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Efficacy and Biochemical Evaluation of Two Nano materials in Control of Cotton Leaf worm, *Spodopetra littoralis*

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Abstract

This study aimed to investigate the insecticidal activity of two Nano materials (Nano Silver and Nano Silica) against the Cotton Leaf Worm, ***Spodopetra littoralis*** in Agriculture research farm, during season 2020/2021. The two Nano materials were characterized by SEM and TEM. Four concentrations were applied for each Nano material (4000, 3500, 3000 and 2500 ppm). The tested two Nano materials significantly reduced the pest population compared to control. The result showed that nano silver material had a toxic effect against 2nd instar larvae of cotton leaf worm specially at 4000 ppm reached to 85.00 % after 12 days of treatment to 6.6 % in un treated also decreased adult emergency to 10.00% compared with 93.34 % in un treated, this mean it had broken life cycle of leaf wormwhile nano Silica recorded mortality 80.00 at 4000 ppm compared with control 6.66 . In pupation % we find a reverse relationship between it and conc. All tested materials had initial & latent toxic effect and developmental effect, this effect increased as both period after treatment and concentration increased. Nano silver showed the highest effect followed by silica, while silver showed the highest antifeedant effect followed by silica. Although silver showed the highest effect, silica is suitable for application for economic consideration and for its local availability.

Keywords: *Spodopetra littoralis*; Nano Silver; Nano Silica; Cotton Leafworm; insecticidal activity

1. Introduction

The cotton leaf worm, *Spodoptera littorals* (Bosid.) (Lepidoptera: Noctuidae) was and still now considered one of the most serious and destructive pests, not only for cotton plants, but also other vegetable, ornamental and field crops in Egypt. Intensive use of broad-spectrum chemical insecticides for controlling this pest usually leads to adverse effects on non-target organisms and development of high levels of resistance in the pest to organophosphates, carbamates and pyrethroids. Organo phosphorous (i.e., parathion, chlorpyrifos and traitorhoods) and pyrethroids (i.e., Cypermethrin, Deltamethrin and Cyfluthrin) pesticides are commonly used for controlling Spodoptera Littoralis [1]. Although, the agronomic and economic benefits of these pesticides, the overuse of such traditional organic pesticides causes critical problems to human health and environment [2]. To avoid unfavorable side effects of the hazards of pesticides on non-target organisms and environment, alternative materials have been initiated recently using safe and effective insect pathogens such as microbial insecticides [3]. Nanotechnology opens a large scope of novel application in the fields of biotechnology and agricultural industries, because nanoparticles have unique physicochemical properties, i.e., high surface area, high reactivity, tunable pore size, and particle morphology, [4]. Nowadays, the need for new and effective method became necessary after the increase of environmental pollution and insect resistance to chemical insecticides. This study contains Preparation of some nanomaterials (Nano silver and Nano silica). Preparation of nanomaterials as sprayable form solution (wettable powder) study the effect of

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different concentration on the mortality percentage of cotton leaf worm using a laboratory strain of Spodopetra *Littoralis* (Boisd) 4thlarve [5]. Used silver nanoparticles as pesticide carrier by loading the organo phosphorous pesticide profenofos on to their surface. This study tested profenofos (Ag Nps) alone and nano composite profenofos (AgNps @ p) against 2nd and 4th larvae of laboratory and field cotton leaf worm and indicated that the AgNps @ p is more effective on cotton leaf worm than each of profenofos and nano silver alone. Prepared silica nanoparticles from rice husk and studying its impact against cotton aphid and mealy bug during 2017-2018 at center for nanotechnology lab. meta silica nanoparticles and dinotefuran 20%SG was used as insecticides. [6]: investigated the impact of silver nanoparticles aqueous solution against 3rd instar larvae of pericalliaricini (Lepidoptera: Archiodae) compared to commercial neem insecticide (Vijayneem) under laboratory conditions.The application of nanomaterials in the area of plant sciences (i.e., nutrients and/or pest control) has been extensively investigated to overcome the expected increases of global population without negative impacts to environment and/or public health [7-9]. For example, Ag, CuO, MgO, and ZnO nanoparticles were presented as effective antimicrobial agents [10-14]. Amorphous silica nanoparticle (SiO2 NPs) showed biological entomologic effects, although it is inert in nature and considered as a biocompatible material according to US Food and Drug Administration [15]. the present investigation was planned to study the efficacy of some Nanoparticles on some biological and biochemical parameters of S. littorals under laboratory conditions. Also, in this research we will studying the resistance phenomenon of *Spodopetra Littoralis* to nanomaterials through determination of some enzymes activity related to the insect resistance.

