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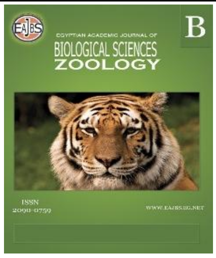

Semi-Artificial Diet Without Agar as a New Approach with Low Cost for Rearing the Cotton Leafworm and the Fall Armyworm as Lepidopteran Insect Pests

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ABSTRACT

The cotton leafworm (CLW), *Spodoptera littoralis* (Boisduval) and the fall armyworm (FAW), *Spodoptera frugiperda* (JE Smith), inflicting significant economic harm by consuming the majority of Egypt's crops. An economical, semi-artificial diet devoid of agar was required to facilitate ongoing biological study on these pests to mitigate their expansion. The survival parameters of the two pests were analyzed during three successive generations on a standard diet of fresh castor bean leaves, *Ricinus communis* L., and a novel experimental diet. Acquiring data that effectively substantiates the technique will facilitate its development into a feasible strategy for managing pest lepidopterans. The expense of growing 1000 newly hatched CLW and FAW larvae to the pupation stage using artificial feeding components is 33 L.E., around $0.70. This is approximately 80% more economical than agar-based alternatives. This technique facilitates future research on more cost-effective gelling chemicals for growing lepidopteran insect pests, simplifying the study and manipulation of these insects.

INTRODUCTION

Cotton (*Gossypium hirsutum* L.), maize (*Zea mays* L.), and *Spodoptera* species (Lepidoptera: Noctuidae) are the most important cultivated plants in the world; the most important pests that attack them are lepidopteran insects. *Spodoptera littoralis* (Boisduval), often known as the cotton leafworm (CLW), is one of the most damaging polyphagous pests of the cotton plant, capable of infesting over 100 different crops and vegetable species (Shonouda and Osman 2000). Furthermore, *Spodoptera frugiperda* (J. E. Smith), the fall armyworm (FAW), has been identified as one of the most important noctuid maize pests worldwide (Malak 2023). According to a recent study on FAW host plants, larvae have the potential to devour 353 different plant species from 76 botanical families (Montezano *et al.*, 2018). Recently studies showed that they can destroy yields and harm crops, severely compromising food security worldwide (Sundufu *et al.*, 2023).

CLW and FAW can cause rapid and significant losses to many valuable crops because of their potent movements, migratory and reproductive capacities, and extensive plant host ranges (Jin *et al.*, 2020). Measuring biological parameters is critical for understanding the fundamental variables for growth, development, and reproduction, as well as the quantity and quality of the food consumed, it is crucial to measure biological parameters (Truzi *et al.,* 2021).

The results of these studies on artificial diets will greatly impact the creation of new integrated pest management (IPM) programs that will stop the spread and destruction of pests. (Pinto *et al.,* 2019). As a result, artificial diets for laboratory insect rearing have developed quickly in response to various scientific and commercial objectives (Ge *et al.,* 2022).
An artificial diet may specifically increase insect fitness compared to insects raised on natural foods. This is because natural feeding is only available during certain seasons. Furthermore, an artificial diet makes it possible to keep raising insects in the lab, which has benefits in terms of quality and time, cost, and space for different lab tests (Ashok *et al.*, 2021; Sayed *et al.,* 2021). Raising insects on their natural host plants is difficult due to seasonal availability, high costs, and fluctuating host plant quality over time (Alfazairy *et al.*, 2012). A number of studies were conducted to establish a rearing technique that could produce a large number of insect individuals of high biological quality utilizing artificial food (Silva and Parra 2013). According to (Hervet *et al.,* 2016), a variety of Lepidoptera species were cultivated on artificial diets as an appropriate medium to establish and maintain a continuous laboratory culture.

