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RESEARCH ARTICLE

SELECTION AND OPTIMIZATION OF A SEMI-SYNTHETIC DIET FOR MASS REARING OF *Helicoverpa armigera* IN LOCAL LABORATORY CONDITIONS, SRI LANKA

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Abstract

The effective mass rearing of *Helicoverpa armigera* under laboratory conditions is essential for numerous entomological research and pest management programs, such as biocontrol studies, resistance identification, toxicology bioassays, and integrated pest management strategies. This study aimed to develop and optimize a cost-effective, nutritionally balanced semi-synthetic diet for rearing *H. armigera* larvae at the Horticultural Crops Research and Development Institute (HORDI). Initially, four semi-synthetic diets with wheat, mung, chickpea, and corn flours as the main ingredients were evaluated alongside cabbage leaves and soaked-chickpea natural diets. Growth and reproductive parameters such as larval and pupal durations, larval and pupal weights, pupation and moth emergence percentages, and moth fecundity were assessed. Results revealed that mung bean and chickpea flour diets significantly outperformed the others, resulting in shorter larval and pupal durations, higher survival rates, and greater fecundity. Although mung bean flour yielded slightly better results than the chickpea flour diet, the chickpea diet was selected as the optimal main ingredient for the semi-synthetic diet due to its lower market cost. Subsequently, the chickpea diet was modified by replacing costly laboratory-grade ingredients, such as yeast extract, sucrose, and agar, with food-grade alternatives, such as instant dry yeast, sugar, and gelatin or agar-agar, in two modified diets. Of the two modified diets, the one using agar-agar (modified diet 1) showed comparable results to the original in all measured parameters, while the gelatin-based diet (modified diet 2) showed slightly delayed development and lower moth emergence, possibly owing to its stickiness, due to instability in room temperature. The study concludes that the chickpea-based semi-synthetic diets, particularly the modified version using cost-effective alternatives and food-grade agar-agar, offer a reliable, economical, and efficient solution for mass rearing *H. armigera* larvae under local laboratory conditions.

Keywords: Chickpea flour, Cost-effective formulation, Insect culture, Insect diet, Tomato fruit borer

INTRODUCTION

Helicoverpa armigera (Hübner) (Lepidoptera: Noctuidae) is a highly destructive polyphagous pest that attacks major crops, including cotton, tomato, chickpea, and various legumes, in tropical and subtropical regions (Rajapakse and Walter, 2007; Sullivan and Molet, 2007). In Sri Lanka, it was first reported in pigeon pea (*Cajanus cajan)*, *Vigna* spp., and cotton (Fellowes and Amarasena, 1977; Keerthisinghe, 1982) and now it is widespread across the country, especially on tomato and mung bean cultivations in Kandy, Matale, Bandarawela, Welimada, Anuradhapura, Ampara and Kegalle districts (Personal communication with Agriculture Extension Officers, 2025). Its high fecundity, migratory behavior, and ability to develop resistance to chemical insecticides pose major challenges to crop protection (EPPO, 2025). Consequently, control measures for this pest, including integrated pest management (IPM) strategies such as the use of biocontrol agents and the development of host-plant resistance, are highly sought. Therefore, laboratory mass rearing of *H. armigera* is essential for studies on pest biology, resistance monitoring, and biocontrol development (Beukeboom, 2017).

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Over the years, many artificial diets have been developed to rear important insect species such as Spodoptera frugiperda, Helicoverpa armigera, Alsophila pometaria, and Macrolophus caliginosus, etc., continuously in the laboratory (Ahmed *et al*., 1998; Cohen, 2001; Castane and Zapata, 2005). While these diets have been somewhat successful in supporting multiple generations, they often come with drawbacks such as reduced fitness and reproduction, leading to longer development times and lower fecundity (Coudron *et al*., 2002). Additionally, preparing artificial diets can be expensive, which makes it difficult for researchers in developing countries with limited funding (Ahmed, 1983; Prasad and Mukhopadhyay, 2020).

