EFFICACY OF ANTIMICROBIALS IN BIOLOGICAL PERFORMANCE OF THE TWO-SPOTTED SPIDER MITE *TETRANYCHUS URTICAE* INFECTING TOMATO IN THE DESERT ENVIRONMENT

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ABSTRACT
The red two-spotted tit, *Tetranychus urticae*, has a wide family range and the possibility of spreading in a wide range of temperatures and humidity, and it has a high ability to show resistance against many chemical pesticides. Therefore, this study aimed to use antimicrobials (Microcin, Chitosan) as environmentally friendly control agents against this pest. The results of the study showed that the Microcin had a significant effect in reducing the rate of egg hatching (the vitality of eggs) to 8%, and it led to a significant decrease in female fertility (the rate of laying eggs) by 28%, it also increased the mortality of nymphs and adults by 90% and 62%, respectively, compared to the control treatment, which resulted in 48%, 52%, 5%, and 10%, respectively. The results also showed that the use of Nano-chitosan significantly reduced the rate of hatching eggs and the rate of laying eggs, and increased the mortality rate of nymphs and adults. The two-spotted spider mite compared with the control treatment. The study showed the possibility of using natural materials such as antimicrobials (Microcin, Chitosan) as alternatives to the use of chemical pesticides in controlling stubborn pests, including *T. urticae*.
Keywords: IPM, biological control, plant mites, tomato

The tomato (*Solanum lycopersicum* L.) belongs to the nightshade family, which ranks second among vegetables globally (FAOTAT, 2015; Ajlan et al., 2007), where the annual production of the tomato crop is estimated at about (183) million tons from an area of (4.85) million hectares. In Iraq, the annual production of tomato is estimated at 6.2 million tons from an area of 22,892 hectares, representing 3.27% of the total global production (FAO, 2019). The nature of the climate and soil in Iraq makes it a suitable environment for growing tomatoes during the seasons year, especially when using modern technologies, hybrid seeds, pesticides, fertilizers and greenhouses. Tomato plants are affected by many pests, the most important of which is the red two-spotted mite *T. urticae*. It is one of the most harmful agricultural pests in the world, and it is one of the most toxic phyto-legged arthropods and feeds on more than 1,100 plant species from more than 150 types of crops of economic importance (Santamaria et al., 2020). The symptoms of the infection begin initially on the leaves of the host plant, especially at the base of the blade and next to the main veins, where pale green spots appear on the upper surface of the leaves, and corresponding to them on the lower surface are the different roles of the mite. With the continuation of feeding and the increase in the severity of the infection, the color of the spots on the upper surface of the leaf turns red-violet. Many control agents have been used to reduce the effect of this dangerous pest that may limit the cultivation of tomato plants.

Microcin is a low molecular weight (10,000Da) bacteriocin secreted by Enterobacteriaceae individuals, and it involved in microbial competition within the gut (2002, White and Fields). The wide diversity of microcin structures results in a variety of mechanisms of action, such as inhibition of vital enzymatic functions and damage to the inner membrane (Duquesne, al., 2007). In addition, interference with intracellular enzymes responsible for DNA structure and synthesis (Duquesne et al., 2007). Morin (2011).

Chitosan is found naturally in the shells of all crustaceans and insects, and some other organisms such as many fungi, algae and yeast. Chitosan is one of the most common polymers found in nature (EPA 2003). One of the most important properties of chitosan is that it is chemically stable, in the form of a white to pale yellow powder or flakes (Polysciences, 2003). Chitosan is insoluble in water, and chitosan can be treated with acid to make it soluble in water. Therefore, this study aimed to use antimicrobials (Microcin, Chitosan) as environmentally friendly control agents against the two-spotted spider mites *Tetranychus urticae*.

MATERIALS AND METHODS

Breeding (culture) the two-spotted spider mites

A primary colony of *T. urticae* was obtained from tomato fields (*Solanum lycopersicom* L) on 9/10/2021 in Al-Haydaria sub-district in the northern part of Al-Najaf governorate. Seeds of eggplant *Solanum melongena* and tomato *S. lycopersicum* were sown, then the seedlings were transplanted in 3 Kg plastic pots, maintained in a plastic house and artificially infected. The infected plants were transferred by transferring part of the upper leaves of the infected tomato plant to a healthy plant for the purpose of obtaining colonies far from the effects of natural enemies and chemical pesticides. The colony was constantly renewed every week. The two-spotted spider mites were reared on tomato leaf discs using the Leaf Disc method (Kondo and Takafuji, 1985) with minor modifications according to the method of Al Suwaidi (2003).

