PREPARATION OF THE ENVIRONMENTALLY FRIENDLY GREEN NANO INSECTICIDE USING COPPER SALTS

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ABSTRACT

The nanoparticle was prepared using aqueous extract of naringin Citrus aurantium and copper salts, which are 100% local materials to combat the white fly Bemisia tabaci, which have the ability to transmit and spread plant diseases at a very high rate and pose a great threat to global food production. In tropical and subtropical regions, the insect problem used in the study has become one of the most serious problems facing crop protection. The economic losses are estimated in the hundreds of millions of dollars. The prepared nanoparticles were characterized by the following assays:UV-visible spectrophotometry, SEM, AFM and FTIR. The results of the study showed that the nanoparticles of copper salts were more effective in the mortality rates of nymphs, eggs, and whole insects of the whitefly at concentrations of 100, 200 and 300 ppm, in addition to that the effectiveness of the nanocide led to higher mortality rates for the third, fourth and virginal stages of whitefly at 300 ppm concentrations compared to the results Aqueous extract of Citrus aurantium and copper salts.

KEYWORDS

Nanoparticles, Bemisia tabaci, SEM, AFM, FTIR, and UV-Visible.

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1. INTRODUCTION

In Iraq, orchids have recently been exposed to infestation with a large number of different insect pests, which led to various damages in severity and size depending on the elements of the prevailing climate and among these insect species are the Aleuroclava Jasmine fly and the jasmine fly on citrus trees. In addition to the white fruit fly (Mediterranean fly), spiders and powdery mildew insects of citrus trees are widely spread in the central regions of Iraq's governorates such as Diyala, Baghdad, Salah al-Din, Karbala, Babil and Wasit, which has exacerbated the damage and the deterioration of trees and their total production[1,2].Thus the use of chemical pesticides is one of the most important applied methods for its rapid effect in reducing high population density and reducing its damage to agricultural crops. It was pointed out the importance of using a pesticide to control white flies on citrus fruits in Iraq and the midacloprid, carbosufan and methyl pesticides were also given the highest rates to kill the whitefly[3, 4].The second half of the twentieth century witnessed an increase in the use of chemical pesticides in controlling various agricultural pests, and it was later found that many pesticides have high toxicity and affect human and animal health as well as negative effects on the entire ecosystem. Likewise, the discovery and use of techniques such as encapsulation and controlled release led to a radical change in the use of pesticides, as many pesticide companies produced products containing nanoparticles with sizes ranging from 100 to 250 nanometers that could dissolve in water more than it is in current pesticides [5,6].In addition, the nanoparticles have a role in treating pollution from chlorine solvents, organic chlorine pesticides, organic dyes and many inorganic compounds [7].The amount of agricultural pesticides used in Iraq is frightening and requires a serious pause to limit its use or the use of advanced modern pesticides because it causes many serious diseases, including kidney failure, cancers of all kinds, congenital and reproductive malformations and neurological diseases[2,8].Also, farmers' failure to use the recommended concentrations and relying on their personal experiences in using these preventive and professional pesticides led to a reduction in safety methods, and the occurrence of injuries and poisoning for farmers [1,2, 9].Previous experiments demonstrated the possibility of synthesizing copper oxide nanoparticles using plant extracts and testing their efficacy as a preservative for wood by using extracts from neem leaves (Azadirachta indica, Pongamia pinnata, Lantana camara and Citrus reticulata) this is for the synthesis of copper [10].While another study showed by summarizing the data of plants used to synthesize CuNPs nanoparticles and opening a new path for researchers to explore those plants that had not been used in the past [11].Also another study showed the development of a non-toxic and cost-effective biological method for the synthesis of CuNPs, which is a green method for the synthesis of copper nanoparticles within 15-20 minutes and its high effectiveness against many pathogenic microbes [12,13,14]. Therefore, this study aimed to arrive at a preparation of an environmentally friendly insecticide using copper nanoparticles and to replace the chemicals in preparing copper nanoparticles using the active ingredients of naringin (Citrus aurantium) extract to control the whitefly.

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2. MATERIAL AND METHODS

2.1. PREPARATION OF AQUEOUS EXTRACTS

Naringin (Citrus aurantium) leaves was prepared by taking 20 grams of previously washed leaves and cutting them into very small pieces and placed in a volumetric flask of 250 ml and adding 100 ml of distilled water to them and the mixture was boiled for 10-12 minutes, then it was filtered and stored in the refrigerator on 4 o C until experiment and chemical analyzes are performed.

