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Evaluating the effect of selected plant extracts on Citrus mealybug, Planococcus citri (RISSO) (Homoptera: Pseudococcidae) attacked different Citrus cultivars in Kurdistan Region

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ABSTRACT

The plants *Eucalyptus camaldulensis*, *Ocimum basilicum*, *Alhaji maurorum*, and *Mentha longifolia* extracted in ethanol at different concentrations (0.5, 0.75, and 1%) were examined under laboratory conditions against Citrus mealybug, *Planococcus citri* (Risso) which is dangerous and widely distributed insects infesting most citrus cultivars in Iraq including Erbil province, Kurdistan region. The concentrations with the control (ethanol solvent only) were evaluated under laboratory conditions, and the lethal effectiveness of all extracts was examined after twelve and twenty-four hours of spraying. Data demonstrated that the Eucalyptus extracts and camel thorn grass had the highest reduction of the mealybug nymphs in both exposure times of spraying from treatment with the evaluated concentrations. The mortality effects of dark opal basil and mint extracts were also significant but in a mild manner. Exposure periods had a significant impact on the reduction of mealy bug nymphs too. The conclusions supported using of plant extracts as an alternative method to properly control this insect from the traditional chemical methods.

1. Introduction

Mealybug, *Planococcus citri* (Risso) is the most economically important sucking insects which is mainly attached to citrus trees and other cultivated plants that not only cause direct damage to plants through sucking plant juice but also habitually transmit devastating microbial problems (Engelbrecht and Kasdorf, 1990; Cabaleiro and Segura, 1997). Control measures for sucking insects such as mealybugs need to spray a large amount of chemical insecticides. As a result of the varied and excessive use of these chemicals, their population densities rise due to resistance incidence (Oomasa, 1990; Shibao, and Tanaka 2000; Jhala *et al*. 2010, and Ghosh, 2020). The natural waxy layer that covers all stages of the insect has led to a reduction in the absorption of chemical pesticides, this is especially proper for the clusters. As a result, their uses are reduced and just the environment will be polluted (Ganjisaffar *et al*., 2019). Meanwhile, the use of natural plant extracts can keep the risks to the environment and human health (Isman, 2006; Wimalawansa and Wimalawansa, 2014; Laxmishree and Nandita, 2017). Nymphal stages and adult females suck the plant sap, and low levels of saps lead to loss of plant strength, slow growth, twigs dieback, and leaf drop, in the case of severe infestation there will be important economic damage to host plants (Williams, 2004). Mealybugs also cause indirect damage by entangling foliage with their honeydew excretions which leads to the growth of sooty mold fungi on the leaves, and fruits which affects plant growth, blemished fruit, fruit quality, and decreased yield (Miller *et al*., 2002; Sirisena *et al*., 2013). Chemical insecticides have traditionally been used to control this pest, but there are depraved residues on the environment, therefore, other modern methods must be found for controlling this dangerous pest. Insecticidal properties of plant extracts can be used as a potential alternative way against a range of sucking insect pests (Srijita, 2015). The present study aimed to find out the extract effectiveness of examined plants on the reduction of mealy bug infestation under laboratory conditions. Besides, different concentrations of extracts and exposure times were evaluated separately.

2. Materials and Methods

2.1. Collecting of the Plant Samples

The plant specimens were collected from the Grdarasha research station, College of Agricultural Engineering Sciences, Salahaddin University, Erbil. The plant specimens were identified by comparison with the preserved specimen in the College of Education, Department of Biology, Salahaddin University, Erbil.

2.2. Preparation of Plant Extracts

Soxhlet techniques and steam distillation were used for the process of extraction (De Silva *et al*., 2017). Initially, the examined plant parts were cleaned and washed with water and then air-dried in the laboratory. The sample was then processed into a powder using a mortar device. The powder was sieved and then kept in a dark bottle for subsequent screening of chemical repellency tests.

