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ARAŞTIRMA MAKALESİ **RESEARCH ARTICLE**

Carbon Nanotubes (CNTS) and Frankincense Nanoparticles as Promising Insecticides to Control Onion Thrips

Abdulla N. Ali[1*], Akram A. Mohammed[2], Sahar M. Jawad[3]

Abstract

Thrips tabaci Lindeman (Thysanoptera: Thripidae) is one of the most common and devastating onion pests which is capable of causing substantial harm to onion crops. Synthetic pesticides are mainly used to control onion thrips. *T. tabaci* requires alternative, low-impact control measures since there are numerous difficulties with utilizing chemical pesticides, including pesticide resistance. This study aimed to evaluate the effectiveness of the nanomaterial compounds on adults and nymphs of the *T. tabaci* in vivo and study their physiological changes caused by pesticides. The findings demonstrate that using nanomaterials, such as carbon nanotubes (CNTs) and frankincense nanoparticles (FNPs), significantly impacts the number of onion thrips. It also has the potential to lower the risk of pesticide resistance. According to the preliminary results, using carbon nanotubes (CNTs) considerably increased the mortality rate of adults and nymphs of *T. tabaci* and decreased egg-hatching success. Carbon nanotube (CNTs) and frankincense nanoparticles showed a high death rate in adult and nymphal stages at a concentration of 0.05 percent. Carbon nanotubes (CNTs) demonstrated exceptional mortality rates in adult and nymphal stages, with 90 and 50 percent at 5 mg/mL concentrations. Frankincense nanoparticles (FNPs) treatment demonstrated a high adult mortality rate of around 60 percent compared to the control treatment. Eggs of onion thrips showed different hatching success rates after treatment with CNTs and FNPs. The egg hatch rate did not exceed 40 percent of hatched eggs in the CNTs treatment compared to 90 percent in the control treatment. On the other hand, number of laid eggs per female did not differ significantly, indicating that none of the treatments affected the fecundity of the females. The ability of thrips to develop resistance to CNTs and frankincense compounds requires additional investigation. These natural products could be a suitable alternative to control destructive pests like onion thrips.

Keywords: Nanomaterials, Eco-friendly insecticides, Onion thrips**,** Frankincense, Carbon nanotubes (CNTs).

[1*] **Sorumlu Yazar/Corresponding Author:** Abdulla N. Ali, University of Kufa, Kufa, Najaf Governorate, Iraq. E-mail: abdullah.alqaseer@uokufa.ediu.iq OrcID: 0000-0002-5733-1789

[2] Akram A. Mohammed, University of Kufa, Kufa, Najaf Governorate, Iraq. E-mail: akrama.abodarb@uokufa.edu.iq OrcID: 0000-0001-7814-6112

[3] Sahar M. Jawad, University of Kufa, Kufa, Najaf Governorate, Iraq. E-mail: saharm.alkarawy@uokufa.edu.iq OrcID: 0000-0002-8130-6719.

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1. Introduction

Thrips tabaci (Thysanoptera: Thripidae) is a common pest of onion and other crops that can cause severe crop losses and economic impact owing to feeding on green leaf parts by the adults and nymphs (Ananthakrishnan, 1973; Lewis, 1997). This tiny insect destroys onion epidermal tissue (Chisholm and Lewis, 1984). They pierce surface tissues and absorb the contents of living cells (Koschier et al., 2002). When *T. tabaci* feeds on onions, it produces silvery leaf spots that evolve into white blotches, then silvery patches along with the leaves, which reduces the plant's ability to photosynthesize, lowering the weight of onion bulbs (Diaz-Montano et al., 2011). Onion thrips are most recognized for their ability to transmit numerous illnesses to onions (Gill et al., 2015). In warm and dry conditions, the population of onion thrips can multiply rapidly, leading to an outbreak (Rueda et al., 2007). *T. tabaci* has been observed to develop pesticide resistance, which might lead to control failures, agricultural losses, and environmental issues (Adesanya et al., 2020). Furthermore, recent research indicates that synthetic pesticides are being used more frequently, which has implications for public health, food safety, and the national economy (Lougraimzi et al., 2022). Consequently, discovering alternate materials to manage onion thrips is a crucial objective (Diaz-Montano et al., 2012). Alternative approaches to control pests are urgently needed because utilizing chemical pesticides can be extremely detrimental to the environment (Tayat and Özder, 2023). Especially in a warm location like Iraq, where the population of onion thrips might develop rapidly due to insufficient pest control and excessive chemical usage.

