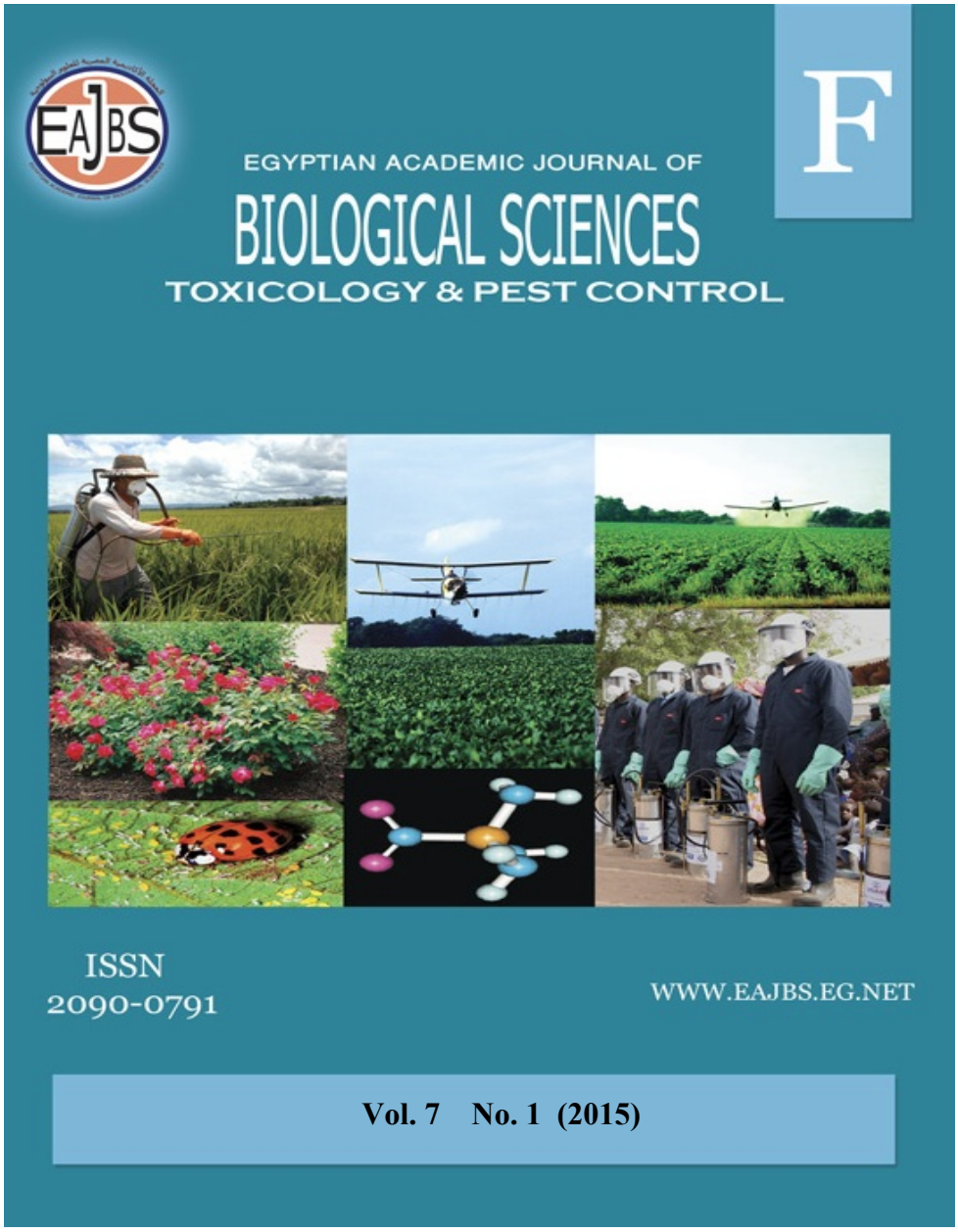

The journal of Toxicology and pest control is one of the series issued twice by the Egyptian Academic Journal of Biological Sciences, and is devoted to publication of original papers related to the interaction between insects and their environment.

The goal of the journal is to advance the scientific understanding of mechanisms of toxicity. Emphasis will be placed on toxic effects observed at relevant exposures, which have direct impact on safety evaluation and risk assessment. The journal therefore welcomes papers on biology ranging from molecular and cell biology, biochemistry and physiology to ecology and environment, also systematics, microbiology, toxicology, hydrobiology, radiobiology and biotechnology.

www.eajbs.eg.net

Egypt. Acad. J. Biolog. Sci., 7(1): 163-175 (2015)

Egyptian Academic Journal of Biological Sciences
F. Toxicology & Pest control
ISSN: 2090 - 0791
www.eajbs.eg.net

New Approach Based on Nanotechnology in Baculovirus Protection

A. El-Helaly[1] and W. A. A. Sayed[2]

1-Department of Economic Entomology and Pesticides, Faculty of Agriculture Cairo University, Giza,

2-Biological Application Department, Nuclear Research Center, Atomic Energy Authority, Abo-Zaabel, Egypt & Middle Eastern Regional Radioisotope Center for Arab Countries, Dokki, Giza, Egypt.

ARTICLE INFO

Article History
Received:1/11/2015
Accepted:15/12/2015

Key words:
Nano Aluminum Oxide
Nano Zinc
*Spli*MNPV
Spodoptera littoralis
squash plants
electron microscope

ABSTRACT

Antioxidants proved decade ago to be promising protective additives to Baculoviruses against deleterious effect of UV in sunlight , Present semi field experiments' tests the role of nano antioxidants in providing better protection for baculoviruses. The treatments with *Spodoptera littoralis* nuclear polyhedrosis virus (*Spli*MNPV), consisted of (Nano Aluminum Oxide with or without *Spli*MNPV LC_{90}, and Nano Zinc Oxide with or without *Spli*MNPV LC_{90}) at five different concentrations.100, 200, 300, 400 and 500 ppm for different investigation periods of artificial UV source for maximum 5 then 10 hours in laboratory then 50 ml / squash plant with five replications, on which neonate larvae of *Spodoptera littoralis* were exposed daily. Larval mortality was recorded till the 15th day post application. Results are based on laboratory and leaf-bioassays to test Lethal Infectivity Time to 50% (LIT_{50}) of population. The results showed that LIT_{50} 82.759 hours and 52.500 hours for additive nano zinc oxide and nano aluminum oxide; respectively at 500 ppm concentration while it gave 14.482 with virus alone treatment and 100.788 hours with Cacao 5% as positive control, the mechanism of protection was studied through transmission electron microscope. The total antioxidants activity was determined using DPPH (2, 2-diphenyl-1-picrylhydrazyl) antioxidant assay technique as a first record to use this technique at this type of investigations. The obtained result suggested the possible capability of nano antioxidants in prolonging the virus activity on plant foliage under small scale semi-field application besides it suggests to do DPPH antioxidant assay technique first in the future with any candidate before the bioassay due to its clear image about the antioxidants activity.

