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RESEARCH ARTICLE

The value of applying a myrrh extract to manage and control date palm aphids in Saudi Arabia and their classification

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ABSTRACT

The date palm can be affected by aphids resulting in the development of sooty mould. Extracts from myrrh plants were utilized as biological insecticides on aphids to learn its impact. Myrrh extracts had been evaluated using four separate amounts, namely 500, 1000, 5000, and l0000 ppm. They were applied two times and then inspected at 12 and 24 h after the exposure to be contrasted to the unexposed samples. The aphids eating date palms were subjected to myrrh extracts and categorized. The bio-insecticidal effects have been investigated based on specific dynamics and genetic evaluation of both exposed and unexposed aphid samples. The biologically insecticidal effects are relevant ($P < 0.05$) regarding all the four selected factors. Moreover, the genetic evaluation indicated that unfinished genetic sequences have dramatically increased upon elevating amounts of myrrh extracts (0, 500, 1000, 5000, and 10000 ppm). Meanwhile, the average genetic distance has reduced with enlarged amounts of myrrh extract in the 24-h period in the case of the exposed aphids in contrast to unexposed samples. Myrrh extracts are very strong and perspective biological insecticides to be applied in the agricultural field. The plant can effectively substitute chemical insecticides in fighting date palm aphids.

Keywords: Date Palm; Aphids; Myrrh Extract; Biological insecticides; Riyadh; Saudi Arabia

INTRODUCTION

Aphids (*Hemiptera: Aphididae*) are recognized as prevalent pests in agriculture, gardening, and urban gentrification (Blackman et al, 2000). There are almost 4700 species globally related to this family of insects (Remaudière & Remaudiere, 1997). Their activity damages plants and their phloem and contributes to the spread of diverse plant viruses, as insects insert toxic saliva elements and excrete toxic residues leading to sooty mould (Blackman & Eastop, 2007). There can be unique adult phenotypes among aphids, although classification distinguishes between winged and wingless grown-up females based on parthenogenesis (Blackman & Eastop, 2007). Morphological characteristics might be diverse among species, implying that each specimen should be subjected to slide-mounting with recording exact host plant domain to ensure accurate and proper classification. As various polyphagous species exist, aphids tend to feed on a specific type or family of plants. Hence, identification keys heavily depend on (Blackman & Eastop, 2019) exact data of a host plant. Databases of host plants (Holman, 2009) are essentially beneficial for this purpose in extending our insight over aphid-host relations, ideas about securing plants and opportunities of aphid species' identification and classification. Aphids' attacks on crops are associated with direct damage with insects sucking the plant sap; yet indirect damage is also present, paving the path for microorganisms that provoke serious diseases (Lins et al, 2020).

As a rule, palm plants are mostly infested by two common types of aphids: *Cerataphis brasiliensis* (Hempel) and *Cerataphis lataniae* (Boisduval) (Brumley, 2020). These insects are typically characterized by the setae of a dagger form, which is a very unstable morphological factor presented among *Cerataphis brasiliensis*. Due to this trait, some taxonomy scientists doubt whether these two species are different types (Brumley, 2020). Confusion with identifying two Cerataphis species and *Cerataphis orchidearum* (simply the orchid aphid) are relatively common. The palm aphids' primary target is growing landscape palms, particularly coconut palms (*Cocos*

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nucifera L.). An insect can usually impact a certain crop related to a group of crop hosts; however, some insects can affect various plant families (Brumley, 2020; Sorensen, 2009). The so-called polyphagous aphids refer to sibling species; this means they are morphologically similar, but they still differ in the karyotype (Anwar et al, 2017; Sorensen, 2009). In common, these aphids represent anholocyclic clones, which means they are biotypes with special priorities over a host plant, multiple mechanisms of transmitting diseases, or/and abilities to resist pesticides (Sorensen, 2009). Aphids' impact on agricultural industry and crops is multi-factorial. Insects can form highly dense populations, deprive plants of their natural nutrients, and cause harm by sucking vital sap, thus provoking decay and plant's death. The transmission of plant viruses is the most serious danger brought by palm aphids (Anwar et al, 2017). Plants infected by the virus tend to demonstrate a yellowing that attracts aphids and provokes an increased release of amino acids, so virus transmission becomes a biological benefit for aphids. The integrated pest management (IPM) approach becomes a significant subject of current studies to manage aphids. IPM directed towards aphids reduces the impact on non-target insects and species (Sorensen, 2009). An action plan usually incorporates cultural control approaches, for instance, decreasing ant populations, applying ultraviolet devices to repulse alatae, or make cross-planting of pollen and nectar source plants within crops to encourage the growth of natural repellents and insecticides (Anwar et al, 2017; Blackman & Eastop, 2007).

