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An artificial diet with local ingredients for codling moth, *Cydia pomonella* (L.) mass rearing in Syria

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Abstract

A new artificial diet with local ingredients for codling moth, *Cydia pomonella* (L.), mass rearing in Syria is described. It is a modification of a diet originally developed by Brinton et al. (1969). Gluten was replaced by a 1:1:1 mixture of corn, barley and wheat flour. Wheat germ was replaced by barley germ and canola meal was replaced by soybean meal. Paper pulp and sawdust were replaced by cotton seed hull and wheat straw, respectively. The amount of water was also readjusted. Adult moths produced on the diet were significantly heavier than those reared on green apples. Their fecundity, fertility, longevity, flying ability, rate of development and survival to the adult stage were unaffected.

Keywords: Insecta, *Cydia pomonella*, artificial diets, mass rearing

1. Introduction

Syria has a large apple production with a total apple acreage of about 45,000 ha [1]. The codling moth, *Cydia pomonella* (L.), is a devastating pest of apple in most parts of the world [2, 3] and a key pest of apple in Syria [4-6]. The insect is also a serious pest on pear, quince and walnuts, and causes millions of dollars in losses to the fruit industry every year. In Syria, the pest has 2-3 generations a year [5] and, without control measures, the pest could destroy 100% of the apple crop. In fact, even with control, the losses could reach 10% [7]. In Syria, infestation rate in neglected apple orchards is 80-100% [5, 6, 8] which makes it impossible to grow apple commercially without effective control measures. In addition, this species is quarantined in some countries which are potential markets for apple exports such as Japan, Korea and parts of China and South America which makes it difficult or impossible for Syria to export apple to these countries.

To control the codling moth in Syria, about 6 chemical treatments are usually applied every year [9].

Chemical control of this pest has many drawbacks such as insecticide resistance [10-12], reduced predator parasite complex population [13, 14] and creation of secondary phytophagous mite problems [15]. In Syria, it also caused difficulties in exporting the country surplus of this crop due to high insecticide residues [16]. An alternative method of control, a sex pheromone-based mating disruption system, has been developed [17-19]. A number of factors, however, limit the success of this method. Some of these factors include high population density and immigration of gravid females from neighbouring orchards [18, 19]. In addition, physical features of the orchard has been reported to play an important role; orchards with many missing trees and steep slops are problematic [18, 19]. Consequently, supplemental control measures are often necessary.

The heavy losses to apple production caused by codling moth infestation in Syria and difficulties in exporting fresh fruits has led to consideration of the sterile insect technique (SIT), a non chemical method for insect pest control, to control or even eradicate this pest from apple producing regions in the country.

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One of the most important prerequisites for the success of this method is the ability to mass rear the insect involved [20]. Techniques for codling moth mass rearing are available [21, 22] and a rearing diet on a commercial scale has been developed [23]. This diet is a sawdust type, relatively dry particularly by the time larvae reach maturity, which enables them to pupate inside the diet. A slightly modified version of this diet is being used at the Canadian codling moth mass rearing facility (Osoyoos, B. C., Canada) to produce millions of moths/week [24, 22, 25]. In this modified version, canola meal and wheat bran were used to replace the more expensive casein in [23] diet. Formaldehyde and methyl paraben were also added to the diet to reduce microbial growth. Although this modified diet is very suitable for codling moth mass rearing on a large scale, some of its ingredients are either too expensive in Syria (paper pulp, gluten) or not commercially available (canola meal, wheat germ).

In this paper, we report on a new diet with local ingredients for codling moth mass rearing in Syria and assess its effects on codling moth fecundity, fertility, longevity, weight, flight ability and rate of development.

2. Materials and Methods

2.1. Laboratory colony

Insects used in these experiments were obtained from a codling moth laboratory colony that was maintained in a growth chamber (3.5x4.2x3.2 m) under constant temperature and relative humidity. The colony originated from larvae obtained from infested apples collected at several locations near the city of Damascus (Syria). Rearing conditions were set at 27±2 °C, 40-60 % RH and light was controlled by an automatic time clock at a photoperiod of 16L:8D cycle. For maintaining vigour, *C. pomonella* males from the wild populations were introduced into the colony several times every year. Larvae were fed on small green apples 2.5-3.5 cm in diameter in transparent plastic boxes (35x20x20 cm). Each box contained about 200 apples and was inoculated at a rate of about 200 eggs/box. Pieces of corrugated cardboard paper were distributed between the apples to collect mature larvae searching for pupating sites. Corrugated paper strips carrying the pupae were collected and transferred to the emergence cabinet which is a wooden box (90x60x145 cm) divided into a lower and an upper part. The lower part consists of 4 separate compartments (40x23.5x100 cm) where pupae were placed. On the ceiling of the upper part, an electric light was installed above each compartment. The top of each compartment has a small hole, 2 cm in diameter, where light coming from the light bulb could get through. Emerging moths attracted to the electric light fly through the holes and get trapped inside a transparent cylindrical plastic container (9.5x14 cm) placed at the top of each hole. Emerging moths, less than 24 h. old, were transferred to an oviposition cage similar to that reported by Proverbs and Logan (1970) and eggs were collected daily.