INTRODUCTION

Spodoptera littoralis as a common pest on many crops (Bulmer *et al*. 2009, Zhang and Xiao-zhen 2010, Cloyd and Bethke 2011) and considered as one of the most series pest for many different crops in Africa and Europe (Horowitz *et al.*, 1994 and Smagghe and Degheele, 1997) was selected in the present study as a model to assess the prolonging in viral activity. Nanotechnology is emerging as a highly attractive tool for formulation and enhancing and offering new active ingredients Leiderer and Dekorsy, 2008.

Enhanced resistance towards insecticides and environmental hazards along with the restrictive use of many pesticides it is important to find out new strategies to replace older ones which are perceived to carry higher safety and environmental risks (Kida *et al.,* 2007). Nanoparticles possess distinct physical, biological and chemical properties associated with their atomic strength (Roy 2009). They can be arranged or assembled into ordered layers, or mine layers (Ulrich *et al.,* 2006). Nanotechnology, a promising field of research of pesticides and pest control (Matsumoto *et al*. 2009 and Harper 2010). Nano-pesticides and nano-encapsulated pesticides are expected to reduce the volume of application (Gojova *et al*. 2007 and Pan *et al.,* 2009). Nano antioxidants found to be folded stronger than natural one (Vardeman *et al*., 2007). The action of nano oxides is greatly depending on its mineral composition, type, insect species, or environmental conditions (Subramanyam and Roesli, 2000). The mode of action of nano aluminum and zinc oxides has not yet been elucidated, and detailed toxicity studies are needed to understand how it works and also to determine whether it constitutes a good alternative for insect pest control. Recently, novel types of nano particulate material, nano aluminum and zinc oxides have been found to induce mortality in insects (Stadler *et al*., 2010 and Debnath *et al.,* 2001). Therefore the present study was conducted to the current interest on nanomaterial-based technology for both baculovirus prolonging period of action and as a synergistic effect.

MATERIALS AND METHODS

Insect

Spodoptera littoralis (Boisd.) was used as test insect and reared under laboratory conditions on a semi synthetic diet of Shorey and Hale 1956 26°C±2 and 12 hours D/N duration lightening.

Virus Inoculum

A Local isolates of (*Spli*MNPV) was originally isolated in Egypt by Abul Nasr1956.

Nano materials

Nano Aluminum Oxide and Nano Zinc materials were tested supplied by Nano Tech. Egypt.

Laboratory Irradiation test

Simulated sunlight UV (SUV) was used where a set of four UV lamps (Ultra-Vitalux, OSRAM, Germany) (Huber and Ludcke, 1996) was established at unit of virology, department of economic entomology and pesticide, Faculty of Agriculture Cairo University. Virus with or without additives resembling 200 fold LC_{90} were spread inside a Petri dish. After air drying, the dishes with the virus film on surface were exposed to the UV irradiation sources. Screening trial divided into two progressive steps. The virus after irradiation was re-suspended according to Cisnero *et al*. (2002. and the bioassay according to (Fritsch and Huber, 1985). The plates were incubated at 26±2ºC and 60±5% R.H. under the laboratory conditions.

Semi Field Experiment

200 pots of squash were cultured in glass house, 140 squash pots only were used, 5 replicates for each concentration and for single period located at the unit of virology, Faculty of Agriculture, Cairo University. One concentration of nano aluminum oxide and nano zinc oxide additives was prepared 500 ppm and (5% w/v) of cacao and kept in the fridge till these additives mixed with virus to give final concentration of LC_{90}, Virus suspension treatments were applied separately to squash foliage using one liter hand sprayer. Leaves were randomly collected from treated / untreated pants at zero time, 10, 24, 48, 96, and 168 hours post application and kept individually.

Each leaf was placed into a glass bottle, on which 10 starved neonate larvae were allowed to feed for 3hr only, before transferred daily to plastic cubs with diameters of 3 cm in radius base and height of 5 cm and full of semi artificial diet of Shorey and Hale 1956 till its half. Then covered with double layer of soft paper tissue and the larval mortality were recorded till death or pupation this was modification of (Shapiro *et al.,* 2008) method.

DPPH assay

The measurement of the DPPH radical scavenging activity was performed according to methodology described by Brand-Williams *et al*., 2008. The samples were reacted with the stable DPPH radical in an ethanol solution. The reaction mixture consisted of adding 0.5 mL of sample, 3 mls of absolute ethanol and 0.3 mL of DPPH radical solution 0.5 mM in ethanol. When DPPH reacts with an antioxidant compound, which can donate hydrogen, it is reduced. The changes in color (from deep violet to light yellow) were read [Absorbance (Abs)] at 517 nm after 100 min of reaction using a UV-VIS spectrophotometer (DU 800; Beckman Coulter, Fullerton, CA, USA). The mixture of ethanol (3.3 mL) and sample (0.5 mL) serve as blank. The control solution was prepared by mixing ethanol (3.5 mL) and DPPH radical solution (0.3 mL). The scavenging activity percentage (AA %) was determined according to Mensor *et al.,* 2005.

Electron microscope

*Spli*NPV mixed with nano aluminum or with nano zinc oxide, and then emerged each sample above sucrose gradient, and then the resulted bands were washed twice in distilled water and Scanning electron microscope (SEM). For SEM (JOEL-JSM 5600, JAPAN)., the OB suspensions were prepared using negative staining . The coated samples were mounted in and visualized and photographed at various magnifications. The sizes of the OBs were measured directly from the amplified photograph using a scale and dividing the value by the magnification of the photograph (Rabindra *et al.,* 2003)**.**

Statistical analysis

Concentration-mortality regressions were calculated to determine the effectiveness of tested material as UV additives for the *Spli*NPV. Slope and LC_{50s} values were calculated according to the method described by Finney (1971).

The potential of the material to prolong the virus persistence as described by (Muro and Paul, 1985). to insure the potential of the tested material to prolong the virus persistence .for DPPH The experiment was done in triplicate for each substance. The results were expressed as percentage decrease with respect to control values and compared by one-way ANOVA and Turkey's test. A difference was considered statistically significant if $p<0.05$.

RESULTS AND DISCUSSION

Two nano materials dissolved in water additives containing antioxidants were evaluated in three progressive steps, first one was under artificial UV sunlight for 300 min as maximum, followed by another experiments for 600 min as maximum to give clear picture of these additives and their role in protection. Finally these materials examined under Egyptian sunny field conditions. The results were as following: Table (1) shows that % of mortality 5 hours later gave 8.69 % only with virus alone treatment while it gave 60.00, 62.50, 67.34, 75.51, and 72.00 % with nano aluminum oxide at different concentrations of 100, 200, 300, 400 and 500 ppm ; respectively while cacao give 54.00 %.

