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Comparison between the Efficacy of Two Nano-Particles and Effective Microorganisms on Some Biological and Biochemical Aspects of *Spodoptera littorals*

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***Abstract* – The cotton leaf worm, *Spodoptera littorals* (Bosid.) (Lepidoptera: Noctuidae) is considered the major important pest of cotton plants as well as other field crops and vegetables, causing severe injuries to the plants in all phonological crop stages. The overall objective of this investigation was to look for new control strategy through evaluating the effect of Silica (SNp) and ,Zinc oxide (ZNp) Nano -particles as well as an effective microorganisms (EMs)on the 2nd larval instar of *S . littoralis* under laboratory conditions . To study the effect of the tested materials on some biological aspects, pupation and adult emergence percentages, larval and pupal weight were estimated. Effect of the tested materials on some biochemical parameters; total carbohydrates, total proteins ,Lactate dehydrogenase ,chitinase ,phenol oxidase alkaline phosphatases GPT and GOT activities of the 6th larval instar were also determined . Results clearly showed that Nano-Silica was the most effective compound ,followed by Nano-Zinc oxide, then EMs , respectively .It causing high toxicity against this pest up to 12 days post exposure .In addition all the tested materials exhibited latent effect via producing high reduction in both pupation and adult emergence rates decreasing both larval and pupal weight of this pest The all tested materials showed reduction in the estimated enzymes activity , except phenol oxidase moreover , decreasing both total carbohydrates and proteins were noticed . Obtained results suggest that using Silica , and Zinc oxide Nano -particles as well as EMs would be a useful and eco- friendly components for controlling *S. littoralis* among Integrated Pest Management strategy.**

Keywords* – Biochemical, Biological, Microorganisms, Nano Particles, *Spodoptera littoralis.

I. Introduction

The cotton leaf worm, *Spodoptera littorals* (Bosid.) (Lepidoptera :Noctuidae) was and still now considered one of the most serious and destructive pest ,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 [1], [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]. Microbial insecticides have been reported to provide inadequate control of *S.littoralis* and prime candidates for use in Integrated Pest Management Programme (IPM). They have high pathogenicity for target pests, safe for most non-target organisms, and have good integration with other pest control methods [4]. Use of effective microorganisms (Ems), as potential biological control agents based on previous reports of their ability to control plant pathogens through competition exclusion has become a new approach. Nodaway nanotechnology has become one of the most promising new approaches for pest control. Nanoparticles represent a new generation of environmental remediation technologies that could provide cost –effective solution to some of the most challenging environmental clean-up problems [5]. Nanoparticles help to produce new pesticides, insecticides and insect repellants [6],.Nanotechnology is emerging as a highly attractive tool for formulation and delivery of pesticide active ingredient as well as enhancing and offering new active ingredients ,such as Nano capsules based on polymers are being designed for controlled release of active ingredient as well as enhanced delivery through improving penetration through leaves [7], [8] showed that Nano alumina could be successfully used to control stored grain pests .Silica (SNp) and Silver nanoparticles (AgNp) can also be used as valuable toll in pest management programs of cowpea seed beetle, *Callosobruchus maculates* [9]. Also hydrophobic Nano Silica was effective against *S. littoralis* and could be useful component of an integrated pest management strategy on tomato plants [7], Therefore the present investigation was planned to study the efficacy of some Nano -particles and Effective microorganisms on some biological and biochemical parameters of *S. littorals* under laboratory conditions.

II. Materials and Methods

A. Tested Insect:

A laboratory strain of *Spodoptera littorals* was obtained from Plant Protection Research Institute, A.R.C, Giza, Egypt, it was reared on castor bean leaves under constant conditions at 25 ± 2 ^{0} C and 65±5%RH. Using the method described by [10]. The 2nd larval instars were used in all laboratory experiments.

B. Test compound

- *Nano -particles:* Zinc oxide and Silica Nano -particles with purity of 99.99% were produced from Egypt Nanotech company Limited, El-Wahaat Road, 6 th October, Cairo Egypt.

- *Microbial agent:* Effective Microorganisms (Ems) bio -formulation used in this study was obtained from the Egyptian Ministry of Agriculture, Cairo , Egypt. This formulation contained 60 species of beneficial microorganisms grow media and produced locally under supervision of Japanese EMRO Scientific Organization.

