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Biology and management of early instar larvae of Fall armyworm, *Spodoptera frugiperda* (Lepidopter: Noctuidae) with selected plant *extracts*

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The Fall armyworm, *Spodoptera frugiperda,* is an invasive pest of maize worldwide. *S. frugiperda* has a wide range of host plants, but maize has been recorded as the preferable host. The current study was aimed to study the life cycle of *S. frugiperda* on maize. The present study results showed complete metamorphosis while the incubation period was 2.01 to 3.05 days. Six instar larvae were recorded whose developmental period was completed in 13.32-20.01 days. The mean average developmental period of first, second, third, fourth, fifth, and sixth larval instar was 2.76 ± 1.00, 2.43 ± 2.23, 2.67 ± 1.10, 3.85 ± 1.21, 2.32 ± 1.61 and 5.01 ± 1.98, respectively. The body length of first, second, third, fourth, fifth and sixth instar larva was 1.65 ± 0.12, 3.10 ± 0.32, 5.91 ± 0.26, 8.98 ± 0.43, 15.76 ± 1.10 and 31.43 ± 1.25, respectively. The pupa was obtect. The pre-oviposition, oviposition, and post oviposition periods were 3.11 to 4.06, 2.01 to 4.09, and 4.00 to 5.11 days, respectively. *A. indica* was found to have more toxic extract, followed by *J. curcas* and *E. globulus. A. indica, J. curcas,* and *E. globulus* caused the highest mortality (53 - 60%) after 24 h while 76 - 83% after 48 and 69 - 98% after 72 h of post-treatment. *E. globulus* caused 5.51, 9.76, and 29% larval mortality after 24, 48, and 72 h, respectively. The study concluded that plant-based extracts are the central part of IPM at the national and international level and should be tested by small land farmers to manage the pest population worldwide, especially in the study area.

Keywords: Maize, Polyphagous pest, *Spodoptera frugiperda*, plant extract, insecticides, Integrated pest management, Pakistan

INTRODUCTION

Agriculture is the backbone of Pakistan economy and plays an essential part in the gross domestic product (GDP). Maize is the third most annual cross-pollinated important Kharif cereal crop globally, while Pakistan is the fourth high yielding cereal crop after wheat, cotton, and rice (Arif et al. 2012). Maize is grown well in soil with a 6.5 – 7.5 pH range. It is a rich source of nutrients such as proteins (10%), starch (72%), fatty acid (10%), vitamins (3-5%), and sugar (3%) (Adnan and Bilal, 2020). It is also used as the staple food for humans after potato, fodder for animals, and oil in running industries (Kumar and Jhariya, 2013; Bari et al. 2018). Maize is grown on 1.3 million hectares in diverse ecologies ranging from 30 meters above sea level in all provinces of Pakistan. It has been reported that Khyber Pakhtunkhwa (KPK) contributes 56%, Punjab contributes 39%, while Sindh and Baluchistan contribute 5% of the total area (Qadir et al. 2013). Maize (*Zea mays*) production is decreasing annually due to various biotic and abiotic factors. Among biotic factors, insect pests (maize stem borer, maize shoot fly, cutworms, and another lepidopteran) and pathogens are the severe threat to maize production all over the world (George et al. 2003), including Pakistan, China, Nepal, the Philippines, Africa, Brazil, Thailand, India, Vietnam, and Indonesia (Kim et al. 2020; Zarkani et al. 2020). Both vegetative and reproductive stages of *Z. mays* are highly affected by several insect pests (Khan et al.

2016). Recently, a new *Spodoptera* species, *S. frugiperda* had reported in many maize growing countries of the world, causing substantial crop losses in those countries (Goergen et al. 2016; Prasanna et al. 2018; Sharanabassapa et al. 2018; Deole and Paul, 2018; Igyuve et al. 2018; Chormule et al. 2019; Nagoshi et al. 2019) even in Pakistan (Bhatti et al. 2021; Ramzan et al. 2021). This species is becoming a significant threat to food security in Pakistan, so there is a need to develop an effective and best management strategy to control this notorious and destructive pest in the country to protect the economy of Pakistan by minimizing crop losses.

Keeping in view the high crop losses and considering the insecticides an effective tool, the current study was conducted to control this most dangerous pest using insecticides. Before this study, no such research has been conducted on this pest (Pakistan), which will be the baseline for further studies. The current study results will be fruitful for future researchers and at the farms level.