Plants and herbs contain potent compounds such as phenols, aldehydes, and alkaloids that have many therapeutic applications against various diseases caused by bacteria, molds, or viruses. There has been worldwide interest in their use (Al-Harrasi et al., 2018). The Boswellia tree is known for its fragrant resin, which is used to make frankincense. Frankincense resin (FR) is inexpensive, renewable, and non-toxic (Shi et al., 2012) and has many therapeutic uses. Various species of *Boswellia* that grow in tropical parts of Africa, Asia, and the Americas are used to make frankincense with varying properties (Al-Harrasi et al., 2018). *Boswellia sacra* is a good source of high-quality frankincense containing bioactive compounds with a variety of functions, including anti-cancer (Efferth and Oesch, 2020). Carbon nanotubes (CNTs) are nanomaterials made of rolled-up graphene sheets with a tube-like shape (Maruyama, 2021). In addition to their unique nanostructures, they have extraordinary characteristics; some are derived from graphite-like features, and others from their one-dimensional aspects. CNTs are used as nanofillers in polymer-based nanocomposites because they have great electrical, magnetic, and mechanical properties (Kaseem et al., 2017). Indeed, considerable research published in peer-reviewed journals demonstrates that adding CNTs to a polymer matrix reinforces mechanical qualities. Carbon-based nanoparticles have also been shown to have high antibacterial activity (Kang et al., 2008). Early research suggested that fullerenes, such as single-walled carbon nanotubes, have powerful microbicidal capabilities. These novel allotropic kinds of carbon were found in the previous two decades and have subsequently been utilized in various scientific applications (Cataldo and Da Ros, 2008). This paper’s contribution and novelty are as follows: seeking unique and novel substances that can reduce populations of onion thrips, aiming to keep them below the economic threshold, thereby obviating the necessity for chemical pesticides. This study also aims to determine the mechanism of action of these chemicals on *T. tabaci*’s physiological function. The use of these novel insecticides may also inhibit or slow the development of pesticide resistance in onion thrips.

2. Materials and Methods

2.1.Onion Thrips population

Thrips samples were collected from green onions in the desert region of Najaf Provence, Iraq, during the winter months of 2021 and 2022 (November–March). Infested green onion plants were grabbed and brought from onion farms to the laboratory using a plastic bag. Onion plants were examined for the presence of onion thrips. It was ensured that no pesticides had been sprayed on the plants two weeks before collecting onion plants. The populations were maintained using green onion seedlings prepared previously for this purpose at 25±1°C, and 70-80% R.H. Experiments were carried out under the same conditions in an incubator. The host plant used in the experiments was also green onion. Following the collection of onion thrips, all onion thrips assays were conducted immediately. Three replicates with ten adults and nymphs of onion thrips were applied for each concentration, and a control treatment consisted of just distilled water.

2.2. Synthesis of carbon nanotubes (CNTs)

The carbon nanotubes were acquired from the Chemical Science Department, Faculty of Science, University of Kufa laboratory and prepared as mentioned in Ordoez-Casanova et al. (2013) and Lafta et al. (2016). Non-volatile carbonaceous compounds (derived from date palm seeds) must be formed as a gas phase, which needs the utilization of energy sources or devices from the substrate. MWNTs with a purity of roughly 85% and a diameter ranging from 166 to 200 nm were employed as support in this study, which were generated by modified CVD on ceramic boats without the use of a catalyst. The first phase of this segment was treated and placed in the center of the tube furnace (XIN YOO electronic components Co. Ltd.), which is the best site for heat recovery for precipitation. Ceramic boats were used as a support. The prepared seed samples were placed in the combustion chamber with a complete connection to the rest of the reactor The nitrogen gas was purged to complete the removal of air from all reaction chamber systems prior to operating the furnace. The reaction temperature was reached once the furnace was turned on. The synthesis was carried out at 750° C under atmospheric pressure, with a normal reaction time of 30 minutes in a nitrogen environment and a flow rate of 100cm^3/min. The nitrogen gas flow was gradually lowered to 50cm^3/min when the furnace achieved the necessary temperature. A waste date palm sample was then added to the reaction by turning on the combustion heater and running it in batches The furnace was turned off and allowed to cool to ambient temperature under a continuous nitrogen flow after precipitation. The product was then collected for purification before going through the characterization process. The purification of the manufactured CNTs was done in two steps: The first phase was to heat the product for 4 hours in an oven, and the second was to oxidize the residual product 30 percent with H_2O_2 at 50˚C for 4 days 24. Scanning electron microscopy (SEM) and Powder X-Ray Diffraction (PXRD), (EDX), Raman Spectroscopy, and FTIR spectra were used to characterize the produced CNTs. (Kumar and Ando, 2010). All The chemicals used in the experiment, including zinc chloride ($ZnCl_2$), ethanol, and all other substances, were of analytical purity and came from Merck (Merck and Co., Inc.). All glassware was washed with sterile distilled water and dried in an oven before use.

