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Research Paper

Green gallic acid nanoparticles to reduce metabolic pesticides' resistance of some mites

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ABSTRACT

Studies on metabolic resistance to several pesticides are on the increase all over the world. Therefore, there is a desideratum to get green available material, which could be adjusted to reduce pesticides' resistance in some pests, such as tetranychid mites: *Tetranychus urticae*, *Panonychus ulmi* and *Oligonychus mangiferus*. In this study, they were found to be resistant to Fenvalerate (Pyrethroids) with high resistance ratios (28.57, 32.46 and 37.99 -folds) and to Spinosad (Spinosyns) with (27.33, 31.87 and 56.28 -folds), respectively before treatment with green nanoparticles of gallic acid. After treatment, all resistance ratios (RRs), that is, for Fenvalerate (27.36, 29.80 and 35.13 -folds) and Spinosad (26.55, 30.53 and 27.21-folds), respectively decreased gradually. Cognitions among prosperous decrease of metabolic pesticides' resistance, increase in the activity of mono amine oxidase (MAO) and the augment ratios of ascorbate peroxidase (APX), as a reactive oxygen scavenger (ROS), appeared distinctly and resulted in susceptibility to pesticides. The results of this study provide a raising hope to reduce metabolic resistance by eco-friendly implementation and preservation of the ecosystem.

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Key words: Nanoparticles, gallic acid, mites, resistance, pyrethroids and spinosyns.

INTRODUCTION

There are many pesticides used widely to control tetranychid mites. Spinosad, a fermented product from *Saccharopolyspora* sp., is a good example of such pesticides. It is lethal by ingestion and contact, and has selective activity on the nervous system of insects (Van Leeuwen et al., 2005). This compound has an action on different insects' orders (Williams et al., 2003) and in the same time it has accepted LC50 over 5000 mg and therefore it was consigned to Class I (Florim and Nakano, 1997), so it is too safe for non-target organisms.

Another pesticide is Fenvalerate (Pyrethroids), an old group of pesticides, utilized for a long time and some pests are able to develop resistance against it. Therefore, many scientists are currently focus on proffering solution to pesticides' resistance quandary, such as that in cucumber (Kim et al., 2013; Samuoliené et al., 2010).

Concerning the presented solution, green nanoparticles

have been obtained from GallaeRhois, which resulted from aphids feeding, primarily *Schlechtendaliachinensis*, on *Rhusjavanica* L. Semi-polar extract showed strong, dose-dependent and radical-scavenging consequences for sundry radicals (Lee et al., 2012).

The harmful impacts of NPs can be ascribed to infinitesimal size and gigantically expanded surface territory, which augment synthetic reactivity and puncturing in living cells (Gojovaet al., 2007; Pan et al., 2009). Beside, gallic acid has been used as a reducing and stabilizing agent for composites of GA-AgNPs (Li et al., 2015). However, reactive oxygen species (ROS) increased the capacity of the antioxidant of the cell, which could lead to adverse biological consequences. They are generated during photo-activation, particle surface reactions, or during interaction' between particles and cellular components (Dreher, 2004; Hirakawa et al., 2004).

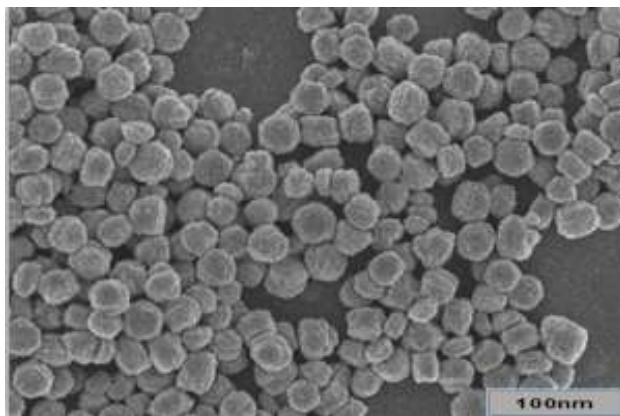


Figure 1: Green nanoparticles of gallic acid (100nm) by scanning electron microscope (SEM).

