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Biology and Morphometrics of Fall armyworm (*Spodoptera frugiperda*) in Chitwan, Nepal

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Abstract

The Fall armyworm (FAW) *Spodoptera frugiperda*, indigenous to the American continents, is an important invasive and polyphagous pest threatening cereal production. Abiotic and biotic factors, including climatic uncertainty and insect pests like *S. frugiperda*, pose threats. Detected in East Africa in 2016 and reported in Nawalpur, Nepal, on May 9, 2019, FAW has led to a 34% decline in maize yields. Studying FAW's biology and morphometrics is crucial for understanding its habitat and ecology, essential prerequisites for adopting integrated management strategies. Biological parameters such as incubation period, larval and pupal duration, pre-oviposition and, oviposition periods, male and female adult longevity, larval and pupal mortality, sex ratio (male: female), and morphometrics of egg, larvae, pupa, and adult were measured. The incubation period (2-3 days), larval and pupal periods (13-18 and 9-12 days) with overall life duration (31-45 days) were recorded in maize. The head capsule width measurements for the first through sixth instars were documented as follows: 0.52±0.05 mm, 0.73±0.05 mm, 1.1±0.05 mm, 1.74±0.12 mm, 2.04±0.15 mm, and 2.91±0.23 mm, respectively. The average body lengths for distinct larval instars were recorded as follows: 3.33±0.26 mm for the first instar, 6.92±1.07 mm for the second instar, 13.11±1.45 mm for the third instar, 16.82±1.57 mm for the fourth instar, 28.68±1.75 mm for the fifth instar, and 34.56±1.99 mm for the sixth instar.

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Introduction

Maize (*Zea mays* L.), also known as the "queen of cereals," is a highly versatile crop used for a range of purposes such as food, feed, fodder, and diverse industrial products (Ranum et al., 2014) (Prasanna, 2012). Due to its tremendous genetic variability, maize is one of the most extensively cultivated crops globally i.e. tropical, subtropical, and temperate climates (Ramirez-Cabral et al., 2017). Maize cultivation spans over 170 countries worldwide,

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covering a total area of 196 million hectares. Global production stands at 1148 million metric tons, with a productivity rate of 5.85 tons per hectare (FAO STAT, 2019). It is the second most important crop in Nepal in terms of area (957,650 ha), having an annual production of 2.8 million metric tons with an average productivity of 2.96 t/ha (MOALD, 2021). Maize plays a role in the National Gross Domestic Product (GDP), contributing approximately 3.15%, and in the Agricultural GDP, accounting for 9.5% (Pandey & Koirala, 2017). However, Nepal's maize productivity of 2.96 tons per hectare is significantly lower than the global average of 5.85 tons per hectare (MOALD, 2021; FAO STAT, 2019).This reduced productivity is attributed to various factors, with insect pests and diseases being significant hindrances (Prasanna, 2012; Ramirez-Cabral et al., 2017; Bastola et al., 2021).

The Fall armyworm (FAW) *Spodoptera frugiperda* (J.E. Smith) native to America holds significance as invasive polyphagous pests (Sharanabasappa et al., 2018; Yigezu & Wakgari, 2020). Cereal production is threatened by several factors, namely climatic variability and invasive pests including *S. frugiperda*, which is a dangerous pest of cereals detected in Africa in 2016 (Tendeng et al., 2019). Initially documented in Nepal in the Nawalpur district on May 9, 2019, as reported by (Bajracharya, et al., 2019). FAW is among the most severe threats to annual crops in tropical areas due to its extensive range of host plants (Praveen & Mallapur, 2019). FAW larvae feed on a variety of plant species, posing a significant threat as destructive insect pests. They cause damage to economically vital cultivated grasses such as maize, rice, sorghum, and sugarcane, along with other crops (Cock et al., 2017). A total of 353 host plant species for FAW larvae across 76 plant families have been reported (Montezano et al., 2019; KC et al., 2015; Bhatti et al., 2021).

Almost all the stages of Maize are susceptible to this insect causing severe losses when whorls are destroyed thus reducing the photosynthetic area and compromise the grain yield. Attack on the lower part of the ear leads to damage in the grain favouring an environment suitable for microbial attack (Cruz et al., 1999). FAW does not have a diapause phase, allowing it to overwinter in warmer climates and enabling multiple generations to overlap within a single crop cycle when conditions are suitable (Harrison et al., 2019).

