Nanoparticles as Attractants of Scolothrips sexmaculatus to Its Treated Prey, Tetranychus urticae

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NANOPARTICLES AS AN ATTRACTANTS OF *SCOLOTHRIPS SEXMACULATUS* TO ITS TREATED PREY, *TETRANYCHUS URTICAE* KOCH

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Abstract

Nanoparticles showed their efficiency to control the two spotted spider mite, *Tetranychus urticae* Koch, directly or indirectly. Directly, LC50s of Zinc and Copper nanoparticles against the adult females of *T.urticae* (green form) recorded 10. 94 μLL^{-1} and 4.25 μLL^{-1} while they were 12.64 μLL^{-1} and 5.43 μLL^{-1} against *T.urticae* (red form), respectively.

Scolothrips sexmaculatus Pergande attracted to both morphs of *T.urticae* before and after treatments with significant difference. The indirect effect of nanoparticles could be shown by the predation of *S. sexmaculatus* on the treated *T.urticae*. *S. sexmaculatus* in direct olfactory tests recorded 96.67% and 80% on the green form treated with ZnO and CuO nanoparticles in comparable with the control (56.33%).However, the predation ratios were 100% and 90.34% on the red form, while the control recorded 66.67% . Besides, tests concerning Y-tube olfactory attraction were done in the presence and absence of 30 adult females and males of green and red forms of *T.urticae.* The inhibition of monoamine oxidase in the treated *T.urticae* was contributed in the attraction of *S.sexmaculatus* to the red form more than green form. There was a strong relation between accumulation of biogenic amines in the treated mites with nanoparticles and *S.sexmaculatus* attraction to the certain prey.

KEY WORDS: *Tetranychus urticae*, *Scolothrips sexmaculatus,* nanoparticles, Predation, Monoamine Oxidase (MAO)

INTRODUCTION

The two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae), is a widespread agricultural pest, causing severe damage on a variety of greenhouse and field crops (Cranham 1985). Spider mites are difficult to control with pesticides (Nahar et al., 2005) due to inaccessibility of lower leaf surfaces, short life cycle, high reproductive capacity, and ability to develop resistance to miticides (Cranham and Helle 1985; Georghiou 1990).

Biological control, using natural enemies, is an alternative strategy to manage mites in agricultural systems. Natural enemies play a major role in the ecology of spider mites, including ladybird beetles (Coleoptera: Coccinellidae) (Obrycki and Kring 1998; Mori et al. 2005), which generally accept a large number of prey species and frequently show a preference for one species (Hodek 1973), and predatory mites (Acari: Phytoseiidae) (Gotoh et al. 2004; Friese and Gilstrap 1985). In addition, acarophagous thrips (Thysanoptera: Aeolothripidae, Thripidae) are important natural enemies, and have various degrees of specialization on various mites; however, many species of *Scolothrips* commonly known as acarophagous ladybird beetles, are predators of agricultural crop pests and significantly contribute to the control of spider mite pests (Lewis 1973; Gilstrap and Oatman 1976; Roy et al., 2002; Gotoh et al., 2004).

The six-spotted thrips *Scolothrips sexmaculatus* Pergande is one of the important predators of spider mite. Its adult consumes about 1000- 3000 *T. urticae* during its lifetime (Hoddle, 2004). *Scolothrips sexmaculatus* Pergande and *Stethorus punctillum* Weise are the important predators of two-spotted spider mite (TSSM), *Tetranychus urticae* Koch.

Nanoparticles possess distinct physical, biological and chemical properties associated with their atomic strength (Leiderer and Dekorsy, 2008).Nanoparticles (which are 1-100 nm in diameter) are agglomerated atom by atom, and their size (and some-times shape) may be maintained by specific experimental procedure (Roy 2009).They can be arranged or assembled into ordered layers, or mine layers (Ulrich 2006).

Such self-assembly is due to forces such as hydrogen bonding, dipolar forces, hydrophobic interactions, surface tension, gravity and other forces. Thus nanotechnology deals with the targeted nanoparticles as and when the particles exhibit different physical strength, chemical reactivity, electrical conductance and magnetic properties (Nykypanchuk et al., 2008).

Nanotechnology, a promising field of the scientific research opens up in the present decade a wide array of opportunities and is expected to give major impulses to technical innovations in a variety of industrial sectors in the future. Nowadays, nanotechnology has being embraced in the world of pesticides and pest control (Harper 2010) and has a potential to revolutionize modern day agriculture pest control, different groups of nano pesticide overcome like insecticides, fungicides, herbicides (Matsumoto et al. 2009) .

