Journal of
NUCLEAR
Technology in Applied Science
ISSN 2314-8209 e-ISSN 2314-8217

J. Nucl. Tech. Appl. Sci., Vol. 11, PP. 1 : 11 (2023)

Ecofriendly Control of the Pink Bollworm, *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae) by Using Chitosan and Spinosad

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Received: 03/04/2023
Accepted: 12/05/2023
DOI: 10.21608/jntas.2023.203777.1055

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ABSTRACT

This study was conducted to assess the susceptibility of the 4th instar larvae of *Pectinophora gossypiella* to different concentrations (0.01, 0.05, 0.1, 0.5, 1, 5, and 10%) of chitosan and spinosad by the application of two treatment tactics on the control of 4th larval instar of *P. gossypiella*. Also, an investigation of the impact of sub-lethal dose LC_{50} on the reproductive potential of insects was determined. The mortality percent was 46.7%, when the larvae were fed on artificial medium mixed with chitosan powder, while was 73.3 % when the larvae were fed on artificial medium mixed with chitosan dissolved in 2ml of glacial acid at the highest concentration of 10% after 6 days post-treatment. On the other hand, the first feeding tactic of spinosad induced 100% larval mortality, while the second spraying tactic induced 66.7% larval mortality at the concentration level of 10% after 6 days post-treatment. There was a reduction in the fecundity, hatchability %, and the resulting adult percent where the adult emergence was 61.9 and 59.9 % at the dose level LC_{50} of chitosan and spinosad, respectively compared to 97.6 % for control. Also, the growth rate of both larvae and the pupae is decreased in spinosad and chitosan compared to the control. We conclude this sub-lethal dose LC_{50} of chitosan and spinosad is recommended for establishment in the control of *P. gossypiella*.

KEYWORDS

Pectinophora gossypiella, Chitosan, Spinosad.

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INTRODUCTION

The Pink bollworm *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae), was described by **Saunders (1843)** in India in 1842 **(Ingram 1994)**. It's one of the serious pest of cotton plants, okra, hollyhock and other malvaceous plants **(Carrière *et al.,* 2005)**. Therefore, it's important to develop an alternative, safe, ecofriendly and economical approach as chitosan and spinosad. Chitosan yields from the deacetylation of the chitin. Chitin and chitosan have gained tremendous interest due to their properties as non-toxic, biocompatible, and biodegradable polymers **(Elieh and Hamblin 2016).** Chitin in nature is present in crab and shrimp shells and remains the primary commercial source, its derivatives of chitosan are made through chemical synthesis, and their insecticidal activities are being reported in an oral larvae-feeding bioassay **(Jiménez and Cecilia 2020; Bakshi *et al.,* 2020)**. Spinosad (Dow Agro Sciences) is a mixture of tetracyclic-macrolide compounds produced by a soil actinomycete and has been classified as a bioinsecticide, spinosad is highly active against Lepidoptera but is reported to be practically nontoxic to insect natural enemies, we assessed the impact of spinosad in a granular maize-flour formulation on a selection of insect predators over periods of 2–14 days **(Cisneros *et al*., 2002).**

The Ministry of Agriculture in Egypt is hoping to find a product safe and low hazard in the environment; with satisfactory killing power, especially for the pink bollworm. So, spinosad was chosen because it is classified as a reduced-risk product and awarded the green chemical challenge award from the white house in the USA, some insecticides with novel modes of action have been introduced for controlling the pest effectively **(Cleveland *et al.,* 2002)**. Therefore the main objective of this work is: (1) to determine the susceptibility of the 4th instar larvae of *P. gossypiella* to spinosad and chitosan to determine the $LC_{50,}$(2) evaluation of the effect of LC_{50} of chitosan and spinosad on some biological aspects of *P. gossypiella.*

MATERIAL AND METHODS

Laboratory rearing technique

The strain *P.gossypiella* was obtained from the central Agricultural Pesticides Laboratory. Agricultural Research Center, Dokki, Giza, Egypt. Newly hatched larvae were reared according to **Gabarty *et al.* (2021)** in glass tubes containing a 2-3 cm artificial diet until pupation. Pupae were separated and kept in tubes until the moths' emergence. Groups of 5 or 10 pairs of emerged moths were placed in a clean glass lamp chimney used as cages (17cm height, 7-12cm diameter) covered with muslin held in place by rubber bands. Adult's nutrition; moths were provided every 48 hr with cotton pieces soaked in 10% sucrose solution, for 3-4 days after emergence was supposed to be egged (egg laying took place).

