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RESEARCH ARTICLE

Chemical assessment of seed oil extracted from *Rhanterium epapposum* Oliv. and its insecticidal efficacy against oleander aphid, *Aphis nerii*

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Abstract

A biopesticide from the seeds of the most abundant desert plant of the Arabian Peninsula, *Rhanterium epapposum* Oliv., was attempted in the current investigation against *Aphis nerii*, a common pest of oleander an ornamental shrub, in laboratory bioassays and its chemical components were profiled through gas chromatography-mass spectroscopy (GC-MS). The oil yielded an average of 2.40 % and its bio efficacy revealed its potency as a bio-pesticide efficient to manage the aphids. While in the bioassay for contact toxicity, the highest concentration of the investigated oil (500 ppm) revealed a 96.70 % mortality on the 3rd day of treatment; in the bioassay for oral toxicity, it was 93.33 %. The trend of mortality was declining for diminishing concentrations of the oil. Further, the toxic regression lines of the highest dose of *R. epapposum* oil provided the LC_{50} and LC_{95} values of 8.67 and 61.78 ppm respectively for oral toxicity and 14.07 and 85.48 ppm respectively for contact toxicity showing the chief toxicity for the highest dosage of *R. epapposum* oil in aphid management. The GC-MS chromatogram exhibited the existence of 17 compounds with 16 possessing insecticidal properties representing 98.72 % of total oil content, extenuating its efficacy to be formulated as a commercial pest control product. 9, 12-octadecadieonic acid (Z, Z)-methyl ester is the compound detected with the highest percent holding 33.19 % followed by 2-methoxy-4-vinylphenol (29.45 %) and hexadecanoic acid (22.57 %). The safety of the oil for both human health and the environment requires further investigations. The study is a baseline that reflects the insecticidal property of the native treasure of Kuwait, *R. epapposum* seeds. Further research to isolate the compound affecting the biopesticidal activity and chemo olfactory investigations on the aphids, to sense out the compounds responsible for the toxicity to be formulated as commercial biopesticides is in demand.

Keywords: aphid; bioactive compounds; botanical pesticide; desert ecosystems; toxicity; *Nerium oleander*; *Rhanterium epapposum*

Introduction

Oleander (*Nerium oleander* L., Gentianales: Apocynaceae), commonly known as oleander or rosebay, is an extremely toxic evergreen ornamental shrub, in other terms a miniature tree, grown in gardens, parks and roadways of the Mediterranean region that offers aesthetic appeal to the landscape. Due to its widespread cultivation, its exact origin has not been determined; nonetheless, suggestions state from the Mediterranean, Asia and Iran (1). Oleander is imperative for traditional medicine as it is utilised as an expectorant, diuretic, emetic, diaphoretic and heart activator (2). Toxic substances such as glycosidase, oleandrine, oleandrigenin, neriine, cardenolides and strychnine present in every part of the oleander plant are the primary sources of acute toxicity (3–5). Oleander can withstand drought and poor soil conditions and is recommended for urban landscaping in the Arab region. The diverse phenotypes of *N. oleander* bearing white, pink and red flowers viz., *N. oleander album, N. oleander carnum, N. oleander roseum* are widely used in urban landscaping (1). Though the plant is highly poisonous, oleander poisons are known to have no effect on certain insects that consume the plant (6). Aphids are one of the only insects that can survive by sucking sap of these plants without suffering any negative effects, though the sap-bearing segments of the plant are poisonous and can lead to gastrointestinal and cardiovascular problems in any creature (7–9). Every portion of the plant, including the lower and top leaves, branches and flowers, is home to aphids.

The oleander aphid, also known as the milkweed aphid (*Aphis nerii*) is a frequent pest of a number of ornamental plants in the Apocynaceae and Asclepiadaceae families (10, 11). The aphid is one of the approximately 5600 species of Aphidomorpha that have been described globally (12, 13). The oleander aphid is a global species that can be found in warm temperate to tropical climates; however, the chief host, oleander is thought to have originated in the Mediterranean region (Fig. 1).

The bright yellow aphids with black appendages live in colonies and ingest the sap from the phloem of the oleander plant through their slender threadlike mouthpart; both the nymph and adult insects consume the host sap. They prefer the soft and tender host tissues, weaken the plant and curl the leaves (Fig. 2). Furthermore, the developing terminals are susceptible to deformation leading to increased leaf malformation and stunted growth (7, 6). The aphids penetrate the root tissue, xylem and

Fig. 1. Aphid conglomeration in the leaves and stem of oleander.

Fig. 2. Aphids sucking the sap of oleander.

phloem tissues of the leaves and stems both intracellularly, but not the epidermal layers. The quantity of aphids on the plant determines the extent of their infestation; the plant may die in case of severe infestation (14). Additionally, as the insect sucks sap from plant tissues, it secretes a honeydew, a sweet sticky liquid that coats the vegetative branches, collects dust particles and grows black sooty molds that impede respiration, photosynthetic activity and the transpiration process. The aphid colonies inflict aesthetic harm due to the copious amount of sticky honeydew that the colony members generate and the black sooty mold that develops on the honeydew (15, 16) (Fig. 3). When the insect has to make honeydew, it exhales too many carbohydrates and the excretions may result in the growth of black fungus that hinders photosynthetic activity and causes chlorosis and a subsequent loss of output (17, 18). Aphid saliva contains the enzyme pectinase that breaks down the cellulose and the intermediate layer between cells and is capable of extracting substances for protein synthesis and surplus carbohydrates to create honeydew droplets. Bright yellow aphids with their cast skins, sticky honeydew and sooty molds are indicators of afflicted plants. These insects harm plants directly by sucking phloem tissue and causing plant malformations and indirectly by causing sooty mold fungal disease due to honeydew production and by spreading viruses (17). The aphid can spread a number of plant viruses as well, that harm the plant and lower its yield. It is responsible for spreading sugarcane mosaic potyvirus and papaya ringspot potyvirus. Apocynaceae (*Nerium* and *Vinca*), Asclepiadaceae (*Asclepias*, *Calotropis* and *Gomphocarpus*), Asteraceae, Convolvulaceae, Euphorbiaceae and Rutaceae are just a few of the plants and families that this aphid can target (12, 19). The aphid affects ornamental nurseries and landscaping with the possibility of stunted growth, with an unsightly appearance due to the recurring heavy infestations all year long.

Since the menace due to the aphid is severe among ornamental landscaping and gardening, leaning on chemical pesticides for their management seemed the initial tactic and the sole option. However, the constant use of chemical pesticides has caused resistance of insects to the insecticides, a resurgence of the pests in large numbers and toxic residues of the chemicals in food commodities leading to environmental pollution (20). Therefore, plant-based botanical insecticides have drawn interest from all across the world in an effort to create innovative and sustainable pest management techniques (21). Due to their benefits on environmental preservation, minimal toxicity to non-target organisms, slower pace of resistance acquisition, botanical pesticides may be helpful as substitute instruments for integrated

Fig. 3. Honey dew secretion, ants and sooty mold infection.

pest control. In order to safely manage the pests, without endangering the ecology, researchers are in search for natural and safe insecticides. A wide range of various chemical components from different plant parts has been investigated for insecticidal, insect repellent, anti-feedant, insect growth inhibition and oviposition deterrent properties. These compounds have an array of physiological and behavioural consequences in insects to avoid the target plant for feeding or oviposition (22). *Rhanterium epapposum* Oliv., is reported to be such a plant pronounced to have insecticidal properties.

Rhanterium epapposum is a desert shrub native to the Arabian Peninsula, locally called as "Al-Arfaj" in Arabic (Fig. 4). The huge bushy shrub with a complicated network of branches, tiny thorny leaves and bright yellow-coloured flowers is a symbol of Arabian deserts. It is the key plant for desert biodiversity and restoration schemes with its remarkable ability to withstand drought, heat and salinity stresses and its potential to accumulate toxins from the soil (23–25). The plant serves as a nourishable fodder for the desert animals viz., camels and sheep (26). The traditional folks use the plants as a fuel source and indigenous medicine to heal skin and gastric infections. The phytochemical screening on the above-ground parts of the *R. epapposum* displayed the presence of flavonoids, tannins, sterols, triterpenes and essential oils (27). The phytochemical screenings of the plant have revealed its antioxidant effects, due to its polyphenolic contents (28). It appears that the plants survive abiotic stresses in the extreme deserts due to the production of phytochemicals in their plant parts to protect themselves from external abiotic restrictions (29), in addition to the presence of beneficial microbes (30) and the diversity of the arbuscular mycorrhizal fungi (AMF) community in association with the native desert plant roots (31). In olden days, the plant was used to repel insect pests as a botanical insecticide (32). The insecticidal effect of *R. epapposum* extract for various insects was reported by various researcher's (33, 34). The essential oil extracted from *R. epapposum* plants, rich in alkaloids, flavonoids, triterpenes, coumarins and tannins, was found to have higher insecticidal properties against the several other agricultural pests such as mosquitoes and other insects (35). As *Rhanterium* communities are predominant in the Arabian Peninsula, the essential oil from the plant can be utilised as a bio-pesticidal formulation for managing the menace of pests on crop production and landscaping. The plant parts of *R. epapposum* were widely analysed for their therapeutic effects by several researchers. However, the seeds have not been investigated to the finest of our awareness. In light of the above, an attempt was taken to investigate the bio-pesticidal effect of the seed oil of *R. epapposum* on the oleander aphids. The aim of the current investigation is to evaluate the bio-components in the seed oil of *R. epapposum* by GC-MS and to determine its aphicidal activity against the oleander aphid in the laboratory bioassay that could lead to the identification of a botanical insecticide for the control of aphids.