So the main target of this research is using nanoparticles not just for controlling the two forms of *T.urticae* but also to estimate the indirect effects of the treated forms with nanoparticles to attract the certain predator, *Scolothrips sexmaculatus.*

MATERIALS AND METHODS

-*Tetranychus urticae* Cultures

The green and red forms of *Tetranychus urticae* Koch were collected from naturally infested cowpea (*Vigna unguiculata*) and strawberry (*Fragaria ananassa*) plants respectively. Then they were reared under laboratory conditions on discs of castor oil bean plants according to Abd El-Wahab (2010) for six months before treatments.

-Nanoparticles

Zinc Oxide (ZnO) and Copper Oxide (CuO) nanoparticles were prepared at NANOTECH Co. at 90% concentration and 100 nanometer volume.

-*Scolothrips sexmaculatus* Pergande Culture

Adults of the predator *S. sexmaculatus* were explored from naturally infested castor oil bean plants. They were collected, brought to the laboratory and identified with a stereo binoculor microscope. After confirming their predation, 50 Adult females and males of each predator were kept under laboratory conditions in the big plastic cells (30*10*5 cm). Moistened pads of cotton with discs of castor oil leaves with both forms of *Tetranychus urticae* Koch on them were presented as prey of the certain predators. They were maintained for six months before testing their efficiency.

-Voracity Assessment

Measurement of the voracity of adult females of predators to *T. urticae,* infested leaves, using a 3 cm diameter cut from the manipulated plants then served as the source of the volatiles for selection circular arena. Treatments depending mainly on Nanoparticles LC50s. Every treatment was triple replicated beside control. The exposure was approx. for 1 hour (Raworth 2001).Every predator had a full choice to go to its preferable prey treated with nanoparticles LC50s.

-Olfactory Response

1-Direct Olfactory Experiments

Infested plants with spider mites, produce a unique blend of volatile compounds to which *Scolothrips sexmaculatus* respond more strongly than they do to uninfested plants (Sabelis and Van der Meer 1986; Dicke et al. 1990a, b) Assessment of the response of the predator adult females to released biogenic amines from two forms of *Tetranychus urticae* Koch as a prey, was done according to (Nachappa et al.2009) with modifications as the following:

2 leaf discs, each (2.2 cm diameter) cut from fresh castor oil bean plants then served as the source of the phyto volatile. Two arenas were setup vertically side by side with a divider which is cardboard (60 cm htX55 cm w) between them to reduce air movement and possible drift of volatiles. In the central of one, there was a leaf disc with a *Tetranychus urticae* form, and on the other there was placed a leaf disc from an uninfested leaf disc as a control.

30 adult females of the predator were starved for 24 hours to join each test and then released at the edge of the arena. The numbers of the predators which found the central leaf disc (source of volatiles) in the arena in the first 10 mins were recorded.

The mentioned experiment was separately done to study the olfaction response of predator to each form of *T.urticae* treated with Nanoparticles LC50s under laboratory circumstances.

2- Y- Olfactory Experiments

Discs with spider mites were placed on the main opening arms of Y-tube in order to test olfaction response of females of both predators to males and females of their preys which treated with Nanoparticles LC50s.

2 leaf discs, each (2.2 cm diameter) cut from fresh castor oil bean plants then served as the source of the phyto volatile. The Y-tube olfaction depending on the determination of the highest predation on females and treated males of the same

form and then compare them with the control. The arrangements were done according (Nachappa et al.2009) with modifications as explained at direct olfaction test.

-Effects on Monoamine Oxidase

MAO-A is a flavin adenine dinucleotide (FAD) containing enzyme which is tightly anchored to the mitochondrial outer membrane. MAO-A inhibition potencies treatments were determined in the homogenates of each treatment. The rate of the MAO catalyzed oxidation of Kynuramine was measured according to Aiyegoro and Van Dyk (2011). Kynuramine is non-fluorescent until undergoing MAO-catalyzed oxidative deamination and subsequent ring closure to yield 4-hydroxyquinoline, a fluorescent metabolite. The concentrations of the MAO-generated 4hydroxyquinoline in the incubation mixtures was determined by comparing the fluorescence emitted by the samples to that of known amounts of authentic 4-hydroxyquinoline at excitation (310 nm) and emission (400 nm) wavelengths. All enzymatic reactions were carried out to a final volume of 500 µL in potassium phosphate buffer and contained kynuramine as substrate, MAO-A (0.0075 mg/mL) and various concentrations of the test inhibitor (treatment). The reactions were carried out for 20 min at 37°C and were terminated with the addition of 200 µL NaOH (2 N). After the addition of distilled water (1200 µL) to each reaction, the reactions were centrifuged for 10 min at 16000 × g. To determine the concentrations of the MAO generated 4-hydroxyquinoline in the reactions, the fluorescence of the supernatant at an excitation wavelength of 310 nm and an emission wavelength of 400 nm were measured (Novaroli et al., 2005).