The most important nutritional factors in artificial diets are the levels of protein and carbohyd rates, which are essential for insects' growth and development (Sarate et al. 2012). Studies on *Helicoverpa armigera* (Hübner, 1808) (Lepidoptera: Noctuidae) and *Plodia interpunctella* (Hübner, 1813) (Lepidoptera: Pyralidae) have demonstrated that low-protein diets encourage changes in the developmental period, larval weight and mortality, pupation rate, adult emergence percentage, and nutritional indicators (Borzoui *et al.,* 2018; Truzi *et al.*, 2019). Thus, the main components used in diets are, soybeans, peas, wheat germ, potatoes, alfalfa, milk powder, yeast powder, vitamins, ascorbic acid, and agar (Alfazairy et al. 2012; Wang *et al.,* 2019). The development of numerous studies on CLWs and FAWs was made difficult by the generally expensive price of some components. Additionally, rice, one of the primary hosts, has been missing from artificial meals (Nagoshi and Meagher 2004). Rice may therefore improve nutrition and consumption, enabling the generation of high-quality insects (Ashok *et al.*, 2021).

The purpose of this study is to compare and asses the biology and fitness of CLW and FAW larvae that are fed artificial diet and fresh castor leaves, which are the food source for these two pest species.

MATERIALS AND METHODS

Insects and Laboratory Conditions:

Rearing was started for *S. littoralis*. and *S. frugiperda* began with the collection egg masses and some individuals found on a maize farm of Nubaria Agriculture Research Station 47 kilometers from Cairo Alexandria Road as well as from a field infested with FAW adjacent to Nubaria, El Beheria governorate, 30°43'46.5''N, 29°57'27.9''E, Egypt. Mass rearing continued for two generations before starting to record data. At Egypt's Plant Protection Research Institute (ARC), the insects' morphological traits were used to identify and confirm them. Daily observations were made during the experiment, which occurred in the faculty of Education laboratory (25°C, 65°RH, and L12:D12 photoperiod). There were two types of diet for feeding; reference one (control) with castor bean leaves (*Ricinus communis*), and artificial one (Alfazairy et al. 2012) with some performs to be more solidification, gelling texture, and more protein content as appeared in Table 1.

Diet Preparation Method:

The diets are prepared using the composition from Table 1 and cooked for 15 minutes, with ascorbic acid and yeast added shortly after the cooker has cooled. The mixture was subsequently mixed in an electric blender. Formalin, was also added, followed by the remaining ingredients, and everything was completely combined. The diet was poured into 24 clear plastic containers measuring (21 x 14.5 x 9.5 cm). After solidifying and cooling, the containers were covered with plastic lids. All rearing containers were stored at a 4-7ºC until needed. The diet was taken out of the refrigerator and, allowed to acclimate at room temperature for 2-3 hours before use. The containers were stacked closely together, and plastic lids were used to secure them. The containers were arranged in a manner that they sealed one another, and plastic covers were used to fasten the containers after they had been perforated with needles to allow for airflow.

Table 1: Ingredient components for semi-artificial diet.

Ingredient	Quantity taken for preparation per 1 diet
Yellow lentils, *Lens culinaris*	350g
Corn flour	20
Soybean powder	20
Rice, *Oryza sativa*	50g
Brewer's yeast powder	38g
Ascorbic acid	6g
Sorbic acid	9g
Sodium benzoate	4g
Formalin (37-40%)	2ml
Distilled water	1500 ml

Every rearing container containing fresh hatching or egg patches was tightly sealed with a cotton cloth and rubber banded, before being inverted for nine days. The larvae of the first three instars tend to approach the diet in such an upside-down position and feed normally as a consequence. After 5 days from hatching each group consisted of 100 CLW and FAW individuals, with three replicates and reared for three consecutive generations in the laboratory (F1, F2, and F3).

The emergent moths were fed by transforming full-grown pupae in the adult rearing cage, (40 × 20 × 10 cm), using little cups that contained cotton wool saturated with 10% sugar. Every day, the collecting egg masses were placed in glass jars and covered with a muslin cloth until they hatched. To avoid the contaminant of food freshness in the experimental results, we changed the semiartificial diets every 3 days, and the castor leaves were changed daily. We recorded the development and survival of the larvae daily until pupation occurred. We determined the larval instar by the number of molts. The pupae were frail at the initial stage, so we weighed them on an electronic balance on the third day post-pupation. We checked the pupae for survival daily until death or eclosion occurred. After adult eclosion, we documented the adult sex ratio and examined the adults for deformities. The biological parameters obtained were the survival percentages and duration of larvae, pupae, and adults, pupal weight, adult sex ratio, egg fecundity, and hatchability.

