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The life cycle of Fall Armyworm (FAW), *Spodoptera frugiperda* and toxicity of botanicals against 2nd instar larvae on maize

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The fall armyworm, *Spodoptera frugiperda* is a sporadic and destructive pest of maize crops worldwide. FAW has a wide range of host plants, but maize has been recorded as the preferable host. The current study aimed to check the life cycle of FAW on maize. The results showed that complete metamorphosis was recorded. The incubation period was 2.01 to 3.05 days. Six instar larvae whose developmental period was completed in 13.32-20.01 days were recorded. The mean average developmental period of the 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 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. 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. The toxicity data showed that *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 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 main part of IPM at national and international levels 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*, Botanical, Integrated pest management, Pakistan

INTRODUCTION

Fall armyworm (FAW), *Spodoptera frugiperda* is considered an invasive alien species and polyphagous pest which attacks various crops belonging to 23 families, especially poaceae. This species is native to tropical and subtropical areas of the world mainly 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 now widely distributed in more than 44 African countries (Goergen et al. 2016) and many others. Within few years of first report, the infestation of larvae has 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 human, feed for poultry and raw material in industry. This third most important cereal crop after wheat and rice is severely attack with FAW in Pakistan. FAW has power to cause 8.3 to 20.6 m tons maize losses annually in African countries (Abrahams et al. 2017) and become risk to food security.

In Pakistan, maize is planted on all four provinces on an area of 0.974 million hectare with 3.707 m tons annual production and 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 one (Julia et al. 2013). Without the absence of management strategies, FAW can cause huge crop losses which ultimately become major threat to food security. The maize productivity cannot be able to meet the national and international needs without its management approaches (Andini and Pribadi, 2019).

In Pakistan, no host plants related study has conducted and scientifically management strategy has still tested against this emerging pest. Farmers on their own behalf using insecticides widely to control this pest which is not only cause the environmental pollution and health hazards but also cause economic losses to purchase these chemicals only for FAW. The application of insecticides in this ratio can also cause insecticides resistance to FAW. There is need to test the host plants and adopt an ecofriendly and alternative strategy such as botanicals to control this pest in the country. The use of an alternative and eco-friendly tool is agenda of entomologists to avoid the harmful effect of insecticides on human, animals, biological fauna and environment. For this purpose, the current study was conducted to check the toxicity of botanicals against 2nd instar larvae of FAW under laboratory conditions. The current findings will be considered base line in the management of FAW on various crops under both laboratory and field conditions. These can prove helpful for the farmers and future researchers in the selection of such strategy.

MATERIALS AND METHODS

Study area and FAW collection

The larvae of FAW were collected from maize plants from different areas of Swabi during 2020 and brought to Rearing Laboratory for rearing purpose and mass culture.

Collection of host plants, rearing of FAW and botanicals

The collected larvae were kept individually in petri dish with fresh host leaves for feeding. Old leaves were replaced with new and fresh leaves till pupation. The pupae were collected and placed in separate plastic container for adult emergence. Adult pair (M: F) was shifted into adult rearing cage for mating and egg lying. A nap liner was placed inside the cage for egg lying site. Twenty eggs were collected and placed individually into petri dish with host leaves to record its life period. 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 2nd instar larvae only on maize leaves. The rearing of pest was continued at three generations and 2nd instar larvae of F3 were used in toxicological studies. The culture was maintained at standard conditions, of 26±5^{0}C, 65±5% R.H. with 14:10 (L.D) photoperiods.

Preparation of botanical extract

The collected leaves of botanicals were brought to 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 distilled water (100 ml) for 24 hours. After 24 hours of soaking, botanicals solution was filtered through a cheese cloth. The filtered plant extracts were left overnight. The method of Sisay (2018) was followed in the preparation of botanical extract. These botanicals were tested on second instar larvae in a Complete Randomized Design (CRD) with three replications. The equal weight (20g) maize leaves were prepared and placed in petri dish and sprayed with 20 ml of each of the botanical extracts. The petri dishes were kept open for 20 min to remove the extra liquid. The leaves treated with distilled water were considered control. Ten second instar larvae were randomly selected from mass culture and released into each petri dish containing the treated leaves.

