RESULTS AND DISCUSSION

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I. The sex ratio of *Pecinophora gossypiella* (Saunders) during the cotton season in 1985 :

Larvae of the pink bollworm were collected from the infested cotton fields of Kalyobia Governorate, weekly during the cotton season of 1985 from June to December. Collected larvae were sexed, and separated into two groups (Normal and abnormal) as explained before.

The sex ratio of male to female larvae at the beginning of the season showed that a greater number of males than females were collected from the infested flowers on June and from green bolls on July, the ratio were (3 ♂: 1 ♀) and (2.09 ♂: 1 ♀) respectively (Table, 1). The reverse was, however, true in larvae collected from green bolls in August and September. It was (1 ♂: 1.8 ♀) and (1 ♂: 1.9 ♀) respectively. Larvae collected from dry bolls in October, also recorded more females than males, the sex ratio was (1 ♂: 1.46 ♀), in November and December, the sex ratio again showed in favor of males for larvae collected from dry bolls as it showed (1.6 ♂: 1 ♀) and (2.5 ♂: 1 ♀) respectively.

First larvae with morphological abnormalities appeared for the first time at the end of August (Table, 1) and amounted to 4 males only among 86 normal males, while all females were normal.

a – Normal and abnormal larvae at the end of the cotton season.

b – Normal and abnormal pupae ensued at the end of the season.

Table (1) : The sex ratio of <u>Pectinophora gossypiella</u> (Saunders) during the period June-December, 1985.

Source of food	Collection date	Sample size	Larvae/Sample Normal ♂	Larvae/Sample Normal ♀	Larvae/Sample Abnormal ♂	Larvae/Sample Abnormal ♀	Sex ratio ♂ : ♀	Abnormal % ♂	Abnormal % ♀
	15.6.85	100	4	-	-	-			
Flowers	22.6	100	3	2	-	-			
	30.6	100	5	2	-	-			
	Total	300	12	4	0	0	12:4	0	0
	8.7.85	100	6	2	-	-			
	15.7	100	8	4	-	-			
	22.7	100	10	6	-	-			
	30.7	100	22	10	-	-			
	Total	400	46	22	0	0	46:22	0	0
	8.8.85	100	26	34	-	-			
Green bolls	15.8	100	10	36	-	-			
	22.8	100	24	40	-	-			
	30.8	100	26	60	4	-			
	Total	400	86	170	4	0	90:170	4.44	0
	8.9.85	100	8	60	6	2			
	15.9	100	18	36	10	4			
	22.9	100	16	44	10	2			
	30.9	100	16	36	14	4			
	Total	400	58	176	40	12	98:188	40.12	6.4

Table (1) : Cont'd.

Source of food	Collection date	Sample size	Larvae/Sample Normal ♂	♀	Abnormal ♂	♀	Sex ratio ♂ : ♀	Abnormal % ♂	♀
	8.10.85	100	44	60	8	4			
	15.10	100	24	44	4	2			
	22.10	100	16	44	6	4			
	30.10	100	20	24	6	6			
	Total	400	104	172	24	16	128:188	18.8	8.5
	8.11.85	100	60	30	2	-			
	15.11	100	54	36	-	2			
Dry	22.11	100	56	34	2	2			
bolls	30.11	100	52	38	4	-			
	Total	400	222	138	8	4	230:142	44.8	2.8
	8.12.85	100	50	30	-	-			
	15.12	100	60	20	-	-			
	22.12	100	58	18	-	-			
	30.12	100	54	24	-	-			
	Total	400	232	92	0	0	232:92	0	0

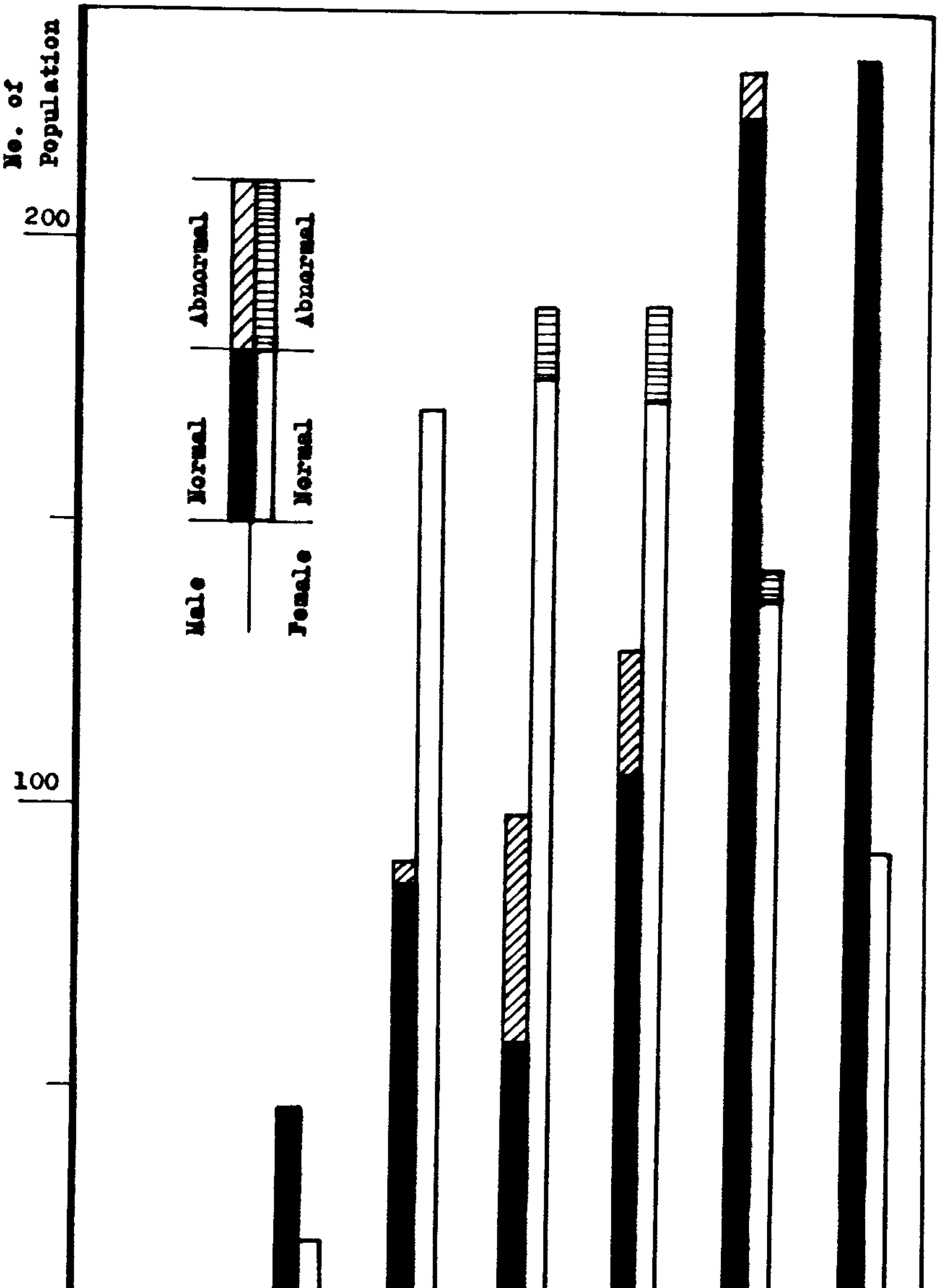


Fig. (1) : Relative distribution of male and female, normal / abnormal larvae of *P. gossypiella* in 1985 season.

In general, the percent of abnormal larvae was greatly skewed in favour of males, and the highest percent of abnormal male larvae (40.82 %) was recorded in September. Whereas the highest percent of abnormal female larvae (8.05) was recorded in October. The abnormal male and female larvae averaged (40.88 % & 6.4 %) on September,(18.75 % & 8.5 %) in October and (4.48 % & 2.8 %) in November. In larvae collected in December, however, no abnormal larvae were found.

From the previous data on the abnormal larvae in table (1), it is apparent that the increase of their number coincides with the increase of rate of infestation, the later being bound to boll formation and maturation (Loftin et al., 1921; Ohlendorf, 1926; Taylor, 1936 and Pearson, 1958).

It was observed in the present work that abnormal larvae failed invariably to enter diapause, this observation was further verified by their complete absence in samples collected in December when all larvae collected were in the diapause stage.

Data on the sex ratio indicated a marked increase in male ratio at the beginning of the season during the months of June and July. The ratio became in favour of females starting from August up to October. At the end of the season, however, in diapausing larvae collected in November and December, the ratio again favoured males. The above results are worth further investigation coupled with field observation on light trap caught moths. It might be an indication for the proper timing for using sex pheromones in a management programmé.

Whether this disproportion is brought about by weather factors can be gleaned from the work of Abdel-Hafez (1982) who pointed out that at 25°, 27° and 30°C more females of <u>P. gossypiella</u> were produced, the reverse occurred at 20°, 37° and 35°C.

Results revealed that at the beginning of the season male larvae appeared ahead of females and at the end of season about (71 %) of resting larvae of <u>P. gossypiella</u> were males. Hunter and Hinds (1904) reported that the sex ratio of <u>Anthonomus grandis</u> was about equal except that more males than females were found among hibernating weevils. Hinds and Yother (1909) found about 54 % of overwintering <u>Anthonomus grandis</u> to be males. Hunter and Pierce (1912) reported almost equal numbers of males and females during the fall before their entery into hibernation.

Diapause was proved to be genetical as early as (1913) by Toyama in the silk worm and later confirmed in well designed laboratory experiments by Barry and Adkisson in (1966) for <u>P. gossypiella</u>.

The results obtained indicated that larval population increased sequentially during the season. This is in line with the findings of(Loftin et al., 1921; Ohlendorf, 1926; Taylor, 1936 and Pearson, 1958). The pink bollworm is primarily a late-season pest of cotton.

Studies of the seasonal history of the insect indicated, in general, that infestations are usually very high during the early fruiting period of cotton and may not become noticeable until the plants reach the bloom stage. After this period has been attained and as bolls become available for oviposition, infestation increase rapidly. All the bolls may become infested with one or more larvae by the end of the season. Andrewartha and Birch (1954), in Chicago, stated that, it is generally true that in multi-voltine life-cycles the incidence of diapause is usually closely related to the season of the year, temperature, humidity, duration of day light and each one may in a particular case, be the stimulus that determines diapause.

The only report on the genetics of diapause of the pink bollworm is that of Barry and Adkisson (1966). But the report of these authors did not refer to sex linkages, if present.

Whether the proportional increase of males at the beginning and end of the season is an indication of sex linkage of the phenomenon is a matter worth of investigation, such knowledge, if available, would be an important idea in designing seasonality patterns and management programms.

II. The effect of different constant temperature on the biology of the fourth-instar larvae of *P.gossypiella* (Saund.) :

In most cotton growing regions in the world, the pink bollworm undergose a facultative diapause which occurs in the 4th (final) stadium. Seasonal changes in photoperiod, temperature, and the host plant are reported to regulate the occurrence of diapause (Adkisson *et al.*, 1964 and Raina and Bell, 1976).

Field collected 4th instar larvae of *P. gossypiella* were divided into patches each of which was subjected to one of three constant temperatures, i.e. 15, 20 and 27°C, and the biology of each patch was studied.

1. Larval weights :

The average weights of the pretreatment larvae are shown in table (2). It is evident that females are 11 mg. heavier than males on the average. When larvae were exposed to the three constant temperatures used, Table (2) : The effects of three constant temperatures on the larval stages and diapausing larvae of *Pectinophora gossypiella* (Saunders).

Sex	Temp. °C	Initial No.	4th instar larvae: Weight (mg) Before treat.		Duration (days)	Diapausing larvae: At 30 days Surviving		After 6 months Surviving		Total mortality	
			Before treat. Mean±S.E.	After 1 week Mean±S.E.	Mean±S.E.	No.	%	No.	%	No.	%
♂	15	60	20.36 ± 3.80	18.28 ± 1.80	105.26 ± 4.24	47	78.3	40	66.6	6	10.0
	20	60	20.36 ± 3.80	19.01 ± 1.24	98.15 ± 1.24	45	75.0	35	58.3	4	6.66
	27	60	20.36 ± 3.80	20.12 ± 3.80	22.12 ± 3.80	17	28.3	0	0.0	0	0.0
♀	15	60	31.39 ± 2.20	22.92 ± 2.50	107.20 ± 1.70	39	65.0	31	51.7	3	5.0
	20	60	31.39 ± 2.20	22.90 ± 1.10	93.30 ± 1.40	37	61.7	30	50.0	1	1.7
	27	60	31.39 ± 2.20	30.00 ± 0.50	22.83 ± 0.90	23	38.3	0	0.0	0	0.0

i.e. 15, 20 and 27°C for one week there was a general loss of weight in all cases and in both sexes.

