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Research Article

In Vitro Comparative Analysis of Fall Armyworm (*Spodoptera frugiperda*) Development: Evaluating Natural Versus Artificial Diets

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ABSTRACT

The fall armyworm (*Spodoptera frugiperda*) is a notorious invasive pest of global economic concern, impacting a diverse array of crops, with a significant preference for maize. Its swift dispersal and capacity to adapt to various environments present substantial obstacles to consistent crop yields and global food supplies. Rearing *S. frugiperda* on natural maize-based diets in laboratory studies is costly, space-dependent, labour-intensive, and prone to microbial contamination, underscoring the need for efficient, consistent, and less resource-demanding alternatives such as artificial diets. This study aimed to compare the developmental performance of *S. frugiperda* larvae reared on a natural maize stem diet versus a formulated artificial diet under controlled laboratory conditions (25 ± 1°C; 65 ± 5% relative humidity). Larvae were subjected to two dietary treatments with three replicates each. Results showed that larvae fed the artificial diet consumed significantly more food (Mean ± SD: 587.6 ± 140.2 mg per larva) than those on the natural diet (432.7 ± 179.3 mg; $p<0.05$). This enhanced nutritional intake reduced the life cycle to 32 days, compared to 41 days on the natural diet. Although both groups exhibited 100% larval survival, pupal weights were significantly higher in the artificial diet group (190.7 ± 29.8 mg) than in the natural diet group (138.3 ± 31.9 mg). The artificial diet also led to markedly lower microbial contamination (5–10%) relative to the natural diet (20–30%). Furthermore, the artificial diet group achieved 100% adult emergence. The resulting adults successfully mated and produced viable eggs, leading to successful larval development. These findings highlight the advantages of artificial diets in ensuring consistent, efficient, and contamination-reduced rearing of *S. frugiperda*, supporting their application in laboratory-based insect toxicology, biological control studies, and mass-rearing initiatives.

Key words: Artificial diet, comparative analysis, fall armyworm, larval development, natural diet, *Spodoptera frugiperda*

INTRODUCTION

The fall armyworm, *Spodoptera frugiperda* (J.E. Smith), is a highly adaptable and destructive global pest with a significant impact on maize and various other crops (Sparks, 1979; De Groote *et al*., 2020; Pal *et al*., 2024), resulting in substantial economic losses worldwide (Khan *et al*., 2020 & 2023; Shehzad & Shahzad, 2022; Wyckhuys *et al*., 2024). Its rapid spread since 2016 has been largely attributed to its strong flight capability and high reproduction potential (Hruska, 2019; Samanta *et al*., 2023).

The larvae of *S. frugiperda* are voracious feeders, causing severe defoliation by consuming leaves, stems, and reproductive structures, directly impacting crop yields (Akeme *et al*., 2021; Kenis *et al*., 2022; Yaméogo *et al*., 2024). Infestation is typically identified by transparent leaf tissue and the presence of holes, affecting all stages of plant growth. Adult females exhibit high fecundity, laying up to 1,500 eggs during their approximate three-week lifespan (Chisonga *et al*., 2023), and the complete life cycle on maize ranges from 30 days in warmer conditions to 60–90 days in cooler temperatures (Deshmukh *et al*., 2021).

A thorough understanding of the biology and physiology of *S. frugiperda* is crucial for the development of effective management strategies. *In vitro* studies, utilizing both natural and artificial diets, serve as essential tools for investigating larval development, nutritional requirements, and the impact of various factors on *S. frugiperda* populations.

Consequently, this study compares *in vitro* development on a natural maize diet versus an artificial diet, evaluating larval growth and survival, pupal development, rearing practicality, contamination risks, cost-effectiveness, and suitability for laboratory studies. The ultimate aim is to optimize rearing protocols for future research on pest biology, insecticide efficacy, and biological control agents targeting *S. frugiperda*.

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MATERIALS AND METHODS

In vitro rearing of *Spodoptera frugiperda*

Egg batches of *S. frugiperda* were sourced from an established colony maintained at the Insect Biological Control Laboratory, CABI, Pakistan, where this species was already being continuously reared. The laboratory environment was consistently regulated at a temperature of 25 ± 1 °C and 65 ± 5% relative humidity throughout the experimental period. Two sterile glass containers were prepared by first washing them with a disinfectant (Glint™), followed by wiping with sterile tissue paper to eliminate any potential contaminants. The collected egg masses were carefully transferred into each of the prepared containers, which were then covered with muslin fabric and tightly sealed with rubber bands to prevent larval escape while ensuring adequate air exchange. Each container was clearly labelled with the date of egg introduction. Regular observation of the containers was conducted to monitor and document the precise timing of larval emergence. Approximately three days after placement, the larvae began to eclose, at which point the muslin covers were carefully removed. Freshly harvested maize stems, serving as the natural food source, were introduced into one of the containers. Concurrently, the prepared artificial diet was placed into the other container, ensuring that each diet type was contained within a separate, sterile environment. Both containers were subsequently re-covered with muslin fabric and secured with rubber bands, thus maintaining distinct feeding regimens for the developing larvae.