2.3.Frankincense nanoparticle synthesis (FNPs)

The aqueous extract of Frankincense resin (FR) obtained from the tree of *Boswellia serrata* was prepared. (5 g) of Frankincense resin (FR) was placed in a 250 ml beaker, adding 100 ml of distilled water. The beaker was placed on a hot plate stirrer for 30-45 minutes at a temperature not exceeding 50 °C. The mixture was then filtered through a filter funnel and set aside for the second day. Frankincense nanoparticle was prepared in the laboratory of the Chemical Sciences Department, Faculty of Science, University of Kufa as mentioned in (Jamdagni et al., 2018).

Preparation ZnO nanoparticles

Zinc oxide synthesis was carried out by dissolving 6.05g in 200 ml of distilled water according to the molarity equation (Mirzaei and Darroudi, 2017).

$$M = \frac{Wt}{MWt \times V/1000}$$

Where (M) represents molar concentration, (Wt) represents the weight of the materials utilized, (Mwt) represents the molecular weight of the materials (g/mole), and (V) represents the volume of distilled water (mL).

Frankincense nanoparticle preparation

The purpose of adding nanoparticles is intended to achieve a high level of adhesion. To prepare frankincense nanoparticles, a mixture of 100 µL hydrochloric acid (to improve solubility), 50 mL of aqueous frankincense extract and 200 mL of 0.2 M zinc chloride were put into a beaker. The beaker was placed onto a hot plate stirrer for one hour at a temperature as little as 80ºC. A magnetic stirrer was used to dissolve the solution at 80°C for 2 hours. The light-yellow tinted precipitate that had formed was then allowed to settle for another 18 hours. Centrifugation at 10000 rpm for 25 minutes was used to remove the precipitate, which was followed by numerous rinses with distilled water to remove impurities and overnight drying in a hot air oven at 90°C. Crystallinity and other organic impurities are removed during the calcination process. The powder was calcined for 2 hours (Jamdagni et al., 2018).

2.4.Treating onion thrips

Individuals of onion thrips were treated by using (Kondo and Takafuji, 1985) disc Leaflet method, with a slight modification. Three discs made from the edges of the uppermost leaves of a tomato plant, each 4 cm in diameter, were placed in a Petri dish 9 cm in diameter and 1.5 cm high. A layer of sponge soaked in water and covered with filter paper was place in the Petri dish under the leave. The petri dish has a sponge layer soaked with water and covered with filter paper, leaving no opening between the edges of the tomato leaflet and the perforations. For replicates in which the disc's outer cover is held in place by pins on all sides. Surround each replicate with cotton and wiping the plastic cover from the outside with a layer of Vaseline to prevent the thrips from escaping. Perforating the dish's outer cover with pins for the purpose of injecting water with a (1 mL) medical syringe from time to time to maintain the leaflet for a longer period and preserving its vitality. Finally, numbering the replicate with a sharpie pen.

Carbon nanotubes (CNTS) and frankincense nanoparticles (FNTs) were applied to adult and nymphal instars, since these instars are active feeding stages, using a manual sprayer in differing proportions (0.05, 0.03, 0.01% and 5,3,1 mg/L, respectively). Distilled water was used as a control treatment in compared to carbon nanotubes, and zinc oxide (as synthesized above) was used as a control treatment in comparison to frankincense. Thrips exhibiting ataxia (active, apparently untidy movement), as well as thrips resting on their backs, legs up, or not moving, were considered dead at 24, 48, and 72 hours (Heinz-Castro et al., 2021). In general, three replicates with ten individuals were used for each treatment within a trial.

