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The development of tobacco budworm, ***Heliothis virescens*** (F.) (Lepidoptera: Noctuidae) on different artificial diets*

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Summary

The development of ***Heliothis virescens*** was investigated on four artificial diets. Studies were conducted at 23°C and 28°C. Larval survival is high on all diets at 23°C but pupal survival is lower on diet I (Casein-wheat germ diet). At 28°C, larval and pupal development is similar on all four diets and the adult emergence is between 26.2 and 27.5 days. On the other hand, at 23°C pupation and emergence time was delayed by 2 days on diet IV (Wheat germ diet). The aim of this study was to investigate the effect of different diets on the development of ***H. virescens.*** Besides, The role of agar and wheatgerm on development of insects is briefly discussed.

Key words: Tobacco budworm, artificial diets, rearing, larvae
Anahtar sözcükler: ***Heliothis virescens***, suni besin, larva gelişimi

Introduction

There has been a great deal of progress in rearing insects in the laboratory on artificial diets in the past 20-30 years. The advantage of artificial diets is that rearing is generally easier; with large numbers of insects being reared economically and simultaneously in a limited space. Insects often can be reared all the year round irrespective of their

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natural food source. Much attention has been directed toward developing techniques for producing high quality insects in large numbers (King and Leppla, 1984).

Several diets, many only slightly modified from that reported by Vanderzant et al. (1962), have been developed for the tobacco budworm, ***Heliothis virescens*** (F.) (Lepidoptera: Noctuidae). One of the most important advances in rearing ***Heliothis*** spp. in the laboratory was the use of wheat germ in the diet (Berger, 1963). Patana (1969) used baby lima beans in the bean diet for rearing several Lepidoptera, including ***H. virescens*** and reported larvae fed more readily on this diet.

Techniques for rearing large numbers of ***Heliothis*** spp. were developed by Raulston and Linger (1972). Subsequent modifications were made by Hartley et al. (1982). A comprehensive review of laboratory propagation of ***Heliothis*** spp. (Raulston and King, 1984) includes information on diet preparation, rearing equipment, techniques and quality control. King and Hartley (1985) developed an artificial soybean flour/wheat germ diet with a high agar content.

The purpose of the research was to investigate the effect of different diets on the development of ***H. virescens.***

Materials and Methods

The ingredients of the diets discussed here in are shown in table 1, 2, 3 with the ingredients reported by Poitout and Bues (1970 - Diet I), Brazzel (1970 - Diet II), U. Bielefeld (Personal communication in ICI Reading - Diet III) and S. Smyth (Personal communication in ICI Reading - Diet IV). Deionised water was used in diet III and IV instead of the distilled water in diet I and II. Furthermore, Alfalfa powder was used instead of cabbage leaf in diet I.

Preparation of diet I (Table 1):

The dietary ingredients were prepared in the following maner. Dry ingredients for the media were weighed separately. Agar and benzoic acid were stirred in the required amount of cold water, heated to boiling point on hot plate and cooled to 56°C. The mixture was then transferred to a waring blender. The remainder of the dry ingredients were then added singly, with 2-3 minutes mixing for each. After blending, the warm mixture was poured into stainless-stell tray 10 x 22 cm, a depth of 3 cm and allowed to set.

Preparation of diet II (Table 1):

Agar was dissolved in the cold water and boiled on hot plate. This mixture was placed in a waring blender. The other dry ingredients were added and blended for 2 minutes. Sorbic acid was dissolved in ethanol. The liquid ingredients were then pipetted into the mixture and blended for 2 minutes. After blending, the hot mixture was dispensed into stainless - steel trays and allowed to solidify.

Table 1. Composition of diet I (Poitout and Bues, 1970) and II (Brazzel, 1970) used to determine the development of ***Heliothis virescens.***

Ingredients	Diet I	Diet II
Agar	5.0 g	25 g
Casein	7.0 g	35 g
Glucose	7.0 g	-
Sucrose	-	35 g
Nipagine	0.2 g	1.5 g
Ascorbic acid	1.0 g	4.0 g
Wheat germ	-	30 g
Cellulose	6.0 g	-
Alfalfa (powder)	10.0 g	-
Chlosterol	0.6 g	-
Wesson salts	2.0 g	-
Brewers yeast	6.0 g	-
Benzoic acid	0.25 g	-
Sorbic acid	-	1.0 g
Choline chloride	-	1.0 g
Alphacel	-	5.0 g
Formaldehyde % 40	-	1.0 ml
Vanderzant Vit. mix.	4.0 g	15 ml
Water	170 ml	850 ml

