

Research Article



Responses of *Cydia Pomonella* (L.) Reared on Different Artificial Diets under Laboratory Condition

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Abstract | Codling moth, *Cydia pomonella* (L.) (Lepidoptera: Tortricidae) is considered as one of the major insect pest for apple growers in Balochistan province, Pakistan. To mass rear codling moth in the laboratory for different research purposes, three artificial diets with different compositions were evaluated for codling moth larvae. Two diets containing wheat germ in their composition as major protein source and one diet without wheat germ were used. Addition of wheat germ in the diet composition for larvae resulted in significantly high percent survival (maximum value) (60 ± 4.56 and 56 ± 2.56) and significantly increased larval body weight of codling moth (0.58 ± 0.04 and 0.42 ± 0.03 gm). While diet without wheat germ resulted in low percent survival (40 ± 5.46) and reduced larval body weight (0.15 ± 0.03 gm). Based on the results, it is presumed that codling moth can be successfully reared on diet containing wheat germ as major constituent.

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Introduction

Apple codling moth, *Cydia pomonella* (L.) is one of the serious insect pests damaging fruits of the family Rosaceae (Shel Deshova, 1967). It can feed on apple, apricot, plum, walnut and other species (Wearing et al., 2001; Boncheva et al., 2006; Soleno et al., 2008). This pest is of Eurasia origin but its adoptability has been reported from cool temperate climates to the Mediterranean climates of the world (Steinberg et al., 1992). Its internal feeding on apple flush makes fruit unacceptable for market use. Insecticidal treatment remained the main control strategy to regulate the population of codling moth over long period of time. However, dependence on chemical control has led towards environmental and health problems; and appearance of resistant

population of codling moth to several widely used insecticides (Reuveny and Cohen, 2004; Knight et al., 1994; Charmillot et al., 1999).

In Pakistan, codling moth has been reported to damage apple crops on hilly areas of Khyber Pakhtunkhwa and Balochistan provinces. Balochistan province is known as fruit basket of the country. The high value of apple crop and minimal acceptance towards pests attack demands the utilization of different management strategies in a compatible manner (Khair et al., 2006). The prevalence of natural enemies associated with codling moth has been reported from different apple growing regions of the world (Falcon and Huber, 1991). These natural enemies can be deployed to regulate codling moth population and conservation of natural ecosystem. They can also provide a safer

management technique to apple growers. In this instance, parasitoids are considered as mostly deployed natural enemies for codling moth (Lloyd, 1958). The synchronization between parasitoid and host species should be considered in selecting its species in biocontrol program (Mills, 2005)

Basic component for assessment of natural enemies is the mass production of their natural hosts under laboratory conditions. Initially, green apples were used for small scale rearing of codling moth under laboratory conditions. They were considered more convenient and more penetrated by the young larvae. While using mature apples as food source becomes problematic under laboratory conditions due to rotting of apple fruits after specific time period. It also results in premature exit of the feeding larvae that may cause mortality in many cases (Dickson et al., 1952; Hamilton and Hathaway, 1966; Pristavko and Boreyko, 1971; Hathaway et al., 1973). Development of some alternate rearing methods can provide appropriate rearing system for mass production of codling moth. Corn meal bread was used as primitive artificial diet for rearing of apple codling moth under laboratory conditions (Therom, 1947).

Rearing techniques for codling moth have been developed with major improvements since last decades. In late sixties, North America and Europe started to develop artificial diets for rearing of codling moth with mixture of some natural ingredients and as well as with nutritional artificial ingredients (Bathon et al., 1991). Later, many researchers provided recipes of artificial diets in a regular sequence (Howell, 1967; 1970; 1972a; 1972b; 1981; Singh, 1977; Ashby et al., 1985; Toba and Howell, 1991; Howell and Neven, 2000). Artificial diets have proved themselves as alternate to natural food for mass rearing of different insect pests under laboratory conditions due to ease of use and cost benefits. These diets provide an essential tool for biological studies of insect pests and their associated natural enemies. They contain different components including proteins, carbohydrates, fats and essential components required for proper development of insect (Blanco et al., 2008).

In this study three different types of artificial diets were evaluated for mass rearing of codling moth in laboratory. This study was aimed to provide a recipe for mass rearing of codling moth in local conditions to conduct different biological studies in terms of its future management with reference to biological control.

Materials and Methods

This study was conducted in Bio-control laboratory of Centre of Agriculture and Biosciences International (CABI) at Directorate of Plant Protection, Agriculture Research Institute, Quetta, Balochistan during the year 2016.