Although FAW exhibits a preference for maize, which is Nepal's primary staple crop, many other major cultivated crops may also be affected. Maize yield declines have been recorded as high as 34 percent as a result of the fall armyworm's feeding (Lima et al., 2010). Given its swift proliferation and unique capacity to inflict substantial harm across diverse crops, the fall armyworm presents a grave menace to the food and nutrition security, as well as the livelihoods of numerous farming households in Nepal. This threat is particularly heightened when compounded with other factors contributing to food insecurity. If left unchecked, the unrestrained spread of this pest could have a profound impact on both farmers and the overall economy.

Since the pest is harmful and also economically important, it is therefore necessary to develop effective strategies for the management of pests against which sustainable crop production can be achieved. In the progression and establishment of insect species, meteorological factors play a vital role. For the development of effective pest management strategies, a detailed knowledge of the biology and morphological features of an insect pest about biotic and abiotic factors is important. Therefore, this research is directed towards studying the detailed biology and morphometrics of this newly introduced invasive pest.

Materials and Methods

The colony of FAW was commenced with larvae gathered from maize fields of NMRP Rampur Chitwan district of Nepal during March-April. The research laboratory is located at a latitude of 27°40'36" and longitude of 84°21'24", with an altitude of 173 meters above sea level (masl). Larvae were reared on maize leaves (Rampur Composite) in Petri-dishes of size 8.5 cm diameter and 1.2 cm height until pupation. After Pupation, they were transferred to a plastic rearing jar (6 cm diameter and 7 cm height) covered with muslin cloth for adult emergence. The adults (one pair each male and female) were kept inside each plastic rearing jar in which, paper towels were used as the oviposition substrate, and the upper side was covered with nylon mesh. Adults were provided with a daily replacement of cotton pads soaked in a 10% honey solution with a diameter of one cm. The newly hatched neonate larvae were housed in Petri-dishes (same as above) with a paper towel at the bottom to supply moisture under controlled temperature of (25 ± 1ºC) and a relative humidity of (70 ± 10%), and photoperiod (14h daylight).

A total of (n=60) newly hatched neonate larvae batches were individually transferred to each Petri dish. Thirty neonate larvae were provided with maize leaves as food and the rest 30 were fed on Napier (*Pennisetum purpureum*) leaves (tender or newly formed leaves) to study the comparative biology on a major host of FAW i.e. maize crop and major pull crop i.e. Napier, which is a major trap crop utilized within the push-pull system of FAW management. Maize and Napier leaves were sterilized with 4% sodium hypochlorite solution, washed 2-3 times with distilled water, dried for 1 hour before feeding to larvae, and reared in glass plates until pupation.

In the initial stages tender leaves (15-20 days old) of Maize variety; Rampur composite, were fed to the developing larvae while the later instars were fed on the whorl portions of mature maize plants. Similarly, tender Napier leaves were given to the larvae, raised on Napier from the NMRP, research farm. Leaves were changed on alternate days with fresh leaves and the paper towel was replaced daily to maintain a clean culture environment to avoid contamination.

Data were recorded on the incubation period, period of larval development, pupal stage, pre-oviposition phase, oviposition phase, and fecundity, egg hatchability, male adult longevity, female adult longevity, larval mortality, pupal mortality, sex ratio (male: female), and morphometrics of egg, larvae, pupa, and adult.

Measurement of eggs, first and second instar larvae were recorded using a compound microscope fitted with a stage micrometer scale of 2 mm and ocular micrometer of 19 mm diameter, having a 10 mm linear scale (100 divisions). Images of various growth stages of the insects were captured with a Leica GZ6 Stereo Zoom Microscope with 10 X and 40 X magnification using a smartphone (Redmi Note 5 Pro). A digital Vernier caliper (0-150 mm) was used to measure larvae in the third, fourth, fifth, and sixth instars, pupae, and adults. Measurements were taken for the length and width of larvae, the head capsule width, the length of pupae from the head to the tip of the abdomen, and the width of its broadest segment. The head capsule of the first and second larval stages were measured using a compound microscope mounted with stage and ocular micrometer, and later instars were measured using a digital Vernier caliper. Male and female adults were mounted with pins, and their wings were extended and allowed to dry. The wing expansion of the forewings and the body length, measured from the head's tip to the abdomen's tip, were recorded. Measurements were recorded for ten individuals in each stage of the insect's lifecycle.

Microsoft Office package 2016 i.e. Microsoft Word, and Microsoft excel was used for data entry and management.