Therefore, this study aimed to present an incipient trend to reduce pesticides' resistance in certain tetranychid mites with a felicitous application of green nanoparticles of gallic acid.

MATERIALS AND METHODS

Used pesticides

Fenvalerate (Pyrethroids) Insecticide 20% EC
Spinosad (Spinosyns)Insecticide 2.5% SC

Green nanoparticles

They were formed mainly of GallaeRhois, which is known as a scavenger of free radicals. Milled galls were collected from certain infested plants leaves obtained from Shenyang, China. Extraction of gallic acid was done from GallaeRhois according to procedure of Kubo et al. (2003). Aqueous ethanol extraction was treated until yellowish residue was obtained. Partition of ethanol crude extract was done by different solvents of polarity and then evaporation occurred. Subsequently, the polar piece of the ethanol extractable portion of the white powder comprised solely of gallic acid. Transformation of gallic acid to nano form was done following the procedure of Abd EL-Wahab (2010) with a high-pressure homogenizer. The resulted nano molecules were scanned and appeared as shown at Figure 1.

Maintenance of tetranychid strains

Susceptible strains

Rearing of colonies of tetranychid mites, *Tetranychus urticae*, *Panonychus ulmi* and *Oligonychus mangiferus*, was

done at Plant Protection Research Institute, Mansoura branch, for several years without exposure to pesticides.

Resistant strains

The original colonies of the tetranychid mites, *T. urticae*, *P. ulmi* and *O. mangiferus* were reared at the laboratory (25°C and 60%RH) to evaluate the activity of Fenvalerate (Pyrethroids) and Spinosad (Spinosyns) adult females. The leaf-dip technique described by Dittrich (1962) was used as follows: Several concentrations of all tested pesticides were prepared. Castor oil leaf discs (2 cm diameter) were dipped in each concentration for 10 s, and left to dry. The discs were placed onto cotton wool pads in Petri-dishes (9 cm diameter). 10 adult females of each tetranychid mites were transferred to the treated castor oil leaf-discs, to each replicate, by using camel hair brush with the aid of stereomicroscope. All of treatments were left under laboratory conditions. Each treatment was replicated three times. In addition, the control discs were dipped in water only. Observations were taken after 24 h. Mortality percentages were determined and corrected using Abbott's formula (1925). The obtained data were subjected to probit analysis according to LeOra software (1994). Selection, according to the procedure of Yang et al. (2002), was done depending on LC50s through thirty generations and compared with the susceptible strains. Selection began with 1000 adult females of this colony. For every two generations, the LC50s were evaluated. Incipient LC50s were applied as subsequent selection pressure. Mortality percentages were recorded. Each new selection was transferred to untreated leaves. Comparisons of LC50s were effectively depended on the resistance ratio (RR) equation.

-Exposure to green nanoparticles

Resistant strains of tetranychid mites reared on discs of castor oil plant leaves were exposed to 10 µl/l of green nanoparticles by spraying. Each resistant strain was maintained after the 30 generations for 10 days during larval and nymphal durations. Then resulted adult females were amassed and the resistance ratio was calculated for each resistant strain.

Monoamine oxidase determination

Monoamine oxidase (MAO) is a catalyzed oxidation of kynuramine. 4-Hydroxyquinoline is the main product of excitation and emission at 310 and 400 nm, respectively. Mites' microsomes were homogenized in potassium phosphate buffers. All required determinations and procedure were done as described by Strydom et al. (2010), Novaroli et al. (2005) and Cheng and Prusoff

Table 1: LC50s and resistance ratios (RRs) of certain pesticides before and after treatments against certain tetranychid mites.