Experimental design

This study employed a controlled experimental design featuring two distinct treatment groups, each replicated in triplicate to ensure the reliability and statistical rigor of the findings. The treatment groups were as follows: (a) *S. frugiperda* larvae raised on a natural diet of maize stem, and (b) *S. frugiperda* larvae raised on a formulated artificial diet. This replicated structure enabled a robust comparative evaluation of larval development under the two different dietary conditions.

Artificial diet-preparation

The artificial diet for rearing *S. frugiperda* larvae was carefully formulated to provide a consistent and nutritionally balanced medium. The complete list of components, including their specific quantities and suppliers, comprised: 200 g of flour (derived from either kidney bean, chickpea, or maize), 30 g of yeast powder (Trinetra™), 1 g of Sorbic acid (DAEJUNG™), 3.5 g of L-(+)-Ascorbic acid (DAEJUNG™), 2 g of Methyl-p-hydroxybenzoate (Sigma-Aldrich®), 2.5 mL of Formaldehyde solution (AnalaR NORMAPUR®), 1 mL of Castor Oil (Prays Pharmaceuticals), 10 g of Agar powder (Agar extrapure, Agar reinst, Agar tres pure, Agar puriss) (bioWORLD®), the contents of two multi-vitamin capsules (Optilets-M®), and 820 ml of distilled water. A visual representation of the ingredients and the prepared diet portions is provided in Figure 1.

Fig. 1. Ingredients used in the formulation of the artificial diet (background) and the resulting formulated artificial diet portions ready for *Spodoptera frugiperda* larva rearing (foreground). (Photo credits: Asma Talib, CABI Insect Biological Control Laboratory, Pakistan).

The preparation commenced by accurately weighing the specified quantity of the chosen flour using a calibrated electronic balance (Mettler Toledo, model B204-S) placed on a level surface. The weighed flour was then transferred into a clean, heat-resistant mixing vessel (a stainless-steel pot of 2 L capacity). Subsequently, all other dry ingredients—yeast powder, sorbic acid, L-ascorbic acid, methyl-p-hydroxybenzoate, and agar powder—were precisely weighed and added to the mixing vessel. Concurrently, formaldehyde solution and castor oil were carefully measured using separate graduated cylinders to ensure accurate liquid volumes. The contents of the multi-vitamin capsules were then expelled and thoroughly ground into a fine, homogeneous powder using a sterile ceramic pestle and mortar. Finally, the distilled water was precisely measured using a graduated cylinder. All pre-measured wet and ground ingredients were then added to the mixing vessel containing the dry components. The entire mixture was thoroughly combined using a sterile stirring rod or an electric mixer until a completely homogeneous suspension was achieved, ensuring an even distribution of all nutrients and preservatives within the diet.

The prepared mixture was then heated over a medium flame on a laboratory hot plate (IKA RCT basic) while being continuously stirred. Heating continued for 5 to 10 min, or until the mixture attained a consistently viscous, paste-like consistency, indicative of the agar fully dissolving and thickening the solution. This continuous stirring was crucial to prevent scorching of the mixture at the bottom of the vessel. Upon reaching the desired viscosity, the thickened mixture was immediately removed from the heat source and aseptically transferred into pre-sterilised containers (autoclavable polypropylene containers or glass jars, sterilised by autoclaving at 121°C and 15 psi for 15 min). The filled containers were then allowed to cool undisturbed at ambient

room temperature (approximately 22-25°C) until the diet solidified. Once completely cooled, the solidified diet portions were sealed and stored under refrigeration at 4°C to maintain their quality, prevent microbial degradation, and preserve their nutritional integrity until required for *S. frugiperda* larval rearing.

Protocol for insect rearing under natural and artificial diets

To evaluate the impact of distinct feeding regimens on the larval development of *S. frugiperda*, two primary rearing protocols were employed: a natural diet-based system and an artificial diet-based system. For the natural diet treatment, fresh maize stems were harvested from field-cultivated plants. The leaf blades were excised, and the remaining stems were precisely cut into small, uniform segments using sterilised scissors to prevent contamination. One gram (1 g) of maize stem tissue was accurately weighed using a calibrated electronic balance and provided as the exclusive food source for each larva within its respective Petri dish. For the artificial diet treatment, a pre-prepared artificial diet (composition as per standard laboratory formulation) was retrieved from the refrigerator at 4°C, and sectioned into uniform 1 g portions using a sterilised scalpel to maintain sterility and ensure consistent food provision. One gram (1 g) of the artificial diet was then placed into each Petri dish containing a larva.

