

A SURVEY AND FIELD STUDY TO EVALUATE THE EFFICACY OF NANO-LOADED TOBACCO PLANT EXTRACT IN CONTROLLING DUBAS PALM BUG *OMMATISSUS LYBICUS* DE BERG

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Abstract

Survey field experiments were conducted to determine the number density of the different stages of the Dubas bug *Ommatissus lybicus* De.Berg, and other field experiments were conducted to evaluate the efficiency of some compounds in controlling the Dubas insect on palm trees. A field survey was conducted for the two generations of the insect, Autumn 2021 and Spring 2022, in Karbala Governorate, for date palm groves in the districts of Al-Husseiniyah, Al-Jedol Al-Gharbi, Al-Hindiyya and Al-Khairat. The results showed that the highest intensity of infection with the insect in the districts of Al-Hasaniya district was (15 ± 3 to 40 ± 2) nymph \ Khoussa, and the percentage of infection with the insect increased to (90 to 100%) for the same province compared to the rest of the provinces. The field results of egg treatment also showed that the effectiveness of the extract exceeded. The water of tobacco plant loaded on Nano-chitosan caused the highest mortality rate as it reached (71.8%) after (28) days at a concentration of (15) ppm, and that the same extract and at the same concentration caused the highest mortality rate of *O. lybicus* day when it reached (81.5%).

Keyword : Dubas bug; *Ommatissus lybicus*; Nano; Plant extract; Palm

Introduction

Dubas palm *Ommatissus lybicus* De. Berg is one of the most important pests that affect palm trees, causing great damage and losses in all areas of its cultivation (Ali, 2010), as the infection increases in orchards near rivers, in which palm trees are planted closely, and the insect infects all varieties of palm trees (Abdul Hussein et al., 1963). As the insect sucks the plant sap and secretes the honeydew that covers the green leaves (khus), so it looks bright. The insect causes indirect damage through the accumulation of dust and mold, which hinders the process of photosynthesis, respiration and transpiration in the palm trees, and leads to a general weakness in the infected palm trees (Al-Jubouri, 2000). Severe infection with this insect hinders the growth of date palms and decreases production. The infected trees give fruits of poor quality and the amount of sugar in them is low, and the fruits sometimes fall before reaching the stage of maturity. Its contamination and damage as a result of the adhesion of dust to it and the growth of mold on it, which results in a decrease in its marketing value or its unfitness for human consumption. The damage also extends to other planted under trees because of honeydew drops falling on these crops (Abd-Allah, 1998). Research and studies on date palms indicate that the first controls for this insect were adopted by the Iraqi Dates Authority in the years 1934 and 1935, where a mixture of nicotine powder, inflorescence, and ash was used (Al-Bakr, 1972), and then DDT, malathion, Diptrex, and Diazinon

were used by ground spraying. Al-Jubouri, 2000), while (Guglielmino, 1997) indicated the lethal effect of nicotine sulfate and mineral oil on all stages of the insect, and the same source also confirmed that the use of dimethoate gave encouraging results in the orchards of Baghdad. And DDT, and DDVP was a very effective substance in combating dubas, so the Ministry of Agriculture decided to adopt the pesticide and it was used in air control in 1966 on a large scale and because of its high effectiveness. Airplanes relied on aerial spraying of Dubas pest, and several pesticides were used, including Malathion ULV, at a ratio of half a liter R per acre. In recent years, a new group has been added, such as Realdan, Dasis, Dursban, Sumex, Alisan, and others. Alsidual pesticide (Fenthwaite) was used for the first time in Iraq in 1999, and it also proved highly efficient in controlling insects. After years of using chemical pesticides and confirming their damage and side effects, the need was forced once again to return to natural products and use biotechnical insecticides, which began three decades ago and widely in developed countries. Effective extracts were obtained from different parts of plants, being efficient and non-toxic. (Wilson and 1992, Hamad, 2012) where flowers, leaves, fruits, roots and seeds of some plants containing toxic, lethal or repellent substances were used directly (Al-Quraishi, 1990, Saleh, 2009). Or by using vegetable powder or after extracting them with organic solvents and from these materials Nicotine, which was extracted from the leaves of the tobacco plant (*N.tabacum*), was discovered in 1748 (Al-Qusayr, 2010, Hashem, 2014), which is considered the main active substance in the tobacco plant. Also, alkaloids were used to inhibit the enzyme choline esterase through their effect on insect neurotransmitters, as these compounds directly interfere and are associated with nerve tissues in the nervous system (Wink, 2000 and Mukherjee, 2007). A study by Al-Rawi and Al-Hamidawi (2003) proved that 0.2% nicotine sulfate with 1% mineral oil is a very effective insecticide against all stages of the Dubas palm insect, *O. lybicus* De Berg.

