Global Scientific JOURNALS

GSJ: Volume 9, Issue 1, January 2021, Online: ISSN 2320-9186
www.globalscientificjournal.com

# THE TOXIC EFFECT OF NANO SILVER ON *SPODOPTERA LITTORALIS*, *TUTA ABSOLUTA* AND *LIRIOMYZA SATIVAE*

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## ABSTRACT

This present study aimed to evaluate the effect of nanosilver material against larvae of cotton leafworm, *Spodoptera litoralis*, tomato leafminer, *Tuta absoluta* and leaf miner flies, *Liriomyza trifolii.* These pests are considered the most serious pests all over the world on many vegetables especially tomato. Because of their infestation, the crops lose their quality and yield. Because of the problems caused by chemical pesticides to all organisms and environment, the natural control replaced pesticides. Nanomaterials control is one of newly effective methods used for pests control, especially nanometals that proved good results in pest control. Nanosilver is very effective against *L. trifolii* more than *T. absoluta* and *S. litoralis* with $LC_{50}$ 7.41, 7.72 and 9.93 ppm for *L. trifolii, T. absoluta* and *S. litoralis*, respectively, in laboratory. Also, in the field, reduction percentage was 73.5% for *L. trifolii*, 65.1% for *T. absoluta* and 54.8% for *S. litoralis.*

**Key words:** *L. trifolii*, *T. absoluta* and *S. litoralis*, tomato plant, nanosilver.

## INTRODUCTION

Tomato, *Lycopersicon spp.* (Family: Solanaceae) is one of the most important vegetables **(Polston and Anderson, 1999)** and it is one daily consumed crops which spread all over the world due to high nutritional value **(Singh, 2017).** Tomato is attacked by various insect pests as cotton leafworm, *Spodoptera littoralis* (Lepidoptera: Noctuidae) tomato leafminer, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) and leaf miner flies, *Liriomyza trifolii* (Diptera: Agromyzidae), **(Patra *et al*., 2016)**. The cotton leafworm, *S. littoralis*, is the most destructive pest of several crops which infests more than 180 plant species (Arumugam *et al*. 2015) such as cotton, tomatoes, cabbage, cauliflower and other crucifers **(Kumar *et al.* 2014)**. *T. absoluta* is one of the major pests that attack tomato in Egypt **(Temerak, 2011)**. Several attacks of tomato fruits lose their commercial value. Fifty to hundred per cent losses have been reported on tomato

**(EPPO, 2005; Desneux *et al*., 2010).** However, *Liriomyza sp.* are destructive pests for many crops especially tomato and their damage caused by both larvae and adults; the adult females puncture the leaf epidermis with their ovipositor to feed and lay eggs, then larvae mine the palisade mesophyll and consumed the parenchyma tissue and this cause falling the leaves and bacteria and fungi attraction **(Parella *et al*., 1985; Matteoni & Broadbent, 1988)**.

The chemical control is the most common method for controlling the populations of pests but this method is considered harmful to human, environment and natural enemies **(Picanço *et al*. 1998)** as well as insect resistance **(Siqueira *et al*. 2000)**. Nanotechnology has promised new approaches for pest control **(Bhattacharyya *et al*., 2010)**. The nanoparticles possess insecticidal property due to novel characteristics like chemical reactivity, electrical conductivity and extraordinary strength which released slowly but efficiently to a particular host plant against an insect pest **(Scrinis & Lyons, 2007).** Nanoparticles could be classified as inorganic and organic. Inorganic nanoparticles incorporate semi-conductor nanoparticles (like ZnO, ZnS and CdS), metallic nanoparticles (like Au, Ag, Cu and Al) and magnetic nanoparticles (like Co, Fe and Ni); while organic nanoparticles subsume carbon nanoparticles (like fullerenes, quantum dots, carbon nano tubes). Gold and Ag (noble metal) nanoparticles furnish superior characteristics with useful flexibility **(Rafique *et al*., 2016).** Metal nanoparticles are unique because they show the potential to change their surfaces in order to introduce specific functionalities for environmental applications **(Haick, 2007)**.

