Bio-ecology of the Fall Armyworm, *Spodoptera frugiperda* (J. E. Smith) on Maize and its Molecular Characterization

मक्का में फॉल आर्मीवर्म, *Spodoptera frugiperda* (J. E. Smith) की जैव-पारिस्थितिकी एवं इसका आणविक निरूपण

DEEPIKA KALYAN

Thesis

Master of Science in Agriculture
(Entomology)

2020

DEPARTMENT OF ENTOMOLOGY
RAJASTHAN COLLEGE OF AGRICULTURE
MAHARANA PRATAP UNIVERSITY OF AGRICULTURE AND TECHNOLOGY
UDAIPUR-313 001 (RAJASTHAN)

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Thesis

Submitted to the

Maharana Pratap University of Agriculture and Technology, Udaipur

in partial fulfillment of the requirement

for the degree of

Master of Science in Agriculture

(Entomology)

By

DEEPIKA KALYAN

2020

CERTIFICATE-I

CERTIFICATE OF ORIGINILTY

The research work embodied in this thesis titled **"Bio-ecology of the fall armyworm, *Spodoptera frugiperda* (J. E. Smith) on maize and its molecular characterization"** submitted for the award of degree of **Master of Science in Agriculture** in the subject of **Entomology** to Maharana Pratap University of Agriculture and Technology, Udaipur (Raj.), is original and bonafide record of research work carried out by me under the supervision of **Dr. M. K. Mahla**, Professor and Head, Department of Entomology, Rajasthan College of Agriculture, Udaipur. The contents of the thesis, either partially or fully, have not been submitted or will not be submitted to any other Institute or University for the award of any degree or diploma.

The work embodied in the thesis represents my ideas in my own words and where others' ideas or words have been included, I have adequately cited and referenced the original source. I also declare that I have adhered to all principles of academic honesty and integrity and have not misrepresented or fabricated or falsified any idea/ data/ fact/ source in my submission. I understand that any violation of the above will be cause for disciplinary action by the University and can also evoke penal action from the sources which have thus not been properly cited of from whom proper permission has not been taken when needed.

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CERTIFICATE-II

Date: 26/08/2020

This is to certify that this thesis entitled **"Bio-ecology of the fall armyworm, *Spodoptera frugiperda* (J. E. Smith) on maize and its molecular characterization"** submitted for the degree of Master of Science in Agriculture in the subject of Entomology, embodies bonafide research work carried out by **Miss Deepika Kalyan** under my guidance and supervision and that no part of this thesis has been submitted for any other degree. The assistance and help received during the course of investigation have been fully acknowledged. The draft of this thesis was also approved by the advisory committee on 20/08/2020.

The manuscript has been subjected to plagiarism check by software Urkund. It is certified that as per the check, the similarity index of the content is 6% and is within permissible limit as per the MPUAT guidelines on checking Plagiarism.

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This is to certify that the thesis entitled entitled **"Bio-ecology of the fall armyworm, *Spodoptera frugiperda* (J. E. Smith) on maize and its molecular characterization"** submitted by **Miss Deepika Kalyan** to the Maharana Pratap University of Agriculture & Technology, Udaipur in partial fulfillment of the requirement for the degree of **Master of Science in Agriculture** in the subject of **Entomology** after recommendation by the external examiner was defended by the candidate before the following members of the examination committee. The performance of the candidate in the oral examination held on 13/08/2020 was found satisfactory, we therefore, recommend that the thesis be approved.

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This is to certify that **Miss Deepika Kalyan,** student of **Master of Science in Agriculture, Department of Entomology** has made all the corrections / modifications in the thesis **"Bio-ecology of the fall armyworm, *Spodoptera frugiperda* (J. E. Smith) on maize and its molecular characterization"** which were suggested by the external examiner and the advisory committee in the oral examination held on / / 2020. The final copies of the thesis duly bound and corrected were submitted on / / 2020 are enclosed here with for approval.

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Acknowledgement

I take it to be my proud privilege to avail this opportunity to express my sincere and deep sense of gratitude to my major advisor ***Dr. M. K, Mahla****, Professor & Head, Department of Entomology, Rajasthan College of Agriculture (RCA), Udaipur for his stimulating guidance, constructive suggestions and incessant encouragement during the entire period of investigation as well as, critically going through the manuscript.*

I owe my special thanks to the members of my advisory committee; ***Dr. S. Ramesh Babu,*** *Assistant Professor, Department of Entomology,* ***Dr. Devendra Jain,*** *Assistant Professor, Department of Molecular Biology & Biotechnology and* ***Dr. R. N. Bunker****, Assistant Professor, Department of Plant Pathology, RCA, Udaipur as the DRI Nominee for their generous help and valuable suggestions in planning and execution of this study.*

I wish to express sincere and deep sense of gratitude to ***Dr. Anil Vyas,*** *Assistant Professor, Department of Entomology, RCA, Udaipur for providing me the necessary facilities during the course of investigation.*

I am privileged to express my earnest gratitude to ***Dr. R. Swaminathan,*** *ICAR-Emeritus Scientist, Department of Entomology, RCA, Udaipur,* ***Mrs. Tatiana Swaminathan*** *and* ***Dr. A. K, Meena*** *for their generous help and valuable guidance during the study period and, also for providing me the necessary facilities during the course of research work.*