Preparation of different concentrations of Microcin and Chitosan

Microsine was isolated and purified from Citrobacter spp. according to the method of Piskunova (2017) cited by Jawad et al. (2021). Microcin was prepared at different concentrations, where 0.5, 1.5 or 2.5 mg/ml of prepared microcin was taken and dissolved in 100 ml of distilled water, and the mixture was placed on a magnetic stirrer for 30 minutes at 50-60°C.

Natural chitosan was prepared according to the method of Jawad (2022), where chitosan was isolated and purified from marine shrimp shells. Nano-chitosan was obtained from commercial sources. Different concentrations of chitosan were prepared, where 1 gm of nano-chitosan was taken and dissolved in 0.5 of oxalic acid in 100 ml of distilled water and the mixture was placed on a magnetic stirrer for 30 minutes at 50-60 °C for the purpose of dissolution. From the stock solution, concentrations 25, 50, and 100 mg/ml were prepared for use in subsequent experiments.

Effect of microcin and chitosan on the two-spotted spider mite *Tetranychus urticae*

Tomato leaf tablets were used and 2 adult females of *T. urticae* were placed in each replicate, left for 24 hours. After that, the adults were removed and 10 eggs were left on the surface of each leaf, as the excess eggs were removed using a soft brush (Alwan, 2015). Leaf tablets were sprayed with the previously prepared microcin at concentrations 0.5, 1.5, or 2.5 mg/ml, or chitosan at concentrations 25, 50, or 100 mg/ml using a hand sprayer (Lu et al. (2017). Each concentration was used for 3 replicates and the control treatment were sprayed distilled water for the microcin treatments or sprayed with oxalic acid in case of chitosan treatments. Then, the dishes were incubated at (2 ± 27) and a relative humidity of 5 ± 65%. The percentage of eggs hatching was recorded daily for 5 days using light microscope. As for the effect of the treatments on the adults and nymphs of spider mites, the method was carried out with the same steps of the hatching experiment using the adults of the red two-spotted mite *T. urticae*. A 10 adult females or nymphs on each leaf disk were used to be sprayed with microcin or chitosan at the same concentrations formerly used (Lu et al. (2017). Each treatment was replicated 3 times for each concentration. Dishes were incubated at 2 ± 27 °C and a relative humidity of 5 ± 65%. Data (mortality) were taken daily for five consecutive days of treatment.

Statistical analysis

JMP16 Pro® was used for analysis of varience (ANOVA) (SAS, Carrie, North Carolina, USA). The laboratory study was according to Factorial Experiments based on completely Randomized Design. The Tukey's Test HSD and the Least Signicant Difference (L.S.D.) at level 0.05 were used (Al-Rawi and Kahlaf-Allah, 2000) and the perishing percentage was corrected according to the Abbott Formula equation (, 1925 Abbott).

RESULTS AND DISCUSSION

The results showed that the Microcin had a significant effect in reducing the rate of eggs hatching of the two-spotted spider mites compared to the control treatment. Where, the lowest rate of hatching eggs was 18% when using microcin at concentration 2.5 mg/ml compared to the control treatment, which resulted in hatching of 48% (Figure 1).

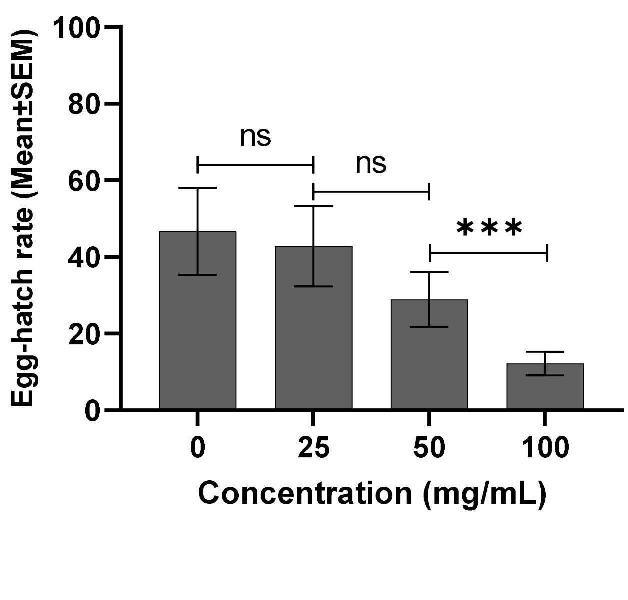

Figure.1 Effect of microcin at different concentrations (Mean ± SEM) on eggs hatching rate of the two-spotted spider mites *T. urticae* (P> 0.0001) **= significant, ***= highly significant, ns= non-significant