Materials and Methods

Rhanterium epapposum seed collection

The capitula of the native plant *R. epapposum* was harvested from 5 diverse sites in a conserved area characterised as the desert plain ecosystem in the Kuwait Institute for Scientific Research (KISR's) Station for Research and Innovation (KSRI), situated in Kabd, the southern part of Kuwait (Fig. 4) (36). There are 1–10 achenes in a capitulum. The achenes are not scattered separately in nature and so the entire capitula is used for the extraction of the oil. The field brought seeds were cleaned and the healthy seeds were meticulously selected for oil extraction. The excess seeds were tagged with a registration number and stored in an airtight container in KISR's seed bank.

Extraction and isolation of *Rhanterium epapposum* seed oil

The seeds of *R. epapposum* were pulverised in the lab mill and 50 g of the powdered seed were taken for oil extraction. The powdered seeds were covered in layered tissue paper and kept in the thimble of the Soxhlet apparatus and the bioactive compounds in the oil were extracted with 50 mL of methanol for 8 hr. The solvent (methanol) was boiled at 65 °C, which is its boiling point, that vaporises and condenses in the condenser and drips back in the *R. epapposum* seed sample in the thimble. The solvent saturated with the extracted components then siphons back into the flask and the cycle is repeated for 8 hr. The seed extracts in methanol were condensed in a rotavapour for the essential oil yield. The extracted oil was stored at -4 °C until use (Fig. 4).

Calculation of yield

The yield of essential oil was determined and expressed on a dry weight basis using the following formula:

Oil yield (%) = Volume of the essential oil obtained (mL)/mass of dry matter (g) x 100 (37).

The extracted *R. epapposum* oil is diluted to various concentrations viz., 5, 10, 20, 40, 50, 100, 200 and 500 ppm levels for bioassay against the aphids. The essential oil was dried with anhydrous sodium sulphate ($Na_2H_2SO_4$) and then stored at -4 °C in the dark.

Gas chromatography-Mass spectrometry analysis

Gas chromatography-Mass spectrometry analysis of the methanolic oil extract of seeds was analysed using standard protocols with a high-resolution gas chromatograph mass spectrometer-double focusing sector (GC-MS DFS-03030) (ThermoScientific, Waltham, MA, USA) furnished with a DB-5MS capillary column to detect the phytochemical components in them. The operating conditions included an initial temperature of

Flower | Single capitulum | Capituli | Single seed

Fig. 4. *Rhanterium epapposum.*

15 °C for 1 min, trailed by elevating it to 280 °C over a total analysis time of 56 min. The seed extract samples of 1 µL volume were injected in a splitless mode injector. Mass spectrometry employed electron impact ionisation mode with 70 eV energy and helium grade-5 served as the carrier gas. Compound identification in the extract was done by comparing retention times of chromatographic peaks using the National Institutes Standard and Technology (NIST) library database, to compare and identify the compounds (38).

Bioassay

Aphis nerii (Aphids) was mass cultured in potted plants under greenhouse conditions. The efficacy of *R. epapposum* seed oils was examined in bioassays viz., leaf dip and dry film method under laboratory conditions using fresh leaves of the oleander. Diverse concentrations of the oil viz., 5, 10, 15, 20, 40, 50, 100, 200 and 500-ppm concentrations were examined for the oral and contact toxicity effects and compared with a standard chemical for sucking pests, Thiacloprid 240 SC (at 0.6 mL L^{-1}).

Oral toxicity (Leaf dip method)

The botanical formulations were prepared in separate beakers and the fresh leaves of oleander were dipped in the formulations for a minute and left to dry on filter paper in open condition in the laboratory. The leaves were placed in a slightly moistened filter paper in Petri dishes for the leaves to be turgid. The leaves were dipped in water for control treatment. Ten apterous aphids maintained in the same size were released in each Petri dish using a fine camel hairbrush. The bio-formulation for each concentration was replicated 3 times (Fig. 5). The experimental setup was maintained in the laboratory setting at 23 ± 5 °C and RH 30 % and 12:12 (light: dark) photoperiod. The insect mortality was recorded at 24, 48, 72, 96, 120 and 144 HAT. The mortality of the insect was confirmed by the immobility of the insects and alteration in the yellow colour of the insect body. The corrected percent mortality was worked out by using Abbott's

$$\text{Percent corrected mortality} = \frac{\text{Percent test mortality} - \text{Percent control mortality} \times 100}{\text{Percent control}}$$

correction (39).

Contact toxicity (Dry film method)

The botanical formulation was deposited on the surface of the Petri dish (5 cm diameter) and the aphids were released in the Petri dish to test the efficacy. 1 mL of the botanical formulation is uniformly spread on the inner sides of the Petri dish by swirling it gently and allowed to dry at room temperature. The universal solvent water acted as a control for dipping the leaves. The 10 apterous aphids maintained in the same size were released in each Petri dish using a fine camel hairbrush. Each concentration of the bio-formulation was replicated thrice (Fig. 5). The experimental setup was maintained in the laboratory settings at 23 ± 5 °C and RH 30 % and 12:12 (light: dark) photoperiod. The insect mortality was recorded at 24, 48, 72, 96, 120 and 144 HAT. The morbid insects were identified by the immobility of the insects and alteration in the yellow colour of the insect body. The corrected percent mortality was worked out by using Abbott's correction (39).

Statistical analysis

The statistical analysis was done by using the data on aphid mortality obtained during the study using one-way ANOVA followed by Duncan's post hoc test to detect the significance at a probability level of ($p \leq 0.05$) using the software, SPSS 26 IBM SPSS Statistics for Windows, version 26 (IBM Corp., Armonk, N.Y., USA) (40).

Results

Oil yield

The methanolic seed extracts of *R. epapposum* yielded an average of 2.40 % of essential oil. The highest yield was being 2.80 % and the lowest was 1.88 % (Table 1).

GC-MS analysis of oil extract

The quantitative seed analysis of the extracts of *R. epapposum* exhibited the existence of 17 compounds between 8.14–30.00 min retention times (Table 2). The compounds were compared and identified by NIST GC-MS library. The chromatogram of *R. epapposum* seeds constitutes 17 components (Fig. 5) namely

Table 1. Average extract of oil from methanolic extract of *Rhanterium epapposum* seeds

Location KSRI	Volume of oil (mL)	Percentage of extraction	Average yield of the extract (%)
Site 1	1.4	2.80	
Site 2	1.21	2.42	
Site 3	0.94	1.88	2.392
Site 4	1.29	2.58	
Site 5	1.14	2.28	

Table 2. Chemical constituents of *Rhanterium epapposum* seed oil extracts by gas chromatography-mass spectrometry analysis

Apex RT	Area	Area (%)	Chemical component	Formula	Molecular weight	Group
8.15	6559123.249	1.78	Phenol, 4-ethyl-2-methoxy- (CAS)	$C_9H_{12}O_2$	152	Methoxyphenols
8.84	108352127.8	29.45	2-Methoxy-4-vinylphenol	$C_9H_{10}O_2$	150	Methoxyphenols
10.86	1629487.941	0.44	Cyclohexanebutanal, 2-methyl-3-oxo-, cis-	$C_{11}H_{18}O_2$	182	Ketone, Aliphatic aldehydes
13.28	9254124.795	2.52	1,4-benzendiol, 2-(1,1-Dimethylethyl)-5-(2-Propenyl)-,	$C_{13}H_{18}O_2$	206	Hydroquinones
14.22	1057578.346	0.29	Naphthalene, 2-decyldecahydro- (CAS)	$C_{20}H_{38}$	278	Aromatic benzene
14.49	2169733.682	0.59	5,9,13-Pentadecatrien-2-one,6,10,14-trimethyl-	$C_{18}H_{30}O$	262	Acyclic diterpenoids
16.06	2967204.804	0.81	α-Cadinol	$C_{15}H_{26}O$	222	Sesquiterpenoid
16.3	681609.09	0.19	β-eudesmol	$C_{15}H_{26}O$	222	Sesquiterpenoid
20.43	1367778.177	0.37	2-Pentadecanone, 6,10,14-trimethyl-	$C_{18}H_{36}O$	268	Sesquiterpenoids
20.74	598013.157	0.16	L-Tyrosine, n-acetyl- (CAS)	$C_{11}H_{13}NO_4$	223	Non-essential amino acid
22.14	83036076.87	22.57	Hexadecanoic methyl ester	$C_{17}H_{34}O_2$	270	Fatty acid methyl ester
22.66	5163601.732	1.4	Phthalic acid, butyl 2-pentyl ester,	$C_{17}H_{24}O_4$	292	Phthalic acid ester
25.29	122001721.2	33.16	9,12-Octadecadienoic acid (Z, Z)-, methyl ester	$C_{19}H_{34}O_2$	294	Aliphatic acid methyl ester
25.4	5780933.342	1.57	9-Octadecenoic acid (z)-, methyl ester	$C_{19}H_{36}O_2$	296	Aliphatic acid methyl ester
25.92	11319455.45	3.08	Octadecanoic acid, methyl ester	$C_{19}H_{38}O_2$	298	Aliphatic acid methyl ester
29.63	1235226.853	0.34	4,8,12,16-tetramethylheptadecan-4-olide and 9(2H)-	$C_{21}H_{40}O_2$	324	Terpenoid
30	4717117.515	1.28	9(2H)-, Anthracenone, 1,3,4,9a-tetrahydro-9a-methyl-	$C_{15}H_{16}O$	212	Anthracenone

