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Enhanced Insecticidal Activity of Orizon and Oxymatrine by Silica Oxide Nanoparticles to control *Tuta absoluta* (Lepidoptera: Gelechiidae)

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ABSTRACT The tomato Leaf miner, *Tuta absoluta* (Lepidoptera: Gelechiidae), is one of the extreme agricultural pests. Nanoparticles have been developed as an alternative tool for pest management currently. In this study commercially silicon oxide nanoparticle (SiO-NPs) was used alone or with a Orizon or Oxymatrine nanocomposite were synthesized and we conducted laboratory and greenhouse experiments to evaluate their effect on 4th instar larvae of *Tuta absoluta* on tomato, *Solanum lycopersicum* L. In the laboratory experiment, three bioassay experiments with 4th instar larvae of *Tuta absoluta.* Firstly, applying SiO-NP at 25, 50 and 75 ppm caused 34, 38 and 49% mortality, respectively. Secondly, using SiO-NPs at 25, 50 and 75 ppm with Orizon at the recommended concentration nanocomposite caused 46, 55 and 66% mortality, respectively. Thirdly, using SiO-NPs at 25, 50 and 75 ppm with Oxymatrine at the recommended concentration nanocomposite caused 46, 55 and 66% mortality, respectively at 72 h after treatment. while the Orizon and Oxymatrine treatments gave larval mortalities of 34 and 36%, respectively. In the greenhouse experiment, the SiO- Oxymatrine treatment recorded the lowest numerical density of 4^{th} instar larvae, which was 0.91 larva/leaf, and the average larval density decreased as a result of the treatments SiO –Orizon, Oxymatrine, Orizon and SiO NPs to 0.96, 1.07, 1.15 and 1.25 larva/leaf, respectively, compared to the control treatment reached 4.31 larva/leaf at 14 day post-treatment. the results also showed highest density was recorded on the Omnia reaching 2.31 larva/leaf, while recorded Oula, Jeda and Kantor hybrids recorded less numerical density were 1.93, 1.52 and 0.79 larva/leaf, respectively These results suggest that the application of SiO-Oxymatrine and SiO –Orizon at 5 ppm can be used as an eco-friendly controlling strategy of *T. absoluta*.

Introduction

Tomato, *Solanum lycopersicum* L. (Solanaceae) is economically important crop, which is included in the daily food in many countries of the world because of the value of this crop especially for vitamins (Liu et al., 2022). The annual global production of tomato crop is assessed at 187 million tons on 5.05 million hectares (FAO 2020). The annual production of tomato in Iraq is assessed at 7.2 million tons from a total area of 31,892 hectares, approximately 3.95% of the total universal production in 2020 (FAO 2020). Tomato crop are exposed of many pests, the tomato Leaf miner, *Tuta absoluta* (Lepidoptera: Gelechiidae) is one of the primaries of these pests (Desneux et al. 2010). it is a serious pest that threatens economically important crop species like tomato, potato, eggplant and bean plants (Sylla et al. 2019). Suitable weather conditions of the insect's living, resulting in 10-12 generations in the year, it has a high fecundity, as the adult female lays 260 eggs during her life, its adaptation to the local environmental conditions of the countries it has entered (Biondi et al., 2018; Cocco et al., 2015), and the presence of alternative plants such as eggplant, furthermore, the larvae of this insect are the source of the infection, which is difficult to control by chemical because it is inside the tomato leaves (Abbes et al. 2012), all these factors involved in the rapid *spread of T. absoluta..*

This pest has dangerous behavior in attacking all parts of the vegetative system and its attacks on the fruits, causing them to rot and reduce their market value (Galdino et al. 2015). In the absence of control strategies, fruit damage can reach 100% (Karut et al. 2011; Polat et al. 2016). The damage on leaf is caused when the larvae feed on the mesophyll, expanding mines and affecting the photosynthetic capacity of the crop, which reduces the yield (Harbi et al. 2012). The repeated, incorrect and random use insecticide resistance by *T. absoluta* developed (Guedes et al. 2019).

Alternative method against *T. absoluta* are needed to avoid crop losses and insecticide resistance development. An emerging technological development uses materials with a physical size of 1 to 100 nanometers to enhance efficacy or reduce the environmental impact of a pesticide (Ahmedani et al. 2007; Barik et al. 2008). Nanoparticles enter insects through contact, ingestion and inhalation (Anandhi et al. 2020).The nanoparticles penetrate the exoskeleton of the insect, and the nanoparticles bind to the sulfur present in the protein or phosphorous present in the DNA inside the cells, which leads to cellular dysfunction and cell death. The nanoparticles also work to dry the insect's body by sticking to the wax layer in the upper cuticle layer and absorbing water, causing the insect's body to dry out (Benelli 2016). Both nanoscale silica oxide and nanoscale aluminum oxide work by the mechanism of adhesion (Rouhani et al., 2013).

