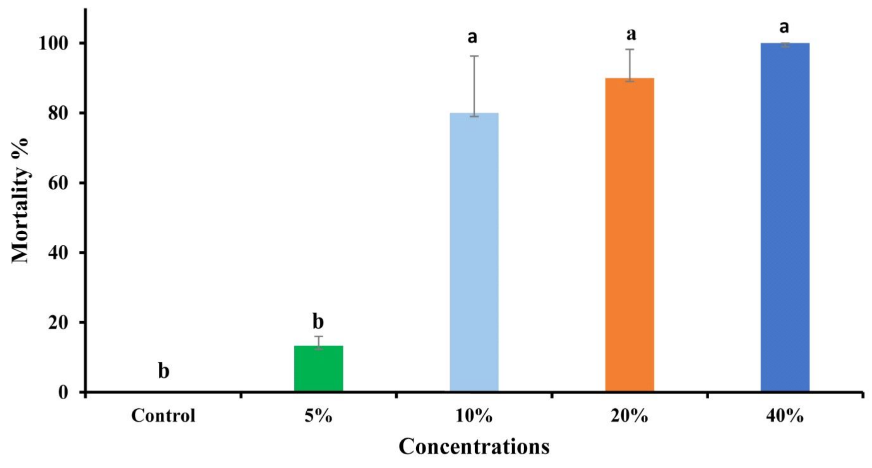


Fig 2: Total phenol, total flavonoids contents of oleander leaves extract.

12 hrs and 40% at 10% after 24 hrs (Table 2; Fig 3). The mortality % ranged from 13.3% at 5% concentration to 100% at 40% after 48 hrs (Table 2). The mortality percentage ranged from 10% at 10% concentration to 73.3% at 40% after 24 hrs (Table 2). The mortality percentage ranged from 13.3% at 10% concentration and 96.7% at 40% concentration after 36 hrs. No mortality was observed in control for all exposed time, or at 5% concentration till 12 hrs. In the current study, the effectiveness of extract concentrations was increased with increasing exposure time (Fig 3). Mamun *et al*. (2008) evaluated the repellence of six botanical extracts against the red flour beetle and found that extracts of all six plants had repellent effects on *T. castaneum* adults, but the neem extracts showed the highest repellent effect with water extract solvent. In addition, they found that the repellency of most plant extracts increases with increasing doses and decreases with increasing time and this agrees with our results, where the mortality rate increases with increased extract concentrations and decreases with increasing exposure time. The effect of four plant extracts was evaluated against *T. castaneum* and *Trogoderma grananium* and they reported that the Rosemary extract was the most efficient against both tested adults causing 58.67% and 80% mortality respectively, after four days (Panezai *et al*., 2019). The results obtained demonstrate the insecticidal activity of the plant extract against stored grain pests and the results agree with our results, where the mortality rate increases with decreasing exposure time.

At 40% concentration, the bioassay has resulted in the highest insecticidal activity with 100% mortality after 48hrs. As well as, the 10 and 20% concentrations also demonstrated insecticidal activity, with 80 and 90% individuals mortality after 48 hrs. The 5% concentration showed the lowest insect mortality 13.3% after 36 hrs and no mortality was observed after 48 hrs. No insect mortality was obtained in control. Overall, no significant differences (P=0.05) were observed between all concentrations after 48 hrs. Comparative toxicity of four plant extracts and spinetoram alone and in combinations were evaluated against *T. castaneum* (Rehman *et al*., 2019), indicating that, the spinetoram alone was more effective with the highest mortality of 79.8%, while in combinations, the neem with spinetoram gave the highest mortality of 84.9%. Also, they found that the mortality increased with increasing extract concentrations and these results are agreeing with our



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).

Fig 3: Total mean (± SE) of mortality % of *Tribolium castaneum* adults at various concentrations.

Table 2: Means of mortality % (± SE) of leaf oleander extracts at various exposure periods (12, 24, 36 and 48 hrs) against *Tribolium castaneum*.

