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LOWER THRESHOLD TEMPERATURE AND THERMAL UNIT OF AMERICAN BOLLWORM, *Helicoverpa armigera* (HÜBNER) REARED ON PEA AND LETTUCE AND ITS REARING ON A NEW MODIFIED ARTIFICIAL DIETS

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ABSTRACT

The present work was conducted to study the relationship between developmental stages of the cotton bollworm, Helicoverpa armigera (Hübner) (ABW) reared in the laboratory on pea and lettuce and the required thermal units (DD's) at constant temperatures, 23, 27 and 30°C ± 1°C. Also, to evaluate the use of two new modified artificial diets (A and B) for mass rearing of ABW and compared with natural food (lettuce). Diets A and B contained dried active yeast, ascorbic acid, sorbic acid, methyl parahydroxy benzoate, liquid milk, vitamin mixture and formaldehyde 34 - 38%. In addition to the essential ingredient, in diet A is kidney beans and wheat grated while, diet B is chickpea and wheat grated.

***Results showed that** there was variation in developmental periods of the ABW in different stages reared on pea and lettuce. Temperature increasing led to an increase in the developmental rate and reduce the development periods of the different stages. The generation period of the ABW reared on lettuce was shorter (32.23 days) than that reared on pea (34.70 days). The lower developmental temperatures were 11.60 and 11.39 °C for the generation and the thermal units required were 467.13 and 511.26 DD's, respectively.*

On the other hand, results showed that highest larval and pupal weight, adult emergence and number of eggs/female (560 eggs/female) and lowest larval and pupal mortality percentage of ABW were observed on diet B followed by diet A and lastly natural food (lettuce).

***In conclusion,** the two new modified artificial diets (A and B) developed in this study has proved to be satisfactory for mass rearing of ABW, Helicoverpa armigera (Hübner).*

Key words: lower threshold temperature & thermal unit, American bollworm, *Helicoverpa armigera*, pea & lettuce, new modified artificial diets.

INTRODUCTION

The cotton bollworm, *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae), is an extremely polyphagous species and a common insect pest of many crops in Egypt (Nada *et al.*, 2004) and many parts of the world (Singh and Mullick, 1997, Reddy *et al.*, 2004). It is causing extensive damage on crops such as cotton, okra and tomato (Sharma, 2001). The thermal requirements (degree-days) of development was often used for estimating developmental times because temperature has a major effect in determining the rate which insects develop (Howe, 1967 and Zaslavski, 1988), the rates of development under natural conditions was largely determined by temperature. Zero developmental temperature (t_0) of *Spodoptera littoralis* (Boisd.) differed according to the host plant fed (Al-shannaf and El-Sayed, 2010). Jallow and Matsura (2001) studied the effect of temperature on the rate of development for *H. armigera* reared on tomato. The duration of different life history stages decreased as temperature increased from 13.3 to 32.5 $^{\circ}$C. A development threshold of 10.5, 11.3 and 13.8 $^{\circ}$C was estimated for the eggs, larvae and pupal stages, respectively. Amer *et al*. (2009) indicated that the lower thermal threshold for generation of ABW was 11.54 °C and the thermal constant was 494.39 DD's

The present work was conducted to calculated lower threshold temperature and thermal unit of ABW reared on pea and lettuce and evaluate of two modified artificial diets for mass rearing of ABW as compared with natural food (lettuce) .

MATERIALS AND METHODS

Insect rearing

One hundred ABW larvae were collected from pea fields in Daqahlia Governorate, Egypt during November of 2014 and brought to the laboratory. Fifteen larvae were fed separately on pea pods, *Pisum sativum* and fifteen larvae were fed separately on lettuce, *Lactuca sativa* in glass tubes (2.5 x 7 cm) covered with absorbent cotton wool. The food was daily renewed until pupation. Pupae were transferred to clean glass tubes and incubated until moth emergence. When adults emerged, moths were sexed and caged till egg laying. Moths were provided with 10% honey solution on a cotton swab. The eggs were separated daily in each previous treatment and placed in glass jars.

