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Responses of desert locust *Schistocerca gregaria* (Orthoptera: Acrididae) to treatment with chemically synthesized zinc and copper oxides nanoparticles

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Abstract:

The desert locust *Schistocerca gregaria* Forsskål (Orthoptera: Acrididae) is harmful insect which cause huge economic losses in agricultural sector all over the world. Nanotechnology is a promising field of interdisciplinary research. Moreover, it provides as ecofriendly and efficient alternatives for the management of insect pests in agriculture. The current work strives for obtaining safe, effective and economic insecticides; Zinc oxide (ZnO) and copper oxide (CuO) as alternative insecticides through the assessment of biological effect on *S. gregaria*. The nanoparticles were chemically synthesized in laboratory scale and Transmission Electron Microscopy (TEM) confirmed the nanostructure. The resulted LT50s were recorded after 10.994, 21.148 days respectively while latent biological responses of desert locusts were recorded in ZnO more than CuO NPs. Also, treatments revealed a notable inhibition in activity of Acetylcholinesterase (AChE.) enzyme.

Introduction

The desert locust *Schistocerca gregaria* Forsskål (Orthoptera: Acrididae) is polyphagous insect with much diversified food mode, including a high number of vegetable plants (up to 400 species) belonging to numerous families.) (Hahn, 2005). It affects vast area of about 28 million km2 that extends from the Atlantic coast of Africa to eastern India and from northern Turkey to Tanzania in the south (Symmons, 2009). Chemical pesticides were an emergency solution to these challenges. Consequently, managing the pathogens and pests need about 2 million metric tons pesticides worldwide per year (worth US $35 billion) (Stephenson, 2003). Given the ambition of scientists and agronomists for protecting agricultural crops from pests, overuse of chemical pesticides created pest resistance problems, and serious Impacts on environment and human (Safi *et al.,* 1993). Sequently, scientists turned to search for alternative solutions; nanoparticles are organic, inorganic or hybrid materials with at least one of their dimensions ranging from 1 to 100 nm (at the nanoscale) (NPs) it represents a new generation in agricultural technology which could be considered new agrochemicals (Owolade *et al.,* 2008). The unique physical and chemical properties make nanocompounds are more effective compared with conventional pesticides and fertilizers, (Chinnamuthu and Murugesa, 2009 and Torney *et al.*, 2007). Finally, it is clear that increasing nanocompounds applications in agriculture take into consideration diversity impacts of using different size of nanoparticls, encouraged scientists to study the changes in eco-system and its biological effects on cells and their components (Nowack and Bucheli, 2007 and Ramesh *et al.*, 2013).

It is worth mentioning that there are no complete studies on use nano compounds in control desert locust. Further, the current study suggests novel strategies for desert locust control, using Zinc oxide (ZnO) and copper oxide (CuO) NPs besides its common usage as fertilizers.

Material and methods

1.Preparation in nanoparticles:

Solvolthermal method was used to synthesize ZnO nanostructures according to Sangari (2015). by reacting of NaOH with Zn (CH3COO) 2.2H2O under continuous stirring to adjust PH value of mixture to (pH.8). A standard procedure was followed to synthesis of CuO-NPs, via chemical precipitation method, described by (Luna *et al.,* 2015) using copper chloride dehydrate as precursor. Morphology (size and shape) of the nanoparticles was obtained by Transmission electron microscope (TEM). The concentration 0.25% (w/v) of ZnO and CuO nanoparticles were used to study the latent effects on tested insect.

2.Experimental insect:

Adult (males and females) of the desert locust, *S. gregaria* were obtained from Plant Protection Research Institute, Dokki, Giza, Egypt. Adults were breed in the laboratory of Pest's Physiology Department, Plant Protection Research Institute, Sharkia branch, under crowded conditions as described by **Hunter-Jones (1961) and Hassanein (1965).**

3. Nymphal treatments:

The newly moulted 4th instar nymphs were isolated from the stock colony and divided into groups. Each group consisted of three replicates of 10 nymphs. the isolated groups were treated with different concentrations previously prepared ZnO and CuO nanoparticles, besides, group of untreated nymphs (negative control) and another group treated with 0.1% tween solution (positive control). Individuals full-spray technique described by **(Simon, 2014**) was applied, where nymphs were placed in 250 ml glass beaker with filter paper at the bottom and sprayed directly by 1ml of treatment suspensions then transferred in 1kg perforated plastic jars covered with gauze to insure high ventilation with maintaining the conditions and feeding that has been followed through the rearing system. Mortality was recorded daily for 14 days. The lethal/time median (LT_{50}) were determined by probit analysis based on the method by **Finney (١٩٧١).**

The same technique that applied in toxicological investigation with same experimental design of replicates. The observations of the mortality and deformities were recorded daily and photographed besides following up vitality and development's period of tested nymphs to adult.

