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Research Article

NOXIOUSNESS OF THREE DIFFERENT ESSENTIAL OILS AGAINST RED PALM WEEVIL UNDER CONTROLLED CONDITION

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Abstract

Asian palm weevil, also known as red palm weevil (RPW), *Rhynchophorus ferrugineus* (Olivier, 1790) (Coleoptera: Curculionidae), has long been regarded as an annoying pest of many palm trees (Arecaceae), particularly date palm. Researchers are very concerned about finding an effective management strategy to control RPW due to its well-known detrimental effects on native date palm, and its economic development. Use of essential oils and botanical extracts is receiving a lot of attention among other control methods. Because they are environmentally safe. However, this study explained the feeding toxicity of three essential oils e.g., clove, black pepper, and eucalyptus seeds against 3rd instar larvae of RPW via feeding bioassay. Results showed that mortality (%) was maximum when treated with a higher dose of clove oil as compared to the other two essential oils. Meanwhile the LC_{50} value of clove oil was minimal which showed a higher toxicity. Our study concluded that clove oil is useful against the immature stages of *R. ferrugineus.* So, we suggest that it should be properly placed in IPM strategies against this pest. To enhance the efficacy of such bioactive compounds in laboratory, more work is necessary.

Keywords: *Piper, clove, eucalyptus, essential oil, toxicity, and feeding bioassay.*
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1. INTRODUCTION

The date palm, *Phoenix dactylifera* L., (Arecaceae: Arecales), is a dioecious and perennial that is extensively cultivated worldwide, particularly in arid areas like the Middle East, North Africa, South Asia, Asia and the Arabian Peninsula (Chao and Krueger 2007, Krueger 2021). Globally, date palm farming has increased dramatically in recent years. Approximately 5000 types of dates are grown globally (Hussain et al. 2020). The date palm is an important fruit tree that contributes significantly to the economies of numerous people in the Arabian Peninsula, North Africa, and the Middle East. Pakistan ranks sixth in date palm production globally. Date palm tree parts are valuable in all forms, including the leaves, date pollen, and seeds (pits and oil). Excellent sources of minerals, dietary fibre, carotenoids, anthocyanin's, vitamins, proteins, and carbs may be found in data palms (Tang et al. 2013). Date palm seeds are leftover by-product of the fruit industry that is either fed to animals or consumed by humans as decaffeinated coffee (Al Harthi et al. 2015, Farag et al. 2023). However,

Data palm is a fruit crop that is widely grown in Pakistan's desert regions (Ata et al. 2012). This crop is grown on 96,514 hectares in all four provinces of Pakistan, yielding about 838251 metric tons annually (GPMNFSR 2023).

Red palm weevil (RPW), *Rhynchophorus ferrugineus* (Olivier, 1790) (Coleoptera: Curculionidae), or commonly called Asian Palm Weevil has been considered a nuisance pest of different palm trees (Arecaceae) especially date palm (Faleiro 2006, Rochat et al. 2017, Matos et al. 2023). This pest native to South Asia and Melanesia and entered the Arabian countries in the mid-1980s (EPPO 2023). It was initially discovered in the Northern United Arab Emirates in 1985, subsequently it moved to Oman. It originated in Eastern Saudi Arabia in 1985 and spread to other nations from there. It was initially found in Iran in 1990, and Egypt found it in 2000. Later, in 1994, it was discovered in southern Spain, and in 1999, in Israel and Jordan. Reported from Italy in 2004, Canary Islands in 2005, Balearic Islands, Cyprus, France and Greece in 2006, Turkey and Malta in 2007 (Mizzi et al. 2009).