Concentration	Mortality (%)			
(%)	12 hrs	24 hrs	36 hrs	48 hrs
Control	0.0±0.0 c	0±0.0 c	0±0.0 c	0±0.0 c
5%	0.0±0.0 c	10±4.7 abc	3.3±2.7 bc	0±0.0 c
10%	13.3±2.7 abc	40±12.5 a	23.3±5.4 abc	3.3±2.7 bc
20%	26.7±11.9 abc	33.3±14.4 ab	26.7±7.2 abc	3.3±2.7 bc
40%	40.0±4.7 a	33.3±16.6 ab	23.3±11.9 abc	3.3±2.7 bc

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.

results. On the other hand, in one study, oleander extract was assayed against *T. castaneum* and showed the least effectiveness than neem extract and Spinosad, that is maybe due to low concentrations (0.5-2.5%) of plant extracts used in the experimentation (Hameed *et al*., 2012). *Lawsonia inermis* L. (Henna) leaves and fruits were applied to evaluate the extracts' activity against adult *T. castaneum* and the leaf extract proved to possess a potential toxicity effect that increased with increasing exposure time till after 48 hrs (Biswas *et al*., 2016) and these results are compatible with recent results study in the concentration part but disagree with exposure times aspect. The jimsonweed extract was evaluated against *T. castanum* and the findings varied according to the plant extract concentrations, where the high concentration reduced the nutritional indices (Abbasipour *et al*., 2011) and these results were compatible with the recent study where the high concentration caused high mortality.

The obtained results showed that there were significant differences between the mortality mean of 40% concentration after 12hrs and at 10% concentration after 24 hrs and the remaining mortality means (Table 2). Also, there were significant differences between control and at 40% concentration after 12 and 24 hrs, respectively. The highest mortality was after 24 hrs at 5, 10, 20% concentrations. The lowest mortality was observed after 48hrs at all concentrations (Table 2). Significant differences ($P<0.05$) were obtained in *T. castaneum* adults mortality percentage among plant extract concentrations (10, 20 and 40%) and at 5% concentration and control (Fig 4). Overall,

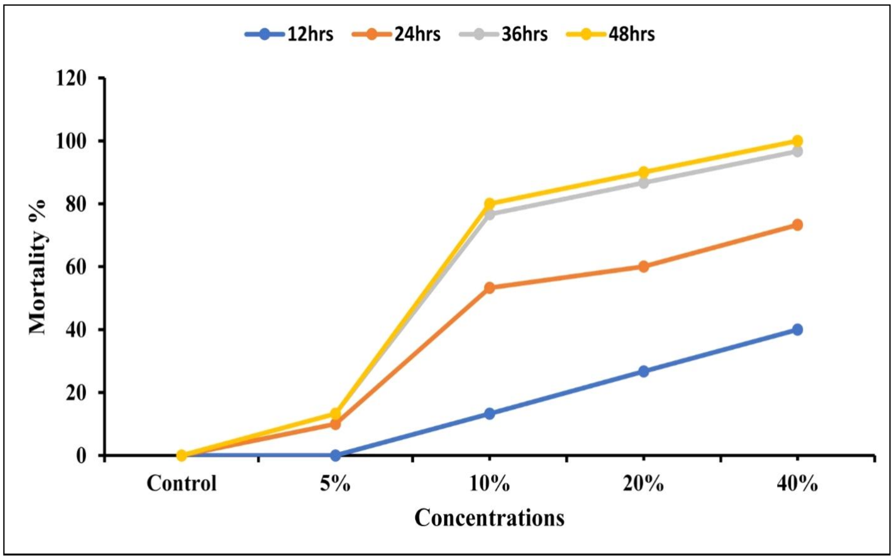


Fig 4: Mortality % of *Tribolium castaneum* assayed with leaf oleander extracts at various exposure periods (12, 24, 36 and 48hrs).

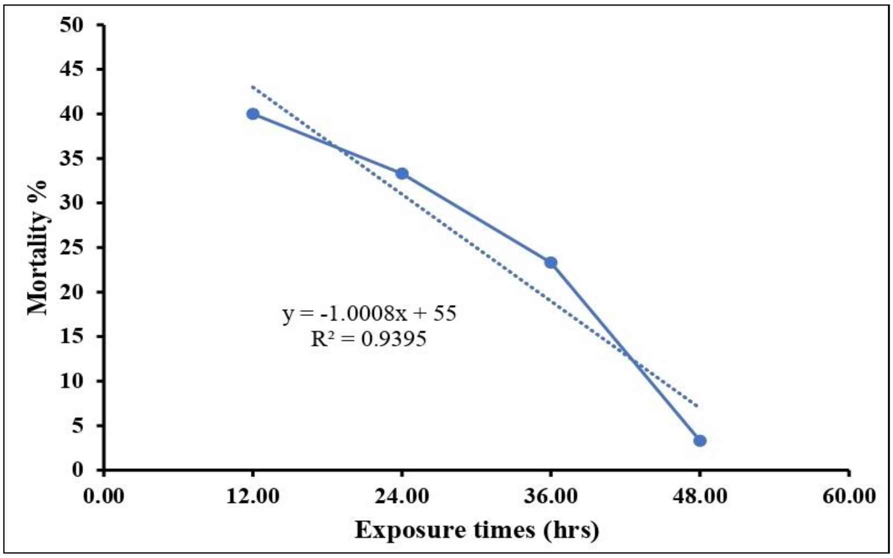


Fig 5: Mean mortality % of *Tribolium castaneum* adults at 40% concentration for various exposure times (hrs).