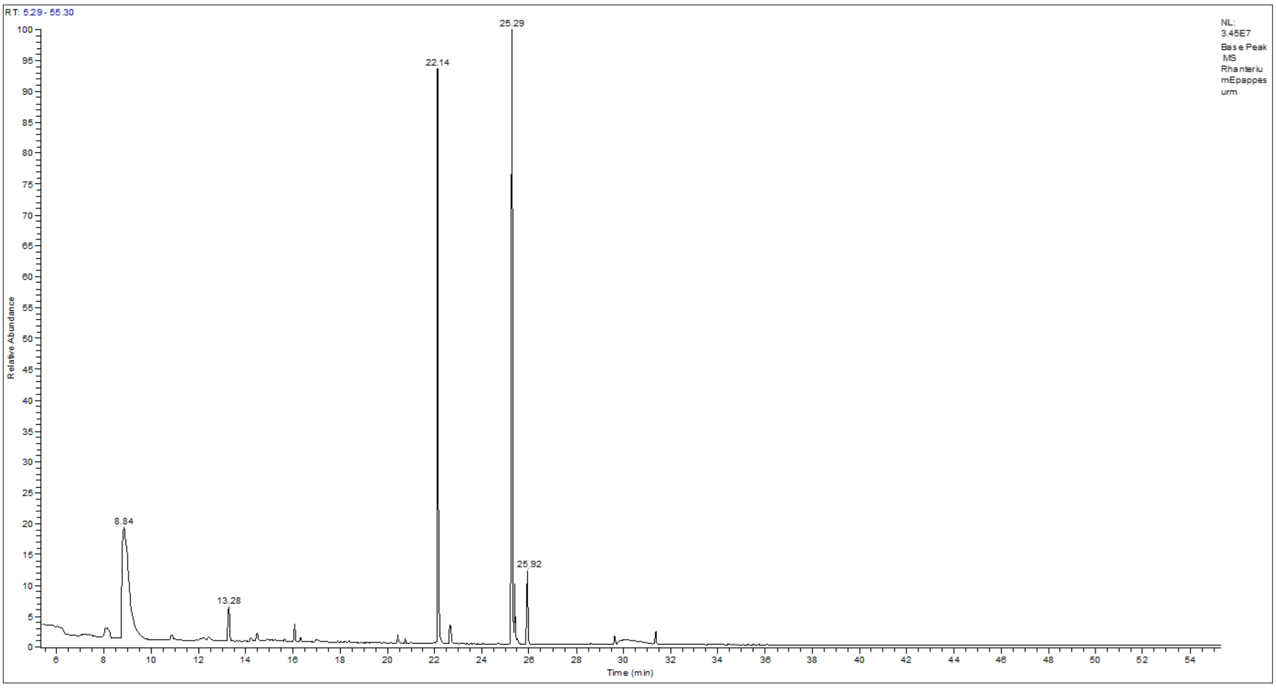

Fig. 5. Chromatogram of *Rhanterium epapposum* seed oil extract.

phenol, 4-ethyl-2-methoxy-(CAS); 2-methoxy-4-vinylphenol; cyclohexanebutanal, 2-methyl-3-oxo-, cis-; 1,4-benzendiol, 2-(1,1-dimethylethyl)-5-(2-propenyl)-; naphthalene, 2-decyldecahydro-(CAS); 5,9,13-pentadecatrien-2-one,6,10,14-trimethyl-, (E, E-)-; α-cadinol; β-eudesmol; 2-pentadecanone, 6,10,14-trimethyl-; L-tyrosine, n-acetyl- (CAS), hexadecanoic methyl ester; phthalic acid, butyl 2-pentyl ester; 9,12-octadecadienoic acid (Z,Z)-, methyl ester; 9-octadecenoic acid (z)-, methyl ester; octadecanoic acid, methyl ester; 4,8,12,16-tetramethylheptadecan-4-olide and 9(2H)-, anthracenone, 1,3,4,9a-tetrahydro-9a-methyl- in 30 min of retention time. The compound 9,12-octadecadienoic acid (Z, Z)-, methyl ester showed the highest area of 33.16 % at 25.29 mins, followed by 2-methoxy-4-vinylphenol at 8.84 mins and hexadecanoic acid methyl ester at 22.14 min (Fig. 5). All the detected compounds possess insecticidal properties according to literature. The compound detected with the highest percent area is 9, 12 –octadecadieonic acid (Z, Z)- methyl ester (33.19 %) and is reported to possess insecticidal properties against sucking pests (29). The second compound detected with highest percent is 2-methoxy-4-vinylphenol holding 29.45 %. The next highest compound is, hexadecanoic acid, methyl ester possessing 22.57 % area.

Bio-efficacy of *Rhanterium epapposum* oil, against oleander aphid, *Aphis nerii*

Dry film method (Contact toxicity)

The bioefficacy of *R. epapposum* oil against oleander aphid, *Aphis nerii,* under laboratory condition by dry film method, revealed the fact that the oil holds capability of a bio-insecticide (Table 3). The highest tested dose (500 ppm) produced better efficacy with 1.67 and one insect being alive after 24 and 48 HAT and attained 100 % mortality from 72 HAT, which is significant to the compared standard chemical, Thiacloprid 240 SC at 0.6 mL L^{-1}, with 2 and 1 live insects at 24 and 48 HAT and attained 100 % mortality further. However, the untreated control recorded 9.67, 8.67, 7.33, 7.00, 6.00, 5.67 and 4.67 live insects at 24, 48, 72, 96, 120, 144 and 178 HAT. The percent reduction over control was 88.23 and 93.79 % for the best dosage of 500 ppm of *R. epapposum* oil on the 3rd and 7th days after treatment. The next highest dose was *R. epapposum* oil at 200 ppm recorded 2.67, 1.33, 0.67 and 0.33 live aphids 24, 48, 72 and 96 DAT and attained 100 % mortality. The next level of *R. epapposum* dose at 100 ppm, kept the investigated aphids alive for 5 consecutive days (4.33, 2.67, 1.33, 0.33 and 1 aphids) from the first till 5th day respectively, with a per cent reduction of 70.58 and 82.54 % after 3 and 7 DAT. In the declining doses of *R. epapposum* oil at 50, 40, 20 and 10 ppm, the aphids were alive for the first 5 days with varying levels of mortality. The percent reduction of aphids over control was registered as 58.82, 52.94, 41.17, 39.17, 27.41 and 76.33, 72.15, 63.92, 63.05, 46.46 % for the doses of 50, 40, 20, 10 and 5 ppm levels of *R. epapposum* oil. The aphid mortality on the 3rd day was registered as 96.7 % for the best dosage of *R. epapposum* oil at 500 ppm, which is significant with the standard check, recording 100 % mortality. The *R. epapposum* oil in the lessening level of dosage viz., 200, 100, 50, 40, 20, 10 and 5 ppm recorded, 93.33, 86.67, 83.33, 80.00,66.67, 63.33, 50.00 % mortality respectively. The live aphids after treatment changed from bright lushy yellow colour to black colour on mortality in varying shades in various stages. The healthy aphids were plumby and dark yellow in colour, whereas the morbid aphids turn pale and black eventually (Fig. 6, 7).