Cannibalism Bioassay of FAW:

One hundred third-instar FAW larvae were selected at random from the rearing cultures, divided into two groups, and fed on either castor bean leaves or an artificial diet. This experiment was conducted to see if castor leaves, or an artificial diet influenced the cannibalism percentage of FAW. Larvae were distributed in groups of four per Petri dish and replicated 25 times for each feeding type. The number of larvae that survived in each Petri dish was counted every day.

Data Analysis:

Data was fed to the computer and analyzed using IBM SPSS software package version 20.0. (Armonk, NY: IBM Corp). Categorical data were represented as numbers and percentages. Quantitative data were expressed as range mean and standard error. Two groups were compared using the student t-test for quantitative variables that were normally distributed and the other groups under study were compared using the One-way ANOVA test, which was followed by Post Hoc test (Tukey) for pairwise comparison. The 5% level was used to assess the results significance.

RESULTS

The biological parameters obtained during the life cycle of the two insect pests, CLW and FAW were shown by All recorded data revealed. All growth stages for the two pests fed the artificial diet were marked by the presence of Plates 1 & 2. During three continuous generations, Fig. 1a showed the larval stages of both species on a reference diet and an artificial one. There were no significant differences between the two types of diet in the longevity of the larval stage in FAW, which had a range of 22-24 days approximately. However, in the CLW case, there was slight difference in F1 and F2 (17, 19, 20, and 23 days) in reference and artificial diet, respectively, and no discernible difference between the two diets in F2 (19-20 days).

Because the cannibalism phenomenon only occurred in FAW in the both diets; there was significance difference in its prevalence between natural diet and artificial diets, (Fig. 1b). In the previous diet, there was a shortcoming, which was that the longevity for the larval stage in the artificial diet was more than the reference one with high significance. By using more soybean as a source of protein, this flow was fixed. Fig. 2a pointed to the pupation stage; hence the artificial diet affects clearly its longevity for both insects by highly significant days; finding data revealed that the numbers of days were increased by 2-4 days compared with the reference in CLW and were twice as long in FAW. In addition, there was no significant difference between the generations in all treatments. By observing the weight of pupae in two insects, there were no differences significant between the reference diet and the artificial one in all generations (Fig. 2b). However, there were differences significant between the two insects, hence the CLW (234-249 mg) weight is lower than FAW (314-345 mg) for three generations.

The results of the adult stage were recorded in Fig. 3; firstly, all the moths of two insects were alive on the reference diet with 100% survival in all generations. However, in the artificial diet their range was (96-97%) for CLW and (91-92%) for FAW (Fig. 3a). According to the duration length of alive moths, in the instance of CLW, there a significant difference in F1 between the two diets, with the artificial being higher. In FAW adults, the artificial diet was significantly higher than the reference diet across all generations, as shown clearly in Fig. (3b).

There was no significant sex ratio in either insect over all generations, which was 1:1 (Table 2). The oviposition period of in CLW was in two diets, and range from (5-7 days), but it was different in FAW; thus, it was (3-4 days) in the reference diet and (4-5 days) in the artificial diet with different significance, as shown in (Fig. 4a).

By studying the fecundity and hatchability of two insects in Fig. (4b-c), the findings revealed that in general, the CLW was higher than the FAW in fecundity, particularly when reared on the castor leaves (2320-2507 egg/moth), whereas it was (2280-2345 egg/moth) when fed an artificial diet (Fig. 4b). We found that the FAW's fecundity ranged from 2200-

2333 eggs/moth in the reference diet to 1890-1989 eggs/moth in the artificial diet. When fed on castor leaves in all generations, the hatching percentage was 100% (Fig. 4c). Furthermore, it was less significant when fed on an artificial diet, with a range of 96–97% in CLW and 94–96% in FAW.

The cost of generating and producing insect-rearing media is unquestionably the most significant financial factor. The estimated cost of the artificial diet ingredients used in this study to raise 1000 freshly hatched CLW and FAW larvae to pupation was 33 L.E., or roughly $0.70.