C. Toxicological and biological studies:

The dipping technique was applied to examine the effect of Zinc oxide, Silica nanoparticles and Effective Microorganisms (Ems) on toxicological and some biological aspects of the 2nd instar larvae of S. littorals. Serial concentration was prepared by dilution the tested compound with distilled water. Castor bean leaves were dipped for 15 second in each concentration, then left to dry at room temperature and were offered to ten 2nd instar larvae, three replicates were carried out for each concentration ,larvae were allowed to feed on the treated leaves for 48 hrs. and then removed, the fresh untreated leaves were provided to the larvae until pupation .Other three replicates were dipped in distilled water for the same period as check. Mortalities were recorded daily and corrected using [11]. To obtain the LC_{50} values for each tested compound to use in biochemical studies, the corrected mortality percentage after the end of larval stage were statistically computed using software program [12]. The different biological aspects such as: pupation and adult emergence percentages, larval and pupal weight were also determined.

D. Biochemical studies :

Total body tissue samples were collected from late 6th larval instars treated as 2nd instars with LC_{50} values of tested three compounds according to the method described by [13]. Determination of total carbohydrates content, total proteins , Lactate dehydrogenase (LDH) ,chitinase, phenol oxidase, alkaline phosphatases, Transaminases Glutamic pyruvic Transaminase (GPT) and Glutamic oxalo acetic Transaminase (GOT) activities were determined calorimetrically according to the methods of [14]- [20], respectively .

E. Statistical analysis:

Mean lethal concentration (LC_{50}' s) and the regression lines were statistically done [12], using probit analysis software computer program.

III. Results and Discussion

A- Efficacy of tested materials on some toxicological and biological aspects of Spodoptera littorals:

The obtained results in Tables (I, II and III) summarized the toxicity and latent effect of candidate materials at different concentrations against the 2 nd larval instar of *S. littorals*. Data clearly showed that the larval mortality % showed positive correlation with the concentrations of the tested materials and time after exposure. The mortalities were increased as concentrations and time after treatment increased. Mortality rate was low during the first three days. The highest cumulative larval mortalities were observed with the maximum conc. level (2000 ppm) ,it produced 86.67 , 83.33 and 50 % larval mortalities after 12 days post exposure for Nano-Zinc oxide (ZNp) ,Nano-Silica (SNp) and Effective microorganisms (Ems) ,respectively as shown in Tables (I,II and III) . Nano-Silica was the most effective compound against the 2nd larvae of *S. littorals*, followed by Nano- Zinc oxide, then Effective microorganisms, respectively according to LC_{50} values and toxicity index (Table IV). The corresponding LC_{50} values were 74.4, 146.79 and 799.62 ppm respectively .On the other hand, the tested Nano -particles and EMs exhibited Latent effect on the larvae of *S. littorals*. The pupation and adult emergence rates resulted from the treated 2nd instars larvae with the different concentrations of candidate compounds gave highly reduction compared to untreated larvae. The highest reduction was observed with the highest concentrations. level (2000 ppm), it produced 13.33, 16.67 and 50 %of pupation compared with 96.67 % in control and 13.33 ,16.67 and 40 % of the adult emergence % compared with 86.67 % in untreated larvae for ZNp ,SNp and EMs ,respectively (Tables I,II and III) .In addition highly reduction in both larval and pupal weight was noticed at all the tested concentrations . The highest concentrations level (2000 ppm) caused the highest reduction in both larval and pupal weight ,which being 0.207, 0.218 and 0.23gm larval weight compared with 0.38 0.392 gm. in control for Nano –Zinc oxide, Nano –Silica and Ems, respectively (Tables I,I I. and III) .