2.1.1. PHYTOCHEMICAL ANALYSIS

The extract of alcoholic naringin prepared for phytochemical detection to reveal the various biological components of the plant such Tannins, Carbohydrates, Glycosides, Phenols, Resins, Flavonoids, Saponins, Alkaloids, Protein, Comarins, Turbines and Steroid was done using standard protocols [11].

2.1.2. PREPARATION OF NANOPARTICLES

It was taken 60 ml of 0.1 M of Cuso4 copper sulfate and added to 10 ml of aqueous extract of naringin Citrus aurantium gradually with constant stirring, after which a few drops of 1 M of NaOH were added and the pH was adjusted to 8 of the solution. Continue magnetic stirring at room temperature for 1 period/ 4 hour until the color changes to a dark black color, which indicates the occurrence of the reaction and the creation of nanoparticles, as in Figure 1, [4,15]

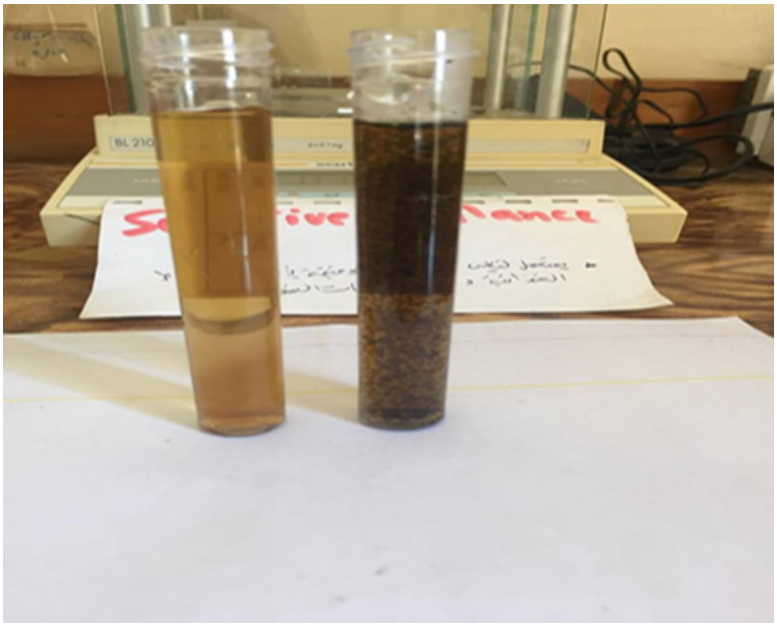

Figure 1. Preparation of CuSo4 nanoparticle

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2.2. CHARACTERIZE THE NANOPARTICLES

The nanoparticles were detected at the Nanotechnology Center / University of Technology/Baghdad by using:

2.2.1. UV-VISIBLE SPECTROMETRY

The ultraviolet rays were used to study the optical properties with a UV-Visible spectrometry (meter teeh sp 8001) in the range of 300-800 nm [16]

2.2.2. XRD ANALYSIS

It was used X-ray (XRD- 700 Shimadza maxima –a) X-Ray diffract meter to determine the crystal phases and estimate the size of the crystals, and that with an electric voltage of 40KV and a current of 30 MA and with a scan range of 100,000) (-20,000 degrees. Also, XRD patterns were recorded within 0.12. Seconds of scanning speed and using Cu tubes with copper wavelength 1.54 Ao. [17,18]

2.2.3. FTIR SPECTROSCOPY

An infrared analysis of copper Nano forms was performed with FTIR - 8400S, SHIMAZW-FTIR spectroscopy with a wavelength ranging between (500 - 4000) cm - 1[19, 20].

2.2.4. SCANNING ELECTRON MICROSCOPE (SEM)

The scanning electron microscope is of the type (TESCAN-VEGA / USA) where the Nano scale particles are scanned with 3 nm beams and 30kv electric voltage knowing that the device is connected and programmed with computer programs to analyze the average particle size in the sample [21].

2.2.5. PARTICLE SIZE DISTRIBUTION ANALYSIS

This device is important for determining fast and accurate size distributions of copper nanoparticles (vision 5.34) with a range ranging from (6- 2 μm nm), and the dynamic light scattering at 90 degrees, at temperatures from (-110-5) m using high power (35 mW) for a laser diode.

