Laboratory Study to evaluate the efficiency of Nano-loaded tobacco aqueous extract on the different roles of *Ommatissus lybicus* De Berg

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Abstract

A laboratory study is conducted to evaluate the efficiency of the aqueous extract of nano-chitosan loaded tobacco plants and laboratory-made. When *O. lybicus* eggs were treated with an aqueous extract of nano- chitosan loaded tobacco plants at two concentrations (10-15) ppm, recorded the highest mortality rates reached (21.2-37) %, respectively. Furthermore, the highest egg mortality rates reached (47.8_66.6) % after (14_21) days. The same concentrations above also affected the mortality rates of first-instar nymphs, which amounted to (30.3_53.3%), respectively. The concentration (15) ppm had the highest significant differences at mortality rate reaching (58.3_70.0) % after (5_7) days. The mortality rates in third-instar nymphs reached (28.9-37.5%) at the concentration of (15) ppm, the highest mortality rates reached (39.9_44.6_40.3) % after (3_5_7) days. The mortality rates in the fifth instar nymphs amounted to (22.6_29.1) %, respectively, as they were the highest mortality rates at a concentration of (15) ppm after (5) days, reaching (39.1) %.

Keyword: Nano-loaded tobacco, *Ommatissus lybicus* De Berg

Introduction

Dubas Bug, *Ommatissus lybicus* de berg, is one of the most important pests that affect date palm trees in all farmlands in Iraq and causes significant economic losses in many countries widely known for growing date palm trees, such as Iraq country (18). It affects all palm tree varieties, especially in provinces situated in the middle of Iraq. The infection is aggravated by plant sap-sucking insects (nymphs and fully grown insects) feeding from green parts of the palm in April, May and June for the spring generation and the months of August, September, October and November for the autumn generation. In addition to honeydew secretion, which leads to reducing leaves' efficiency to perform vital activities and reduce photosynthesis, causes yellowing of these parts, as well as soil and dust accumulation on plant parts, spreading infection with pathogens such as black mold, fungus *Aspergillus niger* (1,6 and 15). In Iraq, the concerned authorities spend enormous sums of money annually within nationwide campaigns to control this pest using aerial and ground spray equipment, and chemical pesticides are the only widely used method in controlling this pest (5). Due to the adverse effects of these pesticides and the resulting disturbances in the natural balance due to targeting natural enemies of insect pests, biological control agents, as well as their impact on the environment and public health. Therefore, it has become essential to find alternative and effective pesticides to reduce the use of chemical pesticides in control due to their adverse effects on the environment public health and the potential of developing pest resistance to those pesticides as a result of repeated use (10). These defensive organic compounds belong to different chemical groups, including phenols, terpenes, alkaloids, glycosides, acetylene complex... etc., which have an anti-effect on varieties of insect pests and many plant extracts used as biopesticides (29).That includes nicotine, as the main active ingredient in tobacco, as well as rotenone and pyrethrum, one of the oldest pesticides of plant origin and the most used since the nineteenth century. They lead to kill and expel insects and inhibit egg-laying, larval development or mating(31 and 4). Moreover, Vandergheynst *et al*. (40) and Adler *et al*.(2) also pointed out that secondary metabolites in plant–based extract function in different mechanisms to affect insect pest cycle-life by being antifeedant, affecting digestion, antimetabolite, acting as toxic substances to insect tissues, affecting chitin biosynthesis and inhibiting larval development and molting.(23) indicated that the tobacco plant, Nicotiana tabacum, contains nicotine compounds used as insecticides, which the plant uses as a defence mechanism against insects and kills them by penetrating the insect's body. Nanocomposites were also used as sexual pheromones carriers to control some pests, as well as studies, have shown that the processing of nanocapsule images from natural polymers caused control and slow flow, which increased the effectiveness at lower doses, as paraquat-loaded chitosan gave 90% flow within 24 hours with no effect on non-target organisms in soil (5). Other studies have shown that nanoformulation of chitosan combined with polyacrylic acid provides an excellent possibility in managing the attack of common pests such as cotton insect pests and bean leaf beetle (*Ceratoma trifurcata*) (6). Based on the above, the study aimed to evaluate the effect of some safe compounds of plant origin and loaded on nanocomposites and conventional chemical pesticides on egg mortality rates and first, third and fifth instar nymphs of *O. lybicus* under laboratory conditions to identify the effectiveness of these substances in inhibiting its population density and thus the possibility of using them as an alternative to traditional chemical pesticides in controlling this insect.