Campbell et al. (2016) reported that insect eggs are difficult to kill due to the unique structure of the eggshell consisting of multiple layers that have evolved to allow the embryo to breathe while at the same time limiting water loss. Eggshells have also been shown to be an excellent barrier to insecticides, fungal pathogens and some toxic fumigants. Some studies have shown that some insect eggs produce high numbers of enzymes to break down pesticides, however, it was found through the current study that microcin has a vital effect in increasing the death rate of eggs of the two-spotted spider mite T. urticae. The activity of microcin is attributed to inhibition of enzymatic functions and damage to the inner membrane (Duquesne et al., 2007). It was also reported that microcin affects cytochromes to inhibit cellular respiration or affects ATP (Baquero et al., 2019; Etayash et al., 2016; Simons et al., 2020).

It was also observed that microcin had a significant effect on the egg laying rate, as it was found that different concentrations of microcin had a different effect. Microcin at a concentration of 2.5 mg/ml led to the highest reduction in the rate of laying eggs to 28% in females of the two-spotted spider mite compared to the highest rate of laying eggs (48.4%) in the control treatment sprayed with distilled water (Fig. 2).

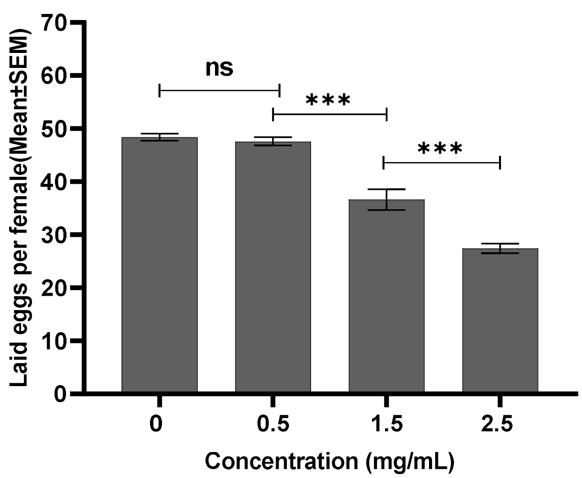

Figure2. Effect of microcin at different concentrations (Mean ± SEM) on eggs laying rate of the two-spotted spider mites *T. urticae* (P> 0.0001), **= significant, ***= highly significant, ns= non-significant

The difference in the rate of laying eggs for T. urticae females treated with microcin may be attributed to the effect of the latter on the growth of female ovaries when treated with microcin in the nymph stage. It is known that the nymph stage is not fully developed in terms of reproductive organs, especially the ovaries. The effect of Microcin may be indirect on the ovaries by affecting enzymatic functions and damage to the inner lining of the intestine (Duquesne et al., 2007). Thus, it affected the supply of the body of dreaming females with the necessary materials for the growth of various organs and body systems, including the reproductive system (ovaries). Duquesne et al. (2007) indicated that the chemical composition of microcin and its physical properties allow it to influence the vital functions within the cells of the organism. Among them is preventing the decomposition of the produced peptides, and thus the cell does not obtain the materials necessary to produce energy for the crisis to carry out its vital functions. Or inhibiting the process of cloning nucleic acids (DNA / RNA) and affecting the production of amino acids or the formation of chromosomes. The process of egg production and embryogenesis that takes place inside the ovaries requires many essential materials, including peptides, enzymes, proteins, amino acids, and lipids (Wheeler, 2009). The ovaries also need the necessary proteins to produce the eggshell or (chorion) necessary to protect the fetus from external factors (Klowden, 2009). The production of these materials may be greatly affected when treating insects or spiders with antibiotic compounds such as microcin.

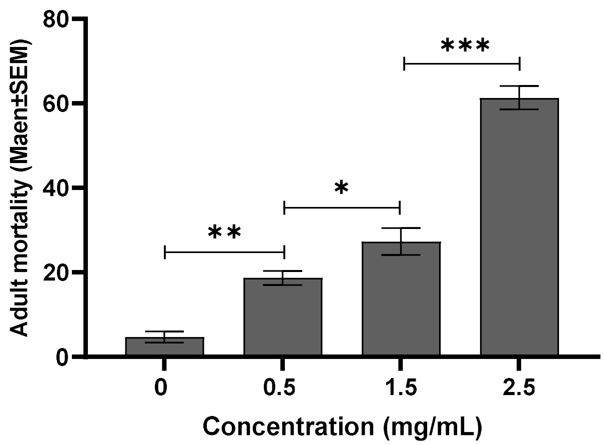

Figure3. Effect of microcin at different concentrations (Mean ± SEM) on adults mortality rate (%) of the two-spotted spider mites *T. urticae* (P> 0.0001), **= significant, ***= highly significant, ns= non-significant