Table I: Influences of different Nano-Silica concentrations on some biological aspects of S. littorals treated as 2nd larval instar

Conc. ppm	% Cumulative larval mortality at indicated days						6th Larval weight (g) ± SE	Pupation %	Pupal weight (g) ±SE	Adult emergence % ±SE
	2	3	5	7	9	12				
2000	16.67	23.33	33.33	50.00	63.33	83.33	0.207±0.024	16.67	0.206±0.019	16.67
500	13.33	20.00	23.33	46.7	53.33	76.67	0.277±0.016	23.33	0.273 ± 0.14	20.00
250	6.67	16.67	20.00	40.0	46.6	73.33	0.263±o.o17	26.67	0.27±.012	23./33
125	3.33	13.33	16.67	36.7	43.33	53.33	0.293±.005	46.67	0.285±.029	.40.00
Control	0.0	0.0	0.0	0.0	3.33	6.67	0.392±0.041	93.33	0.38±0.047	86.67

Results revealed that Silica Nano -particles was the most effective product followed by Zn oxide Nano -particles and Effective microorganisms .SNp beside insecticide is also physically active material. These nanoparticles caused damage to cuticular water barrier of the insect mostly by abrasion as well as to some extent due to adsorption. As particles have enormous surface area at Nano scale compared to their bulk counterpart, Silica caused greater damage to insect exoskeleton. The cuticular water barrier of insect got disrupted, and the insect began to lose water from their bodies and ultimately died. The dead bodies became extremely dehydrated and shrunken in comparison with the live larvae. In previous studies [7], and [21], reported that hydrophobic Nano-Silica when treated against neonates of *S. littoralis* indicated high toxic action at all concentration used after 15 days post application on infested tomato and soybean plants under semi filed conditions. In addition ,it affects the biological parameters of this pest such as , larval and pupal duration ,adult longevity and female fecundity ,thus reducing insect population density ,damages and yield losses to the crop .In contrast , [22], indicated that Silica -nanoparticles showed slightly mortality against the newly hatched larval of the pink bollworm , *Pectinophora gossypiella* .

Table II: Influences of different Nano-Zinc oxide concentrations on some biological aspects of S .littorals treated as 2nd larval instar

Conc. ppm	% Cumulative larval mortality at indicated days						6th Larval weight (g) ±SE	Pupation %	Pupal weight (g) ±SE	Adult emergence % ± SE
	2	3	5	7	9	12				
2000	23.33	36.66	46.67	56.67	66.67	86.67	0.218±0.0138	13.33	0.163±0.035	13.33
500	16.67	26.67	36.67	50.00	56.67	70.00	0.26±0.014	30.00	0.20±0.033	26.67
250	3.33	13.33	16.67	20.00	36.67	63.33	0.273±0.019	36.67	0.22±0.27	30.00
Control	0.0	0.0	0.0	0.0	3.33	6.67	0.392±0.041	93.33	0.38±0.047	86.67

Similarity results also indicated that Zinc oxide Nano-particles showed biological activity against the 2nd Instars larvae *of S. Littorals* may be due to the absorbance of this Nano -particles into the cubicula lipids of the insect, resulting in damage in the protective wax layer and induces death by desiccation (23). The results are going in line with those published by (22), they reported that, Zinc oxide Nano -particles exhibited high efficacy against the pink bollworm (PBW), *p. gossypiella.*

Table III: Influences of different Effective microorganisms (Ems) concentrations on some biological aspects of S. littorals treated as 2nd larval instar

Conc. Ppm	% Cumulative larval mortality at indicated days						6th Larval weight (g)±SE	Pupation %	Pupal weight (g) ±SE	Adult emergence % ± SE
	2	3	5	7	9	12				
2000	20.00	26.67	33.33	36.67	43.33	63.33	0.237±0.0053	36.67	0.22±0.027	33.33
500	16.67	20.00	26.67	33.33	40.00	46.67	0.260±.0.0187	53.33	0.28±0.065	43.33
250	13.33	10.00	23.33	26.67	36.77	43.33	0.275±0.0136	56.67	0.29±0.11	46.67
125	0.0	6.67	13.33	23.33	30.00	33.33	0.29±0.105	66.67	0.296±0.0027	.50.00
Control	0.0	0.0	0.0	0.0	3.33	6.67	0.392±0.041	93.33	0.38±0.047	86.67

Table IV: Toxicity response of the tested materials against the 2nd instar larvae of S. littoralis