The objectives of this study are devoted to investigating the efficacy of SiO-NP with Oxymatrine pesticide or with Orizon pesticide and SiO-NP alone against *T. absoluta* and valuating their enhanced insecticidal activity.

Material and method

Sources of insects and nanoparticles. A colony of *T. absoluta* was obtained from the affected tomato fields from Al-Haidariya sub-district near Najaf Governorate, Iraq, and. The colony was maintained for three generations in a greenhouse before laboratory and field experiments. The seeds of tomato (cv. "Kantor F1") and (cv. "Oula F1") were obtained from the Univert Agricultural Company; we also obtained the seeds of both (cv. "Omnia F1") and (cv. "Jeda F1") from the Debbana Agricultural Company, Najaf, Iraq.

SiO-NP powder was obtained from US Research Nanomaterials Inc. (Houston, TX, USA). Silicon Oxide nano powdered (0.1 g) was dissolved in 3 ml of 10% hydrochloric acid and put the mixture on the magnetic stirrer for 45 minutes at a temperature of 60 ° C for the purpose of dissolution, then complete the solution to 1 liter by adding distilled water and placing the mixture on a hotplate with magnetic stirrer device for 30 minutes (Rasim et al., 2021). The concentration of the prepared solution is 100 ppm. Solution 25, 50 and 75ppm were prepared by dilution 100ppm solution with deionized water.

Preparation of the nanocomposite silica oxide loaded on the pesticide Orizon and the pesticide Oxymatrine

SiO-NPs were loaded onto the pesticide by preparing 1000 ml of a solution of nanoparticles of SiO-NPs at a 100 ppm as a stock solution. The solution at 75ppm was prepared by adding 750 ml distilled water to 250 ml of 100ppm solution to prepare 1 liter at a concentration of 75 ppm. The mixture was placed in magnetic stirrer for 30 minutes at a temperature of 27 °C. 1.5 ml / liter of the pesticide Orizon was added to prepare the Nano composite SiO-NPs loaded Orizon. As for the pesticide Oxymatrine, 1.8 ml / liter was added to the solution of SiO-NPs, and then the mixture was placed in a magnetic stirrer for 30 minutes at a temperature of 27 °C, leaving the solution in the laboratory for one hour at a temperature of 27 °C, then the solution was placed in the water bath for one hour at a temperature of 50 °C, and the same operations were performed for 25,50 ppm concentrations (Jameel et al. 2020)

One sample of composite SiO- Orizon and SiO- Oxymatrine (100 ml) was collected from 100ppm solution and dried at 50°C for 3 d in an oven (UF260; Memmert, Büchenbach, Germany). Samples were sent to University of Kurdistan, Iran, for examination of the diameter of SiO –Orizon and SiO –Oxymatrine an under scanning electron microscope (SEM) (Tescan Mira3 SEM; Tescan, Fuveau, France)

Laboratory experiment: A bioassay test was prepared by placing layer of medical cotton on petri dishes with a diameter of 9 cm and a height of 1.5 cm and quantity of distilled water was added. a circular filter paper was placed on top on it. Fresh and healthy leaf of tomato plant was placed on top of the filter paper. Then the fourth instar larvae were transferred on the leaves. Each concentration was applied in five replicates each consisting of 10 larvae. the treatment was carried out by spraying SiO-NPs at concentrations of 25, 50 and 75ppm at a 1 ml/repeat using a manual spray (Mirza and Abdullah, 2018). As for the comparison treatment, it included two treatments, the first was only spraying the larvae with distilled water only, and the second spraying with distilled water mixed with 3 ml / liter of dilute hydrochloric acid HCL at a concentration of 10%. The dishes were placed in the incubator at a temperature of 2 ± 26 °C and a relative humidity of 5 ± 65%. Then the readings were taken after 24, 48 and 72 hours of treatment, respectively. the second bioassay the larvae were treated with Nano composite SiO-NPs loaded Orizon and the third bioassay the larvae were treated with Nano composite SiO-NPs load Oxymatrine at the same conditions.