To overcome these challenges, semi-synthetic diets have been developed and widely adopted for laboratory rearing of lepidopteran pests. These diets offer a balanced nutritional profile, controlled composition, and ease of preparation, while minimizing external contamination and variability (Sing and Rembold, 1992; Abbasi *et al.,* 2007; Devi *et al.,* 2025). Several formulations based on legume flours, such as chickpea, soybean, and mung bean, have been reported to support optimal growth and development of *H. armigera* (Babu *et al*., 2014; Krishnareddy and Hanur, 2015; Singh *et al.,* 2019). However, the performance of such diets may vary depending on the origin of the insect population, ingredient quality, and cost-effectiveness of the formulation (Abbasi *et al.,* 2007; Assemi *et al.,* 2012; Truzi *et al.,* 2019).

It was required to establish an H. armigera culture at the Horticultural Crops Research and Development Institute (HORDI), Department of Agriculture, Peradeniya, to develop a bio-pesticide for sustainable control of the pest. Initially, natural diets such as cabbage leaves, soaked chickpeas, and tomato fruits were used to rear *H. armigera* larvae. However, these diets presented practical limitations, including high labour requirements, variability in nutritional content, limited availability, and contamination risks from pathogens or pesticide residues. Further, these factors can lead to inconsistent larval performance, affecting the reliability and reproducibility of research outcomes. There is limited information regarding appropriate semi-synthetic diets specifically designed for local *H. armigera* populations. Given economic constraints and the need for sustainable mass-rearing systems, it is essential to formulate an effective, cost-efficient diet using readily available ingredients. Therefore, this study aimed to evaluate flour-based semi-synthetic diets and to develop a cost-effective formulation suitable for local laboratory rearing of H. armigera in Sri Lanka.

MATERIALS AND METHODS

Determination of a suitable semisynthetic diet

A modification of the semisynthetic diet described by Kranthi (2000) was used as the base diet in this study (Table 1).

Table 1: Ingredients and quantities of the four semisynthetic diet treatments

Ingredient	Wheat flour diet	Mung flour diet	Chickpea flour diet	Corn flour diet
Main ingredient	Wheat flour 100 g	Mung flour 100 g	Chickpea flour 100 g	Corn flour 100 g
Yeast extract	10 g	10 g	10 g	10 g
Sucrose	10 g	10 g	10 g	10 g
10% Formaldehyde	2.5 ml	2.5 ml	2.5 ml	2.5 ml
Ascorbic Acid	2 g	2 g	2 g	2 g
Sorbic Acid	0.75 g	0.75 g	0.75 g	0.75 g
Multivitamin tablets (Zincovit)	0.1 g	0.1 g	0.1 g	0.1 g
Antibiotic tablets (Erythromycin Sulphate)	0.01 g	0.01 g	0.01 g	0.01 g
Vitamin E capsule	1	1	1	1
Agar	7.5 g	7.5 g	7.5 g	7.5 g
Distilled water	365 ml	365 ml	365 ml	365 ml

Four different semi-synthetic diets were formulated using wheat flour, mung flour, chickpea flour, and corn flour as the primary nutritional components, modifying the diet described by Kranthi (2000). These flours served as the main sources of carbohydrates and proteins required for larval development. All other diet components, including essential nutrients, vitamin mixtures, preservatives, antibiotics, and solidifying agents, were maintained at constant levels across all formulations to ensure uniformity in the nutritional profile and texture of the diets . Among the ingredients, the main ones were food-grade and sourced from commercial traders. Yeast extract, sucrose, ascorbic acid, sorbic acid, agar, and formaldehyde were of laboratory grade, while vitamins and antibiotics were of medicinal grade.