Results and Discussion

Biology

Egg

Eggs were laid in masses of 20-360 per egg mass by a gravid female. Eggs were creamish white and covered with white scales produced from the female abdomen imparting a fuzzy or mold-like appearance. The color of the eggs transforms from a creamy white to brown and eventually black right before hatching. The incubation period of the egg was recorded at 2-3 days in Maize as shown in Table 1.

Larva

First instar

The first instar larvae were very minute with comparatively large flattened black head. Body of the larvae was whitish with minute hairs on the body surface. The developmental period of first instar larvae varied between 2 to 3 days as shown in Table 1.

Second instar larvae

In the second instar, the larvae displayed an amber-colored head along with a pale white to yellowish-hued body. A tinge of brown was present on the dorsal side along with a development of faint white dorsal and sub-dorsal lines. Developmental period of second larval stage ranged from 2-3 days as shown in Table 1.

Third instar larvae

In this instar, body color is drastically changed from pale white to greenish brown. Black spots become prominent and the dorsal and sub-dorsal lines were clearly evident. The developmental period of this stage is 2 days as shown in Table 1.

Fourth instar larvae

The coloration observed in the fourth instar larvae is olive- brown to dark brown with the mean developmental period of 2 days. White lines on the dorsal and sub-dorsal parts also become conspicuous. Dorsally across the entire body, black tubercles were present, each bearing protruding spines. Larvae possessed some peculiar characteristics such as the presence of a distinct inverted light yellow 'Y' suture on the head capsule and four dark spots located on the dorsal surface of the eighth abdominal segment in square form while in other segments in trapezium form as shown in Table 1.

Fifth instar larvae

In the fifth instar, the larvae exhibit a grayish-brown hue on the dorsal side and a greenish tone on the ventral and sub-ventral sides. The larvae feel smooth to the touch due to the absence of microspines. The developmental period of this stage ranged from 2-3 days as shown in Table 1.

Sixth instar larvae

The sixth instar larvae were cylindrical with smooth, clearly defined and distinct segmentation. The cephalic region was slightly bilobed and black. The larvae exhibited a grayish-brown coloration on the dorsal side, whereas the ventral side was greenish mottled

with reddish brown color. The developmental period of sixth instar larvae is ranged 3-5 days. The overall duration of the larval period ranged from 13 to 18 days as shown in Table 1.

Pupa

Initially, the pupal color was green which gradually changed to reddish-brown color after 12-14 hours. Male and female pupae were differentiated by the gap between their genital and anal openings. The distance was comparatively greater in females than in males. The total pupal period ranged between 9-12 days as shown in Table 1.

Adult

Male and female *S. frugiperda* individuals could be discerned by the distinct wing patterns they exhibited. Male adult longevity ranged from 8-10 days whereas female adult longevity was 9-12 days. The stages of pre-oviposition, oviposition, and post-oviposition ranged from 3-4, 2-3, and 4-5 respectively. The total life cycle of both male and female FAW moths ranged from 30-43 and 31-45 days respectively as shown in Table 1.