Greenhouse experiment. Four hybrids were selected from registered tomato seeds in Iraq for this study. They are Kantor, Oula, Omnia, and Jeda. The seeds of each of these hybrids were sown in plastic dishes designated for seed germination. Each dish consisted of 200 eyes filled with peat moss. After watering the peat moss to settle in its place, a seed was placed in each eye and the seeds were watered until germination. After 45 days of planting, the height of the beginning reached approximately 15 cm. Irrigation process for the land of the greenhouse at the after getting rid of the bush and pathogens in the soil. Then, on 1/12/2021, the tomato plants were transferred

to the greenhouse at Abu Jassim sub-district, Qadissiya Governorate, Iraq (Figure 1). The greenhouse was divided into three blocks; each block
was divided into 24 experimental units. Each experimental unit contained sixth tomato plants, with a distance of 30 cm between plants. The experimental units were arranged in the greenhouse in a randomized complete block.

Figure 1. The greenhouse planted with four hybrids of tomato

the insect was transferred from the infected fields from Al-Haidariya sub-district in Najaf governorate to the greenhouse on 2/23/2021 by transferring larvae of their different stages One leaf disk was placed on each plant. The population was allowed to grow for three generations.
The experimental units were treated with the following treatments: 1- The pesticide Orizon LS. Spray on the plant at a concentration of 1.5 ml / liter of water. 2- Oxymatrine 2.4 LS. Spray on the plant at a concentration of 1.8 ml / liter of water. 3- silicon oxide nanoparticle (SiO-NPs) at 75ppm. 4- the composite of SiO-NPs at 75ppm with Orizon LS pesticide at a concentration of 1.5 ml/liter 5- the composite of SiO-NPs at 75ppm with Oxymatrine 2.4 LS at a concentration of 1.8 ml / liter 6- The comparison was treated with water added to it HCl at a concentration of 3 ml/L . A twenty-liter capacity dorsal sprinkler was used to carry out the spraying process for the treatments. five

leaves were taken from each plant from the upper, middle and lower part, so the sample size for each experimental unit became 30 leaves, in the morning of each sampling date (1 day before treatment, and 1, 3, 5, 7 and 14 days after treatment). The numbers of larvae and eggs were determined under a dissecting microscope.

Statistical analysis

The laboratory experiments were analyzed by using the Prism version 7 program, while the field experiments were analyzed according to the complete randomized block design (RCBD) and the least significant difference (LSD) test was used at the 0.05 probability level to compare the averages of the different treatments and the Genstat 12 program was used in the statistical analysis for data

Results and Discussion

scanning electron microscopy of SiO –Orizon and SiO- Oxymatrine

The images of microscopic examination to determine the the average diameters of of SiO –Orizon and SiO- Oxymatrine were 30.42(Figure 2a) and 21.07 nm(Figure 2b) , respectively.

