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Research Article

Improving mass rearing of *Helicoverpa armigera* (Lepidoptera: Noctuidae) by feeding neonates on chickpea plant

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Abstract: Cotton bollworm, *Helicoverpa armigera* Hübner (Lepidoptera: Noctuidae), is a cosmopolitan polyphagous pest. Researchers need a simple mass rearing procedure to study the life history, behavior, and feeding habits of insects. In the present study, effects of container type (Petri dishes or transparent cubic-containers), the form of positioning the containers (overturned or upright), and diet type (artificial diet or natural diet, i.e., chickpea plants) on the development and survival of neonate of *H. armigera* were assessed. Additionally, effects of container type and ventilation (air circulation in rearing containers) on development and survival of second to sixth instars, percentage of pupation, and adult emergence were assessed. Container type had no significant effect on the development and survival of the neonates and subsequent instars. More neonates survived to the second instar when reared in the containers held overturned, compared to those held upright. The neonates reared on chickpea plants had a shorter larval development and a higher survival rate than those reared on an artificial diet. The percentage of pupation and adult emergence of second instars reared individually in ventilated containers was higher than that of those reared in unventilated containers. As a food source for neonates, chickpea plants could improve the mass rearing of *H. armigera.*

Keywords: *Helicoverpa armigera*, cotton bollworm, mass rearing, survival, artificial diet, rearing container

Introduction

Cotton bollworm, *Helicoverpa armigera* Hübner (Lep.: Noctuidae), is a destructive pest on a wide range of economic crops in many parts of the world (Fathipour *et al*., 2020) with a critical status to many crops, including maize, sorghum, chickpea, tomato, sunflower, cotton, tobacco, soybean, legumes, rapeseed and groundnuts (Fitt, 1989; Ahmed *et al*., 1998; Zalucki *et al*., 2002; Jallow *et al.*, 2004). This insect has a wide host range, high fecundity, facultative diapause (Fitt, 1989; Zalucki *et al*., 2002), capacity to migrate (Jones *et al*., 2019), and ability to develop resistance to insecticides (Kranthi *et al.*, 2002). The early instars are leaf-feeder (Talekar *et al*., 2006; Pannuti *et al*., 2019), while others bore into the fruits or pods (Talekar *et al*., 2006).

Nowadays, *H. armigera is* being reared on an artificial diet to study its life history (Fathipour *et al*., 2021), behavior, feeding habits, resistance to chemical insecticides, and susceptibility to entomopathogens (Abbasi *et al*., 2007;

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Sedaratian *et al*., 2013). So far, many researchers have focused on developing an economical diet type, artificial diet formula, and rearing techniques (Dhandapani and Balasubramanian, 1980; Griffith and Haskell, 1988; Sing and Rembold, 1988; Ahmed *et al*., 1998; Cohen, 2018). Dhandapani and Balasubramanian (1980) evaluated the effect of different host plants on the development and reproduction of *H. armigera*. A new simple rearing technique for *H. armigera* was described by Griffith and Haskell in 1988. They used a semi-artificial diet in conjunction with group rearing at all stages of development and obviated the need for individual handling of eggs, larvae, and adults, based on environmental and spatial considerations. Techniques and methods for rearing all stages of *H. armigera* were described by Armes *et al*. (1992). A new and practical artificial diet for the cotton bollworm, *H. armigera*, containing canned tomato paste, has been developed by Wu and Gong (1997). The developmental time of larvae fed on the diet was considerably shortened because most of them only went through 5 stadia before pupation. Ahmed *et al*. (1998) compared a new modified diet with other diets for mass rearing of *H. armigera.* They used Petri plates (17.5 × 3 cm) for mass rearing and a glass capsule vial (2.5 cm diam. × 5 cm ht.) for individual rearing of neonates.

In such studies, however, different instars in various numbers (usually more than 100) need to be available at any time. The simplicity of rearing procedures, cost, availability of rearing-containers, daily human labor, and saving time is essential in the mass rearing of *H. armigera*. If the artificial diet is refreshed every other day instead of daily, it could save time and human labor and reduce labor charges. On the other hand, a refreshing diet every other day may lead to fungal and bacterial spoilage in the rearing containers, which could be solved by reducing relative humidity via ventilation (air circulation).

Mass rearing of *H. armigera*, especially group rearing of neonates, may confront many difficulties. For instance, many of them disappear, die or escape from rearing containers and do not survive to the subsequent instar, a problem we confronted within our previous study (Allahyari *et al*., 2020). Therefore, the present study was performed in 2 trials. The first trial was conducted to evaluate the effect of container type, a form of positioning containers, and diet type on the development and survival of neonates of *H. armigera*. In this trial, neonate larvae were reared on potted chickpea plants and an artificial diet to determine the best diet type.