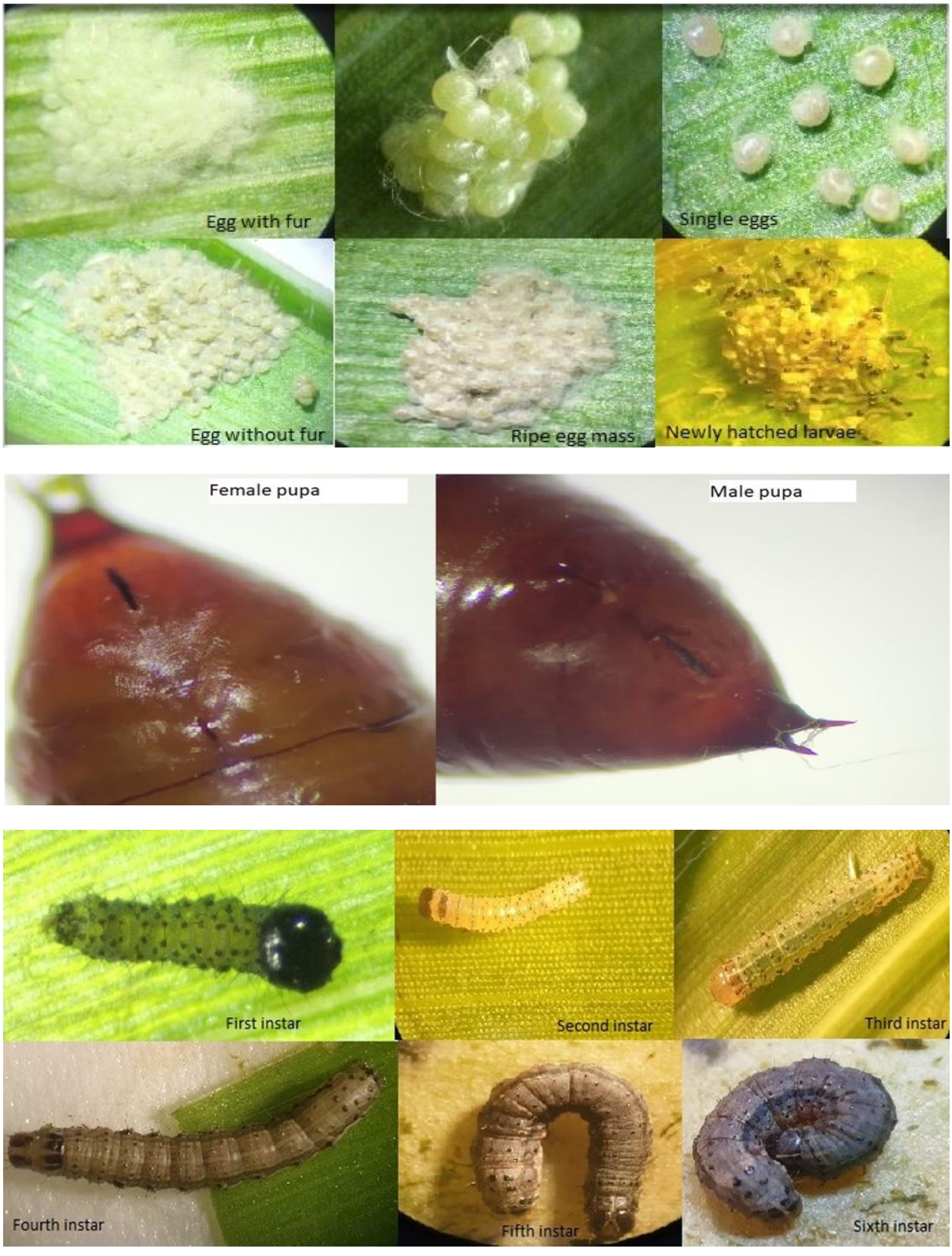
Table 1. Life cycle of FAW at NMRP laboratory Rampur, Chitwan

Stages	Range (Days)	
	Maize (n=30)	Napier (n=30)
Incubation period	2-3	2-3
Larval period	13-18	18-26
I instar	2-3	3-5
II instar	2-3	3-5
III instar	2	3-4
IV instar	2	3-4
V instar	2-3	3-4
VI instar	3-5	6-8
Pupal period	9-12	9-12
Pre-oviposition period	3-4	3-4
Oviposition period	2-3	2-3
Post oviposition period	4-5	3-4
Male adult longevity	8-10	8-10
Female adult longevity	9-12	8-11
Total life cycle (egg to adult)		
Male	30-43	37-51
Female	31-45	37-52

Morphometrics

The mean egg width and thickness of *S. frugiperda* were observed to be 0.50 ± 0.01mm and 0.34 ± 0.02 mm. Head capsule widths were measured at 0.52±0.05, 0.73±0.05, 1.1±0.05, 1.74±0.12, 2.04±0.15, and 2.91±0.23 mm for the first through sixth instars, respectively. Similarly, the average body length for various instars was noted as 3.33±0.26 mm, 6.92±1.07 mm, 13.11±1.45 mm, 16.82±1.57 mm, 28.68±1.75 mm, and 34.56±1.99 mm, corresponding to the first through sixth instars, respectively. Mean pupal length and breadth were documented as 14.52±0.94 mm and 4.09±0.27 mm respectively. Mean adult length of male and female were observed to be 15.54±0.92 mm and 16.00±1.04 mm. In addition, mean wing

span of male and female moths was recorded as 30.36±1.12 and 31.79±1.82 mm. The mean pupal weight was 0.18±0.02 gm. Accordingly, the mean body breadth of different instars were 0.40 ± 0.03, 0.74±0.10, 1.58±0.19, 1.44±0.13, 2.01±0.17, and 3.34±0.22 mm for first, second, third, fourth, fifth and sixth instar respectively. Mean pupal, adult male, and adult female body breadth was recorded at 4.09±0.27, 3.17±0.58, and 3.29±0.48 mm respectively as shown in Table 3.