Recently, attention has turned to the use of nanomaterials in controlling insect pests, as the production of nanocomposites has increased in the form of commercial preparations such as Nano silver oxide, Nano zinc, Nano silica oxide, and others. Or repellents or cause a decrease in fertility (Owolade et al., 2008) and based on the foregoing, the study aimed to use modern nanotechnology with plant extracts in reducing the number density of the different roles of the Dubas insect *O.lybicus* in the field.

Materials and Methods

1- Survey study of the Dubas palm insect *O.lybicus*

A field survey of the insect and the fall and spring generations was carried out in the holy governorate of Karbala, which began in the first week of August 2021 until the end of June 2022, to determine the numerical density and severity of the insect infestation. Each orchard contains approximately (200-250) palm trees, with an age ranging from 10-12 years, with appropriate heights. From each district, 10 random palm trees were chosen, and from each palm 4 palm trees were chosen, and each palm tree was 10 palm trees, taking into account that it is taken in the form of an angle of 45% and from four directions (north). (East, South, West) and taking into account the selection of the same cultivar as much as possible. The trees were marked with colored spray.

The samples were examined by lowering the fronds quietly, then 10 strands were read from each direction on the basis of dividing the fronds into three regions close to the trunk, the middle and the periphery. The density was estimated The number of eggs, then the five stages of the nymph, by taking weekly readings by direct counting of individuals on wicker from the date of their emergence until the disappearance of the insect as a measure of the numerical density of the insect (samples were taken randomly consisting of 40 wicker per palm tree and from the third and fourth frond floors, and thus the total wicker on which the phases were examined is 400 wicker per week. The numerical density curve was drawn by determining the rate of individuals per 40 wicker, and temperature and relative humidity data for the study areas were adopted with a temperature measuring device Hygrothermograph, which was placed in the same study areas (Morris, 1960).

2_ Prepare the aqueous extract of the tobacco plant

The extraction was carried out by following the method of Al-Mansour (1995), based on (Harbone, 1973), with some changes being made by increasing the extraction period to 24 hours, as (10) grams of powdered tobacco leaves were taken and placed in a glass flask with a capacity of (500) ml containing (200) ml of distilled and sterile water, then the contents were mixed by means of a magnetic stirrer for (15) minutes, after that the mixture was left for (24) hours with the provisions of closing the flask well to prevent contamination. The solution was filtered by layers of tulle cloth and then filter paper to ensure that the solution was well filtered. Then the precipitate was discarded and the filtrate was taken and concentrated with a rotary evaporator device, and the filtrate was dried in an electric oven at a temperature of (40_45) degrees Celsius for the purpose of obtaining the dry raw material. (1) gm of the dry material was taken and dissolved in (1000) ml of sterile distilled water, thus the concentration of the solution became The result is 1000 ppm, from which the concentrations (15.10.5) ppm were prepared.

3_ Preparation of commercial nano-chitosan solution

Different concentrations of a commercial nano-chitosan solution were prepared by dissolving (1) gm in drops of acetic oxalate, well dissolved, then 250 ml of sterile distilled water was added to it, then the mixture was placed in a glass beaker, and the contents were mixed by means of a magnetic shaker for 15 minutes at a temperature of 45 degrees Celsius. In order to carry out the process of complete dissolution of the mixture, the solution was added to 1 liter by adding sterile distilled water, then the mixture was placed again on the magnetic vibrator for 30 minutes in order to homogenize the solution more to obtain a nano-chitosan solution with a concentration of (1000 ppm) from which the experimental concentrations (5,10, 15) ppm.