Vitamin and antibiotic tablets were finely ground using a mortar and pestle, and the required quantities were measured with an electronic balance. For each diet, all dry ingredients except yeast extract and agar were mixed separately in bowls. Then, 365 ml of distilled water was added to a beaker along with yeast extract, which was heated (60°C) on a hot plate with a magnetic stirrer till fully dissolved. Agar was then added and boiled until fully dissolved, and the dry ingredients were incorporated, mixed thoroughly and allowed to simmer. Finally, the heat was turned off, and formaldehyde and vitamin E were added and mixed well. Once the mixture was thoroughly combined, it was poured into a clean plastic tray to a height of 1 cm and left to solidify. All semisynthetic diets were prepared in this manner and allowed to solidify, and 1 cm^3 cubes (approximately 0.75g) were cut. For the control treatments, cabbage leaves and chickpeas were used as natural diets. Cabbage leaves were cut into 4 cm × 4 cm squares (approximately 0.25g), avoiding the midrib, and washed twice with tap water, then rinsed with distilled water. The leaf pieces were then dried using autoclaved tissue paper. Similarly, chickpea seeds were washed twice with tap water, soaked in distilled water for 12 hours and dried with autoclaved tissue papers.

Sixty ventilated transparent plastic cups (6.5 cm diameter x 4 cm height) were lined with autoclaved tissues. A 1 cm^3 diet cube was placed on the tissue in each cup. A single, day -old neonate larva obtained from the laboratory culture was carefully placed on the diet cube using a small paintbrush. The top of the cup was covered with cling film, and the lid was placed on top. Sixty such cups were prepared with 20 cups per replicate. All the semisynthetic and natural diet treatments were applied in this manner, and the cups were arranged in a glass cage with wire-mesh sides in a Completely Randomised Design (CRD). Rearing conditions were maintained at 27±1 °C temperature, 70-80 % relative humidity and 12-12 (L:D) photoperiod. All semisynthetic diets were replaced every 5 days, and natural diets were replaced every 2 days. After 10 days, the amount of semisynthetic diet cubes, cabbage leaves, and chickpea seeds given to larvae was increased to 3, since the larvae had grown and consumed more, ensuring each insect had ad libitum access to food. At the 5^{th} larval stage, the weight of each larva was measured by an electronic balance.

When the pupae formed, the remaining diet was carefully removed, and the pupation date was labelled on the cup. Seven days after pupation, the weight of each pupa was measured, and the gender of each pupa was identified by observing the location of the gonophore through a stereo microscope (x10 magnification). Males and females were placed separately in ventilated plastic cups inside plastic containers covered with a muslin cloth. When male and female moths emerged from each diet treatment, they were used for a fecundity test.

Eighteen oviposition containers (22 cm diameter x 28 cm height) were prepared, and two newly emerged male and two female moths were introduced into each container, which was then covered with muslin cloth at the top. Three containers were assigned to each diet treatment for the moths reared on that diet. After three days, the suspended muslin cloth strips and the muslin cloth covering the top of the oviposition containers were removed and transferred to a tray lined with autoclaved tissue paper. Fresh moth feed and clean muslin cloths were then provided. This process of replacing muslin cloths and feed was repeated every other day until the moths died. All used muslin cloths were placed in trays, and the eggs laid on them were counted. Average fecundity per female moth in relation to each diet was determined.

Optimization of selected insect diet

Based on the results, the most suitable semisynthetic diet for *Helicoverpa armigera* larvae was identified. To make the diet more cost-effective, it was modified by substituting some of the original ingredients with more affordable, commercially available alternatives (Table 2). These substitutes were incorporated into the original formulation in the exact quantities.

Table 2: Original and their replacement ingredients in diet treatments

Original diet	Modified diet 1	Modified diet 2
Yeast extract	Instant dry yeast	Instant dry yeast
Sucrose	Sugar	Sugar
Agar	Agar-Agar	Gelatin

The two modified diets were prepared in the same manner as the original semisynthetic diets. For the control, the selected original diet was prepared. Thirty newly hatched *H. armigera* larvae obtained from the original laboratory culture were used per treatment in three replicates. The experiment was conducted in the same way as the initial experiment.