4.Determination of Acetyl-cholinesterase (AchE):

Insect samples were collected after 48 h. after treatments of ZnO and CuO nanoparticles. Samples were homogenized in 5 - 10 ml cold buffer (50 mM potassium phosphate, pH 7.5., 1 mM EDTA) per gram sample weight using glass mortar. Homogenates were perfused in refreeze for a night then were centrifuged at 4,000 rpm for 15 minutes at 4 ° C. The supernatant was subjected to biochemical assay and store on ice. AchE (Acetylcholinesterase) activity was measured according to the method described by **Simpson *et al*. (1964),** using acetylcholine bromide (AchBr) as substrate. The decrease in AchBr resulting from hydrolysis by AchE was read at 515 nm.

Results and discussion

Resulted Zinc oxide (ZnO) was white amorphous powder while copper oxide (CuO) was a dark brown powder. Both Nano-powders showed low solubility in water and were semi-soluble in methanol. Transmission electron microscope showed micrograph of zinc oxide nanoparticles denoted by revealed that they were spherical with little agglomeration. Most of particles were present in the range 10-15 nm in size and possess an average size of 12 nm (Figure,1A) Moreover, TEM micrograph of CuO NPs revealed that the particles were rods in shape and the size came in the average between 11.5 and 13.5 nm which confirmed the formation of Nano structure (Figure, 1 B).

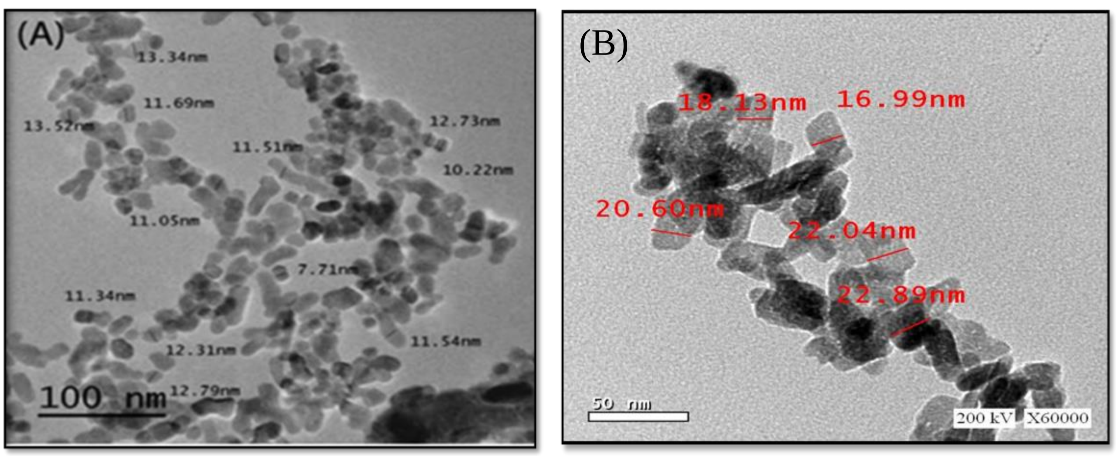

Figure (1): Transmission electron microscope (TEM) morphology; size and shape of ZnO (A) and CuO (B) nanoparticles.

Table (1) represented the LT50 of (4th) instar nymphs treated with the concentration of sub lethal concentration (0.25%) of CuO and ZnO NPS. the resulted LT50s were recorded after 10.994, 21.148 days respectively Furthermore, CuO clued an acute toxicity while ZnO maybe has chronic toxicity. these results lead to that ZnO nanoparticles may resulted in a residual effect more than CuO. results compatible with recent study by (Tuncsoy *et al*., 2019) who described the Insecticidal effect for nanoparticles against insects models by inhalation or ingestion and attributed that the physiological changes, occurring in the larvae were returned to accumulation of NPs in the larval midgut and fat body . On the other hand, biocontrol by microorganisms-based pesticides and plant extracts showed nearly the same latent mortality in *S. gregaria.* (Danyk *et al.,* 2005; Bravo *et al.*, 2011 and Sharma, 2014).