At 15°C, the percentage loss of weight was 10.22 % for males and 27.02 % for females. In larvae kept at 20°C, the loss of weight in males was 6.63 percent, while it was 27.4 percent for females. At a constant temperature of 27°C the loss in males average weight was only 1.18 percent and females lost 3.15 percent of their weights. At the three constant temperatures used, the percentage loss of weight was more pronounced at the low temperature of 15°C in both sexes. This loss of weight was partially alleviated at higher temperature particularly for males at 20°C, the loss of weight, however, was negligible at 27°C.

Loss of weight in diapausing insects was previously reported by Chippendale and Kikukawa (1983), they stated that the diapausing larvae of <u>Homoeos electellum</u> lost about 29 % of their body weight between 20 and 50 days of age and a further 12 % between 83 and 135 days of age.

Accordingly, the conspicuous loss of weight at the constant temperature of 15 and 20°C, may be considered as an indication of a higher percentage of diapause at those temperature.

2. The duration period of the 4th instar larvae :

Data in table (2) gives the duration in days of the 4th instar larval period at different constant temperatures. There is a prolongation of this period at the degree of 15°C, the females requiring a slightly longer period than males. At 20°C, there was a prolongation of this period to a less extend and males requiring a slightly longer period than females. At 27°C, the duration of this period did not reach the threshold of diapause of 30 days (Fife, 1949), and the time required for pupation was more or less equal in males and females (El-Sayed and Rustom, 1960) pointed out that the percentage of resting larvae at any time was inversely proportional to temperature.

The above results indicated that most of the larvae kept at 15°C failed to undergo pupation but remained predominantly as larvae.

It may be concluded that this degree of temperature is not favourable for larvae to complete their development but was accordingly conducive to diapause. The same observation holds true at the degree of 20°C. At the degree of 27°C, however, pupation occurred and duration of the 4th larval instar was more or less the same for males and females. Accordingly, this degree of temperature may be considered favourable for larval development.

3. The pupal stage :

a) Pupation percentage :

Results presented in table (3) indicated that the percentage of pupation increased with the increase of temperature to reach the maximum of 100 % at 27 °C. It is noticeable, however, at the lower temperatures of 20 and 15 °C, that higher percentages of females pupated compared to males.

b) Pupal malformation :

Data in table (3) revealed that malformation of pupae occurred only at the low temperatures of 15 and 20 °C, but was absent at 27 °C.

The percentage of malformation was inversely proportional to temperature. In the two temperatures where the phenomenon occurred, males appeared to be more susceptible to malformation than females.

The above observations emphasise the suitability of 27 °C as a condition for laboratory rearing of *P. gossypiella*.

Table (3) : Effects of three constant temperatures on pupae and moths of Pectinophora gossypiella (Saunders).

Sex	Temp. (°C)	Initial No.	Pupae						Moths		
			Pupation		Malformation		Pupal Period (days)	Pupal Weight (mg)	Emergence	Malformation	
			No.	%	No.	%	Mean±S.E	Mean±S.E.	%	No.	%
♂	15	60	20	33.30	6	30.0	15.4±2.7	17.68±6.4	23.3	2	14.3
	20	60	25	41.67	4	16.0	12.6±2.8	18.10±0.6	35.0	1	4.8
	27	60	60	100.00	0	0.0	7.2±1.6	18.22±0.3	100	0	0.0
♀	15	60	29	48.3	3	10.3	20.7±6.9	20.60±1.3	43.3	2	7.7
	20	60	30	50.0	1	3.3	13.5±2.3	21.12±1.5	48.3	0	0.0
	27	60	60	100.0	0	0.0	8.1±1.2	23.90±1.7	100	0	0.0

c) Pupation period :

As would be expected the length of the pupal period was longer at lower temperatures in males and females. Females appeared to be slightly less affected by low temperature if they were compared to males. Whereas males required 2.75 times the length of their pupal period at 27°C when they were kept at 15°C, females, however, required 2.55 and 1.66 of their pupal period at 27°C at the two above mentioned respective temperatures. It is noticeable, however, that at temperatures of 15 and 27 males required less time for eclosion than females, while the reverse occurred at a temperature of 20°C.

d) Weight of pupae :

The weight of female pupae responded more favourably to a rise in temperature than males. When figures in table (3) were studied, it was noticed that in both males and females, the rise of temperature produced heavier pupae.

In males, the difference in weight between pupae produced at 15 and 20°C was 0.42 mg. The pupae produced at the higher temperature representing 2.37 % increase in weight, while in females the increase was 1.6 mg representing a proportion of 7.76 %.

At 27°C, the male's pupae weighed 0.54 mg more than the average weight of pupae kept at 15°C representing an increase of weight of 3 %. In females, the difference was as large as 3.3 mg representing 16.02 % increase in pupal weight.

According to the above observations, the differences in average weight between males and females increased by the increasing of temperature; females were 2.92 mg, 3.02 and 5.68 mg heavier than males on the average at temperatures of 15°, 20° and 27°C, respectively.

4. Moth emergence and malformation :

The percentage emergence of adults from pupae formed under low temperature conditions was adversely affected in both sexes. Females, however, were affected to a lower extent than males, and the percentage of emergence never reached 50 % in the two low temperatures of 15 and 20°C, while a large percent of the formed pupae of females in the two above mentioned temperatures succeeded in eclosion. At 27°C, however, there were 100 % emergence in pupae of both sexes. Abd El-Hafez (1982) refered to malformed moths whose emergence was incomplete or those with wing and/or leg deformities.

From table (3), it was noticed that deformities almost exclusively occurred at the temperature of 15°C, males being more susceptible than females, their percentage of deformities was almost double that of females at that temperature.

At 20°C, only a small percentage of deformation occurred in both sexes.

The above results agree with those of Thomas (1933) who reported that cases of abnormal metamorphosis, referred to, in his work, as prothetly and metathetly, sometimes occurred after exposure of insects to very low or very high temperatures.

5. The effect of temperature on percentage of diapausing larvae of *P. gossypiella* (Saund.) :

The pink bollworm was reported to remain in diapause when environmental conditions were unfavourable or no food was available (Raina, 1974).

This part of the present studies was to examine the effect of exposure of the 4th instar larvae of pink bollworm to different constant temperatures on the percentage of diapausing male and female larvae. According to Fife (1949), the maximum larval duration of the short-cycle type is 30-days and that larvae which remained alive for a longer period may be safely considered long-cycle or diapausing larvae.

a) <u>Larvae entering diapause</u> :

Data presented in table (2) indicated that of 60 male larvae exposed to the constant temperature of 15°C, 47 were still in their larval stage at the end of 30 days representing 78.3 % of the larvae used in the experiment. Among females, the number of diapausing larvae was conspicuously lower, representing only 65 % of the number of larvae in the experiment.

At 20°C, the number of diapausing larvae was slightly less than at 15°C, with the percentage of diapausing males being higher than those of females.

The examination of larvae kept at a constant temperature of 27°C revealed a much smaller percentage of larvae in diapause in comparison with the above two temperatures. There was, however, a difference between the percentage of males and females, the percentage of diapausing females being higher contrary to the results observed in the above two lower temperatures.

Statistical analysis revealed a highly significant effect of temperature on the percentage of diapause at ($P = 0.05$), while the interaction of sex and temperature was highly significant at ($P = 0.01$). It may be concluded from the above results that the lower temperatures induced more diapause among larvae, so much so among those the proportion of males was lower than females. The above results agree with the findings of Bell (1983) who noticed that in Ephestia elutella larvae maintained in slightly heated or unheated out-building, the summer emergence was poorly synchronised, and males on the average emerged ahead of females.

b) Larvae in extended diapause :

Larvae exposed to different constant temperatures were inspected six months after the commencement of the experiment, results are given in table (2). At the two lower temperatures of 15 and 20°C, 50 % or more of the larvae used were still in diapause, and in both temperatures the percentage of males was higher than females. Statistical analysis of the figures showed that both temperature and sex had significant effects at ($P = 0.01$). Interaction between the two factors was significant at ($P = 0.05$).

At a temperature of 27°C, no larvae of either sex remained in diapause for that period of six months.

The above results agree with previous reports by El-Sayed and Rustom (1960) and Hassanein and Galal (1969) who indicated that the temperature is an important factor governing the initiation of diapause in the pink bollworm. The percentage of resting larvae, at any time being inversely proportional to temperatures.

The present results indicated that the length of diapause was also inversely proportional to temperature, whereas the temperature of 27°C did not induce long cycle diapause, but caused only a relatively small number of larvae to enter short diapause, the lower temperatures, caused proportionately large percentages of diapausing larvae.

The above results, supported by statistical analysis also revealed that in addition to the number of diapausing larvae and the length of diapause, temperature also determined the sex of diapausing larvae at the low temperatures, the percentage of males being higher than females.

All the previous results suggest that a higher population of males would occur at the beginning of the season under laboratory conditions. To verify such speculation is a subject for further field investigations.

6. The effect of temperature on the total mortality :

Field collected 4th instar larvae, obtained from bolls and exposed to the three constant temperatures (15, 20 and 27°C) were inspected for mortalities throughout the six months duration of the experiment, in their larval and subsequent stages.

Results in table (2) revealed that lower temperatures caused more mortalities compared to higher ones; at 15°C ten percent and five percent of male and female larvae died, respectively, while the percentage mortalities in both sexes dropped to 4 % and 1 % at the temperature of 20°C. At the temperature of 27°C, however, no mortalities occurred in both sexes.

The above mentioned figures suggested, moreover, that males were more susceptible to the adverse effect of temperature than females since, at the temperature of 15°C the mortalities among males was twice as large as among females kept at the same temperature, and at the temperature of 20°C, there were four times dead males compared to dead females.

Statistical analysis of the above data revealed a highly significant effect of temperature on mortalities ($P = 0.01$), and the sex was also significant factor on mortalities at ($P = 0.01$).

III. The effect of sublethal doses of insecticides on the biology of the fourth-instar larvae of pink bollworm, *Pectinophora gossypiella* (Saunders) :

Three major pests attack cotton in Egypt namely; the cotton leafworm, *Spodoptera littoralis* (Boisd.), the pink bollworm, *Pectinophora gossypiella* (Saunders) and the spiny bollworm, *Earias insulana* (Boisd.).

Insecticides recommended for treating cotton should therefore control efficiently the three pests. Taso and Lowry (1963) studied the larval dispersal of *P.gossypiella*. Newly hatched larvae moved up and down plants principally along the main stems, but most of them hatched on firm green bolls, and did not disperse but entered the bolls on which they hatched.

As a result, it is concluded that newly hatched larvae of pink bollworm may be exposed to the sprayed insecticides for a short period, then the surviving ones insert themselves into the green bolls to spend their life-cycle. It may be argued that a second exposures ensues when the 4th instar larvae leaves the bolls to find a place for pupation.

Adkisson and Wellso (1962) showed that sublethal doses of DDT or methyl-parathion had stimulating effect on egg production. Conversely, Kuipers (1962) observed that sublethal doses of DDT produced a decrease in the fecundity of the pink bollworm.

The present work was conducted to determine the effect of sublethal doses of insecticides applied to the 4th instar larvae of pink bollworm, at the three different constant temperatures on the biology of treated insects, the nature of interaction between temperature and such latent toxicity effects, in addition to their combined effect on the percentage diapause and diapause duration.

Larvae collected from infested bolls were subjected to topical application by sublethal doses of the three insecticides; fenvalerate, cypermethrin and chlorpyrifos.

1. Log-dose; probit mortality lines (Ld-P) :

It was necessary in this work to determine Ld-P lines for the insecticides used, in order to determine the dose to be applied as a sublethal dose on the basis of a dosage causing ca. 10 % mortality.