Table 1: Average rates of mortality among *S littoralis* neonate larvae treated with *Spli*NPV either alone or in combination with nano aluminum oxide or Cacao at 5 % concentration, all exposed to *different UV irradiation periods.*

Irradiation exposure period	Mortality % among larvae tested viruses						
	*Spli*NPV alone	NPV + nano aluminum oxide additives/ ppm and Cacao at 5%					
		NAO 100	NAO 200	NAO 300	NAO 400	NAO 500	Cacao 5%
Zero	10.00 (49/49)	87.23 (41/47)	95.83 (46/48)	93.47 (43/46)	95.83 (46/48)	96.00 (48/50)	96.00 (48/50)
30	81.63 (40/49)	100.00 (48/48)	91.83 (45/49)	100.00 (49/49)	100.00 (50/50)	100.00 (48/48)	96.00 (48/50)
60	40.81 (20/49)	83.33 (40/48)	100.00 (47/47)	100.00 (50/50)	100.00 (46/46)	97.91 (47/48)	92.00 (46/50)
180	23.40 (11/47)	67.34 (33/49)	74.00 (37/50)	64.58 (31/48)	69.56 (32/46)	69.95 (31/47)	78.00 (39/50)
300	8.69 (4/46)	60.00 (30/50)	62.50 (30/48)	67.34 (33/49)	75.51 (37/49)	72.00 (36/50)	54.00 (27/50)
Control*	0.00 (0/50)	14.89 (7/47)	13.04 (6/46)	20.40 (10/49)	18.75 (9/48)	18.36 (9/49)	0.00 (0/50)
LIT_{50}	64.747	423.404	552.564	421.393	692.380	439.329	406.757

DW + Virus or DW + Virus and nano aluminum oxide

The calculated lethal inactivation time for 50% of the tested *S. littoralis* neonate larvae was 64.747 minutes this activity increased to 423.404, 552.564, 421.393, 692.380 and 439.329 minutes by adding nano aluminum oxide at different concentrations of 100, 200, 300, 400 and 500 ppm; respectively (Fig. 1) while cacao gave 406.757. Nano aluminum oxide 400ppm concentration singled out with 10.693 folds of potency followed by other concentrations giving 8.534, 6.785, 6.539 and 6.508 with 200, 500, 100 and 300 ppm; respectively while it gave 6.282 folds with cacao treatment (Fig. 1). Table (2) shows that % of mortality 5 hours later gave 8.69 % only with virus alone treatment while it gave 68.75, 85.10, 80.00 and 85.41 % with nano zinc oxide at different concentrations of 100, 200, 300, 400 and 500 ppm; respectively while cacao give 54.00 %.

Median lethal inactivation for 50% of population increased with all nano zinc oxide concentration to give 696.477, 726.032, 388.390, 1588.304 and 2369.214 minutes with nano zinc oxide and Potency folds of 6.539, 11.213, 5.998, 24.53 and 36.59 fold; with nano zinc oxide at different concentrations of 100, 200, 300, 400 and 500 ppm; respectively where it gave 406.757 min LIT50 and 6.282 folds of potency with cacao and only 64.747 min with virus alone treatment. (Fig. 2).

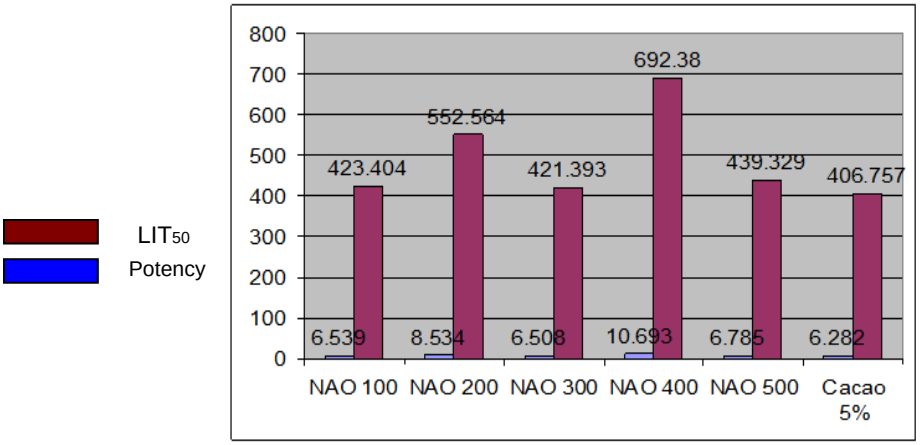

Fig. 1: LIT_{50} (Median lethal inactivation time) and Potency among *S littoralis* neonate larvae treated with *Spli*NPV either alone or in combination with nano aluminum oxide isolates or Cacao at 5% concentration.

Table 2: Average rates of mortality among *S littoralis* neonate larvae treated with *Spli*NPV either alone or in combination with nano zinc oxide or Cacao at 5% concentration, all exposed to different UV irradiation periods.

Irradiation exposure period	Mortality % among larvae tested viruses						
	*Spli*NPV alone	NPV + nano zinc oxide additives/ ppm and Cacao at 5%					
		NZO 100	NZO 200	NZO 300	NZO 400	NZO 500	Cacao 5%
Zero	10.00 (49/49)	100.00 (49/49)	100.00 (50/50)	100.00 (47/47)	100.00 (49/49)	100.00 (49/49)	96.00 (48/50)
30	81.63 (40/49)	100.00 (48/48)	97.95 (48/49)	100.00 (46/46)	100.00 (46/46)	100.00 (48/48)	96.00 (48/50)
60	40.81 (20/49)	94.00 (47/50)	89.58 (43/48)	93.87 (46/49)	100.00 (47/47)	100.00 (47/47)	92.00 (46/50)
180	23.40 (11/47)	83.67 (41/49)	86.00 (43/50)	83.33 (40/48)	81.25 (39/48)	100.00 (48/48)	78.00 (39/50)
300	8.69 (4/46)	68.75 (33/48)	85.10 (40/47)	80.00 (40/50)	85.41 (41/48)	100.00 (49/49)	54.00 (27/50)
Control*	0.00 (0/50)	12.24 (6/49)	6.38 (3/47)	22.00 (11/50)	24.00 (12/50)	22.91 (11/48)	0.00 (0/50)
LIT_{50}	64.747	696.477	726.0322	388.3900	1588.304	2369.214	406.757