Tetranychid mites	Pesticides	Strain	Before treatments		Nano green particles	
			LC50s ($\mu\text{l/l}$)	*RRs	LC50s ($\mu\text{l/l}$)	*RRs
<i>Tetranychus urticae</i>	Fenvalerate	S	73.55	28.57	32.94	27.36
		R	2101.17		901.36	
	Spinosad	S	93.81		30.95	
		R	2563.93	27.33	821.64	26.55
<i>Panonychus ulmi</i>	Fenvalerate	S	92.33	32.46	51.11	
		R	2996.84		1523.23	29.80
	Spinosad	S	101.75	31.87	41.82	30.53
		R	3242.90		1276.93	
<i>Oligonychus mangiferus</i>	Fenvalerate	S	153.73	37.99	66.34	35.13
		R	5840.52		2330.85	
	Spinosad	S	124.39	56.28	33.95	27.21
		R	7000.85		923.91	

*Resistance Ratio (RR) =LC50 value of the resistant strain (R) / LC50 value of the susceptible strain (S).

(1973).

Reactive oxygen scavengers (ROS) determination

Ascorbate Peroxidase (APX) activity levels were assessed as reactive oxygen scavengers (ROS). Peroxidation movement was measured by evaluating the ascorbate oxidation rate (elimination coefficient 2.8 mM/cm). The reaction contained 3 of 50 mM phosphate support (pH 7.0), 0.1 mM H_2O_2 , 0.5 mM sodium ascorbate, 0.1 mM EDTA and extract of enzyme. The adjustment in absorbance was observed at 290 nm and the procedure was done according to that of Nakano and Asada (1981).

Data analysis

SPSS (V.16) was used to show differences before and after treatments with gallic acid nanoparticles. Nonparametric tests were used to test significance among different resistant and susceptible strains of each mite species.

RESULTS AND DISCUSSION

Selection pressure

The activities of Tetranychid strains after selection with LC50s of Fenvalerate and Spinosad were observed. LC50s, after 30 generations selection pressure against *T. urticae*, were 2101.17 and 2563.93 $\mu\text{l/l}$, against *P. ulmi* were 2996.84 and 3242.90 $\mu\text{l/l}$, and against *O. mangiferus* 5840.52 and 7000.85 $\mu\text{l/l}$ (Table 1). On the other hand, all LC50s reduced significantly after treatments with

nanoparticles of gallic acid and the results obtained with Fenvalerate and Spinosad against *T. urticae* were 73.55 and 93.81 $\mu\text{l/l}$, against *P. ulmi* were 92.33 and 101.75 $\mu\text{l/l}$, followed by 153.73 and 124.39 $\mu\text{l/l}$ against *O. mangiferus*, respectively.

Green nanoparticles were the most effective treatment against Spinosad resistant strains of *O. mangiferus* (Table 1), RR was 33.76 -folds, which increase the to deal effectively with decremented resistance of biocides.

The correlations, which depend on Resistance Ratio (RR), were truly viable in deciding pesticides resistance cases, such as resistance ratios of *T. urticae* recorded for Fenpyroximate, Dicofol, Pyridaben, Abamectin, Fenpropathrin, Propargite and Azocyclotin with 182-, 82-, 78-, 6.5-, 9.1-, 6.5- and 5.4- folds respectively (Ramasubramanian et al., 2005).

Effect on MAO activity

There were clear changes in the rate of kynuramine between susceptible and resistant strains of mites' adult females' homogenates (Figure 2). The data generally revealed that MAO activity of Kynuramine, in a case of resistant strains, was higher than in susceptible strains. Friedman Test showed highly significant difference among treatments (Chi-Square=42**) at 1% and in the same trend, Kendall's Coefficient of Concordance=0.875**. Also, Kruskal Wallis Test showed higher significance after treatments (Chi-Square=1.256**) than before (Chi-Square=.641*), and Observed Jonckheere-Terpstra Test recorded higher significance before treatments=25** and then after treatments =23* at Asymp. Sig. (2-tailed). Paired samples test showed highly significant at 1% with Std. Deviation=0.55603 and Std. Error Mean=0.16051. In view