Figures (2, 3 and 4) give the details of the results obtained. The most toxic of the insecticides used was fenvalerate on the basis of LD_{50} or LD_{90} values. A similar result on the high toxicity of this insecticide was reported by Ahmed et al. (1987). Next to fenvalerate was cypermethrin. The value of its LD_{50} was 2.8 times

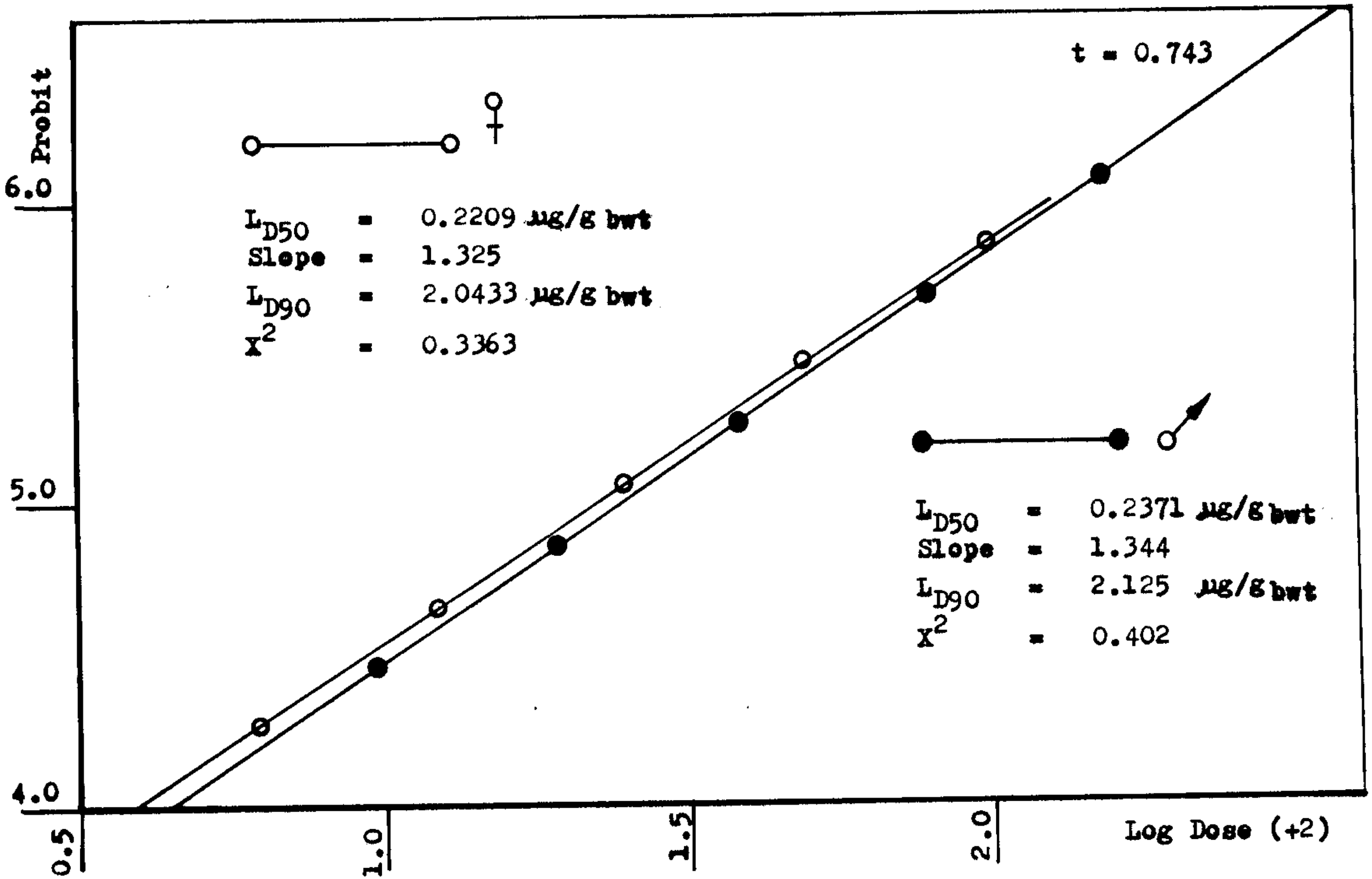


Fig- (2) Log Dose-Probit mortality lines for P.gossypiella larvae treated with Cypermethrin

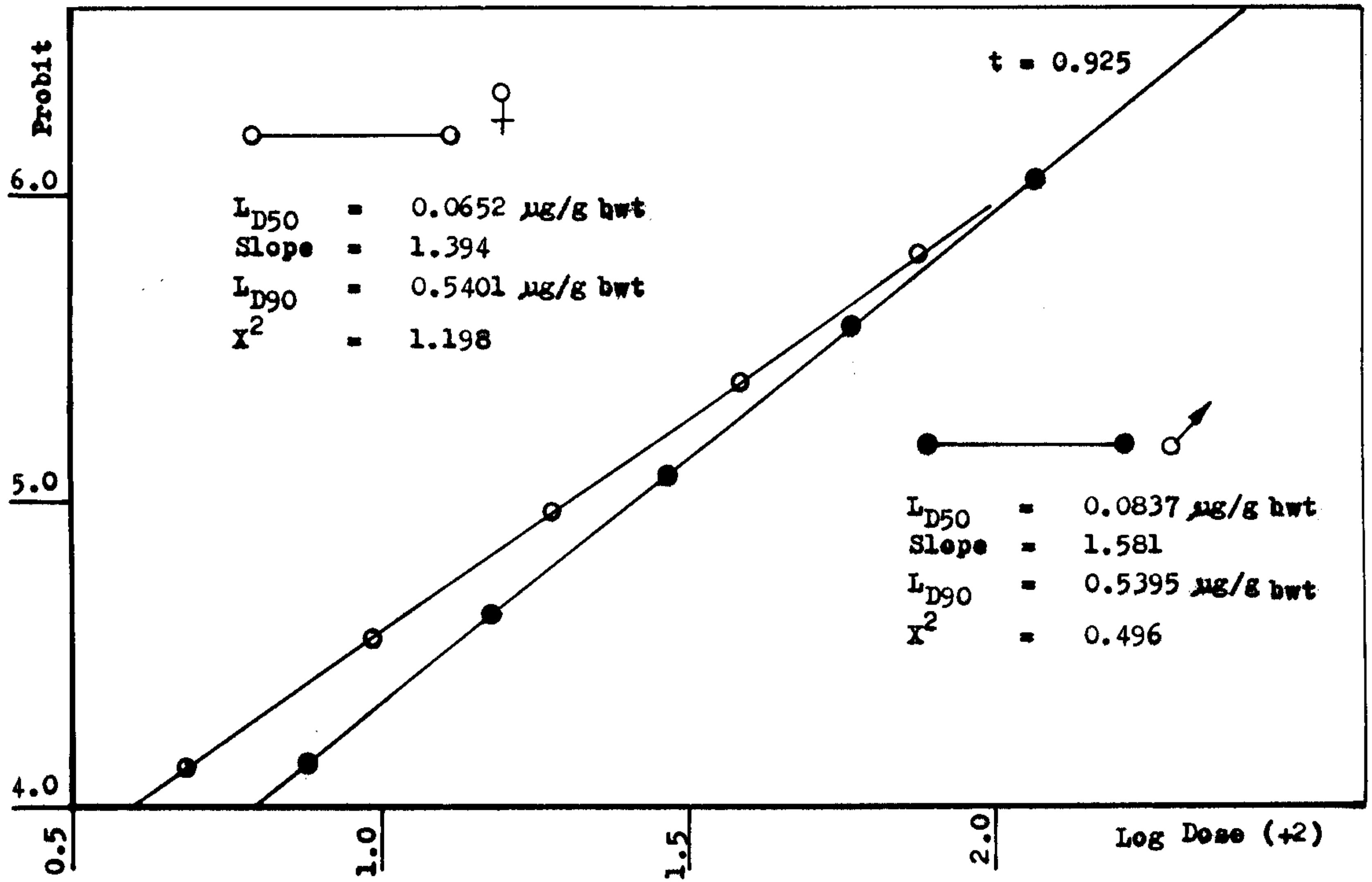


Fig- (3) Log Dose-Probit mortality lines for P.gossypiella larvae treated with Fenvalerate.

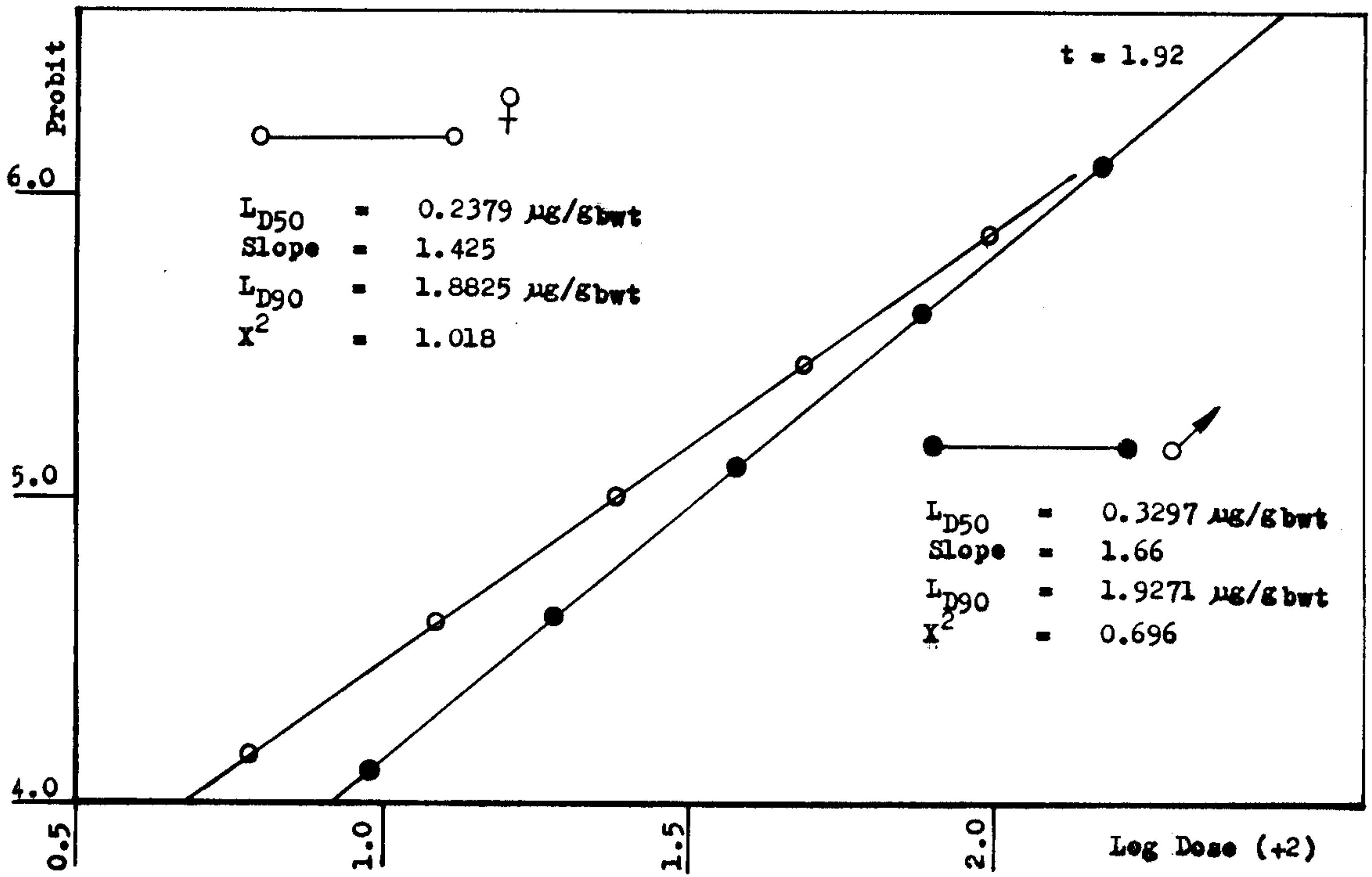


Fig- (4) Log Dose-Probit mortality lines for P.gossypiella larvae treated with Chlorpyrifos.

higher than that of fenvalerate in males and 3.38 times higher in females. The lowest toxicity among the insecticides used was that of chlorpyrifos, where the LD_{50} values showed this toxicant to be comparatively less effective than the two pyrethroids used in this study.

The LD_{50} of chlorpyrifos in males was 3.9 folds higher than fenvalerate and 1.39 times higher than cypermethrin. In females, this parameter was 3.6 folds higher than cypermethrin and 1.07 folds higher than cypermethrin.

The slopes of the insecticides were comparable in chlorpyrifos and fenvalerate trials in each sex separately, while this parameter was slightly lower in cypermethrin in both sexes.

X^2 values were not significant in all trials indicating that the samples used were reasonably homogenous. The figures of LD_{50} for males was generally higher than those of females, but when Student's "t" was calculated, it revealed that these differences were not statistically significant.

The slopes of Ld-P lines for males were always slightly higher than those of females with the result that the differences in LD_{90} values were smaller than the differences observed between the two sexes in LD_{50} values.

Table (4) : The effect of sublethal doses of insecticides on the 4th instar larvae of the pink bollworm (males).

Insecticides	Temp. (°C)	Initial No.	After 24 hrs.		4th instar larvae		
			No. of alive larvae	% of dead larvae	Average wight (mg)		Duration (days)
					Before treatment	After treatment	
					Mean±S.E.	Mean±S.E.	Mean±S.E.
Fenvalerate	15	60	51	15.0	20.4±3.8	12.7±1.2	60.6±4.4
	20	60	49	18.3	20.4±3.8	13.8±2.9	69.0±4.3
	27	60	50	16.6	20.4±3.8	16.2±1.4	20.1±0.6
Cypermethrin	15	60	53	11.6	20.4±3.8	13.4±1.4	63.9±6.1
	20	60	53	11.6	20.4±3.8	13.8±1.7	74.9±3.4
	27	60	59	1.6	20.4±3.8	16.3±1.0	18.7±0.8
Chlorpyrifos	15	60	53	11.6	20.4±3.8	17.6±3.4	78.4±3.4
	20	60	52	13.3	20.4±3.8	17.5±0.5	72.3±4.3
	27	60	53	11.6	20.4±3.8	17.7±1.3	18.2±0.4
Control	15	60	59	1.6	20.4±3.8	18.3±1.8	105.3±4.2
	20	60	60	0.0	20.4±3.8	19.0±0.8	93.1±1.2
	27	60	60	0.0	20.4±3.8	20.1±3.8	22.7±0.8

3. Larval weight after one week of treatment :

When weight of treated larvae were determined one week after treatment, it was found that a loss of weight was evident. Larvae kept at 15°C suffered 42.05 % loss of weight. Loss of weight at that temperature occurred also in the control but it was less than that occurring among treated larvae, in the control the loss was only 26.98 %. The same results occurred among larvae kept at 20°C with minor differences (40.04 % treated and 26.98 % untreated).