2.2.6. AFM EXAMINATION

A three-dimensional image was taken showing the copper sulphate nanoparticles using the deposition method as shown in figure 1.

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2.3. BREEDING WHITEFLY BEMISIA TABACI

Five eggplant seedlings were purchased in pots and placed inside breeding houses covered from the outside with a dull cloth. Seven pairs of whitefly's adults were released into each cage for the insect brought from greenhouses and from both sexes and after 24 hours of laying eggs, the eggs were isolated for each seedling, with the same the method for nymphs, replicates (eggs, nymphs) were treated, duplicates were treated with the prepared pesticide by sprinkler, and the killing numbers readings were taken and recorded after 24, 48, and 72 hours, and the percentage of pesticide efficacy was calculated using the percentage killing percentage according to Abbot's formula [6], after which the percentage of the killed percentage was converted into proportions Angle according to and then subjected to statistical analysis [22]

$$Fatalities\,\% = \frac{Number\,of\,pest\,before\,treatment\,x\,Number\,of\,pest\,after\,treatment}{Number\,of\,pest\,before\,treatment\,x\,Number\,of\,pest\,after\,treatment} \times 100 (1)$$

2.4. FIELD EVALUATION OF THE EFFECTIVENESS OF NANOPARTICLES ON WHITEFLY

The experiment was carried out on eggplant plants in the greenhouse at the Zaafaraniya farm in Baghdad city, with three replicates (a plastic house) for the pesticide under test, and three replicates of the another standard pesticide (deltamethrine) for good comparison between treatments. The population density of whitefly Bemisia tabaci was calculated before the control process was carried out by randomly selecting ten plants for each replicate, in which the numbers of adults were calculated on the plant leaf in three locations for each plant, which are the top, middle and bottom (30 leave). Then the plants were sprayed with Nano pesticide by holder, then the results of killing % were taken after 24, 48 and 72 hours in the same way mentioned above.

2.5. FIELD EXPERIMENT

For each treatment, the numbers of live eggs, nymphs, and adults were estimated before treatment and after spraying one day, three days and one week after the start of the treatment, as the adults were calculated by direct counting method, and the live numbers of adult adults were counted in the statistical analysis in order to take into account the accuracy of the experiment in order to not remain dead adult's adherent to the surface of the leaf.

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Figure 2. life stages of the whitefly Bemisia tabaci

2.6. STATISTICAL ANALYSIS

The killing percentage was corrected according to Abbott's equation to angle ratios [23]. It was subjected to statistical analysis to find out the effect of different factors on the studied traits. The significant differences between the averages were compared with the lowest significant difference test [10].

3. RESULTS AND DISCUSSION

3.1. PHYTOCHEMICAL SCREENING

It was presented the active ingredients in the aqueous extract of naringin Citrus aurantium such as phenols, flavonoids, alkaloids, terpenoid, tannin, protein, charbohydrates, steroids and saponins in Table 1. Results of this study showed a highest content of total phenole, alkaloids and protein, then terpenoid, tannin, charbohydrates and steroides with medium content as in Table 1. Those active ingredients which are a byproduct of the metabolism in all kinds of fruits and vegetables act as a great role in protecting the cell from deterioration and stress and acting as antioxidants to reduce the toxic effects for free radicals [24].

Table 1. The active ingredients in the Citrus Naring aqueous extraction.

Phytochemical test	Results
Total phenol	++++
Alkaloids	+++
Terpenoids	++
Tannin	++
Protein	+++
Carbohydrates	++

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Steroids	++
Saponin	++

Heavy content, ++=medium content=+++

3.2. NANOPARTICLES CHARACTERIZATION

The nanoparticles were described in the Nanotechnology Center / University of Technology / Baghdad followed different ways.

3.2.1. UV-VISIBLE SPECTROMETRY

The ultraviolet rays were used to study the optical properties with a UV-Visible spectrometry (meter teeh sp 8001) in the range of 300-800 nm [25, 26].