Collecting of egg

The deposited eggs were obtained and kept in glass Jars (3.5×12.5cm), under controlled conditions at a temperature of 27±2˚C and 70±10% relative humidity and 12-12 light-dark regime and away from any chemical pressure.

Laboratory bioassay

Laboratory bioassay experiments were carried out to evaluate the relative efficacy of different biocontrol agents; chitosan and spinosad. Selection of the tested agents based on differentiation of natural sources and mode of action. All agents were tested against *P. gossypiella*.

Susceptibility of P. gossypiella *to chitosan*

Chitosan powder was purchased from Chitosan Egypt Company. 7 concentrations of 0.01, 0.05, 0.1, 0.5, 1, 5, and 10% were tested. Two tactics were applied on 4th larval instar of the insect. The first tactic

depends on the mixing of chitosan powder with the food beads (w: w). while the second tactic is used on dissolving the chitosan concentration in glacial acetic acid and then mixing the chitosan solution with the food beads (v: w) then used as food for the larvae. The diet beads were weighted (100 gm) and 10 larvae were introduced onto the diet in a 12 cm petri-dish lined with filter paper. The treated food was used only once at the beginning of the bioassay and the mortality was evaluated daily for 6 days. The experiments were repeated three times for each conc. as a replicate. All dishes were incubated at 27 ± 1˚C with daily examination for 6 days to calculate the percentage of insect mortality.

Susceptibility of P. gossypiella *to spinosad*

Tracer 24 % is a commercial formulation of spinosad produced by Dow Agriculture, England. Spinosad is a bio-insecticide derived from the fermentation of *Saccharopolyspora spinosa*. This formulation is highly active by both contact and ingestion of numerous pests. 7 concentrations of 0.01, 0.05, 0.1, 0.5, 1, 5, and 10% were prepared to evaluate the potency of Spinosad against the 4th larval instar of *P. gossypiella*. In this experiment, 2 application methods (feeding & spay methods) were evaluated**.**

Feeding treatment

100 gm of diet beads immersed in the previously mentioned concentrations and stirred gently for 5 minutes. The diet beads were dried on a nonabsorbent surface at room temperature. After evaporation of the excess water, food beads were placed in Petri dishes 12 cm lined with filter paper. 30 individuals of 4th instar larvae were starved for 24 hours and then introduce to the treated food in separated replicates. The treated food was used only once at the beginning of the bioassay and the experiments were repeated 3 times for each concentration as a replicate. All dishes were incubated at 27 ± 1˚C with daily examination for 6 days to calculate the percentage of insect mortality.

Spray treatment

The tested individuals (4th instar larvae of *P. gossypiella*) were collected from the laboratory colonies. Sterilize Petri-disheses were used. 10 individuals/ dish were treated with 1 ml of each tested concentration using a plastic pipette as a sprayer. The experiments were repeated 3 times for each concentration as a replicate. All dishes were incubated at 27 ± 1˚C with daily examination for 6 days to calculate the percentage of insect mortality.

Mortality percent for all chitosan and spinosad was corrected using Abbott's Formula (Abbott, 1925). LC_{25}, LC_{50}, LC_{75}, LC_{90}, LC_{95} & LC_{99} were calculated according to the method of (Finney, 1971) after 6 days post-treatment for the 4th larval instar post-treatment.

Evaluation of the effect of LC_{50} of chitosan and spinosad on some biological aspects of P. gossypiella

For biological studies, the insects were divided into 3 groups; the first group was reared on a non-treated diet, the second was reared on diet treated with LC_{50} of dissolved chitosan while the third on diet treated with LC_{50} of spinosad to evaluate their palatability and their effects on the biological aspects of this pest under laboratory conditions in 3 separated experiments.