S. *frugiperda* is an invasive alien species and polyphagous pest that attacks various crops belonging to 23 families, especially Poaceae. This species is native to tropical and subtropical areas of the Americas (Prasanna et al. 2018; FAO, 2017, CABI, 2017; Prowell et al. 2004; Clark et al. 2007). Firstly, it has been reported in Africa and is now widely distributed in more than 44 African countries (Goergen etal., 2016). Within a few years of the first report, the infestation of larvae have been recorded on many crops, including rice (Nabity et al. 2011), maize, sorghum, millet (Abrahams et al. 2017; Rwomushana et al. 2018), cotton (Pogue 2002; Nagoshi et al. 2007; Bueno et al. 2010) and several vegetable crops from various other countries including Egypt and Pakistan. It has reported on maize from several other Asian countries, including India (Sharanabasappa et al. 2018a; Mahadevaswamy et al. 2018; Sharanabasappa et al. 2018b), Vietnam, Sri Lanka, Bangladesh, Thailand, China, and Myanmar (Guo et al. 2018; Wu et al. 2019; NATESC 2019a, b; CABI 2019). Maize is a high-yielding staple crop used as fodder for animals, food for humans, poultry feed, and raw material in the industry. This third most important cereal crop after wheat and rice is severely attacked with FAW in Pakistan. FAW has the power to cause 8.3 to 20.6 m tons of maize losses annually in African countries (Abrahams et al. 2017) and becomes a risk to food security.

In Pakistan, maize is planted in all four provinces on 0.974 million hectares with 3.707 m tons annual production and an average yield of 3805 kg/ha. Its production is reducing due to many abiotic factors (irrigation, land, soil type, and climate) and biotic factors (weeds, pests, and diseases) (Reynolds et al. 2015; Sisay et al. 2019). Among biotic factors, invasive alien insect species, especially FAW, are major (Julia et al. 2013). FAW can cause substantial crop losses without management strategies, which ultimately threaten food security. The maize productivity cannot meet the national and international needs without management approaches (Andini and Pribadi, 2019). Synthetic pesticides are used repeatedly to suppress pest populations, but they negatively impact humans and the ecosystem due to their residual effect (Gu et al. 2018; Cai et al. 2017). Due to very modest toxicity hazards and insignificant residual impact on ecosystems, researchers focused on the application of natural chemicals as biopesticides at the turn of the century (Idrees et al. 2016; Idrees et al. 2017; Luo et al. 2018; Qadir et al. 2021; Idrees et al. 2021).

No study of host plants has been conducted in Pakistan, and a scientific management strategy has been tested against this emerging pest. Farmers on their behalf use insecticides widely to control this pest, which causes environmental pollution and health hazards and causes economic losses to purchase these chemicals only for FAW. Applying insecticides in this ratio can also cause insecticides resistance to FAW. There is a need to test the host plants and adopt an eco-friendly and alternative strategy such as botanicals to control this pest. The use of an alternative and eco-friendly tool is the plan of entomologists to avoid the harmful effect of insecticides on humans, animals, natural fauna, and the environment. For this purpose, the current study was conducted to check the toxicity of botanicals against 2^{nd} instar larvae of FAW under laboratory conditions. The present findings will be considered as the baseline in FAW management on various crops under laboratory and field conditions. These can prove helpful for the farmers and future researchers in selecting such a strategy.

MATERIALS AND METHODS

Insect Rearing

The larvae of fall armyworm were collected from different areas of KPK in the maize field during 2020 and brought to the laboratory for rearing. The collected larvae were kept in a petri dish with fresh maize leaves and old leaves replaced with new leaves every day till pupation. Larvae were pupated and the pupae were collected and placed in separate plastic containers for adult emergence. Adult pairs (M: F) were shifted into adult rearing cages for mating and egg-laying. A nap liner was placed inside the cage for the lying egg site. Twenty eggs were collected and put individually into a petri dish with maize leaves to record their life. This procedure is repeated three times. The leaves of botanicals (*Azadirachta indica*, *Jatropha curcas,* and *Eucalyptus globulus*) were collected from different areas of Multan to check their toxicity against second instar larvae. The third generation of FAW is used in toxicological studies. The culture was maintained at 26 ± 2^0C temperature and 65 ± 5% Relative Humidity (RH) with 14:10 (Light: Dark) photoperiods.

Preparation of plant extract

The collected botanical leaves were brought to the laboratory and dried separately under shade. The dried leaves were ground to a fine powder with the help of mortar and pestle. The fine powder of each botanical was soaked in 100 ml distilled water for 24 hours. After 24 hours of soaking, the botanicals solution was filtered through a cheesecloth. The filtered plant extracts were left overnight. The botanical extract was prepared by Sisay et al. 2018. Complete Randomized Design (CRD) with three replications was used in the present study. The 20g of maize leaves were placed in a petri dish and sprayed with 20 ml of each botanical extract. The Petri dishes were kept open for 20 min to remove the extra liquid. The leaves treated with distilled water were considered as a control. Ten-second instar larvae were randomly selected from the uniform mass culture and released into each petri dish containing the treated leaves. The larvae that do not respond to the brush upon touch were considered dead. The data was recorded at 24, 48, and 72 h of post-treatment.