2.2. Preliminary studies

Investigations were started with a diet developed by [23] and in particular a slightly modified version of this diet (Canadian diet) used at the Canadian codling moth rearing facility in Osoyoos, B. C. [24, 22]. Several materials were tested to replace some of its ingredients. Paper pulp was replaced by cotton seed hull and saw dust was replaced by wheat straw. Other materials (corn meal, soybean meal, faba bean and check pea meal, and poultry supper which contains 85% Soya bean meal) were also used to replace canola meal. In addition, gluten was replaced by wheat bran and 1:1:1 mixture of corn, barley and wheat flour. Furthermore, wheat germ was replaced by locally manufactured (impure) wheat and barley germ and the amount of water added to the diet was readjusted. The amount of nutritional ingredients in each formula was kept about the same as in the Canadian diet.

Three different formulae (diets) were tested and compared to the Canadian diet as a standard. From each diet, 4 trays (18x13x2.5 cm), each contained about 800 g of the rearing medium, were prepared. A rectangular sheet of wax paper (17x12 cm) carrying 200 codling moth eggs in the black head stage was placed at the top of each tray. The side of the wax paper carrying the eggs was in contact with the diet. The waxed paper was removed 5 days later to prevent humidity accumulation on the diet surface, examined under a binocular microscope and the number of hatched eggs was recorded. After three weeks of incubation at 27 ± 2 °C, the trays were transferred to the emergence cabinet. Adults emerging from each group of trays were collected separately and their number was recorded daily. Percentage survival to the adult stage was calculated, and the diet produced the largest number of moth was selected for further investigations.

2.3. Effects of the selected diet on moths quality

The selected diet (diet No. 3), referred to hereafter as the local diet, was used to rear codling moth larvae and its effects on moth fecundity, fertility, longevity, weight and flying ability were investigated. The length of immature stages under our laboratory conditions and survival to the adult stage were also determined. The results were compared to rearing on the Canadian diet and green apples. The experimental procedure was carried out as follows and all experiments were repeated 4 times:

2 3.1. Effects on fecundity and fertility

Fecundity and fertility of adults reared on the three different media (Canadian diet, green apples and local diet) was examined. Pairs of moths (20 pairs/replicate) less than 24 h. old were confined in cylindrical transparent plastic containers (16x25 cm) lined with wax paper for 5 days. The moths were provided with water on moistened cotton balls. On day six, the moths were discarded and the wax paper carrying the eggs was incubated at 27 ± 2 °C for 5 days. Following incubation, the wax paper was examined under a binocular microscope and the number of deposited (fecundity) and hatched eggs (fertility) was recorded.

2.3.2. Effects on adult longevity

Moths (0-24 h. old) reared on each of the three different media (20 from each sex/replicate) were placed separately in small transparent plastic containers (16x25 cm) with lids. Moths were provided with water on moistened cotton balls. The number of dead moths was recorded daily and the average life span for males and females was calculated.

2.3.3. Effects on adult weight

Samples of moths (50/sex/replicate) reared on each of the three different media were weighed using an electrical balance with a precision of 0.1 mg. The weight of each moth was recorded and the mean weight/moth (male or female) was calculated.

2.3 4. Effects on survival to the adult stage, flying ability and rate of development

Four trays (18x13x2.5 cm) of each of the local diet, the Canadian diet and four boxes of green apples (35x20x20 cm) were inoculated at a rate of 200 eggs each.

Green apple boxes were provided with pieces of corrugated paper to shelter pupating larvae. Eggs in the black head stage were provided on rectangular sheets of wax paper (17x12 cm) placed at the top of each tray (or box). The wax paper was removed five days later, examined under a binocular microscope and the number of hatched eggs was recorded. The rearing media were incubated at 27 ± 2 °C for 3 weeks after which they were transferred to the emergence cabinet. Emerging moths from each medium were collected separately and their number was recorded daily. The number of pupal exuviae's left in the rearing trays (or boxes) was counted and percentage of flying moths (moths that were able to reach the collection traps) was calculated. The number of days needed for emergence of 50% of the moths was recorded and used to calculate the developmental time to the adult stage.