Plate 1: CLW growth stages from hatching to pupation on a semi artificial diet devoid of agar

Plate 2: FAW growth stages from hatching to pupation on a semi artificial diet devoid of agar

Table 2: Comparing the two different diets that CLW and FAW are fed concerning the sex ratio.

	Sex ratio Male: Female		
	F1	**F2**	**F3**
	Mean ± SE	**Mean ± SE**	**Mean ± SE**
Control CLW	$50^a \pm 0$	$50^a \pm 0$	$50^a \pm 0$
Diet CLW	$50^a \pm 0$	$50^a \pm 0$	$50^a \pm 0$
Control FAW	$50^a \pm 0$	$50^a \pm 0$	$50^a \pm 0$
Diet FAW	$50^a \pm 0$	$51.7^a \pm 1.7$	$50^a \pm 0$
F	–	1.00	–
p	–	0.441	–

F: Generation number
SE: Standard error 3 replica for each group
F: F for One way ANOVA test, pairwise comparison between each 2 groups were done using Post Hoc Test (Tukey)
Data was expressed using Mean ± SE.
p: p value for comparing between the studied groups
*: Statistically significant at $p \leq 0.05$
Means with totally Different letters (a-c) are significant

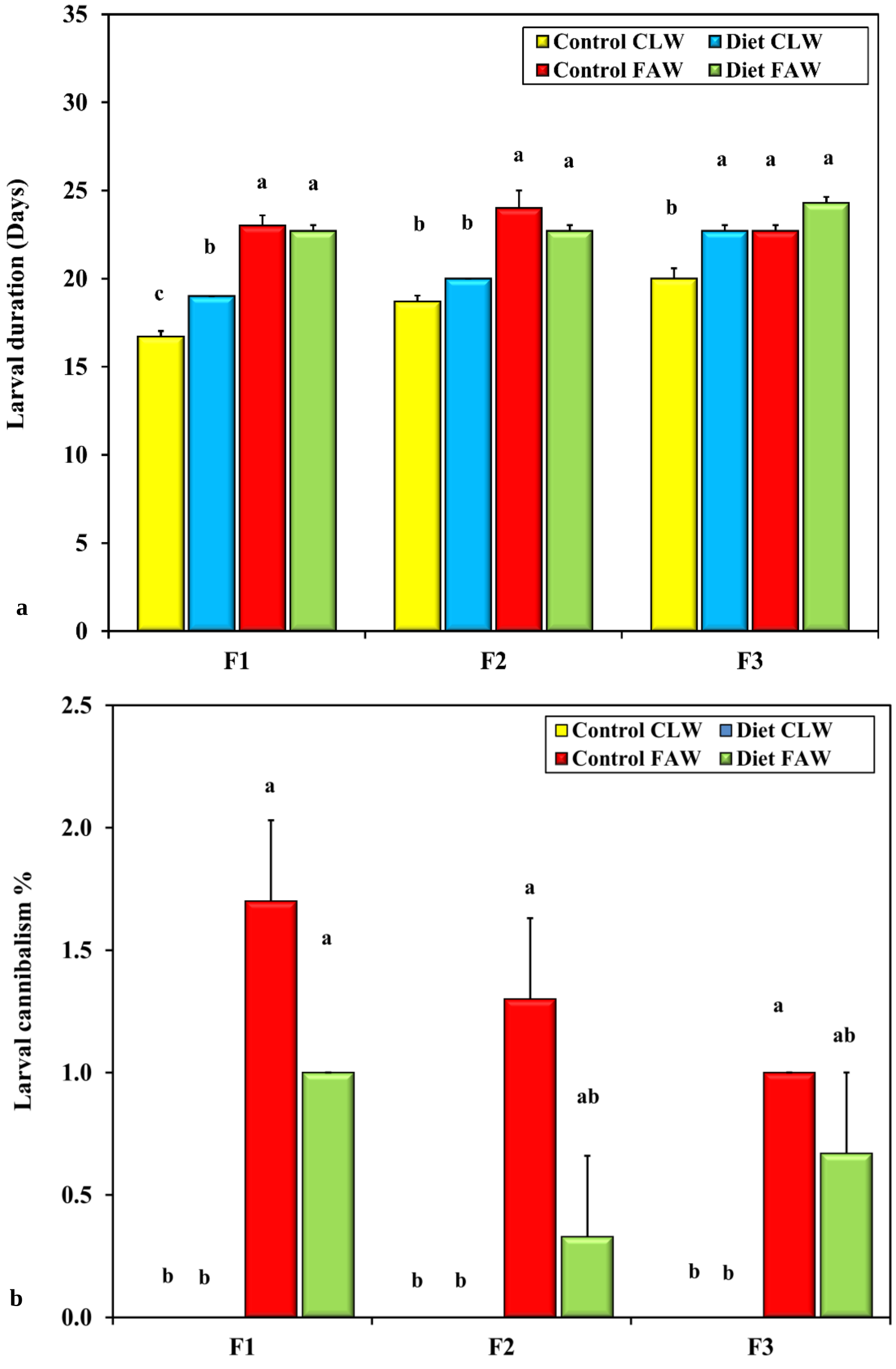

Fig. 1: Compares the two different diets supplied to CLW and FAW during their larval stage. (**a)** The Effect of the two different diets on the larval duration (**b)** the larval cannibalism