2.3. Laboratory Trails

The lethal trials for each plant extract were conducted in disposable Petri dishes under laboratory conditions. Ten active nymphs of citrus mealybug, *Planococcus citri* were placed in a Petri dish with citrus leaves and filter paper. Three concentrations of 0.5, 0.75, and 1% of the lethal efficacy in all tested plant extracts in addition to the control (ethanol only) were used in the evaluation. After 12 hours of spraying, the treated insects were inspected to determine the extracts' impact. The test was repeated after 24

hours of spraying to detect recovery incidence (Hollingsworth, 2005). The test was conducted with four replications for each concentration and each plant. Control readings were obtained by spraying ethanol in the same concentrations without using any repellent before the experiment. Information about plant parts that used their extract in the study is shown in Table 1.

Table 1. Selected plants and parts used for extraction in the Study

Common name	Scientific name	Family	Plant Part used
Eucalyptus	*Eucalyptus camaldulensis* Dehnh.	Myrtaceae	Leaves
Dark opal Basil	*Ocimum basilicum*	Lamiaceae	Leaves and stems
Camel thorn	*Alhaji maurorum*	Fabaceae	Leaves and stems
Mint	*Mentha longifolia*	Lamiaceae	Leaves and stems

2.4. Detection of phytochemical substances

There are numerous methods tested for screening phytochemical activity. The solvent type and the method of extraction are important to find out the chemical components of the extract. (Lapornik *et al*. 2005). Also, the optimal solvent depends on the eventual purpose of the extract. The used parts of selected plants were processed into small portions using a Scissor tool. The portions were placed in a dark bottle and mixed with an absolute ethanol %99 and then filtered. The filtrate was then transferred to a dark vial bottle for subsequent screening of chemical components. The extracts for all plants used in the study were chemically tested for detecting alkaloids, anthraquinones, flavonoids, terpenoids, saponins,
steroids, tannins, and volatile oils.

Alkaloids test

Two milliliters of plant extract were stirred with (2) ml of 10% aqueous hydrochloride acid (HCl), and for Wagner's reagent, one milliliter of the filtrate was treated with a few drops of the reagent. Appearing of a reddish-brown precipitate indicated the presence of alkaloids in the extract (Adejoke *et al*., 2019).

Anthraquinone test

Five grams of the plant powder was mixed with (10) ml benzene, and (5) ml of 10% ammonia solution was added to the filtrate. The mixture was well shaken. A pinkish-red or violet color indicated the incidence of Anthraquinone, glycoside (Duval *et al*., 2016).

Flavonoids test

In this test, a few drops of diluted NaOH (10%) solution were added to the stock solution of selected plants (1) ml, settled down of intense yellow color indicating the presence of flavonoid compounds (Tzanova, *et al*., 2020).

Terpenoids test

In glass test tubes, 5ml stock solution of each plant extract is mixed with 2ml chloroform followed by 2ml concentrated Sulfuric acid. A reddish-brown coloration has proposed the presence of terpenoids (Mujeeb *et al*., 2014).

Saponins test

Two grams of the extract powder was placed into a test tube, (5) ml of water was added and it was shaken intensely. The whole tube was added and it was filled which last for few minutes. Bubbles indicated the presence of saponin (Cheok *et al*., 2014).

Steroids test

Five grams of the powder of each selected plant was dissolved in (5) ml of Chloroform. It was then filtered, and then a few drops of sulphuric acid were carefully added. A reddish brown color ring at the interface indicates the presence of steroids (Dinan *et al*., 2001; Doughari, 2012).

Tannins test

A few drops of Ferric Chloride solution ($FeCl_3$ 1%) were added drop by drop to (2-3) milliliters of each plant extract. A dark green color or blue-black precipitate indicates the incidence of tannins (Jayanegara *et al*., 2009; Bankole *et al*., 2016).

Volatile Oils test

One milliliter of the extract was mixed with diluted Hydrochloric acid (diluted HCl). White precipitation indicated the presence of volatile oils (Politeo et al., 2007).

2.5. Design and Data Analysis

The study was carried out using a Complete Randomize Design (CRD) experiment in the laboratory. The treatments were distributed on the experimental units with four replications, where 10 normal nymphs were placed in each Petri dish as an experimental unit. All the data obtained were statistically analyzed using the SPSS program (version 26), and the experiment was used to show significance between and among the treatments (SPSS, 2018).