2.5.Egg hatching rate (%)

Onion thrips females were given 24 hours to deposit eggs. Following that, the thrips were removed. Eggs were sprayed with (0.05%) frankincense nanoparticles (FNTs) and (5mg/L) Carbon nanotubes (CNTS). Zinc oxide (as mentioned above) was used as a control treatment in comparison to frankincense nanoparticles (FNTs). Distilled water was used as a control treatment in compared to carbon nanotubes (CNTS). The eggs were checked every day over the first ten days under a dissecting microscope to determine the egg hatching rate (%). Hatched eggs were those with a hatching hole or that transformed into nymph, whereas non-hatched eggs were those without a hatching hole or did not develop into nymph. Three replicates, each with ten adults were used for each treatment.

2.6.Female fecundity

Female onion thrips were exposed tocarbon and frankincense nanoparticles (0.05 percent and 5mg/L, respectively), and their fertility was measured after that. Ten females were selected and placed in a Petri dish. All females were placed in Petri dishes under typical circumstances. Each Petri dish with an onion leaf disc represents as one replicate. The number of eggs laid in their lifetime each day was counted until the females dead. Onion leaf discs were changed as needed.

2.7.Statistical analyses

Analysis of variance (ANOVA) was conducted using JMP16 Pro® (SAS Institute, Cary, NC). Two-way ANOVA was used to compare nanomaterials activities on *T. tabaci* population and means were separated by Tukey's HSD test. One-way ANOVA was used for the single trial.Cumulative mortality was corrected for natural death in control using Abbott's formula (Abbott, 1925). An unpaired *t*-test was used to assess which nanomaterial treatment differed significantly from the control in egg hatching rate and female fecundity. A (0.05) significance level was used for all the analyses.

3. Results

The nanomaterial compounds used in this study had varying impacts on the mortality rate, fertility, and hatching rate of onion thrips (both adults and nymphs), as shown below.

3.1.Effect of CNTs in adult and nymphal stages

The overall results from the CNTs trials revealed a signifiacnt effect for CNTs treatments and sexposure time in adult and nyphal instars time (F (11, 22) = 50.14, P<0.0001 and (F (11, 22) = 38.6, P<0.0001), respectively. Theadult mortality rate varied significantlyamong CNTs treatments and the control. There was a significant main effect for treatment, F(3, 22) = 51.5, p < .01, and a significant interaction, F(6, 22) = 11.01, P<0.0001). The mortality rate of nymphal instar also differed significantly across treatments and time of exposure (F (3, 22) = 45.09, P<.0001and F (2, 22) = 94.7, P<0.0001., respectively). Compared to the control treatment, carbon nanotube (CNTs) showed a high death rate in adult and nymphal instars (76.7 and 83.2%), respectivly, (*Figure 1,3*) at concentrations of 0.05% after 72h of exposure.

3.2. *Effect of Frankincense nanoparticles (FNTs) in adult and nymphal stages.*

FNTs trials showed that there was a significant effect for FNTs treatments and exposure in adult and nymphal morilty rate of onoin thrips time (F (11, 22) = 9.8, P<0.0001and (F (11, 22) = 20.7, P<0.0001), respectively. Frankincense nanoparticles (FNPs) treatment at a concentration (5 mg/L) demonstrated a high nymphal mortality rate of around 43.3% (*Figure 2*). Additionally, the concentration (5 mg/L) showed the higher nymphal morility rate with about 565.8% (*Figure 4*). Frankincense nanoparticles (FNPs) also showed a significant impact on nymphal mortality, with around (60%) death (*Figure 4*).

3.3.Effect of CNTs and Frankincense nanoparticles on egg viability

The rate of egg hatching varied significantly between the CNTs and the controls (t= 9.192, df= 4, P = 0.0008). The control treatment had a 90 percent egg hatching rate, compared to around 40 percent of hatched eggs in the CNTs treatment (*Figure 5a*). In contrast, there was no significant difference in egg hatching rate between the frankincense nanoparticles and control treatments (t=1.789, df=4, p= 0.1481) (*Figure 5b*).

3.4.CNTs and frankincense nanoparticles effect on Female Fecundity

Fecundity of adult females in the CNTs treatment did not differ significantly from the control treatment (t=2.152, df=4, p=0.0978) (*Figure 6a*). Similarly, Fecundity of adult females in the FNPs treatment did not differ significantly from the control treatment (t=1.032, df=4, p= 0.3603) (*Figure 6b*).