In each experiment: a transparent plastic cup was cleaned, and provided with 100 gm of diet, and 50 individuals of the 1st larval instar which were isolated from the laboratory colony and introduced into the cup for rearing up to complete the first generation development of each treated diet separately and till the appearance of new insect offspring (eggs) of the next generation. The egg was used to initiate biological studies. Thereafter, the eggs were removed carefully with a softa camel hair brush and left in new 3 plastic cups as replicates, the number of the eggs was not similar in all replicates. The hatching was examined daily under a stereomicroscope and hatch-

ability was confirmed by the presence of the neonate larvae. The development duration was calculated by recording the days that lasted to complete the successful molting process to the next stage until pupation and emerging adult males and females. After adult emergence, 1 : 1 (female: male) were reared in a plastic cup provided with cotton pieces saturated with sugar solution 10% covered with nylon cloth. The cup was checked daily for signs of oviposition. The number of eggs was counted and transferred to a petri-dish until eggs hatched to calculate the hatchability with the calculation of the pre-oviposition period, post-oviposition period, egg deposition, and adult longevity. Adult longevity was recorded as the duration between adult emergence and death. The adult was considered dead if they did not move after being touched with a fine-tip brush. This experiment was repeated 15 times as replicates.

Effects of LC_{50} of chitosan and spinosad on larval growth rate

To evaluate the effects of the tested bio-insecticides on the growth rate of the pink bollworm larvae, 10 replicates of the first larval instar (10 larvae starved for 12 hours/replicate) were reared on an artificial diet (0.5 gm /bead) treated with the LC_{50} of the tested insecticides as a contaminated food for 4 days then replaced with the untreated diet for 10 days to complete the larval stage development under laboratory conditions (27±2°C, 65±10 % RH).

The following biological aspects were:

- The growth rate of larvae according to **Waldbaur (1968)**,

Growth rate (GR) = G / T × A

Where; G = Weight gained of larvae during feeding period /(mg).

T = Duration of feeding period (10) days.

A = Mean weight of larvae during the feeding period (Initial weight + Final weight / 2).

Statistical analysis

Response percentages were corrected using Abbotts' Formula (Abbott, 1925). The (LC_{25}, LC_{50}, LC_{75}, LC_{90}, LC_{95} & LC_{99}) were calculated according to the method of (Finney, 1971). All biological results were statistically analyzed for variance ratios by the method of one-way ANOVA using SPSS program at 5% level.

RESULTS

Susceptibility of Pink Bollworm, P. gossypiella (Saunders) to chitosan and spinosad

This study aimed to determine the effects of different bio-control agents such as chitosan and spinosad against the 4th larval instar of *P. gossypiella***.**

The Pathogenicity of chitosan is indicated by two feeding tactics; in the first feeding tactic, the mortality percentage of the 4th larval instars of *P. gossypiella* as influenced by different concentrations (0.01, 0.05, 0.1, 0.5, 1, 5, and 10%) of chitosan are given in (Table 1). The data obtained indicated that the highest mortality percentage was caused by the highest concentration (10%) as compared to control, where it recorded (0, 33.3, and 46.7%) after 2, 4, and 6 days; respectively, post-treatment. Meanwhile, the low concentration (0.01%) caused a low percentage of mortality (0, 0, and 0) after 2, 4, and 6 days; respectively, post-treatment compared to 0% to the control. At the second feeding tactic the highest mortality percent of the 4th larval instars of *P. gossypiella* were caused by the highest concentration (10%) compared to the control, where it recorded (26.7, 53.3 and 73.3 %) after 2, 4, and 6 days; respectively, post- treatment. Meanwhile, the low concentration (0.01%) caused a low percentage of mortality (0, 0, and 33.3) after 2, 4, and 6 days; respectively, post-treatment compared to 0% to the control. These results proved that; the second tactic is more sensitive than the first tactic.

The pathogenicity of spinosad is indicated by two tactics, the mortality percent of the 4th larval instars of *P. gossypiella* is influenced by different conc. (0.01, 0.05, 0.1, 0.5, 1, 5, and 10%) of spinosad are given in (Table 3). The data obtained of the first feeding tactic indicated the highest mortality percent was caused by the highest concentration (10%) as compared with control, where it was (13.3, 86.7, and 100%) after 2, 4, and 6 days; respectively, post-treatment. Meanwhile, the low concentration (0.01%) caused a low percentage of mortality (0, 20, and 26.7) after 2, 4, and 6 days; respectively, post-treatment compared to 0% for control. In the second spraying tactic; the highest mortality percent of 4th larval instars of *P. gossypiella* as influenced by different concentrations (0.01, 0.05, 0.1, 0.5, 1, 5, and 10%) of spinosad was caused by the highest conc. (10%) compared to control, where it was (6.7, 40 and 66.7%) after 2, 4, and 6 days; respectively,post-treatment. Meanwhile the low conc. (0.01%) caused low mortality percent (0, 6,.7, and 13.3) after 2,4, and 6 days; respectively, post-treatment compared to 0% for control. From the above mentioned results, it's obvious that; the feeding tactic is more sensitive than the spraying tactic.