This study aims to determine the effect of nanosilver on *S. littoralis*, *T. absoluta* and *L. trifolii* in laboratory and field.

## MATERIALS AND METHODS

### I- Laboratory experiements:

### Insects rearing:

**(1) *S. littoralis*:**

A laboratory strain *S. littoralis,* (maintained on above 30 generations) that was initiated from freshly collected egg-masses, supplied from the division of cotton leafworm of Plant Protection Research Institute (PPRI), Dokki, Egypt. The larval stages were reared on castor leaves, which were provided daily, in laboratory under constant conditions of 27±2°C, 65±5% R.H and photoperiod of 14 h light and 10 h dark. Adults were kept separately and mated on 3$^{rd}$ day of emergence in clean jars (4 lb.). They were fed on 10% honey solution and fresh green leaves of tafla, *Nerium oleander* (L.) were provided for egg laying.

**(2) *T. absoluta:***

Tomato leaves including *T. absoluta* were collected form unsprayed farm of Agriculture College, Mansoura University (Dakahlia, Egypt). The larvae were reared for two generations before the beginning of the tests on leaves of unsprayed tomato which were provided daily, in laboratory under constant conditions of 25±2°C, 70±10 % R.H. and photoperiod of 14 h light and 10 h dark. The adults were kept separately and mated on 3$^{rd}$ day of emergence in clean jars (4 lb.). Adults were fed on 10% honey solution and fresh green leaves of unsprayed tomato were provided for egg laying **(Bajonero and Parra, 2017)**.

**(3) *L. trifolii:***

Tomato leaves that carrying larvae of leafminer fly were collected form unsprayed farm of Agriculture College, Mansoura University (Dakahlia, Egypt). The tomato leaves were kept in jars at 27±2$^{O}$C and 65±5% RH. The colony was maintained for two generations before the beginning of the tests. Then, the leaves with the newly larvae of *L. trifolii* were placed in plastic Petri dishes (10 cm. in diameter). Petri dishes were covered with muslin for aeration and the tomato leaves were put on the bottom of dishes **(Madahi and Sahragard, 2012)**. When the leaves appeared discolored, they were replaced with fresh ones.

**Characterization of AgNPs:**

The surface of AgNPs 3lasmon resonance was characterized using UV-2550 spectrophotometer (UV-Visible spectrophotometer Shimadzu, Japan) by operating at the resolution of 1 nm with the range from 190nm to 700nm **(Arumugam *et al*., 2015).** Structural characterization of AgNPs was carried out by transmission electron microscopy (TEM) (JEOL- JEM-2100, Japan). The sample was placed on the carbon coated copper grid, making a thin film of sample on the grid and extra sample was removed using the cone of a blotting paper and kept in grid box sequentially **(Durán *et al*., 2005)**. Malvern Zeta sizer Nano ZS90 (Malvern Instruments Ltd., UK) was used to measure zeta potential of silver nanoparticles. Zeta potential cell was washed with ethanol and deionized water followed by AgNPs sample. The average distribution of nanoparticles based on volume, intensity and number weighting that was studied comparatively **(Amit *et al*., 2014; Sabrien *et al*., 2016).**

**Method of application:**

**Leaf dipping method:**

2$^{nd}$ instar larvae of *S. littoralis* were used to determine the toxicity of the nanosilver material. Tomato leaf discs were dipped into the concentrations for 20 seconds, then left for air dryness, 10 larvae for each replicate were released to each leaf disc placed. Four concentrations and three replicates were used to estimate each concentration-mortality line. The used concentrations were 5, 10, 15 and 20 ppm. The same number of leaf discs per treatment was dipped into dis. water as an untreated check. Before and after treatment, larvae were maintained under laboratory conditions (constant temperature 25 ±2 °C and 70± 5 % R.H. after 24 h of treatment. The percentage of mortality was recorded after one, three, five and seven days. The data were corrected relatively to control mortality **(Abbott, 1925)**.

