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Study of the repellent effect of the insect extract of the mealybug Planococcus cirit on first-phase nymphs of the mealybug Nipaecoccus viridis

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***Abstract*---**The research on field experiments is conducted at (1 March 2021 - 1 January 2022) in the woody plant canopy / Department of Biology / College of Education for Pure Sciences / University of Anbar, and the second part included laboratory experiments that are conducted in the laboratories of the Department of Biology / College of Education for Pure Sciences / University of Anbar to study the repellent effect of the ethanolic extract of Planococcus citri on the mealybug *Nipaecoccus viridis* nymphs.The results have shown that there is an effect of the ethanolic extract of *Planococcus citri* on the repellency of *Nipaecoccus viridis* nymphs, where the higher concentrations of the extract achieved stronger repellency efficacy than the lowest concentrations. The repellency percentage reached at the concentration 10000 ppm (56.7%) after one hour of exposure and 73.3% after 6 hours of exposure, and the lowest percentage of repellency at the 2000ppm concentration is 10.0% after an hour of exposure and 16.7% after 6 hours of exposure. While the 4000ppm concentration achieved a repellency rate of 33.3% after an hour of exposure and 53.3% after 6 hours of exposure. The centrifugal force in these concentrations ranged between the first, third and fourth classes.

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Introduction

Citrus trees are exposed to a number of insect and non-insect pests, and these pests vary in their importance from one region to another. Ebeling has collected nearly 875 species of insects and mites that attack citrus trees around the world, 72 of which are considered major pests in regions Citrus cultivation (Ebeling, 1959). Mealy bugs are a large group of insects belonging to the family Pseudococcidae, above the family Coccoidea, the long-tentacled order Sternorrhyncha, and the hemiptera order. Mealybug carry this name because of the white wax or soft wax that its bodies secrete. It spreads in most regions of the world and infects a wide range of agricultural crops plants, fruit trees, ornamentals and many herbs (Zarkani *et al.*, 2021).

Mealybugs are characterized as invasive insects because they are small in size and often live in hidden environments with their frequent movement on goods in international trade (Michele *et al.*, 2021). The damage caused by the mealybug is related to its feeding on plant sap and its secretion of honeydew, which is linked to the emergence of the black mold (*Aspergillus niger*), including yellowing and falling of leaves and plant weakness leading in some cases to plant death (Fang *etal.*, 2020). In 1996 in Georgia state- USA, the cost of losses due to mealybug infestation that invaded the crops is estimated at approximately $99 million (Chong et al., 2003).

The life characteristics of mealybugs, their lifestyle and reproduction make combating them a difficult and complex process, and greatly weaken the effectiveness of chemical control methods, as they are multi-families of plants. As such, this necessitates the treatment of more than one host when combating them, especially when they are located under the soil or on the roots of these families, as well as their choice of protected places. In addition to its body of cilia and waxy appendages, laying eggs within protected tissues in the form of large gatherings of eggs and moving nymphs reduces the chance of exposure to the control agent and the lack of effectiveness of traditional procedures with it. The high fecundity that characterizes the insect provides it with the speed of colony building and renewal, as well as its great ability to resist and the emergence of resistant strains by inheriting this trait from carrier mothers to their offspring (Dreistadt, 2016; Bettiga, 2013) .Therefore, this study aimed to: Studying the repellent effect of the insect extract of the mealybug *Planococcus cirit* on first-phase nymphs of the mealybug *Nipaecoccus viridis.*

Methods

Preparation of the host plant for rearing citrus mealybugs, *Planococcus cirit* and *Nipaecoccus viridis*

Potato tubers are brought from the local markets in the city of Ramadi. The infected and damaged tubers are excluded. The tubers are washed well with water to remove dust and dirt. Then they are transferred to large plastic containers equipped with a solution of sodium hypochlorite at a concentration of 4-6%, using 25 ml / liter of water for half an hour in order to get rid of fungal and bacterial pathogens, if any. Then, the tubers are placed in plastic molds with dimensions of 45 x 25 x 15 cm in the open air to ensure their dryness. It is placed in the incubator for the purpose of germination at a temperature of 25°C and a relative humidity of 55-60%, for the purpose of breaking the dormancy phase and starting germination and in order to obtain suitable white plants to feed the insect's roles which should be free from chlorophyll and the toxic substance Solanine. Breeding conditions are maintained at absolute dark conditions, and the potato tubers germination stages are followed up weekly until the plants reached the appropriate height (Cocco *et al.*, 2021).