4- Preparation of tobacco plant extract nano-loaded on chitosan.

The compound consisting of tobacco plant extract loaded on nano-chitosan was prepared by taking an amount of commercial nano-chitosan in an amount of (5) gm, mixed with HCL (V/V) 5% in a volume of 100 ml, and the mixture was mixed on a magnetic shaker with continuous stirring for two hours at rpm. 300 Then it was mixed with 100 ml of a previously prepared aqueous tobacco extract solution, and the mixture remained on the thermomagnetic stirrer for three hours, at a temperature between 50-60 degrees Celsius, and the pH was adjusted. The solution is by adding a NaOH sodium hydroxide solution with a titer of 1N dropwise until PH = 9 is obtained, after that

it is washed with sterile distilled water for several times and then dried at a temperature of 45 degrees Celsius for 24 hours, to obtain a dry matter powder, then 0.25 g of the powder is taken and diluted in 250 ml of distilled and sterilized water to obtain a concentration of 1000 ppm, from which concentrations (5,10,15) ppm were prepared (Sumalla et.al, 2020).

5_ Testing the effect of different concentrations of aqueous extract of tobacco plants, a solution of chitosan nanoparticles, and an aqueous extract of tobacco plants loaded on chitosan nanoparticles on the destruction of eggs of the dubas insect on palm trees. lybicus 0 in the field.

The study was carried out in an orchard with an area of 10 dunums, located in District 10 of Al-Bashiya of the Western Table Agriculture Division, during the spring season of 2022. The area of the orchard is (5) dunums, the number of palm trees (250).) a palm tree randomly and marked with labels and spraying by colored spray. Experiments were carried out with 3 treatments, each treatment with three concentrations (5_10_15) ppm and each concentration with three replications, in addition to the comparison treatment with sterile distilled water and vacypermethrin at the concentration recommended by the Ministry of Agriculture 3 ml / 10 liters water, which was also carried out with three replications for each treatment. Three fronds were selected from each replicate and from the third and fourth frond stages in different directions. These fronds were marked with colored bands. The number density of eggs was estimated one day before spraying by direct counting of live eggs for every 10 tufts. From each frond by means of a field microscope, the average number of eggs per 30 tufts was extracted from each replicate. On the next day, the trees were sprayed with the above-mentioned treatments, just as the trees of the comparison treatment were sprayed with distilled water only. With the hand of vacypermethrin, by using a sprayer with a capacity of 16 liters and equipped with a motor that increases the pumping power to control the delivery of the substance to all possible points, the readings were recorded for the percentage of egg mortality in the field after (1_7_14_21_28), days and by the previous method, i.e. by direct counting of live eggs, then they were modified using the Henderson equation to determine perishable percentage.

6. The effect of different concentrations of aqueous extract of tobacco plants loaded with chitosan nanoparticles on the percentage of killing of nymphs of the Dubas palm insect O.lybicus in the field.

The experiment was carried out in the same orchard in which the eggs were prepared in the previous experiments in the spring season 2022. 15 palm trees were randomly selected and marked with labels and colored sprays at three concentrations (10-15-5) ppm, and each concentration had three replications, in addition to the comparison treatment with sterile distilled water. And the pesticide Facypermethrin, at the recommended concentration, the numerical density of nymphs was read 24 hours before spraying, at the rate of three fronds for each repeater and 10 tufts for each frond, and taking the rate of nymphs per 30 tufts for one repeater, which was read randomly and by the direct counting method, which is the method approved by the General Authority for Plant Protection to determine The numerical density of the insect, then the trees were treated on the next day with a 16-liter backpack sprinkler, spraying was done, and the readings were taken

after (1-3-5-7-14) days for the live nymphs and by the direct counting method to calculate the numerical density of the live nymphs.

7_ Statistical analysis

The field study experiments were carried out according to the factorial experiments and with a completely randomized design, and the data were analyzed using the GenStat statistical program, and the least significant difference (L.S.D) was used under the level (0.05) to indicate the significance of the results (Rawi and Khalaf, 2000), and the efficiency was evaluated. The materials used in the experiments are according to the Henderson equation (Henderson and Tilton, 1955).

