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Eco-friendly synthesis of ZnO nanoparticles from *Citrus reticulata* peels and their insecticidal effects on *Aphis fabae* Scopoli (Hemiptera: Aphididae)

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ABSTRACT
This study investigates the synthesis of ZnO nanoparticles from *Citrus reticulata* (mandarin) peel extracts and their characterization through XRD, SEM, and EDX to determine their size and composition. The results show that the ZnO nanoparticles possess a hexagonal structure with varying diameters and contain elements such as Ca, Cu, P, K, O, and Zn. Field emission scanning electron microscopy revealed that the nanoparticles exhibit semi-spherical, spherical, and bacilliform shapes. Various concentrations of ZnO nanoparticles were tested on adult *Aphis fabae*, with mortality rates ranging from 11% to 100%. The 2 mg/L concentration proved to be the most effective, achieving 96.66% toxicity, followed by 1.2 mg/L with 80% mortality, and 0.4 mg/L with 34.07% on the 4th day after application. Furthermore, the study revealed that all three concentrations showed the highest insect mortality on the 7th day post-application. These findings suggest that ZnO nanoparticles from *Citrus reticulata* could be a promising tool for pest control, particularly in managing *Aphis fabae* populations.

Key words : *Aphis fabea* , insectical , nanaparticles , ZnO, *Citrus reticulata* .

INTRODUCTION

Nanotechnology can be defined as the science and engineering involved in the design, synthesis, characterization, and application of materials and devices whose smallest functional organization, in at least one dimension, is on the nanometer scale or one billionth of a meter (Saini , 2010) ; it is based on the synthesis and modulation of nanoparticles (Singh *et al*., 2016).

Nanoparticles have significant applications in various fields such as environment, agriculture, food, biotechnology, biomedicine, pharmaceuticals, etc. such as; waste water treatment, environmental monitoring (Rassaei *et al*., 2011), as functional food additives (Chen *et al*., 2011), and as antimicrobial agents (Thakur *et al*. ,2020a, b; Vijayakumar *et al*., 2019).

Recently, nanotechnology has been utilized to create nanofertilizers and nanopesticides aimed at controlling pests of economic significance, thereby boosting crop yields and supporting efficient pest management. (Koul, 2019; Hilal et al., 2021; AL-Taey et al., 2021)

Zinc oxide nanoparticles (ZnO NPs), one of the most important metal nanoparticles, are widely used in various fields due to their physical properties and specific chemistry. They have shown several therapeutic activities against cancer, diabetes, microbial infections, inflammation and can be used as drug vectors, imaging tools and biosensors. Similarly, they displayed a significant antioxidant potential (Jiang *et al*., 2018; Deka *et al*., 2022).

The extraction of ZnO nanoparticles from natural sources has become an emerging area of research as it avoids conventional chemical synthesis methods, which are often expensive, toxic and generate hazardous waste.

The peel of *Citrus reticulata*, commonly known as tangerine peel, is a natural source rich in bioactive compounds, some of which have shown potential for the synthesis of nanoparticles. Determining the morphology, size and function of nanoparticles with regulated structures is essential for biological activities.

The aim of this research is to investigate the synthesis of ZnO nanoparticles from peel of *Citrus reticulata* for determining their insecticidal properties against Aphis *fabea* . This area of research has great potential for the development of new sustainable and environmentally friendly methods for the synthesis of ZnO nanoparticles by exploiting available natural resources.

MATERIAL AND METHODS

Sample collection and preparation

The plant material used in this study is mandarin peel (*Citrus reticulata*), collected in January 2023. Before use, the peels were washed, air dried for several weeks and then ground with a laboratory blender till a consistant and fine powder was obtained. The drying time may vary depending on factors such as humidity and peel thickness. Air-dried and powdered mandarin peels were macerated in distilled water (1:5 W/V) for 24h at room temperature on stirrer plate (Cole-Parmer ™ Stuart). The extract was filtered several times through filter paper , until a clear filtrate free of pulp and unwanted particles is obtained.