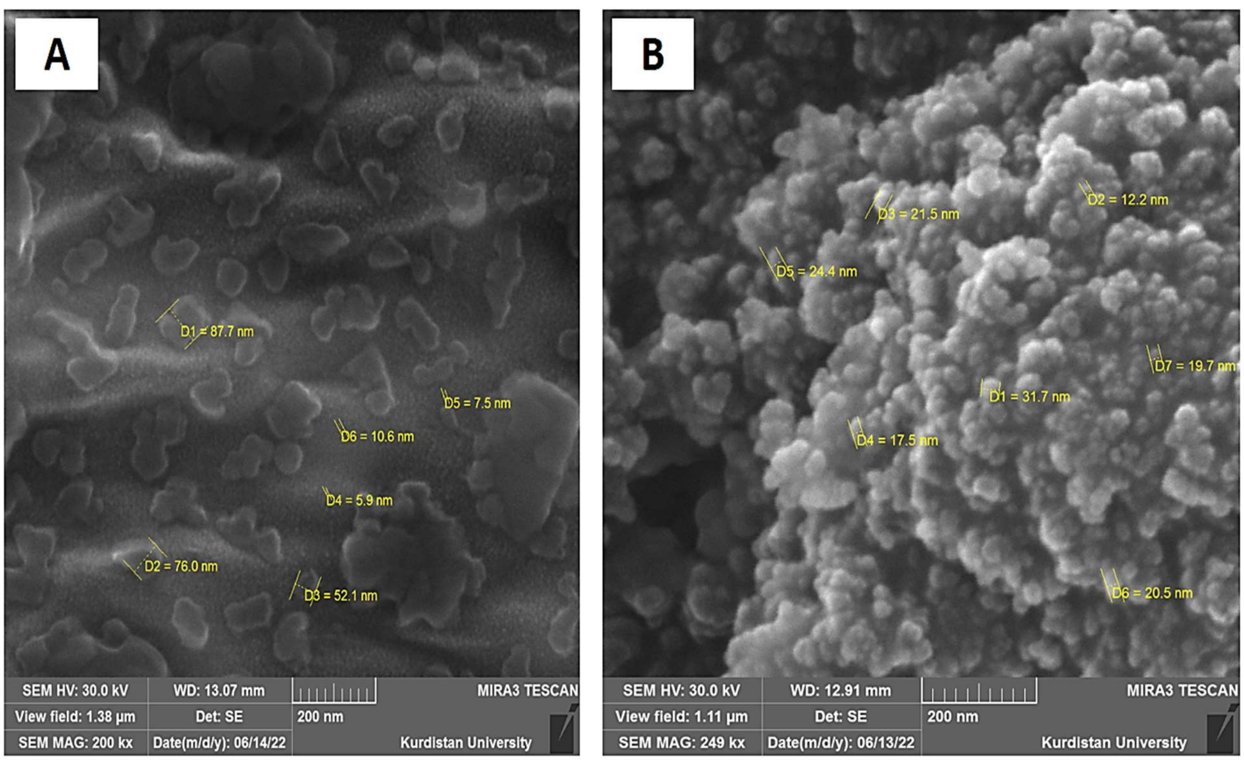

Figure 2. Silicon oxide nanoparticle loaded on Orizon (a) and on Oxymatrine (b) under scanning electron microscope.

Effect of tested different concentrations of silica oxide nanoparticles on the fourth instar larvae of *T. absoluta*

The results revealed that there is insecticidal activity at different concentrations (25, 50 and 75 ppm) of silicon oxide nanoparticle against 4th instar larvae of *T. absoluta* (Figure 3)**.** There is a clear trend of increasing mortality percentage of larvae with increasing of the concentrations of SiO-NPs, the mortality percentages reached 34, 38 and 49% at 25, 50 and 75 ppm, respectively after 72 hours. The results show that there are significant differences between the treatments of different concentrations of SiO-NPs and the control treatments ctr1, ctr2, where the percentage of mortality in the two control treatments was only 8%, while there is no significant difference between the two control treatments ctr1, ctr2. The efficacy of SiO-NPs for the insect *T. absoluta* may be due to the absorption of the lipid nanoparticles in the cuticle layer, which leads to damage to the protective waxy layer that acts as an effective barrier to prevent water loss in the insect's body, causing its dehydration and death (Derbalah et al. 2012; Athanassiou et al., 2009) (Figure 6C). Nanoparticles are considered more acceptable because they are safe for plants and less polluting to the environment compared to conventional chemical pesticides (Rahman et al., 2009; Mewis et al., 2008). Moreover, the application of nanoparticles to leaves and stems does not affect the processes of photosynthesis and respiration in many plants (Singh et al., 2017; zhao et al., 2020). It was also found that the SiO-NPs has a role in improving the condition of the plant treated with it (Derbalah 2012).