Results and discussion

Field survey and calculation of the population density of the Dubas palm insect *O.lybicus*.

The results of the field survey of date palm in the Holy Karbala Governorate as figure 1. which are located within the areas of Al-Husayniyyah, Al-Jaddol Al-Gharbi, Al-Khayrat and Al-Hindiyah, showed that there were differences in the numerical density of the pest in these areas, as the severity of the infection in Al-Husayniyyah district ranged between severe to very severe, with a rate of $(15 \pm 3$ to $40 \pm 2)$ nymphs. \ Khoussa and in the western creek area, the severity of the infection ranged from severe to moderate $(20 \pm 2$ to $9 \pm 2)$ nymph \ Khoussa. The percentages ranged between 90-100% for the Al-Husayniyyah and (60-80) % in the Al-Jaddol Al-Gharbi, while the infection rate ranged between (40-65) % for each of the Al-Khayrat and Al-Hindiyah, the numerical density of palm trees and the high humidity as well as to the convergence and entanglement of fronds and the density of trees planted under palm trees in these areas, which are considered a suitable environment for insects, as well as poor service and agricultural operations (Abdul Hussein and others, 1963, Al-Jubouri, 2000).

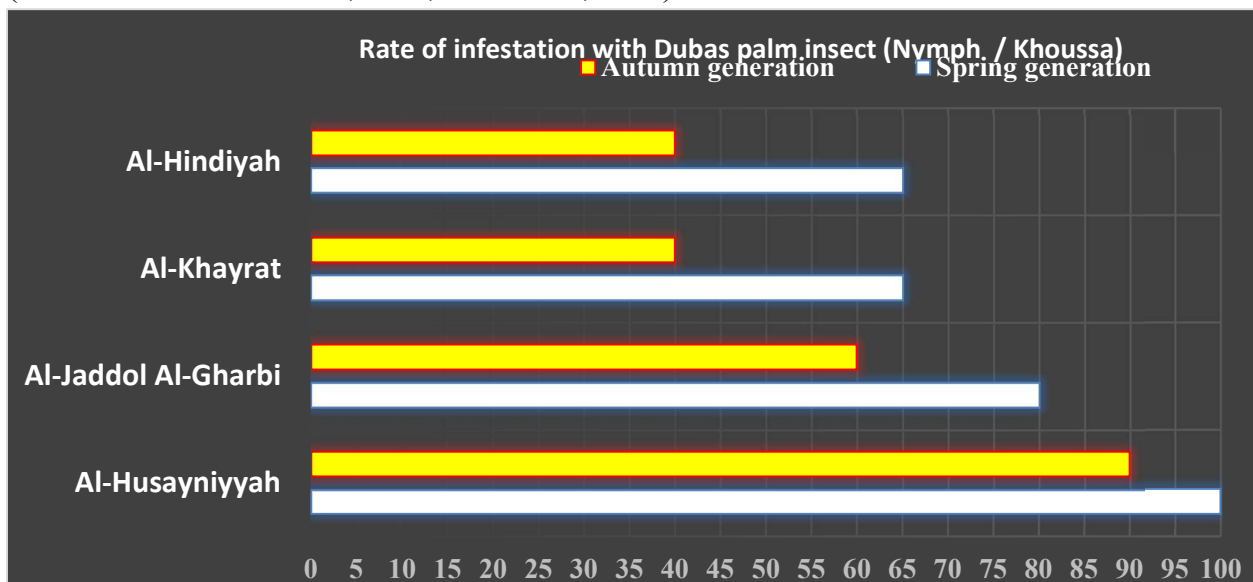


Figure (1) shows the percentage of infestation with the Dubas palm insect (nymph \ Khoussa) for the spring and autumn seasons

Examination of the effect of different concentrations of aqueous extract of tobacco plant, Nano-chitosan solution and Nano-loaded tobacco extract on chitosan in mortality rates of Dubas palm insect *O.lybicus* in the field.