Materials and methods

1. Preparation of aqueous extract of tobacco plant.

   The extraction process was performed by following the method of Harborne, J. B(17), with some modifications being made by increasing the extraction period to 24 hours, as (10) grams of powdered tobacco leaves were taken and placed in a 500 ml glass flask containing 200 ml of sterile distilled water. The components were mixed using a magnetic stirrer for 15 minutes. After that, it was left for (24) hours, and the glass flask was sealed tightly to prevent contamination, then filtered twice using tulle (netting) fabric and filter paper to ensure thorough filtration. After removing the precipitate, the filtrate was taken, concentrated using a rotary evaporator, and dried up in an electric oven at a temperature of 40_45 °C to obtain the dry raw material. A gram of the dry material was taken and dissolved in (1000) ml of sterile distilled water. Thus the concentration of the resulting solution became ppm1000, from which the concentrations (15.10.5) ppm were prepared.
2. Prepare action of the commercial nano-chitosan solution.

   Different concentrations of a nano-chitosan solution were prepared by dissolving (1)g in drops of well-dissolved acetic oxalate, and then 250 ml of sterile distilled water was added. This combination was placed in a glass flask, and a magnetic stirrer was used to mix it for 15 minutes at a temperature of 45°C. To entirely dissolve the mixture, the solution was completed to 1 liter by adding sterile distilled water and then replaced in the magnetic stirrer device for 30 minutes to be well mixed and obtain a nano-chitosan solution at a concentration of (1000ppm).
3. Preparation of nano-loaded extract

   The compound consisting of nano-chitosan loaded tobacco plant extract was prepared by taking a (5) g of commercial nano-chitosan mixed with 5% of 100 mL HCl (V\V), and the entire mixture was mixed in a thermomagnetic stirrer at a constant speed of 3000 rpm for two hours. After that, it was mixed with 100 ml of the pre-prepared aqueous tobacco extract solution and remained in the thermomagnetic stirrer for three hours at a temperature between 50-60 °C and the pH solution was modified by adding (1N) NaOH drop by drop until PH=9 was obtained, then sterile distilled water used for several times and then dried for 24 hours at 45°C. To obtain the dry substance powder, 0.25 g of powder was taken and diluted in 250 ml of distilled and sterile water to obtain a concentration of 1000 ppm, from which the concentrations (of 5,10,15) ppm were prepared (39).

4. Effect of different concentrations of aqueous extract, nano-chitosan solution and nano-loaded extract on egg mortality of date palm tree insect, *O. lybicus*.

   The insect eggs were collected from date palm groves in Karbala province for the spring generation randomly by 10 wickers from each palm of the third and fourth front row and different sides and these samples were brought to the laboratory after being placed in nylon bags. After examining the samples, the wickers were isolated by 10 eggs per replicate on each wicker, with the remaining eggs eliminated. The wickers were inserted in a moist cotton swab with a height of 3 cm that was positioned at the bottom of cup to ensure that the wickers remained moist by (5) replicates per concentration of the plant extract, chitosan solution and nano-loaded extract, as well as two control treatments (Alpha pesticide and sterile distilled water). The replicates were sprayed with (5, 10 and 15) ppm concentrations prepared in advance for all materials by 1.5 ml per replicate.