Additionally, to determine if the container type affected the development and survival of neonates, the neonates were reared in 2 different types of containers (Petri dishes and cubic-transparent containers). Moreover, two forms of positioning in containers were compared to determine which state of having the containers (overturned or upright) resulted in the maximum number of survived neonates. The second trial was conducted to evaluate the effect of container type and ventilation (air circulation in rearing containers) on the development of subsequent instars (second to six instars) and pupa formation and adult emergence of such instars under laboratory conditions.

Materials and Methods

Plants

Plastic pots (15 cm diam. × 13 cm ht.) were prepared with a mixture of soil (30%), coco peat (30%), peat moss (30%), and perlite (10%). The pots were immediately transferred to a greenhouse, set at 23 ± 3 ºC, and watered every 2 to 3 days. Afterward, 15-20 chickpea seeds (Cicer arietinum L.; cv. Bivanij) were sown in each pot. The chickpea plants were used in the experiments after 10-14 days when they had 6-8 true leaves; 7-8 plants per pot remained after thinning.

Rearing procedures

A colony of *H. armigera* was maintained under laboratory conditions at 28 ± 1 ºC, 60 ± 5% RH, and a photoperiod of L16:D8. The colony was established by collecting *H. armigera* larvae (ca. 250) from chickpea fields in Ilam Province, Iran, in the spring of 2018. Each larva was fed

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individually an artificial diet based on that of Twine (1971) (with a modification: 700 ml water was used) in a ventilated plastic container (4 × 6 × 8 cm) until pupation. Ventilation was insured using a hole (3 cm diam.) in the lid, covered with a wire mesh. The pupae were transferred to a transparent Plexiglas ovipositional chamber (20 × 30 × 30 cm), 30-40 per container. After emergence, adults were fed daily with a 10% honey solution provided on cotton balls in open Petri dishes (6 cm diam.). Fine mesh nets were used to cover the top and walls of the chamber as an oviposition substrate. Mesh nets were replaced daily, and those on which eggs were laid were transferred to plastic bags. The newly hatched larvae were either used in the experiments or transferred to transparent containers (as described above) filled with a thin layer (3 mm) of artificial diet and kept overturned after their lids were closed. Neonates reared 30-40 per container up to second instars. The second instars were reared individually in ventilated containers as above. The larvae were fed with cubes of an artificial diet (1 cm^3) until pupation. The diet was refreshed every other day. The sixth generation of *H. armigera* was used in the experiments.

Preparing artificial diet

Based on that of Twine (1971), 205 g bean flour, 35 g yeast, 30 g wheat germ, and 1.1 g sorbic acid were mixed with 350 ml of distilled water (80-90 °C), then 2.5 ml formaldehyde 37% and 5 ml vegetable oil were added and mixed thoroughly with the flowable mixture. Methyl-para-hydroxybenzoate (2.2 g) was dissolved in another 350 ml of boiling distilled water (100 °C), then agar (14 g) was added gradually to prepare a gel-like compound. All were combined with the first flowable mixture, 3.5 g ascorbic acid was then added. A thin layer (3 mm) of the prepared diet was poured in plastic Petri dishes (6 cm diam.) and cubic-transparent containers (as mentioned above) to feed neonates in the main experiments. The prepared containers were held at 6 °C until use. Additionally, enough amount of the artificial diet was poured into a 1 l plastic container, then cut into cubes of 1 cm^3 after solidification to feed the second to sixth instars.

Experimental procedures

Comparing two types of rearing containers

The first trial was conducted to determine the best way for rearing, which would result in the maximum number of neonates surviving and developing to the second instar. For this purpose, an experiment was conducted to compare two container types to rear neonates on an artificial diet.

The containers used were:

I) Petri dishes (6 cm diam.)

II) Transparent cubic containers with lid (4 × 6 × 8 cm).

Using a fine brush, groups of neonates were transferred to the Petri dishes coated with a thin layer of artificial diet (3 mm) as described above (30 neonates per Petri dish; 150 per treatment). Meanwhile, neonates were transferred to the cubic containers coated with a thin layer of artificial diet (3 mm) as above (50 neonates per container; 200 per treatment). The larval density in Petri dishes and cubic containers was approximately 1 neonate/cm2. Every 12 h, the cubic containers and Petri dishes were checked, and the number of larvae molted to the second instar was counted.