Words can hardly register the sincere and heartfelt feeling which I have for all the ***teachers*** *and other* ***staff members*** *of Department of Entomology for their kind co-operation as and when needed.*

I am highly obliged to thank ***Mr. Sankararaman,*** *Ph. D. scholar, Annamalai University, Tamil Nadu and* ***Mr. Ranjith,*** *Ph. D. scholar, Calicut University, Kerela for identifying the parasitoids recorded during my studies.*

I am very much thankful to my seniors ***Dr. Ashok Bishnoi, Mr. G. Chhangani, Mr. K, C. Ahir and Mr. Kuldeep Sharma*** *who extended their constant help during the investigation.*

I am extremely thankful to my dearest friends especially ***Surabhi, Chetana, Deeksha, Khushboo and Swathi*** *for their constant support and encouragement during the study.*

With most humble sense of regards and reverence, I would like to acknowledge the people who mean the world to me, ***my parents, Dr. R, K, Kalyan*** *and* ***Mrs. Tara Kalyan*** *whose endless support, affection and encouragement brought the present task to completion. I don't imagine a life without their love and blessings. I would also like to express my respect to all my* ***family members*** *for their selfless support and encouragement during the course of this work.*

Last but not the least, I appreciate the help rendered to me during the period of my study by all those whose name could not be mentioned.

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Date: 26/08/2020

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1. INTRODUCTION

Maize (*Zea mays* L.) is an important cereal crop in the world's agricultural economy. Worldwide, it is popularly known as the **"Queen of cereals"** because of its high genetic yield potential and wider adaptability. In addition to being a staple food for human beings and quality feed for animals, maize also serves as a basic raw material for a number of industrial products. Globally, maize is cultivated in an area of 192.14 million hectares with a production of 1,113.02 million tonnes and productivity of 5,790 kg ha^{-1} (Anonymous, 2020a). The major maize producing countries of the world are USA, China, Brazil, Europe, Argentina, Ukraine, Mexico and India, with the hegemony of USA. India ranks 4^{th} in area and 7^{th} in production of maize in the world. In India, the area under maize is 9.30 million hectares with a production of 28.50 million tonnes and productivity of 3060 kg ha^{-1} (Anonymous, 2020a). The important maize growing states in India are Karnataka, Madhya Pradesh, Bihar, Tamil Nadu, Maharashtra, Telangana, Andhra Pradesh, Uttar Pradesh and Rajasthan. In Rajasthan, maize is cultivated in an area of 8.87 lakh hectares, with the production and productivity of 1.22 million tonnes and 1378 kg ha^{-1}, respectively (Anonymous, 2020b).

In recent years, maize has gained popularity among farmers and the area under this crop has also increased; however, the production is considerably low due to many biotic and abiotic constraints. Among the biotic constraints, insect pests are an important limiting factor for its profitable cultivation. About 141 species of insect pests have been reported causing varying degree of damage to this plant in India (Reddy and Trivedi, 2008; Kumar *et al.*, 2015). Among these, some of the important ones are the stem borer, *Chilo partellus* (Swinhoe); pink stem borer, *Sesamia inferens* (Walker); armyworm, *Mythimna separata* (Walker) and maize aphid, *Rhopalosiphum maidis* (Fitch) (Jeengar *et al.*, 2010; Saini *et al.*, 2011; Patel and Patel, 2012). An invasive pest, the fall armyworm, *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) has recently been reported from several parts of the world causing significant damage to maize crop (Rakshit *et al.*, 2019).

The fall armyworm (*S. frugiperda*) is native to the tropical and subtropical regions of America and is one of the most economically damaging insect species of

this area (Luginbill, 1928). This pest was reported only in America till 2015, but in 2016, it was recorded from Africa causing serious damage to maize crop (Goergen *et al*., 2016). Within a short span of its introduction into Africa, this pest has been detected from 45 sub-Saharan African countries (Anonymous, 2019). In India, it was reported for the first time from Shivamogga (Karnataka) on maize in the month of May, 2018 (Sharanbasappa *et al*., 2018a) and, within a few months, its infestation spread to several other states of India (CABI, 2019). *S. frugiperda* consists of two morphologically identical but genetically distinct strains, a Corn (C) strain that is primarily associated with maize and sorghum, and a Rice (R) strain which is preferably found in rice and turf grass (Pashley,1986). These strains are region specific and differ with regard to their dispersal pattern, sexual behaviour and response to pesticides (Pashley *et al*., 1992). These variations between both the strains play a significant role in formulating pest control strategies (Adamczyk *et al.,* 1997) and host plant resistance breeding programs (Lu and Adang, 1996); hence, strain identification becomes necessary for its management.

Being a polyphagous pest, *S. frugiperda* causes major damage to economically important cultivated crops such as rice, sorghum, sugarcane, cabbage, beet, peanut, soybean, alfalfa, onion, cotton, pasture grasses, millet, tomato and potato (CABI, 2016). A total of 353 host plants have been recorded belonging to 76 plant families, principally Poacae (106), Asteraceae (31) and Fabaceae (31) (Montezano *et al.,* 2018); however, its maximum incidence and damage has been reported on maize crop. It can infest all the stages of maize, right from emergence to tasseling, silking and cob formation leading to an annual yield loss of 8.3 to 20.6 million tonnes in maize (Day *et al*., 2017).

