EFFICACY OF SILICA NANOPARTICLES ON COTTON LEAF WORM LARVAE, *SPODOPTERA LITTORALS* (BOSID.) (LEPIDOPTERA: NOCTUIDAE)

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**Abstract**

Cotton leaf worm, *Spodoptera littoralis* is a polyphagous insect pest, causes a huge damage to many crops. Silica nanostructure with various physicochemical characteristics were prepared by sol–gel method. The aim of this study is use less expensive sources to prepare silica nanoparticles and evaluate effect silica nanoparticles on second in star larvae of *S. littoralis* and their effects to larval enzyme activity. Types of silica nanoparticles hydrophilic (TSiNP HPHI), silica nanoparticles hydrophobic (TSiNP HPHO) were prepared from Tetraethyl orthosilicate (TEOS), silica nanoparticles hydrophilic prepared from sodium silicate (SSiNP HPHI) in addition traditional silica (TSi) prepared from sand by top down method. The obtained silica powders were characterized by using spectroscopy with high resolution transmission electron microscope, dynamic light scattering particle size analyzer (DLS), X-ray diffraction (XRD), and atomic absorption spectrophotometer Fourier transform infrared (FTIR). These four types by utilizing surface contact were applied against second in star larvae of *S. littoralis*. Laboratory studies were conducted to determine mortality percentages of the second instar larvae of *S. littoralis* at treatment with 10, 20, 300, 4 & 60 g/L concentrations of materials. Our results showed that the increase in the concentration led to the increase in mortality. The LC₅₀ obtained were 10.48, 10.76, 14.01 & 42.99 g/L of TSiNP HPHO, SSiNP HPHI, SSiNP HPHI, and TSi, respectively. Results showed that, the TSiNP HPHO, SSiNP HPHI & TSiNP HPHI had high effects on biochemical parameters, while TSi had no statistically significant effect. Accordingly, we can synthesis silica nanoparticles from low cost sources such as sodium silicate which can be an alternative to the commercial insecticides which have numerous health hazards.

**Key words:** Silica nanoparticles, microsilica, *Spodoptera littoralis*, biochemical.

**Introduction**

The cotton leaf worm, *Spodoptera littoralis* (Bosid.) (Lepidoptera: Noctuidae) was and still is considered one of the most serious and destructive pests. In Egypt, *S. littoralis* attacks more than 112 host plants. Control of cotton leaf worm is complicated due to the adverse effects on non-target organisms and the high levels of resistance developed against organophosphates, carbamates and pyrethroids (Osman et al., 2015). Recently, the global occurrence of cotton leaf worm resistance has resulted in the need for more effective and acceptable control methods such as alternative safe pesticide that has minimal impact on the environment. Nanotechnology is considered to be a promising trend for crop and foodstuff protection. Nanoparticles (NPs) are preferentially harnessed because they offer a higher surface area and circulate more easily and therefore reduce of dosage and repeated applications of conventional pesticides which led to the rapid development of insect resistance to pesticide and adverse effects on human health and environment (Shoaib et al., 2018). The physical properties of nanoparticles are different from the properties of bulk material. In the past decade, nano materials (NMs) have provided a wide range of novel pesticide formulations or pesticide metallic NPs such as nanoemulsion, nanocapsules, nanosuspension, and metallic oxide NPs. These materials had higher efficacy on pest control and less harmful impacts on the environment compared with the traditional materials (Buffle 2006). (Sabbour 2012)
used alumina oxide (Al₂O₃) and titanium oxide (TiO₂) as an insecticide against stored grain insects, *Sitophilus oryzae*. Under laboratory and store conditions, the results showed that Nano alumina oxide were highly effective against *S. oryzae*, while nano TiO₂ was only moderately effective. (Sabbour 2013) studied the effects of Silica gel (Cab-O-Sil-750 and silica gel Cab-O-Sil-500) on *S. oryzae*: the results showed that, under laboratory and store conditions, the mortality of *S. oryzae* was significantly increased. Also, the nano-particle Zinc oxide (ZnO) was more effective in decreasing the infestation of *S. oryzae* under laboratory and store condition (Sabbour 2013b). The amorphous nanosilica can be used as a novel insecticide by the agricultural sector. Silica nanoparticles (SNPs) have gained much attention in scientific research because of its easy preparation and its wide range of industrial as well as biological applications (Xu et al., 2003). (Sabbour and Hussein 2016) reported that both silica gel and nano silica gel decreases the infestation with *Tuta absoluta* under laboratory, green house, and field conditions. The variety of chemical and physical modification possible with silica increases its versatility and its biocompatibility makes it suitable for biological application (Debnath et al., 2012). Hypothesized that amorphous SNPs having narrow size distribution will show insecticidal property and these will be effective in all climatic conditions. Several methods, most of them based on Tetraethyl orthosilicate (TEOS) as a source to prepare silica nanoparticles. Silica nano particles with various characteristics were prepared by ammonia-catalyzed reactions of TEOS with water in low molecular weight alcohol; but this source (TEOS) is of high cost (Essien et al., 2012). The aim of this work is to use alternative sources to obtain SNPs with low cost and compare its effects with SNPs created from TEOS against second instar larvae of *S. littoralis* and its effects to larval enzyme activity.