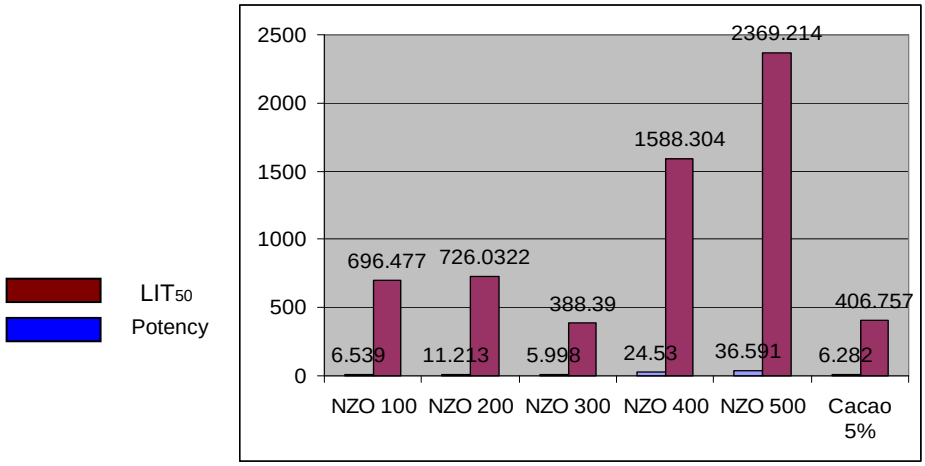

Fig. 2: LIT_{50} (Median lethal inactivation time) and Potency among *S littoralis* neonate larvae treated with *Spli*NPV either alone or in combination with nano aluminum zinc isolates or Cacao at 5% concentration.

The nano aluminum oxide showed to single out as a strong protective material where it give 0.00% of reduction at all UV periods of investigation (Table 3) while it gave 18.37, 59.19, 76.60 and 91.13 with 30, 60, 180 and 300 min with virus alone treatment; respectively and 0.00, 0.00, 026.05, and 24.00 with nano aluminum oxide and finally it gave 0.00, 4.00, 18.00 and 42.00 with cacao at the same previous periods; respectively.

Table 3: Average rates of reduction among *S littoralis* neonate larvae treated with *Spli*NPV either alone or in combination with nano aluminum oxide, nano zinc oxide or Cacao at 1% concentration, all exposed to different UV irradiation periods.

Irradiation exposure period (min)	Mortality % among larvae tested with			
	*Spli*NPV alone	NPV + The indicated additives		
		NAO 500 ppm	NZO 500 ppm	Cacao 5%
30	18.37	0.00	0.00	0.00
60	59.19	0.00	0.00	4.00
180	76.60	26.05	0.00	18.00
300	91.13	24.00	0.00	42.00

Prolonged period of UV application up to 10 hours at second stage showed that virus mixed with cacao gave 11.584 LIT_{50} hours while it gave 8.338 hours with virus mixed with nano aluminum oxide treatment and the protection reached to 134.251 with virus mixed with nano zinc oxide treatment while it give only 1.626 hour with virus alone treatment. (Table 4 & Fig. 3) the mortality % gave 23.40, 2.38, 2.08 and 0.00 with virus alone treatment 3, 5, 7 and 10 hours post investigation; Respectively while it gave 80.00, 80.00, 57.44and 38.00 with nano aluminum oxide treatment mixed with virus 89.79, 81.25, 88.00 and 78.00 % with nano zinc oxide mixed with virus and 92.00, 80.00, 74.00and 54.00% with cacao mixed with virus treatment.

Table 4: Average rates of mortality and reduction in virus activity among *S littoralis* neonate larvae treated with *Spli*NPV either alone or in combination with nano aluminum oxide, nano zinc oxide or Cacao at 5% concentration, all exposed to different UV irradiation periods.

Irradiation periods /hours	**Mortality % among larvae tested with**							
	***Spli*NPV alone**		**NPV + indicated additives**					
			NAO		**NZO**		**Cacao**	
	M%	**R%**	**M%**	**R%**	**M%**	**R%**	**M%**	**R%**
Zero time	100.00 (48/48)	----	100.00 (48/48)	----	100.00 (47/47)	----	100.00 (50/50)	----
3	23.40 (11/47)	76.6	80.00 (40/50)	20.00	89.79 (44/49)	10.21	92.00 (46/50)	8.00
5	2.38 (1/42)	97.62	80.00 (40/50)	20.00	81.25 (39/48)	18.75	80.00 (40/50)	20.00
7	2.08 (1/48)	97.92	57.44 (27/47)	42.56	88.00 (44/50)	12.00	74.00 (37/50)	26.00
10	0.00 (0/49)	100	38.00 (19/50)	62.00	78.00 (39/50)	22.00	54.00 (27/50)	46.00
Control DW	0.00 (0/50)	----	0.00 (0/50)	----	0.00 (0/50)	----	0.00 (0/50)	----
LIT_{50}	1.626	----	8.338	----	134.251	----	11.584	----

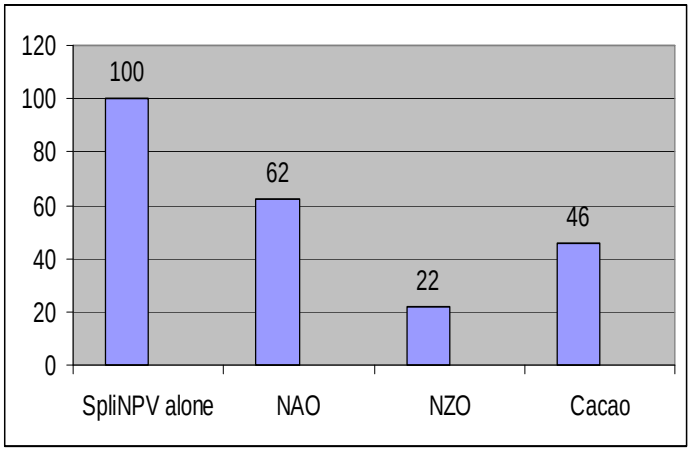

Fig. 3: Average rates of reduction in virus activity expressed in mortality rates among *S littoralis* neonate larvae treated with *Spli*NPV either alone or in combination with aluminum oxide, nano zinc oxide or cacao at 5% concentration, all exposed to different UV irradiation periods.

The reduction was 100% in case of virus alone treatment while it decreased gradually when virus mixed with nano aluminum oxide , cacao and nano zinc

oxide where it gave 62, 46 and 22; Respectively. Third and last evaluation was in semi field under Egyptian sunny conditions investigated on squashes gave the same trend. Virus alone treatment gave 60.00, 42.00, 6.00, 2.00 and 2.00% Mortality 10, 24, 48, 96 and 168 hours post investigation; Respectively while it gave 100.00, 80.00, 78.00, 44.00, 26.00 and 14.00 % mortality with nano aluminum oxide treatment, 93.47, 92.00, 68.00, 47.91and 22.44 with nano zinc oxide mixed with virus treatment and finally it gave 94.00, 80.00, 82.97, 56.25 and 26.53 with cacao mixed with virus treatment at the same previous post investigation periods (Table 5).