The hatched larvae in each previous treatment were transferred individually to glass tubes (2.5 x 7 cm) containing same previous food in each treatment (pea pods or lettuce), each tubes were plugged tightly with absorbent cotton and placed in an incubator at constant temperature of 23,

27 & 30 °C and 65 ± 5 % R.H and light period 14 light:10 dark. Four replicates of 25 larvae/each treatment were used for each tested temperature degree. Larvae were examined daily until pupation to record larval duration and mortality percentage. Pupae were transferred to clean glass jars (1/2 kg) and examined daily until moth emergence to record pupal duration. When adults emerged, moths were sexed and caged to egg laying. Three replicates of five pairs/each treatment were used for each tested temperature degree. Moths were provided with 10 % honey solution. The cages were inspected daily until moth death. The pre-oviposition, oviposition and post-oviposition periods and longevity of adult females and males were calculated.

Eggs laid on the same day (> 24 old) in each tested temperature in each treatment were placed in glass jar and incubated under same constant temperatures (23, 27 & 30 °C) and 65 ± 5 % R.H. Three replicates of 100 eggs/each treatment were used for each tested temperature degree. Incubation period in each tested temperature degree in each treatment was calculated.

Modified artificial diets

Two diets used in this work for rearing ABW (Table 1) were modified from that of Amer (2015). The modifications as follow : Kidney beans, *Phaseolus vulgaris* replaced chickpea, *Cicer arietinum.* Also, the quantities of wheat grated, ascorbic acid and methyl – p -hydroxy benzoat, are different from those used by Amer (2015).

The two new modified artificial diets (A and B) tested for rearing ABW in laboratory and compared with natural food (lettuce) at 27 °C and 65 ± 5 % R.H and 14 L:10 D. The preparation of two modified artificial diets were similar to that described by (Amer, 2015) the rearing technique of the insect on both diets and natural food (lettuce) were similar to that described by (Amer and El-Sayed, 2014).

Statistical analysis

The effect of host plants, artifical diet and temperatures degrees on developmental time of ABW was determined by analysis of variance (ANOVA). The relationship between temperature and mean developmental rate of each stage and generation under tested temperature was determined using liner regression. For tested temperature degree in each treatment, developmental rate (DR) was calculated as reciprocals of development time (D) for each stage (DR= 1/D). The relation between developmental rate and temperature (T) was determined using liner regression equation: DR= a + bT, whereas: a and b parameters of the liner regression. The lower developmental threshold (t0) was determined: t0 = -a/b. On the other hand,

Table 1: Component of the tested diets for rearing ABW.

Ingredients	Diet A	Diet B
Kidney beans (g)	-	250.00
Wheat grated (g)	110.00	125.00
Chickpea (g)	215.00	-
Dry active yeast (g)	49.00	49.00
Ascorbic acid (g)	5.00	5.00
Sorbic acid (g)	2.25	2.25
Methyl parahydroxy benzoate (g)	2.25	2.25
Liquid milk (ml)	100.0	100.00
A mixture of vitamins(ml)	8.00	8.00
Formaldehyde 34-38%(ml)	2.50	2.50

thermal units for completion development of each stage was calculated according to Arnold (1959):

$$\text{Thermal units (DD's)} = D*(T-t0)$$

Where, D- development time of a given stage, T- temperature in degree centigrade and t0- lower developmental threshold.

RESULTS AND DISCUSSION

Duration of different stages:

Data in Table (2) showed the duration periods of ABW different stages reared in the laboratory on pea and lettuce at three constant temperatures 23, 27 and 30°C.