In larvae kept at 27°C, however, there was smaller reduction in weight among treated insects, but still noticeable, i.e. 28.77 %, while the reduction in control was negligible (4.3 %). When loss of weight was compared in males and females, it was evident that females showed greater percentage loss when treated with the insecticides compared to the percentage loss in males. This was most evident in the case of the organophosphorus chlorpyrifos.

The loss of larval weight when exposed to low temperature has been demonstrated previously in this work. This loss was large at lower temperature but negligible at 27°C. When the effect of sublethal doses of fenvalerate was added, the reduction of weight was increased, and this was most obvious at 27°C.

Loss of weight through lowered temperatures may be attributable to the increased stress on adipose tissues for a source of energy to keep the larvae active. The effect of fenvalerate may be attributable to either loss of water reported by earlier authors to accompany exposure to pyrethrins (Ingram, 1955), or an added stress on the metabolism brought about by detoxication processes and requiring extra energy obtainable from the adipose tissues of the larvae.

4. Duration period of the 4th instar larvae :

The average duration period of the 4th instar larvae (in days) in males and females are shown in tables (4 & 6). As shown previously in this work, lower temperatures caused prolongation of the larval period.

The time required for pupation, however, was always shorter among treated larvae in both sexes. At temperature of 15°C, the duration of this stage was 23 days shorter than control in females and 45 days shorter in males, at 20°C, the differences were 22 days in females and 24 days for males. At the non-diapausing (Tables 5 & 7) temperature of 27°C, the differences were rather small ranging between 1 and 3 days in females and 2 to 4 days in males.

Table (5) : The effect of the sublethal doses of insecticides on pupae and moths of the pink bollworm larvae (males).

Insecticides	Temp. (°C)	Initial No.	Pupae						Moths	
			No.	% Pupation	Malformed		Pupal		Emergence %	Malformation %
					No.	%	Period (days) Mean±S.E.	Weight (mg) Mean±S.E.		
Fenvalerate	15	60	12	20.0	5	41.7	19.8±6.4	12.6±1.7	11.7	57.1
	20	60	14	23.3	4	28.7	16.5±2.5	12.6±1.7	16.7	50.0
	27	60	37	61.7	4	10.8	5.9±0.9	14.2±0.6	55.0	45.5
Cypermethrin	15	60	8	13.3	1	12.5	20.1±6.0	12.6±1.6	11.7	71.4
	20	60	10	16.6	1	10.0	20.1±6.0	12.1±1.3	15.0	44.4
	27	60	37	61.7	3	8.1	5.8±0.6	13.9±1.5	56.7	41.2
Chlorpyrifos	15	60	14	23.3	7	50.0	19.4±5.5	16.8±0.5	11.7	42.9
	20	60	15	25.0	4	26.7	17.2±1.5	15.4±1.1	10.3	36.4
	27	60	38	63.3	1	2.6	6.0±0.8	16.7±0.7	61.7	35.1
Control	15	60	20	33.3	6	30.0	15.4±2.7	17.7±0.5	25.3	14.3
	20	60	25	41.7	4	16.0	12.6±2.7	16.1±0.7	35.0	4.8
	27	60	60	100.0	0	0.0	7.2±1.6	16.2±0.3	100.0	0.0

Table (6) : The effect of sublethal doses of insecticides on the 4th instar larvae of the pink bollworm (females).

Insecticides	Temp. (°C)	Initial No.	After 24 hrs. No. of alive larvae	After 24 hrs. % of dead larvae	4th instar larvae: Average weight (mg) Before treatment Mean±S.E.	4th instar larvae: Average weight (mg) After treatment Mean±S.E.	4th instar larvae: Duration (days) Mean±S.E.
Fenvalerate	15	60	53	11.6	31.4±2.2	18.2±2.8	84.2±9.3
	20	60	54	10.0	31.4±2.2	18.8±3.0	71.3±4.4
	27	60	54	10.0	31.4±2.2	22.9±1.1	21.3±0.9
Cypermethrin	15	60	53	11.6	31.4±2.2	18.3±0.9	72.3±4.9
	20	60	53	11.6	31.4±2.2	15.6±1.2	80.9±2.0
	27	60	54	10.0	31.4±2.2	23.8±2.4	20.9±1.2
Chlorpyrifos	15	60	53	11.6	31.4±2.2	19.4±2.2	90.1±1.2
	20	60	52	13.3	31.4±2.2	20.6±1.1	78.3±2.7
	27	60	53	11.6	31.4±2.2	24.9±1.4	19.6±0.6
Control	15	60	59	1.6	31.4±2.2	22.9±2.5	107.2±1.7
	20	60	59	1.6	31.4±2.2	22.9±1.1	93.3±1.4
	27	60	60	0.0	31.4±2.2	30.0+0.5	22.8±0.9

Table (7) : The effect of the sublethal doses of insecticides on pupae and moths of the pink bollworm larvae (females).

Insecticides	Temp. (°C)	Initial No.	Pupae						Moths	
			No.	% Pupation	Malformed		Pupal		Emergence %	Malformation %
					No.	%	Period (days) Mean±S.E.	Weight (mg) Mean±S.E.		
Fenvalerate	15	60	15	25.0	7	46.7	21.6±7.2	16.7±1.5	13.3	37.5
	20	60	16	26.7	5	31.3	19.8±6.9	17.3±1.6	18.3	57.1
	27	60	45	75.0	3	6.7	6.2±0.8	18.0±1.1	70.0	57.1
Cypermethrin	15	60	11	18.3	5	45.5	21.9±7.7	13.8±1.5	10.0	66.7
	20	60	12	20.0	6	50.0	19.1±7.1	14.1±1.3	18.3	63.6
	27	60	54	90.0	8	14.8	6.3±0.9	17.9±1.6	80.0	52.2
Chlorpyrifos	15	60	18	30.0	7	38.9	22.3±7.5	17.6±0.7	18.3	57.1
	20	60	18	30.0	4	22.2	19.7±6.9	17.8±0.8	23.3	36.4
	27	60	43	71.7	7	16.3	6.0±0.8	17.9±1.3	60.0	55.6
Control	15	60	29	48.3	3	10.3	20.7±6.9	20.6±1.3	43.3	7.7
	20	60	30	50.0	1	3.3	13.5±2.3	21.1±1.5	48.3	0.0
	27	60	60	100.0	0	0.0	8.1±1.2	23.9±1.7	100.0	0.0

It appears from these results that treatment with pesticides at the toxicity level applied, did not prevent diapause if the low temperature induction factor was at work. But there was a noticeable reduction of that period at low temperatures in both males and females. This reduction is more pronounced among males and in treatments with fenvalerate. This reduction of the diapause period is worthy of further studies.

5. Pupal stage :

a) Percent of pupation :

The percentage of pupation under the different experimental conditions applied are given for male and female larvae in tables (5 & 7). The deleterious effect of low temperature was augmented to various degrees by pretreatment with pesticides. Without exception, the percentage of pupation was lower in males and females exposed to insecticides at the larval stage in comparison with the untreated control. The differences, however, varied from one insecticide to another.

The lowest percentage of pupation at the two diapause inducing temperatures of 15 and 20°C occurred in both males and females exposed in their larval stage to cypermethrin (Tables 8 & 9). When fenvalerate and chlorpyrifos were compared, it was noticed that while

Table (8) : The effect of sublethal doses on diapausing male larvae and total mortality of the pink bollworm.

Insecticides	Temp. (°C)	Initial No.	Diapausing larvae					
			At the end of 30 days		At the end of 6 months			
			Surviving		Surviving		Total mortality	
			No.	%	No.	%	No.	%
Fenvalerate	15	60	17	28.3	9	15.0	44	73.3
	20	60	29	48.3	12	20.0	38	63.3
	27	60	14	23.3	0	0.0	27	45.0
Cypermethrin	15	60	21	35.0	9	15.0	44	73.3
	20	60	27	45.0	12	20.0	39	65.0
	27	60	13	21.7	0	0.0	26	43.3
Chlorpyrifos	15	60	24	40.0	14	23.3	39	65.0
	20	60	22	36.7	14	23.3	35	58.3
	27	60	12	20.0	0	0.0	23	53.3
Control	15	60	47	78.3	40	66.7	6	10.0
	20	60	45	75.0	35	58.3	4	6.7
	27	60	17	28.3	0	0.0	0	0.0

Table (9) : The effect of sublethal doses on diapausing female larvae and total mortality of the pink bollworm.

Insecticides	Temp. (°C)	Initial No.	Diapausing larvae: At the end of 30 days, Surviving No.	%	At the end of 6 months, Surviving No.	%	Total mortality No.	%
Fenvalerate	15	60	20	33.3	15	25.0	37	61.7
	20	60	35	58.3	15	25.0	34	56.7
	27	60	17	28.3	0	0.0	18	30.0
Cypermethrin	15	60	34	56.7	15	25.0	39	65.0
	20	60	29	48.3	19	31.7	35	58.3
	27	60	14	23.3	0	0.0	14	23.3
Chlorpyrifos	15	60	29	48.3	17	28.3	32	53.3
	20	60	25	58.3	16	26.7	30	50.0
	27	60	11	18.3	0	0.0	24	40.0
Control	15	60	39	65.0	31	51.7	3	5.0
	20	60	37	61.7	30	50.0	1	1.7
	27	60	23	38.3	0	0.0	0	0.0

the reduction in pupation of males was more or less equal in the treatment of the two insecticides, the figures of pupal formation of females was slightly higher in chlorpyrifos treatments than in fenvalerate.

When the normal laboratory breeding temperature of 27°C was considered it was noticed that a conspicuous reduction of percentage pupation occurred among treated larvae in comparison with control. The above results suggest that pre-exposure to insecticides excerted an additive effect on pupation of the treated larvae at low temperatures. When the percentage of pupation in males and females under the different conditions of the experiment were compared, it was evident that males suffered more reductions than females under any given condition.

The only exception was the untreated at 27°C where the percentage pupation was 100 %.

This may be taken as an indication that male larvae, although not significantly tolerant to the acute effect of insecticides than females, but they had a large survival potential when it comes to the comparison of the fate of survival of exposure to sublethal doses.

b) <u>Percentage of malformed pupae</u> :

The diapause inducing temperatures of 15 and 20°C produced some malformation in the resultant larvae in both males and females. The percentage of malformation was highest at the low temperature of 15°C and malformations among males was more than in females.

When the effect of sublethal doses of insecticides was introduced, the percentage of malformations in most cases increased among treated insects. At 15°C, there was a greater increase in this percentage, particularly with chlorpyrifos applied to male larvae. Surprisingly, the percentage of malformations in males treated with fenvalerate at 27°C, was less than control.

There were no big differences between males and females, with the exception of the case of fenvalerate and males, the insecticides used produced more or less similar proportions of deformities in pupae and was proportional to the decrease of temperature in both cypermethrin and chlorpyrifos, while the results of fenvalerate demonstrated a need for further studies.

The incidence of malformed pupae after treating larvae of P. gossypiella was reported by Metwaly et al. (1987). In other insects, Smith (1971) referred to pseudo-juvenilizing effects produced by Lannate in the spruce budworms, Choristoneura occidentalis and Staal (1972) reported that pupae of Tenebrio showed juvenile hormone response to fenthion. Recently, Gomaa et al.

(1987) described dose-dependent morphogenetic effects of deltamethrin on the desert locust, Schistocerca gregaria (Forsk.) treated in the 5th nymphal instar with the toxicant.

It is evident from the above results and literature cited that some sort of juvenile hormone mimic action takes place in larvae of P. gossypiella when treated with sublethal doses of insecticides, this effect was more evident at lower temperatures and was more or less the same with all insecticides investigated except fenvalerate and male larvae.