The median lethal inactivation gave 14.482 hours only with virus alone treatment while it increase gradually with cacao where it gave 100.788 and it gave 82.759 and 52.500 hours with nano zinc oxide and nano aluminum oxide, respectively. (Table 5) at the same trend both cacao and nano zinc oxide gave the lowest reduction % where they gave 69.47 and 75.47% of reduction in order while it increased to 86.00 with nano aluminum oxide mixed with virus and 96.00 with virus alone treatment. (Fig. 4)

Table 5: Average rates of mortality and reduction in virus activity among *S littoralis* neonate larvae treated with *Spli*NPV either alone or in combination with nano aluminum oxide, nano zinc oxide or Cacao at 5% concentration, all exposed to different natural sunlight irradiation periods.

Irradiation periods /hours	Mortality % among larvae tested with							
	*Spli*NPV alone		NPV + indicated additives					
			NAO		NZO		Cacao	
	M%	R%	M%	R%	M%	R%	M%	R%
Zero time	98.00 (49/50)	----	100.00 (49/49)	----	97.91 (47/48)	----	96.00 (48/50)	----
10	60.00 (30/50)	38.00	80.00 (40/50)	20.00	93.47 (43/46)	4.44	94.00 (47/50)	2.00
24	42.00 (21/50)	56.00	78.00 (39/50)	22.00	92.00 (46/50)	5.91	80.00 (40/50)	16.00
48	6.00 (3/50)	92.00	44.00 (22/50)	56.00	68.00 (34/50)	29.91	82.97 (39/47)	14.00
96	2.00 (1/50)	96.00	26.00 (13/50)	74.00	47.91 (23/48)	50.00	56.25 (27/48)	39.75
168	2.00 (1/50)	96.00	14.00 (7/50)	86.00	22.44 (11/49)	75.47	26.53 (13/49)	69.47
Control DW	0.00 (0/50)	----	0.00 (0/50)	----	0.00 (0/50)	----	0.00 (0/49)	----
LIT_{50}	14.482		52.500		82.759		100.788	

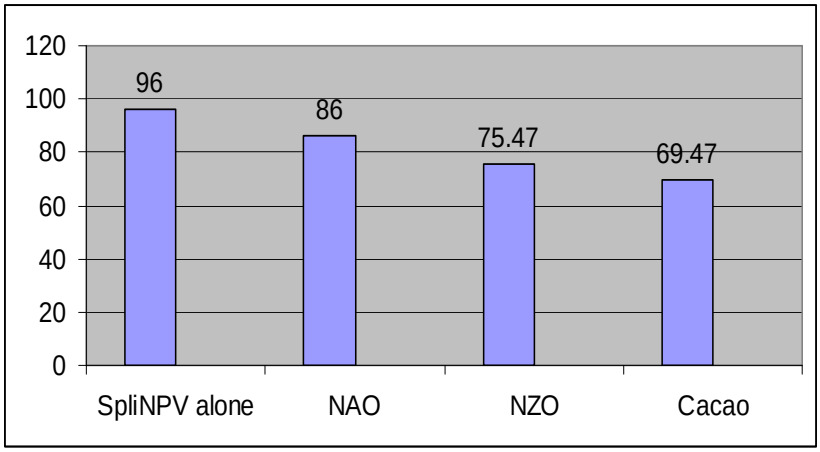

Fig. 4: Average rates of reduction in virus activity expressed in mortality rates among *S littoralis* neonate larvae treated with *Spli*NPV either alone or in combination with aluminum oxide, nano zinc oxide or cacao at 5% concentration, all exposed to different periods of natural sunlight.

As it is illustrated in tables 1 and 2 both nano aluminum oxide and nano zinc oxide alone caused death to *Spodoptera littoralis* neonate larvae where it gave 14.89, 13.04, 20.40, 18.75 and 18.36 mortality % with 100, 200, 300, 400 and 500 ppm concentrations of nano aluminum oxide alone treatment, and 12.24, 6.38, 22.00, 24.00 and 22.91 mortality % with the same concentrations of nano zinc oxide while it gave 0.00% for both distilled water and cacao alone treatment. Further investigation on this point was done, and the role of these materials to enhance virus alone treatment studied and the results showed in Table 6.

Table 6: Average rates of mortality among *S littoralis* neonate larvae treated with *serial concentrations of Spli*NPV either alone or in combination with nano aluminum oxide, nano zinc oxide.

The virus concentration	Mortality % among larvae tested viruses						
	*Spli*NPV alone	NPV + indicated additives					
		300 ppm		400 ppm		500 ppm	
		NAO	NZO	NAO	NZO	NAO	NZO
1 X 10^6	94.00 (47/50)	98.00 (49/50)	100.00 (48/48)	100.00 (48/48)	100.00 (50/50)	100.00 (47/47)	100.00 (50/50)
1 X 10^5	88.00 (44/50)	100.00 (49/49)	94.00 (47/50)	88.00 (44/50)	95.83 (46/48)	97.91 (47/48)	92.00 (46/50)
1 X 10^4	38.00 (19/50)	34.00 (17/50)	40.00 (20/50)	40.81 (20/49)	43.75 (21/48)	44.89 (22/49)	58.00 (29/50)
1 X 10^3	22.00 (11/50)	38.00 (19/50)	46.00 (23/50)	34.00 (17/50)	40.00 (20/50)	38.00 (19/50)	54.00 (27/50)
1 X 10^2	12.00 (6/50)	22.44 (11/49)	18.36 (9/49)	22.00 (11/50)	26.00 (13/50)	34.00 (17/50)	44.00 (22/50)
control*	0.00 (0/50)	20.40 (10/49)	22.00 (11/50)	18.75 (9/48)	24.00 (12/50)	18.36 (9/49)	22.91 (11/48)
LC_{50}	8761.472	3335.458	2705.959	4077.694	1688.323	1868.256	520.407

*DW + Virus or DW + Virus and nano aluminum oxide or nano zinc oxide

The median lethal inactivation dose found to be 8761.472 PIBs with virus alone treatment while it decreased regularly nano zinc oxide mixed with virus to give 3335.458, 4077.694, and 1868.265 with 300, 400 and 500 ppm concentrations and 2705.959, 1688.323 and 520.407 only with nano zinc oxide mixed with virus treatment, besides nano zinc oxide mixed with virus gave 44% mortality % and 34.00% with nano aluminum oxide mixed with virus with sublethal dose 1 X 10^2 PIBs where it gave only 12% mortality % with virus alone treatment. Remarkable effort have been done previously in order to protect baculoviruses (Shapiro et *al*., 2007a, b; Shapiro *et al.,* 2008 and El Salamouny *et al.,* 2009, Deotale et al., 2007 Hong et al, 1996; Mahajan and Sharma 2004 and Nautiyal and Venkataraman 2005, El-Helaly *et al*., 2009 , El-Helaly 2013 and El-Helaly *et al.,* 2013) This work is the first record to use nanotechnology in Baculovirus protection or DPPH assay as a parameter.