Owing to its remarkable dispersal ability, high reproductive capacity, no diapause and wide host range, it is one of the more severe economic pests and an immediate focus of research on this pest is the need of the hour; therefore, the present investigation entitled, **"Bio-ecology of the fall armyworm, *Spodoptera frugiperda* (J. E. Smith) on maize and its molecular characterization"** was proposed with following objectives:

1. To study the biology of *S. frugiperda* on maize.
2. To analyze the population dynamics of *S. frugiperda* on maize.
3. To characterize *S. frugiperda* on a molecular basis.

2. REVIEW OF LITERATURE

Pertinent literature on the present investigation, **"Bio-ecology of the fall armyworm, *Spodoptera frugiperda* (J. E. Smith) on maize and its molecular characterization"** has been reviewed and presented under the following sub heads:

2.1 Biology of fall armyworm:

Spodoptera frugiperda (J. E. Smith) has the ability to complete several generations in an year, with the life cycle comprising of egg, six to seven larval instars, pupa and adult. The eggs are oblate-spheroidal, circular in cross section, greenish grey when freshly deposited and turn dark or blackish before hatching. The height and width of the eggs measure 0.39 and 0.47 mm, respectively. The six larval instars attain a length of 1.7, 3.5, 6.4, 10.0, 17.2 and 34.2 mm, respectively. The pupation normally occurs in soil at a varying depth of 1-3 inches with an average pupal period of 9 days. The average adult longevity of male and female is 10 and 11.4 days, respectively (Walton and Luginbill, 1917; Luginbill, 1928). Completion of the life cycle usually takes about 4 weeks, but can also take up to 12 weeks during periods of low temperatures (Vickery, 1929). A similar trend in the life cycle was noticed by Sparks (1979) and Hogg *et al.* (1982), who observed that temperature and other environmental conditions affect the duration of larval development (hatching to pupation), that can range between 14-50 days.

According to Pitre and Hogg (1983), *S. frugiperda* has six larval instars whose mean development time was determined to be 3.3, 1.7, 1.5, 1.5, 2.0 and 3.7 days, respectively. The head capsule widths were also recorded which were 0.35, 0.45, 0.75, 1.3, 2.0, and 2.6 mm for 1-6 instars, respectively. The total larval period was about 14 days during summers and 30 days during winters. Similarly, the pupal period was about 8-9 days during summers but 20-30 days during winters. The pupal length and width were measured to be 14-18 and 4.5 mm, respectively. The duration of the adult stage ranged from 7-21 days, with an average of 10 days. The life cycle, according to Ashley *et al.* (1989), normally completes in 28 days.

Murua and Virla (2004) studied the biology of *S. frugiperda* on maize and recorded the incubation, larval, pupal, pre-oviposition, oviposition and post oviposition period which were 3.53, 26.97, 10.35, 3.8, 8.5 and 1.5 days, respectively.

The adult longevity was of 16 days while, the entire life cycle completed in 36.8 days. Santos *et al.* (2004) studied the biology of *S. frugiperda* on three different corn genotypes *viz.*, ELISA, BR 400 and BR PAMPA and recorded the oviposition period (7.55; 6.5; 6.11 days), number of ovipositions per female (6.33; 5.25; 4.88), eggs per oviposition (216.72; 215.03; 226.02), egg viability (59.02; 69.76; 60.81 %), male longevity (15.22; 13.62; 13.29 days) and female longevity (16.33; 14.06; 15.65 days) for each of the genotype. Murua *et al.* (2008) noticed that the fall armyworm takes 2.63, 18.18 and 9.28 days to complete its incubation, larval and pupal period, respectively. Female adult longevity was of 12.7 days; while, male adult longevity was of 16.6 days. The sex ratio (F:M) was also observed which turned out to be 1.2:1. Rosa *et al.* (2012) also studied the biology of *S. frugiperda* on five different strains of corn and recorded the incubation, larval, pre-pupal and pupal period of 2.8-3.3, 10.7-21.7, 1.1-1.3 and 2.1-10.1 days, respectively; while, the adult longevity was recorded to be of 14.7-32.3 days.

Silva *et al.* (2017) studied the biological parameters of *S. frugiperda* on maize and observed the pre-pupal, pupal and larval-adult period to be 1.89, 8.54 and 21.41 days, respectively. Sharanabassapa *et al.* (2018b) studied the life cycle of *S. frugiperda* on maize and observed the incubation, larval and pupal period to be of 2-3, 14-19 and 9-12 days, respectively. The mean development time of the six larval instars was 2.60, 2.20, 2.0, 2.0, 2.40 and 4.50 days, respectively. The head capsule widths of the five larval moults were 0.34, 0.48, 0.81, 1.22 and 1.96 mm, respectively. The pre-oviposition, oviposition and post oviposition period ranged from 3-4, 2-3 and 4-5 days, respectively. The male moths survived for 7-9 days; while, the females for 9-12 days. The fecundity ranged from 839-1169 with an average of 1064.80 eggs. The male individuals completed their life cycle in 32-43 days and the female individuals in 34-46 days. According to Venkateswarlu *et al.* (2018), the incubation and larval period of *S. frugiperda* ranges from 2-3 and 14-28 days, respectively. The adults are nocturnal in nature and are most active during warm, humid evenings (Igyuve *et al.*, 2018).