5. Effect of different concentrations of aqueous extract of tobacco plant, nano-chitosan solution and nano-loaded extract on the mortality of *O. lybicus* (first instar nymph)

   In this experiment, nymphs from the same palm tree groves were gathered from infected date palm trees during the beginning of the first week of April for the spring season of 2022, when hatching began. The nymphs were isolated from the wickers directly at the ages of the first instar nymph and placed in Petri dishes by Sabbour *et al.*(35) nymphs for each replicate and five replicates for each concentration of pre-prepared materials and concentrations (15, 10 and 5) ppm with pieces of wet green wicker placed in each dish to feed these insects. In addition to the two control treatments, the nymphs were treated by spraying method at a rate of 1.5ml per replicate, and the dishes were closed with perforated lids for ventilation. Then, they were placed under laboratory conditions and temperature and humidity measurements by hygrothermograph and over the treatment period at a temperature ranging between (+30-3, 25±3) and humidity of 30-50% (32 and 28). The readings were taken after (1,3,5 and 7) days with recording mortality rate of first instar nymphs.

6. Effect of different concentrations of aqueous extract, nano-chitosan solution, and nano-loaded extract on the mortality of *O. lybicus* (third instar nymphs).The third instar nymphs were collected from infected date palm trees, as mentioned in paragraph 5 above and treated similarly with the same concentrations of materials previously prepared, taking into account the same laboratory conditions, and then the mortality rates of the third instar nymphs were recorded.

7. Effect of different concentrations of aqueous extract, nano-chitosan solution and nano-loaded extract on the mortality of *O. lybicus* (fifth instar nymphs). The fifth instar nymphs were collected from the infected date palm trees as mentioned in paragraph 5 above, and treated similarly with the same concentrations of materials previously prepared, taking into account the same laboratory conditions, and then the mortality rates of the fifth instar nymphs were recorded. The experiments were carried out according to the design of factorial trials for Completely Random Design CRB, and the results were analyzed statistically using the statistical program Genstat.12, the differences between the means (coefficients) were compared based on the least significant difference between them (LSD 0.05).

Results and discussion

1. The effect of different concentrations of aqueous tobacco plant extract, nano-chitosan solution, and nano-chitosan loaded tobacco plant extract in lab on the mortality rate of *Ommatissus lybicus*' eggs. Table (1) indicates that the two treatments of the aqueous tobacco plant extract loaded with nano-chitosan at a concentration of (10,15) ppm were significantly higher than the mortality rates of *O. lybicus* eggs over all treatments, reaching (21.2-37.0) %, respectively. When compared to the two control treatments (alpha-cypermethrin pesticide and sterile distilled water) amounting to (0_4.2) %, respectively. While there are no significant differences between the aqueous tobacco extract and nano-chitosan treatments at the same concentration (15) ppm, reaching (14.5_15.8) %, the treatments for other concentrations showed the least mortality rates. At the impact level of days, the rates recorded a highly significant difference in the mortality rates after (the 7-day, 14-day, and 21-day) rest of the rates, as it reached (13.7-19.8-18.9) %, respectively, compared to (1-28) days, which amounted to (2.55-9.26) %, and no significant differences were recorded among the rates after (14-21) days. At the level of interaction, it is observed that there is a highly significant difference in the treatment of aqueous tobacco plant extract loaded with nano-chitosan at a concentration of (15) ppm after (21)days , reaching (66.6) %, compared to the rest of the concentrations, days, and the two control treatments. Based upon table (1) results, it can be concluded that nano-loaded extract significantly lowered the rate of egg hatch inhibition, which increased eggshell encapsulation, prevented the eggs from breathing completely, or increased the entry of toxic compounds into the eggs, which inhibited embryo formation. Nanocarrier systems can encapsulate the component through ionic or covalent bonds between molecules or place them in a polymeric matrix of chitosan to develop the effectiveness of the material's nano conductivity (21). The mortality rate of eggs can also be attributed to the entry of toxic