Table (1) : *Pathogenicity of chitosan against 4th larval instar of P. gossypiella.*

(Conc.mg /100 gm diet)	Corrected mortality % (First feeding tactic)			Corrected mortality % (Second feeding tactic)		
	2 days	4 days.	6 days.	2 days.	4 days.	6 days.
0	0	0	0	0	0	0
0.01 %	0	0	0	0	0	33.3
0.05 %	0	0	13.3	6.7	20	33.3
0.1 %	0	0	20	6.7	26.7	33.3
0.5 %	0	13.3	26.7	13.3	20	40
1 %	0	13.3	26.7	13.3	33.3	60
5 %	0	20	40	20	40	66.7
10 %	0	33.3	46.7	26.7	53.3	73.3

Table (2) : *Lethal concentrations of chitosan against 4th larval instar of P. gossypiella after 6 days post the 2nd feeding tactic.*

Treatments	(LC) values in (mg / 100gm diet)		Slope ± S.E.
Chitosan	LC_{25}	20.9012	0.4236± 0.1298
	LC_{50}	817.3537	
	LC_{75}	31963.0184	
	LC_{90}	866522.4194	
	LC_{95}	6242992.6063	
	LC_{99}	253465502.6322	

Table (3) : *Pathogenicity of spinosad against 4th larval instar of P. gossypiella.*

(Conc.ml / 100ml H2O)	Corrected mortality % (Feeding tactic)			Corrected mortality % (Spraying tactic)		
	2 days	4 days.	6days.	2days.	4 days.	6 days.
0	0	0	0	0	0	0
0.01 %	0	20	26.7	0	6.7	13.3
0.05 %	0	20	33.3	0	6.7	26.7
0.1 %	0	33.3	53.3	0	20	33.3
0.5 %	6.7	46.7	73.3	0	20	33.3
1 %	13.3	66.7	80	6.7	33.3	46.7
5 %	13.3	73.3	100	6.7	33.3	60
10 %	13.3	86.7	100	6.7	40	66.7

Table (4) : *Lethal concentrations of spinosad against 4th larval instar of P. gossypiella after 6 days post spraying tactic.*

Treatments	(LC) values in (*ml/100ml H2O*)		Slope ± S.E.
Spinosad	LC_{25}	0.0195	0.8506 ± 0.2278
	LC_{50}	0.1212	
	LC_{75}	0.7524	
	LC_{90}	3.8926	
	LC_{95}	10.408	
	LC_{99}	65.8396	

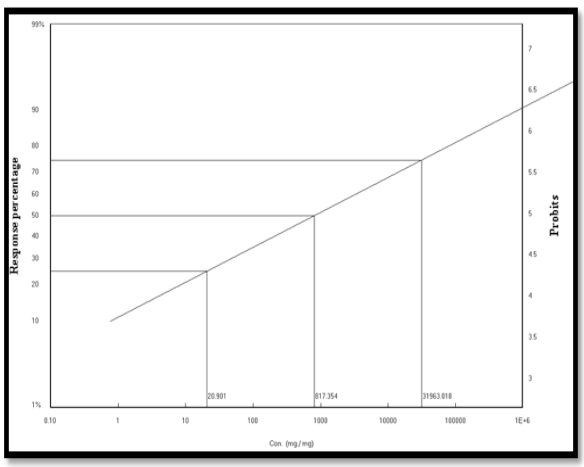

Fig. (1): Concentration-Mortality probit lines of 4th instar larvae of P. *gossypiella* against chitosan after 6 days post 2nd feeding tactic.