Deole and Paul (2018) carried out an experiment to study the biology of fall armyworm on maize and recorded the incubation, larval, pre-pupal, pupal and adult period to be of 2-3, 14-16, 1-2, 6-8 and 5-7 days, respectively. Prasanna *et al.* (2018) estimated the average adult life of 10 days. Bhavani *et al.* (2019) recorded the

incubation, total larval and pupal period of 2-3, 13-14 and 8-9 days, respectively. The female adults survived for an average of 10 days; whereas, males survived for an average of 8 days. The total life cycle was also recorded which ranged from 32-36 days for female individuals and, 30-34 days for male individuals. Malo and Hore (2019) recorded the larval, pupal and adult period to be 14-22, 8-9 and 7-21 days, respectively. Similarly, Manjula *et al.* (2019) also observed the life cycle of *S. frugiperda* and recorded the incubation, larval, pupal and adult duration to be 2-3, 14-30, 9 and 7 days, respectively. The length attained by the larvae during the six instars was 0.7, 3.5-4.0, 7.0, 16, 22 and 28 mm, respectively; and their respective head capsule widths were 0.16, 0.5, 2.0, 2.5, 3.0 and 3.0 mm.

2.2 Population dynamics of fall armyworm:

The literature on the population dynamics of *S. frugiperda* is very scanty; however, available and relevant literature has been compiled and presented here. Waddill *et al.* (1982) recorded the seasonal abundance of fall armyworm at four different locations in Florida *viz.*, Gainesville, Sanford, Bradenton and Homestead, and observed the peak incidence in the month of August (Gainesville), July (Sanford) and October (Bradenton and Homestead). Silvain and Ti-A-Hing (1985) studied the seasonal periodicity of *S. frugiperda* in French Guiana and recorded the maximum larval population in the 4th week of February. Nagoshi and Meagher (2004a) conducted an experiment to study the incidence of fall armyworm in southern Florida. The peak incidence was recorded during spring (March-May) and autumn (October-December) season while the least incidence was recorded during the months of July-October.

Murua *et al.* (2006) recorded the incidence of fall armyworm and its parasitoids infesting maize, and the impact of abiotic factors on their incidence. The incidence of fall armyworm started when the plants achieved V1 stage, while the maximum population was observed during the vegetative stage (V3-V6). Abiotic factors such as atmospheric temperature and rainfall significantly and positively affected the pest density; whereas, the parasitoid population was significantly and positively affected only by temperature. Ayala *et al.* (2013) reported that the incidence of *S. frugiperda* was more during the months of December-February (1.65 larvae/plant) as compared to September-November (1.16 larvae/plant).

Mallapur *et al.* (2018) recorded the incidence of *S. frugiperda* on maize in northern Karnataka and recorded that the crop sown during the first fortnight of September suffered higher incidence while the lowest incidence was recorded in the crop sown during the first fortnight of May. Kuate *et al.* (2019) revealed that the mean incidence of *S. frugiperda* was the highest during the months of October-November (60.7%) followed by February-March (58.4%) and May-June (40.6%). Visalakshi *et al.* (2019) noticed the first incidence of fall armyworm in maize on 10th August, 2018 with an infestation level of 7.34%. Chormule *et al.* (2019) recorded the incidence of *S. frugiperda* on maize, sweet corn, sorghum and sugarcane in five districts of Maharashtra. The incidence was noticed up to 60 days of the crop stage and drastically reduced, thereafter. Fonseca-Medrano *et al.* (2019) observed that the infestation of fall armyworm was significant and positively correlated with minimum temperature and relative humidity, and negatively correlated with maximum temperature; whereas, was non-significant and positively correlated with rainfall. Kumar *et al.* (2020) reported that larval fall armyworm population was positively correlated with maximum temperature and negatively correlated with minimum temperature, relative humidity and rainfall.

As for the biotic factors influencing the incidence of *S. frugiperda*, various natural enemies have been reported from across the world, which have been listed in the table below:

Table 1: Natural enemies of *S. frugiperda*:

S. No.	Natural enemies	Order: Family	Reference
Parasitoids:			
1.	*Meteorus laphygmae* Viereck	Hymenoptera: Ichneumonidae	Hoballah *et al.* (2004)
2.	*Ophion flavidus* Brulle	Hymenoptera: Ichneumonidae	,,
3.	*Pristomerus spinator* (Fabricius)	Hymenoptera: Ichneumonidae	,,
4.	*Campoletis sonorensis* (Cameron)	Hymenoptera: Ichneumonidae	,,
5.	*Cotesia marginiventris* (Cameron)	Hymenoptera: Braconidae	,,
6.	*Homolobus truncator* (Say)	Hymenoptera: Braconidae	,,