substances, alkaloids (nicotine), into tobacco through the hilum or eggshell. Therefore, it leads to the mortality of embryo or incomplete Embryonic development, or some compounds disrupt the process of gas exchange inside eggs (13). Richardson (33) indicated that nicotine sulfate at a concentration of 0.07% was toxic to grain moth eggs. Moreover, Al-Zubaldi *et al*.(12) indicated the high effect of nicotine compound extracted from the industrial waste of cigarette industry plants in Iraq on egg-laying and mortality rates of lemon moth insect, as the highest rate of the mortality rate reached 88.98% at a concentration of 10% compared with (20.81) % in the control treatment . the study (11) also confirmed that the raw alkaloid extract of tobacco plant wastes led to mortality of eggs and *culex pippins* pupae, as the highest egg mortality rate was 94% at a concentration of 5 mg/ml compared to 1% in the control treatment, while the mortality rate of pupae was 98% compared to 1.6% in the control treatment. Furthermore Sabbour *et al*.(35) also indicated that nano-loaded extracts of camphor, rosemary, anise, and garlic tested on eggs and larvae of date moths proved highly effective against the insect.

Table 1. Mortality rate of *O. lybicus* eggs.

Materials	Concentrations	Number of days					Effect of the control
		1 day	7 days	14 days	21 days	28 days	
Control	0	0	0	0	0	0	0
Aqueous extract of tobacco plant	5	4	12.6	11.6	7.2	0	7.08
	10	2	16.4	19.2	8.5	2.8	9.78
	15	4	18.8	25.2	20.6	4	14.5
Chitosan solution	5	0	4	10.4	11.3	10.6	7.2
	10	0	8	19.3	18.5	12.2	11.6
	15	4	20.8	20.6	19.9	14	15.8
Nano-loaded tobacco	5	4	12.4	21.3	18.2	6.6	12.5
	10	4	22.8	29.8	37.6	11.6	21.2
	15	4	26.8	47.8	66.6	40	37
Pesticide	Recommended	2	8.2	11	0	0	4.24
Effect of days		2.55	13.7	19.7	18.9	9.26	
L.S.D Treatment	5.93	L.S.D	Days	4			
L.S.D Interaction	13.3						

2. Effect of different concentrations of aqueous tobacco plant extract, nano-chitosan solution, and aqueous tobacco plant extract loaded on nano-chitosan on the mortality rate of *Ommatissus lybicus* (first instar nymphs)

Table (2) shows that the two treatments of the aqueous tobacco plant extract loaded nano-chitosan at two concentrations (10,15) ppm were significantly higher in mortality rates of the first instar nymph over all treatments, amounting to (30.32-53.03) %, respectively. Followed by the alpha pesticide treatment at the recommended concentration, which reached (24.48) %, while the results showed that the two treatments of aqueous tobacco extract and nano-chitosan at a concentration of (15) ppm were significantly high over the rest of the concentrations of the same materials, amounting to (20.78_19.11) %, respectively, compared to the control treatment. While the effect level of days, the mortality rates after (3,5) days were significantly higher than the rest of the periods, amounting to (19.78-22.59) %, respectively, compared to the mortality rates after (1_7) days, when the mortality percentage was (13.75_16.14) %, respectively.

The interaction level between concentrations and days, the treatment of nano-loaded aqueous tobacco extract at a concentration of (15) ppm achieved the highest mortality rates for nymphs, reaching (70.0) % after (7) days, as it was significantly higher than all concentrations and the two control treatments. In general, the treatment of aqueous tobacco plant extract loaded with nano-chitosan at a concentration of (15) ppm was significantly higher than all other concentrations of aqueous tobacco extract and nano-chitosan, achieving the highest mortality rates compared to the two control treatments on all day

Table 2. Mortality rates of the first instar nymphs.

Materials	Concentrations	Days 1	3	5	7	Effect of the control
Control	0	0.00	0.00	0.00	0.00	0.00
Aqueous extract of tobacco plant	5	6.00	12.88	14.50	9.48	8.35
	10	16.00	19.16	18.20	12.32	15.71
	15	18.00	26.94	25.86	5.34	20.78
Chitosan solution	5	0.00	10.00	15.10	5.34	7.61
	10	10.00	20.10	24.72	12.32	16.79
	15	12.00	20.38	25.42	18.64	19.11
Nano-loaded tobacco	5	14.00	18.60	17.54	16.32	16.62
	10	26.00	34.96	37.00	23.32	30.32
	15	38.00	45.78	58.32	70.00	53.03
Pesticide	Recommended	24.00	25.56	29.36	18.98	24.48