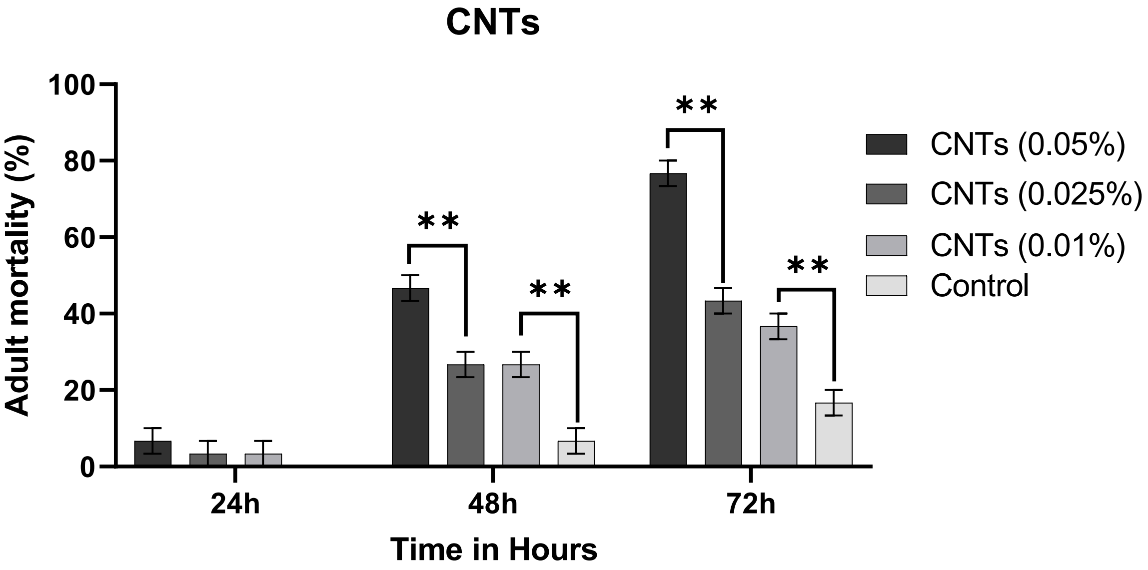

Figure (1). Onion thrips Thrips tabaci adult mortality rate (Mean ± SEM) after exposure to different concentrations of carbon nanotubes (CNTs) vs. the control (distilled water only) and exposure time in hours. P<0.0001, **= significant Bars without connectors were not significantly different.