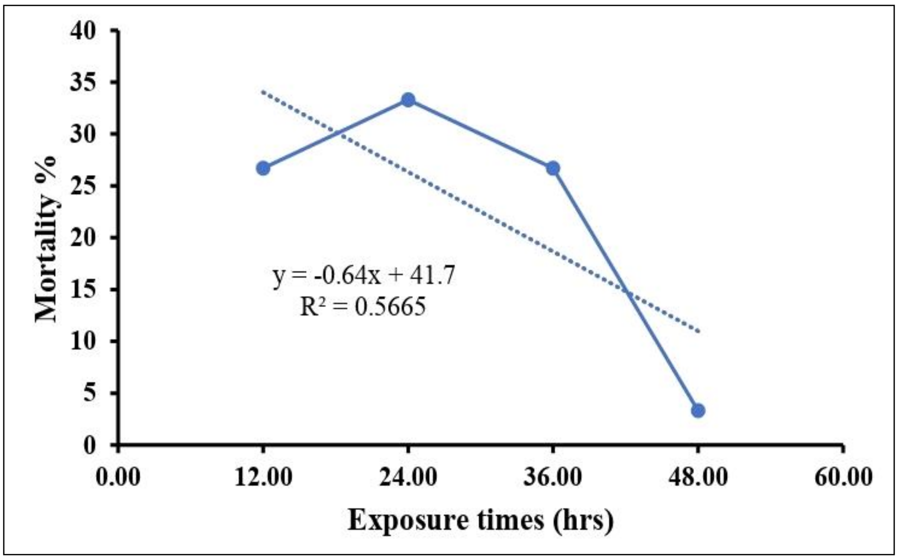


Fig 6: Mean mortality % of *Tribolium castaneum* adults at 20% concentration for various exposure times (hrs).

no significant differences were observed between all concentrations except at 5% concentration (Fig 4). Many studies have indicated that plant-derived pesticides are effective against tested insects, eco-friendly and can potentially be useful in managing stored grain insects (Mamun *et al*., 2008; Al-Ghannoum and Karso, 2015; Buxton *et al*., 2018; Rehman *et al*., 2019). As shown in Fig 3; Table 2, toxic compounds in oleander, even at low concentrations, affected the mortality percentage; that means the increase in extract concentration increased the toxicity and affected the movement of the tested individuals followed by death.

The mortality percentage of the red flour beetle adults decreased with increasing exposure periods (Fig 5). The mortality % was highly negatively correlated with exposure time (R= -0.97, P=.0001; R= -0.80 and P=.0001) at 40 and 20% concentrations, respectively (Fig 5; Fig 6). Whereas the correlation coefficient at 10% was moderated negatively (R= -0.40 and P= 0.0001) and weak negatively at 5% concentration (R= -0.2 and P= 0.0001). 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.

CONCLUSION

It is concluded that the oleander leaf extract can give an effective control against the red flour beetle adults. However, the effectiveness of plant extract was enhanced when used in the optimum concentration, especially in the case of a 40% concentration of *N. oleander*. Therefore, it is suggested that the application of oleander leaf extracts at 40% concentration along with longer exposure periods can be an effective alternative to synthetic insecticides for an eco-friendly control method of stored grain insect pests, but with the prerequisites that one should take much care when handling and when applying the compound.

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Conflict of interest

All authors confirm no potential conflict of interest to declare.

REFERENCES

Abbasipour, H., Mahmoudvand, M., Rastegar, F., Hosseinpour, M.H. (2011). Bioactivities of jimsonweed extract, *Datura stramonium* L. (Solanaceae), against *Tribolium castaneum* (Coleoptera: Tenebrionidae). Turk. J. Agric. For. 35(6): 623-629.

Aboelhadid, S.M., Youssef, I.M. (2021). Control of red flour beetle (*Tribolium castaneum*) in feeds and commercial poultry diets via using a blend of clove and lemongrass extracts. Environ. Sci. Pollut. Res. 28: 30111-30120.