Preparation of diet III (Table 2):

The diet was prepared in the following manner. Group I- components were boiled. Group II- dry ingredients were first ground to a fine powder in a waring blender. Group III- deionised water was placed in the Waring blender. Wheat germ oil, linseed oil, formaldehyde, potassium hydroxide, group IV, were separately pipetted into blender bowl. Then the dry ingredients were added and blended on setting 1 for 3 minutes. The group I vanderzant's vitamin mixture and ascorbic acid were dissolved in the water. This solution was mixed well and added to blender bowl. Finally, the mixture was blended on setting 2 for 5 minutes. After a final mixing, the complete diet was poured into the trays.

Table 2. Composition of diet III (U. Bielefeld, Pers. com.) used to determine the development of ***Heliothis virescens.***

Ingredients (for 188 mini pots)			
Group I		Group III	
Deionised water	1000 ml	Deionised water	400 ml
Agar	40 g		
Sucrose	68 g		
Group II		Group IV	
Casein	63 g	Wheat germ oil	8 ml
Wheat germ	88 g	Linseed oil	2 ml
Brewers yeast	30 g	Formaldehyde	3 ml
Alphacel	12 g	KOH % 45 sol.	4 ml
Sorbic acid	3 g	Group V	
Methyl paraben	4 g	Vanderzant Vit. mix.	6 g
Wesson salts	18 g	Ascorbic acid	10 g
Alfalfa (powder)	21 g	Deionised water	100 ml

Preparation of diet IV (Table 3):

Firstly, all the harricot beans were ground. Agar was weighed out, added to the required amount of water and stirred well until all powder was mixed. This mixture was cooked for 1 hour and 15 minutes on hot plate and stirred frequently i.e. every 15 minutes. The remainder of the water was measured out and into a waring Blender. Ampicillin powder was then added. The cooked agar was added to the blender bowl and mixed on setting 1 for 5 minutes. After the agar and water had mixed for 5 minutes, the rest of the ingredients were added. The hot mixture was blended on setting 1 for a further 5 minutes. Formaldehyde was then added and blended on setting 2 for final 2 minutes. The completed diet was dispensed into the trays, allowed to cool and solidify.

Rearing of larvae:

Eggs and first instar larvae were obtained from ICI - Reading - UK.

A single, active, newly-hatched larva from the petri dish was aseptically transferred to individually a test plastic cup containing diet with a camel hair brush, which had previously been sterilised in formalin and washed with sterile distilled water. Seventy five larva were used in each diet. The larvae were held in incubators at 23°C and 28°C with 30-40 % relative humidity. The incubators were maintained a light cycle of 18 hours of light followed by 6 hours of dark.

Table 3. Composition of diet IV (S. Smyth, Pers. com.) used to determine the development of ***Heliothis virescens***.

Ingredients quantity	(for 50 mini pots)
Alfalfa (powder)	14.8125 g
Haricot beans	14.8125 g
Wheat germ	14.8125 g
Brewers yeast	6.6250 g
Sorbic acid	0.2060 g
Methyl paraben	0.4125 g
Ascorbic acid	0.6625 g
Ampicillin	0.0150 g
Agar	2.6625 g
Formaldehyde	0.2400 ml
Water	125.0 ml

The cooled and solidifed diets were cut into 3x3x1.5 cm blocks and they were placed in clear plastic cups, 3x4 cm. After pupated, pupae were removed and then placed on vermiculite in plastic trays. Trays were kept to emerge at 23°C and 28°C, 30-40 % relative humidity with 18 h photoperiod.

Observations were made daily for each individual on the development and the rate of survival of larvae, pupae. Each cup was numbered so that records could be maintained for each larva and pupa during the entire developmental period. Moreover, the results of all experiments were evaluated by using F-test ad Duncan's Multiple Range Test (DMRT).

Results and Discussion

In this study, eggs hatched in 2.91 and 4.18 days at 28°C, 23°C respectively. Shorey and Hale (1965) reported that the average of egg incubating was 3.3 days at 27°C. El Sayed and Graves (1969) found that egg hatched in 5 days at 25°C but Most eggs in 3 days. As for Patana (1977) reported that egg hatching occurred in 3 days at 28.9°C.