Form of positioning the containers (overturned or upright)

To determine which form of positioning the containers (overturned or upright) would result in greater numbers of survived neonates, groups of neonates were transferred to the Petri dishes coated with a thin layer of artificial diet (3 mm) as above (30 neonates per Petri dish, 350 per treatment). Then, the infested Petri dishes were kept in 2 different positions: overturned and upright.

Every 12 h, the Petri dishes were checked, and the number of larvae molted to the second instar was counted and recorded. The data were analyzed to evaluate the effect of the two forms of positioning or arrangement of rearing containers on the development and survival of neonates.

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Diet type

To evaluate the effect of diet type on the development and survival of neonates, neonates were reared on two types of diet: artificial diet and natural diet, i.e., chickpea plants. Therefore, using a fine brush, groups of neonates were transferred to the potted chickpea plants, cultured, and thinned as described before (15 neonates per pot; 150 per treatment). Meanwhile, neonates were transferred to the Petri dishes coated with a thin layer of artificial diet (3 mm) as above (30 neonates per Petri dish, 150 per treatment). Then the infested plants and Petri dishes were kept under laboratory conditions. Every 12 h, the chickpea plants, and containers were checked, and the number of larvae molted to the second instar was counted and recorded.

It should be noted that the Petri dishes were kept upside down because the overturned containers in this posture had a better efficiency in rearing neonates based on analyzing data of previous experiments. Finally, neonates fed a natural diet (chickpea plants) or artificial diet were analyzed to determine the best diet for rearing neonates.

Effect of container type

To evaluate the effect of container type on the development and survival of *H. armigera* larvae (second to six instars), second instars (4 days old) were reared in the two kinds of containers up to pupation:

I) Petri dishes (6 cm diam.)

II) Transparent cubic containers with lid (4 × 6 × 8 cm).

To prevent cannibalism, the larvae were reared individually, and every other day they were provided with pieces of artificial diet (1 cm^3) until pupation. The larvae excrements in the containers were cleaned by a brush to prevent fungal and bacterial spoilage. Sixty larvae (4 days old) were used in each treatment. Developmental time, the number of pupae formed, and adults emerged were recorded.

Effect of ventilation in containers

To evaluate the effect of ventilation (air circulation in rearing containers) on the development and survival of *H. armigera* larvae (second to six instars), second instars (4 days old) were reared in ventilated or unventilated containers up to pupation (120 larvae per treatment).

Statistical analysis

An independent-samples t-test (α = 0.05) (SPSS, v. 22; SPSS, 2013) was used to evaluate effects of container type, the form of positioning the containers and diet type on developmental time and survival of neonates of *H. armigera*, as well as to assess the impact of container type and ventilation on developmental time, pupae formation and adult emergence of subsequent instars (second to sixth instars).

Results

Effects of container type on development and survival of neonates

As shown in Table 1, container type (Petri dishes or cubic containers) had no significant impact on the developmental time and survival of neonates.

Table 1 Effect of rearing container (Petri dishes or cubic transparent containers) on developmental time (± SE) and survival (± SE) of neonates of *Helicoverpa armigera* reared in groups on artificial diet.

Treatments	n	Duration of first larval instar (day)	n	Survived to second instar (%)
Cubic container	200	4.2 ± 0.08 ns	128	64 ± 3.4 ns
Petri dish	150	4.0 ± 0.07	93	62 ± 4.0
t (df)		1.321 (219)		0.381 (319)
P		0.244		0.725

ns: Non-significant using independent-samples t-test.

Effects of the form of positioning the containers on development and survival of neonates

The form of positioning containers (overturned or upright) had no significant effect on the developmental time of neonates but had a significant impact on the survival of neonates. Neonates reared in overturned containers had a higher survival rate compared to those reared in upright containers (Table 2).

Table 2 Effect of form of positioning container (overturned or upright) on developmental time (± SE) and survival (± SE) of neonates of *Helicoverpa armigera* reared in groups on artificial diet.

Treatments	n	Duration of first larval instar (day)	n	Survived to second instar (%)
Overturned containers	350	4.1 ± 0.05 ns	221	63.1 ± 2.6 ***
Upright containers	350	4.1 ± 0.07	156	44.6 ± 2.7
t (df)		0.241 (321)		5.021 (698)
P		0.812		< 0.001

ns: non-significant; ***: significant using independent-samples t-test.

Effect of diet type (natural or artificial) on development and survival of neonates

Diet type (chickpea plants or artificial diet) had a significant effect on the developmental time and survival of neonates. Duration of the first larval instar was significantly shorter for those fed on a natural diet (chickpea) than those fed an artificial diet. Moreover, the neonates fed a natural diet had a higher survival rate than those fed an artificial diet (Table 3).

