EFFECT OF ALCOHOLIC EXTRACT OF *CARISSA MACROCARPA* LEAVES ON TWO SPOTS MITS *TETRANYCHUS URTICAE* UNDER LABORATORY CONDITION

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Abstract:

A laboratory study was conducted at the Musayyib Technical College / Department of Biologecal control Techniques for the period from 10/1/2021 to 1/11/202 In order to know the evaluation of the efficiency of the alcoholic extract of Charisia leaves. Carissa macrocarpa in the control of Tetranychus urticae under laboratory condition.The results showed that the alcoholic extract of C. macrocarpa leaves had a significant effect on all stages of the two-spot mits at all concentrations, as well as increasing the mortality rates of eggs, larvae, nymphs and adults by increasing the concentration and increasing the time period. The highest mortality rate for eggs (60.67%) at the concentration of 20 mg/ml compared to the control treatment which was (0.00%) for the same concentration during 72 hours of treatment, while the lowest concentration of the extract 5 mg/ml gave the eggs destruction rate amounted to (35.00%). during 72 hours of the treatment, while the comparison treatment was (0.00%) for the same period .As for the larval stage, the highest larval mortality rate was (69.33%) at the concentration of 20 mg/ml compared to the control treatment, which amounted to (0.00%) for the same concentration during 72 hours of treatment . While the lowest concentration of the extract 5 mg/ml gave the larva mortality (32.00%) during 72 hours of treatment, while the comparison treatment was(0.00%) for the same period. As for the nymph stage, the highest mortality rate of nymphs was (65.67%) for the concentration of 20 mg/ml compared to the control treatment, which reached (1.00%) for the same concentration during 72 hours of treatment , While the lowest concentration of the extract 5 mg/ml gave the percentage of nymph mortality (43.67%) during 72 hours of treatment, while the comparison treatment was(1.00%) for the same period , As for the adults, the highest mortality rate of mite adults was (67.67%) for the concentration 20 mg/ml compared to the control treatment which amounted to(0.00%) for the same concentration during 72 hours of treatment, while the lowest concentration of the extract 5 mg/ml gave the death rate for the adults amounted to (40.33%) during 72 hours of the treatment, while the comparison treatment was(0.00%) for the same period.

Keywords : two spots mits , Charisia leaves , alcoholic extract .

INTRODUCTION

The two-spot mite *Tetranychus urticae* is a widespread pest that infects many plants, including fruit trees, field crops, vegetables and ornamental plants, and causes great economic damage, especially when infected in the initial stages of growth [1] **.**this pest has a high potential to attack more than 1100 species of plants of economic importance and cause them many losses [2], as it causes direct damage by absorbing plant juices, causing the appearance of yellow spots on the leaf tissue that leads to a reduction of chlorophyll pigment by 60%, which it leads to a decrease in the efficiency of the photosynthesis process in the leaf [3]. as for the indirect damage, it is

the transfer of viral pathogens and their injection into the healthy plant, as it has piercing and absorbent mouth parts [4]. in view of the amount of losses caused by this pest on economic crops, as well as the speed of its reproduction and the multiplicity of its generations per year [5], and because of its high resistance to chemical pesticides [6]. this necessitated the search for alternatives to chemical compounds harmful to humans and the environment, for example, the control with plant extracts, which are characterized by containing inhibitory or pest-killing compounds and reduce the development of resistance to them [7]. it also contains materials that are non-toxic to humans and are environmentally safe. the study aimed to evaluate the efficiency of the alcoholic extract of *C. macrocarpa* leaves on all stages of two spot mits *T.urticae* in laboratory conditions.

MATERIALS AND METHODS

Laboratory Diagnosis and breeding of two spot mits *T.urticae* in

The two-spot mite T.urticae was obtained from infected eggplant plants and was diagnosed by Dr. Razzaq Shaalan Akl at the Natural History Museum and Research Center / University of Baghdad. The mites were bred in the laboratory according to the paper disc method for researchers [8]By taking the leaves of the castor plant, *Ricinus communis*, containing all the stages of the two-spotted mits and placing them upside down in a plastic petri dish diameter 9 cm containing a layer of medical cotton. Saturated with distilled water to ensure that the leaves are moistened for as long as possible, the dishes were placed in an insect breeding incubator at a temperature of ± 25 2 °C and a relative humidity of 65 ± 5% [9].

Preparation of alcoholic extract of carisia leaves *C. macrocarpa*.

