

**EFFECT OF NEEMAZAL, SPINOSAD AND DIMILIN ON SOME  
BIOLOGICAL AND PHYSIOLOGICAL ACTIVITIES OF PINK  
BOLLWORM, *Pectinophora gossypiella* (SAUND.)**

**BY**

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**ABSTRACT**

Laboratory experiments were directed to study the effect of three compounds, NeemAzal T/S (1 %), Spinosad and IGR diflubenzeron (Dimilin) on the fecundity, fertility and longevity of PBW *Pectinophora gossypiella* (Saund.) adult females. Also, biochemical analyses were conducted to show the effect of the different compounds on adult females and males. Results show that the pre-oviposition periods were 4.1, 2.7, 3.0 and 3.57 days for females treated by NeemAzal, Spinosad, Dimilin and control, respectively, while the average period of oviposition were 10.96, 8.85, 18.47 and 15.38 days, respectively. The mean numbers of eggs laid by all treated females were decreased than the untreated, the total number of eggs were 131.2, 104 and 154.38 for treatments compared with 190.66 in untreated, the reduction in No. of eggs laid ranged between (30.4 – 52.0 %). The biochemical analysis of treated adults shows high reduction in total content of hemolymph proteins and lipids, while GOT and GPT enzymes activities were increased in all treatments, while carbohydrate enzymes were fluctuated.

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**Key words:** PBW, *Pectinophora gossypiella*, NeemAzal T/S, Spinosad, Dimilin, Biochemical analysis.

**INTRODUCTION**

The pink bollworm, *Pectinophora gossypiella*, is one of the most serious insect pests of cotton, causing losses in both yield and quality. New approaches are conducted to control cotton pests using plant extracts, oils, microbial agents and chitin inhibitors.

NeemAzal formulations are considered one of the most effective neem preparations. Some of Lepidopteran insect species were found to be susceptible to its formulation as *Spodoptera littoralis*, *Bombyx mori*, *Earias insulana* and *Pectinophora gossypiella* El- Sayed (1982), El- Sayed *et al.* (1991), Kelany *et al.* (1996), El-Meniawi *et al.* (1999), and Hewady *et al.* (2002).

El-Sayed (2001) mentioned that, NeemAzal extract caused mortality of newly hatched larvae, prolonged larval duration and reduced moth emergence and longevity of *P. gossypiella*. Also, the biochemical effects of AZA on total

carbohydrate, lipids, protein and nucleic acid were studied by Hewady *et al.* (2002). Spinosad is naturally occurring in mixture of two active components (spinosyn A&D) produced by fermentation of soil *Actinomyces saccharopolyspora spinosa*. Several authors studied the effect of Spinosad on different lepidopterus as cotton leafworm and pink bollworm (Raslan, 2002 and Amer, 2004). Also, Flint *et al.* 2000, Natarajie *et al.*, 1985, and Welland and Vitco, 1997, reported that the chitin inhibitor (Dimilin), is effective in the control of cotton bollworms.

The objective of this work is to study the effect of NeemAzal T/S, Dimilin and Spinosad on fertility, fecundity and other biological aspects of *P. gossypiella*. In addition, biochemical studies were directed to estimate the effect on total lipids, total protein, carbohydrates, GOT & GPT of the treated moths.

### MATERIALS AND METHODS

#### Rearing technique:

The moths of *P. gossypiella* used in this study were supplied from the successful mass rearing cultures of Bollworms Research Department, Plant Protection Research Institute (PPRI). The mass rearing of the larvae occurred on the kidney bean diet that previously described by Rashad & Ammar (1985) under constant temperature  $27 \pm 1^\circ\text{C}$  and relative humidity of 80-85%.

#### Materials used:

##### NeemAzal T/s (1%); Spinosad and Dimilin:

The present evaluation of NeemAzal T/s (1%) against the newly emerged moths of *P. gossypiella* depend on  $LC_{50}$  of NeemAzal T/s estimated by Hewady *et al.* (2002)  $LC_{50} = 130.459$  ppm with lower and upper limits 49.72 and 179.71 at 95% respectively).  $LC_{50}$  of both Spinosad & Dimilin was estimated by pilot experiments directed the authors before beginning the present work.  $LC_{50}$  of Spinosad = 0.2 ppm with lower and upper limits 0.031 & 1.0 and 0.043 ppm with lower and upper limits (0.0047 - 0.3) for Dimilin.