Synthesis of ZnO Nanoparticles

ZnO Nanoparticles synthesis was determined according to the method described by (Kausar et al. 2016), with minor modification. Three different doses of Zn sulfate solution (0.4 g, 1.2 g and 2 g) were added to 200mL of the obtained filtrate. The reaction mixture was stirred at 350 rpm and

85 °C for 1 h, and then dried at 100°C for 24h. After the reaction was completed, the mixture was centrifuged for 5 min at 2800 rpm in triplicate, the supernatant was then removed, while the precipitate was collected, dried and incinerated in a furnace oven at 400 °C for 2 h

Characterisation of NPs ZnO

Two distinct approaches was used to characterise ZnO nanoparticles : X-ray diffraction (XRD) and scanning electron microscopy (SEM) in Laboratory of Phrases Transformation , Physics Department, Constantine1 University, Constantine, Algeria.

Insects rearing and Collection

A stock culture of *Aphis fabae*, originated from field collection of infesting broad bean (Vicia faba L.) plants in the experimental area of Plant Protection Department , Faculty of Agriculatur , Isparta Applied University , Turkey, during 2024 was established. The pest was reared in 30 × 20 × 40 cm cages at 22 ± 1 °C and 16 h photoperiod for several generations .

Entompathogenicity test

The direct inoculation method was used to determine the biocidal properties of the nanoparticles. As a standard method recommended by the FAO, it focused on detecting and measuring aphid resistance to insecticides and, in particular, on measuring the toxicity of NPs by contact. The *Aphis fabea* 's aphids were directly immersed for 10 s in each ZnO NPs solution (0.2, 0.4 and 1.2). Dishes were kept at room temperature (25 °C and 60% R.H). The mortality rates of the aphids were evaluated on 1st, 3rd, 5th, and 7th day after application .

5. Statistical analysis

All the treatments were repeated six times, a one-way analysis of variance (ANOVA) was used to assess significant differences between samples. SPSS®(version 17.00 Software. 2006 SPSS.Inc.Chicago.il, USA) was utilized for the statistical analysis. The percentage effects of the three concentrations were calculated and corrected by Abbott's (1925) formula:

Abbott's corrected mortality% =[(mortality in control−mortality in treatment)\(mortality in control)]x100

RESULTS

Structural analysis of powders synthesised by XRD

In Figure 1 we present the X-ray diffraction (XRD) results of the whole series, consisting of five samples prepared with different concentrations of a zinc sulphate salt, while the other operating conditions were kept constant. In this characterisation, the diffraction angle (2θ) was varied in the range [0, 90°]. As can be seen in this figure, which groups together all the diffractograms of the samples prepared at different salt mass concentrations ranging from 1 to 11.25 g/l. The materials (powders) are all polycrystalline and show several peaks positioned at the same diffraction angles. The preliminary study confirms that the crystallites are well crystallised, with no amorphous phase, and that the strongest peaks are those of the ZnO phase, as confirmed by the ASTM data sheet (code N° 1397- 089-01). The selected peaks are positioned according to the diffraction angles (31.66; 34.34; 36.16; 47.45; 56.49; 62.75; 67.83; 69.04°) corresponding to the [h,k,l] orientation planes as shown in the same Figure A: (001; 002; 101; 102; 110; 103; 112; 201). We also note the existence of other peaks of very low intensity that are not ZnO but can be assigned to other secondary phases that probably derive from the constituents of the mandarin: CaO, MgO, CuO, ...