Nanomaterials including polymeric nanoparticles, iron oxide nanoparticles, gold nanoparticles, and silver ions have been exploited as pesticides. (Al-Samarrai 2012) and their potential for use in insect pest management (Bhattacharyya *et al*., 2010) such as *Helicoverpa armigera* (Vinutha *et al.,* 2013), Synthesized silver nanoparticles possessed excellent antilice and mosquito larvicidal activity (Jayaseelan *et al.,* 2011) and cotton lef worm *Spodoptera littoralis*(El-bendary and El-Helaly 2013) have been reported. So the present paper tried to test other nanoparticles and tested their effects alone or in combination with *Spli*NPV. Our gained results were in the same trend

with (Nel *et al.,* 2006). They suggested that nanomaterial may control *Sitophilus granaries.* Mode of action occur destruction of the natural water Barrier (Leiderer and Dekorsy, 2008). Consequently, increase of zinc dose in its normal molecule size, led to the accumulations of zinc in the larval hemolymph and fat body, and more zinc was accumulated in fat body than in hemolymph of *Spodoptera litura* Fabricius. Powell *et al*., 2005 found that The mode of action can be mentioned in specific points as a result of interaction of free radicals with DNA. Besides there are some findings of role of nano antioxidants to cause damage of all components of a cell (Gurr *et al*. 2005, Nel *et al*. 2006 and Ashe 2011) Consequently, while the exact mechanisms of the antibacterial action had not yet been clearly understood, it had been suggested that the rule of reactive oxygen species (ROS) generated on the surface of the particles, zinc ion release, membrane dysfunction, and nanoparticles internalization were the main cause of cell swelling (Nair *et al*., 2008)..

Electron microscope

Electron microscopic (EM) studies revealed typical baculovirus OBs of type Nuclepolyhedrovirus (NPV) with polyhedral structures and rod shaped nucleocapsids (NCs) Fig 5-1 and 5-3). Under SEM the OBs of viruses appeared bacilliform shaped of dimensions 277.7 × 41.6 nm (Fig. 5-3). elongated with parallel sides and two straight ends, measuring the sizes of 277.7 × 41.6 nm (*Spli*NPV) coating the virus resulted into great large diameters (Fig. 5-4, 5-5, and 5-6). These findings agreed with Rabindra *et al.,* 2003 and BaiHuiMin *et al.,* 2011besides in fig 5-3 the virogenic stroma was closely conjugated with nano zinc oxide, which may play a role in baculovirus protection.

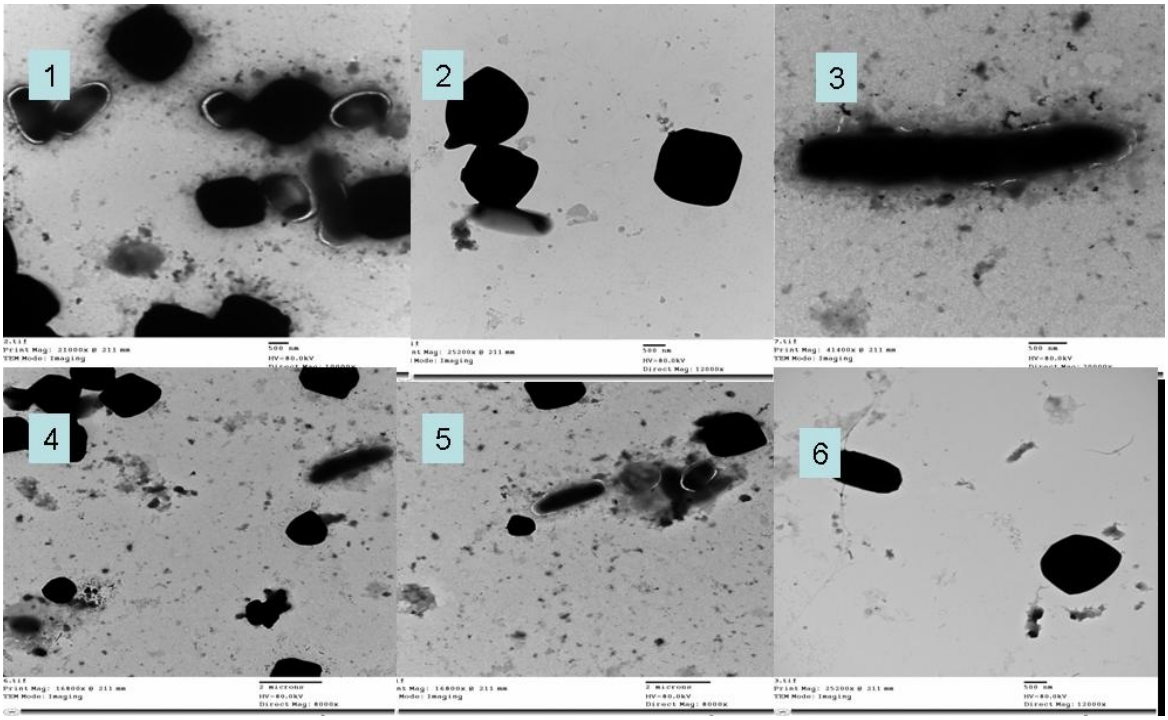

Fig. 5: *Spli*NPV virus in combination with nano aluminum oxide (2, 4, 5) or nano zinc oxide under scanning electron microscope (1. 3 and 6).

DPPH assay

All nano oxides exhibited antioxidant activity with higher values of antioxidant activity for nano zinc oxide (95.7%) followed by nano aluminum oxide, ($p>0.05$). (74.1%) where cacao gave only (34.2%) The DPPH free radical assay can be considered reliable and reproducible because in all products the coefficient of variation is lower as the DPPH assay is a spectrophotometric method. Variations in plant material, extraction method, processing and antioxidant assays employed might affect the concentrations of active compounds that could be reflected in the antioxidant activity Mensor *et al.,* 2005**.** Previous findings explain why nano zinc oxide was singled out and could be a proper protective and synergistic material to baculoviruses

REFERENCES

Abul-Nasr S. (1956). Polyhedrosis-virus disease on cotton leaf worm, *Prodenia litura* F. Bull. Entomol. Soc. Egypt, Econ, Ser., 40:321-332.

Al-Samarrai A M (2012). Nanoparticles as alternative to pesticides in management plant diseases-a review. Int. J. Scient. Res. Publ. 2 (4):1–4.