#### -Procedure:

During mass rearing of PBW, pupae were isolated singly in glass tubes (7X2 cm) capped by cotton wool until emergence of adults. The newly emerged adults were sexed and paired in chimney glass cage, the upper and lower surfaces of each cage were covered with muslin cloth secured by rubber bands. Eighty pairs of newly emerged adults (♂ and ♀) were divided into four groups each group containing (20 pairs of male and female moths). One group was fed on 130 ppm of NeemAzal T/s (1%) solution for 24 hrs and changed to only 10% sucrose solution soaked on cotton wool. Second group was fed on 0.2 ppm solution of Spinosad for 24 hrs. and changed by 10% sucrose solution soaked on cotton wool. The third group fed on 0.043 ppm of Dimilin solution for 24 hrs and changed also by 10% sucrose solution soaked on cotton wool. The fourth group used as control fed only on 10% sucrose solution. All these groups were used to study the effect of NeemAzal T/s, Dimilin & Spinosad on the pre-oviposition, oviposition, post-oviposition and longevity of females and males in comparison to the untreated group.

For the biochemical studies, four groups of adult moths ((40♂ and 40♀) were used The 1<sup>st</sup> fed on 130 ppm of NeemAzal T/s, 2<sup>nd</sup> group fed on 0.043 ppm Dimilin, 3<sup>rd</sup> group fed on 0.2 ppm Spinosad for 24 hrs and changed by 10% sucrose solution and the fourth group fed only on 10% sucrose solution all over the experimental period. Adults were collected 10 days after treatment, separated & placed in clean glass tubes (10X3cm) and kept in a refrigerator ( -5°C) for biochemical analysis.

Evaluations of total protein, lipid and carbohydrate digestive enzymes (amylase, invertase and trehalase) were conducted as mentioned by Hewady, *et al.* (2002).

## RESULTS AND DISCUSSION

The newly emerged adults of PBW were sexed, caged and treated by NeemAzal T/S, Dimilin and Spinosad to study the effect of the three compounds on longevity and fecundity. Pre- oviposition, oviposition and post- oviposition, total number of deposited eggs and total number of hatching larvae were recorded for all treatments.

Data summarized in Table (1) show that the pre-oviposition period was prolonged (4.1 days) for females treated by NeemAzal T/s. These data are in agreement with Badawy (1995), who recorded that the pre-oviposition period of *Spodoptera littoralis* was prolonged when treated by *Boussingaultia gracilis* acetone extraction. While, when the females treated by Spinosad, the pre-oviposition period recorded short period (2.7 days), and this period recorded intermediate time when female treated by Dimilin (3.0 days) compared to control 3.57 days.

### Oviposition period

Data in Table (1) indicated that treating the adult females for 24 hrs. with NeemAzal and Spinosad reduced the oviposition period 1.4, 1.74 times less than the control, respectively. While this period increased to 1.2 times more than control when females treated with Dimilin. The two tested compounds (NeemAzal and Spinosad) show highly significant effect on treated females compared with the untreated.

The average oviposition periods were 10.96, 8.85 and 18.47 days for females treated by NeemAzal, Spinosad and Dimilin, respectively in comparison to 15.38 days for control. Badawy (1995) indicated that the high doses of *Boussingaultia gracilis* acetone extraction caused high decrease in the oviposition period of *S. littoralis* (from 4.8 to 2.6 days) than the untreated.

### Post – oviposition period:

Data in Table (1) recorded high significant effects of adult treatments on the post – oviposition period. It recorded 3.1, 5.2 and 2.7 days when females treated by NeemAzal T/S, Dimilin and control, respectively. While, no significant effect on Spinosad this period was 1.8 days. The longest period was 2.0 times

when females fed on Dimilin. The total numbers of deposited eggs (fecundity) and total No. of hatching larvae from the eggs (fertility) were recorded in Table (1).