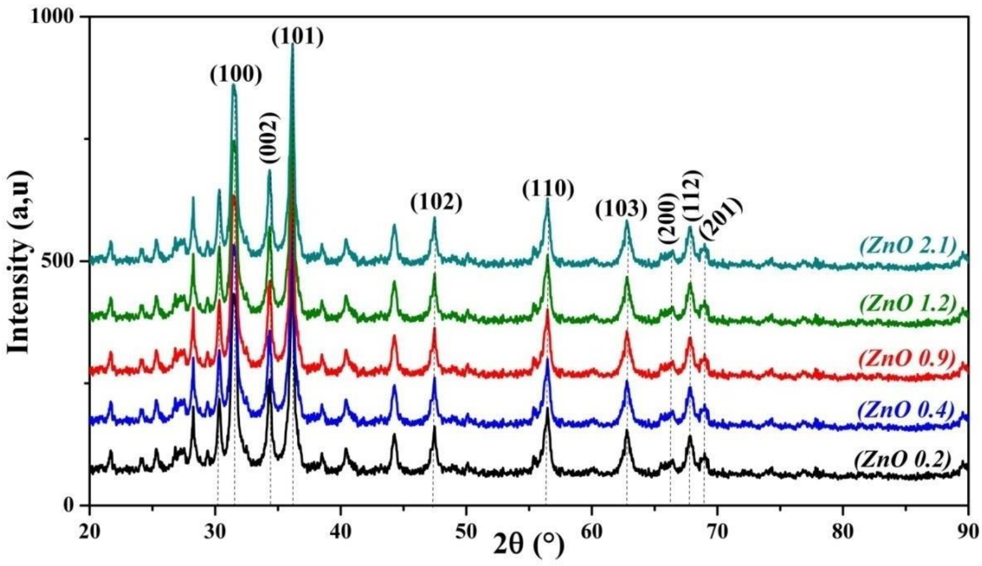

Figure 1: X-ray diffractograms of powdered ZnO samples synthesized with different ZnSO4 concentrations (0.2, 0.4, 0.9, 1.2, 2) and mandarin peels crushed and annealed at 500°C for 3 hours

The results of the changes in the intensities of each peak as a function of the concentration of the ZnSO4 precursor are grouped in Table 1. It seems that the intensities of the peaks increase with the concentration of the zinc precursor salt. In addition, the growth of the crystallites is produced in the first place according to the preferred direction of the plane (101), which has the highest intensity corresponding to the lowest energy of formation, then those of directions (100), (002), ... etc.

Table 1: Presentation of the peaks relating to the ZnO phase with their orientations and the evolution of their intensities as a function of the ZnSO4 concentration of each sample.

Ech.	0.2 *(1g/l)*		0.4 *(2g/l)*		0.9* *(11.25g/l)*		1.2 *(6g/l)*		2 *(10g/l)*	
pic	2θ (°)	I (u.a)	2θ (°)	I (u.a)	2θ (°)	I (u.a)	2θ (°)	I (u.a)	2θ (°)	I (u.a)
(001)	31,66	332,55	31,64	346,73	31,59	398,24	31,67	457,90	31,61	517,99
(002)	34,34	184,71	34,28	249,55	34,20	231,61	34,33	305,70	34,29	435,63
(101)	36,16	458,59	36,08	516,48	36,07	537,92	36,10	660,07	36,07	873,83
(102)	47,45	86,75	47,34	107,80	47,35	96,18	47,44	117,56	47,37	156,59
(110)	56,49	123,18	56,47	159,82	56,41	168,56	56,49	217,07	56,48	255,00
(103)	62,75	80,80	62,76	107,30	62,74	109,51	62,75	150,21	62,67	190,69
(112)	67,83	67,07	67,78	105,86	67,81	83,08	67,88	116,83	67,76	168,22
(201)	69,04	29,79	68,89	58,76	69,00	45,99	68,98	64,51	68,99	77,31

Morphological and compositional analysis of the synthesized ZnO NPs by SEM and EDS In this second analysis by scanning electron microscopy (SEM) equipped with EDS (energy dispersive X-ray spectroscopy), three powder samples were prepared on a conductive support (aluminium foil) in order to evacuate the electrons on the surface during the measurement. The samples selected for this study were prepared with zinc sulphate concentrations of 0.4 g/l, 1.2g/l and 2 g/l

SEM imaging of the synthesized ZnO NPs
The images are obtained after electron bombardment of the sample by an energetic primary electron beam which emits secondary electrons. This emission is used to obtain images as a result. Several images were taken at different magnifications to observe the mapping of each of the powders, as well as the details and accuracies of the nanometric shapes in the material.