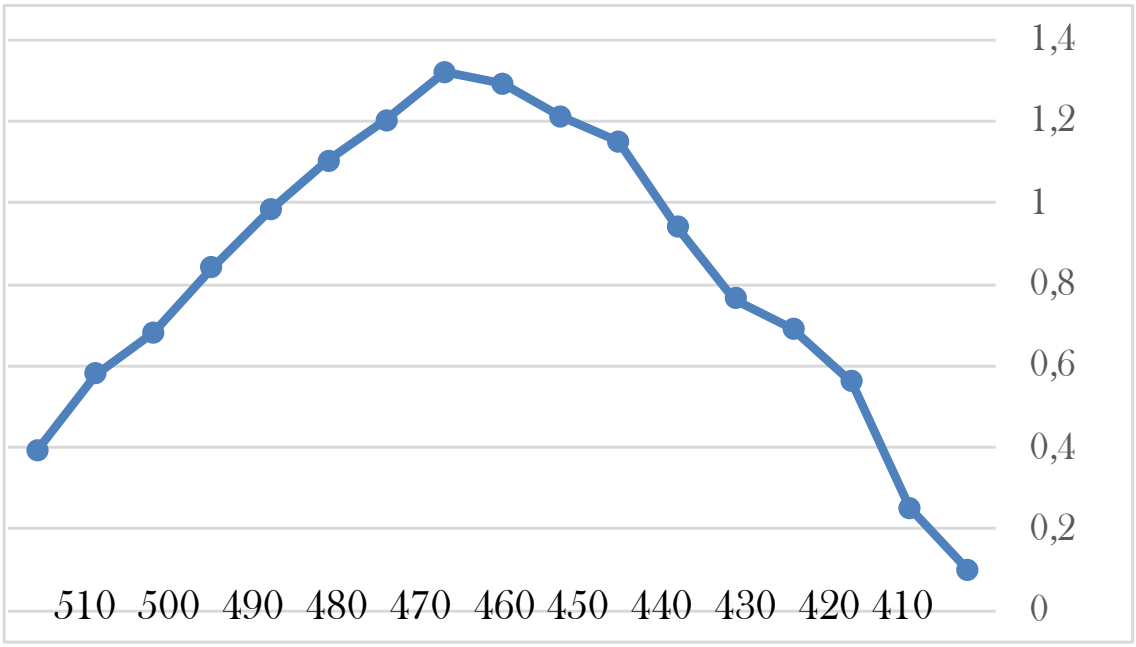

Figure 3. The synthesis of copper nanoparticles using UV-Visible radiation.

3.3. X-RAY DIFFRACTION XRD ANALYSIS

It was used X-ray (XRD- 700 Shimadza maxima –a) X-Ray diffract meter to determine the crystal phases and estimate the size of the crystals with an electric voltage of 40KV and a current of 30 MA and with a scan range between 100,000) (-20,000 degrees. Then, XRD patterns were recorded within 0.12 degrees, seconds of scanning speed and using Cu tubes with copper wavelength of 1.54 Ao. [27, 28].

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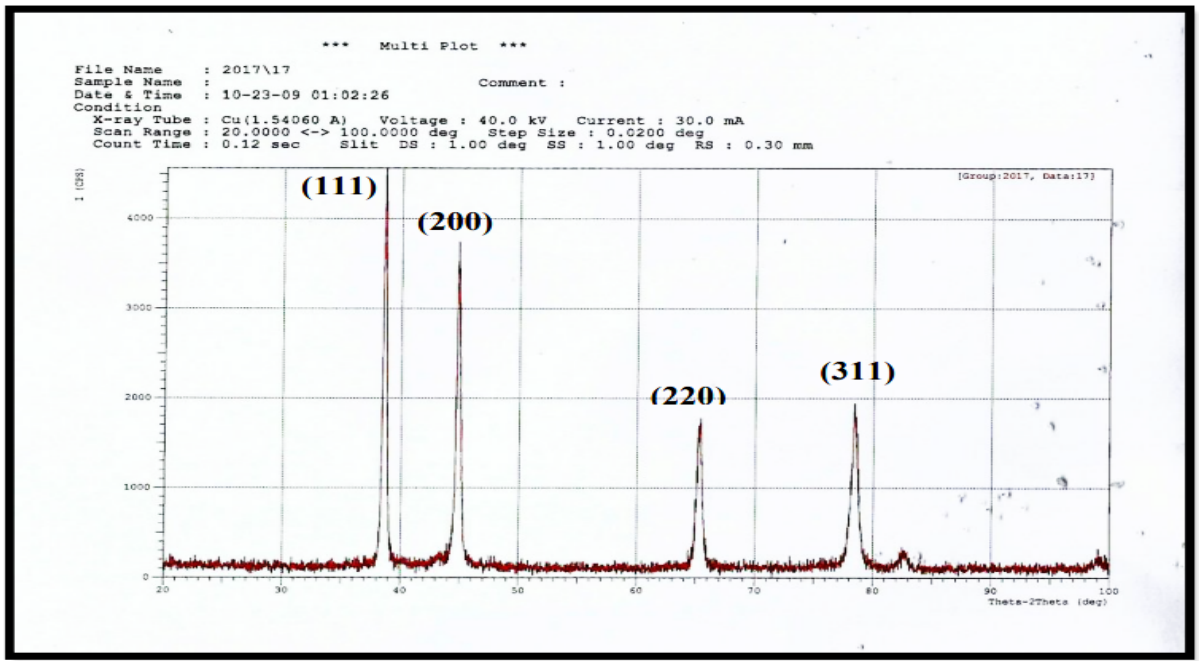