#### **Fecundity:**

The total mean number of eggs laid by all treated females was fewer than the check. The total number of eggs was 131.2, 104.0, 154.38 and 190 eggs / female treated by NeemAzal T/S, Spinosad, Dimilin & control, respectively (Table 1).

It is clearly obvious that the females fed on Spinosad highly affected and laid fewer eggs than control, total deposited eggs were reduced by 40.05 % than check, while the females fed on the Dimilin laid eggs less than the check, the total number of eggs decreased by 19.1 % than the control.

During the oviposition period of adult females feeding on NeemAzal T/S, Spinosad and Dimilin, the average daily deposited eggs/female was 6.9 eggs on the NeemAzal T/S, 7.2eggs on Spinosad and 9.1eggs on Dimilin compared with 15.3 eggs /female.

Fig. (1) shows the influence from high to lower number of eggs from the 4<sup>th</sup> day until 15<sup>th</sup> days and this rate decreased gradually until 0.2 to 0 eggs/females in all treated during the life span.

#### **Fertility:**

Analysis of variances of the data given in Table (1) indicated highly significant difference for fertility of eggs deposited by PBW moths fed on all tested compounds. Treatments reduced hatchability than the check. The reduction ranged between 30.4 and 52.0 %, Dimilin produced the heights reduction.

This result is in agreement with Sexena (1989), Shalaby *et al.* (1997), Hewady *et al.* (2002) and Rao *et al.* (1993) who reported significant decrease in the number of eggs and the percent of hatchability per female of *P. gossypiella* treated by NeemAzal or neem extract up to oviposition repellency effects by prior treatment of larvae with neem extract. Also, Schmutterer and Ascher (1984) who found that females resulted from treated larvae of *S. littoralis* with neem seed extract laid only about 12% the number of eggs laid by untreated ones.

#### **Longevity of adults:**

##### **- Females:**

Data summarized in Table (1) show that the females longevity of PBW affected when fed on NeemAzal T/S and Spinosad. This period decreased to 18.933 and 12.3 days, respectively, while, when female fed on Dimilin the longevity increased to 25.8 days compared with 21.57 days for control. This data indicated that Spinosad decreased longevity of female than the untreated by 0.43 time. In contrast, when females fed on Dimilin, the longevity of females increased by 1.2 times than control.