Data collection and analysis

Data on larval duration, larval weight at the 5th instar stage, pupal duration, pupal weight, number of eggs laid per moth, percentage pupation and percentage moth emergence were recorded for each diet treatment in both experiments. Statistical analyses were performed using IBM SPSS Statistics 22. Normality of the data was assessed using the Shapiro–Wilk test, and, once confirmed ($P>0.05$), differences among treatments were evaluated with one-way ANOVA followed by Tukey's test.

RESULTS AND DISCUSSION

Determination of a suitable semisynthetic diet

Different tested diets had a significant impact ($P<0.05$) on the evaluated growth and reproductive parameters, including mean larval duration, mean larval weight at the 5th instar, mean pupal weight, mean pupal duration, % pupation, % moth emergence, and moth fecundity (Table 3).

Table 3: **The effect of diets on the growth and reproductive parameters of *H. armigera***

Diet	Mean larval duration (Days)	Mean larval weight (g)	Mean pupal duration (Days)	Mean pupal weight (g)	Mean fecundity/ moth	% pupation	% Moth emergence
Wheat flour diet	19.24 ^{c} ±1.43	0.35 ^{a} ±0.02	18.5 ^{c} ±1.81	0.24 ^{a} ±0.01	1014.33 ^{a} ±20.52	81.67 ^{a} ±2.88	87.73 ^{a} ±0.40
Mung flour diet	15.05 ^{a} ±1.16	0.43 ^{c} ±0.02	12.44 ^{a} ±1.09	0.33 ^{d} ±0.02	1543.00 ^{d} ±98.19	93.33 ^{b} ±2.88	98.23 ^{b} ±3.05
Chickpea flour diet	15.27 ^{a} ±1.22	0.42 ^{c} ±0.02	12.88 ^{a} ±1.36	0.32 ^{d} ±0.01	1476.67 ^{cd} ±97.35	91.67 ^{b} ±2.88	96.46 ^{ab} ±3.05
Corn flour diet	18.35 ^{b} ±1.45	0.40 ^{b} ±0.02	15.52 ^{b} ±0.87	0.29 ^{c} ±0.02	1124.00 ^{ab} ±28.21	86.67 ^{ab} ±2.88	94.33 ^{ab} ±5.55
Cabbage leaves	19.18 ^{c} ±1.27	0.36 ^{a} ±0.01	18.43 ^{c} ±1.59	0.24 ^{a} ±0.03	1065.67 ^{ab} ±29.67	81.67 ^{a} ±2.88	95.96 ^{ab} ±3.49
Soaked chickpea seeds	17.49 ^{b} ±1.20	0.39 ^{b} ±0.05	16.11 ^{b} ±2.75	0.28 ^{b} ±0.42	1263.67 ^{bc} ±152.05	89.67 ^{ab} ±2.88	96.23 ^{ab} ±3.15

In a column, means followed by similar letters are not statistically different ($P>0.05$).

Among the tested diets, larvae reared on mung flour and chickpea flour exhibited the shortest larval durations (15.05 ± 1.16 and 15.27 ± 1.22 days, respectively) and pupal durations (12.44 ± 1.09 and 12.88 ± 1.36 days), indicating faster development compared to those reared on other semi-synthetic and natural diets. Corn flour diet recorded shorter larval and pupal durations (18.35 ± 1.45 days and 15.52 ± 0.87 days) compared to cabbage leaf diet (19.18 ± 1.27 days and 18.43 ± 1.59 days) and wheat flour diet (19.24 ± 1.43 days and 18.5 ± 1.81 days), however, natural chickpea diet revealed shorter larval duration (17.49 ±1.20 days) compared to corn flour diet, wheat flour diet and the cabbage leaf diet. The wheat flour diet gave the longest larval and pupal durations in relation to other diets.