Figure 4. The XRD diffraction of copper nanoparticles

3.4. FTIR- SPECTROSCOPY

An infrared analysis of iron Nano forms was performed with FTIR-8400S, SHIMAZW-FTIR spectroscopy with a wavelength ranging between (500 - 4000) cm -1 (Huang, et al., 2018). The use of FT-IR is to find the particles and their functional groups present in the prepared copper nanoparticles. The peaks that appeared are: 3387, 3377.39, 2922, 1645, 15237, 1377, 1246, 1153, 1028, 1028, 9335, 852, 761, 659, 574, 659, 574, 437, 395, 375, that refers to: Alcohol (OH, Alkane (C-H), Alkene (= C-H), Amine (C-N), Nitro compounds (N-O), Stretch-Acid (OH) and Ester (C=C) [28,29].

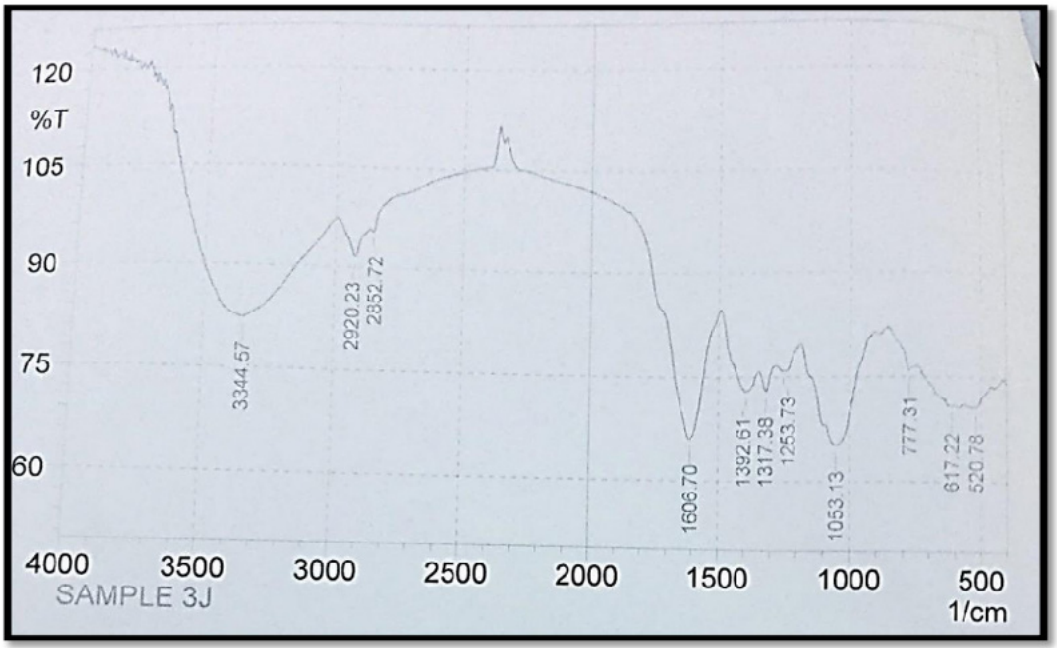

Figure 5. shows the results of the FT-IR examination.