Table (1): LT_{50} of CuO and ZnO NPs 0.025% against 4th instar of *Schistocerca gregaria.*

Treatments	LT_{50} (Day)	Confidence limits		LT_{90} (Day)	Confidence limits		Slope
		lower	Upper		lower	upper	
CuO	10.994	9.287	14.103	33.192	22.568	70.991	2.671
ZnO	21.148	14.978	52.58	79.122	37.39	683.301	2.236

Effect of sub lethal concentration of NPs on Nymphal mortality was initially observed during 4th instar where both ZnO and CuO NPs resulted in a considerable mortality compared to untreated controls. Mortality rates attained 10.34%, 16.67% respectively, whereas the residual mortality recorded during the 5th instar didn't exceed 12% for CuO and 4.4% for ZnO compared to untreated controls (Table, 2). Furthermore, paralysis and decreased locomotion, trembling of abdominal segments were noted in treated nymphs, demonstrating the effect of nanoparticles on the nervous system. This phenomenon is often noted in insects treated with neurotoxic insecticides (Proux *et al*., 1993).

Furthermore, the effect of NPs on Nymphal duration was verified. At ZnO treatment duration of nymphal instars was longer as insects developed into a more advanced stage. While exposure to cuo resulted in reversed findings on the duration of nymphal instars which was shorter compared with untreated controls. Nymphs attained the longest duration (7.85 and 8.45 days) in 4th and 5th instar that respectively with ZnO treatments. Moreover, nymphes treated with CuO recorded 5.3 and 7.09 days in 4th and 5th instar respectively. Additional findings were reported in Table (2) and Figure (2). Nymphal development was affected by treatments with ZnO NPs which revealed a notable deficient molting during development from 4th instar to 5th instar reached 12.4% of survival nymphs likewise, 18.5% deficient molting recorded in development from 5th to adult stage. No deficient development was recorded with CuO treatment. Controversial reports regarding the toxicity of the ZnO in the living cells, particularly in mammalian cells. Some of the reports have shown that ZnO is biocompatible and nontoxic (Zvyagin *et al*., 2008), while some studies have recently reported both in vivo and in vitro toxicity of the ZnO on mammalian cells (Tian *et al*., 2015). It can be elucidated from such studies that the toxicity of ZnO depends upon the concentration and its nano size. On the other hand, there were no studies that can be considered to understand the CuO NPs effects on living organisms.

Table (2): Biological effects of CuO and ZnO NPs (0.025%) on nymphalid stage of *Schistocerca gregaria.*

	4th instar			5th instar		
	Mortality%	Duration (Day)	Deficient-molting %	Mortality%	Duration (Day)	Deficient-molting%
Control	0.0c	6.50b	0.0a	0.0b	7.72b	0.0b
Control-tween (0.01%)	0.0c	6.75b	00.0a	0.0b	7.63b	0.0b
ZnO	10.344b	7.85a	11.489a	4.412b	8.45a	18.793a
CuO	16.670a	5.3c	0.0a	12.00a	7.09c	0.0b
$LSD_{0.05}$	2.996	0.219	9.477	2.145	0.149	5.438
P	0.0000 ***	0.0000 ***	0.0943 ns	0.0000 ***	0.0000 ***	0.0002 ***