**Materials and Methods**

**Insects culture**

A laboratory strain of *Spodoptera littoralis* was obtained from Syngenta Agro Egypt (S.A.E), Kaha research station. It was reared at National Research Center Dokki, Egypt. Insects were reared under controlled conditions in the incubator at 26 ± 2°C and 60±5% RH. Larvae reared on castor bean leaves *Ricinus communis* L., as a source of food. Larval jars were supplied with castor leaves and provided daily until transformed to pupae. Pupae were kept in clean jars (500 g) tell adult emergence. Emerged adults were collected in chimney glass cages and fed on 10% honey solution fresh. Green leaves of *Tafla, Nerium* oleander (L.) were provided for egg laying inside cages (Osman et al., 2015).

**Preparation of Silica nanoparticles forms**

**Preparation of Silica nanoparticles hydrophilic (TSiNP HPHI)**

Sol gel method was used for the preparation of Silica nanoparticles at chemical’s laboratory at national research center. 180 ml Tetraethyl orthosilicate (TEOS) hydrolyzing in flask content of 2000 ml of ethanol absolute and 1800 ml of distilled water then stirred for 30 min to homogeneity. Aqueous ammonia solution added dropwise to the mixture as the catalyst of the reaction mixture. Upon addition of ammonia, the reaction mixture remained clear for some time and slowly turned turbid due to the formation of Silica gel. The reaction was completed in 2 hours. The white powder product was dried overnight at 100°C and thereafter milled to form nano powders (Stober et al., 1968; Debnath et al., 2012).

**Preparation of Silica nanoparticles hydrophobic (TSiNP HPHO)**

The core SNP, on which surface capping was synthesized slightly modifying by Stober et al., 1968; Debnath et al., 2012. TSiNP HPHO was prepared through mix of 2500 ml of ethanol with 2000 ml of distilled water and 7 ml of HCl 0.075(M). This mixture was stirred for 10 minutes then 180 ml of Tetraethyl orthosilicate (TEOS) added to this mixture. The mixture was stirred for 30 min at a temperature of 70°C, then 90 ml of Hhexa methyl disilazane (HMDS) was mixed with solution content of 900 ml of ethanol absolute, 110 ml of distilled water and 7 ml of HCl 0.075(M). Then the solution which contains HMDS was added to the solution which contains TEOS. Reaction mixture with constant stirring for 2h. Finally, the reaction mixture was centrifuged at 12000 rpm for 15 min and washed with ethanol several times to remove the excess of HMDS. The final product was dried at a temperature of 90°C then grinded.

**Preparation of Silica nanoparticles from Sodium metasilicate (SSiNP HPHI)**

500 ml of H₂SO₄ (2M) added to 500 ml of Liquid sodium silicate which is used in the manufacture of detergents under magnetic stirring at room temperature for 2 hours. Deionized water was added successively to the gel for wash and remove sodium sulphate (Na₂SO₄). The washed gel was dried in an oven at 120°C for 1 day, and thereafter milled to form powder_product (Essien et al., 2012).