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3.5. SCANNING ELECTRON MICROSCOPE (SEM)

The scanning electron microscope is of the type (TESCAN-VEGA / USA), in which the nanoparticles are scanned with 3 nm beams and 30 kv electric voltage knowing that the device is connected and programmed with computer programs to analyze the average particle size in the sample [21].

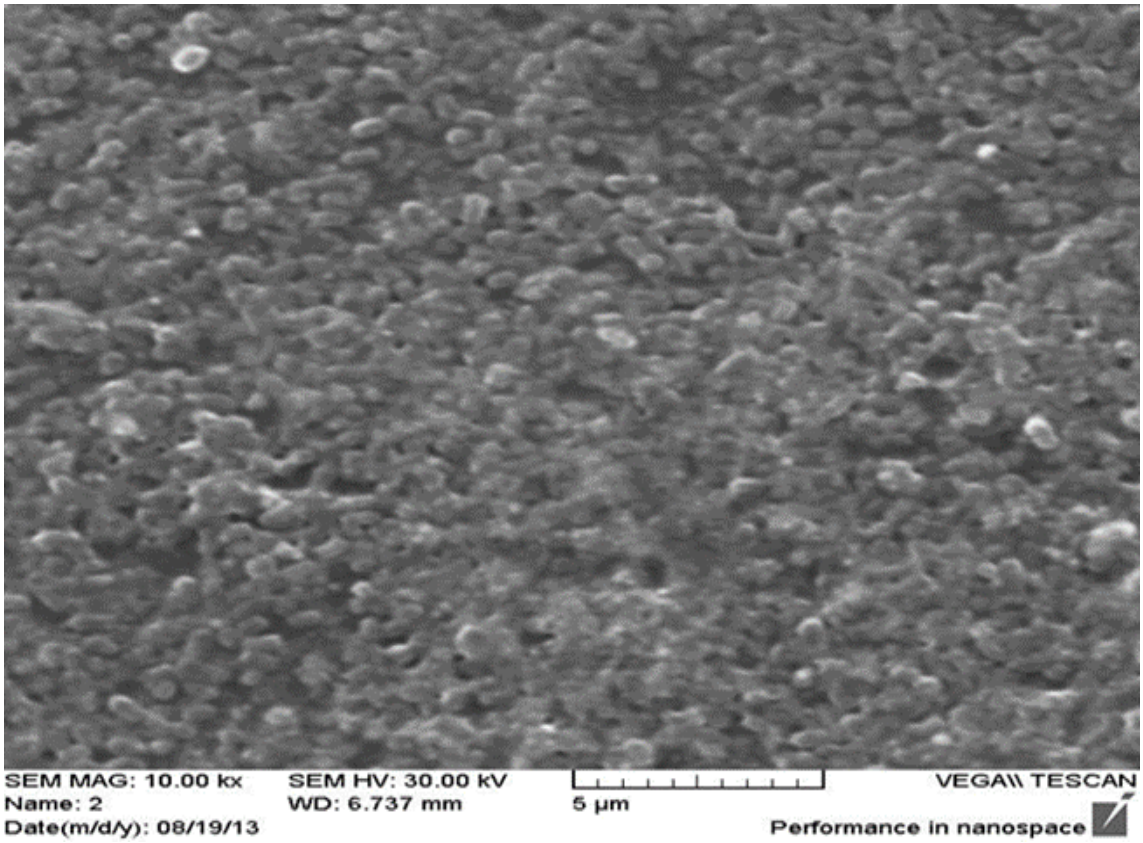

Figure 6. Image of CuSo4 nanoparticles using a scanning electron microscope SEM

3.6. PARTICLE SIZE DISTRIBUTION ANALYSIS

This device is important for determining fast and accurate size distributions of iron nanoparticles (vesion 5.34) with a range ranging from (6- 2 µm nm), and the dynamic light scattering at 90 degrees, at temperatures from (-110-5) m using high power (35 mW) for a laser diode.