Data revealed that the embryonic development periods of ABW reared on pea were 3.3, 2.3 and 2.0 days, whereas it were 3.2, 2.3 and 1.9 days for that reared on lettuce at 23, 27 and 30 °C, respectively. The averages of incubation periods for pea and lettuce were 2.53 and 2.47 days, respectively. Larval durations was shorter, when the larvae reared on lettuce (19.0, 14.5 and 12.0 days) and prolonged to 20.5, 16.0 and 13.0 days at 23, 27 and 30 °C, respectively. The averages of larval duration were 16.5 and 15.17 days on pea and lettuce, respectively. The pupal duration of the ABW were 17.0, 12.0 and 10.5 days, while it was 15.5, 11.0 and 9.5 days for pea and lettuce

Table 2 : Duration of different stages of the American bollworm reared on pea and lettuce at constant temperatures in the laboratory.

Host plants Stage	Pea				Lettuce			
	23°C	**27°C**	**30°C**	**Average**	**23°C**	**27°C**	**30°C**	**Average**
Egg	3.30	2.30	2.00	2.53	3.20	2.30	1.90	2.47
Larva	20.50	16.00	13.00	16.50	19.00	14.5	12.00	15.17
Pupa	17.00	12.00	10.50	13.17	15.50	11.00	9.50	12.00
Pre-ovipostion	3.30	2.20	2.00	2.50	3.30	2.50	2.00	2.60
Ovipostion	9.00	7.50	4.50	7.00	9.50	8.00	4.00	7.17
Post-ovipostion	3.50	2.00	1.70	2.40	3.00	2.00	1.50	2.17
Adult female longevity	15.80	11.70	8.20	11.90	15.80	12.50	7.50	11.93
Generation	44.10	32.50	27.5	34.70	41.00	30.30	25.40	32.23

at 23, 27 and 30 °C, respectively. The averages of pupal duration were 13.17and 12.0 days on pea and lettuce, respectively. The pre-ovipostion periods were 3.3, 2.2 and 2.0 days, whereas it were 3.3, 2.5 and 2.0 days on pea and lettuce at 23, 27 and 30 °C, respectively. Regarding generation period, egg to egg, data indicated that ABW, duration took the longest period on pea and shortest on lettuce with 34.70 and 32.23 days, respectively.

Rate of development:

The ABW rate of development was differed on pea than on lettuce. The developmental rate was increased as temperature increased for pea and lettuce. The average of developmental rate for the ABW generation period on pea was 0.029 while it was 0.031 on lettuce Table 3.

These results are agree with the finding of Vojtech *et al.* (2002), thy reported that the developmental rate help to better understand insect evolution and predict insect population growth rates. Amer *et al.* (2009)

Table (3): Development rate of different stages of the American bollworm reared on pea and lettuce at constant temperatures in the laboratory.

Host plants	Pea				Lettuce			
Stage	23	27	30	Average	23	27	30	Average
Egg	0.303	0.435	0.500	0.395	0.313	0.435	0.526	0.405
Larva	0.488	0.063	0.077	0.061	0.053	0.069	0.083	0.066
Pupa	0.059	0.083	0.095	0.076	0.065	0.091	0.105	0.083
Pre-ovipostion	0.303	0.435	0.500	0.395	0.303	0.400	0.500	0.385
Generation	0.023	0.031	0.036	0.029	0.024	0.033	0.039	0.031

reported that, increasing rearing temperature accelerated the developmental rate and shorted the periods which required completing different stages.

Lower developmental threshold

Data in Table (4) showed the values of lower developmental threshold temperature of ABW different stages reared on pea and lettuce.

The average of lower developmental threshold for ABW different stages ABW reared on pea were 12.14, 10.94, 11.60, 12.14 and 11.39°C for egg, larvae, pupae, pre-oviposition period and generation, respectively, while on lettuce it were 12.77, 11.03, 11.85, 12.31and 11.6 °C for the previous stages, respectively.

Thermal units requirement

Data in Table (5) showed that the thermal units of ABW different stages reared on pea and lettuce. The average thermal units required for egg development till hatching were 35.25 and 32.74 DD's t, for ABW reared on pea and lettuce, respectively. The averages of the thermal units required for larval complete development were 250.66 and 228.88 DD's, respectively. The averages of the thermal units required for pupal development until moths emergence were 190.60 and 170.64 DD's, respectively. While, for pre-oviposition period the averages of the thermal units required were 34.75 and 35.80 DD's, respectively.