The results also showed that Microcin showed a significant effect on the mortality rate of adults and nymphs of the two-spotted spider mite T. urticae (Figure 3). The highest mortality rate for the adult was 60% at a concentration of 2.5 mg/ml, compared to 10% mortality in the control. It was also found that the microcin had a significant effect on mites nymphs resulting in mortality of 90% at a concentration of 2.5 mg/ml while no deaths were recorded in the control treatment after the same period (Figure4).

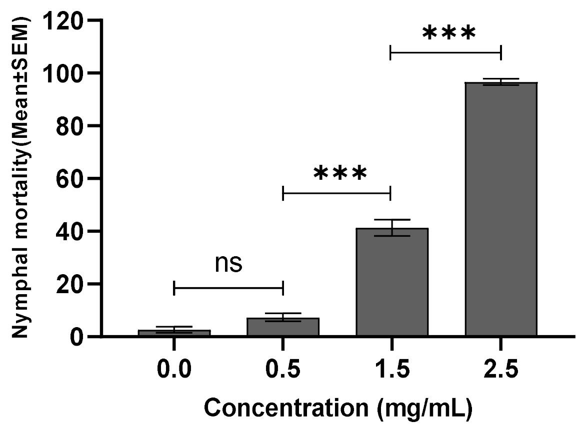

Figure4. Effect of microcin at different concentrations (Mean ± SEM) on nymph mortality rate (%) of the two-spotted spider mites *T. urticae* (P> 0.0001), **= significant, ***= highly significant, ns= non-significant

Effect of Nano-chitosan on the life stage of the two-spotted spider mites *T. urticae*

The results of the study showed that the chitosan nan-ocomposite had a significant effect on the rate of eggs hatching (Fig. 5), and on the rate of egg laying rate produced by the two-spotted spider mites (P>0.0001) T. urticae. Where it was found that the lowest rate of hatching was 18% and egg laying was 45% at in the treatment at concentration 100 mg/ml compared to the control treatment, which resulted in hatching rate of 45% (Figure 5), and 50% egg laying (Figure6).

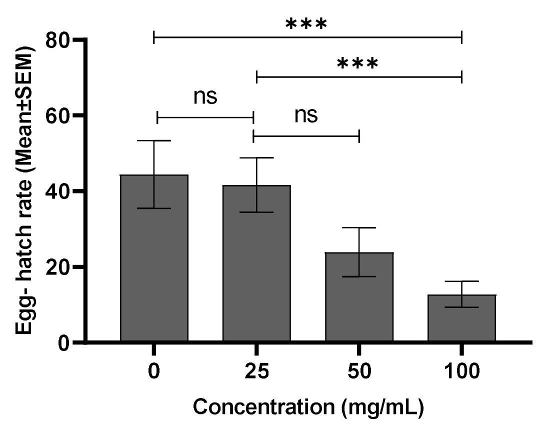

Figure5. Effect of Nano-chitosan at different concentrations (Mean ± SEM) on eggs hatching rate of the two-spotted spider mites *T. urticae* (P> 0.0001) **= significant, ***= highly significant, ns= non-significant

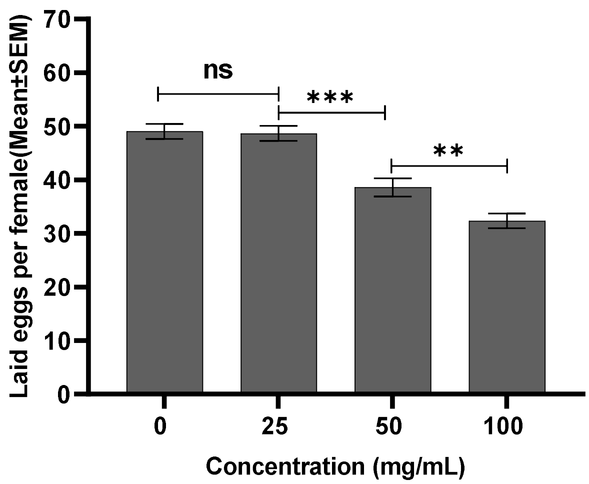

Figure5. Effect of Nano-chitosan at different concentrations (Mean ± SEM) on egg laying rate of the two-spotted spider mites *T. urticae* (P> 0.0001) **= significant, ***= highly significant, ns= non-significant

Eggs are undoubtedly the most difficult stage of insect life to control with insecticides. Regardless of the insecticide application method, the tough eggshell covering the egg prevents entry of many pesticides, including volatile and water-based pesticides, oils, and even some mechanical repellants (Campbell at al., 2016).