Chemical insecticides and the soaps were found to be the optimal exposure method, ensuring 54% of species mortality after 24 h of using 100 ppm amount, at least (Haldhar & Maheshwari, 2018; Shonouda et al, 2000). Insecticides play a crucial role in society and agriculture in particular. Along with the necessity to protect crops, there is a need to protect human health and the health of animals and pets (Pavela, 2007). These risks come from phytophagous insects that cause essential damage to crops, leading to 90% (35–40% on average) of damage (Pavela, 2007; Weinberger & Srinivasan, 2009). The influence of myrrh (*Commiphora molmol*) extract and the sublethal impact of several crop insects, namely profenofos/chlorofluazuron, fenvalerate and pyriproxyfen, on the larvae of *S. littoralis* had been studied (Shonouda et al, 2000). It was revealed that myrrh stimulated the biggest impact after 7 days of use at 10,000 ppm concentration, achieving 44.4% of species mortality. Various types of exposure also illustrated negative influence on pupation, growing adults and larvae's life cycle. The highest amount (10000 ppm) of myrrh extract stimulated 35% pupation (Shonouda et al, 2000).

The current study focuses on the molecular analysis and genetic categorization of aphids (*Cerataphis orchidearum*) that cause harm to the date palms (*Phoenix dactylifera*) in the agricultural domain of Riyadh, Saudi Arabia. Additionally, the study evaluates the opportunity of using a myrrh extract alone as a natural insecticide in the fight against palm aphids to analyse genetic distances.

MATERIALS AND METHODS

The myrrh extracts

The plant's core components (specifically oleo, gum, and resin) have been taken from the stem of the myrrh. One sample includes 7–17% of volatile oil, 25–40% of resin, 57–61% of gum, and 3–4% of other additives. Volatile oil, in turn, incorporates terpenes, sesquiterpenes, esters, cuminic aldehyde, and eugenol. The chemical structure of the plant's resin has not been properly studied to date (Ghazanfer, 1994). The myrrh extract (containing mainly oleo and resin) has been provided by Pharco Pharmaceuticals Company, Alexandria, located in Egypt. The following amounts of extract concentrations were achieved: 0.5, 1.5, 5, and 10%, representing 500, 1000, 5000 and l0000 ppm, accordingly.

Insect sampling and categorization

This research project took place in January 2021 in large date farms located in Riyadh, Saudi Arabia. Aphid insects had been obtained directly from the local date palms (Fig 1).

Specimens of aphids from a single site and population were identified mostly on the date palm's frontal sides (*Crataphidinae*) during the winter season. They were titled *Cerataphis orchidearum* by the efforts of the entomologists from Princess Nourah Bint Abdulrahman University (Table 1). The nymphal periods had been differentiated from the maturing phases by referring to the density and size of their wax cover, specifically on the area of abdomens. Naturally, aphids pass

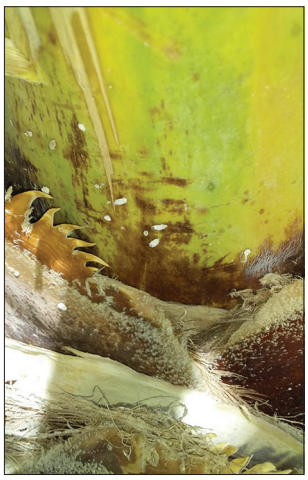

Fig 1. Sooty mould present on the date palm, indicating yellow marks on the frontal side, which is provoked aphids' (*Cerataphis orchidearum*) activity; Riyadh, Saudi Arabia.