Leaf dip method (Oral toxicity)

The bio efficacy of *R. epapposum* oil against oleander aphid, *A. nerii,* under laboratory conditions, in leaf dip method revealed the fact that the investigated aphids have insecticidal properties against aphids (Table 4). The highest dose revealed the best efficacy recording the insect mortality from the 4th day of treatment, with 1.67, 1 and 0.33 aphids alive after 24, 48 and 72 hr of treatment (HAT). However, the standard check, recorded the 100 % mortality on the 3rd day after treatment. The highest dose of *R. epapposum*, recorded an 88.2 % reduction over control on the 3rd DAT and a 92.12 % reduction on the 7th DAT. However, the control treatments witnessed the aphids alive until the 7th day of the investigation with 9.67, 9.33, 8.00, 7.00, 6.33, 5.67 and 4.67 live aphids from the first until the 7th day of treatment. The second-highest dose of *R. epapposum* oil extract at 200 ppm, recorded 3.33, 2.33 and 1 aphid on 24, 48 and 72 DAT, recording an 80.22 % reduction from control on the 3rd day and 88.81 % on the 7th day, with a cent percent mortality on the 4th day. The next highest

Table 3. Efficacy of *R. epapposum* oil, derived from methanol, against oleander aphid, *Aphis nerii* under laboratory conditions – dry film method

Treatment details		Number of aphids alive							Average No. of aphids alive after 3 days	PROC 3 DAT	Average No. of aphids alive 7 DAT	PROC7 DAT	Mortality percentage 3 DAT
		24 HAT	48 HAT	72 HAT	96 HAT	120 HAT	144 HAT	178 HAT					
T1	*R. epapposum* oil 5 ppm	$7.67^{f} \pm 0.57$	$6.00^{e} \pm 1.00$	$5.00^{e} \pm 1.00$	$3.67^{d} \pm 1.115$	$2.00^{c} \pm 1.00$	$1.00^{b} \pm 0.00$	$0.00^{a} \pm 0.00$	$6.22^{e} \pm 0.84$	27.41	$3.71^{e} \pm 0.59$	46.46	$50.00^{f} \pm 10.0$
T2	*R. epapposum* oil 10 ppm	$6.67^{e} \pm 0.57$	$5.00^{d} \pm 0.00$	$3.67^{d} \pm 0.577$	$2.00^{bc} \pm 0.577$	$1.00^{bc} \pm 0.577$	$0.00^{a} \pm 0.577$	$0.00^{a} \pm 0.00$	$5.11^{d} \pm 0.19$	39.17	$2.86^{d} \pm 0.33$	63.05	$63.33^{e} \pm 5.77$
T3	*R. epapposum* oil 20 ppm	$6.67^{e} \pm 0.57$	$5.00^{d} \pm 1.00$	$3.33^{d} \pm 0.577$	$1.67^{b} \pm 0.577$	$0.67^{b} \pm 0.577$	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$	$5.00^{d} \pm 0.66$	41.17	$2.50^{d} \pm 0.36$	63.92	$66.67^{e} \pm 5.77$
T4	*R. epapposum* oil 40 ppm	$5.92^{d} \pm 0.57$	$4.75^{bc} \pm 0.577$	$2.83^{c} \pm 0.00$	$1.67^{b} \pm 0.000$	$0.67^{b} \pm 0.577$	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$	$4.08^{c} \pm 0.39$	52.94	$1.93^{cd} \pm 0.22$	72.15	$80.00^{d} \pm 0.000$
T5	*R. epapposum* oil 50 ppm	$5.33^{d} \pm 0.57$	$3.33^{bc} \pm 0.577$	$1.67^{b} \pm 0.577$	$0.67^{ab} \pm 0.577$	$1.00^{bc} \pm 0.577$	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$	$3.60^{bc} \pm 0.51$	58.82	$1.64^{c} \pm 0.36$	76.33	$83.33^{cd} \pm 5.77$
T6	*R. epapposum* oil 100 ppm	$4.33^{c} \pm 0.57$	$2.67^{b} \pm 0.577$	$1.33^{b} \pm 0.577$	$0.33^{a} \pm 0.577$	$1.00^{bc} \pm 0.577$	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$	$2.99^{b} \pm 0.51$	70.58	$1.21^{bc} \pm 0.25$	82.54	$86.67^{bcd} \pm 5.77$
T7	*R. epapposum* oil 200ppm	$2.67^{b} \pm 0.57$	$1.33^{a} \pm 0.577$	$0.67^{a} \pm 0.577$	$0.33^{a} \pm 0.577$	$0.00^{a} \pm 0.577$	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$	$1.56^{a} \pm 0.38$	80.35	$0.86^{ab} \pm 0.33$	87.59	$93.33^{abc} \pm .5.77$
T8	*R. epapposum* oil 500 ppm	$1.67^{a} \pm 0.00$	$1.00^{a} \pm 1.00$	$0.33^{a} \pm 0.577$	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.577$	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$	$1.11^{a} \pm 0.51$	88.23	$0.43^{a} \pm 0.16$	93.79	$96.70^{ab} \pm 5.77$
T9	Thiacloprid 240 SC at 0.6 mL L^{-1}	$2.00^{ab} \pm 0.57^{a}$	$1.00^{a} \pm 0.577$	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$	$0.89^{a} \pm 0.51$		$0.40^{a} \pm 0.23$		$100.00^{a} \pm 0.00$
T10	Control	$9.67^{g} \pm 0.57$	$8.67^{f} \pm 0.577$	$7.33^{f} \pm 0.577$	$7.00^{e} \pm 1.00$	$6.00^{d} \pm 1.00$	$5.67^{c} \pm 0.577$	$4.67^{b} \pm 0.577$	$8.50^{f} \pm 2.3$		$6.93^{f} \pm 0.65$		$25.00^{g} \pm 5.77$

*Mean of 3 replications.

Values are given as mean ± standard deviation.

In a column, mean followed by column letter(s) are not significantly different by DMRT ($p = 0/05$).

Table 4. Efficacy of *R. epapposum* oil, derived from methanol, against Nerium aphid, *Aphis nerii* under laboratory conditions – leaf dip method

	Treatment details	No. of aphids alive							Average No. of aphids alive after 3 days	PROC 3 DAT	Average No. of aphids alive 7 DAT	PROC 7 DAT	Mortality percentage 3 DAT
		24 HAT	48 HAT	72 HAT	96 HAT	120 HAT	144 HAT	178 HAT					
T1	*R. epapposum* oil 5ppm	$7.67^{f} \pm 0.57$	$6.33^{e} \pm 0.57$	$4.67^{c} \pm 0.57$	$3.33^{c} \pm 0.57$	$2.33^{c} \pm 0.57$	$1.00^{b} \pm 0.00$	$0.00^{a} \pm 0.00$	$6.22^{e} \pm 0.38$	30.88	$3.62^{d} \pm 0.33$	50.01	$55.50^{c} \pm 0.57$
T2	*R. epapposum* oil 10 ppm	$6.67^{e} \pm 0.57$	$5.67^{de} \pm 0.57$	$4.33^{c} \pm 0.57$	$3.00^{c} \pm 1.00$	$1.33^{b} \pm 0.57$	$0.67^{b} \pm 0.57$	$0.00^{a} \pm 0.00$	$5.56^{e} \pm 0.51$	38.22	$3.10^{cd} \pm 0.50$	57.18	$56.67^{c} \pm 0.57$
T3	*R. epapposum* oil 20ppm	$6.33^{e} \pm 1.00$	$6.00^{e} \pm 1.00$	$4.67^{c} \pm 0.57$	$3.00^{c} \pm 0.00$	$2.00^{bc} \pm 0.00$	$1.00^{b} \pm 0.00$	$0.00^{a} \pm 0.00$	$5.67^{e} \pm 0.58$	37.00	$3.29^{d} \pm 0.25$	54.55	$55.00^{c} \pm 0.57$
T4	*R. epapposum* oil 40 ppm	$5.33^{d} \pm 0.57$	$4.67^{d} \pm 0.57$	$4.00^{c} \pm 0.00$	$2.67^{c} \pm 0.57$	$1.33^{b} \pm 0.57$	$0.67^{b} \pm 0.57$	$0.00^{a} \pm 0.00$	$4.67^{d} \pm 0.33$	48.11	$2.67^{c} \pm 0.22$	63.12	$60.00^{c} \pm 0.00$
T5	*R. epapposum* oil 50 ppm	$4.67^{c} \pm 0.57$	$3.67^{bc} \pm 0.57$	$2.67^{b} \pm 0.57$	$1.33^{b} \pm 0.57$	$0.33^{a} \pm 0.57$	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$	$3.67^{c} \pm 0.57$	59.22	$1.81^{b} \pm 0.36$	75.00	$73.33^{b} \pm 0.57$
T6	*R. epapposum* oil 100 ppm	$3.67^{b} \pm 0.57$	$2.67^{b} \pm 0.57$	$1.33^{a} \pm 0.57$	$0.33^{a} \pm 0.57$	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$	$2.50^{b} \pm 0.51$	72.22	$1.14^{a} \pm 0.28$	87.10	$86.67^{a} \pm 0.057$
T7	*R. epapposum* oil 200ppm	$3.33^{b} \pm 0.57$	$2.33^{a} \pm 0.57$	$1.00^{a} \pm 0.57$	$0.00^{a} \pm 0.57$	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$	$2.22^{ab} \pm 0.38$	75.33	$0.95^{a} \pm 0.16$	86.80	$90.00^{a} \pm 0.00$
T8	*R. epapposum* oil 500 ppm	$2.67^{a} \pm 0.57$	$1.67^{a} \pm 0.57$	$1.00^{a} \pm 0.00$	$0.00^{a} \pm 0.57$	$0.00^{a} \pm 0.57$	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$	$1.78^{ab} \pm 0.38$	80.22	$0.81^{a} \pm 0.22$	88.81	$90.00^{a} \pm 0.00$
T9	Thiacloprid 240 SC at 0.6 mL L^{-1}	$2.67^{a} \pm 0.57$	$1.67^{a} \pm 0.57$	$0.67^{a} \pm 0.57$	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$	$1.67^{a} \pm 0.57$		$0.71^{a} \pm 0.28$		$93.33^{a} \pm 0.57$
T10	Control	$9.67^{g} \pm 0.57$	$9.33^{f} \pm 0.57$	$8.00^{d} \pm 0.00$	$7.00^{d} \pm 1.00$	$6.33^{d} \pm 1.15$	$5.67^{c} \pm 0.57$	$4.67^{b} \pm 0.57$	$9.00^{f} \pm 0.33$		$7.24^{e} \pm 0.36$		$20.00^{d} \pm 0.00$

*Mean of 3 replications.