Statistical analysis

The collected data were analyzed statistically using Duncan's Multiple Range test DMRT (P ≤ 0.05).

RESULTS

Biology of FAW

Eggs: Eggs laid in batches on the upper and undersides of the leaves under the fields conditions while some laid on the walls of the rearing cage and napelliner under laboratory conditions. According to Early et al. (2018), mating occurred at night time, and creamy white eggs in one or more than one layer were laid on the lower side of the maize leaves. The color and shape of newly laid eggs color and shape were pale green and flat, respectively. After one day, eggs were changed into golden yellowish, turning black before hatching. The embryonic period has lasted 2.01-3.05 days (Table 1) while (FAO, 2018) reported 4-6 days.

Table 1: Biological parameters of *S. frugiperda* on maize under standard controlled conditions

Stages	X ± SE	Range (days)
Eggs		
Embryonic period	2.50±0.50	2.01-3.05
Larvae		
1st instar	2.76±1.00	2.21-4.10
2nd instar	2.43±2.23	3.11-4.20
3rd instar	2.67±1.10	2.00-3.00
4th instar	3.85±1.21	2.22-4.29
5th instar	2.32±1.61	3.06-4.03
6th instar	12.42±2.64	13.32-20.01
Pupa		
Pre-pupa	1.12±0.34	1.00-2.00
Pupa	9.34±2.65	7.32-13.01
Total pupal period	10.43±1.33	12.01-13.05
Ovipositional period		
Pre-oviposition	3.40±0.12	3.11-4.06
Oviposition	2.90±0.67	2.01-4.09
Post-oviposition	4.60±0.76	4.00-5.11
Fecundity		
Number of eggs /Female	898.79±165.66	776.12-1055.43
Egg hatchability (%)	88.97±3.64	90.00-97.00
Longevity		
Male	5.99±1.89	6.00-10.00
Female	9.57±2.46	10.00-12.00
Total life cycle from egg to adult		
Male	33.43±7.10	30.00-41.00
Female	38.33±6.54	35.00-45.00

Larva: The color of the first instar larva was greenish, which turned into greenish-brown in the second instar. The third and fourth to sixth instar larvae were brownish and brownish-black, respectively. A white inverted Y was found on the front of each instar. The body of the larvae was hairy with white dorsal lines. The mean average developmental period of first, second, third, fourth, fifth, and sixth larval instar was 2.76±1.00, 2.43±2.23, 2.67±1.10, 3.85±1.21, 2.32±1.61, and 5.01±1.98, respectively. Each larva passed six instars, and the total developmental period of 1st to 6th larval instar was completed in 13.32-20.01 days (Table 1). The body length of 1st, 2nd, 3rd, 4th, 5th, and 6th instar larva was 1.65±0.12, 3.10±0.32, 5.91±0.26, 8.98±0.43, 15.76±1.10, and 31.43±1.25, respectively (Table 3).

Pupa: The full-grown larvae stopped the feeding, minimized the movement, and turned greenish to bright brown in color near pupation. The pupal period lasts 7 to 13 days. Similar results (8 days) about the pupal period were recorded by Débora et al. (2017) on maize crops. The pupa of pest was obtect. The main difference between male and female pupa can be determined based on the distance between the anal slot and genital opening. This distance can be more in the case of females. Sharanabasappa et al. (2018) have reported similar findings of sex differentiation at the pupal stage.

Adult: Forewings of the male are brown and gray, while the female's forewings are grayish-brown in color. A circular spot and triangular white patch are present at the forewings' apical region and center. The hindwings of both sexes are silver-white with a narrow dark border. The morphological traits such as Wingspan, wing, and FAW's body length are given in Table. 2, are almost similar to previous study findings (Oliver and Chapin, 1981). Sharanabasappa et al. (2018) reported that a single female laid 835-1169 eggs in her life period, similar to the current study findings in which 776 to 1055 eggs were recorded.

In the present study, 3.11 to 4.06, 2.01 to 4.09, and 4.00 to 5.11 days were recorded pre-oviposition,

oviposition, and post oviposition periods, respectively (Table 1). It was observed that females lived long as compared to males under the same control conditions. The total life cycle of females and males was 35-45 and 30-41 days, respectively.