7.	*Aleiodes laphygmae* (Viereck)	Hymenoptera: Braconidae	,,
8.	*Euplectrus plathypenae* Howard	Hymenoptera: Eulophidae	,,
9.	*Trichogramma atopovirilia* Oatman & Platner	Hymenoptera: Trichogrammatidae	,,
10.	*Campoletis grioti* (Blanchard)	Hymenoptera: Ichneumonidae	Murua *et al.* (2006)
11.	*Chelonus insularis* Cresson	Hymenoptera: Braconidae	,,
12.	*Archytas* sp.	Diptera: Tachinidae	,,
13.	*Meteorus arizonensis* Muesebeck	Hymenoptera: Ichneumonidae	Magali *et al.* (2015)
14.	*Campoletis flavicincta* (Ashmead)	Hymenoptera: Ichneumonidae	,,
15.	*Pristomerus* sp.	Hymenoptera: Ichneumonidae	,,
16.	*Lespesia* sp.	Diptera: Tachinidae	,,
17.	*Archytas marmoratus* (Townsend)	Diptera: Tachinidae	,,
18.	*Trichogramma* sp.	Hymenoptera: Trichogrammatidae	Shylesha *et al.* (2018)
19.	*Campoletis chlorideae* Uchida	Hymenoptera: Ichneumonidae	,,
20.	*Telenomus* sp.	Hymenoptera: Scelionidae	,,
21.	*Glyptapanteles creatonoti* (Viereck)	Hymenoptera: Braconidae	,,
22.	*Charops ater* Szepligeti	Hymenoptera: Ichneumonidae	Sisay *et al.* (2019)
23.	*Coccygidium luteum* (Brulle)	Hymenoptera: Braconidae	,,
24.	*Chelonus curvimaculatus* Cameron	Hymenoptera: Braconidae	,,
25.	*Cotesia icipe* Fernandez-Triana & Fiaboe	Hymenoptera: Braconidae	,,
26.	*Palexorista zonata* (Curran)	Diptera: Tachinidae	,,
27.	*Eriborus* sp.	Hymenoptera: Ichneumonidae	Sharanabasappa *et al.* (2019)
28.	*Odontepyris* sp.	Hymenoptera: Bethylidae	,,
29.	*Coccygidium melleum* (Roman)	Hymenoptera: Braconidae	,,

30.	*Exorista sorbillans* (Wiedemann)	Diptera: Tachinidae	,,
31.	*Chelonus formosanus* Sonan	Hymenoptera: Braconidae	Gupta *et al.* (2019a)
32.	*Cotesia ruficrus* (Haliday)	Hymenoptera: Braconidae	Gupta *et al.* (2019b)
33.	*Coccygidium transcaspicum* (Kokujev)	Hymenoptera: Braconidae	Gupta *et al.* (2020)
Predators:			
1.	*Doru taeniatum* (Dorhn)	Dermaptera: Forficulidae	Wyckhuys and O'Neil (2006)
2.	*Podisus maculiventris* (Say)	Hemiptera: Pentatomidae	Magali *et al.* (2015)
3.	*Forficula* sp.	Dermaptera: Forficulidae	Shylesha *et al.* (2018)
4.	*Eocanthecona furcellata* (Wolff)	Hemiptera: Pentatomidae	Shylesha and Sravika (2018)
5.	*Andrallus spinidens* (Fabricius)	Hemiptera: Pentatomidae	,,
6.	*Harmonia octomaculata* (Fabricius)	Coleoptera: Coccinellidae	Sharanabasappa *et al.* (2019)
7.	*Coccinella transversalis* Fabricius	Coleoptera: Coccinellidae	,,
Pathogens:			
1.	*Beauveria bassiana* (Balsamo) Vuillemin	Hypocreales: Cordycipitaceae	Magali *et al.* (2015)
2.	*Nomuraea rileyi* (Farl.) Samson	Hypocreales: Clavicipitaceae	Shylesha *et al.* (2018)
3.	Nuclear Polyhedrosis Virus (NPV)	Baculoviridae	Manjula *et al.* (2019)
4.	Cytoplasmic Polyhedrosis Virus (CPV)	Baculoviridae	,,

2.3 Molecular characterization of fall armyworm:

The fall armyworm species consists of two morphologically indistinguishable subpopulations, designated as the corn strain (C strain) and the rice strain (R strain) (Pashley *et al.*, 1985), which can be reliably differentiated using molecular markers.

Meagher and Meagher (2003) used two molecular markers *viz.*, mitochondrial DNA (mtDNA) RFLP marker and mitochondrial cytochrome oxidase subunit I (mt*COI*) gene PCR-RFLP marker to identify the host strains of fall armyworm. Nagoshi and Meagher (2004b) also used molecular markers to determine the host strain (corn or rice) of male moths captured in sex pheromone-baited traps placed in

different habitats in the overwintering areas of southern Florida. Their results showed that the rice strain moths were primarily observed in naturalized pasture and wetlands; whereas, the corn strain moths were observed in areas associated with golf courses, agriculture, or urban development. Hence, they concluded that the corn strain moths are limited in their habitat choice while the rice strain moths have a substantially broader range in southern Florida. Lewter and Szalanski (2007) developed a molecular diagnostics protocol using polymerase chain reaction, restriction fragment length polymorphism (PCR-RFLP) to identify *S. frugiperda* using the restriction enzymes Dra I, Alu I and Nla III.

Machado *et al.* (2008) distinguished the two strains of *S. frugiperda* using PCR-RFLP of the mitochondrial gene cytochrome oxidase I (*COI*) with restriction enzymes, *Msp*I and *Sac*I. A similar method of differentiation using PCR-RFLP of *COI* and PCR of the gene FR (for Rice Strain) was used by Cano-Calle *et al.* (2015). As per their observations, the corn strain was more abundant in corn, cotton, sorghum, sugarcane, and sweet sorghum; whereas, the rice strain was more abundant in grass and rice.