**Preparation of traditional silica (TSi) from sand**

Traditional silica is prepared by top-down method from breaking up the bulk materials. The sand was collected
from south Sinai government then crushed, milled for 20-30 min by using the Agate mill equipment. Obtained powder was sieved under 175µm using shaking machine (Khater et al., 2017).

**Characterization of different silica nanoparticles**

Determination of size, morphology and composition of Silica nanoparticles (SiNP HPHI, SiNP HPHO and SSiNP HPHI) were performed by high resolution transmission electron micrograph JEOL 2010. Determination of the particle size distribution of the powder by Dynamic light scattering (DLS) (HR-TEM), X-ray diffraction (XRD), Fourier Transform-Infrared Spectroscopy (FTIR).

**Efficiency of Silica nanoparticles on the 2nd instar larvae of S. littoralis**

All bioassays were carried out at 26 ±2°C with 70% ±5% RH and a light–dark cycle of 16:8 h. The bioassays were performed in plastic cylindrical container (5 × 7.2 cm) using leaf dipping methods (Ayoub et al., 2017). Silica nanoparticles solutions with different concentrations were prepared using distilled water. These concentrations were 10, 20, 40 and 60 g/L. In addition to, the same concentrations of traditional silica were used as a positive control. Leaves discs of castor (3 cm in diameter) were sprayed with the test solution and other leaves discs were treated with distilled water as a control. After five minutes the surface of leaves discs dried. Three leaf discs were placed in a plastic container with a perforated cover for aeration. The second instar larvae were used in all experiments. Four replicates for each concentration were performed, and 25 larvae were utilized for each replicate. After 24h exposure the number of dead the larvae were recorded and calculated the mortality percentages per treatment.

**Effect of the silica nanoparticles on the activity of some enzymes of treated larvae**

Treated larvae with LC$_{50}$ values of tested four compounds and control were prepared as described by (Ishaaya et al., 1974). A known weight of frozen larvae was homogenized in distilled water (1mg/2ml) using a chilled glass Teflon tissue homogenizer. Homogenates were centrifuged at 8000 rpm for 15 minutes at 2°C in a refrigerated Centrifuge. The deposits were discarded and the supernatants, which is referred as enzyme extract, can be stored at least one week without appreciable loss of activity when stored at 5°C. Determination of total proteins, chitinase, amylase, protease and phenol oxidase activities were determined calorimetrically according to (Reitman et al., 1957).

**Statistical analysis**

Duncan post hoc test was adopted for calculating the mortality rates of larvae and validate significant differences for all treatment and were performed with SPSS computing program (Version 16, SPSS Inc., Chicago, IL, USA) data on the effect of silica nanoparticles on the 2nd instar larvae were subjected to probit analysis as described by (Finney 1971). The LC$_{50}$ values were also calculated using the computing program developed by (Noack and Reichmuth 1978). The results are displayed as mean ± standard error with a 95% confidence level.

**Results and Discussion**

**Characterization of Silica nanoparticles**

**Determination of size Silica nanoparticles**

Transmission electron microroscope (TEM) and dynamic light scattering particle size analyzer (DLS) reveals that both the particles are spherical in shape and distinct uniformity. After analyzing data found that nanoparticles size was in the range 0.5:20.0 (Fig. 1.a, d), 5:20.0 (Fig. 1.b, e) and 5.0:25.0 (Fig. 1.c, f) nm. SSiNP HPHO was lower size, SSiNP HPHI medium size and TSiNP HPHI big size, respectively. While the average particle sizes were 175 µm of TSi which obtained by sieve shaker machine.

**Determination of crystalline and amorphous silica nanoparticles**

Powder XRD pattern is obtained from Bragg’s Law $\lambda=2d \sin \theta$ using CuKα radiation. Each of the TSiNP HPHO, SSiNP HPHI and TSiNP HPHI were amorphous form (Fig. 2). This result indicates that SNPs suitable for biological application depending on United States Department of Agriculture (USDA) has already approved non-crystalline silica as safe (Stathers et al., 2004).