It is challenging to observe adults on the palm trees. Larvae live and feed in the plant, which shields them from harsh weather. It is necessary to dissect palm in order to detect and observe RPW inside date palm (Rochat et al. 2017). Nearly all of the commercial date palm varieties grown in the Pakistan are susceptible to damage, with certain kinds such as Aseel, Hillawi, Karbalain, Mozawati, Dhaki, Kechnanr, etc (Shar et al. 2012). Date palms have been suffering financial losses due to this invasive weevil for the past 30 years (Güerri-Agulló et al. 2010). Infestations via RPW have reported in 50% date palm producing countries with yield losses ranging from 0.7 to 10 tons/ha (Yasin et al. 2019, Manzoor et al. 2020). There have been multiple reports of RPW in orchids in Pakistan's date palm-producing areas of Sindh and Baluchistan, as well as in recently constructed residential complexes where date palm is widely grown as an ornamental (Wakil et al. 2015). Losses due to red palm weevil in date palm plantations in Pakistan sometimes surpass 10%-20% (Qayyum et al. 2020).

Several methods of controlling red palm weevil have been employed in the past, including appropriate phyto-sanitation, pheromone trapping (Abraham et al. 2001), the use of microbiological agents (Qayyum et al. 2020) and bio-control (Nurashikin-Khairuddin et al. 2022), as well as the application of fumigation, injection, and spraying techniques (Azam and Razvi 2001) to lower RPW populations (Sutanto et al. 2023). Traditional methods are insufficient to control this pest due to its hidden behaviour, and insecticides are heavily condemned for causing resistance development, environmental risks, and health issues (Wakil et al. 2015, Said Al Waheibi 2019). Approximately 90% of the time, farmers use pesticides to control pests (Naveed et al. 2023). Overuse of harmful materials, pesticides, and insecticides leaves residue in the water, soil, air, and food. These herbicides also have an impact on soil enzymes, which regulate soil quality (Ali et al. 2022). On the other hand, botanicals are less likely to cause insect resistance development, more dependable, and easily biodegradable (Jaleel et al. 2020, Khamiss et al. 2023). The production of botanicals is relatively inexpensive (Johnston-Monje and Lopez Mejia 2020). The majority of botanicals are specialized and have minimal effect on the survival of their natural adversaries (Soares et al. 2019). Therefore, under the Integrated Pest Management program, botanical pesticides are more effective means of controlling the red palm weevil.

Botanicals are best antifeedant and when applied to host of adult insects e.g., a study showed that croton weed flower oil had antifeedant properties against red palm weevil (Shukla et al. 2012). Orange, lemon, eucalyptus, basil, and castor oils have been

used to kill the immature stages of red palm weevil (Ali et al. 2019). The effectiveness of purslane's nano-formulated essential oil against immature red palm weevil was comparable to that of mustard and castor nano-formulated essential oils (Abdel-Raheem et al. 2020). Black pepper oil significantly reduced feeding performance when added to the artificial diet for immature *R. ferrugineus*, indicating its potential use as an environmentally benign biopesticide against red palm weevils (Hussain et al. 2017). Natural insecticide e.g., extracts of cardamom and clove shown best repellent and toxicant against red palm weevil (Mona 2020). Black pepper has recently been employed as a deterrent and toxicant for number of pests in order Hymenoptera, Lepidoptera, Coleoptera, and Diptera (Samuel et al. 2016b, Jaleel et al. 2020). However, the literatures regarding feeding toxicity of essential oil obtained from clove, black pepper, eucalyptus and their combination against the adults of *R. ferrugenius* are limited. Here, we evaluated the toxicity of clove, black pepper, and eucalyptus oil against adult *R. ferrugineus* recognizing the importance of recently emerging invasive pest and its safe management methods.

2. MATERIALS AND METHODS

2.1. Collection of Weevils

Different stages of larvae of RPW were collected from date palm orchard located at Horticultural Research Station, Bahawalpur, Punjab, Pakistan. Recently RPW-infected trees were selected for collection of immatures. In laboratory, collected population of RPW were placed in plastic container (10 cm in length, 7 cm in width, and 7 cm in height) with soft palm pieces (inner and inside the trunk or part of date palm, where immature of red palm weevil feeds) as a source of food. The laboratory environment maintained by considering the outside environment e.g., temperature 25 ± 2°C, 65-70 % humidity with 24 h dark period. Because larvae of RPW feeds inside the trunk of date palm. Third instar of red palm weevil were separated via camel hair brush. Sixty third instar larvae were used for one concentration via feeding bioassays.