Table No. (1) showed the effect of aqueous tobacco extract loaded on Nano-chitosan on the numerical density of eggs of the Dubas palm insect *O.lybicus*, as it gave the lowest rate of egg density at a concentration of 15 ppm, which amounted to (5.5) eggs / Khoussa, which was significantly superior to the aqueous tobacco extract. And a solution of nano-chitosan at the same concentration, as the numerical density of the eggs averaged (7.2-7.5) eggs/Khoussa compared to the averages of the numerical density of eggs in the two control treatments of vasapermethrin and water, which amounted to (8.5-8.6) eggs/Khoussa.

As for the time periods, the lowest numerical density of eggs appeared on day (28), when it reached (5.98) eggs/ Khoussa, compared to the time periods of days (1-7-14-21), when it reached (9.3-8.8-7.6-6.6) eggs/ Khoussa, respectively.

As for the level of interaction between the concentrations and the time periods shown in the table, the lowest rate of numerical density of eggs appeared in the treatment of aqueous tobacco extract loaded with Nano-chitosan at a concentration of (15) ppm, reaching (2.3) eggs/egg after (28) days, compared to the two control treatments, as The average number of eggs was (7.7-7.7) egg/ Khoussa.

Table (1) Effect of different concentrations of aqueous extract of tobacco plant, Nano-chitosan solution, and Nano-loaded tobacco extract on chitosan in mortality rates of eggs of Dubas palm insect *O.lybicus* in the field

	concentrations	Day						
		Before	1	7	14	21	28	Effect
Comparison	0	10	9.5	9.1	8.7	8.2	7.7	8.64
Watery tobacco	5	9.7	9.1	8.5	8	7.4	6.8	7.9
	10	9.8	9	8.2	7.5	6.8	6.2	7.5
	15	10	9	8.1	7.1	6.3	5.8	7.2
Nano chitosan	5	10.2	9.6	9	8.6	8	7.1	8.4
	10	10.1	9.4	8.8	7.8	7	6.3	7.9
	15	9.8	9.1	8.5	7.3	6.6	6.1	7.5
nano tobacco	5	10	9.4	8.6	8	7	6	7.7
	10	9.6	8.8	7.6	5.9	4.9	3.8	6.1
	15	10.6	9.7	7.6	4.7	3.3	2.3	5.5
exterminator	Recommended	10.1	9.4	9	8.5	8.1	7.7	8.5
days effect		9.9	9.3	8.8	7.6	6.69	5.98	

	Treatments LSD=0.3233	day LSD=0.2388
L.S. D	Interfere LSD=0.7988	

Where the highest death rates for eggs were recorded at concentration (15) ppm, reaching (71.8)% after (28) days, compared to all treatments and the two control treatments with water and alpha pesticide, which amounted to (0_0.9)%, respectively as showed in figure 2 .