2 4. Effects of continuous rearing on the local diet

Effects of continuous rearing on the local diet on moths fecundity, fertility, weight, and longevity were examined in 4 consecutive generations (10, 15, 20 and 25). Twenty pairs (males and females) of moths/replicate were examined. The rate of development of immature stages (larvae and pupae) was also examined in the same generations. Experimental procedures was done as explained earlier and results were compared.

2.5. Data analysis

Data from the various experiments were subjected to analysis of variance. Means (adult emergence, fecundity, fertility and survival of F_1 insects to the adult stage) were separated by Fisher's protected least significant difference test (PLSD, 5% level of probability).

3. Results and Discussion

The use of the SIT for insect pest control and/or eradication depends largely, among other things, on the development of an artificial diet for mass rearing of the insect involved. Two requirements for an artificial diet must be considered [26]. The first is that it should be chemically and physically acceptable. The second is that it contains the necessary nutritional ingredients for normal development. To prove that both requirements are being met, normal development must continue on a diet for many generations until any nutritional reserves that are carried through the egg are exhausted [27].

Development is considered normal if fecundity, fertility, longevity, adult weight and growth rate were comparable to those of the insect reared on the natural food [26].

The first work on artificial diets for codling moth was done by [28]. Ever since, numerous diets for rearing this insect have been developed. Some of these were reported by [29, 30, 21, 24, 31 , 22]. However, [23] diet is the most commonly used one for codling moth mass rearing particularly when large numbers of insects are required [29]. It is inexpensive, easily prepared and has fewer problems with fungi.

A modified version of this diet (Canadian diet) is used to produce millions of moth every day for the Canadian codling moth control program [24, 22, 25].

In this paper, we report on a new version of [23] diet with local ingredients for codling moth mass rearing in Syria. Basically, it consists of wheat straw, cotton seed hull, barley germ, soybean meal and a 1:1:1 mixture of corn, wheat and barley meal. Other nutritional ingredients (vitamins and salt mixture) remained about the same as in the Canadian diet. Parameters to measure success were moth fecundity, fertility, longevity, weight, flying ability, survival to the adult stage and rate of development.

Table 1. The artificial diets tested for codling moth mass rearing and their effect on percentage survival to adults.

Diet	Amount (g/ml) of each ingredient			
ingredients	Canadian. diet	Diet No. 1	Diet No. 2*	Diet No. 3
Distilled water	605	800	750	770
paper pulp	8.2	-	-	-
Cotton seed hull	-	16	16	16
Saw dust	70.5	-	-	-
Wheat straw	-	26	23	21
Canola meal	86	-	-	-
Corn meal	-	32	15	30
Soya bean meal	-	49	25	85
Faba & chick pea meal (1:1)	40	-	-	
Poultry supper	-	-	84	-
Gluten	3.5	-	-	-
A (1:1:1) mixture of corn, barley and wheat flour	-	-	-	35
Wheat bran	-	10	15	-
Wheat germ	5.8	-	-	-
Raw wheat germ	-	10	16	-
Barley germ	-	-	-	31
Whole wheat flour	44	35	30	-
Choline chloride	1.3	1.3	-	1.3
Sugar	17.2	17.2	17.2	17.2
Fumaric Acid	5.1	5.1	5.1	5.1
Wesson's salt mix.	3.5	3.5	1.5	3.5
Vitamin mix. (Bio-Serv)	3.7	3.7	1	3.7
Methyl parben	1	1	1	1
Formaldehyde	0.3	0.3	0.3	0.3
% Surv. to adults ± SD	56.6 ± 4.4^{a}	43.3 ± 5.3^{b}	29.8 ± 4.8^{c}	58.3 ± 5.4^{a}

-Data are the average of 4 replicates.

-Means, within a raw, followed by the same letter are not significantly different (P>0.05, Fisher's LSD test).

*The amount of vitamins and salt mixture in formula No. 2 was reduced due to the presence of high quantities of these products in the poultry supper added to this formula.

Effects of the local diet on moth fecundity, fertility, longevity and weight are presented in Table 2. The results show that the local diet has no adverse effect on any of the above characteristics.

In fact, moths reared on the local diet were significantly heavier than those reared on green apples ($P<0.05$) and females reared on the three different media were also heavier than males.

Table 2. Effects of the local diet on codling moth fecundity, fertility, longevity, weight, rate of development, flying ability and survival to adults.