2.2. Oil Extraction

Seeds of clove, black pepper, and eucalyptus were purchased from the local market of Bahawalpur City, Pakistan. Seeds (1000g) of each plant was dried in oven and were put in a Clevenger-type apparatus for 8 h for hydro-distillation by following the methodology of (Rizvi et al. 2018). Each sample's seeds were split into two portions, such as 500g and 800ml, and then each sample was mixed with 800ml of distilled water. Oil of each sample was collected and dried over anhydrous sodium sulfate, and then placed in clear glass vials to be preserved at 4°C or below for feeding bioassay.

2.3. Feeding Bioassay

Different treatment doses of 20, 10, 5, 2.5, and 1.25 µg/ml of clove, black pepper, and eucalyptus oil were prepared in 15 % w/w acetone (water). A 15 % w/w acetone water used as the control. After 12 h starvation, the 3rd instar of red palm weevil shift into jar that placed in dark (Jaleel et al. 2021). Ten larvae of RPW (with six replicates; 60 RPW in total) were placed in another plastic jar with date palm pieces which were treated with a single dose of each oil treatment.

2.4. Mixing oil dose with soft tissue of date palm

Date palm pieces that had been peeled were kept in a prepared oil solution for a five min to make them seem prettier, and then they were allowed to dry on fresh tissue paper at room temperature. And plastic canisters were filled with RPW larvae and dried peeled palm piece.

2.5. Statistical Analysis

Dead 3rd instar of RPW were counted in order to evaluate the mortality % data. After 24, 48, and 72 hours, the third instar mortality percentage of RPW was tallied and reported as a percentage. Using "Probit" software, the LC_{50} were calculated for each plant oil using the methodology described by Finney (Finney 1971). The

mortality (%) was calculated in MS Excel 2019.

3. Results

Mortality was directly correlated with treatment concentrations and exposure time for all treatments. Only percentage mortality at highest concentrations of each data will be illustrated, while LC50 values for each treatment at each interval of recording i.e. 24, 48 and 72 hours will be presented.

3.1. Percentage Mortality

In case of Clove oil, highest concentration (20 ppm) caused maximum mortality i.e. 51.67, 60.00 and 88.33% exposed after 24, 48 and 72 hours respectively (Figure 1). Highest concentration (20 ppm) of Black pepper oil resulted extreme mortality i.e. 48.33, 56.67 and 80% after 24, 48 and 72 hours respectively (Figure 2). Eucalyptus caused 46.67, 55.00 and 65.00% mortality of after 24, 48 and 72 hours at highest concentration (20 ppm) (Figure 3). In case of combined treatments, highest concentration (20 ppm) of Clove Plus Black Pepper Oil ascribed the highest mortality i.e. 53.33, 65.00 and 93.33% after 24, 48 and 72 hours respectively (Figure 4).

3.2. Toxicity results

In case of Clove, LC 50 values were recorded as 11.375, 6.431 and 1.688 µg/ml after 24, 48, and 72 h of feeding respectively (Table I).

Black pepper oil caused toxicity in exposed to *R. ferrugineus* with LC50 values of 21.018, 6.982 and 1.720 µg/ml after 24, 48 and 72 hours. LC50 values of Eucalyptus oil were observed as 26.730, 12.730 and 4.215 µg/ml after 24, 48 and 72 h respectively. In case of combined botanicals, Clove plus Black Pepper Oil appeared with LC50 values of 13.314, 4.215 and 1.485 µg/ml after 24, 48, and 72 hours of feeding respectively.

Over all, highest LC50 with 95% Cl (µg/ml) values after 24 hours of exposure was recorded as 26.730 in case of Eucalyptus. While lowest LC50 values with 95% Cl (µg/ml) after 72 hours was recorded as 1.485 in case of Clove plus black pepper oil, that represents the high toxicity.