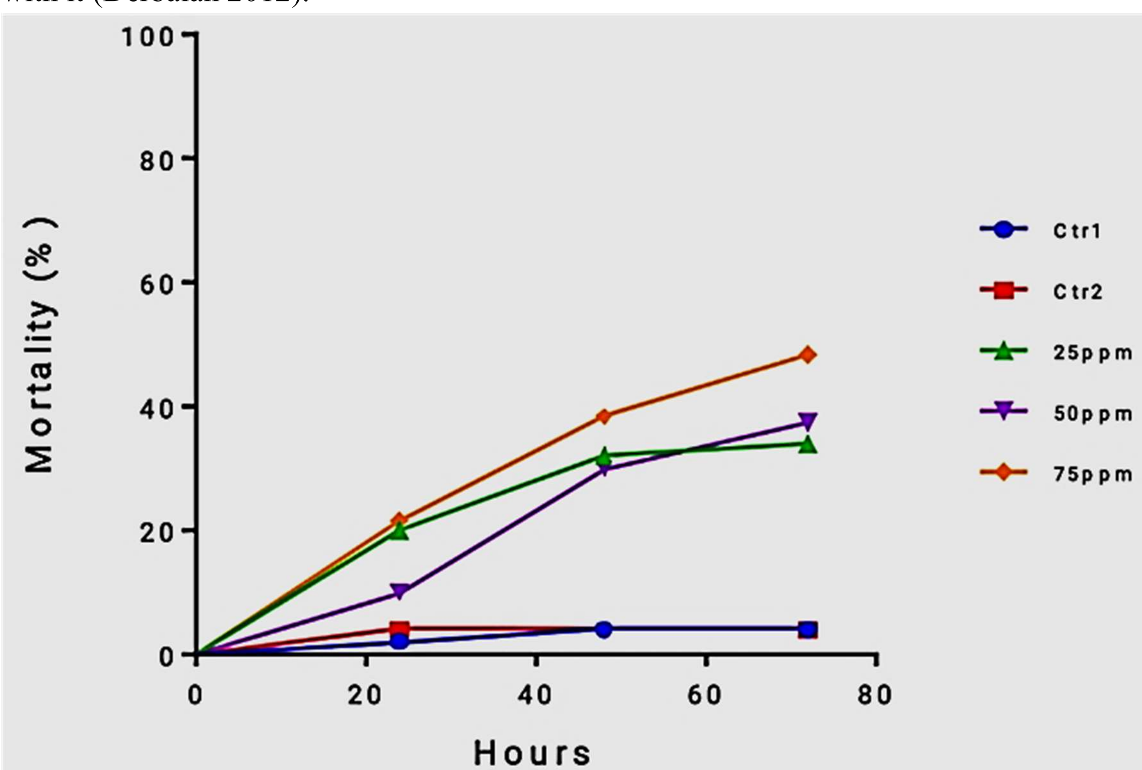

Figure 3. Mortality curves of *T. absoluta* larvae spray by different concentrations of silica oxide nanoparticles ($p < 0.05$; Prism v.7). Control 1 = larvae sprayed by water, control 2 = larvae sprayed by water+Hcl 10%.

Effect of tested different concentrations of silicon oxide nanoparticle loaded on Oxymatrine at the recommended concentration on the fourth instar larvae of *T. absoluta*

The result showed the effect of different concentrations of SiO-NPs loaded on the pesticide Oxymatrine at the recommended concentration and the effect of the exposure time on the percentages of mortality on the 4^{th} instar larvae of *T. absoluta* (Figure 4). The results showed the highest mortality percentage was 69% at 75ppm of SiO-NPs loaded on the pesticide Oxymatrine. While there were 50, 62.5 % at 25, 50 ppm, respectively. While there was 8% mortality for both controls after 72 hours. These mortalities were significantly different between the treatments of different concentrations of SiO-NPs loaded on Oxymatrine at the recommended concentration and the control treatments (ctr1, ctr2). while there is no significant difference between the two control treatments ctr1, ctr2. Also, the use of the pesticide Oxymatrine alone at the recommended concentration caused a mortality percentage was 34% (Figure 6D). The results also show that the highest larval mortality recorded in treatment of SiO-NPs loaded on the pesticide Oxymatrine, and these results due to synergistic action between the pesticide and nanoparticles. The surface area to volume ratio of nanoparticles is large and thus provides a better distribution of them on the bodies of insects, which increased the penetration on the insect body resulting in the efficiency of insecticides (Ziaee et al., 2014). Silica nanoparticles enhances the distribution of adhesion and permeability of pesticides on target insects. In a similar study in improving the action of the pesticide using nanosilica oxide mentioned by Satehi et al. (2018) silica nanoparticles can improve the action of Chlorpyrifo in controlling *Rhyzopertha dominica* and *Tribolium confusum*.