Goergen *et al.* (2016) collected and studied various samples of *S. frugiperda* from different regions of African continent and confirmed its identity based on the DNA barcoding molecular technique using the primer pair LepF1 (5'- ATTC AACC AATC ATAA AGAT ATTGG -3') and LepR1 (5'-TAAA CTTCTGGA TGT CCAA AAA ATCA-3'). Sisodiya *et al.* (2018) identified *S. frugiperda* by PCR amplification of mt*COI* gene using the universal primers *viz*., forward primer: (LCO1490 5'-GGTCAACAAATCATAAAGATATTGG-3') and reverse primer: (HCO2198 5'-TAAACTTCAGGGTGACCAAAAAATCA-3').

According to Mahadeva Swamy *et al.* (2018), the populations of *S. frugiperda* collected from Karnataka, Tamil Nadu, Andhra Pradesh, Telangana, Madhya Pradesh and Maharashtra aligned with Rice strain with minimal genetic diversity. Their results showed the prevalence of Rice strain in India. Nagoshi *et al.* (2019a) distinguished the two strains of *S. frugiperda* by polymorphic sites in the *COI* and *Tpi* genes. Nagoshi *et al.* (2019b) examined the specimens of *S. frugiperda* collected from India and South Africa and their study indicated a genetic homogeneity between the populations tested.

Assefa (2019) did the molecular identification of the invasive *S. frugiperda* in Swaziland and identified it as the Rice strain, using the mt*COI* gene molecular technique. Babu *et al.* (2019) confirmed the identity of *S. frugiperda*, collected from maize fields of Banswara and Dungarpur, using mt*COI* gene, and reported their *COI* gene sequence to be of 657 and 664 bp in size, respectively.

3. MATERIALS AND METHODS

The present investigation entitled, **"Bio-ecology of the fall armyworm, *Spodoptera frugiperda* (J. E. Smith) on maize and its molecular characterization"** was carried out at Agricultural Research Station, Banswara; Department of Entomology and the Instructional farm, Rajasthan College of Agriculture, MPUAT, Udaipur during *Kharif*, 2019. The materials used and methodologies adopted during the course of investigation for conducting each experiment have been detailed below:

3.1 Biology of *S. frugiperda* on maize:

The biology of *S. frugiperda* was studied in the laboratory under controlled conditions of 25 ± 2˚C and 70-75 % RH, for which, its larvae were collected from the Instructional farm, RCA, Udaipur and cultured. The culture was maintained in glass jars (1000 ml capacity) and provided fresh maize leaves and stem, of variety Pratap Makka-3, daily until pupation (Plate 1). The pupae so developed were transferred into clean jars, covered with muslin cloth and fastened with rubber bands, until the emergence of moths. The adults that emerged were paired and allowed to mate in separate glass jars of similar capacity/size. The moths were fed with 10% honey solution soaked on cotton pads for proper egg laying. The eggs thus laid were used for further studies. After hatching, the larvae (n = 30) were transferred into similar sized new glass jars and reared individually on fresh maize leaves that were changed daily. To study the biology, the observations listed below were recorded as per the procedure suggested by Sharanabasappa *et al.* (2018b). Necessary morphological traits were also measured such as: length of different larval instars, pupae, adults and their wing span.

3.1.1 Observations:

a) **Incubation period:** The time taken by the eggs to hatch was recorded.

b) **Larval period:** The time taken by larvae to complete each instar was recorded

c) **Pupal period:** The observations on the pupal period were recorded.

d) **Pre-oviposition period:** After eclosion, the number of days before laying eggs was recorded.

Plate I: Rearing of fall armyworm in laboratory

e) **Oviposition period:** The number of days in which oviposition was carried out was recorded.

f) **Post-oviposition period:** The number of days after oviposition till the death of adults was recorded.

g) **Fecundity:** The number of eggs laid by the female individuals was recorded.

h) **Sex ratio (%):** The number of male and female individuals was identified on the basis of their morphological differences and recorded.

9.2 Population dynamics of fall armyworm:

9.2.1 Site and location of the experiment:

The field experiment was conducted at the Instructional farm, Rajasthan College of Agriculture, Udaipur and the laboratory work was carried out in the Department of Entomology, Rajasthan College of Agriculture, Udaipur. Udaipur is situated at 23.4˚N Longitude and 75˚E Latitude at an elevation of 579.17 MSL in Rajasthan state.

9.2.2 Climate and weather conditions of the location:

The region has a typical sub-tropical climatic condition characterized by mild winters and hot summers. The average annual rainfall of this zone (IV a) ranges between 450-650 mm, most of which is received between July-September with occasional rains during the winters. During summers, the temperature may rise up to 45.5˚C, while in winters it may fall to as low as 4˚C.

9.2.3 Field preparation:

The allotted experimental field was prepared by one deep ploughing through cultivator followed by cross harrowing and planking to improve the field condition for proper soil aeration and easy germination of seeds.

9.2.4 Layout and sowing:

The experiment to study the population dynamics of *S. frugiperda* infesting maize was laid out in uniformly sized plots measuring 7 m × 4.5 m replicated four times. Maize variety Pratap Makka-3 was sown in the prepared field during first week of July, 2019 with row to row and plant to plant spacing of 75 cm × 25 cm, respectively (Plate 2).

Plate II: A view of the experimental field for population dynamics of fall armyworm during *Kharif* 2019

9.2.5 Other agronomic practices:

All other recommended agronomic practices such as thinning, hoeing, weeding and irrigation, except insecticidal sprays, were followed as per the package of practices (Zone IV a) to raise a good crop.