4. Discussion

Researchers are concerned about finding effective management strategies, one of the most destructive insect pest of palm. Ecologists strongly advise using botanicals

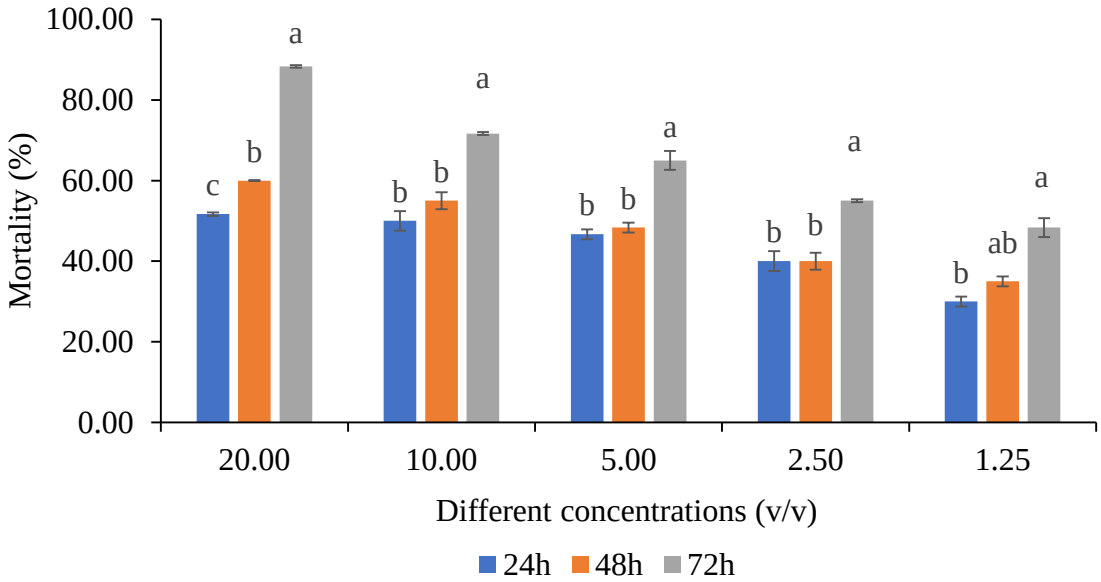

Figure 1. Mortality (%) of 3rd instar of R. ferrugineus after 24, 48, and 72 hrs, when treated with essential oil of clove seeds.
Different small letters on bars among time for each concentration, followed by a common letter does not differ statistically (P≤0.05).

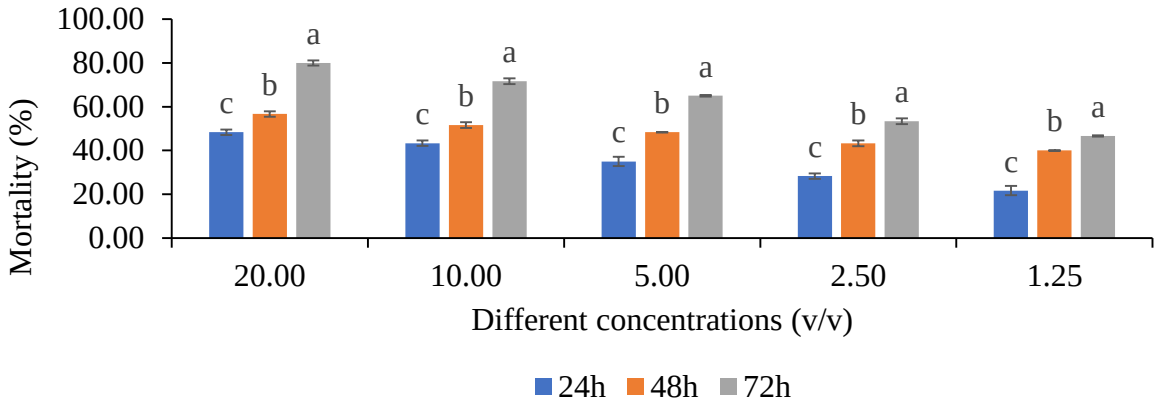

Figure 2. Mortality (%) of 3rd instar of R. ferrugineus after 24, 48, and 72 hrs, when treated with essential oil of black pepper seeds.
Different small letters on bars among time for each concentration, followed by a common letter does not differ statistically (P≤0.05).