Ashe B. (2011). A Detail investigation to observe the effect of zinc oxide and Silver nanoparticles in biological system. M Sc thesis Department of Biotechnology & Medical Engineering, National Institute of Technology Rourkela-769008, Orissa, India. pp.110.

Bai H. M. Wang Yun L. X. Mao H. T. ; Li Y. Han S. Shi Z. and Chen X. W. (2011). Isolation and characterization of a novel Alphanodavirus. Virology Journal, 8(311).

Bhattacharyya A. Bhaumik A. Rani P. U. Mandal S. and Epidi T. T. (2010). Nanoparticles – a recent approach to insect pest control. Afr. J. Biotechnol. 9 (24): 3489–3493.

Brand-Williams W. Cuvelier M. E. Berset C. (1995). Use of a free radical method to evaluate antioxidant activity. Lebenson Wiss Technol, 28:25-30.

Bulmer M. S. Bacheletb I. Ramanb R. Rosengaus B. and Sasisekharan R. (2009). Targeting an antimicrobial effector function in insect immunity as a pest control strategy. PNAS. 106(31):12652-12657

Cloyd R. A. and Bethke J. A. (2011). Impact of neonicotinoid insecticides on natural enemies in greenhouse and interiorscape environments. Pest Manage. Sci. 67(1): 3-9.

Deotale R. O. Dawane P. N. Biswane K. D. and Borker S. L. (2007). Effectiveness of UVprotectants on the activity of NPV against Helicoverpa armigera(Hubner) on chickpea. Journal of Entomology Research. 31(1): 33-35.

El Salamouny S. Shapiro M. Ling K. S. and Shepard B. M. (2009). Black tea and lignin as Ultraviolet protectants for the beet armyworm nucleopolyhedrovirus. Journal of Entomological Science, 44(1): 50-58.

El-bendary H. M. and El-Helaly A. A. (2013). First recordnanotechnology in agriculture: Silicanano-particlesa potential new insecticide for pest control. App. Sci. Report.4 (3): 2013:241-246

El-Helaly A. (2013). Additives For A Baculovirus Against Ultraviolet Effect. Applied Science Reports. 4 (1):187-191

El-Helaly A. El-Salamouny S. Khattab M. El-Sheikh M. and Elnagar S. (2009). Preliminary evaluation of natural antioxidants as UV-protectants of *Spodoptera littoralis* nucleopolyhedrovirus, (Baculoviridae). *4th conference on recent technologies in agriculture"*

challange of agriculture modernization" Cairo university, Faculty of Agriculture.1:7-13.

El-Helaly M. Khattab A. S. El-Salamouny M. El-Sheikh S. Elnagar M. (2013). Promising additives to protect the activity of Baculovirus biocontrol agent under field – sunlight conditions in Egypt.*Journal of Life Sciences 7: 495-500.*

Finney D. J. (1971). Probit analysis. 3rd ed., *Cambridge Univ., Cambridge U.K.*

Fritsch E. and Huber J. (1985). Inactivation of codling moth granulosis viruses by ultraviolet radiation and temperature. Nachrichtenblatt des deutshen pfanzenchutzdientes, 37(6): 84-88.

Gojova A. Guo B. Kota R S. Rutledge J C. Kennedy I M. and Barakat A I (2007). Induction of inflammation in vascular endothelial cells by metal oxide nanoparticles: effect of particle composition. Environmental Health Perspectives, 115, 403–409.

Gurr J. Wang A. A. S. Chen C. and Jan K. (2005). Ultrafine titanium dioxide particles in the absence of photoactivation can induce oxidative damage to human bronchial epithelial cells. Toxicol., 213: 66 –73.

Harper S. (2010). New Approaches Needed to Gauge Safety of Nanotech-Based Pesticides, Researchers Urge. Phys. Chem. 4(33): 2010-2012.

Hong T. D. Linington S. Ellis R. H. (1996). Seed storage behaviour: a compendium.Handbooks for Genebanks: No. 4.

Horowitz, A. R.. Forer G. and Ishaaya I. (1994). Insecticide resistance management as a part of an IPM strategy in Israeli cotton fields. In Challenging the future, Proc. of the World Cotton Research Conference, I, ed. G. A. Constable and W. W. Forresater. Csiro, Australia, 1994, pp. 537- 544.

Huber J. and Ludcke C. (1996). UV-inactivation of baculoviruses: the bisegmented survival curve. Bulletin OILB/SROP. 19(9): 253-256.

Jayaseelan C. Rahuman A. A. and Rajakumar G. (2011). Synthesis of pediculocidal and larvicidal silver nanoparticles by leaf extract from heartleaf moonseed plant, Tinospora cordifolia Miers. Parasitol Res. 109(1):185–194

Kida T. Oka M. Nagano Y. Ishiwata X. and Zheng J. (2007). Synthesis and Application of Stable Copper Oxide Nanoparticle Suspensions for Nanoparticulate Film Fabrication Fabrication.J. Amer. Cera. Soc., 90(1): 107-110.

Leiderer P. and Dekorsy T. (2008). Interactions of nanoparticles and surfaces Tag derm Äundlichen PrÄufung: 25. April. URL: http://www.ub.unikonstanz.de/kops/ volltexte /2008 / 5387/; http://nbn-resolving.de/urn:nbn:de:bsz:352-opus-53877

Mahajan S. Sharma Y. K. (2004). Production of rayon grade pulp fromMoringa oleifera.Indian Forester. 110(3): 303-306.

Matsumoto S. Christie R. . Nishiyama N. Miyata K. and Ishii A. (2009). Environment-responsive block copolymer micelles with a disulfide cross-linked core for enhanced siRNA delivery. Biomacromol. 10: 119-127.

Mensor L. L. Menezes F. S. Leitao G. G. Reis A. S. dos Santos T. C. Coube C. S. (2001). Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. Phytother Res 15: 127-130.

Muro E. M. Paul J. I. (1985). Laboratory evaluation of new ultraviolet absorbers for protection of Douglas-fir tussock moth (Lepidoptera: Lymantriidae)

baculovirus. Journal of Economic Entomology. 78. 951-957.

Nair S. Sasidharan A. and Raina S. (2008). Role of size scale of Zn O. nanoparticles on toxicity toward bacteria and osteopath cells. J. Mater Sci. 20: 235-241.

Nautiyal B. P. Venkataraman K. G. 2005. Moringa (Drumstick)- An ideal tree for social forestry; growing conditions and uses- part I: Myoforest.23(1):53-58.

Nel A. Xia T. Madler L and Li N (2006). Toxic potential of materials at the nano level. Science, 311: 622–627.

Pan Z. Lee W. Slutsky L. Clark R A. Pernodet N. and Rafailovich M. H. (2009). Adverse effects of titanium dioxide nanoparticles on human dermal fibroblasts and how to protect cells. Small. 5: 511-520.