For each dietary treatment, three independent replicates were established. Within each replicate, ten sterile Petri dishes (90 mm diameter) served as individual rearing chambers. Second-instar *S. frugiperda* larvae were carefully selected and individually transferred to each Petri dish using a fine camel hairbrush to minimise physical damage. Petri dishes were inspected daily to monitor larval feeding activity and developmental progression. The respective diets were replaced with fresh portions as required to ensure a continuous nutritional supply, and larval excrement was periodically removed to maintain sanitary conditions within the rearing units. Throughout the experiment, larval development was documented, specifically recording the duration for each larva to reach the pupal stage and the subsequent emergence of adult moths. This entire process, from the initial setup to the recording of developmental milestones, was replicated identically across all three replicates for each treatment to ensure the reproducibility of findings and facilitate robust statistical analysis.

Statistical analysis

All data were statistically analyzed using R software (version 4.4.2; released 2024-10-31, ucrt) to compare the growth and developmental traits of *S. frugiperda* larvae reared on either a natural maize stem diet or a formulated artificial diet under controlled laboratory conditions. Descriptive statistics, including means and standard deviations, were calculated for total larval consumption, sixth instar larval weight, and pupal weight. To evaluate differences in total larval consumption between the two diet treatments, a two-sample t-test assuming unequal variances was employed. Statistical significance was determined at the 5% level ($p<0.05$).

RESULTS AND DISCUSSION

The comparative analysis of *S. frugiperda* larval consumption on natural (maize stem) and artificial diets, as detailed in Table 1 and visually presented in Figures 2A & 2B, reveals distinct feeding patterns under controlled laboratory conditions at the CABI Insect Biological Control Laboratory, Pakistan. A quantitative assessment of total larval diet consumption (1st-6th instar) demonstrated a significantly lower intake by larvae reared on the natural maize diet (Mean ± SD: 432.7 ± 179.3 mg per larva, calculated from replicate means) compared to those fed the artificial diet (Mean ± SD: 587.6 ± 140.2 mg per larva). This trend was consistently observed across all three experimental replicates (Table 1). Specifically, mean consumption on the natural maize diet ranged from 416.0 ± 169.0 mg to 453.3 ± 178.0 mg per larva, while larvae on the artificial diet exhibited a higher range of 552.9 ± 160.0 mg to 624.0 ± 150.0 mg per larva. Larval consumption was measured as the difference between the initial dry weight of the diet provided and the dry weight of the remaining diet, ensuring that water content fluctuations did not influence the measurements. This could be attributed to the optimised nutritional balance and consistent physical properties inherent in the artificial diet formulation. In contrast, natural maize tissues can exhibit variability in nutrient composition and contain secondary metabolites that may function as feeding deterrents or reduce palatability (Scriber & Slansky, 1981). The standardised nature of artificial diets has been previously associated with more predictable and potentially higher feeding rates in insect larvae (van Emden & Service, 2004).

Table 1. Comparison of total larval diet consumption (1st-6th instar), 6th instar larval weight, and pupal weight of fall armyworm (*Spodoptera frugiperda*) fed natural (maize) and artificial diets in a controlled laboratory setting at CABI Insect Biological Control Laboratory, Pakistan

Diet	Replicates	Sample Size (n) Larvae/Pupae	Total Larval Consumption (mg) (Mean ± SD)	6th Instar Larval Weight (mg) (Mean ± SD)	Pupal Weight (mg) (Mean ± SD)
Natural (Maize)	R1	10	416.0 ± 169.0	59.1 ± 4.1	143 ± 19
	R2	10	428.9 ± 191.0	60.9 ± 2.2	137 ± 38
	R3	10	453.3 ± 178.0	61.8 ± 4.3	135 ± 33
Pooled Mean ± SD			**432.7 ± 179.3[b]**	**60.6 ± 3.8[b]**	**138.3 ± 31.9[b]**
Artificial	R1	10	552.9 ± 160.0	71.0 ± 4.9	191 ± 24
	R2	10	624.0 ± 150.0	75.7 ± 4.5	199 ± 44
	R3	10	586.0 ± 106.0	72.0 ± 5.0	182 ± 17
Pooled Mean ± SD			**587.6 ± 140.2[a]**	**72.9 ± 4.9[a]**	**190.7 ± 29.8[a]**

Foot Note: Date of Egg Batch: 16-07-2024; Date of Larvae Emergence: 19-07-2024

Values with different letters in a column are significantly different from each other (independent samples t-test, $P<0.05$).