Table 2: Male and female morphological traits on maize leaves

Parameters	Male		Female	
	Mean ± SE (mm)	Range (mm)	Mean ± SE (mm)	Range (mm)
Body length	14.90±1.23	13.10-16.87	14.21±1.16	13.05-16.19
Wing length	12.54±0.63	11.93-14.62	12.32±0.34	11.27-14.02
Wing span	30.12±2.10	27.55-34.76	29.43±1.37	28.0-33.03

Table 3: The body length of different instar on maize under laboratory conditions

Parameters	The body length of different instar larvae (mm)	
	Mean ± SE	Range
1st	1.65±0.12	1.23-2.01
2nd	3.10±0.32	2.97-3.21
3rd	5.91±0.26	4.99-6.32
4th	8.98±0.43	7.01-9.11
5th	15.76±1.10	13.65-16.10
6th	31.43±1.25	29.05-34.09

Table 4: Mean percent mortality of larvae after 24, 48, and 72 h of post-treatment

Treatments (Botanicals)	Mean percentage mortality		
	24 h	48 h	72 h
Azadirachta indica	60.00 ± 2.12a	83.65 ± 1.99a	98.05 ± 0.09a
Jatropha curcas	53.3 ± 5.17b	76.7 ± 5.17a	85.05 ± 6.69b
Eucalyptus globulus	5.51 ± 0.10b	9.76 ± 0.04ef	29.00 ± 7.75ef
Control	0.00 ± 0.00ef	0.00 ± 0.00ef	5.00 ± 0.64ef

Note: Means within a column followed by different letters are significantly other at P < 0.05 (Tukey test).

Toxicity of plant extract to second instar *S. frugiperda* larvae

In the present study, three plant extracts showed different toxicity to second instar *S. frugiperda* larvae. Significant differences were recorded among all the tested plant extracts in causing larval mortality. *A. indica* was found to have more toxic extract, followed by *J. curcas* and *E. globulus. A. indica, J. curcas,* and *E. globulus* caused the highest mortality (53-60%) after 24 h while 76-83% after 48 and 69-98% after 72 h of post-treatment. *E. globulus* caused 5.51, 9.76, and 29% larval mortality after 24, 48, and 72 h, respectively. Only 5% mortality was recorded in control after 72 h of treatment. The current study showed that *E. globulus* was least toxic while *A. indica* was the most toxic extract (Table 4). revealed that *A. indica, Schinnus molle,* and *Phytolacca dodecandra* were proved more toxic plant extracts than *J. curcas, Chenopodium ambrosoids, Nicotina tabacum,* and *E. globulus*. In the current study, three botanicals were tested, and among them, *A. indica* was recorded as more lethal than others while *C. ambrosoids* and *N. tabacum* were not tested in the current study. Silva et al. (2015) reported similar findings of the toxicity of plant extracts extracted from plant seed not extracted from leaves as done in the current study. The larval growth and feeding were reduced after feeding the leaves treated with *A. indica.* Our results align with the findings of Martínez et al. (2017), who reported that the movement of larvae reduced by eating the treated maize leaves. Many other researchers have used plant-based insecticides against FAW (Ce'spedes et al. 2000). In the previous studies, some repellents have used to check their repellency at low and high doses against larvae of FAW (Silva-Aguayo et al. 2017). Extracts of *Cedrela salvadorensis* and *Cedrela dugessi* caused larval mortality (Ce'spedes et al. 2000).

The current study showed that plant-based chemicals (botanicals) are the main component of integrated pest management under controlled and uncontrolled conditions, especially against FAW. This technique should be adopted at the national and international levels. Small farmers who cannot purchase expensive chemicals and technologies to manage the risk of pest on crops due to lack of resources should use these techniques at their farms. These plants are readily available free of cost at each farmer's farm and could be tested as an alternative and eco-friendly approach in managing FAW.

CONCLUSION

The current study showed that the biological parameters of the present pest in Pakistan are almost similar as recorded in other countries such as Africa, India, and America on maize crops. The current study findings are the basic information about pest biology, morphology, and clue to determine its life cycle on other host plants, which can become the significant hosts in the absence of maize crop in the study area. Due to high reproductive and migratory potential, FAW has spread widely in all maize growing areas of Pakistan; the complete elimination of this pest is complicated due to the lack of suitable and proper management strategies against this. In Pakistan, most farmers rely on insecticides to manage FAW on their crops, which ultimately pollute the environment and harm natural fauna such as predators and parasitoids. Plant-based insecticides like botanicals are the eco-friendly pest management strategies that give maximum pest mortality (FAW) should be tested. Further studies such as host plant resistance, reproductive biology, identification of its natural enemies, and botanicals should be checked, which could be proved helpful in its management.

CONFLICT OF INTEREST

Authors declare no conflict of interest.

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AUTHOR CONTRIBUTIONS

All authors equally contribute to carry out the present study; review and write the manuscript. All authors have read and agreed to the published version of the manuscript.

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