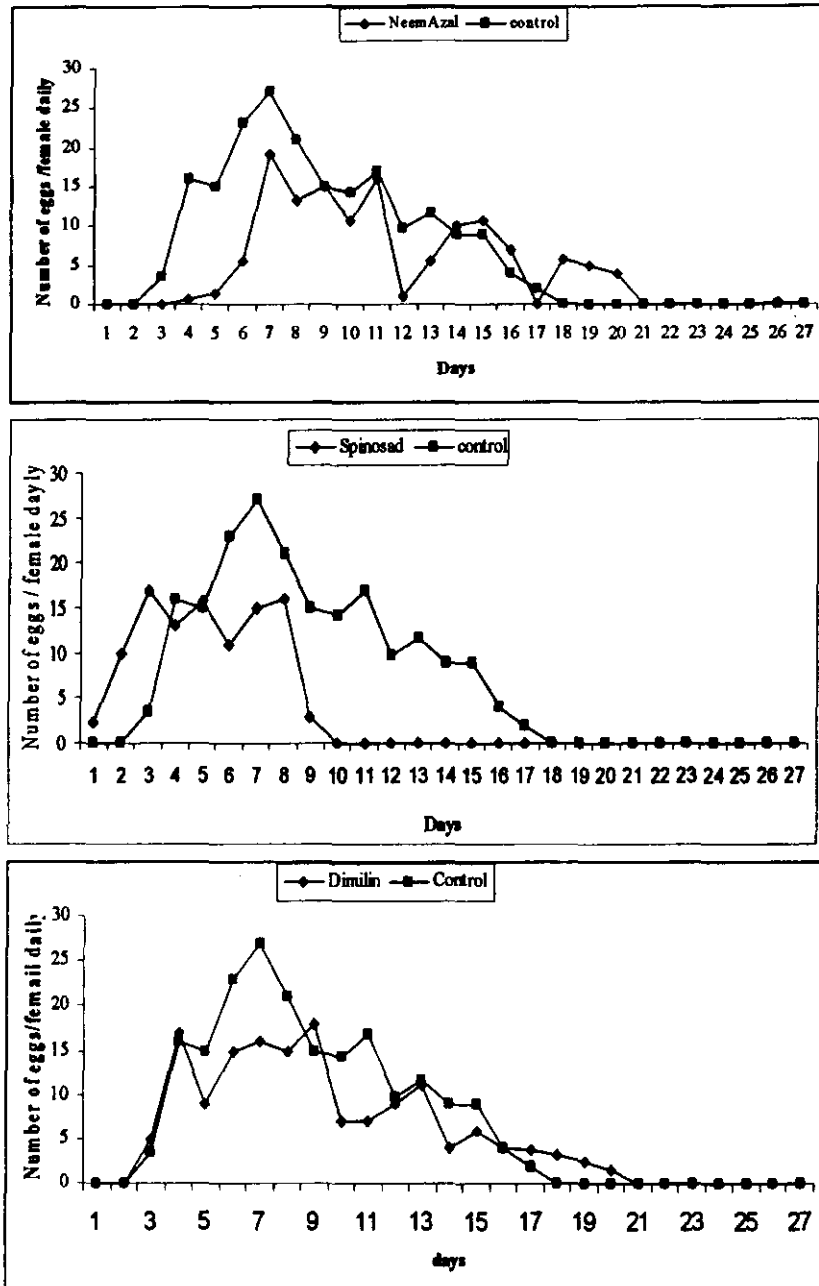


Fig. (1): Effect of NeemAzal, Spinosad and Dimilin on daily deposited eggs of *Pectinophora gossypiella* females moths.

Table (1): Effect of NeemAzal, Spinosad and Dimilin on biological aspects of *P. gossypiella* (Saund.) treated moths under constant conditions.

Treatment	Pre-oviposition (days) ± (s.e)	Oviposition (days) ± (s.e)	Post- oviposition (days) ± (s.e)	Total No of eggs / ♀	% Hatch- ability of eggs	Longevity (days)	
						♀	♂
NeemAzal 130 pp++++m.	4.1± 0.16 (3-5)	10.96±0.620 (3-15)	3.1±0.631 (1-5)	131.24±36.5 (88-156)	65.2%	18.933±2.8 (9-25)	11.33±0.67 (6-18)
Spinosad 0.2ppm	2.7 ±0.16 (2-4)	8.85±0.95 (4-19)	1.8±0.368 (0-4)	104.77±1.15 (30-120)	50.93 %	12.3±1.223 (9-26)	10.25±0.99 (3-17)
Dimilin 0.043 ppm	3.0 ± 0.22 (2-3)	18.47±1.32 (9-23)	5.2±0.378 (2-7)	154±5.12 (139-203)	43.52 %	25.8±1.03 (13-32)	17.9±2.04 (10-25)
Control	3.57±0.38 (2-4)	15.38±0.563 (13-17)	2.71±0.522 (1-4)	190.66±15.9 (90-239)	95.626 %	21.57±1.6 (18-25)	16.74 ± 1.9 (8-23)
F value	3.773	10.77	12.12	15.86	--	32.18	0.427
P	0.059	0.035***	0.0024***	0.001***	--	0.0000***	0.73
L.S.D	ns	4.139	1.314	31.184	--	3.168	ns

**-Males:**

Table (1) shows that the males longevity of PBW affected significantly with NeemAzal T/S and Spinosad. This period decreased to 11.3 and 10.2 days comparing with 16.7 for control. When males fed on Dimilin the longevity of males elongate to reach 21.57 days. From this data indicated that two compound of NeemAzal and Spinosad decreased longevity of male than untreated by 0.33 and 0.6 time, respectively, in contrast, when male treated by Dimilin the longevity of male increased to 1.3 time than control.

**Biochemical analysis of *P. gossypiella* adults treated with the tested compounds:**

**-Proteins.**

Data in Table (2) recorded that feeding adults of *P. gossypiella* on 130 ppm of NeemAzal T/S, 0.043 ppm of Dimilin and 0.2ppm of Spinosad for 24 hr. caused reduction in total content of haemolymph proteins to 3.27, 2.32 and 1.93 mg/ml for female and 0.77, 0.34 and 0.34 mg/ml for male, respectively, compared with 4.6 & 2.9 g/ml for untreated female and male, respectively.