Values are given as mean ± standard deviation.

In a column, mean followed by column letter(s) are not significantly different by DMRT (p = 0/05).

Fig. 6. The experimental setup.

Fig 7. Stages of Aphid Mortality.

dose at 100 ppm, attained 100 % mortality on the 5th day, with the alive insects declining from 3.67, 2.67, 1.33 and 0.33 on 24, 48, 72 and 96 DAT, recording 75.33 and 86.80 % reduction over control. The dose of next level, 50 ppm registered cent percent mortality in the 6th day with a percent control over reduction of 59.22 and 75 % on the 3rd and 7th day. The declining doses, viz., 40, 20, 10 and 5 ppm recorded mortality of the entire lot on the seventh day with 48.11, 37 and 38.22, 30.88 % reduction on the 3rd day and 57.18, 54.55, 63.12 and 50.01 % reduction on the 7th day after treatment. The overall mortality percent ranged from 90.00 to 55.55 % from the highest to the least dose investigated.

Comparative bio efficacy of both bioassays

While comparing both the bioassays in their efficacy in controlling aphids, the outcomes of the dry film method are more tangible in comparison to the leaf dip method. The mortality rate on the 3rd day of treatment in dry film technique for the highest dose (500 ppm) was 96.7 %, whereas it was 90.0 % in leaf dip method. At 100-ppm concentration, the mortality rate was similar in both the bioassays (86.67 %) on the 3rd day of treatment (Fig. 8). The percent reduction over control was 88.23 % on the 3rd day for 500 ppm concentration in dry film method, whereas it was 80.22 for leaf dip bioassay and the same trend was noticed on the 7th day as well exhibiting 93.79 and 88.81 % in dry film and leaf dip bioassays (Fig. 9). Accordingly, the number of aphids alive was roughly less than 2 and 1 after 3 and 7 days in dry film and leaf dip bioassays (Fig. 10), substantiating the maximum effect of contact toxicity more than systemic effect (Fig. 11).

Acute toxicity of *Rhanterium epapposum* oil, against oleander aphid, *Aphis nerii* under laboratory condition

In order to further compare the toxicity of the *R. epapposum* essential oils in the bioassays, toxicity regression lines of the various doses of *R. epapposum* oil were calculated based on the number of live aphids after 24, 48, 72, 96, 120, 144 and 178 HAT for 8 concentrations of the oil (500, 200, 100, 50, 40, 20, 10 and 5 ppm). The LC_{50} and LC_{95} values of the aphid, *A. nerii* to various levels of *R. epapposum* oil in both the bioassays were depicted in Table 5. The median lethal concentration (LC_{50}) was 8.67, 13.96, 23.14, 28.25, 35.29, 40.06, 41.46 and 56.09 % for the doses 500, 200, 100, 50, 40, 20, 10 and 5 ppm levels, declaring the highest toxicity of 500 ppm of *R. epapposum* oil. A similar trend is noticed for the median lethal dose of *R. epapposum* oil at 95 % level as 61.78, 74.29, 101.9, 120.32, 184.79 and 182.26. 210.01 and 242.74 %, for the doses 500, 200, 100, 50, 40, 20, 10 and 5 ppm levels reflecting the highest toxicity level as 500 ppm.

Discussion

Oleander (*Nerium oleander* L.), a tough drought tolerant plant, is widely utilised in urban landscaping in the Mediterranean region (40, 41). Due to its profuse flowering that is long lasting with moderate hardiness the plant is used for hedging along highways, beaches, gardens and parks. It is grown as an indoor plant in some parts of the region. In addition, the plant holds ethno medicinal value (42). It embraces therapeutic properties such as antimicrobial, antibacterial, anti-inflammatory, antinociceptive

Table 5. Acute toxicity of *Rhantarium epapposum* oil, derived from methanol, against *Nerium* aphid, *Aphis nerii* under laboratory conditions

Oil	Concentration	LC_{50} (ppm)	95 % fiducial limit		LC_{95} (ppm)	95 % fiducial limit		Regression equation	X^2
			LL	UL		LL	UL		
R. epapposum oil (Dry film method)	5 ppm	56.09	36.69	81.31	242.74	107.08	550.28	Y= 2.5854x + 0.4784	5.58
	10 ppm	41.46	25.21	68.18	210.01	75.84	581.55	Y = 2.3346x + 1.2234	3.96
	20 ppm	40.06	24.83	64.64	182.26	73.32	453.07	Y = 2.4999x + 0.9934	3.25
	40 ppm	35.29	20.19	61.66	184.79	68.29	500.03	Y = 2.2876x + 1.4596	3.21
	50 ppm	28.25	15.92	50.15	120.32	50.63	285.93	Y = 2.6141x + 1.2066	1.78
	100 ppm	23.14	11.69	45.78	101.90	43.91	236.49	Y= 2.5548x + 1.5145	1.48
	200 ppm	13.96	4.311	45.18	74.29	33.94	162.61	Y = 2.2653x + 2.4067	0.59
	500 ppm	8.67	1.19	62.99	61.78	25.67	148.677	Y = 1.9293x + 3.1898	0.63
R. epapposum oil (Leaf dip method)	5 ppm	57.23	39.77	82.36	255.52	114.08	572.32	Y = 2.5315x + 0.5505	3.40
	10 ppm	46.44	29.78	72.42	234.19	99.97	548.6	Y = 2.341x + 1.0978	3.96
	20 ppm	47.87	29.98	76.45	286.40	104.83	782.47	Y = 2.1174x + 1.4426	4.32
	40 ppm	35.74	19.06	67.05	290.68	106.91	790.29	y = 1.8336x + 2.1563	3.59
	50 ppm	26.18	11.74	58.38	181.39	53.04	620.54	Y = 1.9568x + 2.2253	3.48
	100 ppm	20.15	8.49	47.83	107.17	39.70	289.35	Y = 2.2663x + 2.0441	1.86
	200 ppm	18.46	7.28	46.82	94.05	32.93	268.59	Y = 2.3265x + 2.0539	2.40
	500 ppm	14.07	3.77	52.45	85.48	28.23	258.83	Y = 2.0995x + 2.589	1.98

Eight concentrations of *Rhantarium epapposum* oil were used to determine the toxicity regression lines (5, 10, 20, 40, 50, 100, 200 and 500 ppm) considering the mortality for 3 days after the treatment n = 3 replicates (10 aphids/ per replicate) per concentration. All lines are significantly a good fit ($p < 0.05$).

Fig. 8. Healthy and dead aphid.