2. Experimental

I. Preparation of the tested nanomaterials

A. Preparation of Silver Nano-particles

Preparation of silver nanoparticles by chemical reduction method by putting 80 ml of $AgNO_3$ in a beaker and heated it to 60 ^{0}C. then, in anther beaker we put Poly Vinyl Pyrolidene (PVP) and glucose as a reducing agent with NaOH as accelerator and 60 mL distilled water then heated this beaker to 60 ^{0}C and stirred for 30 min. Then, Added the beaker containing $AgNO_3$ to beaker in which the Poly Vinyl Pyrolidene (PVP) and then add glucose dropwise. The Mixed solution was stirred for 20 minutes and centrifuge to separate Ag nanoparticles. Figure 1.A shows the transmission electron microscope (TEM) photograph of the nano silver dispersed in distilled water.

B. Preparation of Silica nanoparticles:

The preparation of silica nanoparticles by surface – modification in situ in aqueous solution by using sol-gel method [16-24]. Stoichiometric amount of Sod. Meta silicate was dissolved in deionized water in a reactor equipped with a condenser and two isopiestic dropping funnels. A solution of HCL dissolved in deionized water was placed into one funnel. The weighed hexamethyl disilazane (HMDS) dissolved in absolute alcohol as the modifier was placed into the other funnel. At first, we added half of the hydrochloric acid solution to the reactor with stirring at room temperature. Then, the rest of the hydrochloric acid and the modifier were added dropwise to the reactor simultaneously. When the concentration of sodium meta-silicate was equal to that of hydrochloric acid, the reaction solution appeared turbid. Gradually, an amount of foam appeared on the surface of this aqueous solution. The suspension was heated to 60 ºC and was stirred for 4 h at this temperature. The obtained solution was put into a separating funnel. The suspension was separated into two layers quickly, and a layer of white floc settled down of the separating funnel. The floc was collected by filtration and washed repeatedly using a mixed solution of deionized water and alcohol until Cl^- could not be detected using silver nitrate solution by visual examination. Here, Cl^- and Na^+ were removed at the same time. The filtered cake was redisposed into a quantity of mixed solution of deionized water and alcohol at a volume ratio of 1:1 to form emulsion. Finally, the emulsion was spray-dried and loose SiO_2 nanoparticles were obtained (with a yield rate of over 97 % measured based on the Si of sodium meta- silicate and HMDS). A typical formula was used as follows: 0.3 mol / L sodium meta silicate, 0.72 mol / L hydrochloric acid, and 0.05 mol / L modifier. The size of nanoparticles can be conditioned on the concentration of the sodium meta silicate and the modifier. By controlling the reaction conditions, we can obtain nano silica particles. Figure 1.B shows the transmission electron microscope (TEM) photograph of the nano silica dispersed in distilled water.

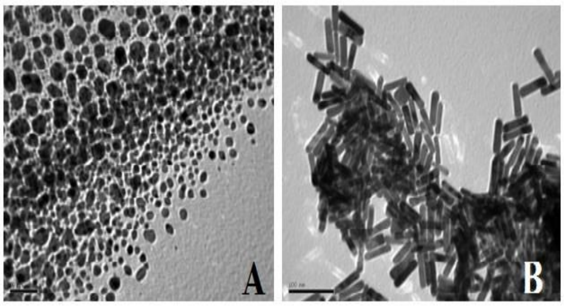

Figure 1. The transmission electron microscope (TEM) image of A) Nano Silver and B) Nano Silica.