As shown in the SEM images (sample 0.4), we observe a spongy shape formed by interconnected agglomerates of non-uniform size and shape. The pore size is not regular, varying from µm to 10 µm. In addition, at higher magnifications of x50,000 and above, we observe the formation of ZnO nanorods or nanowires with 1 to 3 µm lenght ,~~and~~ nanometric diameters (20-100 nm) and different directions. We also observe agglomerations of small nanometric grains of spherical and hexagonal shape.

For the sample with concentration 1.2, we also observe agglomerates made up of small nanometric grains adhering to each other, with nanorods attached in different directions. These have hexagonal sections with different sizes and directions, which is consistent with ZnO. We found that the density of the nanotubes increased with the concentration of the precursor.

The last sample of concentration 2 shows a different aspect from the others. It is made up of leaves (chites) and nanoparticles, with a total absence of nanorods and the appearance of a few nanoneedles of low density. The dispersion of the chites is practically homogeneous and has no typical shape. The images obtained at high magnification (x100000) show that the surfaces are more or less rough and vary from one area to another (Figure 2).

The last sample, with a concentration equal to 2, has a different appearance from the others: the material is made up of sheets (chites) and nanoparticles with the total presence of nanorods, although there are some nanoneedles with a low density. The dispersion of the sheets is practically homogeneous and shows no typical shape. A large grating of x100000 reveals surfaces that are more or less rough, with a roughness that varies from one area to another.

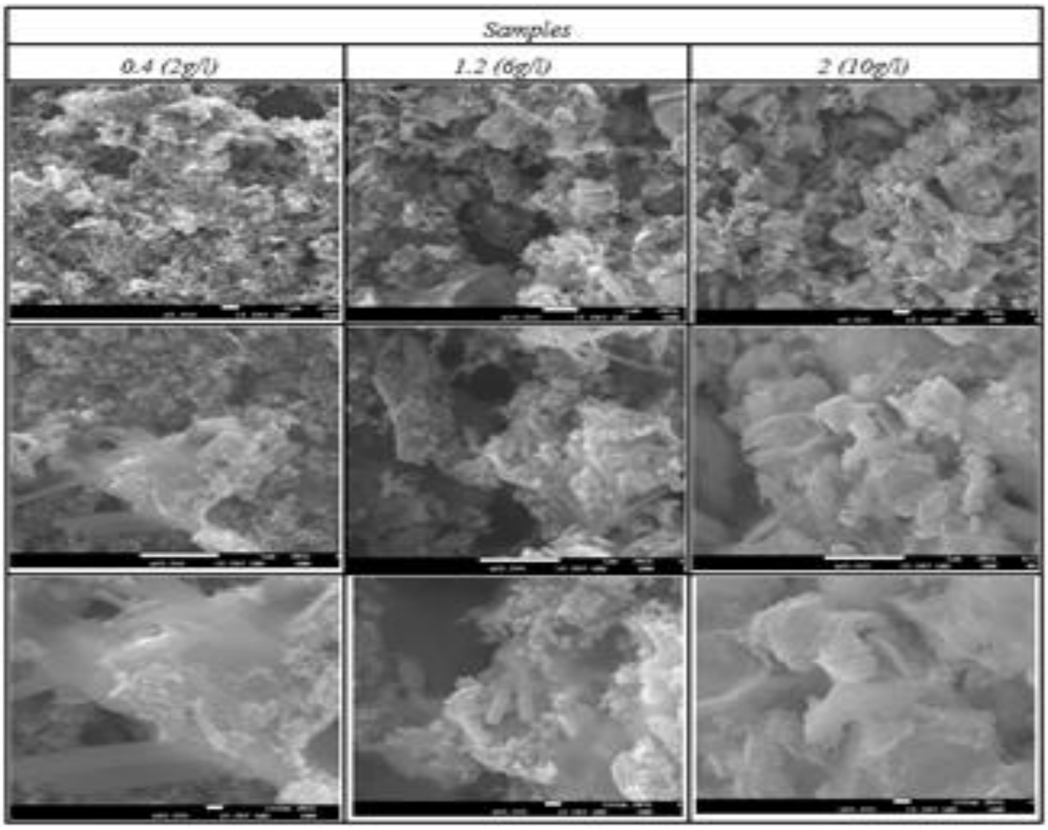

Figure 2: SEM images of the different samples of powders (0.4), (1.2) and (2) taken at different magnifications.