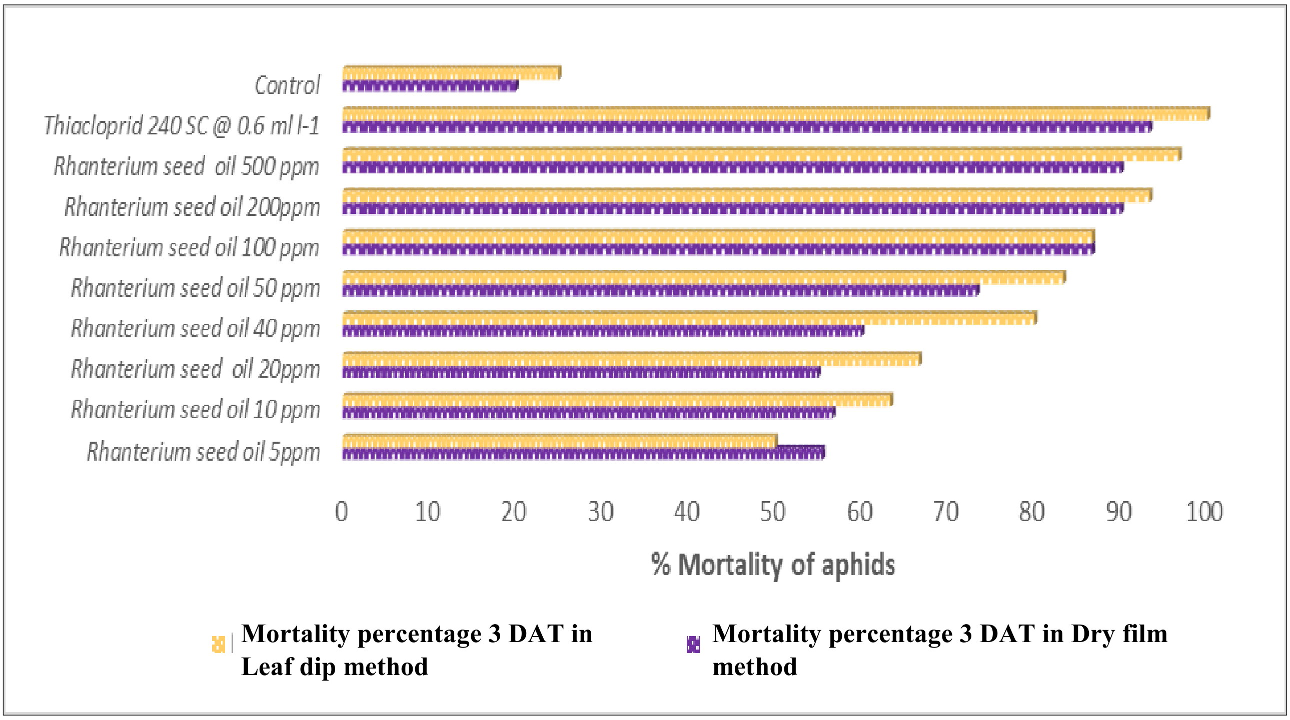

Fig. 9. Comparative efficacy of both the bioassays in aphid management based on percent mortality at 3 DAT.

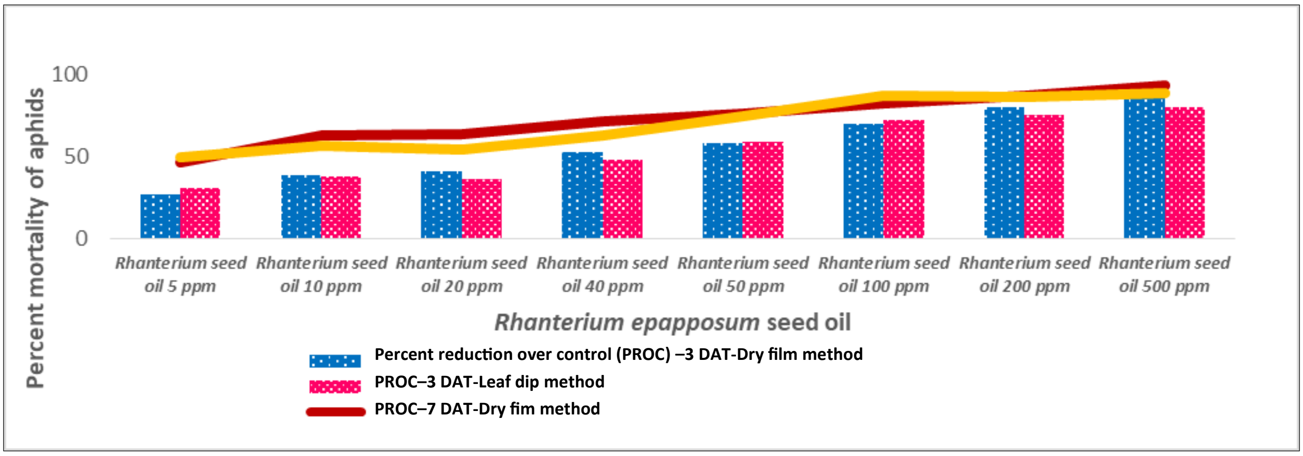

Fig. 10. Comparative efficacy of both the bioassays in aphid management based on percent reduction of aphids over control.

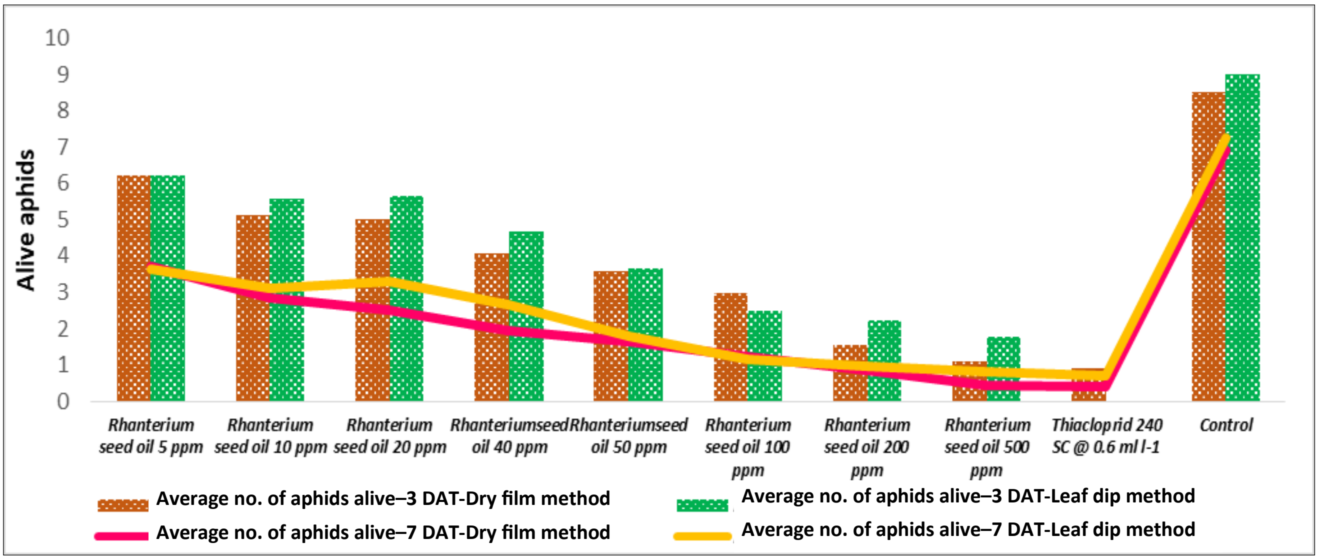

Fig. 11. Comparative efficacy of both the bioassays in aphid management based on alive aphids.

and antitumor activities (43, 44). In spite of its insect-resistant biological properties, some specialised pests attack *N. oleander* and the foremost among them is the notorious aphid, *A. nerii.* Generally, aphids are considered as major pests of various crops of economic importance due to their high reproductive potential and short lifecycle. *Aphis nerii* is cosmopolitan and distributed in diverse regions of the globe (45). The plant is an important ornamental shrub, widely used along roadways in Kuwait. Therefore, the seedlings of the plant are largely produced in the nurseries, where the aphid creates menace by feeding on the cell sap that severely damages the vigor and the plant. The insect injects toxins with its saliva that cause discoloured spots and yellowish discoloration of leaves (46). As the world is towards 'Go Green', with the aim to safeguard the environment from toxins, a safe insecticide for the management of the aphid was attempted in the study.

Rhanterium epapposum, a native desert plant of Arabian Peninsula, is traditionally used to cure diseases and to repel insects by the ancient folks (32, 47). The seeds of any plant inherit higher bio-components possessing biological properties (29). The seeds of *R. epapposum* extracted to examine its bio-pesticidal property yielded 2.4 % of oil from the methanolic seed extracts. Nevertheless, a lower percentage of 0.085 % v/w essential oil from the aerial parts of *R. epapposum* plants was obtained by hydrodistillation in a previous investigation (48). However, 11.3, 10.7 and 10.2 % extracts from an analogous species of *Rhanterium*, *R. suaveolens* was obtained in a similar investigation (49). The bio efficacy of the methanolic extracts of *R. epapposum* seed oil against *A. nerii,* under laboratory conditions, revealed it as a potential aphicide. The insecticidal effect of the plant extracts was corroborated in several studies previously that substantiated the current findings. The plant extracts repelled mosquitoes, *Aedes aegyptii* L. at a minimum effective dose (MED of 0.35 ± 0.010 mg/cm^2) (48). In addition, high larvicidal efficacy of *R. epapposum* leaf extracts against the larvae of the same mosquito species, with a lower LC_{50} value of 168.15 ppm after 24 hr of exposure was reported in an analogous investigation (40). Furthermore, investigations revealed a high toxicity of the *R. epapposum* plants against the adults of the khapra beetle (*Trogoderma granarium*) with a LD_{50} value of 22.3 µg/mL causing 70 % mortality (35). Several researchers (50, 51) demonstrated the insecticidal properties of *R. epapposum* plant parts. In an investigation to evaluate various biorational insecticides including plant extracts in managing the aphid, *A. nerii*, the Neem leaves, *Azhadirachta indica* A. Juss. at 5 mL/L demonstrated 100 % mortality followed by the other plant extracts investigated viz., aqueous leaf extract (5 %) of Kalmegh or the creat, king of bitters (*Andrographis paniculata* (Burm.f.) Wall. ex Nees) and Indian bael or golden apple (*Aegle marmelos* (L.) Corrêa) (52).