Table (4): Lower threshold temperature for different stages of the American bollworm reared on pea and lettuce.

Host plants	Pea			Lettuce		
Stage	Regression equation		Lower threshold temperature (°C)	Regression equation		Lower threshold temperature (°C)
	a	B		a	b	
Egg	-0.345	0.028	12.14	-0.390	0.031	12.77
Larva	-0.044	0.004	10.94	-0.048	0.004	11.03
Pupa	-0.061	0.005	11.60	-0.061	0.006	11.85
Pre-ovipostion	-0.345	0.028	12.14	-0.344	0.028	12.31
Generation	-0.022	0.002	11.39	-0.025	0.002	11.60

Table (5): Thermal unit for different stages of the American bollworm reared on pea and lettuce.

Host plants Stage	Pea				Lettuce			
	23°C	27°C	30°C	Average	23°C	27°C	30°C	Average
Egg	35.84	34.18	35.72	35.25	32.73	32.73	32.74	32.74
Larva	247.23	256.96	247.78	250.66	227.43	231.57	227.64	228.88
Pupa	193.80	184.80	193.20	190.60	172.83	166.65	172.43	170.64
Pre-ovipostion	35.84	32.69	35.72	34.75	35.28	36.73	35.38	35.80
Generation	512.00	507.33	511.78	510.37	467.40	466.62	467.36	467.13

The averages of thermal units required to complete generation period were 510.37 and 467.13 DD's for pea and lettuce, respectively. Clement (1992) reported that the required thermal units is constant for each strain of organism. Jallow and Matsura(2001) studied the effect of temperature on the rate of development for *H. armigera* reared on tomato. The duration of different life history stages decreased as temperature increased from 13.3 to 32.5 °C. A development threshold of 10.5, 11.3 and 13.8 °C was estimated for the eggs, larvae and pupal stages, respectively . Amer *et al*. (2009) indicated

that the thermal constant for generation of ABW was 494.39 DD's. Zero developmental temperature (t_0) of *Spodoptera littoralis* (Boisd.) differed according to the host plant fed(Al-Shannaf and El-Sayed, 2010) .

Biological aspects of American bollworm reared on lettuce (Control) and tested diets:

Larval stage:

The results of the effect of tested diets on developmental time of larval stage of the ABW are shown in Table 6. The mean larval durations were non-significantly affected by diets A & B as compared with control diet (Lettuce). The longest larval duration (16.00 days) was on diet B and shortest (14.50 days) on Lettuce, whereas 14.5 days on diet A, non-significantly differences were found between larval weight when fed on tested diets and Lettuce. The larvae fed on the diet B gave the highest larval weight (0.500 g) while those fed on Lettuce gave the lowest weight (0.420 g). Highly significant differences were found between the mortality percentages for tested diets and Lettuce. The mortality percentages of larval stage were 12.00, 8.00 and 20.00 % for diets A, B and Lettuce, respectively.

Pupal stage:

Data presented in Table (6) showed that the pupal duration was shorter when the larvae fed on lettuce (11.00 days) and prolonged to 12.00 and 11.50 days on diets A & B, respectively. The lowest weight per pupae (0.300 g) was recorded on lettuce. The highest Pupal weight (0.360 g.) was observed on diet B. Pupal weight on diet A was 0.340 g.. Tested diets have highly significant effect on the pupation percentage as compared with lettuce. The pupation percentages were 88.00, 92.00 and 80.00 % for diets A, B and lettuce, respectively. Significant differences were found between pupal mortality percentages resulted from larvae fed on tested diets and lettuce. The highest percentage of pupal mortality (15.00 %) was observed on lettuce, while the lowest percentage of pupal mortality (9.78 %) was obtained on diet B, whereas diets A showed intermediate effect on percentage of pupal mortality.

Adult emergence:

Data given in Table (7) showed that the percentages of adult emergence were 89.78, 90.22 and 85.00 % for diets A, B and lettuce, respectively.