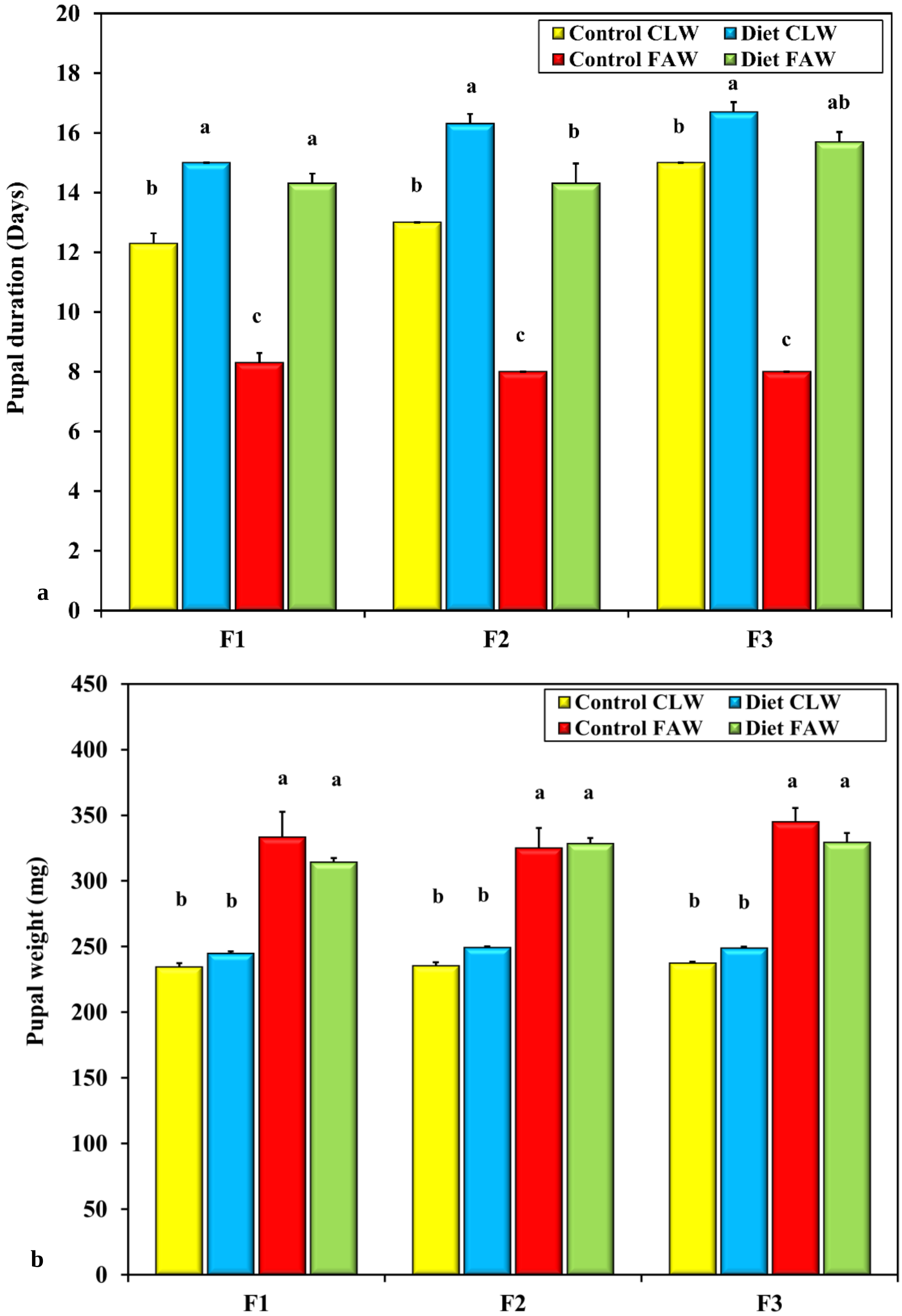

Fig. 2: Comparison of the two different diets fed to CLW and FAW during their pupal stage. (**a)** The impact of the two diets on the pupal duration time and **(b)** the pupal weight