The insecticidal effect any plant is due to the secondary metabolites in their plant parts that distress the insect metabolism by acute toxicity, enzyme inhibition and disruption of food consumption (53–56). The insecticidal property of *R. epapposum* seeds is presumed to be due to the bioactive components present

Table 6. Chemical profile of *R. epapposum* seed extracts and their insecticidal effects

Sl. No	Area (%)	Compound	Group	Insecticidal effect	Reference
1	33.16	9,12-octadecadienoic acid (Z, Z)-, methyl ester	Aliphatic acid methyl ester	Insecticidal activity against sucking pests Natural pesticides to control cotton aphid, *Aphis gossypii* Insecticidal activity against mealybug, *Phenacoccus solenopsis* Insecticidal activity against cabbage aphid, *Brevicoryne brassicae* Insecticidal activity against cotton leafworm, *Spodoptera littoralis* and aphid, *A. gossypii* Insectifuge and nematicidal properties	(57–60)
2	29.45	2-methoxy-4-vinylphenol	Methoxyphenols	Insecticidal activity against the aphid, *Myzus persicae* Insecticidal activity against beet army worm, *Spodoptera exigua* Insecticidal activity against larvae of gypsy moth, *Lymandria dispar* Insecticidal activity against *Spodoptera frugiperda* Insecticidal activity against the mosquito, *Aedes aegypti*	(61–63)
3	22.57	Hexadecanoic acid, Methyl ester	Fatty Acid Methyl Ester	Insecticidal activity against the aphid, *A. gossypii* Larvicidal activity against *S. littoralis* and *S. frugiperda.* Pupicidal activities against *Musca domestica* and *Culex pipens* Adulticidal activity against the red flour beetle, *Tribolium cataneum* Insecticidal action on the mosquito, *Aedes aegyptii* Insect attractant and oviposition enhancement activity on the groundnut bruchid, *Caryedon serratus* Nematicide against *Meloidogyne incognita*	(60,64–66)
4	3.08	Octadecanoic acid, Methyl ester	Aliphatic acid methyl ester	Insecticidal activity against, aphids, *A. gosypii* Insectifuge and nematicidal activity Nematicidal property Natural pesticide for the management of fall armyworm, *S. frugiperda*	(60,67, 68)
5	2.52	1,4-benzenediol, 2-(1,1-dimethylethyl)-5-(2-propenyl)-	Hydroquinones	Nematicidal activity against the *cabbage butterfly larvae, Pieris rapae and beetle, Pentodon alerinus* Insecticidal activity against the larva of *Chironomus riparius*	(69,70)
6	1.78	Phenol, 4-ethyl-2-methoxy- (Cas)	Methoxyphenols	Insecticidal activity against termites, fall armyworm and fireants Attractant for bean weevil (pulse beetle), *Bruchus chinensis* Termicidal activity on termites Larvicide to fall army worm, *S. frugiperda* Semisynthetic insecticide for fall army worm, *S. frugiperda;*	(70–73)
7	1.57	9-octadecenoic acid (Z)-, Methyl ester	Aliphatic acid methyl ester	Insectifuge and nematicidal properties Pupicidal activity against *Helicoverpa armigera* Larvicidal effect against *A. aegypti* and *Culex quinquefasciatus* Insecticidal activity on American cockroach, *Periplanata americana* , American grasshopper*, Schistocera americana* and African malaria mosquito, *Anopheles gambiae*	(60, 67, 68)

8	1.4	Pthalic acid, Butyl 2-pentyl ester	Phthalic acid ester	Larvicidal activity on mosquitoes Insecticidal action against *Gryllus bimaculatus* and darkling beetle, *Tenebrio molitor*	(74)
9	1.28	9 (2h) – anthracenone, 1,3,4-9a-methyl	Anthracenone	Insecticidal activity on maize storage pest, *Sitophilus oryzae* Fumigant, repellent and anti-oviposition activity on whitefly, *Bemisia tabaci*	(75, 76)
10	0.81	α-Cadinol	*Sesquiterpenoid*	Insecticidal properties against pulse beetle, *Callosobruchus maculatus* Fumigant against the rice weevil, *Sitopilus oryzae* Potential natural repellent and contact insecticides against *T.. castaneum* Fumigant, contact and repellent effects against *T. castaneum* and *Rhyzopertha dominica* Contact toxicity against *T. castaneum* Contact toxicity to *S.littoralis* Strong antimite activity against house dust mite (*Dermatophagoides farinae* and *D. pteronyssinus*)	(77, 78)
11	0.59	5,9,13-pentadecatrien-2-one,6,10,14-trimethyl-	Acyclic diterpenoids	Larvicial and pupicidal activity against mosquitoes *A. aegypti, Anopheles stephensi* and *Culex quinquefasciatus* Insecticidal activity against the lepidopteran larvae, the *S. frugiperda* and the *Plutella xylostella* Insecticial activity against the aphids, oleander aphid, *A. nerii A. fabae and* cowpea aphid, *A. craccivora*	(79)
12	0.44	Cyclohexanebutanal, 2-methyl-3-oxo-, Cis-	Ketone, Aliphatic aldehydes	Biocontrol agent for mosquito, *C. quinquefasciatus*	(80)
13	0.37	2-pentadecanone, 6,10,14- trimethyl	*Sesquiterpenoid*	Larvicidal efficacy, malaria vector *Anopheles coluzzii*	(81, 82)
14	0.34	4,8,12,16-tetramethylheptadecan-4-olide	Terpenoid	larvicidal efficacy, *Anopeles coluzzi* and *C. quinquefasciatus*	(83)
15	0.29	Naphthalene, 2-decyldecahydro- (CAS)	Diol/Triol derivatives	Insecticidal activity against *A.saegyptii* Insecticidal activity against Angumois grain moth, *Sitotroga cerealella* Insect repellent	(84, 85)
16	0.19	Β-eudesmol	*Sesquiterpenoid*	*Contact* toxicity *book lice, Liposcelis entomophila* and *T. castaneum*. Insecticidal activity against aphids Insecticidal and fumigant effects against *Sitophilus zeamais and T. castaneum.* Insecticidal activity against *A. aegypti* Pesticidal activity against root-knot nematodes (*Meloidogyne incognita*) Insecticidal activity against *S. littoralis*	(86)
17	0.16	L-tyrosine, N-acetyl- (CAS)	Non-essential amino acid		

in the seed extracts (Table 6). The chemical profile of the oil extract demonstrated 17 components and the abundant one being 9, 12 - octadecadienoic acid (Z, Z)-, methyl ester, also known as linoleic acid, methyl ester, is reported to possess insecticidal properties against sucking pests (57). The bio-pesticidal efficacy of *R. epapposum* seed oil is corroborated by the presence of 9, 12 - octadecadienoic acid (Z, Z)-, methyl ester, due to its presence in hefty magnitudes. The current results are in line with researchers highlighting the insecticidal effect of the compound against cotton and cabbage aphid, *Brevicoryne brassicae* Linnaeus (58). Furthermore, the efficacy of the compound in managing cotton mealy bug, *Phenacoccus solenopsis* Tinsley was corroborated in investigations (57). The seed extract of black locust tree, *Robinia pseudoacacia* L., was efficient in managing cotton aphid, *A. gossypii* Glover and it was encouraging to note that 9, 12 - octadecadienoic acid (Z, Z)-, methyl ester, was the major component in the 51 compounds detected in the seed extracts (59). In addition, ethyl acetate extracts of the entomopathogenic fungi *Beauveria bassiana* and *Trichoderma harzianum,* much effective against cotton leafworm, *Spodoptera littoralis* Boisduval and aphid, *A. gossypii*; exposed the occurrence of 9, 12-octadecadienoic acid (Z, Z)-, methyl ester (60). 2-methoxy-4-vinylphenol, is another compound present in higher quantities in *R. epapposum* oil extract and is reported to possess insecticidal properties against diverse insect pests such as larvae of gypsy moth, *Lymandria dispar*. The bio-pesticidal efficacy of famous Chinese medicinal herb, *Coptis chinensis* Franch on the aphid, *Myzus persicae* Sulzer and *Pinellia ternate* Thunb on beet army worm, *Spodoptera exigua* and *S. frugiperda* is related to the presence of 2-methoxy-4-vinylphenol (61, 62). The *Pinellia ternata* plant extracts distressed the physiology of the larvae by changing the activity of the enzymes viz., detoxification enzymes, digestive enzymes and protective enzymes (62). The extracts of the ornamental shrub and weed in some parts of the world, *Lantana camara* L. constitute 2-methoxy-4-vinylphenol, posing effectiveness against the notorious mosquito, *Aedes aegypti* L. (63). The next compound identified in excess quantities is hexadecanoic acid, methyl ester and its toxicity to insects is substantiated in literature. It acts as a natural pesticide against the aphid, *A. gossypii* and other sucking pests (60) and has a larvicidal effect against the larvae of the fall army worms, *S. littoralis* and *S. frugiperda* (64). The compound abundantly present in the emulsion form of geranium oil has displayed larvicidal and pupicidal activities against *Musca domestica* L. and *Culex pipens* L. and adulticidal activity against the red flour beetle, *Tribolium cataneum* Herbst (65). Hexadecanoic acid methyl ester is the chief component in the nut extracts of *Areca catechu* Linn and was reported to be much more effective against *Aedes aegyptii* L. (66). The aliphatic methyl ester, octadecanoic acid, methyl ester, trails hexadecanoic acid, methyl ester; however, was perceived in minor quantities comparatively. The compound was reported to possess insecticidal activity against the aphid, *A. gossypii*, that substantiates the bio-pesticidal effect of *R. epapposum* oil by its presence (60). The compound is one of the major bioactive components in *Cyperus iria* L., in Iraq and reported to possess insectifuge and nematicidal properties (67). In addition, GC-MS analysis of the ethyl acetate extracts of the liquid culture of fungal strain, *Cladosporium cladosporioides* revealed the presence of octadecanoic acid, methyl ester or stearic acid, that acts as a natural pesticide for the management of larva of the fall army worm and *S. frugiperda* (68). The research investigations corroborated with the current findings with octadecanoic acid, methyl ester as a component in the *R. epapposum* oil effective in managing oleander aphids.