The leaves of C. macrocarpa were collected from gardens and diagnosed by Dr. Khansa Rashid Majid at the Research Center and Museum of Natural History/University of Baghdad, They were ground by an electric grinder and the method was followed [10] to extract the plant samples with organic solvents, where (20) gm of C. macrocarpa leaves powder was taken. It was placed in a filter funnel and placed in the Soxhlet extractor, and poured 200 ml of 99% ethyl alcohol. The device was set at a temperature of 40 ° C for a period of 24 hours. The filtrate was taken from the glass beaker and it was concentrated and disposed of the ethyl alcohol residues by placing the filtrate in a Rotary evaporator at a temperature of (40) C. Then the sample was dried in an electric oven at a temperature of (40-45) C until it became a dry substance, For the purpose of preparing the required concentrations from the extracted sample we followed the method [11] by taking (4) g of the extracted substance and dissolving it with (3) ml ethyl alcohol by stirring the sample with alcohol and completing the volume to 100 ml with distilled water, thus making the stock solution concentration equal to 4% It is equivalent to (40) mg per 1 ml, and from it we prepared concentrations (5, 10 and 20) mg / ml with the addition of 3 ml of diffuser Tween [12].

Effect of alcoholic extract of *C. macrocarpa* leaves on two-spot mite *T.urticae* eggs under laboratory conditions.

Twenty adults of the two-spot mites, 10 males and 10 females, were transferred to castor leaves placed in petri dishes with a diameter of 9 cm, prepared in advance, and left for 48 hours for the purpose of mating. The adults and the excess eggs were removed with a soft brush [13] and 10 eggs were left, Dishes were sprayed with alcoholic extract of Charisia leaves by a small hand sprayer with a capacity of 30 ml, at concentrations (20-10-5) mg/ml at a rate of 1 ml for each dish and 3 replicates for each concentration. As for the control treatment, it was sprayed with distilled water only. The dishes were incubated in the incubator at a temperature of 25±2°C and a relative humidity of 65%. The percentage of eggs perishing was calculated after 24, 48 and 72 hours of treatment, the rates were extracted

and the values were corrected according to the Abbot equation presented in [14].

$$\text{Corrected Percentage of Loss} = \frac{\text{\% death in treatment - \%death in comparison}}{\text{100 - \%death in comparison}} 100\%$$

Effect of alcoholic extract of *C. macrocarpa* leaves on the motile stages of *T.urticae* (larva, nymph, and adult) under laboratory conditions.

We placed 10 individuals from the motile stages for each stage separately (larva - nymph - adult) on castor leaves placed upside down in petri dishes with a diameter of 9 cm prepared in advance and sprayed with alcoholic extract of carisia leaves by a small hand sprayer with a capacity of 30 ml and concentrations (20 - 10 – 5) mg/ml for each mobile phase separately, at a rate of 1 ml for each plate, and 3 replicates for each concentration. As for the comparison treatment, it was sprayed with distilled water only The dishes were also inspected and the percentage of damage was calculated in the same way as before.

Statistical Analysis.

The results of the study were statistically analyzed using the program GenStat (2011) according to a factorial experiment with a completely randomized design (C.R.D) completely randomized design, and the least significant difference (L.S.D.) test was adopted under the 0.05 probability level to test the significance of the results [15].

RESULTS AND DISCUSSION

Effect of alcoholic extract of *C. macrocarpa* leaves on two-spot mite eggs *T.urticae* under laboratory conditions.

The results in Table (1) indicated the effect of all concentrations used of the alcoholic extract of C. macrocarpa leaves in reducing the percentage of egg mortality, as they differed significantly from the control treatment. And the concentration of 20 mg/ml was superior in reducing the percentage of dead mite eggs with two spots, which amounted to (39.00%, 42.67%, 60.67%) for the time periods (24, 48, 72) hours, respectively, which gave statistically significant differences over the comparison treatment that amounted to (0.00%) for all time periods, The lowest mortality rate for the concentration was 5 mg/ml, as it gave (28.33%, 33.00%, 35.00%) for the same previous period, which gave significant differences from the comparison treatment. It was noted that there were significant differences between all the concentrations used and for all periods, as it was noted that the percentage of eggs perishing By increasing the concentration and time, the highest mortality rate for the concentration was 20 mg/ml (47.44) for all periods, and the highest death rate for the 72-hour period was (34.92) for all concentrations, with a significant difference from all rates for the previous periods. These results are consistent with previous studies, where it was found that the leaves of the karisia plant contain many compounds that belong to different chemical groups, where it was found [16] that the leaves of the karisia contain compounds (glycosides, terpenoids, flavonoids, phenols), It was also found [17] that they contain compounds (saponins, tannins, carbohydrates) that these substances have the ability to penetrate the eggshell and affect the protoplasm and death of the fetus [18]. This study also agrees with another study, where it was found that the hatchability rate of eggs decreased to 90% when using pepper extract [19], and another study found that garlic extract had a clear effect in increasing egg mortality. Integrated control of agricultural pests, one of these pests is the two-spotted dream [20].