**Spray method:**

The leaves with larvae of *T. absoluta* and leaves of *L. trifolii* were used for application. Four concentrations were used as well as three replicates for each concentration. 10 individuals of each of *T. absoluta* and *L. trifolii* for each replicate were applied to estimate the mortality line. Different concentrations were sprayed directly on the larvae. The concentrations used were 5, 10, 15 and 20 ppm. The percentage of mortality was recorded after one, three, five and seven days and the data were corrected relatively to control mortality **(Abbott, 1925)**. $LC_{50}$ values were determined using probit analysis statistical method of **Finney, (1971)**.

**Equation: Sun, 1950 (**to determine $LC_{50}$ index**)**

**Toxicity index for $LC_{50}$ =**

$$\frac{LC_{50} \text{ of the most effective compound}}{LC_{50} \text{ of the least effective compound}} \times 100$$

**II- Field experiements:**

$LC_{90}$ of nanosilver for the three pests was applied on invested leaves of tomato crop and reduction percentage was evaluated according to **Hendrson and Tilton formula, (1955)** after 3, 5 and 10 days from spraying for *T. trifolii,* 5, 7 and 10 days for *T. absoluta* and 2, 4 and 6 days for *S. littoralis* by assigned treatments according to the formula:

$$\textbf{Reduction (\%mortality)} = \left[\left(\frac{C_b}{C_a} \times \frac{T_a}{T_b}\right) - 1\right] \times 100$$

Where:

$C_b$= number of alive pest individuals in control before treatment.

$C_a$= number of alive pest individuals in control after treatment.

$T_a$= number of alive pest individuals after treatment.

$T_b$= number of alive pest individuals before treatment.

# RESULTS AND DISCUSSION

## I- Laboratory experiements:

### (1) Effect of nanosilver on mortality rate of *S. littoralis, T. absoluta* and *L. trifolii*:

In **Table (1)**, results showed that the nanosilver affected on the larvae of *S. littoralis, T. absoluta* and *L. trifolii*. This effect caused mortality in all concentrations of nanosilver but the highest effect was on *L. trifolii* then *T. absoluta* followed by *S. littoralis.*

**Mohammed *et al.* (2019)** proved that nano materials were more effective that materials in ordinary case against *L. trifolii*. Also, **Khaled *et al.* (2019)** found that, nanosilver material is effective material against *S. littoralis*. Also, **Hany *et al.* (2016)** showed the effect of nano silica against *T. absoluta* and the results showed the great effect of nano silica on it.

**Table (1): Corrected mortality % of larvae of *S. littoralis, T. absoluta* and *L. sativae* treated with nanosilver under laboratory conditions 27±2 ◦C and 65±5% RH.**

| Pests | Conc. (ppm) | Mortality after treatments % | | | | Total Mortality % |
|---|---|---|---|---|---|---|
| | | One day | Three days | Five days | Seven days | |
| *S. littoralis* | 5 | ----- | 6.67 | 10 | 13.33 | 30 |
| | 10 | 3.33 | 23.33 | 20 | 3.33 | 50 |
| | 15 | 3.33 | 26.67 | 16.67 | 13.33 | 60 |
| | 20 | 6.67 | 43.33 | 16.67 | 6.67 | 73.33 |
| *T. absoluta* | 5 | 6.67 | 16.67 | 3.33 | 6.67 | 33.33 |
| | 10 | 10 | 30 | 20 | 3.33 | 63.33 |
| | 15 | 13.33 | 33.33 | 16.67 | 6.67 | 70 |
| | 20 | 20 | 30 | 26.67 | 3.33 | 80 |
| *L. trifolii* | 5 | 10 | 10 | 10 | 3.33 | 36.67 |
| | 10 | 13.33 | 16.67 | 13.33 | 13.33 | 56.67 |
| | 15 | 10 | 20 | 23.33 | 23.33 | 76.67 |
| | 20 | 20 | 30 | 30 | 10 | 90 |

### (2) Efficiency and toxicity index of nanosilver against larvae of *S. littoralis, T. absoluta* and *L. trifolii:*

However, **Table (2)** and **Fig. (1)** indicated that, although nanosilver material was very effective against the three tested pests, but the more effect was against *L. trifolii* with $LC_{50}$ 7.41 ppm and $LC_{90}$ 23.81 ppm. Then *T. absoluta* was affected with $LC_{50}$ 7.72 ppm and $LC_{90}$ 32.55 ppm then *S. littoralis* with $LC_{50}$ 9.93 ppm and $LC_{90}$ 50.11 ppm.