-Data Analysis

SPSS (V.16) was used to show differences among treatments and test significance among groups through experiments in the present research.

RESULTS

Data revealed that nanoparticles have a direct and indirect effect on the two forms of *Tetranychus urticae*.They can cause direct mortality and when treatments were done by nanoparticles LC50s, then they were able to work successfully as attractants of *Scolothrips sexmaculatus* Pergande to *Tetranychus urticae* forms, especially red form.

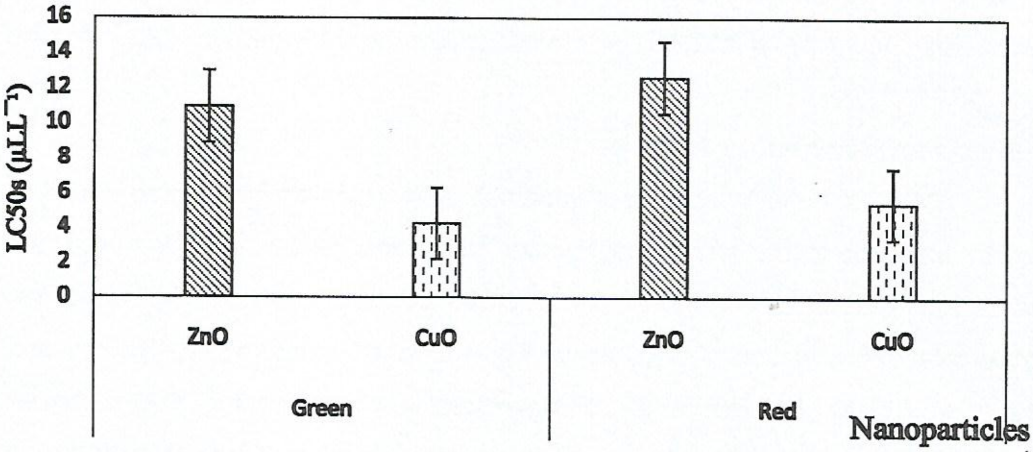

Figure (1) Nanoparticles LC50s against the Two Forms of *Tetranychus urticae* Koch

- **Voracity**

Table (1) showed the voracity values of *Scolothrips sexmaculatus* on both treated forms had a significant difference depending on Kendall's Coefficient of Concordance=.848* and Chi-Square=10.182* at 5%.There was a significant correlation at the 0.05 level (2-tailed) between voracity of *S. sexmaculatus* and treatments with nanoparticles. Estimated correlations were Pearson Correlation=-.875*, Spearman's rho=-.837* and Kendall's tau_b=-.745*. Paired Samples Test at the 0.01 level (2-tailed) was used to show that was a significant relation between voracity and treated form (t=11.541**) and also the same in the relation of voracity and nanoparticles treatment voracity – nano (t=10.771**) with little difference than previous.

- **Direct Olfactory**

Direct Olfactory and Effect of nanoparticles treatments on *Scolothrips sexmaculatus* results were recorded at Table (1) .Among treatments there was significant difference at 99% depending on Paired Samples Test (t=13.006***,Corr -.899) and the mentioned relation was more significant than olfactory and *Tetranychus* forms relation (t=14.019**,Corr .346),Table (1).

In the interaction with nanoparticles, it was clear that treated red form attracted the predator significantly (t=10.902***) more than treated green form (t=8.462*). To get the confirmed evidence that Olfactory response differences in data were mainly revised to the nanoparticles' treatments, the Jonckheere-Terpstra (Std. Deviation of J-T Statistic) = 2.291* and Kruskal Wallis (Chi-Square=1.190*).