Table 4 shows that at 28°C, the average of larval development duration is between 12.74 and 13.50 days and of pupal developmental duration is between 10.59 and 11.29 days. In addition, the average duration from egg to adult emergence is between 26.24 and 27.47 days. On the other hand, Table 5 shows that at 23°C the larval and pupal developmental duration is between 20.04 and 22.82 days; 16.41 and 17

days, respectively. The average duration from egg to adult emergence is between 40.63 and 43.74 days. Larval developmental duration and the average duration from egg to adult emergence is longer in diet IV.

It was found out that according to the results of statistically, all diets at 28°C have no effect on the average development duration of larva ($p < 0.05$). But the average development duration of pupa is shorter in diet II than the others. Besides, the rate of pupa survival is less in diet I than the others while the rate of larval survival is similar to four diets. On the other hand, it was obtained that the average duration of adult emergence on all diets is similar days.

As a result of these experiments, the average larval development duration at 23°C is longer in diet IV than the others. However, there was no difference between the diets on the pupa development ($p < 0.05$). Moreover, the results indicated that the rate of larval and pupal survival is less in diet I than the others. On the other hand, the average duration of adult emergence was found at the same days in diet II and III.

Table 4. The data of development for ***Heliothis virescens*** reared at 28°C on the different artificial diets.

	Diet I	Diet II	Diet III	Diet IV
		in days*		
Larva	13.5 ± 1.52 a	12.74 ± 1.24 a	13.09 ± 1.09 a	13.19 ± 1.12 a
Pupa	11.05 ± 0.89 b	10.59 ± 0.68 c	11.29 ± 0.81 b	11.23 ± 0.97 b
from egg to adult emergence	27.47	26.24	27.29	27.33
% larval survival	91.84	93.94	91.84	88.46
% pupal survival	44.44	93.95	82.22	95.65

* The difference of means shown the same letter are not found significant ($p < 0.05$) as to DMRT

Table 5. The data of development for ***Heliothis virescens*** reared at 23°C on the different artificial diets.

	Diet I	Diet II	Diet III	Diet IV
		in days*		
Larva	20.50 ± 1.87 a	20.04 ± 1.83 a	20.08 ± 2.87 a	22.82 ± 2.93 b
Pupa	17.00 ± 1.09 c	16.41 ± 1.33 c	16.43 ± 1.14 c	16.74 ± 1.39 c
from egg to adult emergence	41.68	40.63	40.69	43.74
% larval survival	84.48	93.55	96.15	90.32
% pupal survival	39.49	86.21	60.00	75.00

* The difference of means shown the same letter are not found significant ($p < 0.05$) as to DMRT

Shorey and Hale (1965) found that at 27°C the average larval developmental duration was 18.2 days (male) and 18.3 days (female); the average pupal developmental duration was 13.5 days (male) and 13.1 days (female). In addition, duration from egg to adult emergence was 35 days (male) and 34.7 days (female). Guerra and Ouye (1968) reported that larval and pupal developmental duration was respectively 17 and 14 days at 26.7 ± 2°C. Shaver and Lukefahr (1969) found that larval developmental duration was 12.8 days 27-29°C. Guerra (1970) reported that at 26.7°C the larval development was between 12 and 15 days, the pupal development was between 13 and 15 days. Proshold and Bartell (1970) found that at 27°C the average larval developmental time was 16.7 (male), and 16.9 (female), pupal developmental time was 14.8 (male), 13.4 (female). On the other hand at 24°C, the larva developmental time was 21.2 (male), 21 (female) and pupal developmental time was 17.2 (male), 15.8 (female). All data includes incubation period of eggs.

Shaver and Raulston (1971) reported that at 29.5°C, 50 % RH, the average pupation duration was 12.5, 12.8, 12.9, 12.3 days in casein (Control), toasted soyflour, regular soyflour and soymeal diets, respectively. The emergence time was 22.1, 22.4, 22.5, 21.9 days in same diets, respectively. Guerra and Bhuiya (1977)found that at 27.8°C and 23.3°C, larval cycle was 13, 14, 13 days, pupal cycle was 13, 13, 13 days in soybean-wheat germ, soybean-grain oil diet prepared with wheat germ oil or corn oil diets, respectively. Brewer (1981) reported that at 29.5°C, larval and pupal duration was respectively 10.97, 9.63 days for first generation which reared on Brewer and King's (1977) diet. On the other hand, larval and pupal duration was respectively 11.62, 9.56 days for first generation reared on a diet in which corn oil was substitued for the wheat germ component. In addition, Raulston and Shaver (1970) found that a 2-day delay in pupation was obtained on the low-agar diet which averaged 14.4 days compared with 12.6 days for standard diet. The 2-day delay was exhibited also in emergence time (24.5 day for the low agar diet compared with 22.6 days fort he standart diet). Powell and Hartley (1987) reported that when a low agar diet containing corn cob grits was used, larvae pupated normally, but parasitoids were unable to develop in the host. In this study, approximate 2-day delay in pupation at 23°C was found only in diet IV which included about 2 % agar. The 2-day delay was obtained also in emergence time at 23°C.