The incidence of the phenomenon in different insects and insecticides belonging to different chemical groups may be taken to suggest that the chemical or its detoxication products are not the main factor producing the malformations, but it may be a result of some disruption of the physiology of the insect as a result of exposure to toxicants. However, this is mearly a theoretical explanation which needs more detailed studies on the biochemical effects of sublethal doses of insecticides.

c) Pupal duration period :

From results in tables (5 & 7), it is evident that the application of insecticides to larvae, prolongs the pupal period compared to the control, this phenomenon was more pronounced in males. On inspection of the figures in control, it is evident that the pupation period for females was longer than that of males at all the temperatures investigated, but the difference between treated and untreated was more pronounced in males than in females. It is noticeable, however, that the impact of insecticidal treatment was more pronounced at 20°C than at 15°C. The differences between different insecticides was small and insignificant. The shorter pupation period of males may explain the observation that males appear more abundently at the beginning of the season.

d) Pupal weight :

As it may be expected, small larvae produced small pupae. According to tables (5 & 7), the weights of pupae resulting from larvae treated with insecticides were smaller than those of the control in both males and females at the three constant temperatures investigated. It was noticeable, however, that in females the reduction in pupal weight was more or less equal at the two lower temperatures, while in cypermethrin if we calculate the reduction in comparison to control weights it will be nearly double the reduction in the other two toxicants. At the temperature of 27°C, however, the reductions were more or less equal. It appears from these results that lower temperatures tend to emphasize the effect of pesticides in lowering weights in subsequent stages of survivals, as reported by Alam et al. (1978) in Spodoptera littoralis and Metwaly et al. (1987) in Pectinophora gossypiella. In males the same phenomenon occurred (Tables 5 & 7), but in a somewhat different pattern.

Reduction was most pronounced in cases where pyrethroids, i.e. fenvalerate and cypermethrin were used and it was slight in chlorpyrifos, a similar result was reported by Metwaly et al. (1987) when samples of larvae from Fayoum were treated with different insecticides, the least reduction in pupal weight occurred with chlorpyrifos. The above observation was true for both sexes.

5. Moth emergence and malformation :

Tables (5 & 7) shows the percentages of adult emergence from larvae treated with insecticides under the three constant temperatures investigated for both males and females.

It appears from the table that, as mentioned before, low temperatures reduced the percentages of emergence and in males was more than in females.

It is noticeable that the largest reductions induced by insecticides occurred at the temperature of

27°C in both sexes in most cases; in fenvalerate, the reduction in percentage of emergence was 30 % and 45 % in females and males respectively. In cypermethrin treatments, however, the reduction at that temperature in percentage emergence was only 20 % in females compared with 44 % in males, while in chlorpyrifos the reduction in percentage of adult emergence was more or less equal in both sexes ca. 40 %.

At lower temperatures, the treatment with insecticides brought about an additional reduction in percentage eclusion of adult moths. When these reductions were compared with untreated control, it was noticed that these reductions were of a greater magnitude in females compared with males taking into consideration eclusion percentage in the untreated control. The largest reduction observed occurred in cypermethrin at 15°C where the percentage emergence was reduced by 33.3 % compared with the control at the same temperature, followed by fenvalerate and cypermethrin at 20°C where reductions were 30 %. In chlorpyrifos, reductions at the two low temperatures were more or less similar.

As mentioned before, reductions in male eclusion were less than in females at the two lower temperatures reaching its maximum in cypermethrin at 20°C.

It should be mentioned that this observation does not contradict with the fact that less males would be produced if we take the original impact of low temperature.

Percentages of malformation among the moths are given in tables (5 & 7). From the tables, it is evident that low temperature _per ce_ caused little or no malformations among the resultant adults. When the factor of larval exposure to insecticides was added a relatively high percent of deformities occurred in the emerged moths.

The highest percentage of deformities occurred in female larvae treated with cypermethrin; Metwaly _et al_. (1987) indicated the incidence of deformities in adults emerged from larvae treated with insecticides, and they did not notice substantial difference between insecticides, they experimented with chlorpyrifos, mephospholan, profenofos and fenvalerate.

These results recall the earlier discussion of the juvenoid mimic effect of pesticides when discussing malformations in pupae after exposure to insecticides.

7. Effect on percentage of diapausing larvae :

In laboratory experiments, the objective of our present studies is to determine the latent effect of exposure the 4th instar larvae of _P. gossypiella_ to sublethal doses of the three tested insecticides (fenvalerate, cypermethrin, chlorpyrifos) under the conditions of different constant temperature 15, 20 and 27°C, respectively on reducing the population number of diapausing male and female larvae. According to Fife (1949) who stated that the maximum larval duration of the short-cycle type is 30 days and that larvae which remained alive for a longer period may safely considered long-cycle larvae.

a) Larvae entering diapause :

Table (8) gives the number and percentages of larvae still in their larval stage at the end of the 30th day under different conditions in males and females. It was demonstrated earlier in this work that low temperatures of 15 and 20°C are diapause inducing in larvae of *P. gossypiella*. The figures for untreated larvae in tables (8 & 9) confirm this fact and reveal that diapause at low temperatures 15 and 20°C occurs more in males, compared with females. While the reverse is true at 27°C. The rest of the table reveals that treatments of larvae with pesticides reduced the percentages of diapausing larvae, this reduction varied with sex and insecticides.

In fenvalerate treatments , there was at 15°C only about half the number of larvae was in diapause compared with control in females, while in males the reduction was even more where the number of diapausing treated larvae was roughly one-third of their number in control. With the result that, contrary to control the percentage of diapausing females was higher than males.

The same trend of results occurred again at 20°C, but to a less extent in both sexes, the impact of pesticides being more pronounced in males, the reduction in percentage being 3.4 % in females compared with 26.7 % in males,at 27°C, there was 10 % reduction in resting female larvae compared with 5 % reduction in males.

In cypermethrin, another pyrethroid, again there was some reduction in the percentages of diapausing larvae in both sexes. The differences between larvae exposed to insecticides and control were less than those found in fenvalerate treatment at 15°C. At 20°C, females were more affected than those exposed to fenvalerate, while the effect on males was more or less similar. At 27°C, however, there were slight differences between the two pesticides. When the numbers of diapausing larvae in cypermethrin treatments were compared with control, the percentage of treated larvae in diapause at 15°C was 8.3 % and 43.5 % lower than control in females and males, respectively. At 20°C, the reduction in diapause among treated larvae was 13.4 % and 38.4 % for females and males, while at 27°C the difference was 15 % and 6.7 % for females and males, respectively.

In larvae exposed to sublethal doses of chlorpyrifos an organophosphorus insecticides, the same trend reported above was noticed but to differint intensities. At 15°C, reduction in diapausing larvae compared with control was 16.7 % in females, a reduction rate in between fenvalerate and cypermethrin, while in males the least reduction occurred with this insecticide at that temperature being 38.5 %. At 20°C, there was a small reduction in diapausing females of 3.4 %, while reduction in diapausing males was 38.4 %. At 20°C, however, no female larvae entered diapause among larvae treated with this toxicant, while 20 % male larvae entered diapause.

When the above figures were subjected to analysis of variance, the effect of temperature and of insecticides were found significant at the same level of probability, while the effect of sex was not significant. The interaction of temperature and insecticides were significant at (P = 0.01). The interaction of insecticides and sex was significant at (P = 0.05), while the interaction of temperature and sex was not significant.

It may be concluded from the above results that low temperatures encouraged diapause, in both sexes, while the exposure to sublethal doses of insecticides significantly reduced the percentages of diapause, the percentages being more pronounced at lower temperatures and more reduction occurs in males than in females which explains the significant insecticide sex interaction. The interaction of temperature and sex or sex by itself are not significant in reducing diapause percentage.

b) Larvae in extended diapause :

The number of larvae at the end of six months was recorded at the three constant temperatures used for insecticides treated and untreated larvae in both sexes, results are presented in tables (8 & 9).

The untreated control showed that at the two low temperatures of 15 and 20°C, a substantial proportion of larvae persisted in their larval stage as diapausing larvae in males more than females, this proportion was never less than 50 % while at 27°C there were no diapausing larvae whether males or females.

In larvae treated with sublethal doses of insecticides, there were substantial reductions in the percentages of male larvae in extended diapause much more than females that indicates that male larvae of *P. gossypiella* were more susceptible to the latent effect of insecticides than females. In larvae treated with fenvalerate there was a 26 % reduction in diapausing larvae in females and 51.6 % reduction in that percentage among males at 15°C. At 20°C, the reduction was more or less the same in females and was 36.3 % in males, owing probably to a reduction in diapause among untreated males at that temperature.

When these figures were recorded for larvae treated with cypermethrin they were found to be more or less similar to those of fenvalerate with no difference at all in the percentage reduction in diapausing male larvae and a slight increase in the percentage of diapausing females at 20°C.

Among treatments with the organic phosphate, chlorpyrifos there were also reduction in the percentages of diapausing larvae at 15°C and 20°C. In females, the magnitude of this reduction was more or less the same at the two temperatures mentioned above.

In males, however, the reduction in diapausing larvae was almost double the reduction in females at 15°C and still about 10 % higher than females at 20°C.

In all cases and treatments mentioned above, there were no diapausing larvae at 27°C in males or females, among larvae treated with insecticides or control larvae.

When the findings mentioned above were reviewed it could be deduced that at the low temperatures of 15 and 20°C, the larvae of P. gossypiella may persist in their larval stage, thus entering what may be termed extended diapause, this does not occur at 27°C. Furthermore, the exposure to insecticides reduces the numbers of larvae going into that type of diapause substantially, the reduction being more apparent in male larvae than in females, the insecticides used in this experiment being with more or less equal effect regardless of their chemical structure, thus pointing to the possibility of the incidence of some physiological lesion occurring as a result of sublethal poisoning, which is probably not connected to the chemistry of the compound used or its biodegradation products. A similar trend was reported by Bell (1979) who found that infestation with a cytoplasmic polyhydrosis virus shortened diapause in P. gossypiella.

Such a statement of course is theoretical deduction, which requires further experimentation to prove or disprove.

Apart from the reason of the phenomena, statistical analysis indicated that the effect of temperature on extended diapause was significant at (P = 0.01) and the effect of insecticides on its reduction was also significant at the same level of probability. The interaction between sex and insecticides was significant at (P = 0.05), while the interaction between temperature and sex was not significant.

8. Total mortalities after 6 months :

The aim of insecticidal application in general is to reduce the population of the target insect species. It was thought of interest, therefore, to compare the total mortalities throughout the six months duration of the experiment. Figures in tables (5 & 7) gives the sum-total of mortalities throughout this period for males and females subjected to various treatments. In control treatments, where no insecticides were used, mortalities were slight at 15 and 20°C, and non-existent at 27°C. It was noticeable, however, that the percentage of mortalities among male larvae at the two low temperatures was higher than among females.

In larvae treated with fenvalerate, there were high percentages of mortalities at the three constant temperatures used and at the three temperatures, mortalities among male larvae were higher than females. The effect of the insecticide was most remarkable at 27°C where mortalities in males and females were 30 % and 45 %, respectively, compared with no mortalities in the control in both sexes. At 15°C, the mortalities in treated females was 61.7 % compared with 73.3 % in males, the same trend of higher mortalities among males may be noticed at 20 and 27°C.

Statistical analysis revealed that in treatments with fenvalerate, the raising of temperature significantly reduced mortalities (P = 0.01), the effect of sex was also significant at (P = 0.05), while the interaction between sex and temperature was not significant.

When figures for total mortalities of larvae treated with cypermethrin were inspected, it was remarkable that they were more or less similar to those of fenvalerate with the same trend favouring high mortalities in males compared with females, particularly at 27°C.

Statistical analysis showed that the effect of temperature was significant at (P = 0.01), sex significant at (P = 0.05) and the interaction of sex and temperature was not significant.

Chlorpyrifos treatments showed the highest mortalities at 27°C among the three insecticides tested. Otherwise, the mortalities at the two lower temperature gave somewhat lower mortalities than the other two toxicants and the difference between males and females were rather small. Statistical analysis showed a significant effect of temperature at ($P = 0.01$), while the effect of sex and the interaction of sex and temperature were not significant.

The incidence of what they termed as "latent mortalities" was reported by Metwaly _et al_. (1987) who reported that among four insecticides; chlorpyrifos, produced the largest percentage of latent mortalities among larvae of _P_. _gossypiella_ kept at 27°C.

It is worth mentioning that dead larvae at 15°C in insecticidal treatment were characterized by considerable shrivelling and a hard stoney texture.

IV. Influence of some allelochemics on the biology of the newly hatching larvae *P. gossypiella* (Saund.) :

Gossypol, a yellow polyphenolic pigment found in the pigment glands in plants of genus *Gossypium* has received considerable attention as a source of resistance in some cotton plants to the bollworm. Bottger *at al.* (1964) and Lukefahr *et al.* (1966) suggested that it might be possible to increase the resistance of cotton to insects by breeding a strain of cotton with a higher content of gossypol.

Coumarin, a rodenticide found in Tonka seeds as the odorous principle with no record of being tested against insects is another attractive compound to be tested against pink bollworm.