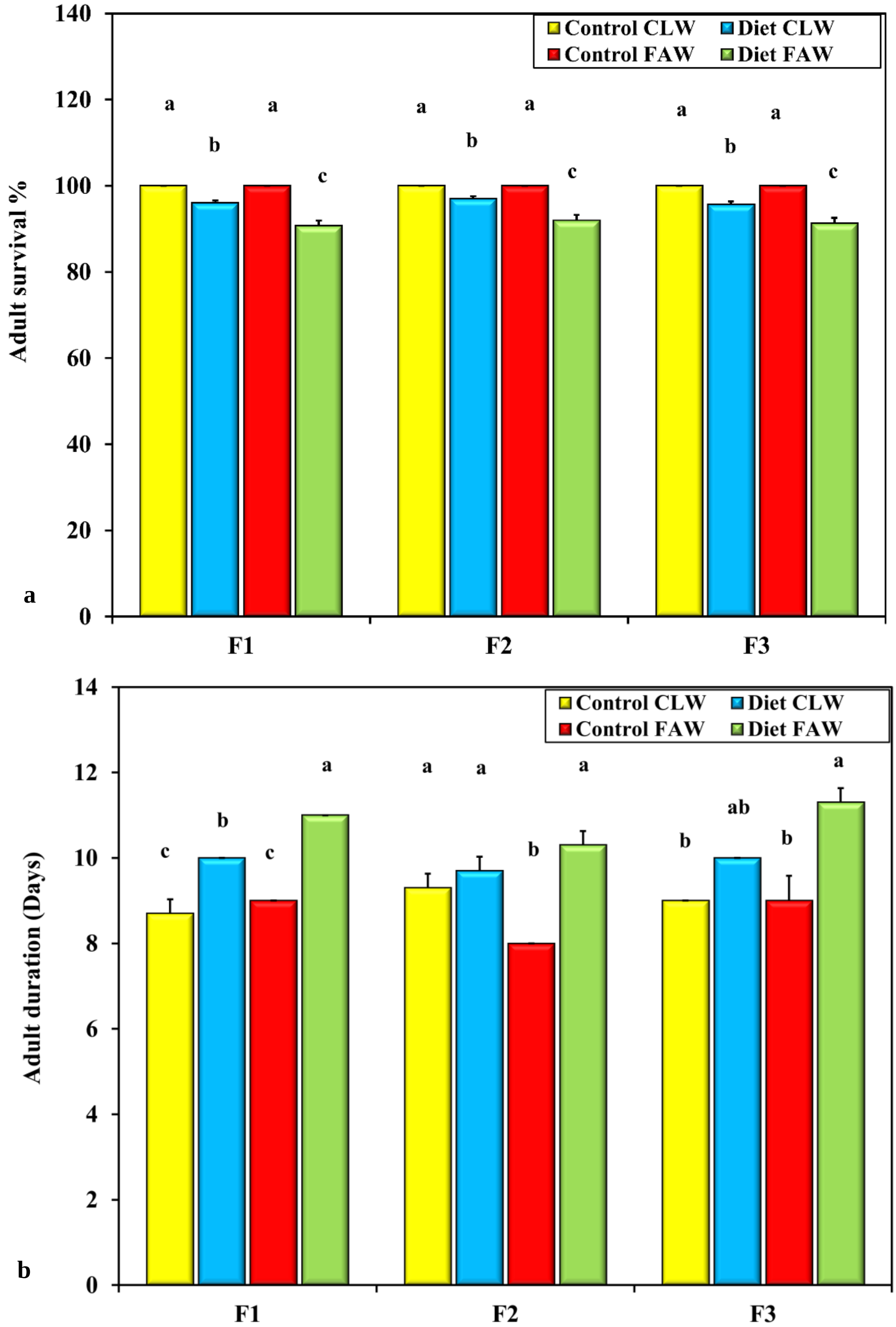

Fig. 3: Comparison the two different diets fed to CLW and FAW at the adult stage. (**a)** The impact of the two different diets on the adult survival and **(b)** adult duration.