Ačanski, J., Gvozdenac, S., Radenković, M., Prvulović, D., Tanasković, S., Horvatović, M. (2022). Effect of four plant extracts on the mortality, population growth and fluctuating asymmetry of *Sitophilus oryzae*. Entomol Exp. Appl. 2023(171): 611-620.

Ainsworth, E.A., Gillespie, K.M. (2007). Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin-Ciocalteu reagent. Nat. Protoc. 2: 875-877.

Al-Ghannoum, M.I., Karso, B.A. (2015). Biological Potency of *Nerium oleander* L. Leaf Extracts on Mortality of the Red Flour Beetle *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae). Egy. J. Biol. Pest Contro. l25(1): 135-138.

Ali, S.M.A, Ammad, A.M., Ahmad, S.K., Bilal, I.M., Abdullah, A., Talal, I., Zulfiqar, M. (2020). Effectiveness of different plant extracts along with new chemistry insecticide against *Tribolium castaneum*. GSC Biol. Pharm. Sci.13(1): 95-100.

Biswas, K.K., Sharmin, N., Rabbi, M.A. (2016). Evaluation of insecticidal activity of *Lawsonia inermis* Linn. against the red flour beetle, *Tribolium castaneum* (Herbst). Nat. Prod. An. Ind. J. 12(1): 8-11.

Borjigidai, A., Cui, X., Pang, Z.R., Cui, J., Kang, F. (2014). Study on extraction process of coumarin in a Mongolian drug Chagan-sorlo (Radix Glehniae). Spectr. Spectr. Anal. 34: 1085-1088.

Bousquet, Y. (1990). Beetles associated with stored products in Canada: An identification guide. Ottawa, Canada: Canadian Government Publishing Centre, 220 pp.

Buxton, T., Owusu, E.O., Kim, C.S. (2018). Bioactivity of cardanol against the rust red flour beetle, *Tribolium castaneum* (Coleoptera: Tenebrionidae). Int. J. Trop. Insect. Sci. 38(4): 353-361.

Chen, Y., Fan, G., Chen, B., Xie, Y., Wu, H., Wu, Y., Yan, C., Wang, J. (2006). Separation and quantitative analysis of coumarin compounds from *Angelica dahurica* (Fisch. ex Hoffm) Benth. et Hook. f by pressurized capillary electrochromatography. J. Pharm. Biomed. Anal. 41: 105-116.

Deb, M., Kumar, D. (2021). Grain protectant behaviour of *Artemisia annua* and reduction in nutritional damage of wheat flour (*Triticum aestivum* L.) caused by *Tribolium castaneum*. Food Cont. 127: 108114.

Farkhondeh, T., Kianmehr, M., Kazemi, T., Samarghandian, S., Khazdair, M.R. (2020). Toxicity effects of *Nerium oleander*, basic and clinical evidence: A comprehensive review. Hum. Exp. Toxicol. 39(6): 773-784.

Farooqui, S., Tyagi, T. (2018). *Nerium oleander*: It's application in basic and applied science: A Review. Int. J. Pharm. Pharm. Sci. 10(3): 1-4.

Gautam, S.G., Opit, G.P. (2015). Phosphine resistance in eggs of *Tribolium castaneum* and *Plodia interpunctella* from almond storage facilities in the Central Valley of California. Integ. Prot. Stor. Prod. IOBC-WPRS Bull. 111: 41-49.

Goktas, O., Mammadov, R., Duru, M.E., Ozen, E., Colak, A.M. (2007). Application of extracts from the poisonous plant, *Nerium oleander* L., as a wood preservative. Afr. J. Biotechnol. 6(17): 2000-2003.

Hameed, A., Freed, S., Hussain, A., Iqbal, M., Hussain, M., Naeem, M, ...Tipu, A.L. (2012). Toxicological effects of neem (*Azadirachta indica*), Kanair (*Nerium oleander*) and spinosad (Tracer 240 SC) on the red flour beetle (*Tribolium castaneum*) (Herbst.). Afr. J. Agric. Res. 7(4): 555-560.

Hamel, D., Rozman, V., Liška, A. (2020). Storage of Cereals in Warehouses with or without Pesticides. Insects. 11(12): 846.

Jamilah, Mujnisa, A., Akbar. (2021). The population of red flour beetle (*Tribolium casteneum*) imago and crude protein of rice bran with an addition of lemongrass (*Cymbopogon citratus*) flour. In IOP Conference Series: Earth and Environmental Science IOP Conference Series: Earth and Environmental Science, Volume 788, The 3rd International Conference of Animal Science and Technology 3-4 November 2020, Makassar, Indonesia.