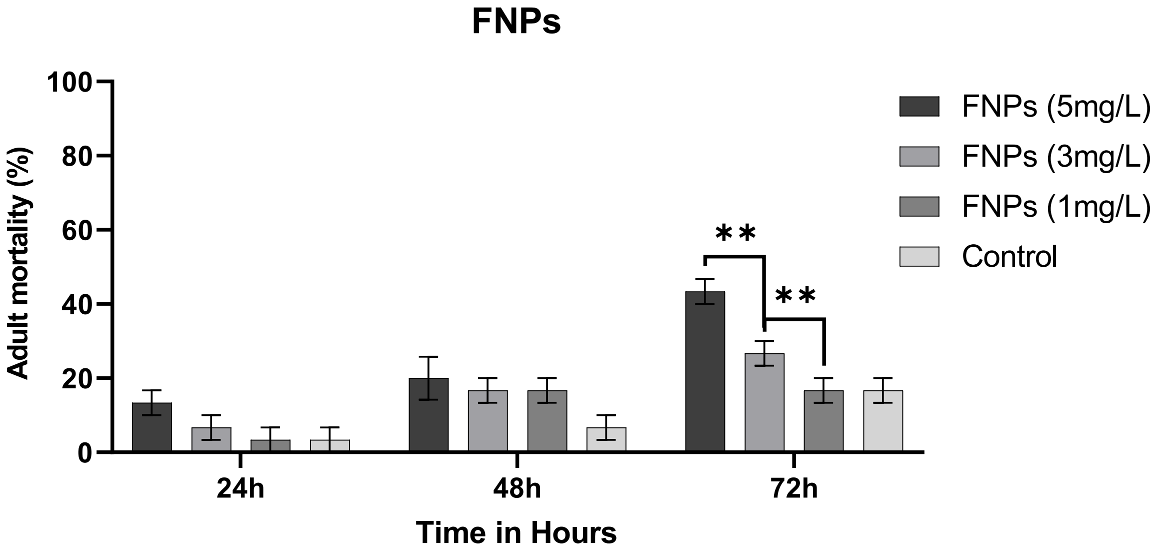

Figure (2). Onion thrips Thrips tabaci adult mortality rate (Mean ± SEM) after exposure to different concentrations of frankincense nanoparticles (FR) vs. the control (ZnO) and exposure time in hours. P<0.0001, **= significant. Bars without connectors were not significantly different.

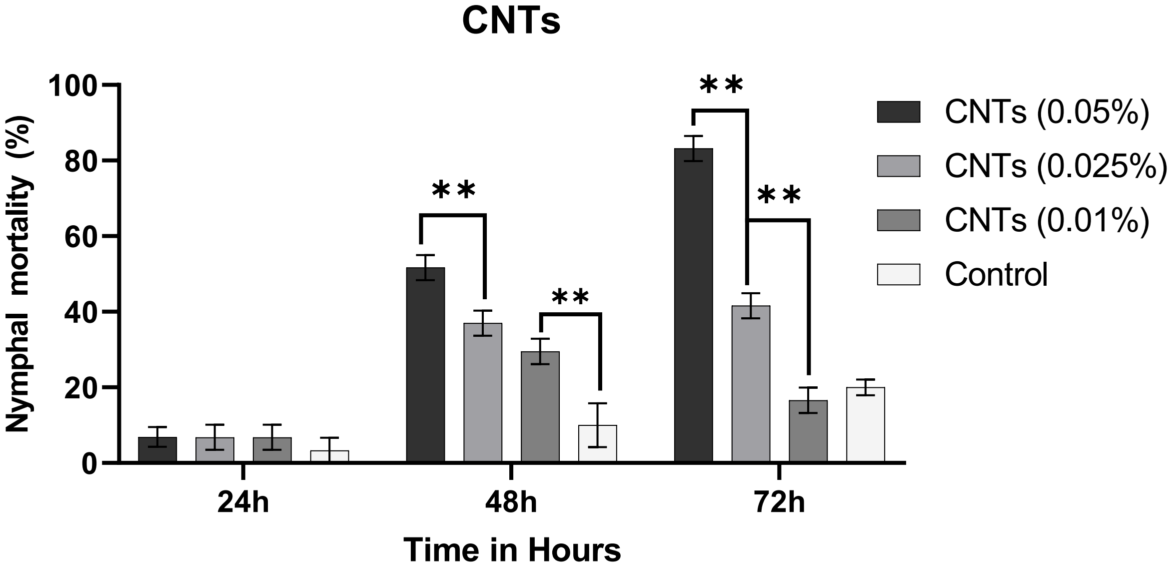

Figure (3). Onion thrips Thrips tabaci nymphal mortality rate (Mean ± SEM) after exposure to different concentrations of carbon nanotubes (CNTs) vs. the control (distilled water only) and exposure time in hours. P<0.0001, **= significant. Bars without connectors were not significantly different.