Character examined		Diet		
		Apple	Canadian	Local
Fecundity		67.8±13.1[a]	69.9±14.2[a]	71.1±13.9[a]
% Fertility		90.4±07.7[a]	89.7±06.4[a]	88.8±07.9[a]
Longevity	Males	10.7±02.8[a]	11.2±02.4[a]	11.1±01.6[a]
(days)	Females	09.6±02.4[a]	10.1±01.8[a]	09.8±02.0[a]
Adult weight	Males	17.9±01.5[a]	20.1±02.0[b]	19.3±02.5[b]
(mg)	Females	25.3±02.8[a]	32.2±03.0[b]	31.8±03.6[b]
% Survival to adults		35.7±06.6[a]	54.3±05.5[b]	53.6±07.3[b]
% Flying moths		96.3±01.8[a]	97.1±01.5[a]	96.8±01.7[a]
Life cycle (days)		34.8±01.8[a]	35.5±01.3[a]	35.2±01.2[a]

-Data are the average of 4 replicates.
-Means, within a raw, followed by the same letter are not significantly different ($P>0.05$, Fisher's LSD test).

Table 3. Effects of continuous rearing on the local diet on codling moth fecundity, fertility, longevity, weight and rate of development.

Character examined		Generation No.			
		10	15	20	25
Fecundity		76.8±13.9[a]	85.8±13.0[ab]	91.2±10.2[b]	90.9±11.0[b]
% Fertility		92.6±06.5[a]	87.6±08.6[a]	92.6±06.1[a]	93.3±06.1[a]
Longevity	Males	09.8±03.3[a]	10.1±02.3[a]	11.2±02.6[a]	10.3±02.4[a]
(days)	Females	09.9±02.7[a]	10.3±02.1[a]	09.9±01.6[a]	11.1±02.7[a]
Adult weight	Males	19.8±02.0[a]	19.4±01.8[a]	20.4±01.6[a]	20.6±01.8[a]
(mg)	Females	30.9±02.2[a]	30.3±02.4[a]	29.7±03.3[a]	30.6±04.0[a]
Rate of development for larvae and pupae (days)		30.4±01.3[a]	31.4±01.3[a]	31.5±01.4[a]	30.6±01.5[a]

-Data are an average of 4 replicates.
-Means, within a raw, followed by the same letter are not significantly different ($P>0.05$, Fisher's LSD test).

Table 2 also shows the average developmental time (in days) needed for neonatal larvae to develop into adults, percentage of 1st instar larvae that were able to reach the adult stage and moths flying ability. The results show that the time required for larval development was shortest on green apples.

However, the difference between developmental periods on the three different diets was not significant ($p>0.05$). Under our laboratory conditions, the egg stage takes about 4 days and the larval and pupal stages lasted for about one month. In total, the life cycle takes about 5 weeks.

Percentage of larvae that were able to reach the adult stage on the local diet was not significantly different from that reared on the Canadian diet.

On the contrary, larval survival on green apples was significantly lower ($p<0.05$) and lying ability was similar for moths produced on the three different diets ($P>0.05$).

Fecundity, fertility, weight, rate of development and longevity of moths reared on the local diet were examined in generations 10, 15, 20, and 25. Results are presented in Table 3. No significant differences in adult weight, fertility, moths longevity or rate of development were observed ($P>0.05$). However, the average number of eggs/female increased significantly up to generation 20 ($P<0.05$).

In general, our results show that the codling moth has been successfully cultured on a local diet for many generations with no adverse effects on any of the most important parameters (fecundity, fertility, flying ability, rate of development and survival to adults). For instance, moths reared on the local diet were comparable to those reared on green apples in their fecundity, fertility, flying ability and rate of development. Adult survival was significantly higher and moths were heavier which reflects the richness of the local diet with nutritional ingredients. Survival rate to the adult stage on the local diet exceeded 50% which is similar to that reported by [24]. The reduced survival rate on green apples could be due to the limited number of apples/larvae and/or their cannibalistic behaviour [32]. Females were also heavier than males which is in general agreement with previously reported data [33, 24].

Since females lay most of their eggs (more than 90%) in the first five days of age [35], the average number of eggs laid by the females in our colony (71/female) is lower than the Canadian one [26, 34, 35, 24]. This could be due to strain differences and/or better adaptation to rearing conditions. In fact, female fecundity increased by about 30% within 25 generations of continuous rearing in the laboratory which is in support of this hypothesis.

Rate of development of larvae on the local diet was similar to that on the Canadian diet and on green apples. Immature stages (larvae and pupae) lasted about one month at 27 ± 2 °C which is in general agreement with data reported by several other authors before [36, 35, 31].

4. Conclusion

In summary, the new local diet is nutritionally adequate and physically acceptable for codling moth mass rearing.

The main nutritional ingredients, in this diet, are: soybean meal; corn, barley and wheat flour and barley germ in addition to vitamins and salt mixtures. Fertility, longevity, adult weight and rate of development remained unchanged for 25 generations while fecundity increased significantly with time.

Compliance with Ethics Requirements. The authors declare that they comply with the Ethics requirements of the journal. The authors declare that they have no conflicts of interest and that all procedures involving human or animal subjects (if any) comply with specific regulations and standards.

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