9.2.6 Meteorological data:

The meteorological data throughout the experimental period for atmospheric temperature (^{o}C), relative humidity (%) and rainfall (mm) were obtained from the Meteorological Observatory at Rajasthan College of Agriculture, Udaipur.

9.2.7 Observations:

(a) The observations for the population of fall armyworm larvae were taken at weekly intervals during morning hours between 6.30 am to 9.00 am on ten randomly selected plants in each plot by visual count.

(b) Observations were taken for the associated natural enemies with a view to estimate the parasitization by various larval parasitoids of *S. frugiperda*, for which the larvae collected from the field were brought to the laboratory and reared in glass jars on maize leaves until the emergence of *S. frugiperda* adult or any parasitoid. The parasitization per cent (Murua *et al.*, 2006) and effective parasitization per cent (Tian *et al.*, 2008) were calculated using following formulas:

$$\text{Parasitization (\%)} = \frac{\text{No. of parasitized individuals}}{\text{No. of total individulas observed}} \times 100$$

$$\text{Effective Parasitization (\%)} = \frac{\text{No. of parasitized larvae}}{\text{No. of parasitized + healthy larvae}} \times 100$$

9.2.8 Statistical analysis:

Population data of *S. frugiperda* thus obtained was subjected to statistical analysis to find out the co-efficient of correlation and multiple linear regression with the prevailing abiotic conditions of the atmosphere using the following formulas suggested by Rangaswamy (2010):

$$r_{xy} = \frac{\sum xy - \frac{\sum x \sum y}{n}}{\sqrt{\left[\sum (x)^2 - \frac{(\sum x)^2}{n}\right]\left[\sum (y)^2 - \frac{(\sum y)^2}{n}\right]}}$$

Where,

r_{xy} = Simple correlation coefficient

x = Variable *i.e.* abiotic component

y = Variable *i.e.* mean number of insects

n = Number of observations

The correlation coefficient (r) values were subjected to the test of significance using t- test:

$$t = \frac{r}{\sqrt{1 - r^2}} \times \sqrt{n-2} \sim t_{n-2} \text{ d.f.}$$

The multiple linear regression equation was computed by using following modal:

$$Y = a + b_1X_1 + b_2X_2 + b_3X_3$$

Where,

a	=	Constant
Y	=	Dependent variable
X_1, X_2 and X_3	=	Independent variables
b_1, b_2 and b_3	=	Partial regression co-efficient

The data of fall armyworm incidence on randomly selected ten plants in four plots taken at weekly intervals was also subjected to various spatial dispersion indices to find out its spatial distribution pattern. The spatial distribution was determined using the Dispersion Index, Aggregation Index, Clumping Index, Mean Crowding Index, Lloyd's Patchiness Index, Mean colony size, Iwao's patchiness regression and Taylor's Power law. To work out these indices, the parameters that were calculated initially include the following:

1. Mean ($\bar{x}$) $= \sum \frac{x}{n}$
2. Variance (s^2) $= \frac{\sum(x-\bar{x})^2}{n-1}$

Where,

x = values of the number of observations

n = number of observations

Further, the following spatial dispersion indices were calculated to determine the distribution pattern:

1. Dispersion Index or variance to mean ratio (VMR), which is calculated by $s^2/\bar{x}$.
2. Aggregation Index (K), which is calculated by $\bar{x}^2/(s^2-\bar{x})$.
3. Clumping Index or David and Moore Index (I_{DM}), which is calculated by $(s^2-\bar{x})-1$.
4. Mean Crowding index (X^*), which is calculated by $\bar{x} + I_{DM}$.
5. Lloyd's Patchiness Index, which is calculated by $X^*/\bar{x}$.
6. Mean colony size (C^*), which is calculated by X^*+1.
7. Iwao's patchiness regression ($X^*=\alpha+\beta\bar{x}$), which gives a linear relationship between mean crowding (X^*) and mean density ($\bar{x}$), where α is the index of basic contagion and β is the density contagiousness coefficient.
8. Taylor's Power law ($s^2=a\bar{x}^b$), which gives a relation between variance and mean, where 'a' is the constant and 'b' is the coefficient of contagion.

9.3 Molecular characterization of fall armyworm:

9.3.1 Species identification:

The larvae of *S. frugiperda* were collected from the Instructional farm, Rajasthan College of Agriculture, MPUAT, Udaipur. They were identified by studying their various morphological characters and confirmed with the earlier findings of Pogue (2002) and EPPO (2015). The male genitalia dissections were also prepared following the methodology suggested by Clarke (1941). The genitalia were dissected with the help of stereo zoom binoculars and imaged using Stemi 2000 C Stereozoom Binoculars of Carl Zeiss. Voucher specimen along with dissected

genitalia were stored in small vials in glycerin and deposited at the Department of Entomology, Rajasthan College of Agriculture, MPUAT, Udaipur.

