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Events Induced By chitosan and Nano Chitosan On the Behavior of *Phthorimaea Operculella* (Lepidoptera: Gelechiidae) Under Laboratory and Field Conditions Materials.

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ABSTRACT

Chitosan and Nano- Chitosan were evaluated on the potato tuber moth *P. operculella*, that data obtained show that the LC50s recorded 34, 48, 69, 99, 122, 123 and 125 for 1st, 2nd, 3rd, 4t, adult male and female respectively. When the target insect pests treated with Nano- chitosan, the LC50s obtained 17, 21, 29,32, 40,43 and 42 for the corresponding stages of *P. operculella* respectively. Under greenhouse conditions the means number of infestations recorded 12.0±2.1, 20.1±5.8, 32.4±7.8 and59.8±7.9 individuals after 20, 50, 90 and 120 day of applications. When potato plats treated with the Nano-chitosan the means number of infestations were significantly decreased to 2.1±4.7, 5.0±1.1, 9.2±6.8 and 11.0±8.1 individuals of *P. operculella* respectively, as compared to 33.2±4.1, 69±5.9, 79±9.9 and 99±9.9 individuals in the control. The field applications were made into two governorates Qalubia and Nobaria , differ in climate and soil, the data recorded that in Qalubia governorate, Potatoes weight significantly increased to 3131± 34.84 and 2839± 29.67 Kg/ feddan in plots treated with Nano-Chitosan and Chitosan as compared to 1893± 49.81 Kg/ feddan in the control. While in Nobaria governorate the weight of potatoes significantly increased to 3331± 76.70, 3109± 66.40 Kg/ feddan in plots treated with Nano- Citosan and Chitosan respectively as compared to 1745± 51.68 Kg/ feddan in the control.

Keywords: Chitosan.Nano. *P. operculella*, potato,biocontrol

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INTRODUCTION

Chitosan (CS)-g-poly (acrylic acid) (PAA) nanoparticles, which are well dispersed and stable in aqueous solution have been prepared by template polymerization of acrylic acid in chitosan solution. The prepared CS-PAA had a white powder shape and was insoluble in water and diluted acid [1]. The mean particles size were found to be around 50nm. FTIR spectra of CS-PAA nanoparticles for CS, the intensities of the amide band were observed clearly. The board peak appeared at 2500cm-1, which confirmed the presence of NH3 +in the CS-PAA nanoparticles. Nanoparticles synthesis is currently intensively researched due to its wide variety of potential applications [2]. As an alternative to chemical manufactured pesticides, use of nanoparticles as an antimicrobial agents has become more common as technological advances have made their production more economical [3,4]. Numerous studies on the antimicrobial activity of chitosan and its derivatives against most economic plant

Pathogens have been investigated [5,6, 7, 8]. Therefore, these compounds are considered as useful pesticides in the control of plant diseases.

The tomato crop, *Lycopersicon esculentum* (Mill) is a considered among vegetable crop of the large importance throughout the world. In Egypt the potato tuber moth, *Phthorimaea operculella* (Zeller) (Lepidoptera: Gelechiidae) is the most economic important pests. Larvae cause severe damage to vegetable crops of family Solanaceae [9, 10, 11].

Nanoparticles synthesis is currently intensively researched due to its wide variety of potential applications. As an alternative to chemical manufactured pesticides, use of nanoparticles as an anti microbial agents has become more common as technological advances have made their production more economical [4].Numerous studies on the antimicrobial activity of chitosan and its derivatives against most economic plantpathogens have been investigated [12,13, 14, 8]. Therefore, these compounds are considered as useful pesticides in the control of plant diseases.

The present study, aims to evaluate the effect of chitosan and Nano chitosan gel against *P. operculella* under laboratory, green house and tomato field conditions.

MATERIALS AND METHODS

The raw materials used for ion exchange preparation are:

- The Chitosan(CS) used in this study was purchased from HAS HMRZEL laboratories LTD (Netherlands)
- The acrylic acid was purchased from Sd .Finc.Chem. Limited (Laboratory grade for synthesis) and was freshly distilled under reduced pressure to eliminate any inhibitors.
- Both initiators (ammonium persulfate and sodium bisulfite) and other chemicals were of analytical reagent and used as received

Insect rearing:

Insects from laboratory colonies of *P. operculella* (potato tuber moth) were included in the study. They were obtained from the insect rearing laboratory, Pests and Plant Protection Department, National Research Centre, Dokki, Giza, Egypt. A disease free culture of the test insect was maintained under controlled conditions (26+28C and 70+5% R.H.) and used for the experiments.