3. Results and Discussion

The results of the present study revealed that the extracts of all selected plants have a great lethal role in decreasing citrus mealy bug individuals at three different concentrations 0.5, 0.75, and 1%, in comparison with the control agent. However, it was difficult to absorb the amount of the control agent from the body of the mealybug nymphs, via the waxy layer that covers whole the body (Figs.1 & 2). entirely, but using selected plant extracts in the current experiment was significant.

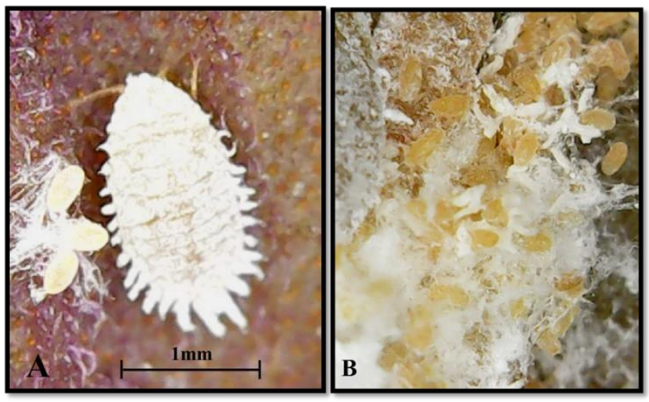

Figure 1

A, Adult female mealybug, *Planococcus citri*; B, Nymphs in the cluster

Figure 2. Damage signs by mealybug infestation on citrus leaves, branches, and fruit

As shown in Figure (3), the death caused by *Eucalyptus camaldulensis*, and *Alhaji maurorum* extract had higher significance than the other extracts in the concentrations 0.75, and 1% respectively after 12 hours. While the *Ocimum basilicum* extract had a rather high lethal effect in concentration 1%. At the same time, there were no significant differences between the concentrations 0.5 and 0.75. Regarding the extract of *Mentha longifolia*, there were low significant effects at 0.75% while no significant differences between the two concentrations 0.5 and 1%.

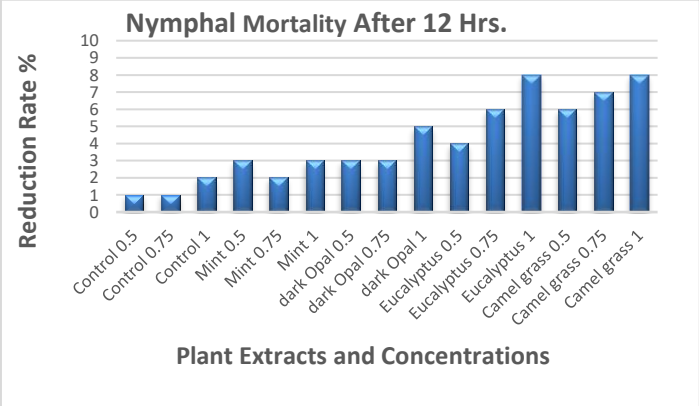

Figure 3. The rate of nymph reduction of Planococcus citri (RISSO) influenced by different concentrations of selected plant extracts after 12 hrs. of spraying

Figure 4, indicated that the mortality rate gradually increased with increasing concentrations, and exposure times, the extracts of *Alhaji maurorum* and *Eucalyptus camaldulensis* caused a higher percentage of reductions that were documented 24 hours after spraying in all concentrations except for the 0.5 concentration of Eucalyptus extract. Moreover, the *Ocimum basilicum* extract had a high reduction rate in the1% concentration, at the same time their effects were equal in the concentrations of 0.5% and 0.75. However, the extract of *Mentha longifolia* had high efficiency in the reduction of mealy bug nymphs in the concentration of 1%, meanwhile, it had the same significant differences in 0.75% concentration.