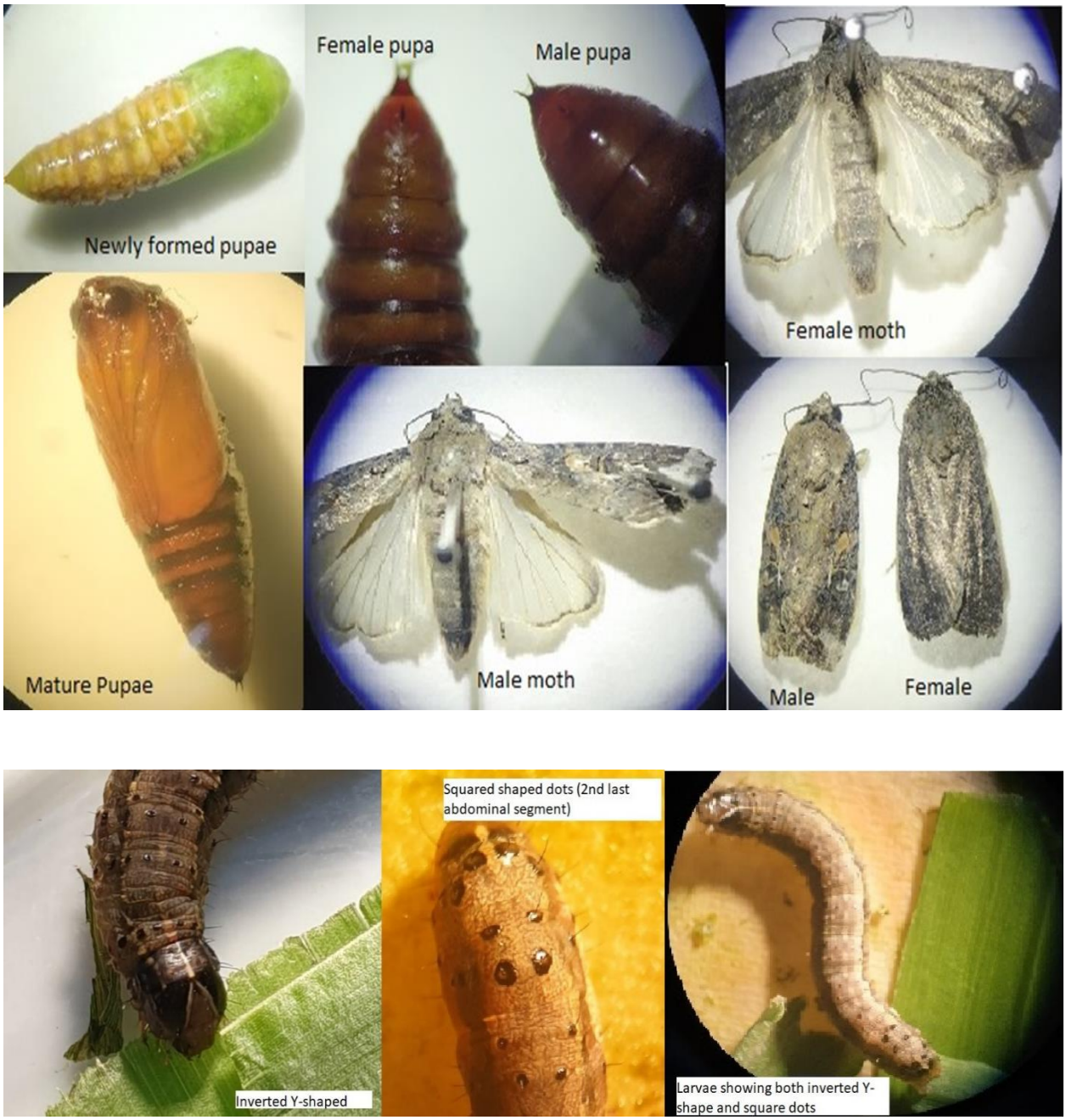


Figure 1: Different stage of life cycle of Fall Army Worm

Table 2. Morphometrics of FAW at Entomology laboratory AFU, Rampur, Chitwan

S.No.	Developmental stages	Parameters	Range (mm)	Mean ± SD (mm)
1	**Egg**	Width	0.49-0.52	0.50±0.01
		Thickness	0.31-0.36	0.34 ± 0.02
2	**Larval stages**			
	I instar	Head capsule	0.50-0.54	0.52±0.01
		Body length	2.9-3.73	3.33±0.26
		Body breadth	0.39-0.45	0.40±0.03
	II instar			0.73±0.05
		Head capsule	0.66-0.84	6.92±1.07
		Body length	5.23-9.06	0.74±0.10
		Body breadth	0.61-0.96	
	III instar			1.1±0.05
		Head capsule	1.0-1.21	13.11±1.45
		Body length	10.17-15.27	1.58±0.19
		Body breadth	1.34-1.92	
	IV instar			1.74±0.12
		Head capsule	1.53-1.96	16.82±1.57
		Body length	13.66-18.48	1.44±0.13
		Body breadth	1.23-1.67	
	V instar			2.04±0.15
		Head capsule	1.86-2.34	28.68±1.75
		Body length	25.6-31.56	2.01±0.17
		Body breadth	1.76-2.31	
	VI instar			2.91±0.23
		Head capsule	2.5-3.2	34.56±1.99
		Body length	30.84-38.46	3.34±0.22
		Body breadth	2.98-3.76	
3	**Pupa**			
		Length	13.03-16.06	14.52±0.94
		Breadth	3.61-4.53	4.09±0.27
		Weight (gm)	0.15-0.21	0.18±0.02
4	**Adult**			
	Male	Body length	13.44-16.81	15.54±0.92
		Breadth	2.74-3.65	3.17±0.58
		Wingspan	28.11-33.48	30.36±1.12
	Female	Body length	15.39-17.18	16.00±1.04
		Breadth	2.92-3.97	3.29±0.48
		Wingspan	29.6-34.75	31.79±1.82

Fall armyworm (FAW) *Spodoptera frugiperda* poses a significant threat to maize and other cereal crops, currently displaying invasive tendencies in Africa and the Indian sub-continent (Bhatti et al., 2021). After its introduction in Nepal in 2019, it has posed a serious threat to thousands of maize growing farmers in hilly regions. To develop an effective management strategy for this invasive pest, biology, and morphometrics of various stages

were studied at laboratory conditions. The mean developmental period of different stages was recorded. Recordings were made for the incubation period, larval duration, pupal stage, and the longevity of adults, which is in line with research conducted by (Siddhapara et al., 2021; (Russianzi et al., 2021; Sharanabasappa et al., 2018; Ramzan et al., 2021). Similarly, the durations of pre-oviposition, oviposition, and post- oviposition were observed as 3-4, 2-3, and 4-5 days respectively which was similar to the findings of (Navasero & Navasero, 2020; Kalyan et al., 2020).