Preparation of Nano- Chitozan:

Chitosan Nanoparticles were synthesized by h y- drolyzing titanium tetra isopropoxide in a mixture of 1:1 anhydrous ethanol and water. 9 ml of titanium tetra isopropoxide is mixed with 41ml of anhydrous ethanol (A). 1:1 ethanol and water mixture is prepared. (B) Solution A is added in drop wise to solute ion B and stirred vigorously for 2hrs. At room temperature hydrolysis and condensation are performed, using 1M sulphuric acid and stirred for 2 hrs. Then the ageing was undertaken for 12hrs. The gel was transferred into an autoclave and tightly closed, and the mixture was subjected to hydrothermal treatment at 353K for 24hrs. After filtration the

solid residue was washed thoroughly with water and ethanol mixture, dried at 373K in an oven and calcined at 773K.

Nanoencapsulation:

The Nanoencapsulation is a process through which a chemical is slowly but efficiently released to the particular host for insect pests control. "Release mechanisms include dissolution, biodegradation, diffusion and osmotic pressure with specific pH" [15] Encapsulated of Nano chitosan tested Nano-emulsion is prepared by high-pressure homogenization of 2.5% surfactant and 100% glycerol, to create stable droplets which that that increase the retention of the oil and cause a slow release of the Nano materials . The release rate depends upon the protection time; consequently a decrease in release rate can prolong insect pests protection time [16].

Efficacy of Chitosan against the target insect pests:

The insecticide Chitosan were tested at the 6 concentrations: 6 mg, 5mg, 4mg, 3mg, 2mg, 1 mg. The insecticide, prepared 6 concentrations (prepared according [17] Percentages of mortality were calculated according to Abbott's formula [18] , while the LC50 values was calculated throughout probit analysis [19] . The experiment was carried out under laboratory conditions at 26±2°C and 60-70% RH

Green house trials:

Tomato plant Winter Variety Platenium 5043 was planted in the green house in 40 plots in each artificial infestation was made by spraying the plant with the chitosan at the concentrations of and 5g/l. Control samples were sprayed by water only. The plants were examined every two days, the percentage of infestation was calculated until the end of the experiment. Each treatment was replicated 4 times. The percent mortality was counted and corrected according to Abott, 1925; while Lc50s were calculated through probit analysis after [25]

Field trials:

The experiments were carried out to study the effectiveness of the tested chitosan and *nano* chitosan against the target insect pests in two different areas. These two areas were: El-Qalubia and Nobaria were the two governorates differ in climates and soil**.** Tomato planted Winter Variety Platenium-5043 planted on the end of September in an area of about 1600 m^2, and divided into 16 plots of 50 m2 each. Four plots were assigned for each silica gel , while 4 plots were treated with water and used as the controls. Silica gel Treated t 5g/ml. Treatments were performed in a randomized plot design at sunset. A five-litre sprayer was used to spray on the treatments. Three applications were made at one week intervals, at the commencement of the experiment. Twenty plant samples were randomly collected at certain time intervals from each plot and transferred to the laboratory for examination. The average number of each of the tested pests/ sample/ plot/treatment was calculated 21, 45 and 120 days after the 1st application. The infestations of target insect pests were then estimated in each case. After harvest, the yield of each treatment was weighed as kgs/feddan.

STATISTICAL ANALYSIS

Data obtained was statistical analysed using Duncan's multiple range tests according to [20]. Efficacy of tested nano- Chitosan applied alone on the mean number of deposited eggs of target insects for conducting the combination tests with Chitosan formulations (0.5 g/kg of grains).The Chitosan alone were used at rate (1.0 g/kg) of grains. Four replicates of 100 g grains for each treatment were used. Each replicate was treated individually with treatments and then shaken manually for 1 min to achieve equal distribution of the dust in the entire formulation quantity and was placed in glass jar. Four replicates jar containing untreated grain served as control. Subsequently, one paired of newly emerged adults were introduced into each jar. The number of deposited eggs on treated or untreated grains/female was counted. The data was analyzed using analysis of variance (ANOVA), where significant differences between the treatments were observed. Mean values were significantly separated by using the least significant difference (LSD) test at 5% level [21].

RESULTS

Data in table 1 show the effect of chitosan on the potato tuber moth *P. operculella* ,which show that the LC50s recorded 34, 48, 69, 99, 122, 123 and 125 for 1st, 2nd, 3rd, 4t, adult male and female respectively. When the target insect pests treated with Nano- chitosan, the LC50s obtained 17, 21, 29,32, 40,43 and 42 for the corresponding stages of *P. operculella* respectively (Table2).

