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## Assessment of cohort laboratory rearing on performance and biology of the fall armyworm, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae)

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### Abstract

Fall armyworm (FAW), *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae), is one of the main threats to food security and farmers' livelihoods worldwide. For developing an effective management against this pest, improving the rearing technique and basic information on its biology is required. Therefore, this study was designed to assess the cohort rearing on each developmental stage and reproductive behavior of FAW feeding on castor oil leaves under laboratory conditions. Results revealed that the larval development lasted (12-19 days) while suffered (13%) mortality in their early instars. The percentage of larvae succeeded to pupal stage was (68%). When larvae reared in cohorts, about (3%) cannibalism only were recorded. The period required for adult emergency was (7.70 days) with (68.04 %) survival. Moreover, the average male to female sex ratio was (1: 1.06) with non-significantly difference. The wing malformation in females (15.67%) was significantly higher than males (1.82%), while the longevity was insignificantly affected. Females emerged earlier and began to lay their eggs through (3.33 days) and the oviposition continued up to (4-7 days). Single fertile female laid about (1225 – 2363 eggs) throughout its lifespan. The incubation period of egg stage was (2.19 days) with (97.92 %) of hatchability. Eventually, the total life period lasted about (23-30 days). Therefore, Cohort-based method is recommended for rearing FAW in laboratory without mandatory effect on their larval cannibalism, survival and hence adult fecundity.

**Keywords:** *Spodoptera frugiperda*, developmental duration, cannibalism, reproductive behavior, cohort-rearing

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### Introduction

Agriculture is the main key of economy in the developing countries. As a result of the transboundary movement of agricultural commodities and trade, traditional quarantine programs, human practices and climatic change; about 1300 invasive alien species (IAS) have been spread fast across 124 countries (Paini et al., 2016) <sup>[1]</sup>. These species caused extensive losses to the agricultural production, threatened food security, exposed farmer livelihood at risk, and ultimately leveled down national economy (Sharanabasappa et al., 2018) <sup>[2]</sup>. Among them, the fall armyworm (FAW), *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) is threatening more than 353 cultivated economical crops (Montezano et al., 2018; CABI, 2020) <sup>[3, 4]</sup>. Recently, FAW was recorded as "A<sub>1</sub> Quarantine pest" due to its high spread ability into new geographic areas, high fecundity rate, long-distance migratory behavior over 500 km (300 miles), polyphagous insect with continuous generations throughout the year, adaptable to climate conditions and strong flier moths up to 100 Km per night (Prasanna et al., 2018; FAO, 2019) <sup>[5, 6]</sup>. Since 2016, it invaded the African continent and spread widely to more than 28 countries (FAO, 2018) <sup>[7]</sup>. Afterwards, the first appearance of FAW in Egypt was in May 2019 in Aswan Governorate, Upper Egypt as reported by the Agricultural Pesticide Committee (APC), Ministry of Agriculture (Dahi et al., 2020) <sup>[8]</sup>. Within a short period of its invading, it has further spread almost all the Governorates of Egypt in 2021.

The economic importance of this new pest led researchers worldwide to study its biology on different host plants or artificial diets in laboratories to manage it before destroying the crop fields or to use it as a factitious host of natural enemies. However, almost of these previous studies based on rearing FAW individually to avoid the cannibalistic behavior between their larvae (Chapman et al., 2000; Busato et al., 2006; Modolon et al., 2017; Montezano et al., 2019; Pinto et al., 2019; Gamil, 2020; Navasero and Navasero, 2020; Ashok et al., 2021; Kranthi et al., 2021; Navasero et al., 2021; Putra and Khotimah, 2021; Ramzan et al., 2021; Salem et al., 2021; Sari et al., 2021) <sup>[9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22]</sup>.

In addition, it is difficult to maintain the FAW cultures on its natural host (maize) for a long period under laboratory condition. Therefore, improving the FAW rearing is required before the effective management. For this purpose, this study assessed the effect of cohort-based method on the performance and biology of FAW when reared on castor oil leaves under laboratory condition. Based on our knowledge, there is no detailed biology information of FAW related to cohort rearing.