Table 3 Effect of diet (chickpea or artificial diet) on developmental time (± SE) and survival (± SE) of neonates of *Helicoverpa armigera*.

Treatments	n	Duration of first larval instar (day)	n	Survived to second instar (%)
Natural diet (chickpea)	150	3.1 ± 0.03	138	92.0 ± 2.2 ***
Artificial diet	150	4.0 ± 0.07 ***	93	62.0 ± 4.00
t (df)		13.845 (229)		6.614 (298)
P		< 0.001		< 0.001

*** significant using independent-samples t-test.

Effects of container type on development and survival of larvae

Container type had no significant impact on the developmental time of larvae (second to sixth instars) nor pupa formation and adult emergence (Table 4).

Effects of ventilation on development and survival of larvae

Ventilation (air circulation in rearing containers) had no significant impact on the developmental time of *H. armigera* larvae (second to sixth instars). However, pupae formation and adult emergence in ventilated containers were significantly higher than unventilated containers (Table 5).

Table 4 Effect of container (cubic containers and Petri dishes) on developmental time, pupa formation, and adult emergence (± SE) of larvae of *Helicoverpa armigera*. Tested larvae were second instars which reared individually up to pupation.

Treatments	n	Duration of larval instar (2nd to 6th) (day)	n	Pupal development time (day)	n	Pupa formation (%)	n	Adult emergence (%)
Cubic container	60	14.7 ± 0.11ns	47	10.6 ± 0.15	47	78.3 ± 5.4 ns	37	61.7 ± 6.3 ns
Petri dish	60	14.7 ± 0.11	46	10.7 ± 0.14 ns	46	76.7 ± 5.5	35	58.3 ± 6.4
t (df)		0.104 (118)		0.522 (91)		0.231 (118)		0.371 (118)
P		0.927		0.641		0.804		0.735

ns: Non-significant using independent-samples t-test.

Table 5 Effect of ventilation on developmental time, pupa formation, and adult emergence (± SE) of larvae of *Helicoverpa armigera.* Tested larvae were second instars which reared individually up to pupation.

Treatments	n	Duration of larval instar (2nd to 6th) (day)	n	Pupal development time (day)	n	Pupal formation (%)	n	Adult emergence (%)
Ventilated containers	120	14.6 ± 0.07	111	10.5 ± 0.09	111	92.5 ± 2.4	92	76.7 ± 3.9
Unventilated containers	120	14.7 ± 0.08 ns	93	10.7 ± 0.10 ns	93	77.5 ± 3.8 ***	72	60.0 ± 4.5 ***
t (df)		1.224 (237)		1.051 (202)		3.314 (238)		2.845 (238)
P		0.223		0.336		0.001		0.005

ns: non-significant; ***: significant using independent-samples t-test.

Discussion

Positioning the containers overturned increased the survival of neonates of *H. armigera* when reared on an artificial diet. However, chickpea plants caused many more neonates to survive and develop to second instar as a food source.

Although the frequency of cannibalism is higher among instars 3 to 6, it was reported that early instars had a low rate of cannibalism (Armes *et al*., 1992; Kakimoto *et al*., 2003). Therefore, the neonates reared on potted chickpea were less subjected to cannibalism than those reared in groups in overturned or upright containers. As a result, the percentage of neonates molted to second instar was the highest in those reared on chickpea plants. Armes *et al*. (1992) reared neonates of *H. armigera* in 300 ml containers (5, 10, and 20 neonates per container) on an artificial diet. They reported that as larval density increased, larval mortality was raised, and the pupation percentage decreased. It is worth noting that the first instars of *H. armigera,* poorly tolerated high environmental humidity compared to subsequent instars (Queiroz-Santos *et al.*, 2018). Therefore, moisture is a key factor that should be considered in rearing neonates.

Sing and Rembold (1988) reported that the larval diet did not affect the pupal period of *H. armigera*. Queiroz-Santos *et al*. (2018) reported that the first instars of *H. armigera* fed on the chorion and then dispersed to find a food source. If no suitable food were found, they would consume unhatched eggs and other less active larvae. When they provisioned pairs of larvae in containers with food, cannibalism was not observed, but some of the larvae prevented the others from reaching the food, leading to their death from starvation. Armes *et al*. (1992) reported that individually rearing of *H. armigera* is time-consuming and more expensive. However, they did not recommend group rearing because infected larvae were attacked and eaten by larger healthy ones, resulting in increased disease problems in the colony. In contrast with our results, Assemi *et al*. (2012) reported that container size had a significant effect on the mass rearing of *H. armigera.* As Cohen (2018) described a suitable container, the containers used in the present study serve the insect's needs, including thermal features, humidity accommodation, and gas exchange. Additionally, the containers we used served the rearing personnel's needs, including the availability of the containers at a more affordable cost, storage accommodation, ease in access to the insects, and cleaning requirements.