The head capsule width for first through sixth instar larvae was documented as 0.52±0.05, 0.73±0.05, 1.1±0.05, 1.74±0.12, 2.04±0.15, and 2.91±0.23 mm, respectively, which was similar to the findings reported by (Navasero & Navasero, 2020; Bhatti et al., 2021). The mean egg width and thickness of *S. frugiperda* was observed to be 0.50 ± 0.01mm and 0.34 ± 0.02 mm which was in line with the findings of (Navasero & Navasero, 2020). In addition to that, the results of pupal length, adult length, and wing span were also in accordance with the findings of (Navasero & Navasero, 2020).

A longer larval period of 18-26 days was observed in larvae reared on Napier compared to Maize leaves of 13-18 days. Similarly, larval mortality of 73 % in Napier grass and 20% in Maize was observed. Similar findings were reported by (Khan et al., 2006) where the larval mortality in Napier grass was up to 90% and larval duration two weeks longer than maize fed larvae. The reason behind this might be the poor nutritional quality of Napier grass, exudation of gummy substance after the herbivore injury, and the presence of hairs which makes it unsuitable for feeding by larvae, although it initially attracts the moths for oviposition.

Conclusion

Biology and morphometrics study is helpful to identify the pest and its behavior for developing management strategy. The incubation period (2-3 days), larval and pupal periods (13-18 and 9-12 days) with overall life duration (31-45 days) were recorded in maize. Similarly, the durations of pre-oviposition, oviposition, and post- oviposition were observed as 3-4, 2-3, and 4-5 days respectively. The mean egg width and thickness of *S. frugiperda* were observed to be 0.50 ± 0.01mm and 0.34 ± 0.02 mm. The head capsule width for first through sixth instar larvae was documented as 0.52±0.05, 0.73±0.05, 1.1±0.05, 1.74±0.12, 2.04±0.15, and 2.91±0.23 mm. Further, average body length for various instars was noted as 3.33±0.26 mm, 6.92±1.07 mm, 13.11±1.45 mm, 16.82±1.57 mm, 28.68±1.75 mm, and 34.56±1.99 mm, corresponding to the first through sixth instars, respectively.

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