Powell C. Swenberg J. S. and Rusyn I. (2005). Expression of base excision DNA repairs genes as a biomarker of oxidative DNA damage. Cancer Lett. 229:1-11.

Rabindra R. J. Swamiappan M. Parthasarathy R. Subramanian, S. Kennedy J. S. Sathiah N. and Rajasekaran B. (2003). Isolation and DNA characterizationof a nuclear polyhedrosis virus from the looperBoarmia (Ascotis) selenaria (Lepidoptera: Geometridae). Pest Management in Horticultural Ecosystems, 9(1): 49-53.

Roy S. C. (2009). There is plenty of holes at the bottom: The other side of Nano. Sci. Cult. 75(1-2): 1-3.

Shapiro M. El Salamouny S. Shepard B.M. (2008). Green tea extracts as ultraviolet protectants for the beet armyworm, Spodoptera exigua nucleopolyhedrovirus. Biocontrol Science and Technology. 18 (6): 591-603.

Shapiro M. Shepard B. M. Lopez R. (2007a). Effect of speices upon activity of gypsy moth (Lepidoptera;Lymantriidae).Journal of Entomological Science.42:82-91.

Shapiro M. Shepard B. M. Lopez R. (2007b). Effect of medicinal herbs upon the biological activity of the gypsy moth nucleoplyhedrosis virus.BiocontrolScience and Technology. 18:605-617.

Shorey H. Hale R. L. 1965. Mass rearing of the larvae of nine noctuid species on a simple artificial medium. *Journal of Economic Entomology. 58:522-524.*

Smagghe G. and Degheele D. (1997). Comparative toxicity and tolerance for the ecdysteroid mimic tebufenozide in a laboratory strain of cotton leafworm (Lepidoptera: Noctuidae). J. Econ. Entomol., 90: 278- 282.

Stadler T. Buteler M. Weaver D.K. (2010). Novel use of nanostructured alumina as an insecticide. Pest Management Science 66: 577- 579.

Debnath N. Das S. Seth D. Chandra R. Bhattacharya, S. Ch. Goswami, A . 2001. Entomotoxic effect of silica nanoparticles against Sitophilus oryzae (L.). Journal of Pesticide Science 84, 99e105.

Subramanyam B. Roesli R. (2000). Inert dusts. In: Subramanyam, Bh., Hagstrum, D.W. (Eds.), Alternatives to Pesticides in Stored-product IPM. Kluwer Academic Publishers, Boston, MA, pp. 321e380

Ulrich S. (2006). Solid-phase micro extraction in biomedical analysis. J. Chromatogr. A. 902: 167-194.

Vardeman E. A. Arthur F.H. Nechols J. R. Campbell J. F. (2007). Effi cacy of surface applications with diatomaceous earth to control Rhyzopertha dominica (F.) (Coleoptera: Bostrichidae) in stored wheat. Journal of Stored Product Research, 43: 335e341.

Vinutha J. S Bhagat D. and Bakthavatsalam N. (2013). Nanotechnology in the management

of polyphagous pest *Helicoverpa armigera*. J. Acad. Indus. Res. 1(10):606–608.

Zhang S. and Xiao-zhen Y. E (2010). Impacts of chemical insecticides on extracellular protease and chitinase activities of Metarhizium anisopliae. J. Fujian College Forest, 4: 289-292.

ARABIC SUMMERY

إتجاه جديد للنانوتكنولوجى فى حماية الفيروسات العصوية المغلفة

ألكسندرا الهلالى – وحيد أحمد عبد الحميد*

قسم الحشرات الإقتصادية و المبيدات – كلية الزراعة – جامعة القاهرة

***قسم التطبيقات البيولوجية- مركز البحوث النووية- هيئة الطاقة الذرية - أبو زعبل – مصر ومركز الشرق الأوسط للنظائر المشعة للدول العربية – الدقى – جيزة- مصر.**

ملخص

أثبتت مضادات الأكسدة من عدة عقود إنها مواد حامية واعدة للفيروسات العصوية المغلفة للتاثير المضاد لأشعة الشمس فوق البنفسجية. الدراسة نصف الحقلية الحالية إختبرت دور مضادات الأكسدة النانوية فى إمداد الفيروسات العصوية المغلفة بالحماية. المعاملات المخلوطة مع فيروس دودة ورق القطن البوليهدروزى النانوى شملت (نانو ألومنيوم أوكسيد مع أو بدون الفيروس *Spli*MNPV LC_{90} أو نانو زينك أوكسيد مع أو بدون الفيروس *Spli*MNPV LC_{90}) عند خمس تركيزات مختلفة ١٠٠، ٢٠٠، ٣٠٠، ٤٠٠و ٥٠٠ جزء فى المليون و تم تعريض التركيزات المختلفة لمصدر ضوء صناعى للأشعة فوق البنفسجية لمدد تصل الى ٥ ثم ١٠ ساعات كحد أقصى فى المعمل ثم تطبيق ٥٠ مللى /نبات الكوسة مع إستخدام خمس مكررات / معاملة، و التى فيها تم تعريض يرقات حديثة الفقس لدودة ورق القطن يوميا. تم تسجيل موت اليرقات يوميا و حتى اليوم الخامس عشر بعد المعاملة. النتائج مبنية على التقدير الحيوى معمليا و على أوراق النبات فى التجربة نصف الحقلية لحساب الوقت اللازم لموت ٥٠% من التعداد (LIT_{50}). الدراسات أظهرت أن LIT_{50} بلغ ٨٢.٧٥٩ ساعة و ٥٢.٥٠ ساعة بالنسبة للمواد المضافة نانو زنك أوكسيد و نانو ألومنيوم أوكسيد على الترتيب، عند تركيز ٥٠٠ جزء فى المليون بينما أعطى ١٤.٤٨٢ مع معاملة الفيروس منفردا و أعطت ١٠٠.٧٨٨ ساعة مع الكاكاو ٥ % كمادة مقارنة إيجابية. ميكانيكية الحماية تم دراستها بإستخدام تكنيك DPPH (2, 2-diphenyl-1-picrylhydrazyl) لتقدير مضادات الأكسدة كتسجيل سباق لإستخدام مثل هذا التكنيك فى مثل هذا النوع من الدراسات. النتائج المتحصل عليها إقترحت إحتمالية مقدرة المواد النانوية المضادة للأكسدة فى إطالة كفاءة الفيروس على أوراق النباتات تحت التجربة نصف الحقلية المصغرة كما تقترح لعمل تقدير لكفاءة مضادات الأكسدة DPPH أولا فى المستقبل مع أى مادة مضافة يتم ترشيحها قبل عمل التقدير الحيوى نظرا للصورة الواضحة الى يعطيها عن كفاءة مضادات الأكسدة.