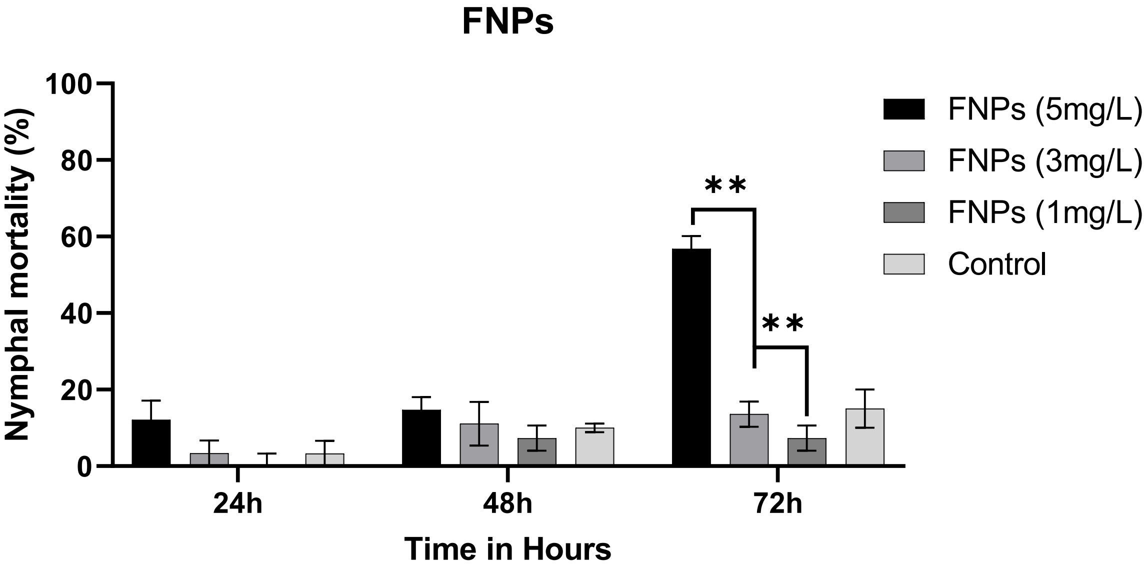

Figure (4). Onion thrips Thrips tabaci nymphal mortality rate (Mean ± SEM) after exposure to different frankincense nanoparticles (FR) concentrations. P<0.0001, **= significant. Bars without connectors were not significantly different.

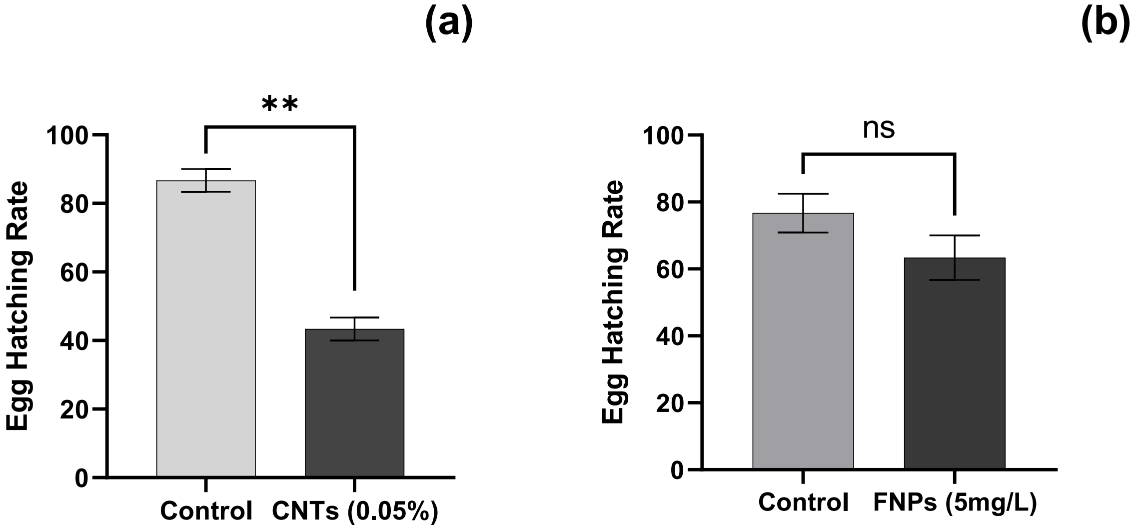

Figure (5). Onion thrips Thrips tabaci egg hatching rate (Mean ± SEM). (a) carbon nanotubes CNTs (0.05%) vs. the control (distilled water only). (b) frankincense nanoparticles FNPs (5mg/L)) vs the control. P<0.05.