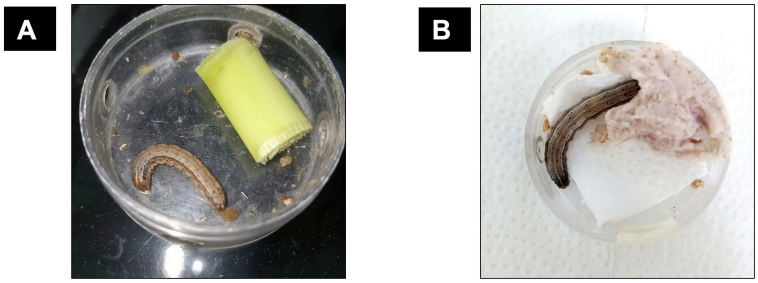

Fig. 2. *Spodoptera frugiperda* larva reared on **(A)** natural (maize stem) diet and **(B)** artificial diet. (Photo credits: Asma Talib, CABI Insect Biological Control Laboratory, Pakistan).

The higher consumption of the artificial diet observed in this study directly influenced larval growth and development. Larvae fed the artificial diet completed their life cycle in an average of 32 days, significantly faster than the 41 days observed for those on the natural diet. This shorter development period aligns with the optimised nutritional profiles of artificial diets, which often result in improved growth rates and condensed developmental timelines in insect rearing (Cohen, 2015). Both diet groups exhibited a 100% larval survival rate. Furthermore, larvae reared on the natural maize diet exhibited reduced pupation rates and yielded lighter pupae (Mean ± SD: 138.3 ± 31.9 mg) compared to those fed the artificial diet (Mean ± SD: 190.7 ± 29.8 mg), as shown in Table 1 and Figures 3A & 3B. These heavier pupal weights associated with the artificial diet group indicate superior nutrient assimilation during the larval stages, leading to enhanced growth and biomass accumulation. This pattern aligns with findings reported for other *Spodoptera* species where artificial diets have positively influenced pupal weight and development (Scriber & Slansky, 1981; Singh, 1983). The observed variability in natural diets can be influenced by factors such as plant age, tissue type, and the presence of defensive compounds (Hunter, 2016).

Fig. 3. *Spodoptera frugiperda* pupae reared on **(A)** natural (maize) diet and **(B)** artificial diet. The artificial diet resulted in a higher rate of successful pupation and greater pupal mass, suggesting improved nutrient processing. (Photo credits: Asma Talib, CABI Insect Biological Control Laboratory, Pakistan).

Furthermore, the artificial diet demonstrated a significantly lower microbial contamination rate (5-10%) compared to the natural diet (20-30%). This reduction in contamination is crucial for maintaining healthy insect colonies and ensuring the validity of experimental results. The accelerated development of larvae on artificial diets, a phenomenon supported by earlier investigations on *Spodoptera* spp. (King & Hartley, 1985; Navon, 2000), also contributes to enhanced cost-effectiveness by shortening generation times and increasing biomass production. Importantly, larvae reared on the artificial diet exhibited a 100% adult emergence rate. The emerged adults successfully mated and laid viable eggs. This demonstrates the *in vitro* life cycle progression of *S. frugiperda* on an artificial diet and highlights the successful reproductive capacity of the females and larval development (Figures 4A-C). These results underscore the value of artificial diets as a practical and advantageous alternative for both fundamental research and large-scale rearing of *S. frugiperda*.

Fig. 4. Successful oviposition and larval development of *Spodoptera frugiperda* on an artificial diet. The figure shows that female adults laid viable egg masses **(A)**, leading to the successful development of larvae within the eggs **(B)**, and the emergence of healthy, first-instar larvae **(C)**. Photo credits: Asma Talib (A & C) and Fazlullah (B), CABI Insect Biological Control Laboratory, Pakistan.

CONCLUSION

In summary, this study unequivocally demonstrates the substantial advantages of artificial diets over natural maize diets for the laboratory rearing of *S. frugiperda*. Larvae fed the artificial diet exhibited significantly increased food consumption, coupled with

higher pupation rates, greater pupal biomass, and remarkably, 100% adult emergence with no morphological abnormalities, enabling successful mating and viable egg laying. These outcomes are indicative of enhanced nutrient assimilation and utilisation. In contrast to the inherent variability and potential contamination risks associated with natural maize, artificial diets offer a standardised and dependable medium conducive to predictable larval development and robust scientific inquiry. The enhanced efficiency and replicability afforded by artificial diets, despite a potentially higher initial cost, strongly advocate for their adoption in both fundamental research and large-scale insect production systems. This finding underscores the significant benefits of artificial diets for the efficient laboratory rearing of this important pest species, aligning with the established advantages of providing consistent and optimised nutrition for insect research and mass production. Future research endeavours should focus on further refining artificial diet compositions to precisely modulate specific biological and ecological traits of this globally significant pest.

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ETHICAL STATEMENT
Not applicable

CONFLICT OF INTEREST
The authors declare no conflict of interest.

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