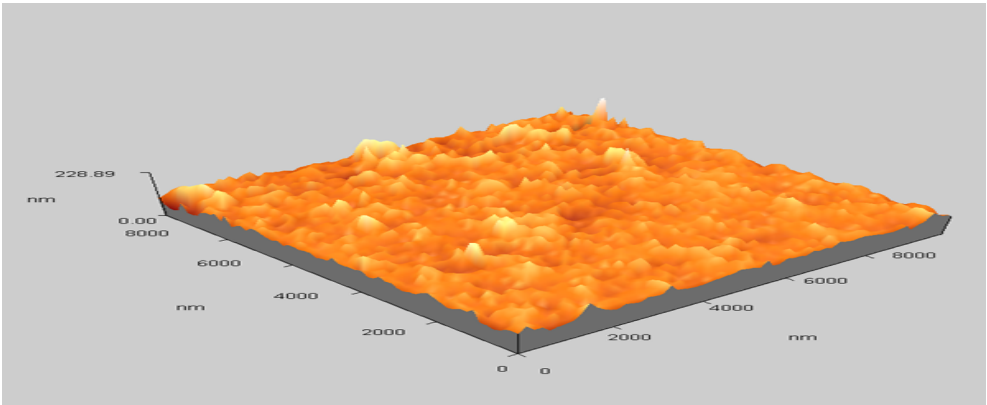

Figure 7a. FAM of synthesized Copper nanoparticles with naringin leaf broth.

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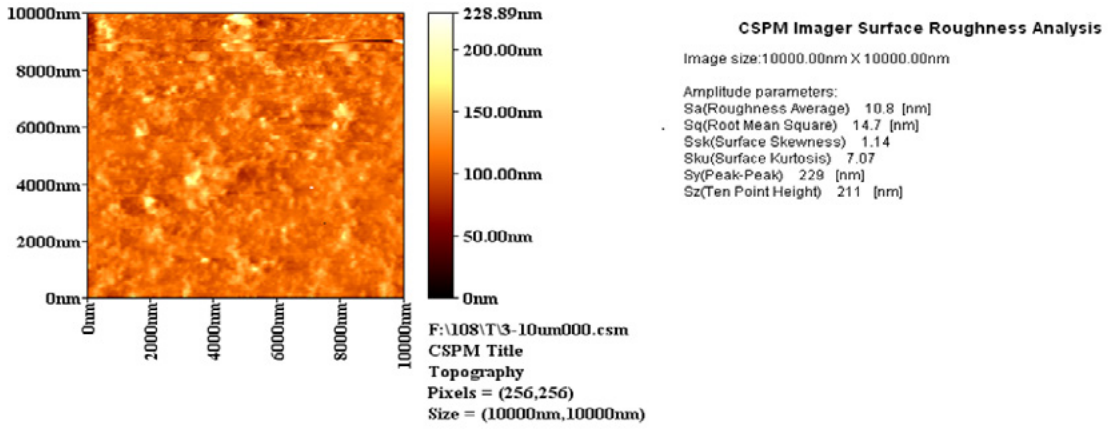

Figure 7b. AFM of synthesized Copper nanoparticles with naringin leaf extract

3.7. EFFECT OF NARINGIN (CITRUS AURANTIUM) EXTRACT ON WHITEFLY STAGES)

Results of this study presented the effect of naringin leaf extract on the mortality percent of whitefly's eggs and nymphs in Table 2. Results of this study were showed by increased the concentrations of extract from 100, 200 to 300 ppm showed a high (P≤0.05) significant effect on whitefly's eggs were 7.6, 18.8 and 26.8 respectively. Also showed a high significant effect (P≤0.05) on whitefly's nymphs were 9.8, 33.5 and 44.8 for 100, 200 and 300 ppm of the copper nanoparticles extract respectively as in Table 2. [28,30].

Table 2. Effect of citruses Naringin leaves extract on whitefly eggs and nymphs.

Extract concentration (ppm)	Mortality %	
	Whitefly eggs	Whitefly nymphs
100 ppm	8.0	9
200 ppm	15.0	27
300 ppm	27.0	44
Average	16.6	27
LSD	4.726 *	6.033 *
* (P≤0.05).		

3.8. EFFECT OF COPPER SALT EXTRACT ON WHITEFLY STAGES

Results of this study presented the effect of copper salt extract on the mortality percent of whitefly's eggs and nymphs in Table 3. Results of this study were showed by increased the concentrations of extract from 100, 200 to 300 ppm showed a high (P≤0.05) significant effect on whitefly's eggs were 0.8, 1.8 and 4.8 respectively. Also showed a high significant effect (P≤0.05) on whitefly's nymphs were 2.4, 7.4 and 11.8 for 100, 200 and 300 ppm of the copper salt extract respectively as in Table 3, [1, 28].

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Table 3. Effect of copper salts on whitefly eggs and nymphs.