Nanomaterials were prepared as wet table powder formulation 15% in pesticide formulation research department, control agriculture pesticides laboratory by mixing nano particle with bentonite as a carrier and Arkobal N 100 as suspending, wetting, and spreading agent.

The culture of ***S. littorals*** used in this study originated from eggs obtained by a susceptible strain established in cotton leaf worm department, Plant Protection Research Institute, A.R.C, Giza, Egypt. This strain was reared in the laboratory under constant laboratory conditions of 25 ± 2 ºC and 70 ± 5 % R.H. according to [25]. egg masses were kept jn petri dishes, 2 egg masses in each dish, until hatching. The hatched larvae were transferred to glass jars (2 liter capacity) covered with muslin cloth secured with rubber bands larvae were provided daily with fresh Castor leaves (*Rieinus communis*). The resulting moths were fed on 20 % sugar solution and allowed to lay their eggs on fresh *Neriumo leader* leaves as a physical surface for moths mating, oviposit ion and resting egg- masses were collected each 2 days and transferred to petri- dishes for another generation.

II. The insecticidal activity of Nano Prepared formulations:

For studying the latent effect, other samples were taken two Nano materials were used (Nano silver and silica) by 4 concentrations 4000, 3500, 2000 and 2500 ppm.). Three replicates for each treatment each have 20 larvae in addition to another untreated check control (treated with fresh water). The larvae used in the experiment 2nd and 4th instar larvae. The leaf dipping technique method was used in this study, where fresh castor oil leaves were dipped for 15 second in one of the prepared concentrations. All the obtain results were statistically analyzed as a complete randomized blocks design and the appropriate methods were used for the analysis data. The percentage of larval mortality were corrected according to Abbott's formula [26]

Corrected mortality % =

$$\frac{\text{observed mortality \%} - \text{control mortality \%}}{100} \times 100$$

-Estimation of LC_{50} according to [27] Known as Probit long paper.

III. Biochemical Evaluation of Some Nano materials:

Homogenate samples were collected from late 4th and 6th instar larvae fed on different nano metal treated leaves by homogenizing in insect physiological saline and collected in cold tubes (on ice) previously coated with crystals of phenylthiourea to prevent melanization. The samples were centrifuged at 2500 rpm for 5 minutes under cooling (4°C) to remove the tissues. After centrifugation, the supernatant fluid was divided into small aliquots (0.5 ml) and stored at – 20 °C until analysis. The Biochemical analysis were the following: a) determination of total protein content [28], b) determination of Chitinase activity [29] and c) determination of non-specific esterases activities [28].

3. RESULTS AND DISCUSSION

I. The insecticidal activity of nano materials on cotton leafworm

Results shown in table 1. about the toxicity and latent effect of nano silver against 2nd instar larvae of cotton leafworm indicated that the toxicity and % of mortality increased by increasing concentration (2500, 3000, 3500 and 4000) and also increased by increasing period of feeding with treated leaves increased. On the other hand, pupation % decreasing by increasing the concentration (15, 18.33, 20 and 26.66 in a concentration 4000, 3500, 3000 and 2500 ppm) and the same thing in % of adult emergency. Results shown the toxicity and latent effect of nano silver against 4th instar larvae of cotton leafworm indicated that the toxicity increased with high concentration (4000, 3500, 3000 and 2500) and also increased by increasing period of feeding with treated leaves increased On the other hand pupation % decreasing by increasing the concentration (20.00, 21.67, 25.00 and 30.00 in a concentration 4000, 3500, 3000 and 2500 ppm) compared with untreated control which recorded 95.00% and the same thing in % of adult emergency which recorded 15.00, 18.00, 21.00 and 26.00 while control recorded 93.34%. Generally, it could said that nano silver material had a toxic effect against 2nd instar larvae of cotton leaf worm specially at 4000 ppm reached to 85.00 % after 12 days of treatment to 6.6 % in un treated also decreased adult emergency to 10.00% compared with 93.33 % in un treated, this mean it had broken life cycle of leaf worm.