## Materials and Methods

### *S. frugiperda* Stock Culture

Larvae of *S. frugiperda* (F<sub>0</sub> generation) used in the study were initially collected from farmers' infested maize fields (Fig 1) in Assuit Governorate, Upper Egypt. Plant whorls with damage symptoms were removed from the field and in suitable bags transferred to Assiut insect rearing laboratory, Plant Protection Research Institute, Agricultural Research Center (ARC), for inspection and identification. Different instars of collected larvae were divided in clean plastic container (25 cm diameter x 15 cm height) and reared on fresh maize leaves (natural preferable host), until pupation. Pupae were collected and placed on wooden cages (35 × 35 × 35 cm<sup>3</sup>) until adult moth emergence. Moths were fed by 10% honey solution soaked on cotton pads, hanged inside the cage and renewed daily till all adult died. The inner walls of the cage were covered with zigzag-sheets of (A<sub>4</sub>) white copy paper and branches of oleander (*Nerium oleander* L.) as ovipositional site, and inspected daily. Neonate larvae (F<sub>1</sub>) were reared on fresh castor oil leaves (*Ricinus communis* L.) until pupation. Then, they were transferred to new cages for adult emergency and egg-laying. The FAW was reared under laboratory conditions (27 ± 1 °C; > 60% RH and 12L: 12D) for using in the experiments.



**Fig 1:** Initial culture of the fall armyworm, *S. frugiperda* larvae obtained from infested maize fields in Assuit Governorate, Upper Egypt.

### Experimental Design for *S. frugiperda* Cohort Rearing and Their Biological Parameters

Egg-masses were collected from the cultures daily to start the experiments, and the others were used to maintain the insect colony. A number of 250 neonate larvae with similar time of hatching were divided into 10 equal groups (25 larvae/ group) and placed in transparent plastic boxes (19 x 14 x 7 cm) supplied with fresh castor leaves for feeding under the aforementioned rearing conditions. These leaves were replenished every two days till reaching to the 3<sup>rd</sup> instar. Thereafter, the abundant leaves were supplied daily because the larvae grew and consumed almost food. The boxes were cleaned from dried food and feces daily to avoid any contamination. Once the full developed larvae (6<sup>th</sup> instars) reached to pre-pupae (non-mobile and non-feeding stage), they were transferred to new plastic boxes filled with sawdust and dried leaves to pupate.

### Larvae

The surviving larvae were checked daily till pupation to determine the durations of each larval instars (developmental period), total larval duration (from neonate to pupae), full grown larval weight (g), dead larvae%, cannibalism%, larval survival% (the number of larvae reached pupation) and malformed larvae% (failed to complete pupation).

### Pupae

Newly formed pupae were collected and placed individually in glass tubes (2 cm diameter x 7 cm height) plugged tightly with cotton. The pupae were checked daily till the moth emergence to calculate the pupal period (number of days for adult emergency), pupal weight (g) at 24h, pupal survival% (the number of emerged adult), and dead pupae %.

### Adults

The emerged moths were observed to calculate their sex-ratio% ( $\text{♂}:\text{♀}$ ), adult weight (g), and adult malformation% either in wings or difficulty in emergence (adults were attached to the pupal exuviae). To investigate longevity and fecundity of adults, each paired (1♂, 1♀) of 15 couples were put in plastic container (2L capacity); allowing moths to mate and oviposit. Each container provided with 10% honey solution cotton pads for feeding, and the sheet of A<sub>4</sub> white paper and *N. oleander* branch for oviposition. The adult container checked daily to determine the pre-oviposition period (the period between female emergence and the first egg-mass incidence), oviposition period (egg-laying period), and post-oviposition period (the period between female stopped laying eggs and the death). Longevity of male and female adults was recorded as the period from adult emergence till their death. Newly laid egg-masses were placed in new plastic cups (7 cm upper diameter x 5 cm bottom diameter x 5 cm height), and the total number of deposited eggs/female during her lifespan (fecundity) were counted with the aid of a binocular light microscope.

### Eggs

Each sample of egg-masses were examined at 12 hrs interval to calculate the time of hatching (incubation period) and the number of neonates from each egg-mass (hatchability %). Total life period (days) from egg-laying to adult emergence was determined.

### Data Analysis

Data regarding the adult stage (body weight, sex-ratio, malformation, longevity) were analyzed by *t*-test at ( $P \leq 0.05$ ) level for comparisons. All calculations were used by Microsoft Excel<sup>®</sup> software according to Fowler et al. (1998) [23].

### Results

FAW successfully completed its development when reared in cohorts based on the detailed results.

