Kafrelsheikh University
Faculty of Agriculture
Genetics Department

GENETIC EFFECTS OF USING SOME NANOPARTICLES IN INSECT PESTS CONTROL

By

Ahmed Fouad Ahmed Thabet

B.Sc. Agric. (Economic Entomology), Fac. of Agric., Kafrelsheikh Univ., 2009
M.Sc. Agric. (Economic Entomology), Fac. of Agric., Kafrelsheikh Univ., 2015

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5. SUMMARY

This study was carried out at Laboratory of Genetics Department, Faculty of Agriculture, Kafrelshiekh University, Egypt as well as Laboratories of Insect Natural Enemies, and Sanitary Entomology, Faculty of Agriculture, Kyushu University, Japan.

The aim of this study was to:

1. determine the efficacy of NPs as an insecticidal agent against *L. trifolii*; *V. faba* main insect pest.

2. investigate the potential genotoxicity of NPs on *V. faba*.

Efficiency of NPs against *L. trifolii* was determined using SiO_2NPs (19.6±5.7nm), TiO_2NPs (13.7±2.2nm) and AgNPs (20.1±5.4 nm) at four different concentrations for each (50, 100, 200 and 400 mg/L) to study their toxic effects on *L. trifolii* biological parameters, total protein content, and expression of CAT and SOD2 genes involved in the response to oxidative stress.

In order to investigate the toxic effects of NPs on *V. faba*, an experiment was performed using three different concentrations (25, 50 and 75 mg/L) of two other nanosized materials; n-SiO_2 (119.1±2.8 nm) and n-TiO_2 (283.6±15.9 nm) which were examined for their effects on seed germination and seedling growth; in addition to their genotoxic effects on root-tip cells (mitotic index and chromosomal abnormalities) and genomic DNA (genomic template stability; GTS).

The obtained results could be summarized as follows:

I. Efficiency of NPs against *L. trifolii* insect pest:

- Concerning effects of NPs on biological parameters, results indicated that SiO_2NPs at low concentrations (50 and 100 mg/L) had the advantage to control *L. trifolii* as they recorded the lowest values of larval feeding velocity and pupal weight, and generated smaller pupae and adult wings in contrast to 200 and 400 mg/L which differed in their impacts. Regarding TiO_2NPs, there was no sign for their entomotoxic effects at all stages of insect life cycle as TiO_2NPs enhanced larval feeding velocity, pupal size and weight in addition to generating bigger adult wings. About AgNPs, it can be used at the concentration of 100 mg/L to control *L. trifolii* as it recorded the lowest values for all tested parameters.

- Silica NPs showed significant increase in total protein content at 100 mg/L and significant decrease at 200 mg/L compared to control and other SiO_2NPs concentrations. Concerning TiO_2NPs, there was no significant differences among the used concentrations. However, total protein content was increased by increasing AgNPs concentration to reach the highest significant value at 400 mg/L.

- Based on qRT-PCR analysis for the relative expression of two oxidative stress genes; CAT and SOD2, significant differences in expression levels were observed for both genes in response to TiO_2NPs and AgNPs concentrations, whereas expression of both genes was not significantly differed among SiO_2NPs concentrations. Expression of CAT was significantly increased at 400 mg/L and significantly decreased at 100 mg/L due to TiO_2NPs treatment, while both 50 and 200 mg/L did not differ significantly compared to control. Meanwhile,

for SOD2, all concentrations of TiO_2NPs; except 100 mg/L. induced higher expression levels than control. The highest activity was recorded at 50 mg/L. With respect to AgNPs, all concentrations; except 100 mg/L, significantly increased the relative expression of CAT compared to control. The highest activity was occurred at 200 mg/L. However, SOD2 activity reached the highest estimate at 400 mg/L.

II. Toxicity of NPs on *V. faba*:

- For effects of n-SiO_2 (119.1±2.8 nm) and n-TiO_2 (283.6±15.9 nm) on germination and plant growth parameters, all n-SiO_2 concentrations decreased seed germination compared to control. However, all concentrations induced shorter shoots (except 50 mg/L) and longer roots than control. The same trend; as shoot length results, was observed for seedling vigor index which recorded lower values than control at all concentrations, except 50 mg/L. With respect to n-TiO_2, it did not affect seed germination at any used concentration while shorter shoots were induced at all concentrations without significant differences. The same trend was observed for seedling vigor index while root length was not affected.

- Concerning cytological analysis, n-SiO_2 significantly decreased the mitotic index of *V. faba* root tip cells at 50 and 75 mg/L; with no significant differences, while 25 mg/L raised it significantly. The three tested concentrations of n-TiO_2 significantly decreased *V. faba* mitotic index than control to reach the lowest value at 75 mg/L with no significant differences between 25 and 75 mg/L. All n-SiO_2 and n-TiO_2 significantly increased the percentage of abnormal cells compared to control treatment. Treatment of *V. faba* with both n-SiO_2 and n-TiO_2 recorded various types of chromosomal abnormalities in root tip cells.

The most frequent aberrations were stickiness, C-metaphase, disturbance, laggards, fragments and bridges.

- According to RAPD-PCR analysis, the 14 used oligonucleotide primers revealed visible changes between control and all of n-SiO_2 and n-TiO_2 concentrations in the number of amplified DNA bands with 53.80% polymorphism. Disappearance of normal bands was the common event arising in the *V. faba* DNA patterns treated with n-SiO_2. The highest number of disappeared bands was recorded at the highest concentration of n-SiO_2; 75 mg/L, which induced the lowest GTS (64.60%). On the other hand, appearance of new bands; compared to the control, was the major event for all n-TiO_2 concentrations. Concentrations of 25 and 50 mg/L n-TiO_2 recorded an equal effect on GTS (58.41%) which was lower than its value at 75 mg/L (61.06%). Generally, all the tested concentrations of n-SiO_2 and n-TiO_2 showed low GTS compared to control indicating that both NPs may interact with DNA causing genotoxic effect.