Table 1: Categorization of aphids attacking the date palms in Riyadh, Saudi Arabia

Categorization	
Phylum	Arthropoda
Order	Hemiptera (True bugs)
Suborder	Sternorrhyncha
Family	Aphidoidea
Genus	*Cerataphis*
Species	*Cerataphis orchidearum*
Common name	Aphids
Date palm species (host)	*Phoenix dactylifera*

Table 1: Myrrh extracts' insecticidal impact on the biological factors of activity of the exposed aphids *(Cerataphis orchidearum)* at the 12-h period since the initial exposure (mean ± SEM)

Biological variables	500 ppm	1000 ppm	5000 ppm	10000 ppm
% Parasitism	57.4 ± 4.39	51.6 ± 5.83	42.5 ± 2.71	32.2 ± 6.12
Development				
Egg-mummy	17.5 ± 2.82	14.5 ± 2.77	9.5 ± 3.45	6.5 ± 1.06
Egg to adult-male	19.6 ± 4.56	16.2 ± 5.08	11.5 ± 4.11	8.8 ± 2.04
Egg to adult-female	22.8 ± 4.43	20.1 ± 3.09	16.8 ± 2.46	9.7 ± 3.89
% Emergence	78.7 ± 4.79	73.4 ± 3.12	56.6 ± 4.32	39.7 ± 3.44

through six stages, namely egg/embryo, four nymphal instars, and full growth of an adult species.

Aphids breeding & exposure

In a study, 114 adult species were selected. Of the total amount, there were 58 males and 56 females; there were 23 winged and 33 wingless specimens. They were marked and held in glass containers (diameter: 4 cm; height: 3 cm) to be further transported to the laboratory. Winged adults have been bred at a temperature of 20ºC, 50 ± 5% RH for 12-h photophase within wooden cavities equipped with healthy fronds of the date palm. In cavities, there was a layer of a 1% agar-water solution added for 1 h; after that, females were taken away. Consequently, the aphid nymphs had been supported within the cavities until the full growth of parasitoids. Stock colonies where aphids passed all life stages (eggs, instars, and adults) had been differentiated into two groups: unaffected (unexposed) group and affected (exposed) group with four amounts of extract usage, namely 500, 1000, 5000, and l0000 ppm. The procedure of exposure was reiterated twice in 12 and 24 h with the following inspections. Stages of the aphid (instars and adults) were particularly investigated in the exposed group, the specimens were distinguished referring to their size and shape. The time of growth from egg to mummy and from egg to grown-up parasitoid had been identified for both groups of insects by using *Diaeretiella rapae* (*D. rapae*), which is parasitoid wasp. The rates of mortality have been calculated by applying Abbott's formula:

$$\text{Corrected mortality (\%)} \frac{\text{\% mortality in treated} - \text{\% mortality in control}}{100 - \text{\% mortality in control}} \times 100$$

The Abbott formula for measuring infestation and efficiency of insecticides.

All the specimens had been kept in 80%-ethanol for the following DNA extraction. Voucher samples were gathered from ethanol and then stored in the Biology Department of Princess Nourah Bint Abdulrahman University (Riyadh, Saudi Arabia) at -20°C temperature for long-term conservation.

Dna extraction and polymerase chain reaction (pcr)

Every sample was derived from the 80% ethanol, subjected to air dry, and then put in a 1.5-mL microfuge tube with special tongs; they were preliminary soaked in 0.5% NaCIO. Overall genomic DNA had been successfully extracted from selected aphids from two groups of investigation (exposed and unexposed) by applying a non-destructive strategy (Rowley et al., 2007). Given the different amounts, the DNA samples have been diluted with the help of sterilized distilled water to get a viable solution of 20–25 ng μl^{-1} purified DNA. A proportion of the overall DNA was kept in glycerol (10%) at -80°C temperature for future use. Routine protocols had been properly complied with while completing PCR, cloning, sequencing of the CO-I section, and alignment of sequences (Hajibabaei et al, 2006; Toda & Komazaki, 2002).