**Determination of functional groups by Fourier Transform-Infrared Spectroscopy (FTIR)**

Fig. 3 shows the FTIR spectra of different silica nanoparticles. In TSi form, Peak obtained at 3432.67 cm$^{-1}$ show the presence of O–H stretching of the surface silanol groups this peak appears in the four forms, at peak 1623.77 cm$^{-1}$ show the presence of C=O of the carboxylic acid group. In TSiNP HPHI form, Peak obtained at 3440.39 cm$^{-1}$ appears for O–H stretching of the surface silanol groups. Characteristic 1631.48 cm$^{-1}$ peak is for the C=O of the carboxylic acid group this peak appears in the four types, peak at 966.16 cm$^{-1}$ is attributed towards Si-O stretching this peak appears in three forms accept SM form. In SSiNP HPHI form, Peak obtained at 3446.17 cm$^{-1}$ appears for O–H stretching of the surface silanol
Effect of silica nanoparticles on 2nd instar larvae of S. littoralis

Table 1 shows that TSiNP HPHO, SSiNP HPHI and TSiNP HPHO could kill 100% of the second instar of S. littoralis larvae at 60 and 40 g/L whereas TSi caused 70% mortality at 60 g/L and 38% at 40 g/L. The mortality percentages were 83, 83, 71 and 22% when treated with TSiNP HPHO, SSiNP HPHI, TSiNP HPHO and TSi respectively at 20 g/L. While, at 10 g/L for TSiNP HPHO, SSiNP HPHI, TSiNP HPHO and TSi cause 46, 40, 32 and 13% mortality, respectively. The results show that the TSiNP HPHO and SSiNP HPHI may be due to the small size of the granules in case of silica nanoparticles which cover the largest area of the body of larvae treated and at the same time possess sharp edges. The results show that the increase in the concentration led to increase in the death rate. It was observed that the dead bodies of the insects became extremely dehydrated. This results agreement with (Debnath et al., 2012) who reported the amorphous silica nanoparticles (SNP) could effectively kill the S. litura larvae at a dosage of 0.5 mg cm\(^{-2}\) and caused damage in the cuticular water barrier as a result of abrasion or to some extent because of absorption of lipids by SNPs present in cuticle. The insects began to lose water from their body and died because of desiccation. The SNPs can used to control the stored grain insects, such as S. oryzae, Callosobruchus maculatus and Corcyra cephalonica (Debnath et al., 2012).

Table 1: The lethal effect of TSiNP HPHO, SSiNP HPHI and TSiNP HPHO on S. littoralis larvae at different concentrations.

<table>
<thead>
<tr>
<th>Concentration (g/L)</th>
<th>TSiNP HPHO (%)</th>
<th>SSiNP HPHI (%)</th>
<th>TSiNP HPHO (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>60</td>
<td>100</td>
<td>100</td>
<td>100</td>
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<tr>
<td>40</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>20</td>
<td>70</td>
<td>71</td>
<td>71</td>
</tr>
<tr>
<td>10</td>
<td>46</td>
<td>40</td>
<td>32</td>
</tr>
<tr>
<td>5</td>
<td>22</td>
<td>22</td>
<td>22</td>
</tr>
</tbody>
</table>

Fig. 1: TEM image of (a) TSiNP HPHO, (b) SSiNP HPHI and (c) TSiNP HPHI Inset showing particle size distribution. d, e and f determine the particle size distribution of the particles.

Fig. 2: X-ray diffraction pattern of (a) TSiNP HPHO, (b) SSiNP HPHI and (c) TSiNP HPHI.

Fig. 3: FTIR spectra of (a) TSi, (b) TSiNP HPHI, (c) SSiNP HPHI and (d) TSiNP HPHO.
Table 1: The mortality percentage of 2nd instar larvae of S. littoralis treated with silica nanoparticles.