From this data estimated reduction in total protein was 28.9, 44.9 and 48.1% for females and 72.5, 88.3 and 88.3% for males, when treated by NeemAzal T/S, Dimilin and, Spinosad, respectively than control. These effects may be the main reason in reducing the total numbers of eggs and or % of hatchability (Table, 1)

**-Lipids:**

Data given in Table (2) indicated that treating adults with the tested compounds affected the total lipids in haemolymph 67.1, 62.5 and 60.8 females and 0.0, 36.1 and 22.65 % for males.

The reduction in total soluble lipids in female ranged from 32.9 to 37.5 %, while, this reduction highly increased in case of male from 0.0 – 64.5%.

**Carbohydrate digestive enzyme activities:**

**Amylase**

Data in Table (3) show that the adult females and males of *P. gossypiella* fed on NeemAzal T/S or Dimilin & Spinosad, increased total haemolymph amylase activity. This highly increased to 0.667 & 0.44 mg glucose/min/ml when females & males fed on NeemAzal T/S and increased to 0.60 mg glucose/min/ml when female fed on Dimilin, in contrast, when males of *P. gossypiella* exposed to Dimilin, the amylase activity nearly equal to control. While, when female and male fed on Spinosad the amylase activity was 0.15 and 0.186 mg glucose/min/ml, respectively.

**Invertase**

The results presented in Table (3) indicated that, when adult females treated by the three compounds, all treatments caused high increase in invertase enzyme activity from 1.158 mg glucose/min/ml for control to 2.6, 3.3 and 3.33 mg glucose/min/ml for females treated by NeemAzal T/S, Dimilin and Spinosad, respectively. The increase was more than control by 2.25, 2.85 and 1.0 times for females.

Also, the haemolymph invertase enzymes activity for males treated by NeemAzal T/S, Dimilin and Spinosad, estimated by 2.74, 2.93 and 2.44 (mg glucose/min/ml) respectively, compared to 1.976 (mg glucose/min/ml) for control male.

Table (2): Changes in total soluble proteins and lipids in haemolymph of adult females and males of PBW treated with NeemAzal T/S, Dimilin and Spinosad.

Treatments	Concentrations (ppm)	Species	Total soluble proteins		
			Total protein mg/ml	Balance % relative control	Reduction
NeemAzal T/S	130	♀	3.27	66.5	28.9
		♂	0.77	23.6	72.5
Dimilin	0.043	♀	2.32	47.1	44.9
		♂	0.34	8.82	88.3
Spinosad	0.2	♀	1.93	37.35	48.1
		♂	0.34	8.82	88.3
Control	--	♀	4.6		
		♂	2.9		
Total soluble lipids					
NeemAzal T/S	130	♀	67.1	32.4	32.9
		♂	0.0	63.8	0.0
Dimilin	0.043	♀	62.5	38	37.5
		♂	36.1	7.22	43.5
Spinosad	0.2	♀	60.8	39.2	34.2
		♂	22.65	28.29	64.5
Control	--	♀	100		
		♂	63.8		

Table (3): Changes in different enzymes in haemolymph of PBW moths treated with NeemAzal T/S, Dimilin and Spinosad

Treatments	Species	%Transaminase enzymes		Enzymes activity mg/glucose/min/ml haemolymph		
		GOT	GPT	Amylase	Invertase	Trehalse
Neemazal T/S 130ppm	♀	27.5	22.22	0.667	2.6	0.61
	♂	0.0	16.00	0.44	2.74	0.16
Dimilin 0.043ppm	♀	206	16.00	0.60	3.3	1.80
	♂	28.26	17.39	0.08	2.93	1.78
Spinosad 0.2ppm	♀	262.5	20.00	0.15	3.33	1.9
	♂	255.813	18.615	0.186	2.44	1.49
Control	♀	198	7.017	0.104	1.158	0.79
	♂	86.8	7.017	0.1	1.967	0.066



**Trehalase:**

Data in Table (3) show that, when adult female and male treated by NeemAzal T/S, Spinosad and Dimilin, the haemolymph trehalase activity was 0.61, 1.8 and 1.9 (mg glucose/min/ml) /female, than 0.79 (mg glucose/min/ml)/ female for control and 0.16, 1.78 and 1.49 (mg glucose/min/ml)/male than 0.066 (mg glucose/min/ml) for control.