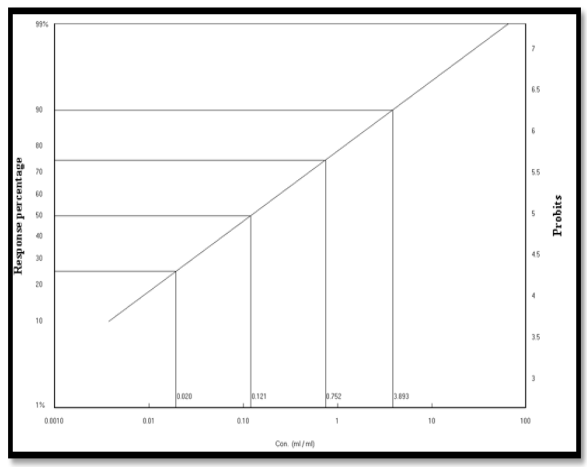

Fig. (2): Concentration-Mortality probit lines of 4th instar larvae of P. *gossypiella* against spinosad after 6 days post spraying tactic.

From the aforementioned results, it is obvious that the lethal doses based on the calculation of the LC_{25}, $LC_{50,}$ $LC_{75,}$ LC_{90}, $LC_{95,}$ and LC_{99} for the chitosan and spinosad have been found in (Table 2) and (Table 4) and illustrated in (Fig 1), and (Fig 2), respectively.

Evaluation of the effect of LC_{50} of chitosan and spinosad on some biological aspects of P. gossypiella

During this biological study, the insect was reared on an artificial diet (three times) the first was on an untreated diet, the second on diet treated with LC_{50} of chitosan while the last on diet treated with LC_{50} of spinosad to evaluate their palatability and their effects on the biological aspects of this pest under laboratory conditions in three separated experiments.

The data given in (Table 5) showed the effect of LC_{50} of chitosan and spinosad on the reproductive potential of *P.gossypiella* compared to the control (untreated diet). As shown from the results in (Table 5) the fecundity of *P.gossypiella* reared on diet treated with LC_{50} of chitosan and spinosad respectively was decreased compared to the untreated insects (control). Where the eggs count laid by insects treated with LC_{50} of chitosan was 58.067 ± 3.81 eggs laid /♀ and by the insects treated with LC50 of spinosad was 62.9 ± 6.34 vs. 186.9 ± 12.6 eggs laid /♀ for control. There was a reduction in the hatchability percent, where it was 87.2, and 77.4 % at LC_{50} of chitosan and spinosad respectively. VS. 94.2% for the control (Table 5).

The pre-pupation % and pupation% are decreased as compared with the control. While the pre- pupation % was 72.2 and 58.5 % at LC_{50} of chitosan and spinosad, respectively compared to 90.4 % for the control. Meanwhile, the pupation % was 69.4 and 62.8 % at LC50 of chitosan and spinosad, respectively compared to 96.03 % for the control (Table 5).

The mean duration of the resulted larvae and pupae treated with chitosan and spinosad was increased as compared with the control. The larvae duration was19.758 ± 0.39 and 21.654 ± 1.21 days at the LC_{50} of chitosan and spinosad, respectively compared to 15.44 ±1.91 days for control, while the pupae duration was 9.176 ±0.85 and 9.992 ± 0.54 days at the LC_{50} of chitosan and spinosad, respectively compared to 6.54 ± 0.54 days for control (Table 6, 7 and 5). There is A reduction in the resulted adults percentage, where the emergence % was 61.9 and 59.9 % at the LC_{50} of chitosan and spinosad, respectively compared to 97.6 % for control, also the results indicated the sex ratio among the progeny (Table 5) where it was 2.26 and 1.5 % at the LC_{50} of chitosan and spinosad, respectively compared to 2.3% for control.

Effects of LC_{50} of chitosan and spinosad on the larval and pupal growth rate of P. gossypila under laboratory conditions

This study is carried out to evaluate the effects of the tested bio-control (chitosan and spinosad) on the growth rate of *P. gossypila* larvae compared to the control. The growth rate of both larvae and the pupae is decreased by the treatment with spinosad and chitosan compared to the control as shown in (Table 6), where the weight of larvae treated by LC_{50} of spinosad and chitosan were 0.087±0.046, and 0.082 ± 0.033 respectively compared to 0.139± 0.045 for the control. While the reduction in the weight of the resulted pupae was 2.128±0.17 and 2.114±0.09 respectively compared to 2.945±0.10 for the control. Also, the growth rate reduction percentage of both the larvae and the pupae was increased in spinosad and chitosan compared to control.