Data in both Table 2 indicated that the effect of nano Silica in pupation % we find a reverse relationship between it and conc. we find that a small pupation % with high conc. 20.00, 23.34,24.00 and 28.67 &28.87, 31.67, 33.34 and 35.00 in both 2nd& 4th instar larvae, compared with untreated control 93.34 &95.00, respectively.

Also, the same thing in adult emergency which recorded 15.00, 18.00, 20.00 and 22.00 & 20.00, 25.00, 28.00 and 30.00 compared with control recorded 93.34 & 93.33, respectively.

Table (1): Effect of Nano silver material on different developmental stages of 2nd and 4th instar larvae of S. littorals

Conc. ppm	2nd instar larvae		4th instar larvae	
	Pupation %	% of Adult emergency	Pupation %	% of Adult emergency
2500	26.66	21.66	30.00	26.66
3000	20.00	16.66	26.00	21.66
3500	18.33	13.00	21.66	18.33
4000	15.00	10.00	20.00	3.33
Control	93.34	93.34	95.00	93.33

Table (2): Effect of Nano silica material on different developmental stages of 2nd and 4th instar larvae of S.littorals

Conc. ppm	2nd instar larvae		4th instar larvae	
	Pupation %	% of Adult emergency	Pupation%	% of Adult emergency
2500	26.66	25.00	35.00	31.33
3000	25.00	21.66	33.34	28.33
3500	23.33	20.00	31.66	25.00
4000	20.00	15.00	26.66	21.66
Control	93.34	93.34	95.00	93.33

The results shown that the toxicity increased as in both concentration and period of feeding with treated leaves increased. The tested compounds were more effective against 2nd instar larvae than 4th instar larvae. Silver showed the highest initial and latent toxicity against both 2nd& 4th instar larvae followed by silica. Results obtained were agree with the study obtained in references [27-29].

II. Antifeedant effect:

The results in Table 3 show the antifeedant effect of the tested nano materials indicated that the percentage of reduction in food consumption which mean protection of treated plant from feeding with this insect increase as an increase in concentration, the effect against 2nd instar larvae was more than 4th instar larvae. Silver gave the highest antifeedant effect than silica. Antifeedant activity was determined according to El-Sisi and Farag formula (1989).

Table 3. Antifeedant effect of the tested materials against 2nd and 4th instar larvae of S. littorals

Treatments	Conc. ppm	2nd instar larvae		4th instar larvae	
		Consumption %	Reduction %	Consumption %	Reduction %
Sliver	2500	81.95	18.05	89.71	10.29
	3000	80.00	20.00	88.92	11.08
	3500	76.334	23.66	88.48	11.52
	4000	71.75	28.25	77.18	22.82
Silica	2500	83.23	16.77	86.34	13.66
	3000	82.00	18.00	85.67	14.33
	3500	77.56	22.44	83.87	16.13
	4000	74.45	25.55	82.13	17.87
Control	100.00	00.00	100.00	00.00	

III. Biochemical studies:

A. Enzymes measurements: Determination of total proteins:

The proteins are the major component of enzymes responsible for multiple biochemical functions in growth of insects. Data in table (4) show that all tested---- have an observed significantly increase in total proteins 222.69, 247.49 for silver, silica respectively, compared with 165.88 for control against 4th instar larvae. However, total proteins were not significantly decrease 249.72,269.94 for silver, silica respectively, compared with 272.83 for control against 6th instar larvae.

Table (4): Effect of nano metal on the total contents of protein (ug protein/min/ml) of late 4th and 6th instar larvae of Spodoptera littoralis.