Chemical composition and proportions of elements in each sample

The results of the analyses (Table 2) show that the sample prepared with a precursor concentration of 2 contains a small amount of carbon, the absence of phosphorus (P) and copper (Cu) in this sample were also noted.

Table 2: Summary table of the chemical elements present in the samples synthesised with different concentrations of ZnSO4: (0.4), (1.2), (2).

Samples	***0.4mg/ml***		***1.2 mg/ml***		***2 mg/ml***	
Elelments	*Mass%*	*Atom%*	*Mass%*	*Atom%*	*Mass%*	*Atom%*
C	//	//	//	//	0.69	2.16
O	23.44	48.88	16.82	41.86	17.66	41.40
P	1.50	1.61	1.18	1.52	//	//
S	8.62	8.97	5.14	6.38	8.39	9.81
K	12.06	10.29	6.67	6.79	9.14	8.77
Ca	7.73	6.43	1.85	1.84	3.00	2.81
Cu	1.42	0.75	1.43	0.90	//	//
Zn	45.23	23.07	66.90	40.72	61.12	35.05
Total	100	100	100	100	100	100

Pathogenicity bioassay

As shown in Figure 3, seven days after treatment, the mortality rate of *Aphis fabae* ranged from 11% to 100%. The findings indicated that the 2 mg/L dosage was the most toxic, achieving a mortality rate of 96.66%, followed by the 1.2 mg/L dose with 80% mortality, and the 0.4 mg/L dose with 34.07% on the fourth day post-application. Furthermore, the study revealed that all three concentrations had the highest impact on insect mortality by the seventh day after application.

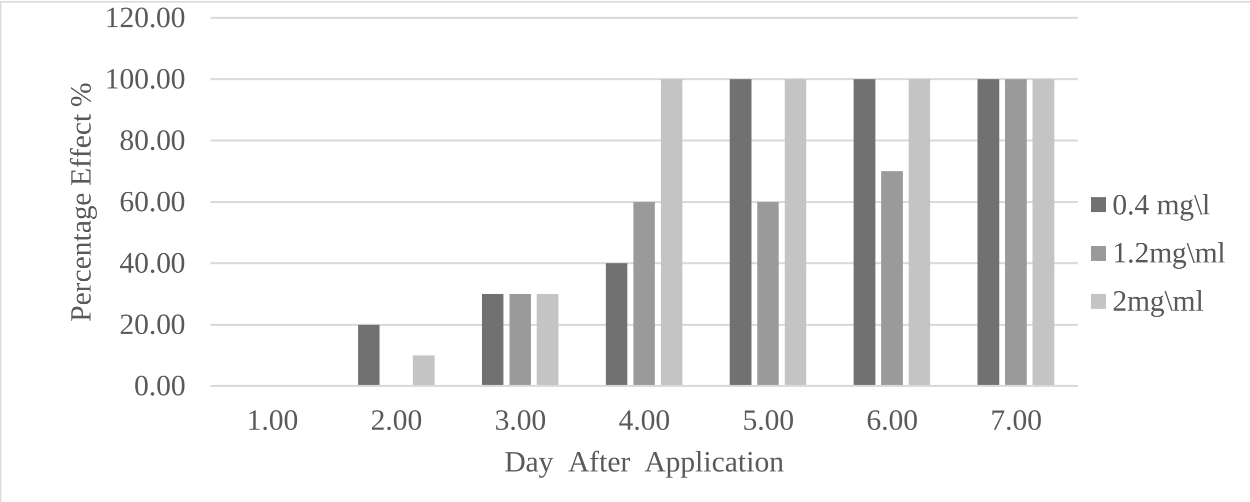

Figure 3: Percentage mortality of three doses on *Aphis fabea*

All doses produced different mortality values among treatment; the highest mortality value was obtained on 7 days post treatment (F7DAA = 32.698, df = 3, p ≤ 0.00) (Table 3 , Figure 4 and 5).