Effect of days	13.75	19.78	22.59	16.14

L.S.D of days 3.81 l. s. d of Treatment 6.318

L.S.D of Interaction 12.636

3. The effect of different concentrations of aqueous tobacco extract and nano-chitosan, and aqueous tobacco extract loaded with nano-chitosan, on the mortality rates of *Ommatissus lybicus* (third instar nymphs)

Table (3) results indicate that the treatment of aqueous tobacco plant extract loaded with nano-chitosan was significantly higher in the mortality rates of third instar nymphs at a concentration of (15) ppm than all treatments, which amounted to (37.5) %. While the concentration (10) ppm for the same substance achieved a mortality rate of (28.9) %, followed by the alpha pesticide treatment, which amounted to (23.5%). The two treatments of aqueous tobacco extract and nano-chitosan did not achieve any significant differences between them at a concentration of (15) ppm, which amounted to (19.1_17.6%), respectively, compared to the control treatment. Concerning the days level effect, there were no significant differences between the mortality rates after (3-5) days, amounting to (19.3-20.2) %, respectively, which were significantly higher than the mortality rates after (1-7) days, which amounted to (12.9) %, respectively. However, the level of the interaction effect of the concentrations and days showed that the highest mortality rates concerning nymphs were at the concentration of (15) ppm, reaching (44) % after five day

Table 3. Mortality rates of the third instar nymphs

Materials	Concentrations	Number of days				Effect of the control
		1	3	5	7	
Control	0	0	0	0	0	0
Aqueous extract of tobacco plant	5	4	12.4	2.2	0	4.6
	10	16	19.5	17.5	0	13.2
	15	20	22.2	22.3	12	19.1
Chitosan solution	5	0	10	15.3	2.2	6.8
	10	6	14.8	22.2	12.8	13.9
	15	10	20.1	23	17.3	17.6
Nano-loaded tobacco	5	14	18.6	14	11.3	14.4
	10	24	28.8	33.9	28.8	28.9
	15	26	40.3	44	39.9	37.5

Pesticide	Recommended	22	25.7	27.9	18.6	23.5
Effect of days		12.9	19.3	20.2	12.9	
L. S. D	L.S.D of Treatment 4.172		L.S.D of days 2.516			
	L.S.D of Interaction 8.344					

4. The effect of different concentrations of aqueous tobacco plant extract, nano-chitosan solution, and aqueous tobacco plant extract loaded with nano-chitosan on the mortality rates of *Ommatissus lybicus* (fifth instar nymphs).

It was found (Table4) that the treatment of tobacco plant extract loaded with nano-chitosan at a concentration of (15) ppm was significantly higher than all treatments, reaching (29.1) %, followed by the treatment of concentration (of 10) ppm, and then the alpha pesticide treatment, which amounted to (22.6 _ 21.5). %, respectively, while the treatment of aqueous tobacco extract achieved a mortality rate of (17.5) % at the same concentration (15) ppm, while the nano-chitosan treatment achieved the least level in the same concentration compared to the control treatment in which the mortality rate was (0%). In the effect level of days for the same experiment, no significant differences were recorded after (3-5-7) days, in which mortality rates reached (14.7-16.5-14.8) %, respectively. Thus, it is significantly higher than the mortality rates after (1) day, which amounted to (9.09) %. At the interaction level, the on-centration treatment (15) ppm achieved the highest significant difference, after (5) days, in the mortality rates, as it amounted to (39.1) %. Generally, the treatment of nano-loaded tobacco extract at a concentration of (15) ppm was significantly higher than all treatments after (1-3-5-7) days compared to the control treatments.