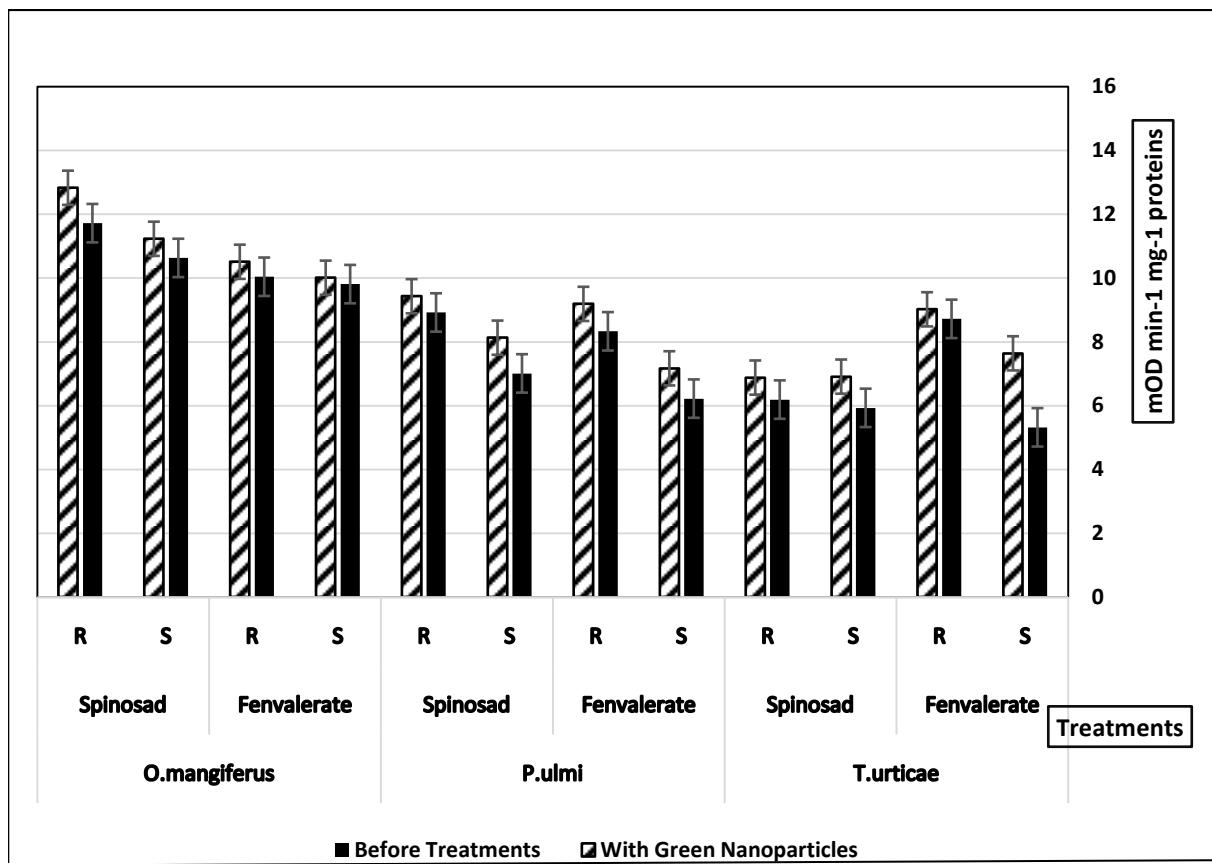


Figure 2: Activity rate of momo amine oxidase (MAO) in tetranychid mites before and after certain treatments with green gallic nanoparticles.

of this enzymatically oxidizable nature of gallic acid, particularly in the safe tetranychid mites, the oxidation value fundamentally increased when the synergist measures of l-DOPA ended up plainly accessible as a cofactor. Thus by increasing the dopaminergic compounds in mites' body, more chitin were formed and appeared in the immature stage, to be used subsequently. In the mature stage, catalysis activity with oxidizable nature of gallic acid was in nano molecules, which helped in the interaction with pesticides molecules and transfer of oxidized metabolite to a new specific site of action. Moreover, this create the impression that gallic acid can be separated as a tyrosinase inhibitor by bioassay-guided fractionation (Conrad et al., 1994), which could be useful in decreasing pesticides resistance in pests. These results support the findings of Yang et al. (2002), who confirmed that esterases, as detoxifying factors of mites' species, assumed a critical part in presenting pyrethroids resistance.