Extract concentration (ppm)	Mortality %	
	Whitefly eggs	Whitefly nymphs
100 ppm	0.8	2.4
200 ppm	1.8	7.4
300 ppm	4.8	11.8
Average	2.5	7.2
LSD	1.152 *	2.894 *
* (P≤0.05).		

3.9. EFFECT NANOPARTICLES CITRUS WITH NARINGIN EXTRACT ON WHITEFLY STAGES

Results of this study presented the effect of nanoparticles citrus with naringin extract on the mortality percent of whitefly's eggs and nymphs in Table 4. Results of this study were showed by increased the concentrations of extract from 100, 200 to 300 ppm showed a high (P≤0.05) significant effect on whitefly's eggs were 2.3, 7.8 and 11.8 respectively. Also showed a high significant effect (P≤0.05) on whitefly's nymphs were 72, 88 and 98 for 100, 200 and 300 ppm of the nanoparticles citrus with naringin extract respectively as in Table 4. Also, it was showed that the average rate of nymphs' mortality was significantly higher 86, compared to average rate of whitefly's egg 7.3. [20, 31-32].

Table 4. Effect of synthesized nanoparticles on whitefly's egg and nymphs.

Extract concentration (ppm)	Mortality %	
	Whitefly eggs	Whitefly nymphs
100 ppm	2.3	72
200 ppm	7.8	88
300 ppm	11.8	98
Average	7.3	86
LSD	2.637 *	7.905 *
* (P≤0.05).		

3.10.NANOPARTICLE EFFICIENCY

The results of nanoparticle efficiency on whiteflies (Bemisia tabaci) tabulated as in Table 5. It was founded, the relative efficiency of the copper nanoparticles of Citrus aurantium leaf extract in controlling white flies in the field was marked highly significant difference (P≤0.05) 96.8%, 97.6% and 100% after 24, 48 and 72 hours, respectively compare with relative efficiency of the standard pesticide Decis on

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Bemisia tabaci which reached 59,0, 48. 5 and 40.2 %, respectively. Note that the recorded fact distinguishes whiteflies as their tolerance and resistance to many chemical pesticides, so the Nanopesticide can be considered very effective and it is recommended to use it to combat this pest. This results identical to [6][3] were founded whiteflies have the ability to develop resistance to both conventional and nonconventional insecticides. Through the results obtained, the nanoparticles from the aqueous extract of naringin (Citrus aurantium) showed a very high significant effect in the mortality rate of the mosquitoes of the third and fourth stages and the virgin in vitro; in addition to its significant effect on the mortality rates of the white fly stages in the laboratory and in the field. These results came close to what he found [28]

Table 5. The relative efficacy of the nanoparticles of citruses naringin leaf extract on adult phase of Bemisia tabaci whiteflies compared to Deltamethrine 2.5% EC.

Examination times (Hours)	Relative efficiency of 2.5% of Deltamethrine pesticide	Relative efficiency of Citrus *aurantium* leaf nanoparticles
24 hr.	59.0	96.8
48 hr.	48.5	97.6
72 hr.	40.2	100
LSD	5.482 *	5.077 NS

* (P≤0.05).

4. CONCLUSION

The results of the study showed the possibility and ease of preparing environmentally friendly nanoparticles synthesis using aqueous extract of naringin (Citrus aurantium) with copper salts. Moreover, the prepared nanoparticles are fixed and unchanging, that is, when the nanoparticles are formed, the color of the extract changes from light green to black and remains constant. Also, the raw materials used to prepare nanoparticles are inexpensive. Also, the nanoparticles do not generate generations of insect resistance as is the case with chemical pesticides. So this idea can be used in the Ministry of Agriculture, and for the benefit of citrus orchard farmers, which are widespread in most governorates of the country, especially central, southern and northern Iraq, in addition to the fact that most of Iraq's lands are suitable for growing fruits, especially citrus fruits. But there are political factors, administrative and economic corruption, mismanagement of the water of the Tigris and Euphrates rivers, the spread of various plant diseases, including powdery mildew and the spread of the white fruit fly, as well as high temperatures and drought in the long summer, which led to the reluctance of farmers to abandon most orchards. Therefore, this study proved the possibility of finding an alternative to traditional chemical insecticides by using green nanotechnology as an environmentally friendly and safe insecticide that would not be used in Iraq beforehand.

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