Adult female longevity:

The pre-oviposition and post-oviposition periods were significantly different among the tested diets and lettuce but the oviposition period was

Table 6: Biological aspects of immature stages of American bollworm reared on lettuce (control) and tested diets.

Foods	Larval duration	Larval mortality	Larval weight	Pupation %	Pupal duration	Pupal weight	Pupal mortality
Lettuce	14.50	20.00a	0.420	80.00b	11.00	0.300b	15.00a
Diet (A)	15.50	12.00b	0.480	88.00a	12.00	0.340a	10.22b
Diet (B)	16.00	8.00c	0.500	92.00a	11.50	0.360a	9.78b
P	NS	***	NS	**	NS	*	**
LSD 0.05		1.997		5.994		0.039	2.825

NS : Non-significant *:Significant **:Highly significant

Table 7: Adult longevity and reproductive potential of American bollworm reared on lettuce (control) and tested diets.

Foods	Adult emergenc	Pre-oviposition	Oviposition	Post-oviposition	Female longevity	Male longevity	No. of egg /female	Hatchability %
Lettuce	85.00	2.500a	8.00	2.50a	13.00	10.0	450.00b	84.00
Diet (A)	89.78	2.33a	8.00	1.50c	11.83	9.50	525.00a	86.00
Diet (B)	90.22	2.00b	8.50	2.00b	12.50	10.50	560.00a	88.00
P	NS	**	NS	**	NS	NS	**	NS
LSD 0.05		0.023		0.475			50.08	

NS : non-significant *:Significant **:highly significant

non - significant by the tested diets. The oviposition periods were 8.00, 8.50 and 8.00 days for diets A, B and lettuce, respectively. The female longevity

of the ABW moths were 11.83, 12.50 and 13.00 days on diets A, B and lettuce, respectively Table 7.

Eggs laying:

Data given in Table (7) showed that the highest number of eggs per female was 560.00 eggs on diet B, followed by 525.00 eggs on diet A and the lowest number was 450.00 eggs on lettuce. The hatchability percentages were 88.00 , 86.00, and 84.0 % for diets A, B and Lettuce, respectively.

In conclusion, the two new modified artificial diets (A and B) developed in this study has proved to be satisfactory for mass rearing of ABW, *Helicoverpa armigera* (Hübner).

REFERENCES

Al-Shannaf and El-Sayed (2010): Effect of host plants on the required heat units of *Spodoptera littoralis* **(**Boisd**.)** (Lepidoptera: Noctuidae). *Bull. Ent. Soc. Egypt*, (87):79-88

Amer, A.E.A.(2015): Economic artificial diets for rearing spiny bollworm, *Earias insulana* (Boisd.) (Lepidoptera: Noctuidae*). J. Plant. Prot. and Path., Mansoura Univ*., 6 (3): 527-534.

Amer, A.E.A.; A.A.A. El-Sayed (2014): Effect of different host plants and artificial diet on, *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae*). J. Entomol.*, 11(5):299-305.

Amer, A.E.A.; A.A.A. El-Sayed and M.A. Nada (2009): Development of american bollworm, *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) in relation to heat unit requirement. *Egypt. J. Agric. Res.*, 87 (3):667-674.

Arnold, C.Y. (1959): The determination and significance of the base temperature in a linear heat unit system. *In Proc. Am. Soc. Hortic. Sci.*, 74:430-445.

Clement, A. N. (1992*):* *The Biology Of Mosquitoes*. Chapman & Hall, London.

Howe, R.W. (1967): Temperature effects on embryonic development in insect. *Ann. Rev. Entomol*., 10:15-42.

Jallow, M.F.A. and M. Matsura (2001): Influence of temperature on the rate of development of *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae). *Appl. Entomol. Zool*., 36(4):427-430.

Nada, M. A., M. G. Ragab and E. S. Mansour (2004): Seasonal abundance and prediction possibility of American bollworm, *Helicoverpa armigera* (Hub.) in relation to pheromone trap catches and heat unit accumulated. *Egypt. J. Appl. Sci*., 19 (11):294-409.