Prepare the required colony of the citrus mealybug Planococcus citri and Nipaecoccus viridis for laboratory experiments

After the potato tuber sprouts reached the appropriate length, the tubers are transferred to the plastic molds in the incubator to grow the mealybug *Nipaecoccus viridis* and *Planococcus citri.* Where infection of healthy tubers is carried out by mechanical transmission, that is, by transferring mealybug roles from infected trees to healthy tubers, then plastic molds are covered and transferred to incubators for breeding at a temperature of 27 ± 2°C, humidity 55-65%, and a duration of (light: dark) (16: 8) hour (Bonierbale *et al.*, 2020). Then the molds are left for a week in the incubator with complete darkness to ensure the stability of the creepers and install themselves on potato plants for the purpose of feeding and preventing their dispersal as long as they are attracted to light. The stages of growth and development of the mealybug on the tubers are monitored daily until the mealybug colonies reached the appropriate age and to ensure their full spread on the potato tubers (Cocco *et al.*, 2021).

Collection and preservation of secretions of mealybug Planococcus citri and Nipaecoccus viridis

The experiment is carried out with four groups:

- The first group represents the honey symposium and the rest of the products of mealybugs of the genus *Nipaecoccus viridis* before convergence of the genus *Planococcus citri.*
- The second group is for the genus *Planococcus citri* also before the convergence of the genus *Nipaecoccus viridis.*
- The third group of the first genus after the approach of the second genus.
- The last group of the second genus after the approach.
- Insect products for each group are placed in small plastic cans (50 ml) by using filter paper and glued to the colony containing the insect secretions. After that, they are placed in cans and closed well, and each group carried the name of the genus, and then kept in the refrigerator at a low temperature (-4) C.

Extraction of the products of mealybugs

The maceration method is used to extract the byproducts of mealybugs. The extraction method is carried out according to the following steps:

- The extraction process is carried out by a mixture of organic solvents, namely ethanol, hexane, diethyl ether in a ratio of.(1 :1 :2)
- The mealybug products with their different samples are transferred to a 1000ml glass beaker containing the mixture of the mentioned solvents.
- The samples are left in an incubator at 30°C for 24 hours with shaking from time to time.
- After 24 hours the extract solution is purified and filtered by Buchner funnel and Whatman No. 1 filter paper under vacuum to obtain a clear filtrate. Then the filtrate is centrifuged at 5000 rpm.
- After obtaining a pure filtrate, the extract is concentrated and the organic solvents are disposed of by a rotary evaporator under vacuum at a temperature of 40°C.
- Collect the extract in glass tubes and store in the refrigerator until use.
- A standard solution is prepared from the mentioned extracts at a concentration of 1% = 10000 ppm.

Preparation of mealybug extract concentrations

The concentrations of the insect extract are prepared using 3 numbered sterile test tubes (1-3). A standard solution of the extract is prepared at a concentration of 1% = 10,000 ppm in the first tube and in the second test tube 20 ml of the first concentration is added and the volume is completed to 100 ml, while the third concentration is by adding 40 ml of the first concentration and completing the volume to 100 ml, as this is done according to the following equation:

$$C2*V2=C1*V1$$

$10000*V1=2000*100$
$V1=20\%$

$100*4000=V1*10000$
$V1=40\%$

C1= original concentration
V1 = the volume taken from C1 to obtain the concentration of C2
C2 = the desired concentration for a volume of V2
V2 = final volume of desired concentration

The repellent effect of the extract of mealybug *Planococcus citri*

An experiment is conducted to test the efficacy of the repellent effect of different concentrations of the insect extract on crawlers of the mealybug *N.viridis*, where the repellents are evaluated using the Area preference method (Obeng-Ofori *et al.*, 1998), where the test areas consisted of filter paper in the middle. 1 ml of the three concentrations of the extract is applied to one half of the filter paper with a pipette and the other half of the filter paper is treated with water alone as a control, the two halves are dried in the air to evaporate the water completely. Then the two halves of the filter paper are stuck together in each Petri dish. 20 mealybug crawlers are inserted into the center of each circular leaf and then the plate is covered. Each treatment is repeated 3 times. The number of insects present on the control and treated areas is recorded after (1 hour-6 hours). Then the repulsion index (Indicator repellents) is calculated by the following equation (Mazzonetto, 2002)

$$IR = 2 G / G + p$$

where: treatment areas (G) viewing areas. (P)
The repulsion index is classified as follows:
Attractive 1 < IR, Neutral 1 = IR, Repelling IR 1 < IR

o The IR value is less than 1, the extract is repellent.
o IR value greater than 1, the extract is Attractant.
o The IR value is 1 and the extract is neutral.