Under greenhouse conditions the means number of infestations recorded 12.0±2.1, 20.1±5.8, 32.4±7.8 and59.8±7.9 individuals after 20, 50, 90 and 120 day of applications. When potato plats treated with the Nano- chitosan the means number of infestations were significantly decreased to 2.1±4.7, 5.0±1.1, 9.2±6.8 and 11.0±8.1 individuals of *P. operculella* respectively, as compared to 33.2±4.1, 69±5.9, 79±9.9 and 99±9.9 individuals in the control (Table 3).

The field applications were made into two governorates Qalubia and Nobaria , differ in climate and soil, the data recorded that in Qalubia governorate, Potatoes weight significantly increased to 3131± 34.84 and 2839± 29.67 Kg/ feddan inn plots treated with Nano-Chitosan and Chitosan as compared to 1893± 49.81 Kg/ feddan in the control. While in Nobaria governorate the weight of potatoes significantly increased to 3331± 76.70, 3109± 66.40 Kg/ feddan in plots treated with Nano- Citosan and Chitosan respectively as compared to 1745± 51.68 Kg/ feddan in the control (Table 4).

Figuer 1 show tat te percentages of *P.* operculella infestations under greenhouse conditions significantly decreased 20% after Nano chitosan treatments as compared to 100% in the control. Under field conditions the percentages of ifestations significantly decreased to 25% and 51% after nano- Chitosan and chitosan treatments as compared to 100% in te control (Fig 2). figure 3 a and b show the chitosan by scanning electron microsopy at 200 nm and 0,5 um

DISCUSSION

[26-29] agree with our results and control a lot of pests with Nano materials. {30-41] have the same results obtained and show that the Nano pesticide is a perfect for controlling many pests and diseases. [40-45] found the insecticidal activity the Nano-silicagel (CS-g-PAA) showed highest effect against the three insect of soybean. As the means number of eggs deposited /female were significantly decreased. Under laboratory and semifield condition, *Aphis gossypii* were significantly decreased to 20.9±9.1 and 28.9±9.2 eggs/female respectively as compared to 97.3±4.9 and 90.3±4.9 eggs/female in the control, respectively. The same trends were also observed against *Callosobruchus maculatus*. Sabbour 2015, a, b, c found that the Nano insecticides of Imidacloprid and fungi strains case a higher mortality for insect infestations. Our results agree with [43,44] who find that the Nano pesticide decrease the infestation percentage of different pests. The tomato leafminer, *Tuta absoluta* Meyrick, (Lepidoptera: Gelechiidae) controlled by the biocontrol agent spinosad and its nano particles. Under laboratory conditions [45-50].The results were matched with those found by [51, 52], when they controlled cereal aphids with entomo pathogenic fungi. They found that the infestation was reduced after fungi applications under laboratory and field conditions. [53,54], found that the fungi reduced insect infestations of cabbage and tomato pests under laboratory and field conditions.[55,56, 57] found that the natural product destruxin which extracted from the fungus *M. anisopliae* gave a good results in controlling insect pests. The finding in obtained by [57] which recorded that the Nano compound could to reduce the insect infestations under laboratory and field conditions

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Table 1: Effect of chitosan against the different stages of *P. operculella* under laboratory conditions.

Insects	LC_{50}	Slope	variance	95%confidence limits
Newly hatched larvae	34	0.1	1.01	30-59
1st larval instars	48	0.1	1.01	32-78
2nd larval instars	69	0.4	1.01	77-117
3rd larval instars	99	0.3	1.01	90-132
4th larval instars	112	0.2	1.01	100-143
Adult males	123	0.1	1.02	111-138
Adult females	125	0.1	1.02	108-135

Table 2: Effect of Nano-chitosan against the different stages of *P. operculella* under laboratory conditions.

Insects	LC_{50}	Slope	variance	95%confidence limits
Newly hatched larvae	17	0.1	1.01	10-30
1st larval instars	21	0.2	1.01	15-68
2nd larval instars	29	0.4	1.01	20-90
3rd larval instars	32	0.3	1.01	27-108
4th larval instars	40	0.2	1.01	31-0
Adult males	43	0.1	1.02	20-70
Adult females	42	0.1	1.02	22-95

Table 3: Effect of chitosan against *P. operculella* under greenhouse conditions

Treatments	Days after treatment	Means of infestations (Means ± S.E.)
Control	20	33.2±4.1
	50	69±5.9
	90	79±9.9
	120	99±9.9
Chitosan	20	12.0±2.1
	50	20.1±5.8
	90	32.4±7.8
	120	59.8±7.9
Nano-Chitosan	20	2.1±4.7
	50	5.0±1.1
	90	9.2±6.8
	120	11.0±8.1
F –test	13.9	
LSD 5%	11.7	

Table 4: Weight of harvested tomato into two Egyptian regions after silica gel and Nano-Chitosan treatment against *T. absoluta* during seasons 2015.