Reddy, K. S.; G. R. Rao; P. A. Rao and P. Rajasekhar (2004): Life table studies of the Capitulum Borer, *Helicoverpa armigera* (Hübner) Infesting Sunflower. *J. Entomol. Res.,* 28: 13-18.

Sharma, H. C. (2001): Cotton bollworm / legume pod borer, *Helicoverpa armigera* (Hubner) (Lepidoptera : Noctuidae) biology and management. *Indian. Entomol*., 59:209-214.

Singh, A. K. and S. Mullick (1997): Effect of leguminous plants on the growth and development of gram pod borer, *Helicoverpa armigera* (Hübner). *Ann. Plant Prot. Sci.,* 17:229-282

Vojtech J.; A. Honek and Anthony F.G. Dixon (2002): Developmental rate isomorphy in insects and mites. *The American Naturalist,* 160(4):497-510.

Zaslavski, V.A. (1988*):* *Insect Development: Photoperiodic And Temperature Control.* Springer- Verlag, Berlin, N.Y. :187 pp.

صفر النمو والوحدات الحرارية لدودة اللوز الأمريكية المرباه على البسلة والخس وتربيتها على بيئات صناعية جديدة معدلة

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تم دراسة العلاقة بين نمو الأطوار المختلفة لدودة اللوز الأمريكية المرباة على البسلة والخس والوحدات الحرارية المتجمعة اللازمة على درجات حرارة ثابتة وهي ٢٣ و ٢٧ و ٣٠ ± ١ درجة مئوية وفترة ضوئية ١٤ ضوء : ١٠ اظلام . وتقييم استخدام بيئتين صناعيتين معدلتين لتربية دودة اللوز الأمريكية مقارنه بتربيتها على عائل طبيعى (الخس) . هذه البيئات **أ ، ب** تحتوي علي خميره جافه نشطه ، أسكوربيك أسيد ، سوربيك أسيد ، ميثيل باراهيدروكسي بنزوات ، لبن سائل ، مخلوط من الفيتامينات وفورمالدهيد ٣٤- ٣٨ %. بالاضافه الي ان المادة الاساسيه في البيئه أ الفاصوليا والقمح المبشور وفي البيئه **ب** االحمص والقمح المبشور .

أظهرت النتائج أن هناك تفاوت في فترات النمو اللازمة لاستكمال مختلف مراحل النمو من البيض حتى الحشرة الكاملة لدودة اللوز الأمريكية المرباة على البسلة والخس ، حيث أدت زيادة درجة الحرارة إلى زيادة معدل النمو وتقليص فترات الأطوار المختلفة ، مما أدي إلي قصر مدة الجيل لدودة اللوز الأمريكية المرباة على البسلة والخس . و قصرت مدة الجيل لدودة اللوز الأمريكية المرباة على الخس (٣٢.٢٣يوم) عن تلك المرباة على البسلة (٣٤.٧٣يوم) . و كانت درجة حرارة صفر النمو لمدة الجيل ١١.٦٠ درجة مئوية للمرباة على البسلة و ١١.٣٩ درجة مئوية للمرباة على الخس. و بحساب الوحدات الحرارية اللازمة لمدة الجيل وجد أنها ٤٦٧.١٣ و ٥١١.٢٦ وحده حرارية للمرباة على البسلة والخس على الترتيب . على الجانب الآخر أظهرت النتائج ارتفاع وزن اليرقات والعذارى و خروج الفراشات وعدد البيض الموضوع / أنثى (٥٦٠بيضة/أنثى) وأقل نسب موت يرقات وعذارى لدودة اللوز الأمريكية شوهدت على البيئة أ تلاها على البيئة ب ثم الغذاء الطبيعى (الخس).

التوصية: من نتائج تلك الدر اسه ينصح باستخدام البيئتين الصناعيتين المعدلتين المستخدمتين في هذه الدراسة (أ ، ب) للتربية الموسعة لدودة اللوز الأمريكية.