PCR has been fulfilled using a thermal cycler (ABI-Applied Biosystems, Veriti, USA) under the fixed cycling properties: denaturation started at 94°C for 4 min; it passed through 35 cycles at 94°C for 30 s; annealing started at 47°C for 45 s; extension started at 72°C for 45 s; final extension started at 72°C for 20 min. The universal CO-I primers were utilized: LCO-1490; 5'-GGT CAA CAA ATC ATA AAG ATA TTG G-3' and HCO2198; 5'- TAA ACT TCA GGG TGA CCA AAA AAT CA-3' (Hebert et al, 2003). The overall reaction amount of 25 µl incorporated ×20 picomoles of every primer, 10 mM Tris/HCl (pH 8.3), 50 mM KCl, 2.5 mM $MgCl_2$, 0.25 mM of each dNTP, as well as 0.5 units of Taq DNA polymerase (Fermentas Life Sciences, UK). The improved outputs had been resolved in 1.0% agarose gel. Afterwards, they were stained using ethidium bromide (10 $\mu g\ ml^{-1}$) and finally visually presented in a specific gel documentation system (UVP).

Sequencing and sequence evaluation

The improved outputs had been eluted with the help of a gel extraction kit (Nucleospin® Extract II, Macherey Nagel, Germany). The procedure was completed in compliance with the manufacturer's guidelines. The outputs eluted had been ligated into a cloning vector of general-purpose,

namely InsT/A clone (Fermentas Life Sciences, UK), also in compliance with the manufacturer's guideline. The blue-white compilation had been fulfilled to isolate plasmids with the help of GenJET™ plasmid MiniPrep kit (Fermentas Life Sciences, UK), following the manufacturer's standards. It was completed based on the overnight culture of positive clones bred inside LB broth. Sequencing procedures had been completed in triplicate based on the clones cultivated using the special autonomic sequencer (ABI prism® 3730 XL DNA Analyzer: Applied Biosystems, USA) based on the M13 universal primers (forward and reverse orientations were used). A homology inspection had been completed thanks to NCBI-BLAST (http://blast.ncbi.nlm.nih.gov/). In the meantime, the alignment of sequences had been implemented with the help of BioEdit version 7.0.9.0 (Hall, 1999). The sequences produced have been stored in the NCBI-GenBank (Supplementary material 1), which means they are available in BOLD. Alignment of CO-I sequences was carried out with the help of the Clustal W software in BioEdit.7.0. In turn, MEGA.5.0 has been used to evaluate the sequences produced (Kumar et al*,* 1993) to identify conspecific and congeneric genetic distances. Finally, Neighbour-Joining (NJ) trees have been developed with the help of the Kimura-2-parameter (K2P) distance model (Kumar et al*,* 1993; Saitou & Nei, 1987).

Data evaluation

All factors (development dynamics, parasitism rates, occurrence and mortality rates) have been evaluated using ANOVA after completing a Tukey test at 5% significance magnitude. To complete this, the SigmaStat program, version 3.5 (Systat Software, San Jose, CA, USA) at 5% significance magnitude (P values < 0.05), had been implemented.

RESULTS

insecticidal impact of myrrh extract

From the total amount of 114 gathered and incubated winged adults of aphids, 112 insect samples were processed with a myrrh extract. The development duration serves in a study as a sign of exposure quality. Hence, it was noted that the growth rates from egg to mummy, from egg to adult-male and also from egg to adult-female ($P < 0.0001$) in the case of *D. rapae* were steadily reducing, yet this trend was rather indicative in adult males than in females (Table 1, 2). Still, Tables 3 and 4 illustrate that insecticidal potency in terms of the concentration-response ratio for a myrrh extract towards winged adults and instars had been determined based on two exposure intervals (12 h and 24 h). The mortality and occurrence rates have been labelled as the affected ones, elevating the amounts of the extract (0, 500, 1000, 5000, 10000 ppm) along with periods of exposure.

Genetic Evaluation

The genetic evaluation has been completed following the 316 sequenced loci, which were varied in length (from 347 to 2,241bp, representing a median of 1,326 bp). The overall length of combined alignment for unaffected aphid insects has been properly sequenced as a single species interacting with the date palm trees from three different sites of Riyadh.

However, several sequences have been found unfinished or missing in the case of the affected/exposed aphids, which means the output availed between 24 and 47bp for loci selected (Fig 2).