The present study was initiated to determine the influence of adding different concentrations of gossypol or coumarin to the artificial diet on the biology of *P. gossypiella*. These experiments were carried out under laboratory conditions at a constant temperature $27 \pm 1\,^{\circ}C$ and 80 ± 5 % R.H. The newly hatched (first instar) larvae were individually transfered to glass vials on the artificial diet which contained different concentrations of gossypol or coumarin compounds.

c - Larvae ensued from media containing coumarin.

d - Malformed pupae ensued from larvae reared on diets containing coumarin.

1. Effect on surviving percentages :

Results in table (10) pointed that the percentages of larvae able to survive and reach fourth instar when exposed to gossypol or coumarin in their artificial diet was remarkably reduced, the reduction being dose dependent.

Figure (5) shows log.dose probit mortality lines for these results calculated on the basis of failure to reach 4th instar according to Finney (1971). This analysis revealed that coumarin was far more toxic than gossypol; LC_{50} for gossypol was 0.057 % with a 95 % confidence limits of 0.044 % and 0.074 % and a slope of 1.546 while in coumarin the LC_{50} was 0.0125 % with a 95 % confidence limits of 0.0057 % and 0.0273 % the slope being 1.323. These figures indicated that as far as toxicity percent of the two compounds, coumarin was about 4.56 times more toxic than gossypol. The results of gossypol are in line with the findings of Shaver and Lukefahr (1969) who reported that gossypol was toxic to the pink bollworm. There are no reports on the toxicity of coumarin to insects to the knowledge of the author.

When an analysis of variance was carried for the figures of gossypol in the table (10), the differences between the treatment and check were found significant at (P = 0.01). L.S.D. values showed that the concentration

Table (10) : The effect of adding gossypol/coumarin to the artificial diet on the biology of *P. gossypiella* (Saunders).

Compound and concentration	Stages											
	Larvae				Pupae					Moths		% Larvae exceeding 30 days
	Larvae % developing to 4th	Duration period (days) Mean±S.E.	Weight (mg) on 10th day ♂ Mean±S.E.	Weight (mg) on 10th day ♀ Mean±S.E.	% Pupation	Pupal weight (mg) ♂ Mean±S.E.	Pupal weight (mg) ♀ Mean±S.E.	Malformed pupae %	Duration of pupal stage	Eclusion %	Malformation %	
Gossypol												
0.25	16	33.75±2.4	6.15±0.78	8.10±0.63	75	4.60±0.5	6.6±0.4	58	12.5±1.10	41.6	40.0	25.0
0.125	29	31.25±2.2	8.10±1.20	11.10±1.20	86	6.27±0.9	9.13±1.0	48	11.8±0.83	52.0	30.8	13.0
0.050	51	29.75±1.9	11.90±1.50	17.30±0.86	94	8.61±0.5	13.5±1.2	41	11.5±1.10	58.3	17.9	5.8
0.025	68	28.75±1.9	16.70±0.85	20.00±0.85	95	13.50±1.8	16.7±0.7	33.8	10.8±0.82	66.2	11.6	4.4
Check	99	17.50±1.1	22.80±2.10	31.90±1.20	99	17.90±2.2	23.3±2.2	1	7.5±1.1	98.9	0.0	0.0
L.S.D.												
(0.05)	1.7	3.436	2.38	1.72								
(0.01)	2.35	4.752	3.29	2.38								
Coumarin												
0.050	15	40.25±4.6	9.25±1.80	16.15±0.83	66	6.54±1.1	12.66±1.4	70	13.5±1.1	30.0	33.3	33.3
0.025	30	39.00±5.0	9.50±0.84	16.50±0.68	83	7.44±0.9	14.00±0.32	68	12.75±1.4	33.3	25.0	16.6
0.0125	56	28.75±2.3	11.70±1.90	18.90±0.85	92	9.14±1.6	16.00±1.4	51	12.50±1.1	48.0	20.0	7.0
0.00625	71	27.25±2.8	16.93±0.60	23.10±1.70	94	13.97±0.9	19.10±0.8	47.8	12.00±1.2	52.2	17.0	5.0
Check	98	17.75±1.9	20.69±0.96	30.67±1.20	100	15.83±1.2	21.38±1.4	2.1	6.50±1.1	97.9	0.0	0.0
L.S.D.												
(0.05)	2.382	6.163	2.35	1.94								
(0.01)	3.295	8.520	3.25	2.68								

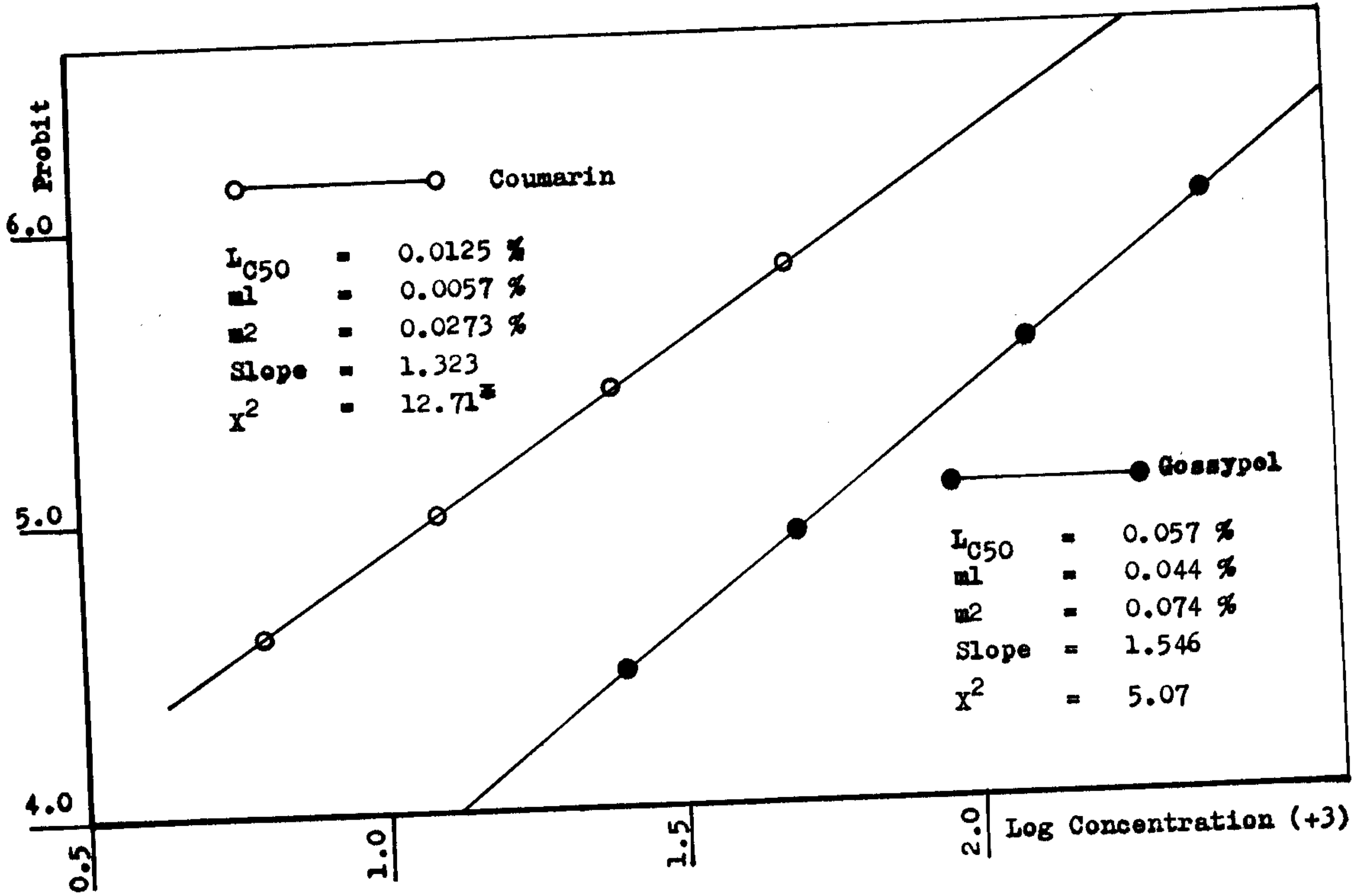


Fig (5) Log Concentration-probit mortality lines for P. gossypiella larvae reared on diets containing concentrations of Coumarin and Gossypol.

of 0.25 % significantly reduced the percentage of surviving larvae at (P = 0.01). Data for coumarin in figure (5) showed a highly significant difference between treatments and control at (P = 0.01) and L.S.D. figures indicated a highly significant level of significance in reducing survival of larvae for the concentration of 0.05 %.

2. Effect on larval duration :

The duration of larvae from 1st to 4th instar when exposed to gossypol are given in table (10). The figures indicated a prolongation of this period in comparison to control. This prolongation was found to be statistically significant by analysis of variance at (P = 0.01). The effect of concentration was not very pronounced, the highest concentration produced an increase of the larval period equal to 91.55 % of the same period in control, i.e. the period was nearly doubled. The next concentration caused a larval elongation period of 77.46 % compared with control. While the two other concentrations caused 69 % and 63.38 % increase, respectively. Accordingly, when the figures for L.S.D. were used, it revealed a highly significant difference between all the concentrations used and control, while the differences between the subsequent concentrations were not significant except between 0.250 % and 0.025 %, where the difference was significant at (P = 0.01).

In larvae exposed to coumarin, the same pattern was encountered, a statistically significant prolongation of this period occurred (P = 0.01). The magnitude of this prolongation was greater than that occurring with gossypol in the two high concentrations of 0.05 and 0.025, where this period was longer by 128.5 % and 119.72 % respectively, compared with the untreated control. In the lower concentrations, however, the prolongation was more or less similar to gossypol treatments. Calculations of L.S.D. revealed that the difference between the period produced by 0.05 % coumarin was significant at (P = 0.01) when compared to control, 0.0125 % and 0.00625 % concentrations. The difference between any of the concentrations used and control was also highly significant.

The above results indicated that the two compounds tested caused a slowing down of the rate of development of the larval stage of P. gossypiella. Other compounds were reported which produced similar results by Lukefahr and Martin (1966) who found that the incorporation of quercetin and rutin in the diet of the larvae of pink bollworm doubled the duration of the larval period.

3. Larval weight :

The survivals of larvae exposed to either of gossypol or coumarin were sexed on the 10th day of exposure and the average weights of males or females were determined.

Figures in table (10) revealed that in both sexes and in the two compounds investigated there was a remarkable reduction in weight, this reduction was proportional to the concentration gradient.

Slight differences, however, were noticed between the effects of the two compounds. In gossypol treatments the reduction in weight was larger than the reduction ensuing from coumarin treatments, and the percentage of reduction was comparable in males and females. In gossypol, the highest concentration used (0.250 %) produced 73.0 % and 74.6 % reduction in weight in males and females, respectively as compared with the respective controls. In the concentration of 0.125 %, the reduction in comparison to weights of control was 64.5 % and 62.2 % in males and females, respectively.

The percentage reduction was lowered to 47.8 % and 45.8 % in males and females, respectively, when the lower concentration of 0.05 % was used. In the lowest concentration used, however, i.e. 0.025 % gossypol in the diet, females suffered a higher reduction in weight, the reductions were 26.73 % for males compared with 37.3 % in females.

In coumarin treatments, there were substantial reductions in weight, but the pattern was different from that outlined above for gossypol in three aspects :

1. The reductions were more pronounced in males in comparison to females with a difference between ca. 8 % - 6 % barring the lowest concentration where the case was reversed.
2. The magnitude of reductions were less than those recorded for gossypol.
3. The relationship between dose and magnitude of reductions was less pronounced in coumarin in comparison to gossypol.

In order to verify the above inductions, analysis of variance was carried out. It revealed a significant difference between weights of larvae reared on gossypol or coumarin containing diets and control at ($P = 0.01$). figures for L.S.D. revealed a significant difference between any concentration and control of ($P = 0.01$) in both males and females compounds (Table, 11).