<table>
<thead>
<tr>
<th>Concentrations (g/L)</th>
<th>Mortality percentages (mean ±SE)</th>
<th>F. value</th>
<th>P. value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Silica nanoparticles forms</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>TSiNP HPHO¹</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>SSiNP HPHI²</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>TSiNP HPHI³</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>TSi⁴</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.0±0.00d</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>46.0±87.8c</td>
<td>32.0±71.4k</td>
<td>13.0±48.6d</td>
</tr>
<tr>
<td>20</td>
<td>83.0±1.32ab</td>
<td>71.0±1.65ab</td>
<td>22.0±29.0k</td>
</tr>
<tr>
<td>40</td>
<td>100.0±0.00e</td>
<td>100.0±0.00e</td>
<td>38.0±96.0k</td>
</tr>
<tr>
<td>60</td>
<td>100.0±0.00e</td>
<td>100.0±0.00e</td>
<td>70.0±64.0k</td>
</tr>
<tr>
<td>F.value</td>
<td>231.832</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P.value</td>
<td>0.000</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Means followed by some letters are insignificant, small letters represent differences between concentrations and capital letters represent differences between treatment materials.

1= silica nanoparticles functionalized, 2= silica nanoparticles from sodium silicat, 3= silica nanoparticles from TEOS and 4= microsilica from sand.

2012).

LC₅₀

Silica nanoparticles with different source were the most effective compound against the 2nd larvae of S. littoralis, compared with microsilica according to LC₅₀ values and toxicity index which were 10.048, 10.076, 10.401 and 40.299g/L for TSiNP HPHO, SSiNP HPHI, TSiNP HPHI, and TSi, respectively this result approaching the results reached by (Ayoub et al., 2017) Where they used four different forms of SiO₂ nanostructures SiO₂-TX, SiO₂-CTAB, SiO₂-PVP and commercial SiO₂ nanostructures against S. littoralis. They found that LC₅₀ values were highest values for commercial silica then nanostructures.

Biochemical studies

Data represented in table 2 showed that total protein content of S. littoralis larvae was significantly decreased in all treatments in TSiNP HPHO -72.67% treatment, SSiNP HPHI -68.34% treatment, TSiNP HPHI -64.92% followed by TSi treatment -18.22% lower than control. Data showed different pattern effect on total protein contents of the different compounds. The effect of TSiNP HPHO, SSiNP HPHI and TSiNP HPHI on 2th in stars of S. littoralis showed marked decrease of the total protein content while in treatment with TSi was mild increased. This due to that protein leakage during intoxication may arise from reduced body weight (Rawi et al., 1995). Conversion of protein to amino acids and with the entrance of these amino acids to TCA cycle as a keto acid, they will help to supply energy for the insect (Etebari and Matindoost 2004). With regard to foreign compounds, proteins help insects to synthesize the microsomal detoxifying enzymes. proteins can bind with some of these foreign compounds and therefore the decrease in proteins may reflect the decrease in activity of these enzymes (Kyung and Kim 1990). Thus, it could be declared that both TSiNP HPHO, SSiNP HPHI and TSiNP HPHI have more significant effect on the reduction in total protein content than treatments with SM. Also, our results were in agreement with the results of (Derbalah et al., 2014). Chitinase activity was significantly increased in nanosilica treatments. The most increase was happened with the TSiNP HPHO (21.37), then SSiNP HPHI (19.93%), followed by and TSiNP HPHI (10.69%) and TSi (1.68%). and were shown to affect feeding indices. While TSi had insignificant effect, this is one possible reason is that they may affect digestive enzyme activities. The hyper chitinase activity might be attributed to degradation of the endocuticle during molting (Samuels and Reynolds 1993). (Sabry et al., 2014) reported that tebufenozide, and methoxyfenozide were led to significant increase in activity of chitinase and phenol oxidase enzymes of 4th instar larvae of S. littoralis. They have suggested that the reason behind deformations and mortality may be a result of changes in chitinase, protease,
Table 2: Influences of total protein and activity of some enzymes in 2nd instar larvae of S. littoralis exposed to LC50 of silica nanoparticles.