The repellency percentage (PR) (Tapondjou et al., 2005) is calculated as:

$$PR = [(NC-NT) / (NC + NT)]* 100$$

(NC): The number of insects in the observed areas
(NT): Number of insects in the areas treated with the extract.

The repulsion value is calculated for each concentration according to the repulsion force and classified into five categories (Jilani and Su 1983) (the repulsion force increases with the increase in the category number, and the fifth category is the most repelling) as follows:

0.1%< PR	Category 0
20% - 0.1%=PR	Category 1
40% -20.1%=PR	Category 2
60% - 40.1% =PR	Category 3
80%-%60.1%= PR	Category 4
100%-80.1%= PR	Category 5

Results and Discussion

Repellent effect of the insect extract of the mealybug *Planococcus cirit* on first-phase nymphs of the mealybug *Nipaecoccus viridis*

The results have shown that the extract had a repellent effect on the first-phase nymphs of the mealybug *N. viridis.* This effect varied according to the concentration gradients and according to the duration of exposure. A greater effect is observed for the insects treated with the extract immediately after exposure. The disturbance of insects is observed in the experiment dishes after two to four hours of treatment. Also, a greater number of insects is recorded in the untreated half after half an hour of exposure, while a relative stability of the number of insects is observed in the untreated halves after six hours of exposure. The results have shown that the higher concentrations of the extract had a stronger repellency efficacy than the lower concentrations in the first-phase nymphs of the insect studied. As the extract achieved the highest rate of repellency at the concentration 10000ppm amounted to 56.68% after an hour of exposure and 73.304% after 6 hours of exposure. Also, the lowest percentage of repellency reached at the concentration of 2000ppm it reached 10% after an hour of exposure and 16.7% after 6 hours of exposure. While the concentration of 4000ppm achieved an repellency rate of 33.344% after an hour of exposure and 53.341% after 6 hours of exposure (Table No. 7).

Table 7
Effect of the insect extract *Planococcus citri* on the percentage of repellency of the first nymph of *Nipaecoccus viridis*

Percentage of repellency after 6 hours	The average number of insects in		Percentage of repellency after 1 hour	The average number of insects in		Insect extract and concentration
	treatment	control		treatment	control	
% 73.304	2.67	17.333	% 56.68	4.333	15.67	10000
%53.341	6.67	13.333	%33.344	4.67	15.333	4000
%16.7	9	11.67	%10	8.333	10.67	2000

When calculating the centrifugal index and evaluating the centrifugal force, it is found that the extract had a repelling effect at the highest concentrations represented by the concentrations of 10000ppm and 4000ppm, and its effect is weak at the lowest concentrations represented by the concentration of 2000ppm of the extract, and the centrifugal force in these concentrations ranged between the first, third and fourth classes (Table No. 8) .The results agreed with what is indicated by (Navarro and Navarro, 2018) in the difference in the percentage of dead insects according to the concentration of the extract, the exposure period and the treated phase, and the higher concentrations achieved the highest death rate. It is reported in a study (Ibrahim and Sisay, 2011) that the extract of the leaves of the eucalyptus plant is repellent at a concentration of 10% Eucalyptus camaldulensi in the adults of the potato tuber moth operculella Phthorimae. The repellent rate reached 90% after 12 hours and 100% after 24 hours, which differ from the results of the current study, as the percentage of repellency is higher during the sixth hour of the treatment compared to the first hour.

Table 8
IR repellency index of the insect extract *Planococcus citri* in the first nymph phase of *Nipaecoccus viridis*

Repellency class	Repellency treatment	Repellency indicator after6 hours	Repellency class	Repellency evaluation	Repellency indicator after 1 hour	Insect extract and its concentration
4	Repelling	0.27	3	Repelling	0.433	10000
3	Repelling	0.47	2	Repelling	0.67	4000

1	Repelling	0.833	1	Repelling	0.05	2000

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