### Larval Stage

FAW larvae developed into 6 instars till pupal formation which lasted about (12-19 days) with a mean of (14.38 days) at 27° C (Table 1). The longest developmental duration was observed in 1<sup>st</sup> larval instar (4-6 days), followed by 6<sup>th</sup> instar (2-5 days) than the other instars. In addition, the majority of mortality occurred during larval growth was noticed in early instars from new hatched (neonates) and 1<sup>st</sup> instar with averaged about (13 %). Full grown larvae (6<sup>th</sup> instar) consumed almost castor leaves and exhibited the heaviest weight (0.26-0.61 g) when reared in cohorts. The percentage of larvae succeeded to complete pupation (larval survival %) was (68%) and (16%) failed (malformed). For the first time, the cannibalistic behavior of FAW was appeared on the 4<sup>th</sup> instar larvae. Thereafter, the most cannibalistic and aggressive instars was the 6<sup>th</sup> one feeding on the younger age larvae than those of the same age, but preferred the pre-pupae (non-mobile stage) (Fig 2). Cohort rearing of FAW led to (3%) cannibalism only between their larvae.

**Table 1:** Larval developmental period (days), larval weight (g), larval survival (%), dead & malformed larvae (%), and cannibalism (%) of fall armyworm, *S. frugiperda* under cohort-rearing method

	Parameters	Mean ± SE	Range
Larval development (days)	1 <sup>st</sup> instar (d)	4.20± 0.06	4-6
	2 <sup>nd</sup> instar (d)	3.39± 0.05	3-4
	3 <sup>rd</sup> instar (d)	2.07± 0.09	1-3
	4 <sup>th</sup> instar (d)	1.58± 0.05	1-2
	5 <sup>th</sup> instar (d)	1.33± 0.05	1-2
	6 <sup>th</sup> instar (d)	3.22± 0.10	2-5
	Total larval duration	14.38± 0.16	12-19
	Larval weight (g)	0.40± 0.01	0.26-0.61
	Larval survival (%)	68.00± 5.15	55-80
	Dead larvae (%)	13.00± 4.06	5-25
	Malformed larvae (failed) (%)	16.00± 3.48	10-20
	Cannibalism (%)	3.00± 0.63	0-10



**Fig 2:** Cannibalistic behavior of *S. frugiperda* larvae fed on castor oil leaves, *R. communis* under cohort laboratory rearing.

### Pupal Stage

The duration required to emerge FAW adult was (7-12 days) with an average of (7.70 days) and the percent of survival (adult emergence %) was (68.04 %) (Table 2). Male and female were distinguished on the formed pupae by the genital opening and anal slot distance which in male pupae was shorter than females (Fig 3).

**Table 2:** Effect of cohort-rearing method of *S. frugiperda* on the pupal period (days), pupal weight (g), pupal survival (%) and dead pupae (%)

Parameters	Mean $\pm$ SE	Range
Pupal period (d)	7.70 $\pm$ 0.12	7-12
Pupal weight (g)	0.17 $\pm$ 0.001	0.12-0.26
Pupal survival (%)	68.04 $\pm$ 3.70	45.45-83.33
Dead pupae (%)	31.96 $\pm$ 4.59	16.17-54.54



**Fig 3:** Male and female pupae of *S. frugiperda* distinguished by the genital opening and anal slot distance.

### Adult stage

When FAW larvae reared under cohort conditions, the body weight of emerged male moths (0.17 g) didn't vary considerably from female (0.19 g) (Table 3, Fig 4). Moreover, the average male to female sex ratio was (1: 1.06)

with non-significantly difference. In the opposite, the malformation appeared in females (15.67%) were significantly higher than males (1.82%) (Fig 5). Longevity of FAW moths was insignificantly affected by rearing in cohort for both male and female. Despite of the females lived slightly longer than the males.