Codling moth culture

Apple orchards at Quetta were looked for pupal stage of apple codling moth. For this purpose, tree cracks and crevices were monitored thoroughly and pupa was collected from apple tree cracks. Pupa were picked up with fine forceps and placed in plastic jars (48 × 15 cm) for transportation to laboratory. Collected pupas were placed in rearing cages (60 × 60 cm) and were kept till adult emergence. After emergence of adult codling moths, they were provided with mixture of honey and water (30:70) as diet. Culture was maintained at 28 ± 2 °C and $70 \pm 10\%$ RH. Rearing containers were provided with small apple leaves and plastic strips for oviposition by females of codling moth. The laid eggs on leaves and small strips were observed until black head was seen inside. Then eggs were cut along their margin and placed on artificial diets for further penetration of neonates into diet.

Diet composition and preparation

Three artificial diets were prepared with different compositions (Table 1). For preparation of diets, small plastic containers were used. Agar was swelled in small quantity of water and was dispersed in a separate stainless steel container having specified amount of water as given in each diet. Container was heated. All other flours and wheat germ (for diet A and C) were mixed in a separate plastic container. Mixture of flours was added in heated container and mixed thoroughly. At the end, other chemicals including antibacterial and vitamin tablet were mixed one by one in it and was heated for 20-30 minutes. Prepared mixture was let down to cool and was poured in small plastic rearing trays. Diet surface of these rearing trays were provided with small holes to assist entry of newly hatched codling moth larvae. Each treatment (diet) was replicated five times.

Seeding of CM eggs on diets

Codling moth adults culture was observed regularly for neonate development inside eggs. After the black head was seen inside egg, the eggs on leaves and small strips were cut along their margins and were placed

on diet surface in rearing trays. Each rearing tray was provided with 25 eggs of apple codling moth.

Table 1: *Composition of different artificial diets (gm or ml).*

Ingredients	Diet A	Diet B	Diet C
Agar	100	128	20
Wheat Germ	500	--	185
Soyabean Flour	--	1006	--
Wheat Flour	--	711	--
Maize Flour	--	355	--
Dried brewer's yeast	125	320	25
Ascorbic Acid	25	32	5
Citric Acid	25	--	5
Methyle Paraben	8.5	20	1.7
Vitamin B-complex	0.35	--	1
Streptomycin	--	--	1
Sorbic Acid	7	10	1.4
Formaldehyde	30	20	--
Water	5600	6400	1200

Percent survival of CM larvae

After seeding of eggs on rearing trays, initially rearing trays were observed after 15 days for monitoring of emergence of mature codling moth larvae. The very first emergence of codling moth larvae from artificial diets was observed after 35 days of seeding. After emergence of first larvae, rearing trays were monitored at five days interval to assess the percent emergence of codling moth larvae. The number of emerged larvae after complete feeding inside artificial diet was counted in each replicate. Percent survival of codling moth larvae in each diet was calculated by using following formula.

$$\text{Percent Survival} = \frac{\text{Emerged CM larvae}}{\text{Total CM eggs provided}} \times 100$$

Body weight of CM larvae

To assess the effect of artificial diets nutrients on larval health, body weight of the each emerged larvae was measured using weight balance machine (gm) and data of each larvae was recorded on its emergence from diet at five days interval. The emergence span of larva comprised of 14 days from emergence of very first larvae to the last larvae emerged from diets. Mean larval body weight in each treatment was calculated by using following formula.

$$\text{Larval Body Weight (Mean)} = \frac{\sum \text{body weight of larvae in a treatment}}{\text{Number of replicates}}$$

Data analysis

One Way ANOVA followed by Least Significant Difference (LSD) tests were used to determine the effect of artificial diets on percent survival and larval body weight of the growing codling moth larvae.

Results and Discussion

Data was analyzed to determine artificial diets (A, B and C) suitable for rearing of apple codling moth in laboratory. For this purpose larval response to these diets composition was considered as major response factor and was evaluated. Results showed that Diet A and C were not significantly different from each other, while Diet B was significantly different from Diets A and C in terms of percent survival and larval body weight after emergence from diet (Figure 1).