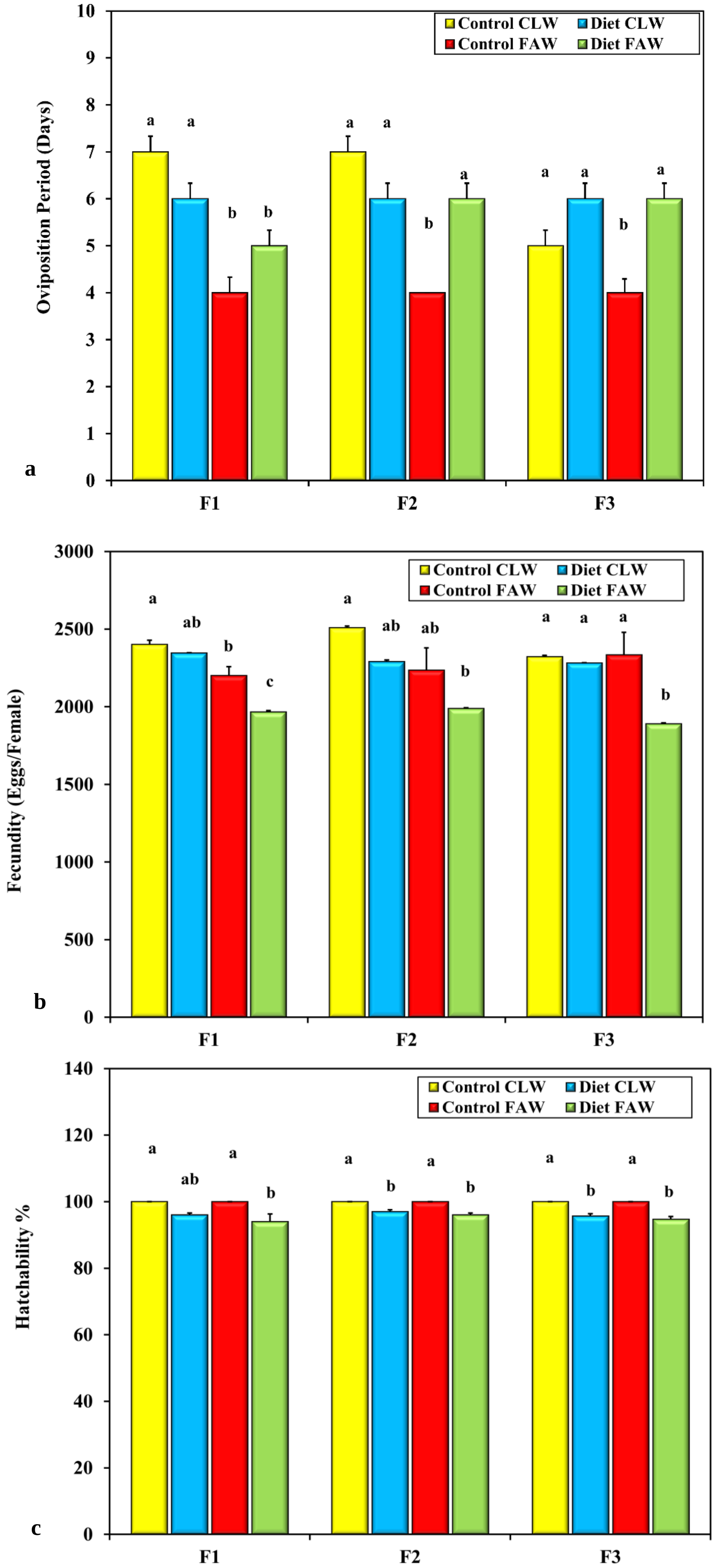

Fig. 4: A Comparison of the oviposition performance of the two distinct diets fed to CLW and FAW. (**a**) How the two distinct diets affected the oviposition period, **(b)** fecundity, and **(c)** hatchability

DISCUSSION

Being provided a natural diet, the FAW and CLW's survival parameters matched those published by (Kotey *et al.,* 2017) and also the results of cannibalism; however, it was not the same as obtained by (Jin et al. 2020) who found there was no cannibalism when served by an artificial diet.

The life span parameters of six lepidopteran species, including FAW, were studied on three artificial diets made up of agar and one natural dried leaf mixed into sorghum, maize, or sugarcane. The percentage of larval survival and pupation was increased across all artificial diets, which is synchronizing with the current study of just the pupal stage. (Strydom *et al.,* 2024).

The larval developmental period on an artificial diet ranged between 12 and 14 days in a study done on four types of artificial agar diets, some of which including soybean among other ingredients. Males and females had varying pupal mass levels, with males weighing 173.5 and 232.2 mg and females weighing 200.0 and 236.8 mg. (Jin *et al.,* 2020) which corresponded with. the findings of this study.

In addition to the pupal period of approximately 10 days, the pupal mass on various artificial diets ranges from 238.0 and 252.7 mg, and the survival rate is 84-90%. The larval survival rate on an artificial diet which, ranged from 40% to 82% (Martínez *et al.,* 2004; Sundufu et al. 2023), which corresponds with the data in this study relating exclusively to the pupal stage. Concerning the current study, the pupal length was reported by (Lynch *et al.,* 1989) as 22-29 days, which is longer than usual.