The percentage of unfinished sequences had been dramatically ($P < 0.001$) higher with elevated amounts of a myrrh extract in the 24-h period since the application to the exposed aphids. This was presented as follows: missing 24 ± 2.64, 27 ± 2.53, 38 ± 3.14, and 47 ± 2.08 bp at the amounts 0, 500, 1000, 5000, and 10000 ppm, respectively. Moreover, the intrageneric rates were found to visibly reduce the mean genetic distance parameters upon elevation of amounts of a myrrh extract (Table 5) in the 24-h period since the application compared to unaffected and unexposed aphid specimens.

DISCUSSION

The date palm remains an important and popular fruit tree in the Middle East, particularly Saudi Arabia (Erskine et al*,* 2004). Damage caused by date palm aphids is extensive, as insects harm the crops through feeding habits and transmit plant viruses via passing toxic saliva and by provoking sooty mould (Blackman & Eastop, 2007). The chemical insecticides used today to protect crops dissolute the aphids' defensive coating, leading to their death (Agnello, 2002). The mechanism of myrrh is associated with causing a smothering effect on soft-bodied insects, while available soaps destroy them by depriving them of natural protective

Table 2: Myrrh extracts' insecticidal impact on the biological factors of activity of the exposed aphids *(Cerataphis orchidearum)* at the 24-h period since the initial exposure (mean ± SEM)

Biological variables	500 ppm	1000 ppm	5000 ppm	10000 ppm
% Parasitism	44.7 ± 3.21	36.3 ± 4.51	24.8 ± 3.42	11.2 ± 3.31
Development				
Egg-mummy	12.9 ± 1.68	10.1 ± 3.09	5.2 ± 2.41	2.9 ± 0.47
Egg to adult-male	14.4 ± 2.11	11.0 ± 2.08	7.6 ± 2.11	3.6 ± 1.01
Egg to adult-female	16.5 ± 1.21	12.4 ± 2.13	8.4 ± 1.33	4.4 ± 1.03
% Emergence	51.5 ± 3.27	46.8 ± 4.01	32.3 ± 2.52	18.5 ± 2.46

Table 3: Myrrh extracts' insecticidal impact on the mortality dynamics (mean ± SEM) of the adult specimens and nymph instars *(Cerataphis orchidearum)* at the 12-h period since the initial exposure

Concentration (ppm)	Dead	Alive	P value	Dead	Alive	P value
	Adults			Instars		
0	2	112	0.000	0	983	0.000
500	29	83	0.000	174	809	0.000
1000	36	77	0.000	332	651	0.000
5000	47	66	0.000	419	564	0.000
10000	88	24	0.000	846	137	0.000

Table 4: Myrrh extracts' insecticidal impact on the mortality dynamics (mean ± SEM) of the adult specimens and nymph instars *(Cerataphis orchidearum)* at the 24-h period since the initial exposure

Concentration (ppm)	Dead	Alive	P value	Dead	Alive	P value
	Adults			Instars		
0	1	113	0.000	0	983	0.000
500	34	78	0.000	174	809	0.000
1000	41	71	0.000	332	651	0.000
5000	53	59	0.000	419	564	0.000
10000	96	16	0.000	846	137	0.000

Table 5: The intrageneric rates demonstrate the mean genetic distance parameters at a 24-h period since applying a myrrh extract (in different amounts

Concentrations	No. of specimens (adults)	Genetic distance (%)
0	100 (alive)	4.96
500	60 (30 dead +30 alive)	3.86
1000	40 (20 dead + 20 alive)	3.12
5000	50 (25 dead + 25 alive)	2.19
10000	30 (15 dead + 15 alive)	1.74