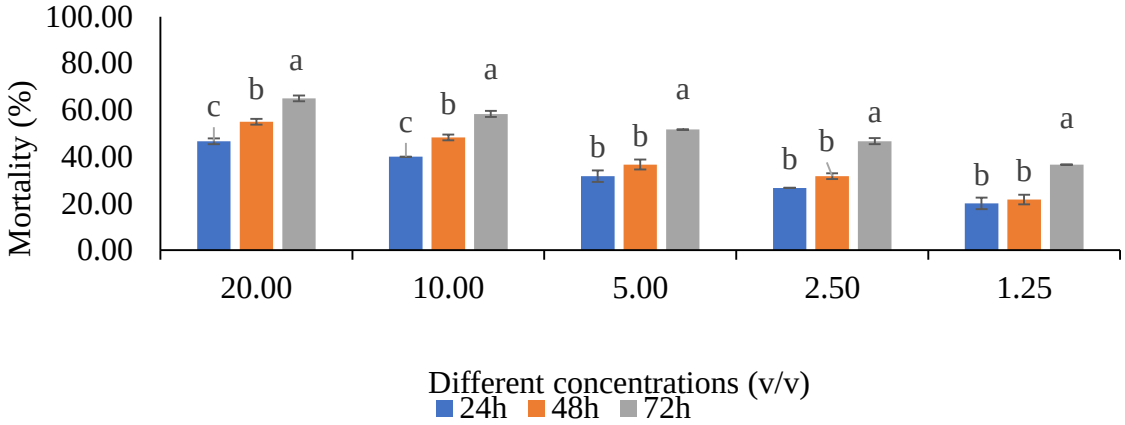

Figure 3. Mortality (%) of 3rd instar of R. ferrugineus after 24, 48, and 72 hrs, when treated with essential oil of eucalyptus seeds.
Different small letters on bars among time for each concentration, followed by a common letter does not differ statistically (P≤0.05).

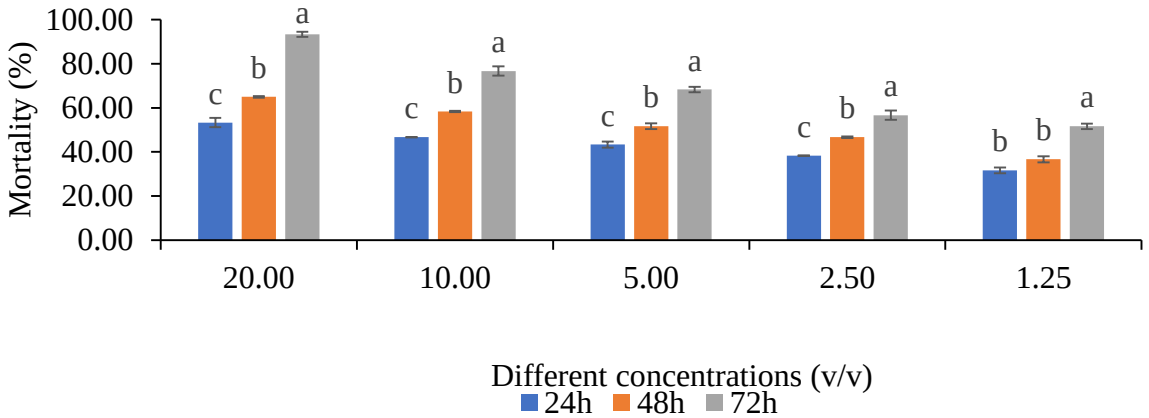

Figure 4. Mortality (%) of 3rd instar of R. ferrugineus after 24, 48, and 72 hrs, when treated with essential oils of clove and black pepper seeds.
Different small letters on bars among time for each concentration, followed by a common letter does not differ statistically (P≤0.05).

Table 1. Feeding toxicity of three different essentials oils against 3rd instar of red palm weevil.