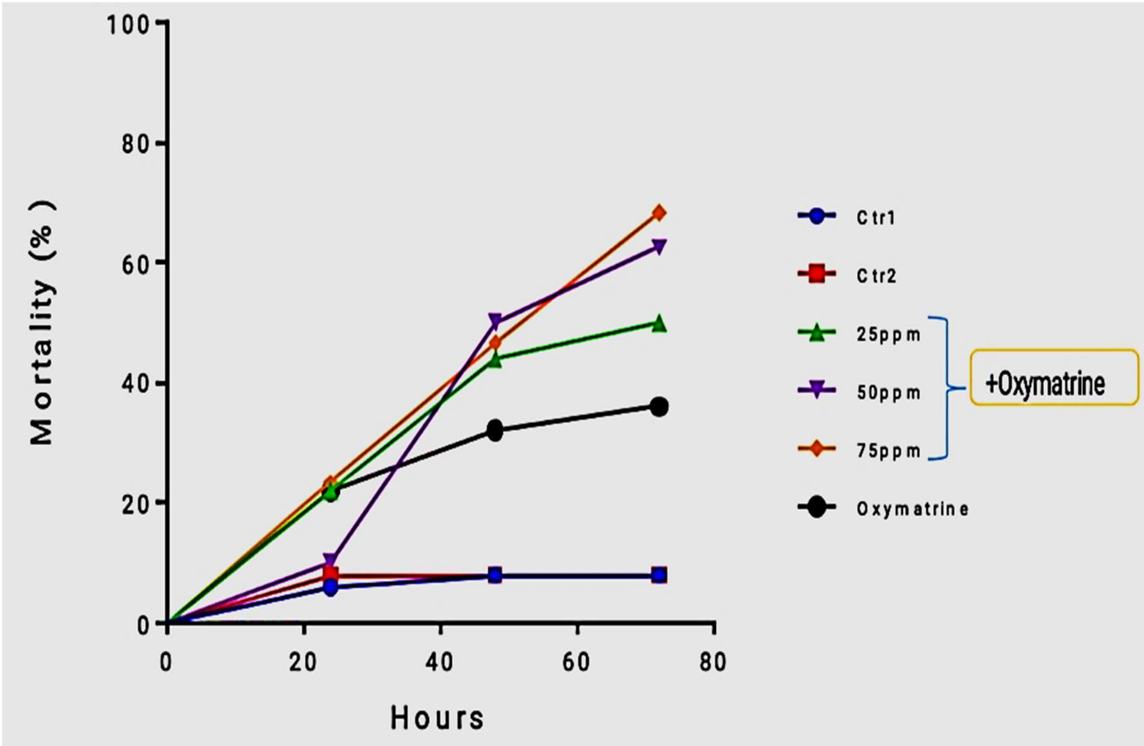

Figure 4. Mortality curves of *T. absoluta* larvae spray by different concentrations of silica oxide nanoparticles loaded on Oxymatrine with the recommended concentration ($p < 0.05$; Prism v.7) . Control 1 = larvae sprayed by water, control 2 = larvae sprayed by water+Hcl 10%.

Effect of tested different concentrations of silicon oxide nanoparticle loaded on Orizon at the recommended concentration on the fourth instar larvae of *T. absoluta*

The results of this study (Figure 5) show that the highest percentage of mortality was 66% For SiO-NPs (75ppm) loaded on Orizon pesticide at the recommended concentration of the 4^{th} instar larvae of *T. absoluta*, where the treatments at 25 and 50 ppm of SiO-NPs loaded on Orizon caused 46, 55 %, respectively after 72 hours. As for the effect of the time period, the time period of 72 hours significantly exceeded the rest of the time periods. Interestingly, they were significant differences between the treatments of different concentrations of SiO-NPs loaded on Orizon at the recommended concentration and the two control treatments ctr1, ctr2, where the percentage of mortality in the two control treatments was only 8% and there are no significant differences between the two control treatments ctr1, ctr2. The use of the pesticide Orizon alone at the recommended concentration caused mortality was 36% (Figure 6 B). It is worth noting that the addition of SiO-NPs with the pesticide Orizon causes a positive synergistic effect, Orizon insecticide contains the active substance Acetamiprid, which works to stimulate acetylcholine receptors located in the nervous system, specifically in the synapses between neurons responsible for transmitting nerve impulses, which affects the behavior of the insect and increases its activity through hyperactivity and repetition of nervous impulses, which leads to its death (Pravinson et al., 2021). In a similar study by Taleh et al. (2020) who observed that the use of the pesticide Acetamiprid on *T. absoluta* larvae caused the mortality of 4th instar larva was 63% after a week under field conditions. Acetamiprid is one of the environmentally friendly pesticides (Yamad and Hatano, 1999). The pesticide also showed low toxicity on honey bees and bio-enemies (Shi et al., 2020 Kim; and Riedl, 2006). Thus, it has been suggested here that using SiO-NPs loaded on Orizon is a novel approach to control the development of *T. absoluta*.