Data recording

Insect mortality data were recorded after 24, 48 and 72 h of post treatment.

Statistical analysis

Data were arranged on Excel Sheet and statistically analyzed by using Statistix 8.1 software. The means of treatments were compared by Tukey's HSD test (at 5% alpha) to check effect of treatments on mortality. Standard error and means of biological parameters were also calculated.

RESULTS

Biology of FAW

Eggs

Eggs of FAW were laid in batches on upper and undersides of the leaves in the fields while on the walls of rearing cage and napelliner in the laboratory. According to Early et al. (2018), mating occurred at night time and creamy white eggs in one or more than one layers were

laid on the lower side of the maize leaves. The color and shape of newly laid eggs was pale green and flat, respectively. After one day, eggs were changed into golden yellowish while turned black prior to hatching. The embryonic period was lasted 2.01-3.05 days (Table 1) while (FAO, 2018) reported 4-6 days.

Larva

The color of first instar larva was greenish which turned into greenish brown in second instar. The color of third and fourth to sixth instar larvae was brownish and brownish black, respectively. A white inverted Y was found on the frons of each instar. The body of 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 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).

Table 1: Biology 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	5.01±1.98	5.01-7.39
Total larval period	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

Pupa

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

Adult

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

In the current 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 period, respectively (Table 1). It was observed that female was long lived as compared to male under the same control conditions. The total life cycle of female and male 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: Body length of different instar on maize under laboratory conditions

Parameters	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 applications

Treatments (Botanicals)	Mean percentage mortality		
	24 h	48 h	72 h
Azadirachta indica	60.00 ± 2.12^{a}	83.65 ± 1.99^{a}	98.05 ± 0.09^{a}
Jatropha curcas	53.3 ± 5.17^{b}	76.7 ± 5.17^{a}	69 ± 6.69^{b}
Eucalyptus globulus	5.51 ± 0.10^{b}	9.76 ± 0.04^{ef}	29.00 ± 7.75^{ef}
Control	0.00 ± 0.00^{ef}	0.00 ± 0.00^{ef}	5.00 ± 0.64^{ef}

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

Efficacy of botanicals

In the present study, each botanical extract showed different level of toxicity against larvae of FAW. The significant differences were recorded between *Eucalyptus globulus, Jatropha curcas* and *Azadirachta indica* in causing larval mortality. The mean percentage mortality of larvae showed that *A. indica* was found more toxic extract followed by *J. curcas* and *E. globulus. A. indica, J. curcas* and *E. globulus* caused 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 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 lethal than others while *C. ambrosoids* and *N. tabacum* were not tested in the current study. Silva et al. (2015) had reported the similar findings about toxicity of plant extracts which 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 are in line with findings of Martínez et al. (2017), they reported that movement of larvae reduced by eating the treated maize leaves. The plant-based insecticides have been used by many other researchers against FAW (Ce´spedes et al. 2000). In the previous studies some repellents have used to check their repellency at low as well as high dose 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 main component of integrated pest management under controlled and uncontrolled conditions throughout the world especially against FAW. This technique should be adopted at national and international level. The small farmers which cannot purchase expensive chemicals and technologies to manage the risk of pest on crops due to lack of resources should be used this techniques at their own farms. These plants are easily available free of cost at each farmer farms and could be tested as an alternative and eco-friendly technique in managing FAW.

The current study showed that biological parameters of the current pest in Pakistan are almost similar as recorded in other countries such as Africa, India and America on maize crop. 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 major hosts in 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 now, complete elimination of this pest is

very difficult due to lack of suitable and proper management strategies against this.

CONCLUSION

In Pakistan majority of farmers relay on the insecticides in managing FAW on their crops which ultimately pollute the environment and become harmful for biological fauna such as predators and parasitoids. The plant-based insecticides like botanicals are the ecofriendly pest management strategies which give maximum mortality of pest (FAW) should be tested. The 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

The authors declared that present study was performed in absence of any 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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