The maximum percent survival ($F = 9.03$; $df = 2$; $P < 0.005$; $\chi^2 1.75$) of apple codling moth larvae was observed in Diet C (average) (48.8 ± 2.65) followed by Diet A (48 ± 4.56) and minimum percent survival was observed in Diet B (25.6 ± 5.46). Maximum larval body weight ($F = 15.9$; $df = 2$; $P < 0.005$; $\chi^2 = 0.18$) was observed on larvae emerging from Diet C (0.46 ± 0.04) followed by Diet A (0.33 ± 0.03) and minimum larval body weight was observed in Diet B (0.15 ± 0.03) (Table 2). These results suggested that addition of wheat germ which is considered as main protein constituent for rearing diets can greatly increase the survival of codling moth and has positive effect on larval body health. While using conventional flours as protein source yields very low number of week larva.

The emergence span of codling moth larvae differed significantly among three diets ($\alpha = 0.05$). The very first emergence of codling moth larvae was observed after 35 days of seeding ($F = 2.60$; $df = 2$; $P = 0.115$) in Diet A and Diet C, while no larval emergence was seen in Diet B. At consecutive five days intervals after 40 and 45 days of seeding, all three diets depicted larval emergence. Furthermore, after 49 days of seeding, Diet A and Diet C showed maximum larval emergence while negligible emergence was seen in Diet B ($P < 0.001$; $\chi^2 = 8.41$). No larva emerged from artificial diets after 49 days (Figure 2).

Brinton et al. (1969) also evaluated different ingredients for selection of suitable diet for mass rearing of codling moth. They replaced agar with wood dust and suggested that addition of sorbic aid and citric acid

Table 2: Effect of artificial diets on rearing parameters of apple codling moth larvae.

Diets	% Survival			Larval Body Weight (grams)		
	Minimum	Maximum	Average	Minimum	Maximum	Average
Diet A	32 ± 4.56a	60 ± 4.56a	48 ± 4.56a	0.22 ± 0.03a	0.42 ± 0.03a	0.33 ± 0.03a
Diet B	12 ± 5.46b	40 ± 5.46b	25.6 ± 5.46b	0.06 ± 0.03b	0.26 ± 0.03b	0.15 ± 0.03b
Diet C	40 ± 2.65a	56 ± 2.56a	48.8 ± 2.65a	0.36 ± 0.04a	0.58 ± 0.04a	0.46 ± 0.04a

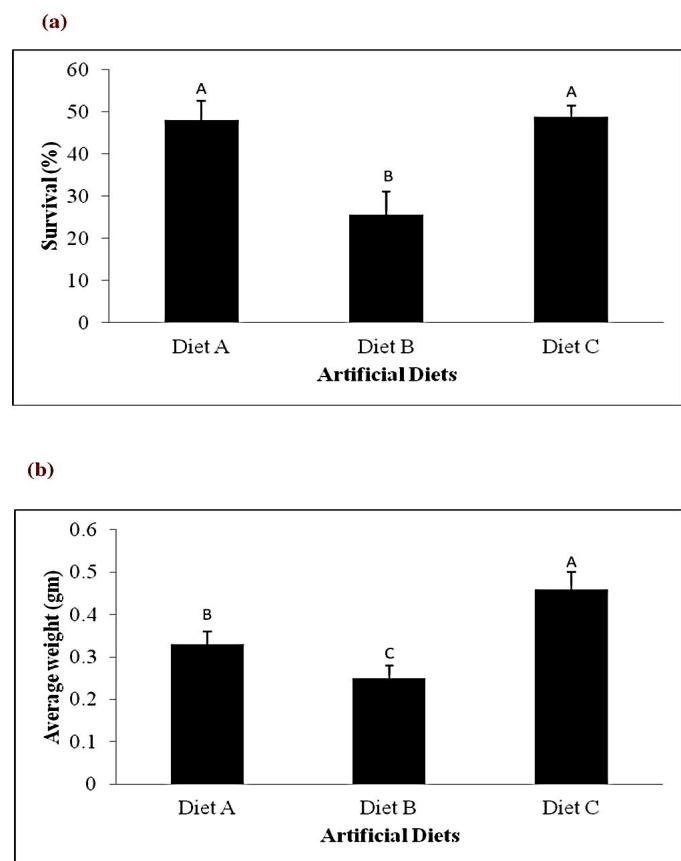


Figure 1: (a) Percent survival (%); (b) mean larval body weight (gm) of codling moth larvae reared on artificial diets.

helped to minimize microorganisms and PH of the diet, which facilitates neonate to complete their development inside egg. They found 52% survival of eggs into adult stage after rearing on artificial diets. They also suggested that larvae reared on artificial diets become almost equal in size as compared to when reared on apple fruit. Moreover, the net reproductive rates of the diet reared larvae and apple reared larvae were also found non-significant to each other. Later, [Howell \(1970\)](#) also evaluated artificial medium for codling moth rearing and found that addition of wheat germ, casein and sugar accelerated the growth of larvae and 88% of the larvae were able to complete their development inside rearing cages. He also suggested that when newly emerged larvae become able to survive on artificial diet and transform into adult stage, the prepared artificial diet must be designated as chemically and physically acceptable by the insect. Agar has always been the ingredient of

different artificial diets and act as binding material. But [Navon and Moore \(1971\)](#) replaced the agar with sodium alginate as binding agent and found the same larval development as was mentioned in case of using agar. The alginate materials are cheaper than agar and reduce the cost of diet.