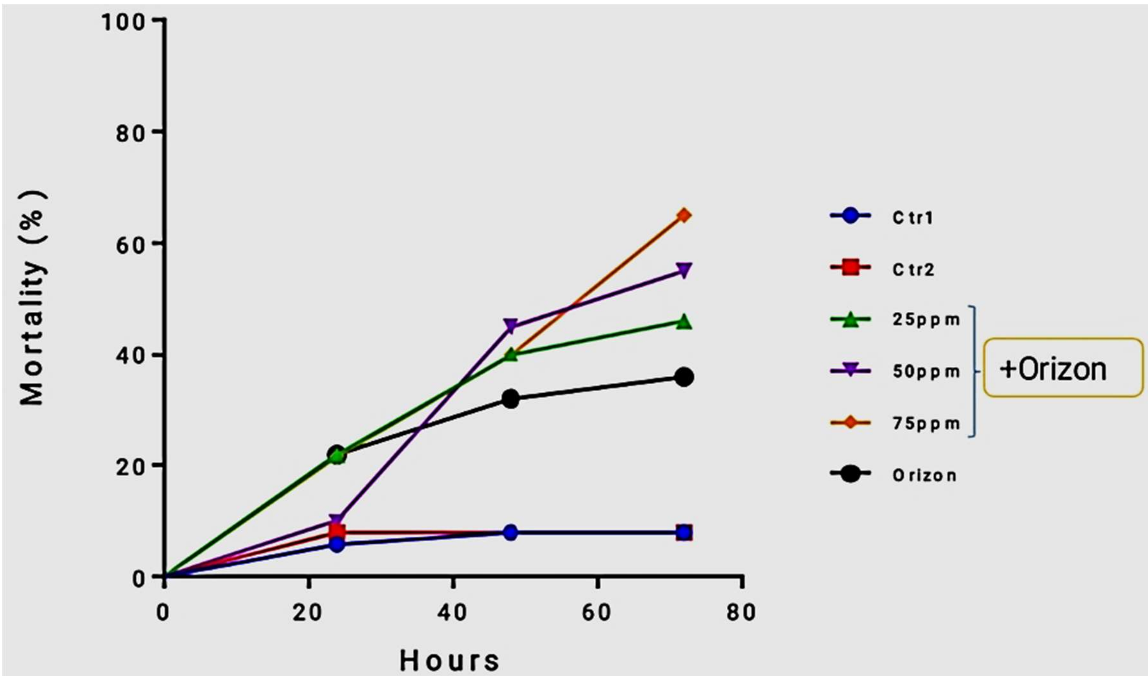

Figure 5. Mortality curves of *T. absoluta* larvae spray by different concentrations of silica oxide nanoparticles loaded on Orizon with the recommended concentration ($p < 0.05$; Prism v.7). Control 1 = larvae sprayed by water, control 2 = larvae sprayed by water+Hcl 10%.

Figure 6. The fourth instar larvae of *T. absoluta* that were untreated (A), or treated with Orizon (B) or 75 ppm silicon oxide nanoparticles (C) or Oxymatrine (D).

Field evaluation of the effectiveness of the pesticides Orizon, Oxymatrine and SiO-NPs and their combinations on the fourth instar larvae of *T. absoluta.*