Susceptibility' reduction in *T. urticae* strains by bifenthrin and lambda-cyhalothrin was related with a 1.3- and 1.1- overlap increment of esterase action, separately. The mean general esterase action was higher in exposed *O. pratensis* and *T. urticae* strains to pyrethroids than in the non-selected strain. In spite of the fact that there was no

huge increment in esterase action in the exposed strain of *T. urticae* to dimethoate, there was a decrease in susceptibility with diminished glutathione S-transferase.

Effect on APX activity

Concerning Reactive Oxygen Scavengers (ROS), pesticides' tetranychid resistant strains showed significantly higher increase than susceptible strains ($P < 0.05$). Ascorbate Peroxidase (APX) activity levels in the resistant strains were higher than in susceptible strains before treatments (Table 2). The activity of free radical scavenging enzymes, such as APX, showed an inverse relationship with pesticides resistance. Therefore, it can be said that green nanoparticles led to more significant increase in scavengers, especially with the Spinosad resistant mites of *O. mangiferus*, and Fenvalerate resistant mites of *T. urticae* with high significant increase ratio of 4.08 and 3.47%, respectively. Green nanoparticles were able to increase even antioxidants effectively in relation with reactive oxygen scavengers (ROS) to decrease resistance in the used pesticides. Kendall's Coefficient of Concordance (Kendall's Wa) = 0.694* and Friedman Test showed that Chi-Square=8.333*. Beside, Wilcoxon test showed that

Table 2: Effect of certain pesticides before and after specific treatments with green nanoparticles on reactive oxygen scavengers (ROS) of tetranychid mites.

Tetranychid mites	Pesticides	Strain	ROS (Reactive Oxygen Scavengers)			
			Ascorbate Peroxidase (APX)			
			Before treatments	% Increase	Nano green particles	% Increase
<i>Tetranychus urticae</i>	Fenvalerate	S	4.91	3.22	8.28	3.47**
		R	20.73		37.05	
	Spinosad	S	3.02	1.38	6.62	1.15*
		R	7.18		16.41	
<i>Panonychus ulmi</i>	Fenvalerate	S	6.72	1.03	9.10	2.15*
		R	13.64		19.54	
	Spinosad	S	5.91	1.51	10.72	2.03*
		R	14.83		32.43	
<i>Oligonychus mangiferus</i>	Fenvalerate	S	4.50	1.05	8.01	0.72*
		R	9.23		28.41	
	Spinosad	S	7.10	1.21	7.01	4.08**
		R	15.66		35.60	

*= Significant at P<0.05.

Z=2.981*.

The data revealed that the biocides are able to gain resistance in mites exposed to them, but at the same time, resistance decreased faster as compared with chemical pesticides upon the ratios of reactive oxygen scavengers (ROS). In a similar pattern, Kubo et al. (2003) reported that gallates showed a practical activity of scavengers in insects, such as pink bollworm (*Pectinophora gossypiella*) larvae.

Nanoparticles could be used in direct pest control as reported by Abd El-Wahab and Anwar (2014). They showed decreased ratio percentages of APX, 19.93, 13.90, 65.73, 31.09, and 54.18% for ZnO, ZnO + Vertimec, CuO, CuO + Vertimec and Vertimec, respectively as compared with control, as a result of the first treatment against the sensitive strain of *Spodoptera littoralis*. Proteins actions could be lost due to the oxidative change that induced carbonyl reproduction. Such produced structures were steady and consistent. Besides, their appearance caused unending changes in the proteins structures (Davies et al., 1999; Dalle-Donne et al., 2003). Although most of the changed structures of the protein, after adsorption on the NP surface, sometimes decreased the thermodynamic stability of the protein, making it more critical to chemical denaturants, such as urea (Shang et al., 2007). Such advanced mode of action is adjusted perfectly against the presented pests, such as *T. urticae*, *P. ulmi*, *O. mangiferus* and other tetranychid mites, which were categorized as high risk in a recent report of European Food Safety Authority (2017).

CONCOLUSION

In conclusion, the green gallic acid, which is used as green nanoparticle, was effective in reducing metabolic resistance to pesticides expeditiously. This depends mainly on the new proclivity of mode of action. The results of this study will provide a new trend of researching into new green nano materials to stop or reduce pesticides' resistance.

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