<table>
<thead>
<tr>
<th>Total protein</th>
<th>Chitinase</th>
<th>α-amylase</th>
<th>Protease</th>
<th>Phenol oxidase</th>
</tr>
</thead>
<tbody>
<tr>
<td>µg/mg bw (10⁻⁶)</td>
<td>µg NAGA/mg bw</td>
<td>µg/mg bw (10⁻³)</td>
<td>µg/mg bw</td>
<td>µg/mg bw</td>
</tr>
<tr>
<td>Mean ±SE</td>
<td>Change (%)</td>
<td>Mean ±SE</td>
<td>Change (%)</td>
<td>Mean ±SE</td>
</tr>
<tr>
<td>Control</td>
<td>4.39±5.84ᵃ</td>
<td>60.44±3.38ᵇ</td>
<td>2.41±3.71ᵇ</td>
<td>102.55±24ᵃ</td>
</tr>
<tr>
<td>TSi</td>
<td>3.59±6.62ᵇ (-18.22)</td>
<td>61.05±3.62ᵇ (1.68)</td>
<td>2.32±23ᵇ (-3.49)</td>
<td>84.9±3.5ᵇ (-17.17)</td>
</tr>
<tr>
<td>TSiNP HPHI</td>
<td>1.54±5.84ᵇ (-69.92)</td>
<td>66.90±6.9ᵇ (10.69)</td>
<td>1.60±39ᵇ (-33.42)</td>
<td>44.4±3.1ᵇ (-65.68)</td>
</tr>
<tr>
<td>SSiNP HPHI</td>
<td>1.39±5.67ᵇ (-68.34)</td>
<td>72.48±3.8ᵇ (19.93)</td>
<td>1.54±67ᵇ (-35.93)</td>
<td>43.7±29ᵇ (-57.37)</td>
</tr>
<tr>
<td>TSiNP HPHO</td>
<td>1.20±5.84ᵇ (-72.67)</td>
<td>73.36±4.9ᵇ (21.37)</td>
<td>1.47±33ᵇ (-38.85)</td>
<td>43.1±29ᵇ (-57.95)</td>
</tr>
<tr>
<td>F. value</td>
<td>3.193E3</td>
<td>1.475E3</td>
<td>321.050</td>
<td>102.55±24ᵃ</td>
</tr>
<tr>
<td>Sig.</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
</tr>
</tbody>
</table>

Means followed by some letters are insignificant.

Phenol oxidase enzymes. Ecdysis is initiated by a polysis, the process that separates epidermal cells from the old cuticle by molting fluid secretion and ecdysial membrane formation. Molting fluid contains protease and chitinase enzymes that digest the main constituents of the old endocuticle. Therefore, they were decreased α-amylase and protease activities. α -Amylase and protease activity decreased by both of TSiNP HPHO, SSiNP HPHI and TSiNP HPHI compared with control, but in case of TSi, α-amylase and protease activities were almost equal with control. These results agreed with those obtained by (Bahrami et al., 2018) who cleared that when S. littoralis larvae feeding on food containing 100 and 200 ppm of caffeic acid (CA) after 10 days α-amylase activities decreased from 132 mu in the control to 62.1 and 55 respectively. Protease activity decreased from 120.8 mu in the control to 61.4 at 100 ppm and 41.13 mu at 200/g 26 (Hill et al., 2005) referred the reduction of digestive enzymatic activities by the lack of food intake. Also our results agreement with (Kantrao et al., 2017) who found that the higher concentration of The leaf extracts of banyan tree, Ficus benghalensis and Peepal tree, Ficus religiosa AgNPs alone had effectively reduced the larval body weight and increased mortality rates and inhibited protease activity. Also, they suggested that decrease in protease activity was due to the protease buried inside the nanoparticles and making no substrate available for binding of protease. Phenol oxidase activity was significantly increased in all treatments TSiNP HPHO and SSiPN HPHI the most increase followed by SiNP HPHI then TSi treatment. Our results agreed with (Kamel et al., 2010), who reported that phenol oxidase had a greater activity level of S. littoralis larvae treated with Agerin followed by Dipel 2X than those of control. Also agreed with (Osman et al., 2015) where applied silica nanoparticles Nano Zno and microorganisms against S. littoralis which reported that there increase in phenol oxidase was compared with control in case silica nanoparticles but lower than Nano Zno and microorganisms.

References


Efficacy of Silica nanoparticles on cotton leaf worm larvae, Spodoptera littoralis (Boisd.)