The results of the effectiveness of the pesticides Orizon, Oxymatrine, SiO-NPs, SiO –Orizon and SiO- Oxymatrine (Table 1) on the 4th instar larvae of *T. absoluta* showed significant differences among the densities of the larvae on the four Tomato hybrids. The SiO- Oxymatrine treatment recorded the lowest numerical density of 4th instar larvae, which was 0.91 larva/leaf, and the average larval density decreased as a result of the treatments SiO –Orizon, Oxymatrine, Orizon and SiO NPs to 0.96, 1.07, 1.15 and 1.25 larva/leaf, respectively, compared to the control treatment reached 4.31 larva/leaf. In addition, the results indicate that significant differences between the two treatments Oxymatrine and SiO- Oxymatrine and between the two treatments Orizon and SiO –Orizon as well as significant differences between the control treatments and all treatments. As for the hybrids, the highest density was recorded on the Omnia reaching 2.31 larva/leaf, while the hybrids recorded Oula, Jeda and Kantor less numerical density were 1.93, 1.52 and 0.79 larva/leaf, respectively. It was found that there are significant differences among the hybrids. As for the days, On the 7th day recorded the lowest numerical density of larvae, which reached 0.88 larva/leaf, followed by 5th day, which recorded 0.98 larva/leaf, It is noted from the results of the interaction between the treatments, the hybrids and the time exposure that the lowest number of 4th instar larvae of *T.absoluta* was recorded in the interaction treatments, SiO-Nps of the hybrid Kantor at 7 day, Orizon treatment of the hybrid Kantor at 5 and 7 day, Oxymatrine treatment of the hybrid Kantor at 5 and 7day and the Oxymatrine-SiO treatment the hybrid Kantor at 5, 7 and 14day. It has been shown that Orizon was more effective on the first and second larval instars, while the insecticide showed Oxymatrine is more effective on the 3rd and 4th instars, and the reason for this is due to the different motor activity of the larvae and the difference in the mechanism of action of the pesticide. It is clear noted from the above results that the effect of the pesticide Orizon and Oxymatrine decreases on the 14th day, and there is a relative increase in the number of larvae density. As for SiO-Nps, it affected all stages. It is considered a safe biocidal for humans according to the opinion of the World Health Organization (WHO) (Barik et al., 2008) and the results of the current study agree with Derbalah et al. (2012) who indicated that SiO-Nps had a significant effect in controlling the tomato leaf miner *T. absoluta* under greenhouse conditions, as confirmed by El-Samahy (2014) who observed that SiO-Nps in controlling *T. absoluta* under field conditions. Our data have demonstrated that the loading SiO-Nps with the pesticide Orizon led to increase larval mortality and increase in the duration of the effect compared with the results of the treatments of Orizon, Oxymatrine and SiO-Nps alone, that this superiority is attributed to the synergistic action of the nanomaterial with pesticides , The present results are consistent with Jameel et al. (2020) mentioned that when loading Zinc oxide nanoparticles on Thiamethoxam led to an increase in the larval mortality of the 4th instar larvae *Spodoptera litura* compared to the treatment of Thiamethoxam or the treatment of Zinc Oxide nanoparticles alone.

The present study demonstrated the effects of silica oxide nanoparticle is a strategy to improve the work of the pesticide and reduce the resistance *T. absoluta* to the pesticides.

Table 1. Effect of the two pesticides Orizon, Oxymatrine and SiO-NPs and their combinations on the population density of on the fourth instar larvae of *T. absoluta.* in the field.

treatment	hybrids	**average Density of 4th instar larvae of *T. absoluta* (larva/leaf)**						Treatment rate	Hybrid rate
		Before treatment	day 1	day3	day 5	day7	day14		
SiO- NPs	Kantor	1.67	1.00	0.16	0.00	0.00	0.34	1.25	Kantor 0.79
	Oula	4.60	1.33	1.00	0.67	0.33	0.77		
	Omnia	5.83	1.67	1.20	0.72	0.67	0.33		
	Jeda	3.72	1.35	0.92	0.81	0.35	0.44		
Orizon	Kantor	1.67	0.32	0.08	0.00	0.00	0.52	1.15	
	Oula	4.60	1.24	1.19	0.83	0.00	0.80		Oula 1.93
	Omnia	5.83	1.28	1.00	0.52	0.41	0.77		
	Jeda	3.72	1.21	0.91	0.00	0.00	0.67		
Oxymatrine	Kantor	1.67	0.41	0.33	0.00	0.00	0.38	1.07	
	Oula	4.60	1.67	0.64	0.21	0.18	0.33		

	Omnia	5.83	1.43	0.47	0.45	0.40	0.36		
	Jeda	3.72	1.00	0.91	0.00	0.34	0.33		
SiO – Orizon	Kantor	1.67	0.20	0.05	0.00	0.00	0.00	0.96	Omnia 2.31
	Oula	4.60	0.96	0.81	0.62	0.17	0.19		
	Omnia	5.83	1.24	1.00	0.00	0.00	0.00		
	Jeda	3.72	0.62	0.41	0.38	0.34	0.33		
SiO-Oxymatrine	Kantor	1.67	0.23	0.04	0.00	0.00	0.31	0.91	
	Oula	4.60	1.00	0.67	0.00	0.00	0.00		Jeda 1.52
	Omnia	5.83	1.12	0.73	0.00	0.00	0.67		
	Jeda	3.72	1.00	0.31	0.00	0.00	0.00		
Control	Kantor	1.67	1.73	1.80	2.15	2.00	2.23		
	Oula	4.60	4.83	4.97	5.73	5.91	4.73	4.31	
	Omnia	5.83	6.07	6.72	6.33	6.02	6.42		
	Jeda	3.72	3.70	4.07	4.13	4.06	4.32		
Days rate		3.96	1.53	1.48	0.98	0.88	1.05		
L.S.D.0.05		time	0.286	hybrid	0.275	treatment	0.186	interaction 0.771	

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