Tested compound	4 th instar larvae		6th instar larvae	
	Total proteins		Total proteins	
	Mean activity (ug protein/min/ml)	Change %	Mean activity (ug protein/min/ml)	Change %
Silver late	222.687	25.51	249.72	-9.25
Silica late	247.492	32.97	269.944	-1.07
Iron oxcide	202.353	18.022	357.64	23.71
Control	165.884	---	272.835	---
LSD 0.05	**1.41**			

This result agreed with [28] observed a conspicuous depletion in total protein content in 6th treated larval instar withLC_{50} of the novel insecticide pyridalyl. The protein pool of the Naphthol /min/ml) of late 4th and 6th instar larvae of Spodoptera *littoralis*

1- Determination of Alpha & Beta esterase activity:

Values of alpha and beta esterase are tabulated in Tables (5,6) against 4thinstar larvae. All tested compounds caused an increase in alpha esterase activity, Silica late recorded the highest value of activation 49.32% followed by Silver late which have 41.29% above the control level, respectively. On the other hand, both Silica late and Silver late increased the activity of beta esterase enzymes 52.38% and 43.05% higher above the control level, respectively. The same results were obtained against 6thinstar larvae. All tested compounds caused an increase in alpha esterase activity, Silica late recorded the highest value of activation 45.92% followed by Silver late which have 38.71% and 27.75% above the control level, respectively.

Also, both Silica late and Silver late increased the Also, both Silica late and Silver late increased the activity of beta esterase enzymes 45.925% and 40.46% higher above the control level, respectively. The obtained results matched with [29] findings in Gharbia, alpha -esterase activity increased. Concluded that both alpha and beta esterase in *S. littoralis* was highly inhibited with hexaflumuron.

It is clearly noticed that Silver late, Silica late and Iron oxcide late may be cause different levels of significant changes in alpha and beta esterase on *S. littoralis*. Haemolymph functions as a reserve source of protein synthesis need for growth and development of the adult stage during pupal life [27]. Wilkinson (1976) stated that protein help to synthesize microsomal detoxifying enzymes which assist in the detoxification of toxicants that enter into the insect body. Proteins are the most important components of biochemical of insect that bind the foreign compounds. In general, the problem of protein synthesis is intimately related to metabolism of nucleic acids.

4. Statistical analysis:

Statistical significance was assessed by Duncan,s and Tukey,s test at p=0.05 (Snedecor& Cochran 1980).

Table (5): Effect of nano metal on the α-Estrase (ug α-Naphthol /min/ml) of late 4 th and 6thinstarlarvae of Spodoptera littoralis

	4 th instar larvae		6th instar larvae	
Tested compound	Alpha esterase		Alpha esterase	
	Mean enzyme activity (ug α-naphthol/min/g .b.wt)	Change %	Mean enzyme activity (ug α-naphthol/min/g .b.wt)	Change %
Silver late	536.05	41.29	452.764	38.71
Silica late	620.99	49.32	513.193	45.92
Iron oxcide late	421.90	25.40	384.112	27.75
Control	314.74	---	277.507	---
LSD 0.05				

Table (6): Effect of nano metal on the β-Estrase (ug β-Naphthol /min/ml) of late 4 th and 6thinstarlarvae of Spodoptera littoralis.

	4 th instar larvae		6th instar larvae	
Tested compound	Beta esterase		Beta esterase	
	Mean enzyme activity (ug α-naphthol/min/g .b.wt)	Change %	Mean enzyme activity (ug α-naphthol/min/g .b.wt)	Change %
Silver late	312.410	43.05	263.447	40.46
Silica late	373.608	52.38	290.065	45.925
Iron oxcide late	230.932	22.96	217.107	27.75
Control	177.90	---	156.852	---
LSD 0.05	1.37			

5. Conclusion

The results of conducted study about nano materials (silver, silica) which prepared as wet table powder formulation against both 2nd and 4th instar larvae of cotton leafworm indicated that all tested materials had initial & latent toxic effect and developmental effect, this effect increased as both period after treatment and concentration increased. Nano silver showed the highest effect followed by silica, while silver showed the highest antifeedant effect followed by silica. Although silver showed the highest effect, silica is suitable for application for economic consideration and for its local availability. More research should be carried out in field to determine the successful concentration required for controlling this serious insect.