Table 4. Shows the mortality rates of fifth-instar nymphs

Materials	Concentrations	Number of days				Effect of the control
		1	3	5	7	
Control	0	0	0	0	0	0
Aqueous	5	4	4	6.4	2.2	4.15
extract of	10	8	15.1	22.4	12.3	14.4
tobacco plant	15	14	18.3	19.9	17.9	17.5
Chitosan solution	5	0	6	10.6	9.1	6.4
	10	4	12.6	16.2	14.3	11.7
	15	4	14.4	19.3	18	9.15
Nano-loaded	5	12	15.8	16.3	13.9	14.5
tobacco	10	18	24.1	25.6	23	22.6

	15	20	27.6	39.1	29.9	29.1
Pesticide	Recommended	16	23.6	24.7	22	21.5
Effect of days		9.09	14.7	18.2	14.8	
L. S. D	L.S.D of Treatment	3.70	l. s. d of days	2.394		
	L.S.D of Interaction	7.940				

It is generally noted from the current study the clear effects of the aqueous extract of the nano-loaded aquatic tobacco plant in mortality rates of *Ommatissus lybicus* nymphs . The tobacco plant contains various chemicals that affect insects, including alkaloid compounds, the most significant of which is nicotine, which is present in high concentrations and affects an insect's nervous system both directly and indirectly, disrupting its ability to move and balance and ultimately causing its death. Their effect is attributed to entry of toxic compounds in the extract into insect's body through mouth and breathing holes and accessing into nervous system and nerve tissues leading to disorder or paralysis (19).

It was confirmed (3) that the alkaloid compounds to which nicotine belongs are neurotoxins and that the effect of the Nicotine compound as a toxic substance on Culex quinquefasciatus, house flies and *Tribolium castneum*. The study complies with (9) that tobacco plant extracts enhanced mortality rates in green peach aphids, and insect mortality rates increased as extract concentration increased.Khan and Saddiqi (23) pointed out that nicotine produced by *N. tabaccum* is used as a plant defence mechanism against insects and kills them by penetrating insect's body; this substance interacts with nerve ganglia of insect central nervous system to produce excitation in low concentrations creating paralysis cases.Koppad and Shivanna (25) indicated that, in high concentrations, nicotine functions to kill insect, since its composition is similar to acetylcholine synthesis because its molecular dimensions are similar to acetylcholine's molecular dimensions. Which is one of the basics in nerve signal transmission in synapse gaps. It combines with acetylcholine receptors at the point where nerves meet muscles, causing tremors followed by paralysis and death as a result of acetylcholine accumulation in nerve synapses . Metspalu *et al.* (17) also pointed out that the effect of tobacco extract on the first instar nymphs is more sensitive due to the low thickness of cuticle layer. The entry of the extract into larva body affects the efficiency of food conversion or prevention larvae from feeding, resulting in the mortality of large numbers of house 'flies' larvae. The present study's results also indicate the effectiveness of nano-loaded tobacco extract over the aqueous extract of tobacco and the nano-chitosan solution separately and in all concentrations, as indicated (40 and 24) that Nano-Extracts possess properties enabling them to penetrate bodies of the target organisms causing death, as well as increase these materials' ability to survive unfavourable environmental conditions. However, the reason behind the mortality of nymphs treated with nano-loaded extracts is also to the fact that nanoparticles function to damage the protective wax covering that envelops the insect's cuticle, which leads to water loss and then dehydration and mortality of insects(38). Moreover, the study is consistent with (26 and 14) findings when using nano-loaded oils of *Artemisia arborescent* in controlling aphids and whiteflies, as it was found to be resistant to degradation compared to ordinary compounds.

Measuring the Nanosizes of a nano-loaded tobacco plant extract using electron microscopy (SEM).