- Y-Tube Olfactory Attraction

Table (1) showed that different types used of nanoparticles caused significant difference in predator's olfaction to be attracted to *T.urticae* forms. Nanoparticles interaction with certain forms showed through One-Sample Test (t=3.464*).There was a significant attraction of the predator to the females of both forms treated with nanoparticles appeared through Kruskal Wallis (1.190**),J-T Statistic (7.000**) and Std. J-T Statistic (1.091).On the other hand,the presence of *Tetranychus* males changed olfaction significantly with Kruskal Wallis (.429*),J-T Statistic (6.000*) and Std. J-T Statistic (.655).While less significant difference showed through the response of the predator to none presence adult females of the two forms of *T.urticae* with Kruskal Wallis (.429*) depending on J-T Statistic (6.000*) and Std. J-T Statistic (.655).But there was no significant effect in the case of olfaction with the none presence of *T.urticae* males. That was proved by Kruskal Wallis (.196) depending on J-T Statistic (5.500) and Std. J-T Statistic (.443).

Table 1. Voracity and Olfaction Percentages of *Scolothrips sexmaculatus* Pergande to the treated two forms of *Tetranychus urticae* Koch with nanoparticles.

Tetranychus urticae	Nanoparticles	% Voracity	Direct Olfactory	% Y-Tube Olfactory Attraction			
				With 30 Adult Females	Without 30 Adult Females	With 30 Adult Males	Without 30 Adult Males
				T.urticae			
Green Form	*ZnO*	83.33	96.67	76.67	50	66.67	40
	CuO	70	80	46.67	33.33	40	30
	Control	56.67	63.33	13.33	0	16.67	0
Red Form	*ZnO*	100	100	100	80	90	60
	CuO	86.67	90	86.67	46.67	83.33	33.33
	Control	63.33	66.67	16.67	3.33	13.33	0

- Effects on Mono Amine Oxidases (MAO)

Effects of nanoparticles on Mono Amine Oxidases (MAO) of *T.urticae* adult Females in both two forms were highly significant in general (Figure 2). With lower MAO activity, the more accumulation of biogenic amines occurred and then attraction of the predator increased as a result.

The specific activity as shown at figure (2) of MAO was higher in the treatments than control. ZnO affected MAO activity with 1.12 and 1.73 mOD min-1 mg-1 proteins while CuO affected MAO activity with 2.03 and 2.94 mOD min-1 mg-1 proteins in comparison with control (3.67 and 5.84 mOD min-1 mg-1 proteins) in green and red forms of *Tetranychus urticae*, respectively.

Partial correlation between nanoparticles and MAO of both treated mites recorded .812*. Paired Samples Correlations (.395) and Paired Samples Test (t=-2.165) between two forms and resulted MAO ratios in comparable with control showed non-significant difference at 95% (Sig. (2-tailed) =.083) which means that the main difference depended mainly on the treatments.

To prove so, Paired Samples Correlations =-.874 with significant difference =.023* and among variables (t=6.708**), MAO (t=4.150**) with the lowest record of MAO in case of red form treated with ZnO

Kendall's tau_b Correlation Coefficient between Nanoparticles and MAO =.-.894*, Spearman's rho=-.956** and Pearson Correlation=-.874* were calculated to confirm results. Morover, R=.874*, R^2=.763, Adjusted R^2=.704.

ANOVA showed that F=12.884* which showed significant relation between MAO and nanoparticles treatments. Mainly calculated Standardized Coefficient of affected MAO is (t= 7.934**)

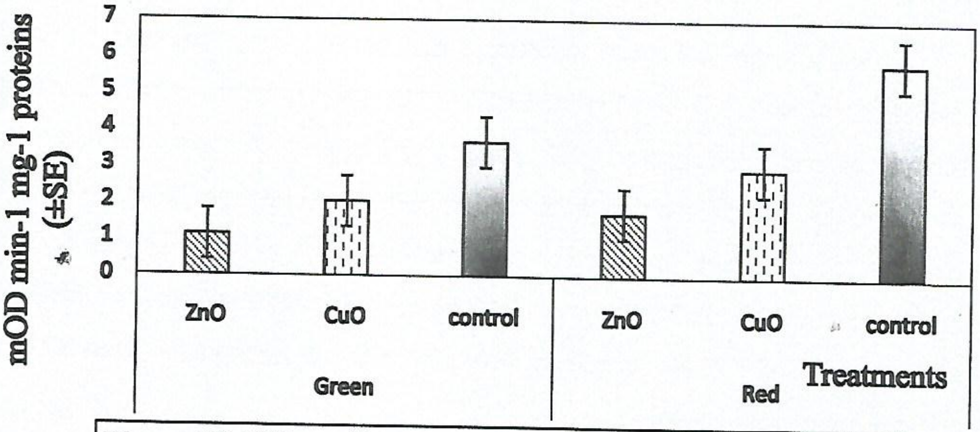

Figure (2) Effect of Nanoparticles on Mono Amine Oxidases (MAO) in The Two Forms of *Tetranychus urticae* Koch

DISCUSSION

It was observed clearly that the attraction of *S.sexmaculatus* to red form of *Tetranychus urticae* was more than green form. There was an explanation related mainly to cyanatelyase encoding gene that might be involved in feeding on cyanogenic plants (Grbić et al.2011) and that was available in strawberry plants.