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7.REFERENCES

[1] Abbott, M. S. (1925): J. Econ. Entomol.18 : 265 – 267.
[2] Abdel-Mageed, M.I., M.G. Abbas, A.I. Gadallah and A. Hanafy(2000): Annals of Agricultural Science (Cairo), 4: 1585-1595.
[3] Ahmed,K.S.,Mikhail,W.Z.A.,Sobhy,H.M.,Radwan,E.M.M.and Salaheldin\,T.A.(2019):.Bulletin of the National Research Center).
[4] Amal A. Abd Allah (2016): Al- Azhar. J. Agric. Res., vol. 27,,297- 303.
[5] Dahi, H.F., A.S. Kamel, N.M. El-Barkey and M.F. Abd-El-Aziz, (2011): J. Amer. Sci., 7(12): 855-863.
[6] El-Defrawi, M., A. Tappozada, N. Mansour and M. Zeid (1964): J. Econ. Entomol. 57 (4): 591- 594.
[7] El- Kholy, R. M. A. (2005): Al-Azhar J. Agric. Res. 42: 79 – 93.
[8] El-Sisi, A. G. and R. M. Farrag (1989): Agric. Res. Rev., 67 (1) : 29 – 35.
[9] Finney, D. J. (1925) : Probit analysis 2 ndedn. Cambridge univ. press, London : 318 pp.
[10] Florkin, M. and C.H. Jeanuiaux,(1964): Academic Press, New York & London., (3): 109-152.
[11] Gavkare, O.; M. U.Palil ; A. V. Kulkarni and S. Gupta(2013):.J.Pop.Kheti.,1(3):34-39.
[12] Gerstl, Z.; Nasser, A.; Mingelgrin, U.(1998): J. Agric. Food Chem, 46, 3797– 3809.
[13] Ishaaya, I. and J.E. Casida (1974): Biochem. Physiol., 4: 484-49.
[14] Lowry, O. H.; Rosebrough, N. J.; Farr, A. L. and Randall, R. J. (1951): J. Biol. Chem., 193: 265-275.
[15] Sahayaraj, K.; M.Madasamy and S. A. Radhika (2016): J. Biopesticides, 9(1), 63-72.
[16] Attia, M. S., Youssef, A. O., Khan, Z. A., & Abou-Omar, M. N. Talanta. 186, (2018)36–43.
[17] Attia, M. S. and Al-Radadi, N. S. Biosens. Bioelect. 86, (2016) 413-419.
[18] Attia, M. S., Khalil, M. H., Abdel-Mottaleb, M. S. A., Lukyanova, M. B., Alekseenko, Yu. A., Lukyanov, B. Int. J. Photoenergy, 2006, 1–9. (2006).
[19] Attia, M. S., Youssef, A. O., Essawy, A. A. Anal. Methods. 4, 2323–2328(2012).
[20] Attia, M. S., Ali, K., El-Kemary, M., & Darwish, W. M. Talanta, 201, (2019)185–193.
[21] Attia, M. S., Mahmoud, W. H., Youssef, A. O., Mostafa, M. S. J Fluoresc. 21, (2011)2229-2235.
[22] Attia, M.S., Ramsis, M.N., Khalil, L.H., Hashem, S.G., J. Fluoresc., 22 , (2012)779–788
[23] Attia M. S., W. H. Mahmoud, M. N. Ramsis, L. H. Khalil, A. M. Othman, S. G. Hashem, M. S. Mostafa, J. Fluoresc. 21 (2011) 1739–1748
[24] Attia, M.S., Othman, A.M., Elraghi, E., Aboul-Enein, H.Y., J. Fluoresc. 21 (2011) 739–745
[25] Nasir.A.Siddique, Mohammed.A.Al, Enezi, Perwez Alam, Anzarulhaque, Mohammed Ali. (2015. Afr J Tradit complement Altern Med.12(3):42-48.
[26] Sneddcor, G.W. and W.G. Conchran,(1980): Statistical Methods. 7th Ed. State University Press, Ames.
[27] Van Asperen, K. (1962): J. Insect Physiol., 8: 401-416.
[28] Wilkinson, F., (1976): Insecticide biochemistry and physiology. Plenum Press, New York.
[29] Xiaohongli,Zhicao,Fengliu,Zhijun.Zhang,and Hongxin Dang(2005): Chinese Academy of sciences.vol(35)94-96.