The average of the numerical density of eggs

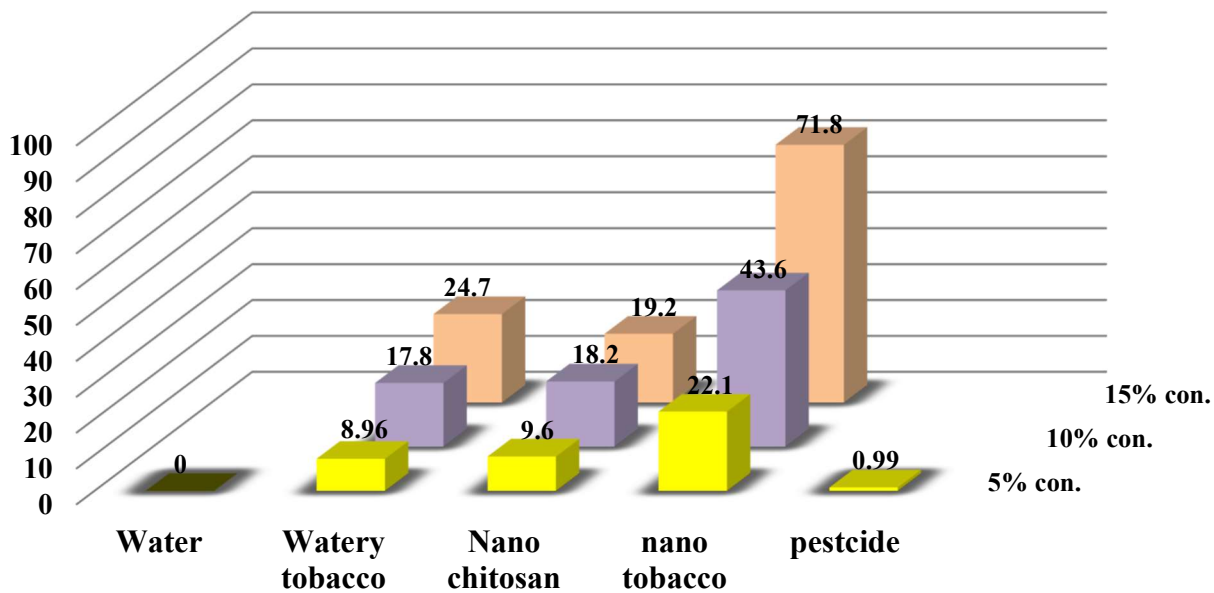


Figure (2) shows the efficiency of the aqueous extract of tobacco plant loaded with Nano-chitosan on the destruction of eggs

We conclude from the results of the above table that the plant extract loaded on the Nano is likely to be effective in destroying eggs, as it increased the encapsulation of the egg shell and prevented it from breathing completely, or increased the entry of toxic compounds into the egg and prevented the formation of the embryo, as the Nano carrier system enables the encapsulation of the component through ionic or covalent bonds between molecules or placing them in a polymeric matrix of chitosan to improve the effectiveness of the Nano conducting material (kashyap, 2016). Or the effect is due to the entry of the extract into the egg, which leads to the failure of the growth and development of the embryo and thus the loss of the ability to breathe (Roxten, 1991), and the destruction of the eggs is also attributed to the entry of toxic substances in the extracts from the hilum opening and the occurrence of a biological disturbance inside the egg, which leads to non-existence of The larvae or nymphs were able to tear the egg membrane and exit naturally, so they died (Batish, 2008).

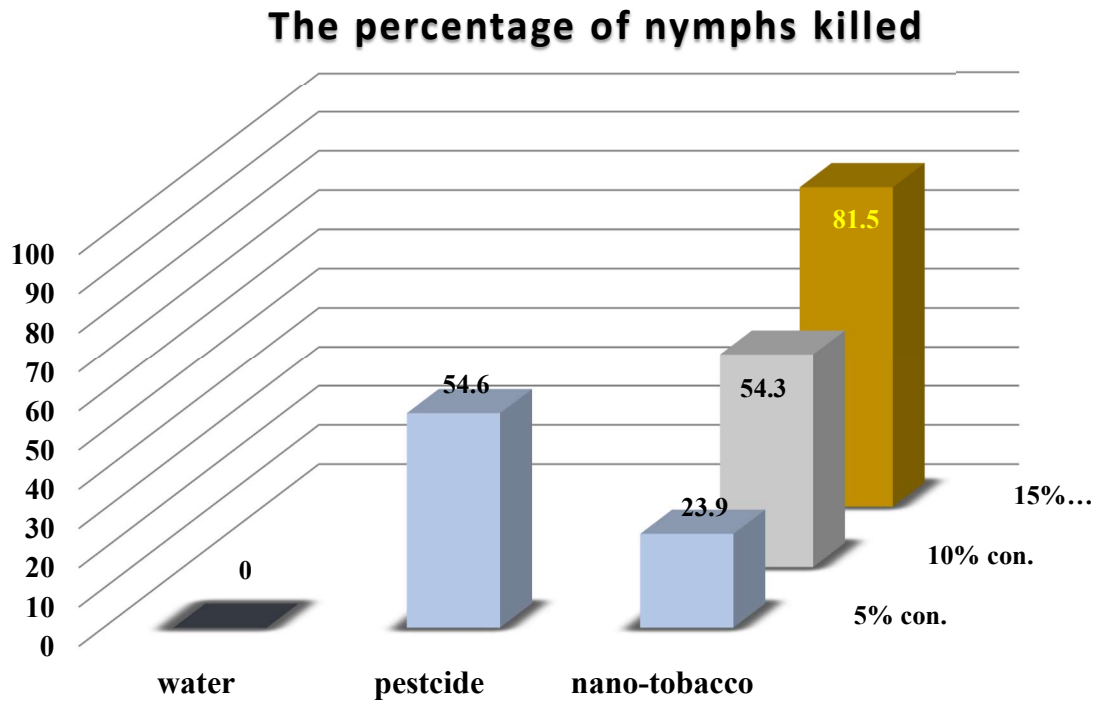
Testing the effect of different concentrations of aqueous extract of tobacco plants loaded with Nano-chitosan on the percentage of death of different nymphal stages of *O.lybicus dubas* in the field.