**Table (2): Efficiency of nanosilver against *S. littoralis, T. absoluta* and *L. trifolii***

| Treatments | Conc. | Corrected mortality % | $LC_{50}$ | $LC_{90}$ | Slope± S.D. | Toxicity index $LC_{50}$ | $LC_{90}$/ $LC_{50}$ |
|---|---|---|---|---|---|---|---|
| ***S. littoralis*** | 5 | 30 | 9.93 | 50.11 | 1.8 ± 0.29 | 74.62 | 5.05 |
| | 10 | 50 | | | | | |
| | 15 | 60 | | | | | |
| | 20 | 73.33 | | | | | |
| ***T. absoluta*** | 5 | 33.33 | 7.72 | 32.55 | 2.05± 0.29 | 95.95 | 4.3 |
| | 10 | 63.33 | | | | | |
| | 15 | 70 | | | | | |
| | 20 | 80 | | | | | |
| ***L. sativae*** | 5 | 36.67 | 7.41 | 23.81 | 2.53± 0.31 | 100 | 3.2 |
| | 10 | 56.67 | | | | | |
| | 15 | 76.67 | | | | | |
| | 20 | 90 | | | | | |

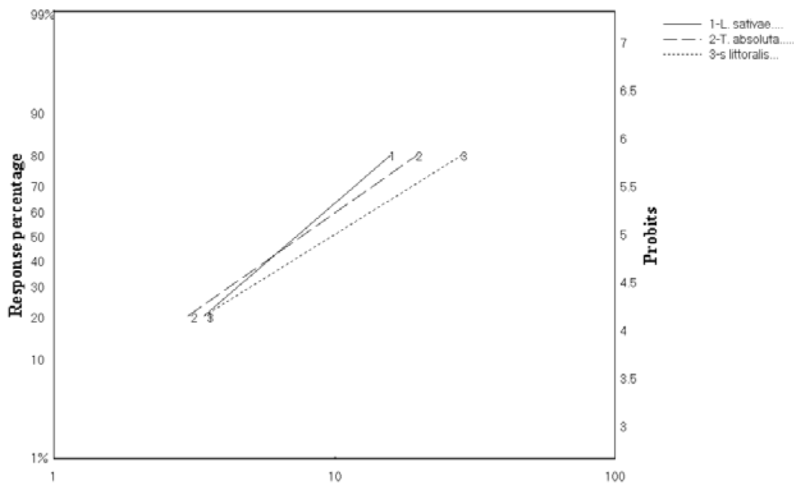


**Fig. (1): LC-P line for nanosilver *of S. littoralis, T. absoluta* and *L. trifolii***

From **Table (1)** and **Fig. (1)** we noticed that, the most effect was against the leafminer insects especially *L. trifolii*, this is may be due to it is more sensitive insect against treatments.

Some denaturations were noticed on the treated larvae of *S. littoralis* and *T. absoluta* that mentioned in **Fig.2** and **Fig. 3.**


**Fig. (2): Denaturation in treated larva of *S. littoralis* treated with nanosilver (a) compared with untreated one (b).**


**Fig. (3): Denaturation in treated larva of *T. absoluta* treated with nanosilver (a) compared with untreated one (b).**

**Prateek *et al.* (2018)** proved that nanotoxicity of nanosilver on mammals while **Khaled *et al.* (2019)** proved the effectiveness of nanosilver in management of 2nd and 4th instars of *S. littoralis*. Also, **Hany *et al.* (2016)** indicated the effectiveness of nano silica on larvae of *T. absoluta*.

## II- Field experiements:

The results in **Table (3)** indicated that, the total reduction caused by nanosilver on *S. littoralis* was higher after 4 days more than other days. **Table (4)** demonstrated that the mean of total reduction percentage was 54.8%.

The results in **Table (5)** indicated that, the total reduction caused by nanosilver on *T. absoluta* was higher after 10 days more than other days. **Table (6)** indicated that the mean of total reduction percentage was 65.1%.