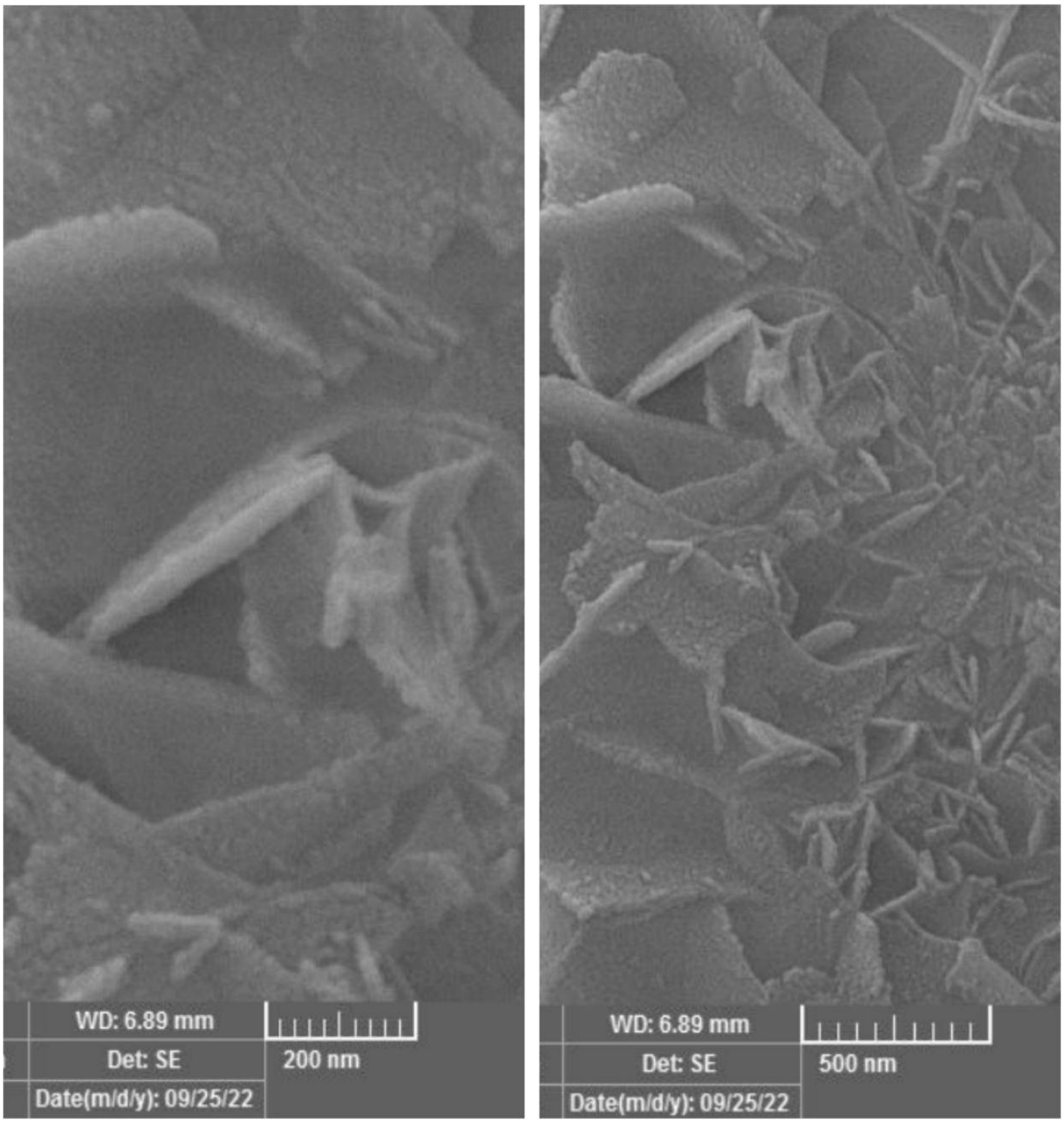

Figure 1. Showed the scanning electron microscopy (SEM) images (magnification at 100,200nm of the nano-loaded tobacco extract used in this study, the nanoparticles dimensions of the nano-chitosan loaded tobacco extract after a storage period of 6 months, and no change was observed in the nanosized under storage conditions.

Conclusion

Seemingly, it was observed during the study that the lab-treated instar nymphs have deformations and die rapidly after or during moulting without completing their life cycle. This is due to the plant extracts containing many compounds that interfere with the physiological processes in the insect during its development.

Conflict of interest

The authors have no conflict of interest.

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