Table (1) shows the mortality percentage of eggs treated with alcoholic extract of *C. macrocarpa* leaves during 3 different time periods.

Con. mg/ml	time periods/hour			Con. rate
	24	48	72	
5	28.33	33.00	35.00	32.11
10	33.67	39.33	44.00	39.00
20	39.00	42.67	60.67	47.44
control	0.00	0.00	0.00	0.00
period rate	25.25	28.75	34.92	
LSD Con.= 3.50 period=3.03 overlap= 6.06				

Effect of alcoholic extract of C. macrocarpa leaves on the motile stages of T.urticae (larva - nymph - adult) under laboratory conditions.

The results in Table (2) indicated the effect of all concentrations used of the alcoholic extract of *C. macrocarpa* leaves in reducing the percentage of mortality of the motile stages of *T.urticae* (larva - nymph - adult), which differed significantly from the control treatment. The concentration of 20 mg/ml was superior to the increase in the percentage of death of the larvae of the two-spotted nipple, which amounted to (69.33%) for the time period (72) hours, and the nymphs amounted to (65.67%), and the percentage of adult death was (76.67%) for the same time period, which significantly differed from The comparison treatment which amounted to (0.00, 1.00 and 0.00) for larvae, nymphs and adults, respectively, for a period of time (72) hours. The lowest concentration of the extract was 5 mg/ml, which gave a mortality rate for larvae (32.00%), nymphs (43.67%), adults (40.33%) and for the time period (72) hours, with a significant difference from the control treatment, which amounted to (0.00%, 1.00% and 0.00%).) for larvae, nymphs and adults, respectively, for a period of time (72) hours. These results agree with previous studies, which found that the active compounds extracted from some plants affect the protease enzyme in the middle alimentary canal, in addition to reducing the level of sugar and protein in the insect's blood [21]. Another study showed that the active chemicals found in plant extracts may combine with some fatty compounds, proteins and digestive enzymes and thus form indigestible chemical complexes, which leads to affecting the metabolism of the insect's body [22], Therefore, we find that the alcoholic extract of Charisia leaves has a clear effect in the moving stages, and the death rates vary according to the plant part used and the concentration [23]. Where a study found an increase in the percentage of death in the moving stages of the two-spot dream with an increase in the time when using the water extract of *Lantana montevidensis*[24]. A study found that the alcoholic extract of Charisia leaves worked to prevent feeding of the two-spot mite, so we find an increase in mortality with increasing time, as in [25]. A study found an increase in the death rate of adults with two-spot mites when using of *Lantana montevidensis* extract after 3 days of treatment [26]. A study confirmed that the use of neem extract led to an increase in the death rate of all stages of the mits, and the percentage of death increased with increasing time, as the

death rate of adults reached 90% after 7 days of treatment [27].

Table (2) shows the percentage of mortality of the motile stages of two-spot mites treated with alcoholic extract of C. macrocarpa leaves during 3 different time periods.

Con. mg/ml	Percentage of larval mortality after/hour			Con. rate	Percentage of nymphs perish after/hour			Con. rate	Percentage of adult mortality after/hour			Con. rate
	24	48	72		24	48	72		24	48	72	
5	17.67	27.67	32.00	25.78	30.33	39.67	43.67	37.89	32.33	38.00	40.33	36.89
10	27.00	36.00	42.33	35.78	31.00	47.33	52.00	43.44	36.00	42.00	53.33	43.78
20	29.00	38.67	69.33	45.00	39.67	48.67	65.67	51.33	43.00	49.00	76.67	56.22
control	0.00	0.00	0.00	0.00	0.00	0.67	1.00	0.56	0.00	0.00	0.00	0.00
Time rate	18.42	25.58	35.92	Time rate	25.25	34.08	40.58	Time rate	27.83	32.25	42.58	
LSD Con. = 1.99 Time = 1.73 overlap= 3.46					LSD Con. = 1.14 Time= 0.99 overlap=1.98				LSD Con.= 1.82 Time= 1.58 overlap= 3.16			

CONCLUSIONS

We conclude from the study that the alcoholic extract of C. macrocarpa leaves had a significant effect on the mortality rates of T.urticae in all its stages under laboratory conditions. The concentration of the extract 20 mg/ml was the best concentration with the highest mortality rates for eggs, larvae, nymphs and adults, and the time period 72 hours was the best period with the highest mortality rates for eggs, larvae, nymphs and adults.

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