The results in **Table (7)** indicated that, the total reduction caused by nanosilver on *L. trifolii* was higher after 10 days more than other days. **Table (8)** demonstrated that the mean of total reduction percentage was 73.5%.



**Table (3): Effect of treatments on infestation reduction percentage of *S. littoralis* after 2, 4 and 6 days:**

| Treatment | 1st replicate Red.% | 2nd replicate Red.% | 3rd replicate Red.% | 4th replicate Red.% | Total reduction % |
|---|---|---|---|---|---|
| | After 2 days | | | | |
| nanosilver | 58 | 56 | 50 | 54 | 54.5 |
| control | ----- | ----- | ----- | ----- | |
| | After 4 days | | | | |
| nanosilver | 60 | 55 | 60 | 55 | 57.5 |
| control | ----- | ----- | ----- | ----- | |
| | After 6 days | | | | |

| | | | | | |
|---|---|---|---|---|---|
| **nanosilver** | **40** | **60** | **60** | **50** | **52.5** |
| **control** | ----- | ----- | ----- | ----- | |

**Table (4): Mean of total reduction percentage of *S. littoralis* infestation:**

| Treatments | Mean reduction of 1st scan | Mean reduction of 2nd scan | Mean reduction of 3rd scan | Mean of total reduction |
|---|---|---|---|---|
| **Nanosilver** | **54.5** | **57.5** | **52.5** | **54.8** |

**Table (5): Effect of treatments on infestation reduction percentage of *T. absoluta* after 5, 7 and 10 days:**

| Treatment | 1st replicate Red.% | 2nd replicate Red.% | 3rd replicate Red.% | 4th replicate Red.% | Total reduction % |
|---|---|---|---|---|---|
| | **After 5 days** | | | | |
| **nanosilver** | **66** | **70** | **60** | **50** | **61.5** |
| **control** | ----- | ----- | ----- | ----- | |
| | **After 7 days** | | | | |
| **nanosilver** | **70** | **75** | **66** | **55** | **66.5** |
| **control** | ----- | ----- | ----- | ----- | |
| | **After 10 days** | | | | |
| **nanosilver** | **70** | **77** | **65** | **57** | **67.3** |
| **control** | ----- | ----- | ----- | ----- | |

**Table (6): Mean of total reduction percentage of *T. absoluta* infestation:**

| Treatments | Mean reduction of 1st scan | Mean reduction of 2nd scan | Mean reduction of 3rd scan | Mean of total reduction |
|---|---|---|---|---|
| **Nanosilver** | **61.5** | **66.5** | **67.3** | **65.1** |

**Table (7): Effect of treatments on infestation reduction percentage of *L. trifolii* after 3, 5 and 10 days:**

| Treatment | 1st replicate Red.% | 2nd replicate Red.% | 3rd replicate Red.% | 4th replicate Red.% | Total reduction % |
|---|---|---|---|---|---|
| | **After 3 days** | | | | |
| **nanosilver** | **80** | **80** | **60** | **55** | **68.8** |
| **control** | ----- | ----- | ----- | ----- | |
| | **After 5 days** | | | | |

| | | | | | |
|---|---|---|---|---|---|
| **nanosilver** | **80** | **77** | **65** | **60** | **70.5** |
| **control** | ----- | ----- | ----- | ----- | |
| | **After 10 days** | | | | |
| **nanosilver** | **90** | **80** | **75** | **80** | **81.3** |
| **control** | ----- | ----- | ----- | ----- | |

**Table (8): Mean of total reduction percentage of *L.trifolii* infestation:**

| Treatments | Mean reduction of 1st scan | Mean reduction of 2nd scan | Mean reduction of 3rd scan | Mean of total reduction |
|---|---|---|---|---|
| Nanosilver | 68.8 | 70.5 | 81.3 | 73.5 |

**Khaled *et al.* (2019)** proved the effectiveness of nanosilver on 2nd instar larvae of *S. littoralis* more than the effect on 4th instar larvae when applied in field and proved that the management by nanosilver might be successful for reducing environmental pollution and the resistance of this pest to many pesticides.

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