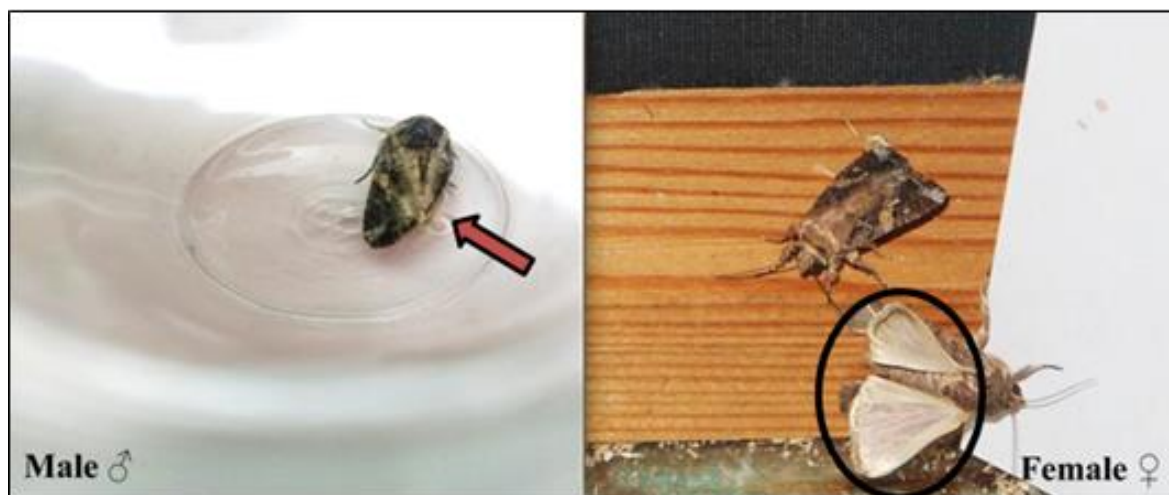
**Table 3:** Effect of cohort-rearing method of *S. frugiperda* on the adult weight (g), sex-ratio (%), adult malformation (%) and longevity (days)

Sex	Adult weight (g)	Sex-ratio (%) (♂:♀)	Adult malformation (%)	Longevity (days)
Male (♂)	0.17± 0.01 <sup>a</sup> (0.08-0.37)	48.63± 3.94 <sup>a</sup> (30-75)	1.82± 1.63 <sup>b</sup> (0-9)	7.12±0.48 <sup>a</sup> (6-9)
Female (♀)	0.19± 0.01 <sup>a</sup> (0.12-0.37)	51.37± 4.06 <sup>a</sup> (25-70)	15.67± 2.17 <sup>a</sup> (8-20)	8.25±0.41 <sup>a</sup> (7-10)

Means ±SE sharing the same small letters in the same columns are statistically insignificant ( $P > 0.05$ ). Numbers in parentheses represent the range.



**Fig 4:** Male and female adult of *S. frugiperda* emerged from cohort laboratory rearing.



**Fig 5:** Male and female adult of *S. frugiperda* showing malformation in the wing.

#### FAW Adult Reproduction and Egg Stage

Females of FAW emerged earlier than males and the mating occurred after (2-3 days) of adult emergency. Then, females began to lay their eggs through (1-2 days) after mating from night till dawn. Sometimes, the females laid eggs during (1-3 hrs) after mating. The mean period from female's emergency to first egg-mass laid was (3.33 days) and the oviposition continued up to (4-7 days) (Table 4). Single fertile female laid eggs in batches with different layers and their numbers counted about (1225 up to 2363) throughout her lifespan. The highest amounts of eggs/female were laid during the first 3 to 4 days of the oviposition period. The incubation period of these

deposited eggs ranged (2-2.5 day) with (97.92 %) of hatchability. Afterwards, the total life period from egg to adult emergence was (23-30 days) with an average of (26 days) under cohort rearing conditions.

**Table 4:** Effect of cohort larval feeding of *S. frugiperda* on their adult reproduction and egg stage

Parameters	Mean $\pm$ SE	Range
Pre-oviposition period (d)	3.33 $\pm$ 0.14	3-4
Oviposition period (d)	5.33 $\pm$ 0.38	4-7
Post-oviposition period (d)	1.67 $\pm$ 0.33	1-3
Fecundity (No. of eggs/female)	1810.33 $\pm$ 379.80	1225-2363
Incubation period (d)	2.19 $\pm$ 0.05	2-2.5
Hatchability %	97.92 $\pm$ 1.39	84.5-100
Egg to adult (d)	26.00 $\pm$ 0.87	23-30