Shorey and Hale (1965), developed a simple artificial diet to facilitate the mass rearing of a variety of noctuid species with a minimum expenditure of time and money. They found that larval and pupal survival of ***H. virescens*** were 80 %, 74 % at 27°C, respectively. El Sayed and Graves (1968) reported that mortality of one day old larvae reared on artificial diet and unirridiated at 27°C was 22.2 %. In addition, mortality of pupae was 13.3 %. Shaver and Lukefahr (1969) reported that larval survival was 88.9 % at 27-29°C on Berger's diet. Raulston and Sharer (1970) found that 89.9 % of the larvae reared on the standart diet (which is Berger's diet) pupated; of those reared on the low agar diet,only 54.1 % pupated at 27-29°C and about 50 % RH.

Shaver and Raulston (1971) found that larval survival of ***H. virescens*** was 82 %, 89 %, 81 %, 86 % and pupal survival was 88 %,83 %, 82 %, 90 %, in casein (control diet), toasted soyflour, regular soyflour and soymeal diets respectively. And Guerra and Bhuiya (1977) reported that larval survival was 79 %, 91.2 %, 89 % in soybean - wheat germ diet, soybean - grain oil diet prepared with wheat germ or with corn oil, respectively. Brewer and King (1979) reported that ***Heliothis*** spp. reared on a soybean flour - wheat germ diet containing corn cob grits prepared with either high or low amounts of agar had similar growth and development parameters, but larval survival was lower with the less agar. Agar is the preferred substance for the hardness of a diet because it is compatible with dietary ingredients. Most diets use about 3 % agar as this study 2-3 %.

Table 1, 2 and 3 shows that only diet I doesn't include wheat germ. One of the most important advances in rearing lepidopteran larvae and other phytophagous insects in the laboratory was the use of wheat germ in the formulation of diets (Singh, 1976). It is said that wheat germ is rich in B vitamins. The need for dietary B vitamins in animals is so universal that it hardly seems necessary to restate it (Vanderzant, 1966). Recently, most diets include wheat germ.

Conclusions

As a conclusion, it may be said that diet II and III have efficiency on the larva and pupa development as larval and pupal survival because of including high wheat germ.

Agar is very important in all diets to control the hardness of a diet because it is compatible with dietary ingredients, and in highly purified

form is satisfactory for use in nutritional experiments. On the other hand, when the low agar is used there may e several disadvantages. It is concluded that agar can be used in suitable rations. Similarly wheat germ which is rich in B vitamins which are necessary for larvae to grow normally and to increase survival. Nowadays, most diets include wheat germ.

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Özet

Heliothis virescens **(F.) (Lepidoptera: Noctuidae)'in farklı suni besinlerde gelişimi**

Dört farklı suni besinde ***Heliothis virescens***'in gelişmesi araştırıldı. Çalışmalar 23°C ve 28°C sıcaklıklarda yürütüldü. Larva canlılık oranı her iki sıcaklıkta ve bütün besinlerde yüksek olmasına karşılık, pupa canlılık oranı ise besin I (Kazein - Buğday embriyo besini)'de düşük olarak bulundu. 28°C'de, larva ve pupa gelişimi dört besinde de aynı olup; yumurtadan ergine kadar geçen süre 26.2 ile 27.5 gün arasındadır. Ayrıca, 23°C'de besin IV (Buğday embriyo besini)'de pupa olma süresi ile ergin çıkış süresi, diğer besinlere göre 2 gün uzun sürmüştür. Bu çalışmanın amacı, farklı suni besinlerin ***H. virescens***'in gelişmesi üzerine etkisini araştırmaktı. Ayrıca bu çalışmada, agar ile buğday embriyosunun böceklerin gelişmesi üzerine etkisi tartışılmıştır.

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