Between concentrations in gossypol, however, the differences were not always significant in males, the difference between 0.250 % and 0.725 % was not

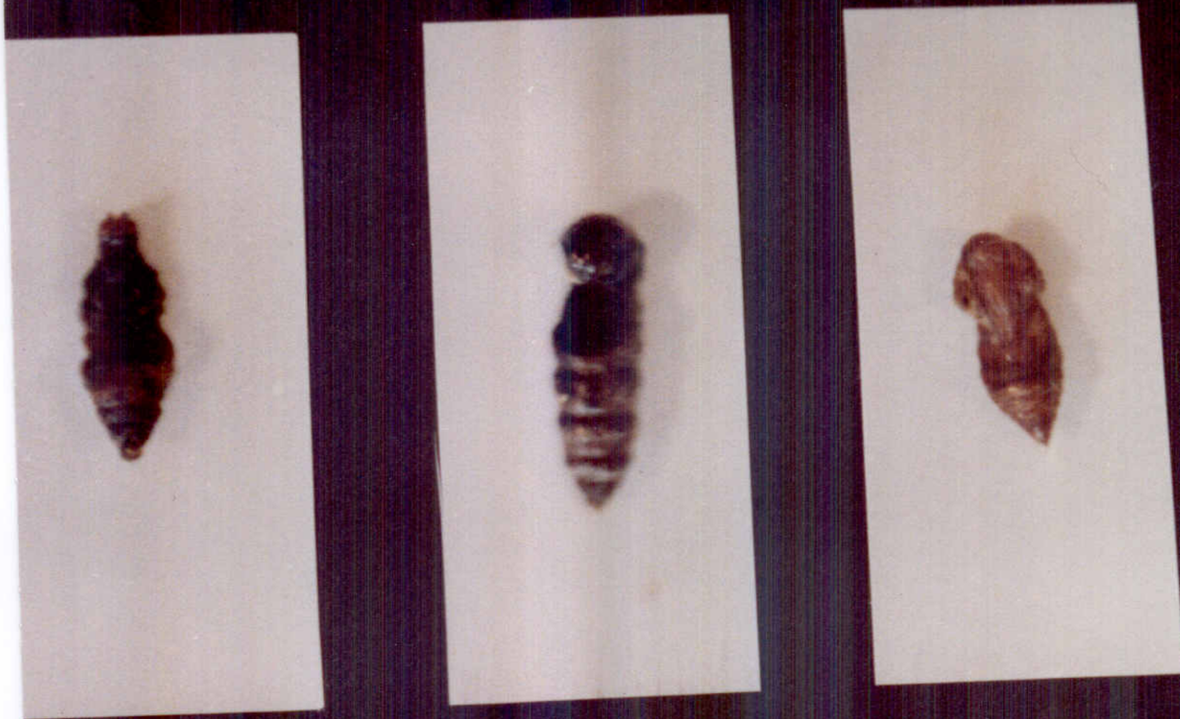
5. Pupal weight :

It was naturally expected that the reduction in weight among larvae exposed to concentrations of gossypol or coumarin in their diets would be reflected in the resultant pupae. Figures in table (10) confirmed this expectation, i.e. lighter larvae metamorphosed into lighter pupae. The percentage of reduction in weight compared with untreated control was more or less similar to the same percentage in larvae in both males and females exposed to gossypol, indicating the absence of additional effect of the compound on pupal weight.

In coumarin treatments at the smallest concentration used, i.e. 0.00625, however, the percentage reduction was less than that encountered among larvae, indicating some sort of recovery of the effect of the compound on weight at pupation. Otherwise the effects were percentage reduction in weight was similar to that of larvae.

6. Percentage of malformed pupae :

Pupae with incomplete thoracic sterna were considered malformed, particularly so since such pupae died without eclusion into adults. Naturally occurring malformed pupae usually form a small percentage under normal conditions. In the control of gossypol treatments, their



d - Malformed pupae ensued from larvae reared on diets containing coumarin.

e - Malformed moths ensued from larvea reared on diets containing coumarin.

proportion was 1 % and in the control of coumarin it was 2.1 %.

In larvae reared on either compound, the percentage of malformed pupae was higher. In larvae reared on gossypol containing diet their percentage ranged between 58 and 33.8. There was a concentration effect of small proportion, the percentage being slightly lowered by lowering the concentration in the rearing diet.

The effect of coumarin, however, was more pronounced in causing malformation among pupae. Initially the concentrations used were about five folds lower than those of gossypol, additionally, the percentages of malformations in pupae were always higher than gossypol. The effect of concentrations did not appear to be linear with response in production of malformed pupae.

7. Duration of pupal stage :

The duration of the pupal stage ensuing from larvae reared on gossypol and coumarin diets are given in table (10). In gossypol treatments, there was a clear elongation of this period when compared with control. The longest period was recorded for the concentration of 0.25 % when it reached 12.5 days. In lower concentrations this period decreased only slightly to become

10.8 days at the lowest concentration of 0.025 %, all that compared with an average of 7.5 days in the untreated control.

In coumarin treatments, the phenomenon was repeated on a some what higher scale, the highest concentration more than doubled the pupal period in comprising with untreated control, 13.5 days at the concentration of 0.05 % compared to 6.5 days in control. In lower concentrations, there were slight reductions in the elongation caused by the compound, but it did not correspond to the reductions in concentration.

The above results indicated that quantities of the two compounds (250 ppm) and (62.5 ppm) caused substantial increments in the length of pupal stage in comparison with untreated control.

8. Moths eclosion :

Table (10) showed the percentages of eclosion from pupae. The figures indicated that pupae formed from larvae reared on diets contaminated with either gossypol or coumarin showed appreciable percentages of failure to produce adults. In gossypol at its highest concentration 58.4 % of the pupae failed to develop adults while at the lowest concentration about one-third of the pupae showed similar failure, while in control 98.9 % of the pupae developed successfully to adults.

In coumarin treatments, the effect was more pronounced, at the highest concentration 70 % of the pupae failed to develop into adults, while at the lowest concentration the percentage failure was 47.8 %. In both compounds the effect of dose was fairly conspicuous.

9. Percentage of moth malformations :

Adults ensuing from the control of this experiment did not show any deformities. In adults formed from larvae exposed to either coumarin or gossypol, however, there was a persistent, dose dependent appearance of malformations which took the form of uncomplete eclusion and/or wing and/or leg deformities.

In all previous effects of the two compounds, coumarin was more potent than gossypol in this case, however, the first two concentrations of gossypol caused more deformities the two corresponding concentrations of coumarin, while the reverse was true in the two lower concentrations.

10. Percentage of larvae exceeding 30 days :

The percentages of surviving larvae which had been reared on the artificial diet containing gossypol or coumarin as a newly hatching of *P. gossypiella* (Saund.) were estimated on 30 day and according to Fife (1949) considered as in short diapause. Data in table (10) pointed that as the concentrations of gossypol or coumarin in diet increased the number of larvae which had been unable to complete their development to the pupal stage in 30 days increased. The range of percentages larvae in resting stage for gossypol treatments was (25 % - 4.4 %) against (0.0) in check. While for coumarin treatments, the percentage range was (33.3 % - 5 %) against (0.0) in check. These results indicated that the presence of these two compounds in larval diet has an important function in determining the percentage of larvae in resting stage. However, results in table (10) indicated that those larvae considered as resting larvae were slower in growth and development to pupal stage than in check, and the average of larval duration period never exceed (40.25 days).

since none of these larvae were able to enter into a long-term diapause which would enable the larvae to stay at rest up to the next season.

11. <u>The influence of gossypol or coumarin on fecundity and fertility of *Pectinophora gossypiella* (Saund.)</u>:

Adult of *P. gossypiella* ensuing from larvae reared on diet containing either gossypol or coumarin were allowed to copulate, lay eggs and eggs incubated till hatching. The average number of eggs laid per female, hatchability, mating percent, number of spermatophores per female and percentage of mating frequency in caged pairs of the pink bollworm are presented in table (11).

The incidence of mating was determined by the number of spermatophores in the bursa copulatrix of the adult female (Ouye *et al*., 1964 & 1965 and Graham *et al*., 1965). Results revealed that the average number of eggs laid by female moths reared on diets which contained gossypol or coumarin was reduced to a marked extent in comparison with control. This reduction was about 62 % of the number recorded for control, and was more or less equals for the two compounds. This reduction may be related to the size of the insect. Large females from check produced more eggs than the smaller ones from coumarin and gossypol treatments.

Analysis of variance revealed a significant difference at (P = 0.01) between treatments and control, L.S.D. values at (P = 0.01) indicated a highly significant

Table (11) : The influence of adding Gossypol/coumarin to the artificial diet on fecundity and fertility of *P. gossypiella* (Saunders).

Compound	No. of eggs/♀ Mean±S.E.	Hatching %	Mating %	No.of spermatophores per ♀ Mean±S.E.	(Range)	Percentage of mating frequency 0	1	2	3
Gossypol	50.0±15.3	67.2	87.5	1.28±0.08	(0-2)	12.5	47.5	40.0	0
Coumarin	50.2±21.6	68.2	80.0	1.38±0.16	(0-2)	20.0	22.5	57.5	0
Check	133.0±16.3	96.0	90.0	2.18±0.15	(0-3)	10.0	7.5	37.5	45
L.S.D. (0.05)	27.69			0.754					
(0.01)	39.39			1.084					

reduction in the number of eggs for gossypol treatments. Wellso and Adkisson (1961) observed that the effect of larval food on the reproductive capacity of the adult and one size of insect are apparently related factors. Large females generally produced more eggs than small ones. This suggests that the reproductive capacity of females populations from any given larval source is proportional to the average size of the individuals produced.

There are no reports given on the effect of coumarin on oviposition of insects, whereas for gossypol, Ahmed et al. (1980) reported that the presence of compound in the diets of adult houseflies did not cause any reduction in the number of eggs laid.

1. Hatchability :

The percentage of eggs that hatched from diets containing gossypol or coumarin is given in table (11) compared with 96 % hatching in control.

The figures indicated a reduction of ca. 28 % in both treatments in comparison to control, and the differences between the two diets was negligible.

These results agree with those of Ahmed et al. (1980) who reported a significant reduction in hatchability in housefly eggs from adults exposed to gossypol in their diet. From the present results it is apparent that coumarin showed similar effects, this indicated the probability that the lowering of hatchability may be a factor resulting from the weakened state of the adults resulting from larvae reared on contaminated diets.

2. <u>The effect of gossypol or coumarin on mating</u> :

Table (11) showed the percentage of mated females in moths developed from gossypol and coumarin treated larvae in contrast to untreated control. Figures indicated a slight insignificant decrease in percentages of mated females in the case of gossypol in comparison with control. The percentage was lower in coumarin treatments than both gossypol and control.

If the average number of spermatophores per female was taken as an indication of mating, it would become evident from table (11) that the normal average of spermatophores per female under the conditions of these experiments would be 2.18 with a range between 0-3, the figures recorded for the untreated control. The means for gossypol and coumarin were 1.28 and 1.38 spermatophores per female respectively with a range of 0-2 in both cases. This may be considered as an indication that while mating took place in all cases, more or less, its frequency was reduced, and analysis of variance revealed a significant difference at (P = 0.05) between treatments and control. L.S.D. calculation revealed a significant difference at (P = 0.05) between both treatments and control, while the difference between coumarin and gossypol treatments was not significant.

3. Percentage of mating frequency :

The percentage of mating frequency is given in table (11) as indicated by the number of spermatophores found in the bursa capulatrix of females. From the table, it is evident that there was a relatively high percentage of unmated females in the coumarin treatment, the percentage being double that of the control. This percentage, indicating unmated females, however, was insignificantly higher in gossypol treatments compared to control, these percentages were 10 % and 12.5 % for control and gossypol respectively.

In females mated once, i.e. with one spermatophore, the highest percentage occurred in the gossypol treatment 47.5 % and the lowest was in the untreated control 7.5 % with this percentage being inbetween the above cases in the treatment of coumarin.

The percentages of females mated twice showed a similar trend with less magnitude than that described for females mated once; both gossypol and coumarin showed higher percentages than the control, but the differences were smaller.

Females mated three times were non existent in gossypol or coumarin treatments, but constituted the highest percentage in untreated control. These results suggested that gossypol and coumarin excreted their effect on the frequency of mating rather than mating <u>per se</u>.

The above results indicated that breeding the newly hatched larvae of <u>P. gossypiella</u> on diet containing gossypol or coumarin resulted in the reduction of fecundity, fertility and mating ability of the moths which succeeded to emerge.

Abd El-Hafez <u>et al</u>. (1982) stated that irradiation caused an obvious reduction in fecundity, fertility and mating ability of the pink bollworm moths which emerged from irradiated 3-day-old pupae. This suggested that exposure of larvae to adverse conditions by irradiation or harmful xenochemics would give similar effects on the biotic potential of the resultant adults.

The obtained results indicated that gossypol or coumarin when incorporated into the diet of newly hatched larvae were toxic, toxicity being dose dependent.

It was noticed in the survivals which were able to form full-grown larvae from both gossypol or coumarin diets that their colour was pale yellow instead of pinkish as usual in normal larvae, in addition of the diminished size and weights in the treated larvae.

The duration of larval periods were approximately twice that in check. Though the percentage of pupation among survivals were not significantly different from control.

Malformations among pupae were high and appeared as incomplete formation the thoracic sterna, the malformed pupae were considered dead so that percentage of emerged moths were reduced much more than in check.

Emerged moths from treatments were smaller in size and with an appreciable percentage of malformations occurring as incomplete emergence and/or wing and leg deformities.

Percentage of larvae exceding 30 days for their development among larvae reared on artificial diet containing gossypol or coumarin were higher than control, but none of those larvae could enter into long diapause.

Pectinophora gossypiella adults ensuing from larvae reared on diet containing gossypol or coumarin had reduced fertility, fecundity and mating ability.