Bioactive compound	Hrs.	LC_{50} (v/v)	d	χ^2	Slope ± SE
Clove	24	11.375	3	0.793	0.456 ± 0.173
	48	6.431	3	0.070	0.551 ± 0.173
	72	1.688	3	1.920	0.925 ± 0.186
Black pepper	24	21.018	3	0.084	0.625 ± 0.179
	48	6.982	3	0.019	0.350 ± 0.171
	72	1.720	3	0.135	0.776 ± 0.180
Eucalyptus	24	26.730	3	0.041	0.626 ± 0.181
	48	12.730	3	0.239	0.746 ± 0.179
	72	4.215	3	0.135	0.579 ± 0.173
Clove + Black pepper	24	13.314	3	0.088	0.442 ± 0.173
	48	4.215	3	0.135	0.579 ± 0.173
	72	1.485	3	3.139	1.051 ± 0.194

Cl: Confidence limit, χ^2: Pearson's chi-squared goodness-of fit test. *df*: Degree of freedom.

as one the many control strategies as they are successful and ecofriendly in addition with no threat to non-target organisms. (Isman 2006). Botanicals are typically utilized as deterrents, tough can be used can be used as gastrointestinal toxins in certain circumstances. We employed essential oils extracted from Clove, Black pepper and Eucalyptus in our study.

Our findings imply that every treatment should promise when tested against test population. During every observation clove oil without any combination seemed to be most harmful against test population. Our results are somehow similar to that of (Mona 2020) which reported that clove seed powder along with petroleum-ether caused 100% mortality against *Rhynchophorus ferrugineus* whereas in this study clove oil induced 88.3% mortality which may be due to the addition of petroleum-ether in that study which increase the efficiency of work of clove oil which caused 100% mortality against *R. ferrugineus*. After clove oil, black pepper oil induced maximum mortality of 80% which matches with the findings of (Su 1977) showed that black pepper powder and its crude ethanol extract significantly reduced rice weevil mortality. (Samuel et al. 2016a) reported that best control measure against *Anopheles arabiensis* (Diptera; Culicidae) was black powder and black piperine our findings somehow match with that study. *E. citriodora* essential oil, has many uses because of its pleasant aroma and possible health advantages. In addition to aromatherapy, it possesses significant antifungal, antibacterial, and anti-acetyl cholinesterase characteristics. This antioxidant quality allows it to be effectively employed as a spray deterrent against pests (Khan et al. 2017). (Kardinan et al. 2023) reported that *E. citriodora* essential oil gave the highest insect mortality (93.3%) against *C. maculatus* but in our study was just 65% after 72 hrs which can be because C. maculatus is stored grain pest while *R. ferrugineus* is field pest and difference in insect activity.

According to our findings mixture of two botanicals black pepper oil and clove oil was best among all the treatments causing 93% mortality and highest toxicity lowest LC50 values with 95% Cl (µg/ml) after 72 hours was recorded as 1.485. The possible reason is that, as Clove oil caused highest toxicity when used without combinations, so when it was used in combinations with black pepper oil, the highest toxicity was possibly ascribed by Clove oil. Synergistic effects are typically produced by combining essential oils, but it depends on a various type of factors, such as the oils' dosage and the intricate nature of the chemical compounds, each of which can act on a different target (Kachkoul et al. 2021), our study also proved that. The black pepper

and clove extract, which operate as natural insecticides, can be used to create superior plans for controlling and preventing insects. Moreover, clove is widely acknowledged to be safe for human health due to its widespread use as a spice and herbal medication (Zheng et al. 1992, Naveena et al. 2006).

5. Conclusion

Our study concluded that clove and black pepper seeds oil are useful for the control of RPW and further study will be conducted on the efficacy of bioactive components as repellent or toxicant against *R. ferrugineus* adults and immatures in both laboratory and field. To enhance the efficacy of such bioactive compounds in laboratory, more work is necessary.

6. Acknowledgments

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7. Conflicts of Interest

The authors declare no conflict of interest.

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