Table (5) : *Effects of LC_{50} of chitosan and spinosad on biological aspects of P. gossypiella.*

Biological parameter		Control Mean ± SD	LC_{50} of Chitosan Mean± SD	LC_{50} of Spinosad Mean± SD
Larvae	Initiation count (larvae)	33.4 ± 6.71	30.6 ± 6.47*	30.6 ± 6.47*
	Larval Developmental duration (day)	4.54 ± 0.35	19.758 ± 0.39*	21.654 ± 1.21*
	Pre-pupation	30.6 ± 6.47	22 ± 4.2*	17.8 ± 3.49*
	Pre- pupation %	91.5	72.2	58.5
pre-pupa	Initiation count (No. of pre-pupae)	30.6 ± 6.47	22 ±4.2*	17.8 ± 3.49*
	Duration of Pre-Pupal Development (day)	15.44 ± 1.91	2.858 ± 0.21*	2.392 ± 0.13*
	Pupation	27.6 ± 5.64	15.4 ± 3.72*	11.2 ± 2.4*
	Pupation %	90.4	69.4	62.8
Pupae	Initiation count (pupae)	26.4 ± 5.04	15.4 ± 3.72*	11.2 ± 2.4*
	Pupal Developmental duration	6.54 ± 0.54	9.176 ± 0.85*	9.992 ± 0.54*
Adult	Adult emergence	25.6 ± 3.93	9.8 ± 3.76*	6.6 ± 1.02*
	Emergence %	97.6	61.9	59.88
	Male count	7.8 ± 2.48	3 ± 1.1*	2.6 ± 0.8*
	Male %	30.4	30.9	38.95
	Male longevity	8.85 ± 0.63	5.394 ± 0.49*	5.684 ± 0.37*
	Female count	17.8 ± 3.12	6.8 ± 2.71	4 ± 0.63
	Female %	69.6	69.1	61.04
	Female longevity	13.89 ± 0.65	9.05 ± 0.32*	8.59 ± 0.47*
	Sex ratio	2.3	2.26	1.5
Fecundity	Pre-oviposition	2.41± 0.08	2.5333 ± 0.34	2.3333 ± 0.3
	Oviposition duration	8.09 ± 0.98	4.4667 ± 0.27*	4.5 ± 0.6*
	Post-oviposition	2.48 ± 0.53	1.7333 ± 0.33	1.8333 ± 0.68
	Egg deposition	186.9 ± 12.6	58.067 ± 3.81*	62.9 ± 6.34*
	Hatching	176 ± 14	48.533 ± 5.18*	48.533 ± 5.18*
	Hatchability %	94.2	87.2	77.4

**The mean difference is significant at the 0.05 level compare with control.*

Table (6) : *Effects of LC_{50} of chitosan and spinosad on larval and pupal growth rate of P. gossypiella.*

Bioagents	Weight of the larvae / mg (n= 30)						Weight of pupae/mg (n= 30)	
	Initial weight (At zero time)	Final weight (10 days post-treatment)	Gained weight (G) (weight of larvae during feeding period)		Growth rate (GR)		Growth rate (GR)	
	Mean ± SD	*Mean ± SD*	*Mean ± SD*	*%reduction*	*Mean ± SD*	*%reduction*	*Mean ± SD*	*%reduction*
Control	0.336± 0.269	1.825±0.48	1.489±0.58	0	0.139±0.04	0	2.945±0.10	**0**
Spinosad	0.323± 0.164	0.766±0.08	0.443±0.18	29.75	0.087±0.04	37.22	2.128±0.17	**27.74**
Chitosan	0.331± 0.152	0.73±0.131	0.399±0.10	26.79	0.082±0.03	41.31	2.114±0.09	**28.21**