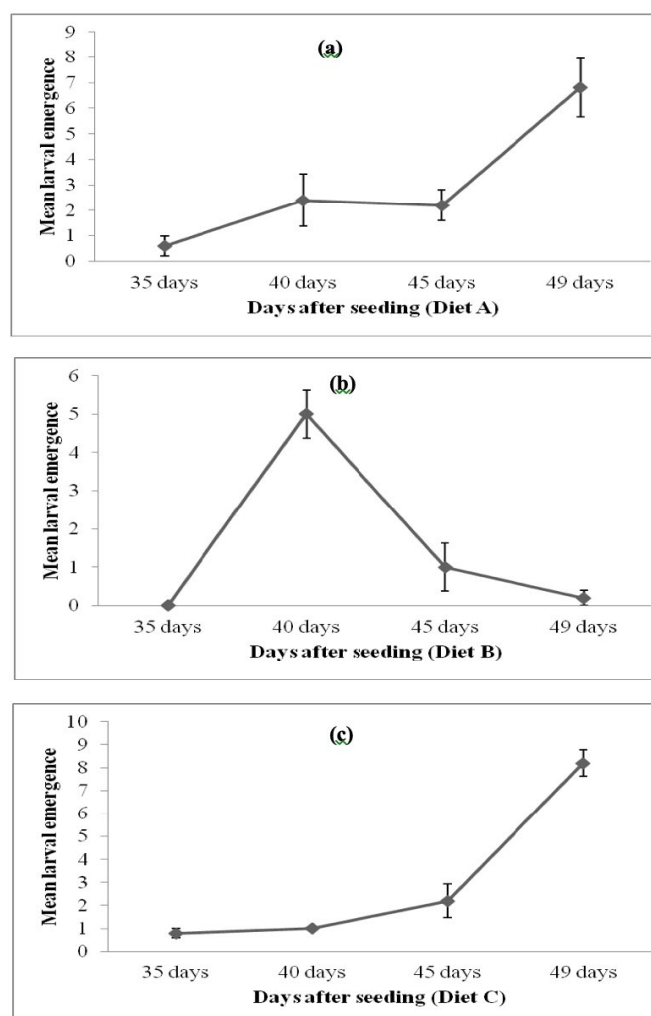


Figure 2: Mean emergence of codling moth larvae after seeding on artificial diets (a-c).

Differences in diet composition can have greater impact on development and feeding preferences of the growing insects ([Moore, 1986](#)). Wheat germ is considered as most essential source of protein in many artificial diets and fluctuation in its quantity within a diet can greatly influence the growth rate of the insect. In some cases, the high quantity of wheat

germ in an artificial diet can negatively affect the percent survivorship of the insects (Cohen, 2004). Manipulation of protein contents in a diet can be used in an easy way to assess their developmental and reproductive parameters. These diets can give more accurate estimates of the nutrients correlation with the insect behavior as compared to their feeding on plants in the field (Blanco et al., 2008).

Based on above facts and results revealed in this study, it is evident that addition of wheat germ can significantly affect survival and body health of the rearing codling moth larvae. This factor can greatly influence the rearing system for codling moth during different biological studies.

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Author's Contribution

Aqsa Khan: Involved to work with the CABI team to complete her degree research work and completed experiments in laboratory.

Sabyan Faris Honey: CABI Entomologist and Co-Supervisor of Ms. Aqsa Khan. He was involved to facilitate technical assistance to Aqsa Khan in implementation of experiments and write up.

Babar E Bajwa: CABI Project Executive. He was involved in this team as a leading role and facilitated team with provision of all financial and laboratory inputs required to conduct experiments. Afterwards, he reviewed the draft manuscript for final submission.

Nelofer Jamil: Supervisor of Ms. Aqsa Khan. She was involved in designing the experiments and prioritizing the key area as per degree discipline.

Muhammad Sohail Mazhar: CABI Project Manager and contributed his role as supervision of overall ongoing research and its implementation. Later, contributed to draft the manuscript.

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