Larval and pupal weights were also significantly higher on mung (0.43 ± 0.02 g and 0.33 ± 0.02 g) and chickpea diets (0.42 ± 0.02 g and 0.32 ± 0.01 g), reflecting better nutrient profiles suited to *H. armigera.* Corn flour diet performed better than wheat flour semi-synthetic diet and natural diets concerning larval (0.40 ± 0.02 g) and pulpal weights (0.29 ± 0.02 g). Furthermore, fecundity was highest in moths reared on mung flour (1543.00 ± 98.19 eggs/moth) and chickpea flour (1476.67 ± 97.35 eggs/moth), while those from wheat flour and cabbage leaf diets produced significantly fewer eggs (1014.33 ± 20.52 and 1065.67 ± 29.67 eggs/ moth, respectively).

Survival to pupation and adult emergence was also higher on mung (93.33% and 98.23%) and chickpea diets (91.67% and 96.46%) than on wheat flour and cabbage. These results indicate that mung and chickpea-based diets are the most suitable among those tested, supporting optimal growth, survival, and reproductive capacity of *H. armigera* under laboratory conditions. However, since the market value of mung bean was higher than that of chickpea, the chickpea flour diet was selected as ideal for rearing *H. armigera* under laboratory conditions. Nevertheless, our results indicate that mung bean flour is also suitable.

In previous research by Singh *et al. (2019),* chickpea flour-based diets yielded shorter larval and pupal durations, higher larval and pupal weights, and higher pupation and moth emergence percentages compared to mung flour and black gram diets. Another study reported that larval duration was shorter, and larval, pupation, and adult emergence percentages were higher on a chickpea flour-based diet compared to a mung bean flour diet (Kumar *et al*., 2018). However, the pupation period was not significantly different between the two semisynthetic diets (Kumar *et al*., 2018). Many other studies have also revealed that chickpea flour-based diets are suitable for rearing *H. armigera* under laboratory conditions (Singh and Remould, 1988; Babu et al., 2014; Krishnareddy and Hanur, 2015; Chakravarthi *et al.,* 2018). However, none of the studies comparing chickpea- and mung bean-based diets have obtained results similar to those in our study (Kumar *et al*., 2018; Singh *et al.,* 2019). The enhanced performance of *H. armigera* larvae on the mung bean–based diet observed in this study may be attributed to host plant adaptation. Unlike chickpea, mung bean is widely cultivated in Sri Lanka, and the local *H. armigera* population is likely to have undergone physiological or behavioural adaptations to the mung crop. In contrast, chickpeas are widely grown in countries such as India, where most comparative studies of the two diets have been conducted.

Optimization of selected insect diet

When preparing the selected diet, some ingredients were more expensive and needed to be replaced with suitable counterparts (Table 4). It was assumed that the use of alternative ingredients would not significantly deviate from the nutrient profile of the original ingredients. The alternative ingredients were readily accessible, commercially available food-grade ingredients. Based on the cost analysis, it was more economical to use locally available commercial food-grade ingredients than laboratory-grade ingredients in the original diet.

Table 4: Price comparison of the original diet ingredient and replacement ingredient (Base year-2025)

Original ingredient	Quantity (g)	Price of original ingredient(Rs)	Source	Replacement ingredient	Price of replacement ingredient (Rs)	Source	Profit for the quantity (Rs)
Yeast extract	10	1121.01	www.sigmaaldrich.com	Instant dry yeast	91.67	Cargills Food City, Sri Lanka	1029.34
Sucrose	10	98.79	www.sigmaaldrich.com	Sugar	2.60	Cargills Food City, Sri Lanka	96.19
Agar	7.5	2338.45	www.sigmaaldrich.com	Agar-Agar	202.50	Cargills Food City, Sri Lanka	2135,95
Agar	7.5	2338.45	www.sigmaaldrich.com	Gelatin	137.50	Cargills Food City, Sri Lanka	2200.95