Also, Carotenoids are organic pigments commonly synthesized by plants, algae and some microorganisms. Through absorption of light energy, carotenoids facilitate photosynthesis and provide protection against photo-oxidation. Altincicek et al. (2012) mentioned that while it was presumed that all carotenoids in animals were sequestered from their diets, aphids were recently shown to harbour genomic copies of functional carotenoid biosynthesis genes that were acquired via horizontal gene transfer from fungi. Their search of available animal transcripts revealed the presence of two related genes in the two-spotted spider mite *Tetranychus urticae*. Phylogenetic analyses suggest that the *T. urticae* genes were transferred from fungi into the spider mite genome, probably in a similar manner as recently suggested for aphids. The genes are expressed in both green and red morphs, with red morphs exhibiting higher levels of gene expression.That was explained the highly saturated colors of *Tetranychus urticae* which were found on plants exposed to LEDs and specifically red *T.urticae* under blue color (Abd El-Wahab and Abouhatab 2014).

Loss of the biological functions of proteins may be due to oxidative modification that leading to the production of carbonyl groups (=C=O).These groups are stable and specific, and their appearance causes permanent changes in the structures of the proteins (Davies et al. 1999, Dalle-Donne et al. 2003). Those changes join oxidative stress which occurred as a result of treatments with Vertimec and increased with joint action of nanoparticles in *Spodoptera littoralis* larvae (Abd El-Wahab and Anwar 2014).So gained results from the present paper were in the same trend of mentioned research.

Abd El-Wahab and Anwar (2014) proved that nanoparticles have an important role to control pests even by directly use or indirectly as an additive to other pesticides. Copper and Zinc Nanoparticles showed significant effect on the 2nd instar larvae of *Spodoptera littoralis* even alone or combined with Vertimec. ZnO, CuO+Vertimec, ZnO+Vertimec, and CuO nanoparticles and Vertimc caused mortality with 100, 100, 86.67, 73.33 and 33.33 %, resp. Some treatments showed malformation and morphological changes by nanoparticles adsorption through the integument of the 2nd larval stage of *Spodoptera littoralis*. Most of the dead larvae malformed and underwent liquefaction. Apoptosis was showed through DNA damage response, which increased with the addition of Vertimec to nano metal particles but nano ZnO recorded the highest effect. Oxidative stress interacted effectively with induced DNA as a result of treatments.

Accumulation of biogenic amines played the specific role in the attraction of *S.sexmaculatus* to *T.urticae* forms and mainly red morph. Biogenic amines could be revealed potential new target sites for the development of future pesticides such as targeted in the present paper by LEDs (Abd El-Wahab et al.2014) and also as mentioned by Fuchs et al. (2014).They recently proved that biogenic amines affected mosquito fertility. Even egg melanisation was regulated by adrenergic signalling, whose disruption caused premature melanisation specifically through the action of tyramine. The strong cumulative negative effect was on mosquito locomotion and survival. Dopaminergic and serotonergic antagonists such as amitriptyline and citalopram recapitulated this effect.

Biogenic amines have a wide variety of functions in both the central and peripheral nervous systems of insects. They can act as neurotransmitters, neuromodulators and even circulating neurohormones. Knowledge of the pharmacology of the receptors that mediate the actions of biogenic amines in insects is increasing, there was only one known example of a pesticide that activates biogenic amine receptors. The knowledge of the mode of action of insect biogenic amine receptors is mediated through second messenger systems. However, Evans (1985) mentioned that no pesticides are known to bring about their actions by directly interfering with second messenger systems in insects, but now and after present research, it can be said that nanoparticles are able to do so action even lonely or with joint action of Vertimec. Beside so, Light Emitting Diodes (LEDs) has highly effects on MAO and they are resembling ecofriendly control tool (Abd El-Wahab *et al.*2014).

Through this paper, it can be recommended that nanoparticles could be used as an attractant factor of insect predators successfully and could be joined in integrated pest management (IPM).Future studies could be depending on the internal effect of nanoparticles on the insect predators. But as a personally hint and through this research, no bad side effects were noticed on the predator individuals.

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