The availability of diet is an important factor in the mass rearing of insects (Cohen, 2003). However, culturing chickpea (as a diet for neonates) was more accessible than preparing an artificial diet. Ahmed *et al*. (1998) concluded that chickpea flour mediated diet and other essential ingredients are very important in the mass rearing of *H. armigera*. The survival and development period of *H. armigera* larvae depends on diet ingredients (Singh and Rembold, 1988). Singh (1999) reared *H. armigera* larvae on an artificial diet prepared with maize, soybean, and chickpea and reported that food consumption and growth of *H. armigera* larvae were minimal on maize. Moreover, the nutritional value of the soybean diet was higher but the consumption rate of larvae was more on chickpea than others. Jha *et al*. (2012) reported that an artificial diet is more suitable than hybrid sweet corn for the mass rearing of *H. armigera.* Late instars consumed much more amount of food and consequently produced much more amount of feces daily. Therefore, much waste caused a high rate of microbial growth in containers. Mass rearing of *H. armigera* on an artificial diet yielded a low number of survived neonates, while chickpea increased their survival. However, rearing second to sixth instars on chickpea plants has some difficulties. For example, planting and preparing enough potted chickpea as a food source is time-consuming and requires high human labor. Therefore, using chickpea plants to feed neonates and an artificial diet to provide next instars could improve the mass rearing of this insect.

Conclusion

Feasibility and low cost of rearing procedures are important in the mass rearing of *H. armigera*. Our results could help researchers improve the efficiency of mass rearing of *H. armigera* by using chickpea plants as a food source for neonates. If neonates are reared on an artificial diet, arranging containers in an inverted position is recommended. Air circulation in rearing containers was an important factor in rearing second to sixth instars, which reduced microbial contamination. Since container type did not affect development and survival, using less costly containers could reduce the rearing cost of this insect.

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بهینه‌سازی پرورش انبوه *Helicoverpa armigera* (Lepidoptera: Noctuidae) با تغذیه لاروهای نئونات از گیاه نخود

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چکیده: کرم غوزه پنبه *Helicoverpa armigera* Hübner (Lep.: Noctuidae) آفتی همه‌جازی و بسیار چندخوار می‌باشد. محققین برای مطالعه زیست‌شناسی، رفتار و عادات حشرات، نیاز به یک روش پرورش ساده دارند. در تحقیق حاضر، اثر نوع ظرف پرورش (پتری دیش یا ظروف شفاف مکعبی)، شیوه نگه‌داری ظرف‌های پرورش (به‌صورت وارونه یا رو به بالا) و نوع غذا (غذای مصنوعی یا غذای طبیعی یعنی گیاه نخود) روی تکامل و بقای لاروهای نئونات *H. armigera* ارزیابی شد. هم‌چنین، اثرات نوع ظروف پرورش و تهویه (جریان هوا در ظرف‌های پرورش) روی تکامل و بقای لاروهای سنین دوم تا ششم، درصد تشکیل شفیره و ظهور حشرات کامل آفت مورد ارزیابی قرار گرفت. نوع ظرف پرورش، تأثیر معنی‌داری روی تکامل و بقای لاروهای نئونات و سنین بعدی لاروی نداشت. تعداد لاروهای نئوناتی که به سن دوم لاروی رسیدند، در ظروف پرورش وارونه، بیش‌تر از آن‌هایی بود که در ظروف رو به بالا پرورش یافتند. لاروهای نئوناتی که روی گیاه نخود پرورش یافتند در مقایسه با آن‌هایی که روی غذای مصنوعی پرورش یافته بودند، دوره لاروی کوتاه‌تر و نرخ بقای بالاتری داشتند. درصد شفیرگی و ظاهر شدن حشره کامل، در لاروهای سن دوم که به‌صورت انفرادی در ظرف‌های تهویه‌دار پرورش یافتند، نسبت به آن‌هایی که در ظرف‌های بدون تهویه پرورش یافته بودند، بیش‌تر بود. گیاه نخود به‌عنوان یک منبع غذایی برای لاروهای نئونات، می‌تواند پرورش انبوه کرم غوزه پنبه را بهبود بخشد.

واژگان کلیدی: بقاء، تولید انبوه، کرم قوزه پنبه، *Helicoverpa armigera*، غذای مصنوعی، ظروف پرورش

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