Compound	LC_{50} ppm	Lower Limit	Upper limit	Slope ±SE	Toxicity index
Nano-Silica	74.40	19.61	135.68	0.719±0.159	100
Nano-Zinc	146.79	81.17	211.803	0.937±0.160	50.69
Ems	799.62	514.74	1631.56	0.666±0.145	9.30

As shown in Table (III) the effective microorganisms (Ems) formulation showed moderately toxic against the larvae *of S. littorals* , this may be due to the presence of several microbial isolates in the formulation that have entomopathogenic activity through production of some toxic secondary metabolites [24], The main species involved in the Ems include : lactic acid bacteria (*Lactobacillus plantarum, L. casei* and *Streptoccus lactis),* photosynthectic bacteria (*Rhodopseudomonas palustrus* and *Rhodobacter spaeroides) ,yeasts (Scacharomyces cerevisiae and candida utilits*) , actinomycetes (*Streptomyces albus and S. griseus) and fermenting fungi (Aspergillus oryza and Mucar hiemalis)* [25], Synthesis using bio- organisms is compatible with the green chemistry principles, and is considered eco-friendly [26].

B- Efficacy of tested materials on some biochemical parameters of S. littorals :

Table V: Total carbohydrates and Total protein content of S. littorals treated with LC_{50} of Nano Zinc oxide, Nano silica and Effective Microorganisms (EMs).

Treatment	Total carbohydrates *(mg/g.b.wt*) M± SE			Total protein content(mg/g.b.wt) M ± SE		
	Mean ±SE	Change %	Activity ratio	Mean ±SE	Change %	Activity ratio
Nano-Zno	10.76±0.80	-19.2	0.807	21.93±1.43	-30.66	0.693
Nano- silica	8.43±0.58	-36.76	0.632	22.43±0.35	-29.08	0.709
Ems	6.73 ±0.56	- 49.51	0.504	28.30±1.13	-10.52	0.894
Control	13.33± 1.11	---------	---------	31.63±1.55	-------	-------

Data represented in Table (V) showed that total carbohydrates content of *S. littoralis* was significantly decreased in all treatments. The most reduction was occurred by the treatments of Ems and Nano silica, the corresponding reduction were (- 49.51), and (-36.76), respectively while Nano Zno caused slightly decrease which being (-19). The decrease in total soluble carbohydrate content of the treated larva could be attributed to metamorphic changes in larva. During this stage, the carbohydrate content supply the body with glucose which provides an energy source for synthesis of larva and adult tissues, especially the cuticle. Carbohydrates are necessary for the normal functioning of the male and female reproductive systems, as well as for the development of the embryo. In males, sugars form an important constituent of the reproductive glands and most of the carbohydrates of reproductive system were present in the testes. In the females system, carbohydrates are necessary for vitellogenesis and for the formation of the glycosaminoglycan present in the vitalize membrane and chorion. Vitellogenesis involves the accumulation within the oocyte of carbohydrate, lipid and protein yolk to meet the structural and metabolic needs of the developing embryo [27], [28], this may explain the high malformation rate and more significant decrease in total carbohydrates content in 6^{th} instars of *S. Littorals* treated with the treatment of EMS .These results are parallel with [29], who reported that *S. marcescence* could be considered as a bio pesticide , and become more effective when used in sequential treatment with either teflubenzuron or *Bacillus . thuringiensis* . Also our results are in agreement with [22], who reported that EMs caused highest decrease in carbohydrate digestive enzymes than zinc oxide, silica and spinsade. Also data from Table (V) showed that total protein content of *S. littoralis was* significantly decreased in all treatments. The most decrease was happened with the% reduction in Nano Zn .oxide (-30.66) treatment and,then Nano silica (-29.08) treatment followed by EMS treatment (-10.52) lower than in the control.Data obtained from Table (V) showed different pattern effect on total protein contents of the candidate compounds. The effect of both Nano Zno and Nano silica on 6^{th} instars of *Spodoptera littoralis* showed marked decrease of the total protein content while in treatment of EMS was mild decreased. [30], whe reported that protein leakage during intoxication may arise from reduced body weight, conversion of protein to amino acids, degradation of protein to release energy or the direct effect of the tested extracts on the amino acids transport of the cell. Thus, it could be stated that both Nano Zno and Nano silica had more significant effect on the reduction in total soluble protein content than treatments with Ems. Similar results were obtained by [31] who found that *B. thuringiensis* var. kurstaki caused a significant reduction in the protein content of *S. littorals* larvae. [32] observed significant reduction in total protein content of *S. littoralis* larvae after treatment with *B. thuringiensis* var. kurstaki. This could be due to the breakdown of protein into amino acids which help to supply energy for the insect**.** [33] indicated that, *B. thuringiensis* resulted in a great reduction in protein content of *S. littorals* larvae and these toxins of *B.thuringiensis* are responsible for the inhibition of protein synthesis by forming a protein complex. The decrease in the protein content of the pupae in the present work in all treatments might be due to the protein was binding with foreign compounds as the compounds used. Our results were also in agreement with the results of [22].