Table 3: different mortality values among treatment

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Model	3738.040[a]	10	373,804	80,742	,000
DOSE	454,458	3	151,486	32,721	,000
DAY	592,419	6	98,736	21,327	,000
Error	337,960	73	4,630		
Total	4076,000	83			

R Squared = .917 (Adjusted R Squared = .906)

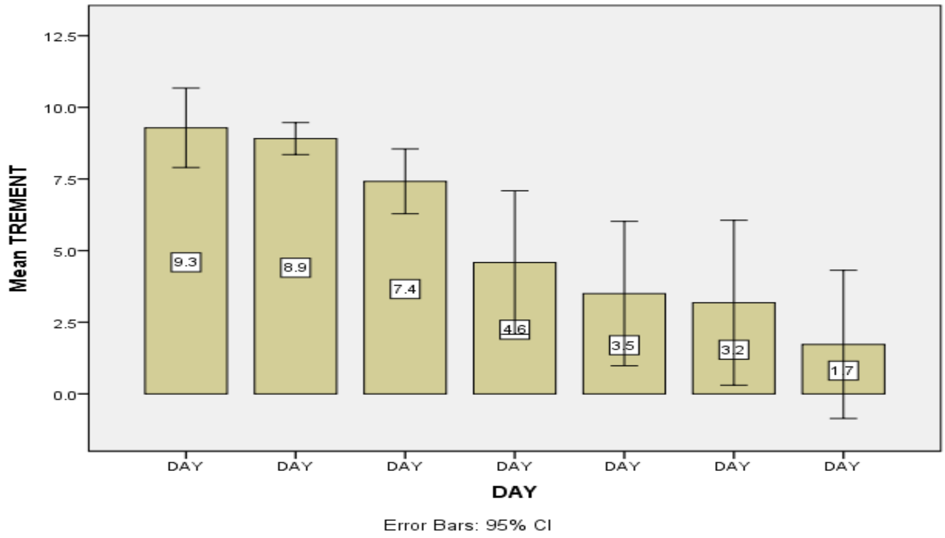

Figure 4 : Different mortality values among treatment

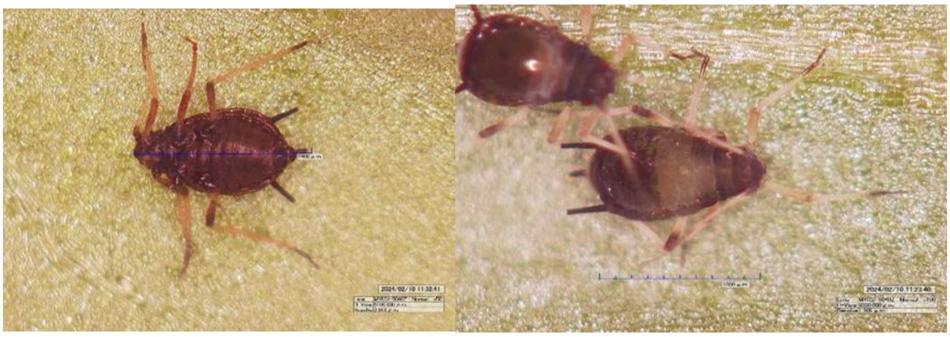

Figure 5 : Efcacy of ZnO NPs against *Aphis fabea*

DISCUSSION

The sustainable development of agriculture has been hampered by the unscientific use of synthetic pesticides, which has had various negative effects on the environment. Nanoparticles have shown great potential in the development of pesticide formulations in recent years, as they can be used as carriers to improve pesticide delivery (Wang *et al* .,2022)

The analysis results show that the sample prepared with precursor concentration contains a small amount of carbon, possibly due to incomplete disappearance of organic matter. The solution to this problem is either an increase in calcination temperature or an increase in calcination time. The absence of Phosphorus (P) and Copper (Cu) in this sample could be due to the fact that they exist in trace amounts or that they remain in the organic part. The removal of sulphur (S) can be solved by replacing the precursor of ZnSO4 with another precursor of Zn, in this case zinc acetate or zinc chloride.