## Discussion

Recently, FAW is one of the most threats to food security and farmers' livelihoods (Sharanabasappa et al., 2018) <sup>[2]</sup> due to their alarming spread in different regions worldwide and larval feeding habitat not only on economic and cash crops but also weeds and non-crop species (CABI, 2020) <sup>[4]</sup>. Mass-rearing of this alien pest is a fundamental and crucial tool for understanding the basic information on its performance and biology as developmental period, susceptible growth stage, mortality, survival, longevity and reproductive behavior before developing an efficient integrated management programs. Until now, almost studies have been done on rearing FAW individually to avoid the cannibalistic behavior of their larvae as mentioned by (Chapman et al., 2000; Busato et al., 2006; Da Silva and Parra, 2013; Modolon et al., 2017; Montezano et al., 2019; Pinto et al., 2019; Gamil, 2020; Navasero and Navasero, 2020; Ashok et al., 2021; Kranthi et al., 2021; Navasero et al., 2021; Putra and Khotimah, 2021; Ramzan et al., 2021; Salem et al., 2021; Sari et al., 2021) <sup>[9, 10, 24, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22]</sup>. However, this rearing method is being unpractical, more efforts needs, high-cost, and time- and resource-consuming, even in small or mass production. A major challenge in rearing FAW is the need for providing sufficient numbers of their certain life cycle stages at a time for the beginning of different experimental tests to improve the solution against them. From this stand point, the present study evaluated the FAW cohort rearing instead of individually on its biology when fed on castor oil leaves. Obtained results revealed the total larval development (12-19 days) at 27° C is consistent somewhat with the observations noted previously by researchers when reared FAW on natural hosts or artificial diets individually (Sharanabasappa et al., 2018; Pinto et al., 2019; Ramya et al., 2019; Tendeng et al., 2019; Kalyan et al., 2020; Lekha et al., 2020; He et al., 2021; Keerthi et al., 2021; Kranthi et al., 2021; Ramzan et al., 2021; Wu et al., 2021) <sup>[2, 13, 25, 26, 27, 28, 29, 30, 17, 20, 31]</sup>. In contrast, the larval duration (21.4 days) was increased at 26° C as reported by (Gamil, 2020) <sup>[14]</sup> on castor leaves; (Salem et al., 2021) <sup>[21]</sup> was (23.36, 23.58 and 22.8 days) on three different hosts of castor leaves, corn and lettuce, respectively; and by (Wu et al., 2021) <sup>[31]</sup> was (21.23 and 25.18 days) when fed on tomato and pepper, respectively. These variations in larval durations may be related to the nutritional quality of each reared diet (Cunha et al., 2008) <sup>[32]</sup> and/or the differences in rearing conditions because almost of these previous findings were obtained from reared FAW individually but the present results was in cohorts. For laboratory studies, a major obstacle of rearing FAW in cohorts rather than individually is avoidance the cannibalistic behavior between their larvae and maintenance the cultures. Cannibalism is a natural behavioral characteristic observed in many animal species (Fox, 1975) <sup>[33]</sup>, but it is common in lepidopteran larvae (Dail and Adler, 1990; Reed et al., 1996) <sup>[34, 35]</sup>; causing substantial levels of mortality and influencing significantly on population structure (Fox, 1975; Richardson et al., 2010) <sup>[33, 36]</sup>. Based on the observation reported by (Chapman et al., 1999a; Bentivenha et al., 2017) <sup>[37, 38]</sup> that cannibalism increased when different larval instars contact with each other competing for resources such as food and space. Indeed, our results confirmed the cohort rearing method led to low level rate in larval cannibalism (3%) and thus didn't affect negatively on their survival. Subsequently, larval cannibalism is not the main mortality factor in rearing FAW in groups as long as nutritional food quantities and space in the containers are available. Additional support to this assumption reported by (Grobler, 2019) <sup>[39]</sup> that cannibalism was less when the FAW larvae in cohort fed on castor leaves but was more frequent on maize. In addition, (Da Silva and Parra, 2013) <sup>[24]</sup> observed this behavior was more on maize rather than on artificial diets and (Chapman et al., 1999b) <sup>[40]</sup> recorded about 40 to 83% cannibalism when the same-age larvae reared on maize leaves. These variations in cannibalism level in FAW larvae fed on castor, maize or artificial diets might be due to castor oil leaves had the required nutritional value more than maize leaves or it may be contain substances which alter the bacterial community within the digestive system of their larvae, and thus could in turn affect the feeding status of the larvae (Dyer et al., 2008; Da Silva and Parra, 2013; Maheshwari and Kovalchuk, 2016; Grobler, 2019) <sup>[41, 24, 42, 39]</sup>. Besides food suitability, the larval cannibalism may be influencing by insect density, temperature, humidity (Raffa, 1987; Richardson et al., 2010) <sup>[43, 36]</sup>, and a specific need to proteins or other nutrients rather than hunger (Alzubaidi and Capinera, 1983; Xiao et al., 2010) <sup>[44, 45]</sup>. Under cohort rearing conditions, the pupal period (7.7 days) is in accordance with those noted by (Tendeng et al., 2019; Kranthi et al., 2021) <sup>[26, 17]</sup> which is lasted about (6.34-7 days); and about (8.5-9 days) by (Débora et al., 2017; Ramya et al., 2019; Kalyan et al., 2020; Keerthi et al. 2021; Ramzan et al., 2021; Russiani et al., 2021) <sup>[46, 25, 27, 30, 20, 47]</sup> when their larvae reared individually on different artificial diets. Slightly different results in the