Treatments	Qalubia Weight Potatoes (Kg/feddan)	Nobaria Weight Potatoes (Kg/feddan
Control	1893± 49.81	1745± 51.68
Chitosan	2839± 29.67	3109±66.40
Nano Chitosan	3131± 34.84	3331±76.70
F –test	34.1	
LSD 5%	18.3	

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Figure 1: Infestation percentages of *P. operculella* after chitosan and Nano-chitosan treatments under greenhouse conditions

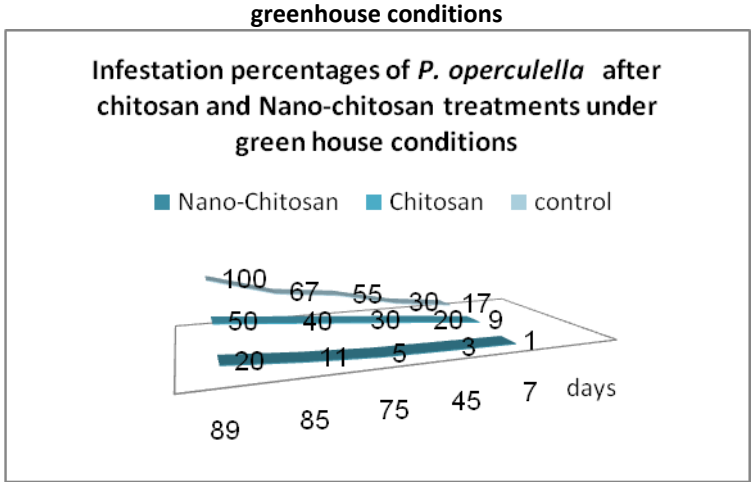


Figure 2: Infestation percentages of *P. operculella* under field conditions after chitosan and nano-chitosan treatments.

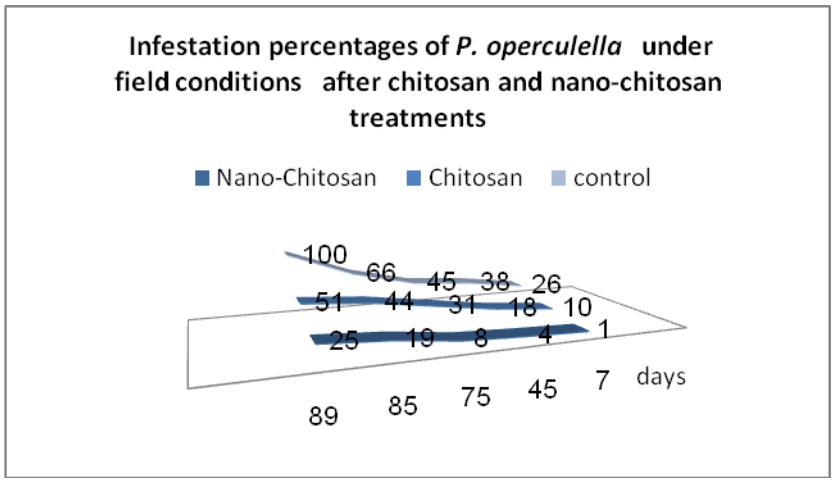
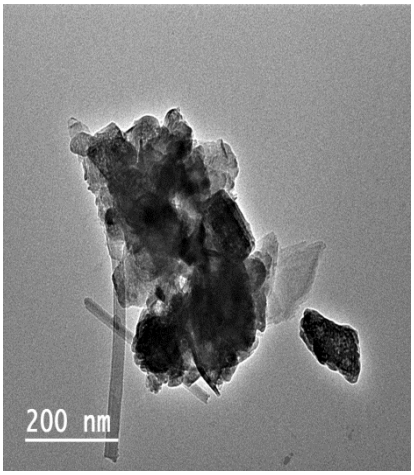


Figure 3: show the particles of the nano silica gel under scanning electron microscopy

Figure 3: scanninigng electron microscopy 200NM of Chitosan Scanning electron microscopy 50 nano meter



b. Scnning electron microscopy 0.5Um nano meter

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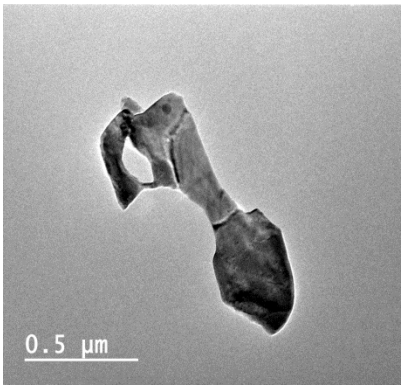

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