The trailing compound, 1, 4- benzenediol, 2-(1,1-dimethyl ethyl)-5-(2-propenyl), the hydroquinones, is reported to possess insecticidal activity on cabbage butterfly larvae, Pieris rapae and the beetle, Pentodon alerinus (69, 70). The following compound in abundance is a methoxy phenol compound, phenol, 4-ethyl-2-methoxy- (CAS), documented as a toxic agent for insects such as termites, fall armyworms, fire ants (*Solenopsis invicta* Buren) and the cowpea aphid, *Aphis craccivora* (71, 72). The aliphatic acid methyl ester, 9- octadecenoic acid (Z)-, methyl ester, also known as oleic acid, methyl ester, was the next compound detected at 1.58 %. The compound detected in maximum quantity 9, 12-octadecenoic acid (Z, Z)-, methyl ester ($C_{19}H_{34}O$) and 9- octadecenoic acid (Z)-, methyl ester, ($C_{19}H_{34}O_2$), differs only by 2 hydrogen atoms and is reported to possess insectifuge and nematicidal properties (73). Phthalic acid, butyl 2-pentyl ester is the trailing component reported to possess insecticidal and insect repellent activity on several insects such as oriental armyworm, *Mythimna seperata* Walker (74). The presence of an anthracenone, 9 (2H) – Anthracenone, 1,3,4,9a-tetrahydro-9a-tetrahydro-9a- 9, the trailing bio-component in the *R. epapposum* seed oil, is also substantiated for its efficacy in toxifying the oleander aphids, as it has exhibited insecticidal efficacy against maize storage pests, *Sitophilus oryzae* L. (75), fumigant, repellent and anti-oviposition activity against the whiteflies, *Bemisia tabaci* Gennadius (76). A sesquiterpenoid, α-cadinol present in meagre levels in *R. epapposum* seed oil extract was reported to possess vast insecticidal activity on various insect pests. The mint, *Mentha longifolia* L. essential oil extract with α-cadinol as an active component was found to inhibit the fecundity, fertility and emergence of mature *C. maculatus* displaying 100 % mortality rates. The bioactive component, α-cadinol is found to present in various plant products and reported to be effective against diverse stored product pest, viz., *Tribolium castaneum* Herbst, *Rhyzopertha dominica Fab.,* adults of wheat weevil, *Sitophilus granarium* L. (77) with biting deterrent property on mosquitoes, *A. aegyptii* (78). An acrylic diterpenoid, 5, 9 ,13-pentadecatrien-2-one,6,10,14-trimethyl-existed in *R. epapposum* seed oil extract possessess larvicidal and pupicidal activity against mosquitoes *A. egyptii*, *C. quinquefasciatus* and *Anopheles stephensi* (79). Cyclohexanebutanal, 2-methyl-3-oxo-, cis-, a ketone, aliphatic aldehydes is reported as a biocontrol agent for mosquito, *C. quinquefasciatus* and *Anopheles stephensi* Liston, as it showed 100 % mortality with LC_{50} and LC_{95} values of 2.476 and 13.207 ppm in the red seaweed, *Portieria hornemannii* that comprises cyclohexanebutanal, 2-methyl-3-oxo as a component (80). The sesquiterpenoid, 2-pentadecanone, 6,10,14-trimethyl- in 0.3 % area is reported to possess an insecticidal response to lepidopterans and sucking pests. The insecticidal activity against the lepidopteran larvae, *S. frugiperda* and *Plutella xylostella L.* was previously reported (81). It could inhibit the infestation of the oleander aphid, *A. nerii* in Oleander, *Aphis fabae* Scopoli in *Hibiscus rosasinensis* L. and *Aphis craccivora* Koch in cowpea (82). The terpenoid, 4,8,12,16-tetramethylheptadecan-4-olide occupied 0.34 % area and is reported to possess larvicidal efficacy on malaria vector, *Anopeles coluzzi* Coetzee & Wilkerson and *C. quinquefasciatus* (83). The trailing component existed in the *R. epapposum* seed oil extracts was naphthalene, 2-decyldecahydro- (CAS), a polycyclic aromatic hydrocarbon, widely utilised as a repellent for urban pests. Napthalene, is commonly used by the people as mothballs to manage urban pests in households and museums. The compound is reported as an insect repellent and is reported to inhibit the emergence of *A. aegypti* (84). Pesticidal effects of some botanicals and naphthalene on anguimous grain moth-inhabiting grains revealed naphthalene as an effective treatment in managing the pest (85). The sesquiterpenoid, β-eudesmol is detected at 0.10 - and is demonstrated to be highly toxic to aphids with LC_{50} at 36 and 126 ppm and LC_{95} at 102 and 198 ppm that justifies the aphicidal effect of *R. epapposum* seed oil extract. The last detected compound is a non-essential amino acid, L-Tyrosine, N-acetyl-(CAS), which does have no insecticidal effect; rather is a healthy compound, for its utility as an herbal diet (86).

The bio-pesticidal effect of *R. epapposum* seed oil is substantiated by its chemical components and their insecticidal effects were highlighted in diverse research investigations by several researchers. The plant with its rich wealth of compounds holding high efficacy in managing oleander aphids in laboratory bioassays necessitates further field investigations to confirm its wide utility as a bio formulation. Furthermore, the bio efficacy is to be examined for various other sucking pests and lepidopterans, to explore the extensive potency of the *R. epapposum* oil in pest management.

Conclusion

Rhanterium epapposum, a native plant of the desert ecosystem and the national flower of the State of Kuwait, reported to possess several biological properties, was investigated for its bio-pesticidal effect in managing the oleander aphids, revealing an overwhelming response. A high mortality rate of 96.7 and 93.33 % was noticed after 3 days of treatment for the highest concentration in the leaf dip and dry film bioassays respectively. Even the lowest concentration was able to manage 50 % of the population approximately, that rationalises the vigorous bio efficacy of the oil. The captituli of *R. epapposum* has a strong pungent aroma that affects the nervous system of the insects causing physiological alterations in their body, leading to mortality. Further, the toxic regression lines of the highest dose of *R. epapposum* oil, provided the LC_{50} and LC_{95} values of 8.67 % and 61.78 %, respectively. The GC-MS analysis of the oil exhibited the existence of 17 chemical components with rich insecticidal effects on diverse sucking pests. The chemical compounds, 9, 12-octadecadinoic acid (Z, Z)-, methyl ester, 2-methoxy-4-vinylphenol and hexadecanoic acid, methyl ester were profusely found in 33.16, 29.45 and 22.57 % area inheriting opulent biological properties. The investigation's findings have demonstrated greater efficacy of the seeds of the desert plant, *R. epapposum* to be an effective biopesticide. However, it is necessary to validate the concentrations under field conditions for additional characteristics such as photostability and safety for non-target organisms. Diverse formulations based on nanotechnology can be experimented with for enhanced activity to combat a number of significant agricultural pests. Further investigations to isolate the biopesticidal chemical component and to ascertain its environmental fate and species toxicity to diverse agricultural pests are warranted. The outcomes of the investigation have demonstrated greater efficacy of the seeds of the desert plant to be a potent biopesticide. The outcomes of the current investigation are pioneering, for the insecticidal effect of the seeds of the desert plant, *R. epapposum*, shedding light on its utilisation in agricultural pest management.

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Authors' contributions

TAM and MKD contributed to conceptualisation and writing of the manuscript. TAM contributed to reviewing, MKD contributed editing and revising. Both authors contributed to validation of the manuscript. Both authors read and approved the final manuscript.

Compliance with ethical standards

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