The physical appearance and texture of the modified chickpea diet were similar to the originally selected diet; however, the modified diet 2 appeared slightly sticky, as gelatin was not stable at room temperature and may have hindered the movements of neonates. In the statistical analysis, it was found that the mean larval duration was significantly influenced by the diet (P<0.05), with larvae fed the modified chickpea flour diet 2 exhibiting the longest development time (15.82 ± 1.44 days), which was significantly higher than that of larvae on the standard chickpea flour diet (15.17 ± 1.16 days). This suggests that the second modification may have introduced nutritional or structural changes that slightly delayed larval development.

However, mean larval weight (0.43–0.45 g), mean pupal duration (12.33–12.60 days), and mean pupal weight (0.32–0.34 g) did not show statistically significant differences across the treatments, indicating that all three diets were similarly effective in supporting growth and metamorphosis. Female fecundity also remained unaffected, with egg production ranging from 1356.00 ± 53.67 to 1516.00 ± 70.37 eggs per moth, demonstrating that the reproductive potential of adults was not compromised by dietary modifications.

The pupation percentage was consistently high (86.67–95.00%) across all diets, with no significant differences observed. However, the percentage of adult moth emergence showed significant variation (P<0.05), with the standard chickpea flour diet yielding the highest emergence rate (96.56 ± 2.97%), significantly outperforming the modified chickpea flour diet 2 (90.40 ± 2.33%). This decline may be attributed to suboptimal conditions during the pupal stage or reduced survivability due to dietary changes. Furthermore, the modified chickpea diet 1 did not significantly deviate from the original diet across any of the parameters mentioned, indicating that it can replace the original diet without compromising its effect on *H. armigera* (Table 5).

Table 5: **The effect of modified diets on the growth and reproductive parameters of *H. armigera***

Diet	Mean larval duration	Mean larval weight	Mean pupal duration	Mean pupal weight	Mean fecundity/ moth	% pupation	% moth emergence
Chickpea flour diet	15.17 ^{a} ±1.16	0.43 ^{a} ±0.02	12.33 ^{a} 1.03	0.34 ^{a} ±0.02	1516.00 ^{a} ±70.37	95.00 ^{a} ±5.00	96.56 ^{b} ±2.97
Modified chickpea flour diet 1	15.25 ^{ab} ±1.16	0.43 ^{a} ±0.02	12.50 ^{a} ±0.94	0.33 ^{a} ±0.01	1441.33 ^{a} ±72.56	91.67 ^{a} ±2.88	94.43 ^{ab} ±5.55
Modified chickpea flour diet 2	15.82 ^{b} ±1.44	0.45 ^{a} ±0.03	12.60 ^{a} ±0.82	0.32 ^{a} ±0.01	1356.00 ^{a} ±53.67	86.67 ^{a} ±2.88	90.40 ^{a} ±2.33

In a column, means followed by similar letters are not statistically different (P>0.05)

CONCLUSIONS

Both mung and chickpea flour diets were the most effective for rearing *H. armigera* in laboratory conditions, supporting faster development, higher survival rates, and greater reproductive output. Although mung bean flour showed better results in some aspects, chickpea flour was selected as the preferred diet due to its lower cost and comparable performance. The use of commercially available, food-grade ingredients to create a modified chickpea diet proved to be a cost-efficient approach without compromising insect growth and development. Therefore, the optimized chickpea-based diet using food-grade agar-agar provides a reliable, low-cost solution for mass rearing of *H. armigera* in Sri Lanka, supporting future IPM and biological control initiatives.

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AUTHOR CONTRIBUTION

WMNKKK, KMDWPN, LN and KSH conceptualised and designed the study. WMNKKK conducted the research and analysed the data. WMNKKK drafted the manuscript, and KMDWPN, LN, and KSH critically reviewed and revised it.

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