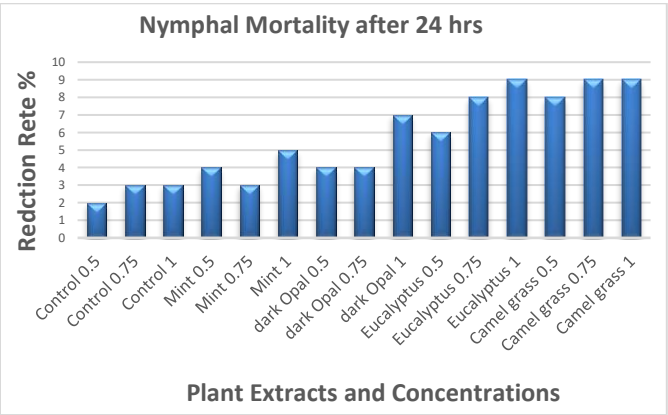

Figure 4. The rate of nymph reduction of Planococcus citri (RISSO) influenced by different concentrations of selected plant extracts after 24 hrs. of spraying

Figure (5) indicates the rate of mortality in which increased in all examined plant extracts in the second observation (24 hrs.) which proved highly significant among different concentrations. Also, results showed that in all treatments the lethal effect increased by around 10 % through two different exposure periods, and there was a significant difference between them.

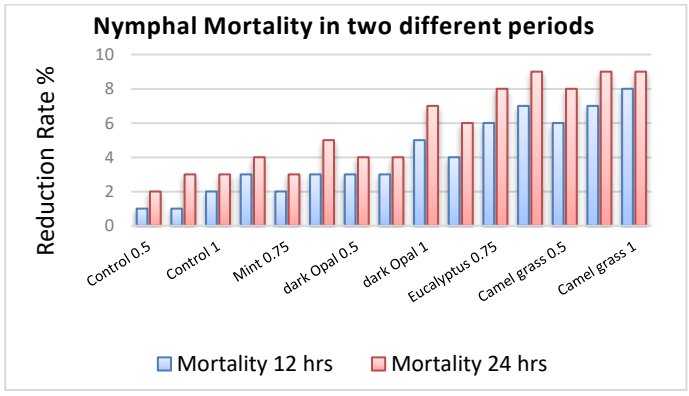

Figure 5. The rate of nymph reduction of Planococcus citri treated with three different concentrations of selected plant extracts during two different periods (12 and 24) hrs.

The use of plant extract as botanical pesticides for protecting orchards, crops, and ornamentals from insect problems is expected more important all over the world due to rising awareness of the dangerous effects of using non-selective synthetic pesticides (Joseph and Sujatha, 2012; Mishra *et al*., 2018). Therefore, this investigation was performed The presence of phytochemical components in the plants, Eucalyptus Dark Opal Basil, Camel thorn, and Mint, as more than seven different compounds discovered in the extracted substances indicated that the foliar parts of these plants are rich in chemical ingredients due to the high amount of phenols, Anthraquinonoids, flavonoids, saponins, and tannins (Kaur *et al*., 2019). The high presence of steroids, flavonoids, saponins, and tannins may be a justification for

using plant extracts as a control agent in insect management planning (Jeyasankar *et al*., 2014). Saponins are active agents and have a main role in the protection mechanism against sucking insects (Golawska *et al*., 2006). Tannins have decent repellency properties (Bernays, 1981; Barbehenn and Constable, 2011). According to Diaz Napal and Palacios (2015), flavonoids are known to kill insects. Accordingly, there may be a scientific basis for the use of camel grass and *Eucalyptus* which is reportedly used for the control of insect outbreak problems.

Conclusions

According to the results of the present study, it concluded that the repellent composition of selected plant extracts had a great impact on the reduction of the Citrus mealybug, *Planococcus citri*; therefore, could be depended as a successful technique in applying pest control management. Camel thorn grass and Eucalyptus extracts had more lethal reduction than the dark pal basil and mint extract, therefore it concluded that their compounds should be formulated into products as a botanical insecticide, then could be used against insect pest infestations that are accessible by smallholder farmers, in which have a positive role in food safety as well as protection of the environment and human health.

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