Table VI: Lactate dehydrogenase (LDH) and chitinase activity of 6^{th} instars of S. littoralis treated with LC_{50} of Nano Zinc oxide, Nano silica and Effective Microorganisms (EMs)

Treatment	(LDH) Mean (mg/g.b.wt) ± SE)			chitinase (ug NAGA /min/g.b.wt)±SE		
	Mean ±SE	Change %	Activity ratio	Mean ±SE	Change %	Activity ratio
Nano-Zno	235.66±6.65	- 48.58	0.514	383.33±12.22	22.34	1.22
Nano- silica	229.66±5.03	-49.89	0.501	325.33±6.42	3.89	1.03
Ems	262.33±6.80	-42.76	0.572	402.33±14.64	28.40	1.28
Control	458.33±19.31	---------	---------	313.33±11.59	-------	-------

Results in Table (VI) clearly showed that Lactate dehydrogenase (LDH) activity of *S. littorals* was significantly decreased in all treatments. The most decrease was happened with the Nano silica treatments, % activity (- 49.89) followed by ZnO treatment (-48.58) and then EMS treatment (-42.76%).Results showed that Lactate dehydrogenase (LDH) activity of *S. littorals* was most significantly decreased with the Nano silica followed by EMs treatment and, then N. Zn O (Table VI). These results were in agree with that recorded by [34], who reported that Emamectin benzoate, resulted in inhibition of LDH of *S. litttoralis* also our results were parallel with results of [35], where spinotram had inhibitory effect on LDH with a range between -46.59 and -55.75% for kalyobia and behiara strains of *S. litttoralis* and the suppression of LDH level due to treatment demonstrating low nutritional efficiency of larvae since LDH is important glycolytic enzymes and can used as indicator of exposure to insecticide [36], Lactate dehydrogenase is an important glycolytic enzyme being present in virtually all tissues [37], it is also involved in carbohydrate metabolism and has been used as an indicative criterion of exposure to chemical stress [38], [36] Also data from Table (VI) showed that chitin's activity was significantly increased in all treatments. The most increase was happened with the Ems treatments, (28.40) followed by Nano Zn o Treatment (22.34) and Nano silica (3.89%).Data showed that chitinase was most increase with the Ems treatments, followed, by N. ZnO and then, N. silica (Table VI). These results were in agreement with [39], who reported that chitinase was widely produced in strains and some of the strains could enhance the toxicity of active *B.*

thuringiensis strain .Our results also parallel with [29], who reported that chitinase in pupal stage of *S. littorals* treated with *B. thureinginsis* caused a significant increases in the chitinase through the pupal stage of *S. littoralis* at all times.

Table VII: Phenol oxidase and alkaline phosphatases (ALP) activity of 6th instars of S. littoralis treated with LC_{50} of Nano Zinc oxide, Nano silica and Effective Microorganisms (EMs)

Treatment	Phenol oxidase activity (O.D units /min/g.b.w)±SE			Alkaline phosphatases activity $Ux10^3$/g.b.wt. ±SE		
	Mean ±SE	Change %	Activity ratio	Mean ±SE	Change %	Activity ratio
Nano-Zno	14.24±0.19	23.50	1.23	268 ±5.29	-7.69	0.923
Nano- silica	11.95±0.2	3.64	1.03	273.33±5.71	-5.85	0.941
EMs	15.36±0.60	33.21	1.33	284 ±6	-2.18	0.978
control	11.53±0.32	---------	---------	290.33±10.59	-------	-------