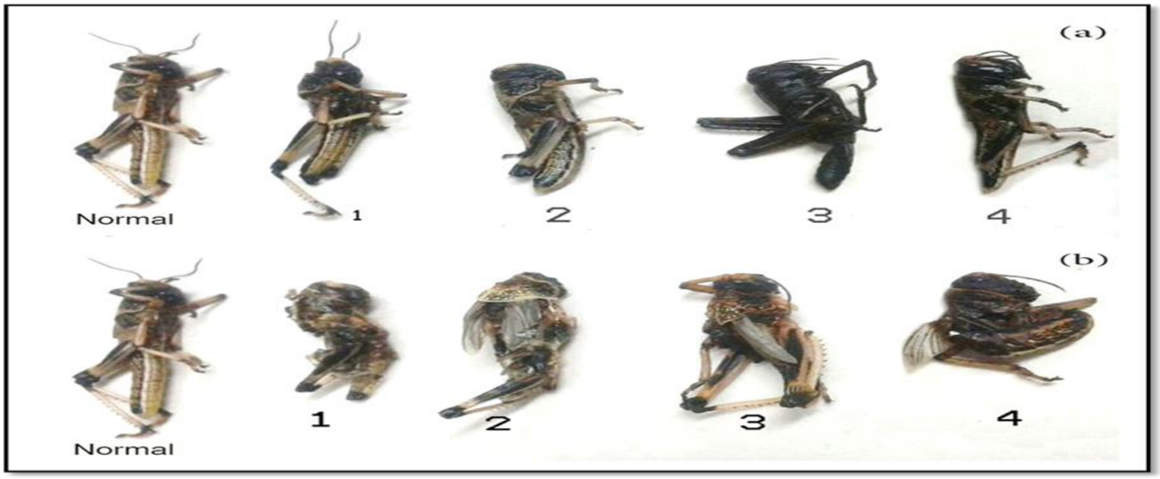

Figure (2): Death symptoms and incomplete molting of *Schistocerca gregaria* treated with CuO (a) and ZnO (b) nanoparticles during nymphal stage.

The activity of AChE. enzyme in Figure (3) showed a marked decrease in both tested nanopartecle where the enzyme activity with ZnO reached (4.781 U/gm), and (9.41 U/gm) with CuO treatment. Comparing to the negative and positive control that recorded (22.48, 15.06 U/gm.) respectively, these obtained results cleared that ZnO was more effective than CuO in inhibition of AChE with a statistically significant difference (LSD=4.566, P=0.0001). this reduction attributed to nanoparticles crossed the blood-brain barrier and gained access to the central nervous system, this led to binding with acetylcholinesterase (AChE) and affected its activity and hydrolyzing the neurotransmitter Acetylecholine into choline and acetic acid in cholinergic synapses as discussed by Hu and Gao (2010).

Our results enhanced by other studies detected that TiO_2 NPs reduced AChE. activity by Yixi *et al.* (2014). Although disagreed with Milivojević *et al.* (2015) that stated increasing in AChE. by exposer bees to ZnO NPs. Where there are many parameters can affect such as chemical, physical and geometrical properties

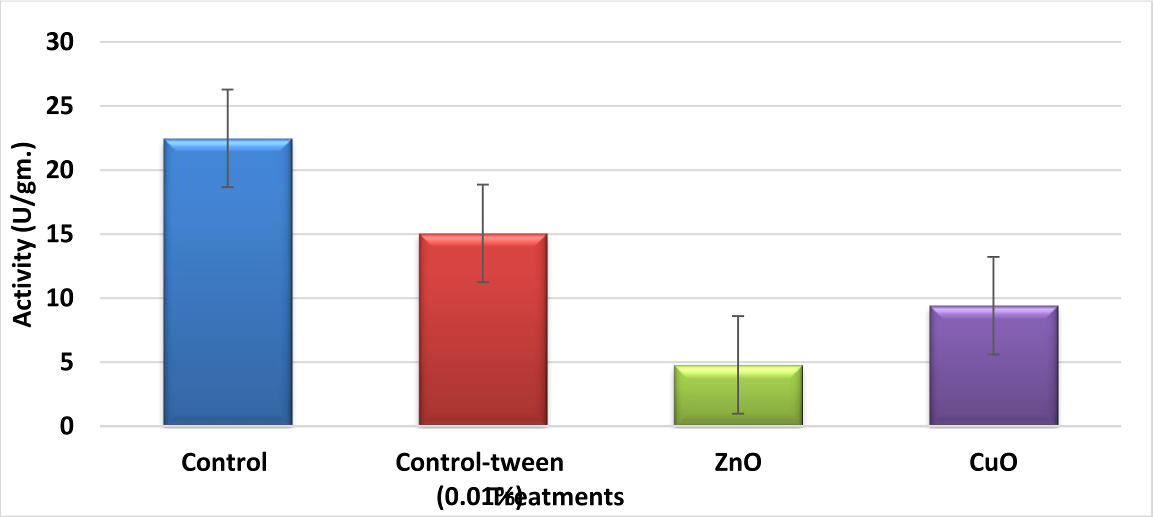

Figure (3): Effect of CuO, ZnO NPs on activity of AChE. enzyme in 4th instar of *Shistocereca gregaria* .

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