Mamun, M.S.A., Shahjahan, M., Ahmad, M. (2008). Laboratory evaluation of some indigenous plant extracts as repellent against red flour beetle, *Tribolium castaneum* Herbst. Banglad. J. Entomol. 18(1): 91-99.

Mostafa, M., Hossain, H., Hossain, M.A., Biswas, P.K., Haque, M.Z. (2012). Insecticidal activity of plant extracts against *Tribolium castaneum* Herbst. J. Adv. Sci. Res. 3(03): 80-84.

Mu, F., Yang, L., Wang, W., Luo, M., Fu, Y., Guo, X., Zu, Y. (2012). Negative-pressure cavitation extraction of four main vinca alkaloids from *Catharanthus roseus* leaves. Molecules. 17: 8742-8752.

Ordonez, A., Gomez, J., Vattuone, M. (2006). Antioxidant activities of *Sechium edule* (Jacq.) Swartz extracts. Food Chem. 97: 452-458.

Padín, S.B., Fuse, C.B., Urrutia, M.I., Dal Bello, G. (2013). Toxicity and repellency of nine medicinal plants against *Tribolium castaneum* in stored wheat. Bull. Insectology. 66(1): 45-49.

Padín, S., Dal Bello, G., Fabrizio, M. (2002). Grain loss caused by *Tribolium castaneum*, *Sitophilus oryzae* and *Acanthoscelides obtectus* in stored durum wheat and beans treated with *Beauveria bassiana*. J. Stored Prod. Res. 38(1): 69-74.

Panezai, G.M., Javaid, M., Shahid, S., Noor, W., Bibi, Z., Ejaz, A. (2019). Effect of four plant extracts against *Trogoderma granarium* and *Tribolium castaneum*. Pak. J. of Bot. 51(3): 1149-1153.

Pavela, R. (2016). History, presence and perspective of using plant extracts as commercial botanical insecticides and farm products for protection against insects-A review. Plant Protect. Sci. 52(4): 229-241.

Plante, K.S., Dwivedi, V., Plante, J.A., Fernandez, D., Mirchandani, D., Bopp, N., ...Newman, R.A. (2021). Antiviral activity of oleandrin and a defined extract of *Nerium oleander* against SARS-CoV-2. Biomed. Pharmacother. 138: 111457.

Regnault-Roger, C., Vincent, C., Arnason, J.T. (2012). Essential oils in insect control: low-risk products in a high-stakes world. Annu. Rev. Entomol. 57: 405-424.

Rehman, H.U., Qurban, A.L.I., Mirza, S., Sharif, S., Hasan, M.U., Yasir, M. (2019). Comparative toxic potential of some plant extracts and spinetoram against *Tribolium castaneum* (Herbst, 1797) (Coleoptera: Tenebrionidae). Turk. J. Entom. 43(2): 201-210.

Rösner, J., Wellmeyer, B., Merzendorfer, H. (2020). *Tribolium castaneum*: A model for investigating the mode of action of insecticides and mechanisms of resistance. Curr. Pharm. Des. 26(29): 3554-3568.

Sagheer, M., Hasan, M., Najam-ul-Hassan, M., Farhan, M., Khan, F.Z.A., Rahman, A. (2014). Repellent effects of selected medicinal plant extracts against rust-red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae). J. Entomol. Zool. Stud. 2(3): 107-110.

SAS, (2008). SAS® for Windows, version 9.2. SAS Institute, Cary, North Carolina, USA.

Souto, A.L., Sylvestre, M., Tölke, E.D., Tavares, J.F., Barbosa-Filho, J.M., Cebrián-Torrejón, G. (2021). Plant-derived pesticides as an alternative to pest management and sustainable agricultural production: Prospects, applications and challenges. Molecules. 26(16): 4835.

Teng, H., Choi, Y.H. (2014). Optimization of ultrasonic-assisted extraction of bioactive alkaloid compounds from rhizoma coptidis (*Coptis chinensis* Franch.) using response surface methodology. Food Chem. 142: 299-305.

Zaid, R., Canela-Garayoa, R., Ortega-Chacón, N.M., Mouhouche, F. (2022). Phytochemical analyses and toxicity of *Nerium oleander* (Apocynaceae) leaf extracts against *Chaitophorus leucomelas* Koch, 1854 (Homoptera: Aphididae). J. Saudi Soc. Agric. Sci. 21(5): 310-317.

Zettler, L.J. (1991). Pesticide resistance in *Tribolium castaneum* and *T. confusum* (Coleoptera: Tenebrionidae) from flour mills in the United States. J. Econ. Entomol. 84(3): 763-767.