It is thought that the effect of ZnO NPs is due to its small size and thus the ease with which they penetrate into the insect cuticle and into the insect cuticle and thus interfere with the physiological processes of the physiological processes such as feeding and moulting. Our study results were similar to those reported by Pittarate *et al*.,(2021) in which a higher mortality rate and effect on female fertility, development and occurrence of malformations in all stages of *Spodoptera frugiperda* were observed after the application of ZnO NPs. Furthermore, these results follow those of Keratum *et al*., (2015), where mortality of the rice weevil, Sitophilus oryzae, increased with increasing exposure time and concentration of ZnO NPs.

Due to the rapid, inexpensive and environmentally friendly nature of the process, green biosynthesis of metal NPs has been widely adopted. Therefore, the green synthesis of ZnONPs using leaf extracts of various plants like *Azadirachta indica, Emblica officinalis, Kedrostis foetidissima, Allium sativum, Rosmarius officinalis* and *Ocimum bassilicum* with particle sizes ranging between 14 and 27 nm (Ahmed *et al*., 2017) and evaluated for efficacy against various pests (Jampilek and Kralova, 2019).

It is also considered environmentally safe and significantly effective against *M. persicae* under laboratory conditions. Other benefits of ZnO NPs include their ability to promote seed germination and root and shoot length in edible crops (Singh *et al.,* 2015), This makes them more suitable for crops that are susceptible to pest attack. However, further research is needed to study the effects of these nanobiopesticides and their impact on humans and the environment.

Ghidan *et al* .,2016 found that copper oxide nanoparticles reduced the of green peach aphid 1st, 2nd, 3rd and 4th nymphal instars by 86% compared to the control.

For the control of *S. litura* and *M. euphorbiae*, ZnO NPs have been shown to be a promising source of safe, yet effective insecticides. In addition, it has been observed that it can control several other insect pests in an environmentally friendly manner, and low doses of about 700 mg mL^{-1} had the highest insect mortality effect. When Aphis nerii infested leaves were immersed in different concentrations of ZnO. This has a considerable impact upon their evolution (Rouhani *et al*. ,2012). A similar result was obtained by Wazid *et al*., (2018), who studied the effect of nanoparticles synthesised from zinc oxide green (ZnO) on *Callosobruchus analis* (Fabricius).

The data from the various concentrations tested by Applied Nanoscience of ZnO nanoparticles indicated that adult mortality rose as concentration and exposure time increased. Of all the concentrations,2mg/ml , showing the highest mortality rate.. These findings align with those of Lakshmi *et al.*, (2020), who recorded a 100% mortality rate in the pulse beetle (*Callosobruchus maculatus*) when exposed to varying concentrations of ZnO NPs. Similar outcomes have been reported in other insects, including *Sitophilus oryzae* and *Trialeurodes vaporariorum*, with mortality rates reaching as high as 91% due to ZnO NP exposure (Khooshe *et al*., 2016).

Nevertheless, further research is needed to understand the residual effects and environmental fate of these nanobiopesticides, to determine their impact on humans and the environment and ensure their effective application under field conditions.

Conclusion

This study reveals the potential of *Citrus reticulata* peels in synthesizing ZnO nanoparticles (NPs), offering an affordable, simple, and effective solution that may also help overcome insect resistance. Additionally, this method is environmentally friendly and demonstrates significant efficacy against *Aphis fabae* in laboratory settings. However, more research is necessary to investigate the effects of these nanobiopesticides on human health and the environment, as well as to explore their residual impact and behavior within ecosystems, to ensure their safe and effective use in field applications.

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