The protein value of FAW is determined by its amino acid content and digestion, efficient and least costly diet for rearing FAWs was the one with the largest percentage of corn flour (17%) out of all the diets. Since maize is the primary host plant for FAW by nature, eating a diet high in corn may improve the nutrition and consumption of FAW (Marcomini *et al.,* 2015; Jin *et al.,* 2020).

The high fecundity, adult survival rate, and intrinsic rate of rise noted in this study may be attributed to corn flour and soybeans, which agreed with reported by (Jin *et al.,* 2020).

Conclusion

To sum up, this study successfully mass-reared CLW and FAW on the agar-free semiartificial diet, demonstrating no discernible tendency toward a reduction in development or reproduction for three successive generations. The method provides a new avenue for future research into less expensive gelling agents and is nearly 80% less expensive than agar-based diets. On this larval feeding medium, additional phytophagous insects can be mass-reared.

Here are the natural rearing settings as well as the life history parameters and conditions revealed in this study, were compared to those published it comparable, studies.

Declarations:

Ethical Approval: The article doesn't include any studies on the harmful exposure of materials to humans or animals.

Competing interests: The author states that there are no competing interests to declare.

Author's Contributions: HA had a key role in developing the concept and the approach for this investigation. MM and HA carried out the experiments and gathered the information. The two insects were raised by MM on a natural diet and by HA on an artificial diet. Writers HA and MM drafted the manuscript. Manuscript writing, review, editing, data curation, and visualization were all done by MM and HA. The final manuscript was read and approved by both writers.

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Availability of Data and Materials: The datasets utilized and analyzed during this investigation are available upon reasonable request from the corresponding author.

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