**The mean difference is significant at the 0.05 level compare with control.*

DISCUSSION

Chitosan is a derivative of chitin's naturally occurring compound and became of great interest as an alternative to chemical insecticides. Chitosan is a derivative form of chitin, which is the major component of the exoskeletons of arthropods and the cell walls of fungi. The antimicrobial activity of chitosan against lepidopterans, aphids, fungi, and bacteria has been extensively investigated by **Raji *et al.* (2018).** This study indicated the effect of chitosan on the mortality percentage of 4th larval instars of *P. gossypiella* as influenced by different concentrations of chitosan, also showed the effect of LC_{50} of chitosan on the reproductive potential of *P. gossypiella;* compared to control. These results agree with **Zhang *et al.* (2003)** who reported that chitosan was active against lepidopterous insects and the mortality was reached 80%. Also, the lethal concentration LC_{50} was determined by using chitinase on the 1st, 2nd, and 3rd instars of *Spodoptera litura*. And showed the highest insecticidal activity **was** 6 µM concentration within 48 h. **(Chandrasekara *et al.*, 2012)**. **Sayed *et al.*, (2014)** reported the insecticidal activity of chitosan against the 4th larval instar of the cotton leaf worm *Spodoptera littoralis* (Boisd.) under laboratory conditions. These findings suggest that chitosan caused a physiological disturbance in *S. littoralis* larvae and may be used in IPM programs for controlling this pest.

The susceptibility of the pink bollworm, *Pectinophora gossypiella* (Saunders) to spinosad was examined under laboratory conditions. The highest mortality percent of 4thlarval instars of *P. gossypiella* was caused by the highest conc. while. the low mortality percentage caused by the lowest conc. after 6 days post-treatment, also the lethal dose LC_{50} of spinosad on the reproductive potential of *P.gossypiella*. These results agree with **Cisneros *et al.* (2002)** reported that the spinosad (Dow Agro Sciences) is a mixture of tetracyclic-macrolide compounds produced by a soil actinomycete and has been classified as a bio- insecticide and spinosad is highly active against Lepidoptera. The susceptibility of Glyphodes pyloalis larvae to spinosad was studied using the leaf dip method. Treatment with sublethal concentrations of LC_{10} and LC_{30} spinosad concentrations on some biological parameters showed that the percentage of larval pupation and female fecundity significantly decreased in the concentration of LC_{30} **(Piri *et al.*, 2014).** Different studies demonstrated the insecticidal activity of spinosad on insects such as **Wang *et al.* (2009)** on *Helicoverpa armigera*; **Vayias *et***

***al.* (2009)** on *Rhyzopertha dominic*, *Sitophilus oryzae* and *Tribolium confusum*; **Wang *et al.* (2013)** on *Spodoptera exigua*. The reason for insects' mortality was illustrated by **Yang *et al.* (2017)** who reported that spinosad, a reduced-risk insecticide, acts on the nicotinic acetylcholine receptors and the gamma amino butyric acid receptor in the nervous system of target insects. However, its mechanism of action in non-neural insect cells is unclear. When he studied the mitochondrial functional changes associated with spinosad in *Spodoptera frugiperda* (Sf9) insect cells. He suggested that spinosad-induced programmed cell death was modulated by mitochondrial dysfunction and cytochrome C release.

Chitosan and spinosad reduced the growth rate of both larvae and the pupae compared to the control this result agrees with **Wang *et al.* (2009)** on *Helicoverpa armigera* by using spinosad.

CONCLUSION

Spinosad and chitosan are naturally occurring compounds and became of great interest as an alternative to chemical insecticides when testing the effect of sub-lethal dose LC_{50} of chitosan and spinosad on the reproductive potential of *P.gossypiella* the following occurred. There was a reduction in the fecundity, hatchability %, and the resulting adult percent where the adult emergence was 61.9 and 59.9 % at the dose level LC_{50} of chitosan and spinosad, respectively compared to 97.6 % for control. Also, the growth rate of both larvae and the pupae is decreased in spinosad and chitosan compared to the control. We conclude this sub-lethal dose LC_{50} of chitosan and spinosad is recommended for establishment in the control of *P. gossypiella*