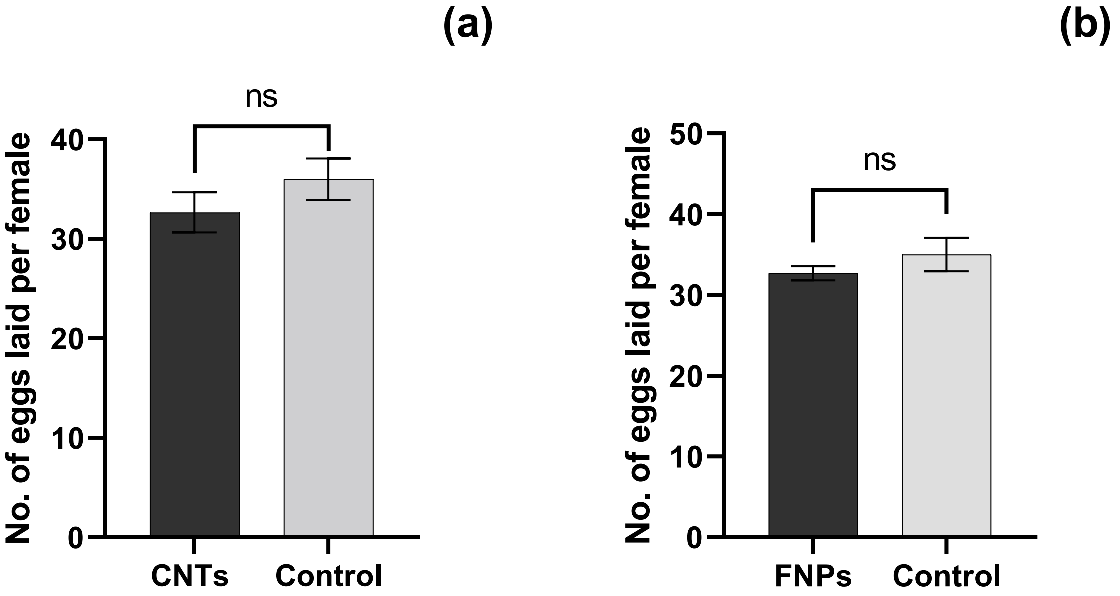

Figure (6). Mean(±SEM) number of eggs laid by Onion thrips Thrips tabaci female after surviving exposure to nanomaterials:(a) carbon nanotube (CNTs) and (b) frankincense nanoparticles (FNPs). $P \geq 0.05$,ns= non-significant.

4. Discussion

Few studies have examined the effectiveness of various synthetic nanoparticle compounds against insect pests. This study tested the effectiveness of two distinct nanoparticle compounds against *T. tabaci* onion thrips. This research found that carbon nanotubes and frankincense are effective nanomaterials against onion thrips. The findings of this study indicate that carbon nanotubes and frankincense are efficient compounds against onion thrips. CNTs with different structural and chemical compositions may have different effects on onion thrips (Martins et al., 2019). Carbon nanotubes (CNTs) were shown to have antibacterial properties against numerous *Escherichia coli* cells (Kang et al., 2008). However, little study has been conducted on the influence of carbon nanotubes on insect biological parameters, and most of it has been done in the lab. One of the first was research on Drosophila (Drosophilidae: Diptera) by (Liu et al., 2009).

Salamn et al. (2021) evaluated the antibacterial activity of crude aqueous extracts of *Boswellia sacra* bark against clinical isolates of periodontitis-causing bacteria in vitro (*Streptococcus orails*, *Gemella morbillorum*, and *Rothia dentocariosa*). They discovered that the concentration (250 mg/L) is more effective than the others. According to a review by Efferth and Oesch (2020), the phytochemical compounds in frankincense have shown promise in treating a wide range of conditions, including osteoarthritis, asthma, psoriasis, erythema, plaque-induced gingivitis, and pain.

The research says nothing about the manufacturing process of zinc oxide, and nothing about safety of any of the compounds tested. We hypothesize that nanomaterials have essential nutrients to the onion thrips (e.g. de Vries et al., 2008). From the results provided here, CNTs were found to have an effect on egg viability. In addition, CNTs and FNPs, at certain concentrations, had an effect on adult and nymphal mortality rates. This could be because CNTs harm the digestive system of adults and nymph or their metabolic efficiency.

Carbon nanomaterials (CNMs) were shown to negatively impact the reproductive, digestive, and metabolic efficiency of *Spodoptera frugiperda*, according to recent research (Martins et al., 2019). In this investigation, CNTs had no insecticidal action against female fecundity. CNMs were also not found to have an influence on female fertility in *Drosophila* larvae (Liu et al., 2009).

5. Conclusions

In conclusion, carbon nanotubes (CNTs) and frankincense nanoparticles (FNPs) exhibited potential eco-freindly insecticidal effects against onion thrips. The use of CNTs had the most significant effect on the mortality of adult and nymph of onion thrips, the fertility and hatching rate of eggs laid by females, and the overall population of onion thrips. More study is required to determine the biological mechanisms of these nanomaterials on onion thrips. This study might aid in the creation of efficient nanoparticle-based treatments.

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