It was shown in Table No. (2) that the tobacco extract loaded with nanoparticles caused the death of nymphs at a concentration of (15) ppm, when the rate reached (5.1) nymphs/female, which was significantly superior to the concentration (5.10) ppm, reaching (8.6-11.7). nymphs/khoussa, respectively, with high significant differences, as well as the two control treatments with vacypermethrin and water only, which amounted to (8.3-14) nymphs/3 yews, respectively. As for the effect of the time period, the lowest numerical density of nymphs appeared on day (14), as the numerical density of nymphs averaged (7.5) nymphs/female, compared to the time periods after (1,3,5,7) days, when it reached (8.5-9). 3-10.4-11.9) nymphs \ khoussa respectively. As for the level of interaction between the nano-loaded tobacco extract and the time period shown in the table, the lowest mean of the numerical density of nymphs appeared at the concentration (15) ppm, when it reached (2.3) nymphs / nymphs after 14 days, as it was significantly superior compared to the two control treatments that gave (6-13.4) nymphs/khoussa, respectively, after 14 days of treatment.

Table (2) Effect of different concentrations of aqueous extract of tobacco plants loaded with nano-chitosan on the mortality rates of different nymphal stages of *Dubas* insect *O.lybicus* in the field

The average number of nymphs								
Materials	concentration	Day						Treatment Effect
		قبل	1	3	5	7	14	
Comparison	0	15.1	14.6	14.3	14.1	13.7	13.4	14
nano tobacco	5	14.8	13.4	12.7	11.5	11.1	10	11.7
	10	14.8	12	9.7	8.5	7	6	8.6
	15	14	8.9	6.3	4.5	3.6	2.3	5.1
Pesticide	Recommend	14.9	10.7	9.4	8.2	7.2	6	8.3
Effect day		14.7	11.9	10.4	9.3	8.5	7.5	
Treatment LSD 0.962		Day LSD 1.054			L.S.D 2.357			

Figure (3) shows that the aqueous extract of nano-loaded tobacco achieved the highest percentage of killing *Dubas* insect nymphs at a concentration of (15) ppm, reaching (81.5)% after (14) days of treatment, followed by alpha pesticide at the recommended concentration of (54.6)%. Then the nano-tobacco extract at a concentration of (10) ppm, which reached (54.3)% after (14) days, compared to the control treatment with water, which amounted to 0%.



The results of the current study indicate the superiority of Nano-loaded tobacco extract over chitosan in nymph mortality rates, and this is consistent with what was found by (Vanderghyest et al., 2007) and (Kitherian, 2017) that Nano-extracts have properties that enable them to penetrate into the bodies of target organisms, causing killing. As well as increasing the ability of these materials to withstand inappropriate environmental conditions. (Lee et al., 1992) indicate that chitosan has a positive ionic charge (the amino group) that has the ability to chemically bind to many compounds, due to the role of the amino group and its association with fats, mineral ions, and proteins present in the insect and its food, and converting them into compounds that interfere with physiological activity or Chemical related to the growth, development and reproduction of the insect, which leads to many changes. The reason for the death of nymphs treated with Nano-loaded extracts is also attributed to the fact that the nanoparticles work to damage the protective wax cover that envelops the insect's cuticle, which leads to water loss and then dehydration and death of insects (Stadler et al., 2012).