The respiratory system in insect eggs differs from the rest of the respiratory systems in other life stages of the insect. The embryo of the egg breathes through a small opening (aeropyles) on the outer shell of the egg, which allows gas exchange and reduces water loss during respiratory activities. The chitosan compound, when transformed into ammonia (NH_3) in an acidic medium (pH 6), turns into a toxic gas that penetrates the egg through aeropyles and thus leads to the death of the embryo. In addition to the chitosan compound in its gaseous form, it penetrates the egg and kills the embryo. Or in its emulsified form, it closes the aeropyles and thus leads to fetal suffocation, as happens when using oily insecticides (Smith et al., 1996).

Nano-chitosan showed a significant effect and increased the mortality rate of adults (Fig. 7) and nymphs (Fig. 8) of the two-spotted spider mite T. urticae (P>0.0001). The highest mortality rate for adults was 80% and nymphs 85% was recorded at a concentration of 100 mg / ml compared to the lowest mortality rate for adults and nymphs (10%) in the control treatment treated with oxalic acid.

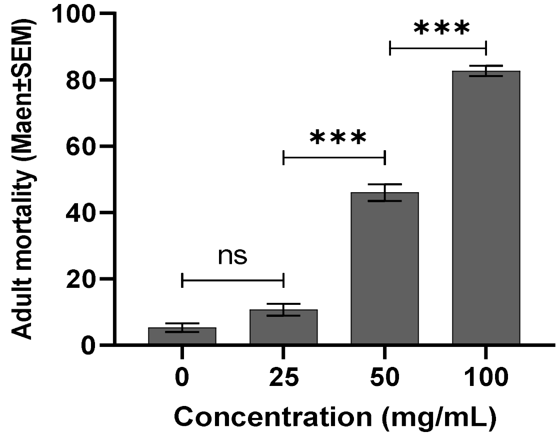

Figure7. Effect of Nano-chitosan at different concentrations (Mean ± SEM) adult mortality rate of the two-spotted spider mites *T. urticae* (P>0.0001), ***=highly significant, ns= non-significant

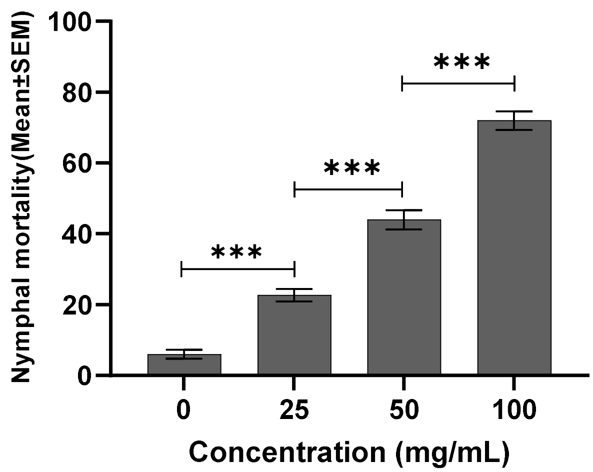

Figure8. Effect of Nano-chitosan at different concentrations (Mean ± SEM) nymph mortality rate of the two-spotted spider mites *T. urticae* (P> 0.0001) **= significant, ***= highly significant, ns= non-significant

Chitosan is a unique linear natural polymer that possesses reactive centers (-OH and NH2) which facilitate reactions and hydrogen bond formation. The amino group (NH2) in chitosan has an acid dissociation constant pKa of 6.5 (Dong et al. 2013). Thus, chitosan converts to a protonated form at pH (pH>6), forming ammonia (NH3+). The resulting cationic polymer may tend to bind negatively charged sites by binding to glutamate chloride ion channels (Glucls). This affects the permeability of the cell membrane, causing hyperpolarization. Thus, paralysis and death of the organism occurs. This is consistent with the results of (Ahmed et al., 2011) when studying the effect of chitosan on the larva of the horse fly G. intestinalis (Ahmed et al., 2011). Chitosan is a natural polymer complex and can be easily isolated and prepared. It comes from natural sources such as the exoskeleton of crustaceans, insects and fungal cell walls. It is also non-absorbable, relatively inexpensive and can be covalently linked to many compounds via its amino groups (Ahmed et al., 2011; Terbojevich and Muzzarelli, 2009).

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