Data from Table (VII) showed that Phenol oxidase activity was significantly increased in all treatments, the most increase caused with the Ems treatments, (33.21), followed by Nano Zno treatment (23.50) and Nano silica(3.64%). upper than in the control

Phenol oxidase is important components of insect immune systems. Phenol oxidase activity has been shown to correlate with resistance to some parasites/pathogens across species [40]. Our results were in agreement with [32], who reported that phenol oxidase had a greater activity level in larvae treated with Agerin followed by Dipel 2x. Than those of control

Data in table (VII) showed that alkaline phosphatases (ALP) activity of 6th instars of *S. littorals was* significantly decreased in all treatments, the most decrease was happened with the Nano Zno, (-7.69) Nano silica (-5.85), then EMS treatments (-2.18%) lower than in the control. ALP and ACP are hydrolytic enzymes, which hydrolyze phosphor monoesters under acid or alkaline conditions, respectively. ALP is mainly found in the intestinal epithelium of animals and its primary function is to provide phosphate ions from mononucleotide and ribonucleic-proteins for a variety of metabolic processes. ALP is involved in the Trans phosphorylation reaction (Etebari *et al.,* 2005)**.** Our results clearly indicated that the alkaline phosphatases activities were highly significantly reduced at all-time interval post – treatment. These results are in conformity with the findings of [41], [42], and [43], [44], who reported that Alkaline and acid phosphatase showed a significant reduction in *Musca domestic* treated with *B. thuringiensis.*

Table VIII: Glutamic pyruvic Transaminase (GPT) and Glutamic oxaloacetic Transaminase (GOT) activities of 6th instars of S. littorals treated with LC_{50} of Nano Zinc oxide, Nano silica and Effective Microorganisms (EMs).

Treatment	GPT			GOT		
	Mean ±SE	Change %	Activity ratio	Mean ±SE	Change %	Activity ratio
Nano-Zno	584.66±15.50	-42.22	0.577	1634.33±28.88	-11.80	0.881
Nano- silica	1005±10.14	-0.691	0.993	1846 ±16.82	0.38-	1.012
Ems	762.66±10.50	-24.63	0.753	1655.5 ±10.81	-10.71	0.893
Control	1012±24.33	---------	-----------	1853.66±55.41	-------	-------

Results in Tables (VIII) showed that (GPT) and (GOT) activity of 6th instars of *S. Littorals* was significantly decreased in all treatments the most decrease was caused with the Nano Znic oxide (-11.80), (- 42.22), EMS (- 10.71), (-24.63), then Nano silica (-0.38), (-0.691) treatments, respectively lower than in the control. Transaminases (GPT and GOT) enzymes help in the production of energy [45], GOT and GPT serve as a strategic link between the carbohydrates and protein metabolism and are known to be altered during various physiological and pathological conditions [46], Our results clearly indicated that GOT and GPT activities were highly significantly reduced at all-time interval post – treatment. These results are in conformity with the findings of [22], who reported that the Nano- silica and pyriproxyfen were the most effective treatments in GOT enzyme activity reduction in the 4th instar larvae, of *P. gossypiella* , followed by spinosad, zinc oxide and EMs, respectively. High, reduction of GPT enzyme compared to control was noticed by pyriproxyfen and silica, respectively. EMs lead; to increase in GPT activity opposite to control. The observed changes in levels of the tested enzymes may be due to the physiological or pathological alterations induced' by treatments and may contribute significantly to their levels in treated larvae [47],

IV. Conclusion

It could be concluded that efficacy of Silica and Zinc oxide nanoparticles as well as effective microorganisms against the cotton leaf worm, *S. littorals* exhibited their potential as a new source of insecticidal materials. Minimizing side effects for being rich source of bioactive chemicals, biodegradable in nature and nonpolluting (eco-friendly). Particulate systems like nanoparticles have been used a physical approach to alter and improve the effective to properties of some types of synthetic chemical pesticides. This study demonstrated that SNp ,ZNp and Ems could be a useful components for controlling *S. littorals* in Integrated Pest Management strategy

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