period extended to (10.89 days) as mentioned by (Wu et al., 2021; Adjaoke et al., 2021; Salem et al., 2021) [31, 48, 21]. Besides, the pupal weight is in general agreement with those obtained from larvae fed individually on natural hosts or artificial diets with different nutritional content (Giongo et al., 2015; Pinto et al., 2019; Adjaoke et al., 2021; He et al., 2021; Salem et al., 2021; Wu et al., 2021) [49, 13, 48, 29, 21, 31]. About 17 % of FAW adults emerged with deformed wing when their larvae fed on castor leaves; however (da Silva Lopes et al., 2008) [50] noted 22% of adult deformation was from cassava leaves. This wing deformation according to (Parra, 2001) [51] may be due to the deficiency in fatty acids (linoleic and linolenic) or by their interactions with temperature. In addition, (Pinto et al., 2019) [13] recorded the highest rate (64.7%) of wing deformation in adult emergence after rearing on 3 artificial diets with different nutritional ingredients, and thus may be related to the relative amounts of palmitic, oleic, and linoleic fatty acids present in the diets. No significant difference observed in adult sex ratio (1: 1.06) and this present observation is in close proximity with that recorded previously by (Navasero et al., 2021) [18]. The fecundity observed in this study (1225 up to 2363) was similar somewhat with that recorded by (Busato et al., 2006; da Silva Lopes et al., 2008; Da Silva and Parra, 2013; Prasanna et al., 2018; Pinto et al., 2019; Gamil 2020; Kalyan et al. 2020; Navasero and Navasero 2020; Salem et al., 2021; Wu et al., 2021) [10, 50, 24, 5, 13, 14, 27, 15, 21, 31]. However, lower fecundity was reported by (Murúa et al., 2008) [52] who counted 955.05, 885.89, 519.83, 978.10, and 758.89 eggs/female from maize, alfalfa, soybean, wheat, and weeds, respectively and 981.76 eggs by (Ramzan et al., 2021) [20]. These inconsistency observed in the fecundity may be due to feed larvae on different diets with varied nutritional contents (quality and quantity of ingested food); thus influencing their reproductive capacity. Longevity of FAW moths was insignificantly affected by the cohort rearing and this concept was in close agreement to those mentioned by (Ramya et al., 2019; Lekha et al., 2020; Kranthi et al., 2021) [25, 28, 17]. Moreover, the period required for egg's hatching (< 3 days) in this study was in full accordance with the previous results by (Ramya et al., 2019; Tendeng et al., 2019; Kranthi et al., 2021; Ramzan et al., 2021; Russiani et al., 2021) [25, 26, 17, 20, 47]; however slightly higher period (> 3 days) recorded by (Kalyan et al., 2020; Lekha et al., 2020; Salem et al., 2021) [27, 28, 21].

### Conclusion

Cohort-based method is recommended for rearing FAW larvae; allowing them to complete development with high numbers and producing high fecund adults similar to the standard spread method of rearing larvae individually. Moreover, larval cannibalism is not the main factor of larval mortality in cohorts as long as nutritional diet quantities and space in the containers are available. Castor leaves are the standard recommended host for FAW rearing instead to natural diet under laboratory condition. This study revealed the detailed information on the performance and development of this invasive pest when reared in cohorts before developing an efficient integrated management programs.

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### Conflict of interest

The authors do not have any conflict of interest regarding the publication of this manuscript.

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