From this data indicated that the haemolymph trehalase activity was reduced in the female treated by NeemAzal T/S by 22.8 % (mg glucose/min/ml)/female haemolymph than the untreated.

In contrast, the treated adults females Dimilin and Spinosad caused increase of Trehalase activity by 2.28 and 2.26 time, respectively than control. In addition, in case of males treated by three tested compounds highly increase in trehalase in haemolymph was observed by 2.42, 26.96 and 22.27 times respectively than control.

**-GPT:**

The haemolymph GPT activity seemed to be affected to some extent after treating adults with the three tested compounds. The females and males treatments caused an increase in activity of GPT enzyme from 2.28-3.16 times than control Table (3). The highest increase was recorded in NeemAzal T/s treatment. In case of males treatments, the haemolymph GPT activity was approximately equal in most treatments. This increased by 2.28 & 2.48 times than control when males fed on NeemAzal T/S and Spinosad, respectively. The increase reached to 2.6 times more than control when males fed on dimilin.

**-GOT:**

Data in Table (3) show that the adult feeding on NeemAzal T/S (130ppm) greatly reduced haemolymph GOT enzymes to 27.5 and 0.0 mg/ml. for females and males, respectively, while GOT activity recorded 198 and 86.8 mg/ml for untreated females and males, respectively .

In the other hand, GOT enzyme highly increased in females and males (206 mg/ml/female & 28.26 mg/ml/male) when, treated by Dimilin to reach 262.5 and 255.8 mg/ml in case of Spinosad, respectively.

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تأثير مركب النيمازال و الأسبينوزاد و الديميلين على بعض الأنشطة البيولوجية  
والفسيولوجية لغراشة دودة اللوز القرنفلية

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تم إجراء تجارب معملية لدراسة تأثير ثلاث مركبات تنتمي لثلاث مجموعات مختلفة وهي مستخلص نبات النيم (النيمازال تى/أس/١%) & المركب الحيوي سبينوزاد ومائع الاتسلاخ الديميلين على كفاءة وضع البيض وطول حياة الحشرة الكاملة من إناث وذكور دودة اللوز القرنفلية وقد تم التحليل للفراشات المعاملة لمعرفة الملائكة مابين تأثير هذه المركبات على البروتين الكلي والإنزيمات المحللة للكربوهيدرات والمحللة للدهون وعلاقتها بوضع البيض وطول حياة الحشرة الكاملة.

وقد أوضحت النتائج أن فترة ما قبل وضع البيض استغرقت ٤,١ ، ٢,٧ ، ٣,٠ و ٣,٥٧ يوما على التوالي عند تغذية الحشرات الكاملة على كلا من النيمازال ١٣٠ والأسبينوزاد ٠,٢ والديميلين ٠,٠٤٣ ppm مقارنة بالكنترول وكذلك استغرقت فترات وضع البيض على نفس المركبات ١٠,٩٦ ، ٨,٨٥ ، ١٨,٤٧ و ١٥,٣٨ يوم كما انخفضت كميات البيض الموضوعة لهذه الإناث المغذاة على هذه المركبات على التوالي ١٣١,٢ ، ١٠٤ ، ١٥٤,٣٨ بيضة لكل أنثى مقارنة بالكنترول ١٩٠,٦٦ بيضة لكل أنثى كما انخفضت نسبة الفقس لكل بيض ناتج من الإناث المعاملة .

كما أوضحت الدراسات البيوكيميائية أن هناك انخفاض في نسبة البروتين مقارنة بالكنترول وقد يكون هذا النقص سبب في قلة إجمالي عدد البيض الموضوع لكل أنثى معاملة وبالعكس حدث زيادة في إنزيمات GPT&GOT بينما حدث تذبذب في نسبة إنزيمات الكربوهيدرات (الأميليز والأنفرتيز والترياليز).