From the above results, it may be deduced that some xenochemics in the larval diet has an important function in determining growth,development, diapause and reproductive capacity of the pink bollworm. Lukefahr and Martin (1966) stated that gossypol caused high mortality and inhibited larval growth. Also they pointed that naturally occurring flavonoid compounds have some potential as a source of resistance in cotton to bollworm and the tobacco budworm.

Whereas for the effect of food quality on the reproductive capacity. Wellso and Adkisson (1961) found that the ovaries of females reared from the wheat germ diet may contain 550 or more eggs in various stages of development at the time the moth emerges from the pupal case. This number greatly exceeds the number of eggs that is generally deposited by an average female pink bollworm moth.

V. <u>Comparison between some body contents in different physiological cases of the 4th instar larvae of P. gossypiella (Saunders):</u>

These experiment were conducted under laboratory conditions in order to compare fresh; dry weights and water percent in different physiological cases of the fourth instar larvae of the pink bollworm. These cases investigated were diapausing; insuing of diapause larvae, abnormal active larvae; and larvae reared on a coumarin containing diet. Males and females of each case were used separately for the above mentioned determinations.

It was thought of interest to investigate the overall amino acids composition of the above mentioned different physiological cases of the 4th instar larvae of *P.gossypiella*.

1. <u>Fresh/dry weight and water percent</u> :

Data in table (12) showed that fresh weights in different physiological cases (diapausing, ensue of diapausing, active, abnormal and coumarin treated larvae) in general were for female much higher than male larvae.

Analysis of variance revealed that those differences were significant at ($P = 0.01$), L.S.D. value indicated that the diapausing larvae of both sexes were significantly heavier than active; abnormal and coumarin treated larvae in both sexes at ($P = 0.01$) for males and (P = 0.05) for females. The active females were significantly heavier than males of larvae treated with coumarin at (P = 0.05). The differences in female weight of active; abnormal and coumarin treated were not significant, also the differences in weight between abnormal males and active ones were not significant.

Data in table (12) pointed that dry weight for the different physiological cases mentioned were for female higher than male larvae. Statistical analysis gave the same trend of fresh weight values. The only exception was that the significant difference at (P = 0.05) of coumarin treated and active males, was not significant when the comparison was on dry weight bases, indicating that the difference was mainly due to water content.

Also data in table (12) showed that the percent of water content of the 4th instar larvae in different physiological cases were varied; these percentages for male and female larvae were lowest for diapausing and ensuing of diapause. In active, abnormal and coumarin treated larvae the percentages were rather high with the exception of coumarin treated males where moisture content was comparable to males ensuing of diapause. Brazzel and Newsom (1959) pointed out that boll weevils in diapause contained less water than reproductively active

Table (12) : Fresh/dry weights and water percentage of the fourth instar larvae of *P. gossypiella* (Saunders) at different physiological cases.

Source of food	Physiological cases	Date	Sex	Weight (mg) Fresh Mean±S.E.	Weight (mg) Dry Mean	Water %
Coumarin/ artificial diet	Treated	22.10.87	♂	6.80±2.82	2.18	67.9
			♀	15.72±5.41	3.25	79.3
Green bolls	Late season (Abnormal) non-diapause	26.10.87	♂	8.63±4.77	2.18	81.5
			♀	16.27±2.92	3.25	79.3
Dry bolls	Diapause	14.12.87	♂	31.13±1.50	11.26	63.8
			♀	43.38±4.97	14.62	66.3
	Ensue of diapause	19.4.88	♂	21.28±5.64	6.58	69.1
			♀	22.28±5.05	7.57	66.0
Flowers	Early season (active)	25.6.88	♂	12.66±2.65	2.87	77.4
			♀	15.76±1.99	4.08	71.9
L.S.D. : (0.05) / (0.01)			♂	4.94/6.69	2.12/2.87	
			♀	5.59/7.56	1.46/1.98	

ones. Decreased water content in diapausing insects has been reported for many species and seems to be a general phenomenon.

A point of interest is the similarity in fresh/dry weight and water content between the early season active larvae and the late season abnormal (non-diapausing) larvae. The lighter weight of those larvae is in agreement with Adkisson and Gaines (1960) who found that adult females reared from green bolls were 3-4 times heavier than individuals fed on squares. But the last season individuals form a constant phenomenon, their small weight in comparison to the majority of larvae about to enter diapause, alongside their inability to enter diapause require further studies and detailed investigations.

2. Amino acids :

The amino acids which were identified and quantified in the hydrolysates of larvae in different physiological conditions are given in table (13). It should be mentioned that the amounts expressed in this table represent the total amino acids in the bodies of larvae, by examining the figures it was noticed that :

Asparatic acid : The content of this acid was highest in diapausing males and females. The amount detected in active males was rather low, compared with active females, consequently the amount of this acid was higher in diapausing, abnormal and coumarin treated male larvae compared with active males.

Threonine : The amount of this acid in active females was nearly twice its amount in active males. In diapausing larvae the amount was equal in both sexes and was similar to that in active males. Results of abnormal larvae and larvae treated with coumarin were similar in both sexes and comparable to diapausing larvae. This acid was reported to be essential for the boll weevil by Vanderzant (1965).

Serine : The only noticeable figure in the estimates of this amino acid is the 2.6 folds titre of serine in active males compared with females.

In the three other cases; diapausing, abnormal and coumarin treated larvae there were only small variations.

Glutamic acid : There was a 3-4 folds increase in this acid in diapausing, abnormal and coumarin treated larvae in comparison with active ones.

A similar increase in this acid was reported in cases of infection of Popilia japonica with Bacillus *lentimorbus* by Shotwell *et al.* (1965). The increased amount of this amino acid may be due to an interference with the functions of GOT and GPT. That these enzyme may be affected by insecticides, the environment of insects was indicated by Abdel-Hafez (1985) and Belal *et al.* (1988). The figures for the amounts of this amino acid may be taken as an indication of the probability that chemicals such as coumarin, different physiological conditions such as diapause, and reduced growth may have their impact on these enzymes by partial inhibition, leading to the accumulation of the acid. The presence of the acid was in a lower titre than active or diapausing males and a noticeable reduction occurred in coumarin treated larvae.

Proline : Proline, though present conspicuously in active males, was undetectable in active females. In diapausing females, it was present, but in a lower titre than males. The same pattern was present, but in lower amounts in abnormal larvae. In larvae treated with coumarin, however, this amino acid was present equally in both sexes.

Proline and glutamate are closely tied up both in biosynthesis and catabolism (Rodwell, 1977), there is some balance in the patterns of both acids in the different physiological states.

Glycine : This amino acid was present in more or less equal quantities in active males and females, it was nearly doubled in diapausing larvae of both sexes. Its titre in abnormal larvae was equivalent to that in active larvae, and a slight rise occurred in coumarin treated larvae.

Alanine : A part from active females, the amounts detected of these amino acid were comparable in active males, diapausing, abnormal and coumarin treated male and female larvae. The titre of this amino acid was rather low in active females.

Valine : In active males there was more valine than in active females, the situation was reversed in diapausing larvae. In abnormal and coumarin treated larvae there was a marked reduction in the presence of this acid.

Isoleucine : There was about four times more isoleucine in females than males in active larvae, while a slight increase occurred in diapausing males over corresponding females. There was a marked reduction of this acid in abnormal larvae, while it was comparable to diapausing larvae in coumarin treatments.

Leucine : Leucine was higher in the active larvae than otherwise. The difference was negligible in diapausing larvae, while a noticeable drop occurred in abnormal larvae and the amount detected in coumarin treated larvae was nearly half that found in active normal larvae.

Valine, isoleuchine and leucine were reported essential amino acids in the boll weevil (Vanderzant, 1965). Their similarity of structure, routes of biosynthesis and catabolism of the carbon skeleton reactions (Harper et al., 1977), together with the more or less similar pattern of their presence in defferent physiolgical conditions reported above would deserve further detailed investigations.

Tyrosine : Males contained more tyrosine than females in the active condition. In diapausing males, the amount was reduced to be equal more or less to females. This amino acid was reported not essential in the boll weevil (Vanderzant, 1965), and is notable for its important metabolic reactions and products (Rodwell, 1977), its depletion in abnormal and coumarin treated larvae is worth of investigations.

Methionine : Methionine was reported to be an essential amino acid in boll weevils (Vanderzant, 1965) and is a well known essential amino acid in mammals. In active males, this amino acid was nine folds higher than in active females, but only 1.75 higher in diapausing males than females.

Phenylalanine and histidine : The differences of the titre of these two essential amino acids for the boll weevil (Vanderzant, 1965) were in all cases rather small and no large scale differences were detected.

Lysine : There was about double the amount of lysine in active males compared to females. The amount of this amino acid recorded a large increase in diapausing females, while in abnormal males and females, its amount was comparable to diapausing larae. There was marked reduction of this amino acid in larvae treated with coumarin. This acid was reported essential for boll weevil.

Argenine : Argenine, reported essential by (Vanderzant 1965) was present in a very high titre (4.5-folds) in active males compared with females. The amino acid was undetectable in diapausing larvae, present in small amounts in abnormal larvae while in coumarin treated larvae, it was present in comparatively high titre in both sexes.

Ammonia : Ammonia was found in trace quantities in all cases, it was found in comparatively large amounts in active males and in coumarin treated larvae.

Table (13) : Comparison of amino acids (%) in different physiological cases of the 4th instar larvae of *Pectinophora gossypiella* (Saunders).

	Amino acid	Active		Diapause		Abnormal		Treated	
		♂	♀	♂	♀	♂	♀	♂	♀
1	Asparatic	1.85	4.56	10.43	6.47	5.51	5.86	4.86	5.58
2	Threonine	2.40	4.23	2.90	2.94	2.57	2.68	1.97	2.11
3	Serine	2.98	7.85	5.02	3.59	3.69	4.80	3.17	3.75
4	Glutamic	3.22	1.52	11.10	9.12	7.20	8.13	10.40	11.10
5	Proline	7.20	*	5.95	3.46	3.11	1.77	5.19	5.32
6	Glycine	2.10	2.66	5.29	5.68	2.29	2.58	3.46	3.79
7	Alanine	3.56	0.31	3.75	2.86	3.10	3.17	4.46	4.40
8	Valine	5.21	3.10	3.02	5.78	1.10	1.37	1.69	1.85
9	Methionine	7.96	0.86	9.92	5.65	4.40	4.68	1.54	2.22
10	Isoleucine	1.49	5.52	5.41	4.20	2.93	2.04	4.50	4.76
11	Leusine	5.14	6.48	4.24	3.30	1.06	1.76	2.54	2.60
12	Tyrosine	10.03	6.03	5.13	6.29	2.55	2.43	4.65	2.90
13	Phenylalanine	3.26	5.23	3.72	3.69	3.10	3.06	3.78	2.90
14	Histidine	3.10	2.83	3.65	3.82	3.08	4.72	3.29	3.66
15	Lysine	9.90	5.90	9.15	12.15	8.36	10.82	5.56	5.32
16	Ammonia	1.60	0.70	0.08	0.17	0.52	0.60	1.26	1.68
17	Arginine	7.20	1.60	*	*	0.67	0.86	4.62	5.52

* : Undetected

The overall picture which appeared from these results, is that active males usually contained higher titres of amino acids than females. In diapausing larvae considerable increases were found compared with active larvae in the amino acids aspartic, glutamic, glycine and methionine. In females, there was an increase in lycine, proline and alanine, while in males there was an increase in serine and iso-leucine. If the figures were calculated for active and diapausing larvae of the amino acids detected, it will be noticed that in the active larvae males had 76.44 %. While females had only 58.68 %. In diapausing larvae, there were 88.65 % in males and 79.0 % in females.

It has been demonstrated that diapause is accompanied by loss of water (Brazzel and Newsom, 1959), and increase in weight. From the present results it appeared that this increase in weight could be partially accounted for in terms of an increase in amino acids, most probably bound in protein forms, though the possibility of an increase in free amino acids cannot be excluded.

These results are in line with those of Jeffery et al. (1974) who reported increase and alterations of ratios in amino acids between diapausing and active pink bollworm.

The other cases investigated included abnormal larvae, these larvae, in general contained a low titre of amino acids. There was a reduction in the valine, leucine and isoleucine content in these larvae compared to active samples. Tyrosine and argenine were also present in smaller amounts. In females, threonine and serine were present in lesser amounts while in males there was an increase in glutamic acid although not as high as that encountered in diapausing larvae. A slight increase was encounted in asparatic and particularly in males. In females there were some increament in the titres of histidine, lysine and methionine.

In larvae reared on a diet containing coumarin, there was an increase in asparatic acid, glutamic acid, glycine and alanine; in females an increase in methionine and argenine were noticed, while reductions were recorded in threonine, valine, leucine and tyrosine. In males there were reductions in proline, methionine and lycine, while in females reduction was in phenylalanine.

The present results revealed the need for detailed studies of the roles of protein synthesis and metabolism and some amino acids particularly glutamic and its metabolism in the induction and duration of diapause and reaction to adverse conditions.