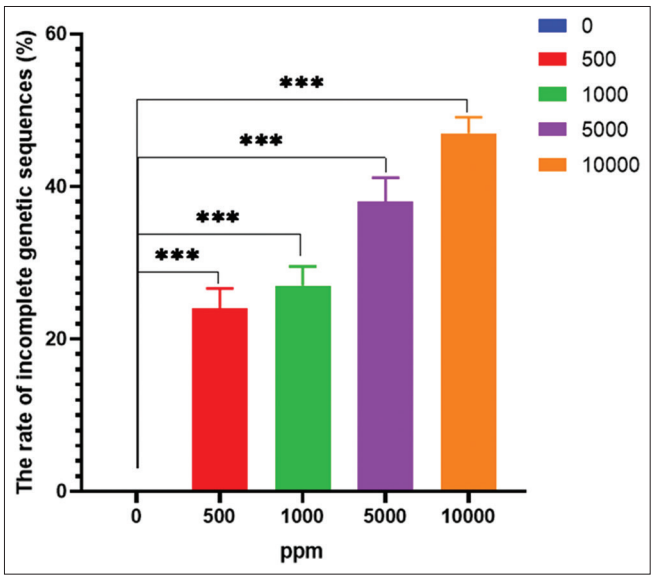

Fig 2. The percentage of unfinished genetic sequences had been dramatically (P < 0.001) higher with elevated amounts of a myrrh extract (0, 500, 1000, 5000 & 10000 ppm) in the 24-h period since the application to the exposed aphids (n = 100). The discrepancies between amounts of bioinsecticidal effects by a myrrh extract have been positioned as relevant at *P<0.05, **P < 0.01, and ***P < 0.001.

"coverage" (Pennacchio et al, 2010; Singh et al, 2015). Myrrh is a widely applied herb that is especially popular in the pharmaceutical industry and pest management areas in the Arab states (Al-Faris et al, 2008; ALLAM & EL-SAYAD, 2001; Kandar, 2021). Even though many scientific sources focused on biological influences of myrrh in the fight against microorganisms (Massoud et al, 2001; Sheir et al, 2001), a limited number of researches have actualized the plant's value in the fight against insects and pests due to the plant's toxicity, high contamination properties and adverse effects [30–33]. (Kandar, 2021; Massoud et al, 2001; Rao et al, 2001; Sheir et al, 2001).

The problem of aphids attacking crops is global and disturbing since this type of pests is prevalent over other insects in the context of population density, biological diversity, host choice, and geographical spread (Foottit et al, 2008). This study has taken the date palm (*Phoenix dactylifera*) located in Riyadh, Saudi Arabia, as the target host for aphids, being the unique research project emphasizing the identification and categorization of aphids in the selected jurisdiction, along with focusing on the myrrh extract's role as a biological insecticide.

To evaluate myrrh's impact, four biological factors (development dynamics, parasitism, occurrence, and mortality rates) have been selected for the study (Al-Shuraym et al, 2020). Here we found that a myrrh extract's impact increased with increasing extract amounts. The findings are correlated with some other studies that have confirmed the potency of myrrh as an antibacterial, antifungal, antiparasitic and bioinsecticidal natural agent (ALLAM & EL-SAYAD, 2001; Kandar, 2021; Sheir et al, 2001). The aphids selected for this project have been classified properly based on DNA barcodes, which was found to be a good practical method. This eventually helped to evaluate and determine the genetic distance after the extract's application with identifying the plant's advantages (Asokan et al, 2011). Hence, the intrageneric rates have visibly reduced the mean genetic distance parameters upon the elevation of amounts and bioinsecticidal impact of a myrrh extract (Table 5) in the 24-h period since the application. It formally implies the identified amounts

utilized in this research cause a strong genotoxic influence on aphids attacking date palms.

CONCLUSION

The application of a myrrh extract studied in this research has marked itself as a useful bioinsecticide (with the involvement of four different lethal amounts) against date palm aphids inhabiting farms of Riyadh, Saudi Arabia. The study helps develop a fast and efficient bioinsecticidal management strategy for handling date palm aphids' attacks and intrusions affecting insects at various life stages and without harming the crops themselves. Additionally, current findings will assist in future research, investigating effects on other insects and pests, contributing to the protection of agricultural, horticultural, and forest domains.

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AUTHOR CONTRIBUTIONS

Conceptualization, Methodology, Software, Formal analysis, Writing - Original Draft, Review & Editing.

CONFLICTS OF INTEREST

The author declares that there is no conflict of interest.

DATA AVAILABILITY STATEMENT

All the data of this study are available within the paper.

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