REFERENCES

- **Abbott, W.S. (1925):** A method of computing the effectiveness of an insecticide. *Econ. Entomol.*, 18(2): 265.
- **Bakshi, P. S.; Selvakumar, D.; Kadirvelu, K. and Kumar, N.S. (2020):** Chitosan as an environment friendly biomaterial–a review on recent modifications and applications. *Int. J. Boil. Macromol.,* (150):1072.
- **Chandrasekaran, R.; Revathi, K.; Nisha, S.; Kirubakaran, S. A.; Sathish- Narayanan, S. and Senthil-Nathan, S. (2012):** Physiological effect of chitinase purified from Bacillus subtilis against the tobacco cutworm Spodoptera litura Fab. *Pestic. Biochem. Physiol*., 104(1): 65.
- **Cisneros, J.; Goulson, D.; Derwent, L.C.; Penagos, D.I.; Hernández, O. and Williams, T. (2002):** Toxic effects of spinosad on predatory insects. *Biol. Cont*., 23(2): 156.
- **Cleveland, C.B.; Mayes, M.A. and Cryer, S.A. (2002):** An ecological risk assessment for spinosad use on cotton. *Pest Manag. Sci.: formerly Pest. Sci.*, 58(1): 70.
- **Elieh-Ali-Komi, D. and Hamblin, M.R. (2016):** Chitin and chitosan: production and application of versatile biomedical nanomaterials. *Int. J. Adv. Res*., 4(3): 411.
- **Finney, D.J. (1971)**. Probit analysis third edition. Cambridge Univ. Press., 333 p.
- **Gabarty, A., Abas, A., Salem, H.M., El-Sonbaty, S.M., Farghaly, D. S. and Awad, H.A. (2021):** Assessment of combining biosynthesized silver nanoparticles using *Bacillus thuringiensis* and gamma irradiation for controlling *Pectinophora gossypiella* (saunders) (Lepidoptera: Gelechiidae). *Int. J. Rad. Biol*., 97(9): 1299.
- **Ingram, W.R. (1994):** *Pectinophora* (Lepidoptera: Gelechiidae). In: Matthews, G.A., Tunstall, J.P.(Eds.), *Insect Pests of Cotton.* CAB International., Wallingford, UK., 107–149.

- **Jiménez-Gómez, C.P. and Cecilia, J.A. (2020):** Chitosan: a natural biopolymer with a wide and varied range of applications. *Molecules*., 25(17): 3981.
- **Piri, F.; Sahragard, A. and Ghadamyari, M. (2014):** Sublethal Effects of Spinosad on Some Biochemical and Biological Parameters of *Glyphodes pyloalis* Walker (Lepidoptera: Pyralidae). *Plant Prot. Sci*., 50(3): 135.
- **Raji, O.; Tang, J.D.; Telmadarrehei, T. and Jeremic, D. (2018):** Termiticidal activity of chitosan against the subterranean termites *Reticulitermes flavipes* and *Reticulitermes virginicus*. *Pest Manag. Sci*., 74(7): 1704.
- **Saunders, W.W. (1843):** Description of a species of moth destructive to cotton crops in India. *Trans. R. Entomol. Soc. Lond*., (3): 284.
- **Sayed, R.M., Khidr, A.A., and Moustafa, H.Z. (2014):** Chitosan Efficacy against the Cotton Leaf Worm *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae). *Egy. J. Biol. Pest Cont*., 24(2): 461.
- **Vayias, B.J., Athanassiou, C.G., Milonas, D.N. and Mavrotas, C. (2009):** Activity of spinosad against three stored-product beetle species on four-grain commodities. *Crop Prot*., 28(7): 561.
- **Wang, D.; Gong, P.; Li, M.; Qiu, X. and Wang, K. (2009):** Sublethal effects of spinosad on survival, growth and reproduction of *Helicoverpa armigera* (Lepidoptera: Noctuidae). *Pest Manag. Sci.: formerly Pest. Sci*., 65(2): 223.
- **Wang, D.; Wang, Y.M.; Liu, H.Y.; Xin, Z. and Xue, M. (2013):** Lethal and sublethal effects of spinosad on *Spodoptera exigua* (Lepidoptera: Noctuidae). *J. Econ. Entomol.,* 106(4): 1825.
- **Yang, M.; Wang, B.; Gao, J.; Zhang, Y.; Xu, W. and Tao, L. (2017):** Spinosad induces programmed cell death involves mitochondrial dysfunction and cytochrome C release in *Spodoptera frugiperda* Sf9 cells. *Chemosphere., (169): 155.*
- **Zhang, M.I.; Tan, T.; Yuan, H. and Rui, C. (2003):** Insecticidal and fungicidal activities of chitosan and oligo-chitosan. *J. Bioact. Compat. Polym.,* 18(5): 391.