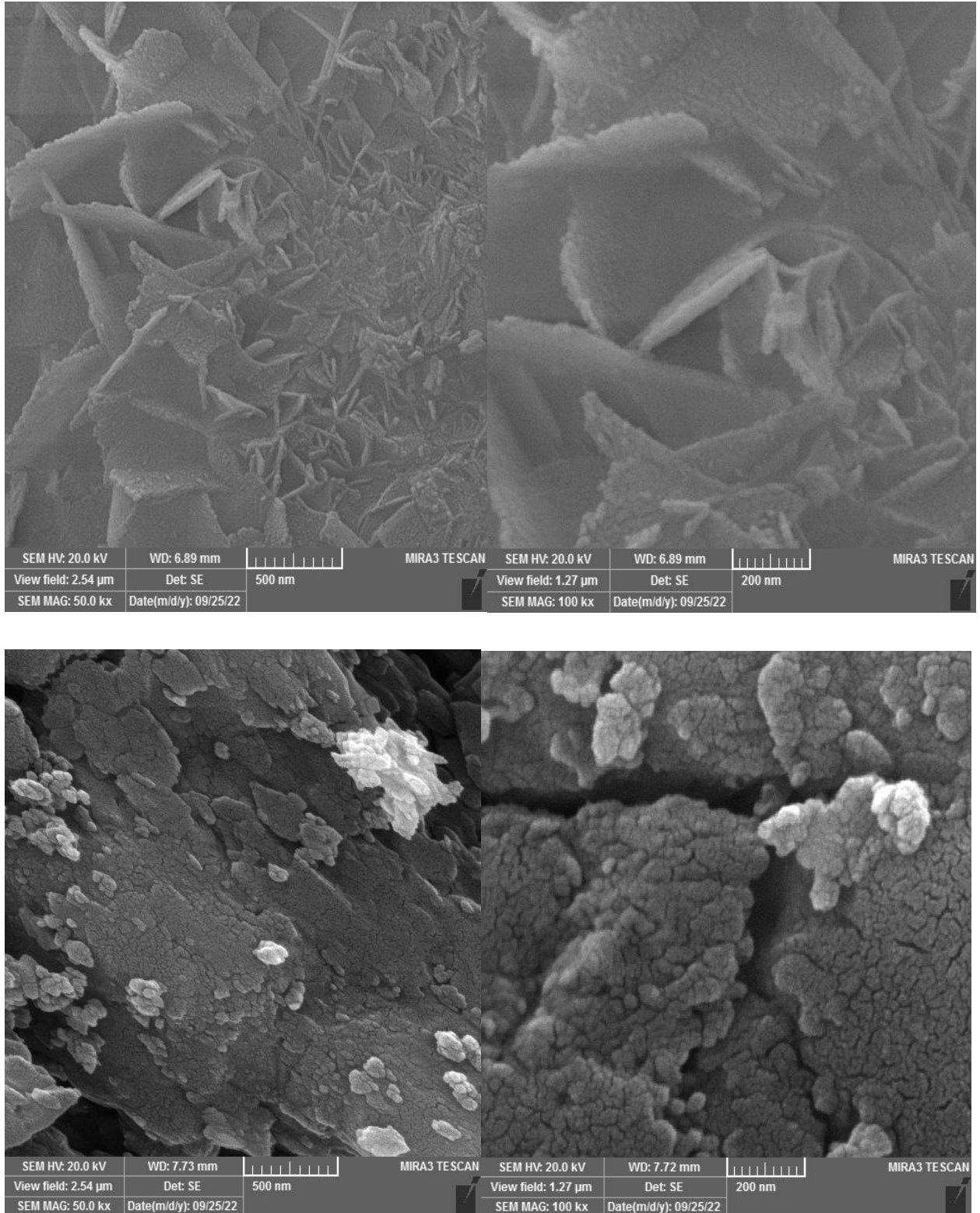


Figure (4) images of nanocomposites under magnification powers of 200 nm and 500 nm using scanning electron microscopy (SEM).

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