9.3.2 DNA extraction, amplification and sequencing:

The identified larvae were then preserved in 95% EtOH and stored at -20 ^{o}C until further use for DNA extraction. A portion of larval tissue was dissected and air-dried for few minutes and then rinsed with sterile molecular grade water to remove the excess ethanol in the sample. Total genomic DNA was extracted from the dissected portion using DNASure Tissue mini kit (Nucleo-pore, Genetix Brand, India), in accordance with the manufacturer's instructions. The intact genomic DNA was visualized using 0.8% agarose gel and quantified by using a Nano-spectrophotometer (NABi, Microdigital, South Korea). After quantification, the DNA samples were diluted with molecular gradient sterile water to get a working solution of 40-50 ng/µl. The extracted DNA was subjected to PCR amplification using the cytochrome oxidase subunit I (*COI*) gene of 658-700 bp region with the universal primers *viz*., forward primer: (LCO1490 5'-GGTCAACAAATCATAAAGA TATTGG-3') and reverse primer: (HCO2198 5'-TAAACTTCAGGGTGA CCAAAAAATCA3') (Folmer *et al*., 1994). PCR amplification was carried out in a final volume of 25µL using 12.5µL of DreamTaq PCR Master Mix (2X) (ThermoFisher, Scientific, UK) 2µL DNA template, 10 pmol of each forward and reverse primer and rest of the volume made up with nuclease free water. Polymerase chain reaction (PCR) was performed with C1000Touch^{TM} Thermal cycler of Bio-Rad, USA with the following parameters, an initial denaturation of 94 °C for 4 min., followed by 35 cycles of denaturation at 94 °C for 30 sec., annealing at 47 °C for 45 sec., extension at 72 °C for 45 sec. and final extension at 72 °C for 20 min. Three replications were carried out for each of the reactions and were sent for sequence analysis. The amplified PCR products were analyzed by electrophoresis on a 1.8% agarose gel with a 100bp DNA ladder used as a molecular weight standard and visualized in a gel Documentation system (Gel Doc^{TM} EZ Imager, Bio-Rad, USA). PCR products were purified by using a GeneJET PCR purification Kit (ThermoFisher, Scientific, UK) in accordance with the manufacturer's protocol. The purified PCR product was sent through outsourcing Agile Lifescience Technologies India Pvt. Ltd, Pune (ABI PRISM 3730xl Genetic Analyzer develop by Applied Biosystems, USA) for sequencing target fragment by using universal primers. The obtained

chromatogram was edited to remove the ambiguous bases and the sequence was compared with authenticated sequences through Basic Local Alignment Search Tool (BLASTn, http://www.ncbi.nlm.nih.gov) search and also from the Barcoding of Life Data system (BOLD; http://www.boldsystems.org/) to confirm the identity of the sequence. The sequence obtained was deposited at the gene bank of National Center for Biotechnology Information (NCBI), USA to obtain the accession number.

9.3.3 Strain Analysis:

The PCR products of the mitochondrial DNA region of *COI* gene of, collected larvae, were subjected to RFLP analysis (Cano-Calle *et al.*, 2015; Nagoshi and Meagher, 2003; Velez-Arango *et al.*, 2008). The PCR reaction was carried out for the amplification of cytochrome oxidase subunit I (*COI*) gene which is of ~570 bp using the forward primer JM76 (5'-GAGCTGAATTAGG(G/A)ACTCCAGG-3') and the reverse primer JM77 (5'-ATCACCTCC(A/T)CCTGCAGGATC-3') (Levy et al., 2002). The PCR reaction was performed with initial denaturation for 3 min. at 94°C, followed by 30 cycles of 1 min. denaturation at 94°C, 1 min. primer annealing at 62°C, 1 min. initial extension at 72°C and a final extension cycle of 10 min. at 72°C. Before restriction enzymes digestion, the PCR products were purified by using GeneJET PCR purification Kit (ThermoFisher, Scientific, UK) and restriction enzyme digestion was done by using the restriction enzymes, *Msp*I and *Sac*I for the obtained purified PCR products. Ten microlitre of the PCR product, 2 µl of 10X Buffer (Thermo Scientific) and in a two different reactions, 10-20 units of the restriction enzyme *Msp*I and *Sac*I were added and this volume was adjusted with nuclear free water the same was incubated at 37°C for 2-3 hrs. All samples were separated by electrophoresis in a 2% agarose gel containing ethidium bromide (0.5µg/µL) for 60 min at 80 V (BIO-RAD, USA) and visualized in gel documentation system (Gel Doc^{TM} EZ Imager, BIO-RAD, USA). PCR band size of about 569 bp is obtained in the rice strain (CO-RS); whereas, in the corn strain (CO-CS) two fragments of size 497 and 72 bp are obtained on digestion with *Msp*I restriction enzyme (Nagoshi and Meagher 2003). A diagnostic banding pattern of 500 and 69 bp is obtained in the rice strain by the digestion with restriction enzyme, *Sac*I (Lu *et al.*, 1994).

4. RESULTS

The results of the investigations carried out on **"Bio-ecology of the fall armyworm, *Spodoptera frugiperda* (J. E. Smith) on maize and its molecular characterization"** are presented in this chapter.

4.1 Biology of *S. frugiperda* on maize:

The biology of *S. frugiperda* on maize was studied in laboratory during the experimental period, the data of which have been presented in Table (2 & 3) and the details of the life stages have been illustrated in Plate 3.

4.1.1 Egg:

The eggs were generally laid in masses of 25-330, which were either laid in a single layer or stacked up in two to three layers. A gravid female laid 4-11 egg masses. They were covered with grayish-white scales from the female abdomen. The eggs were somewhat dome shaped; with a flattened base and rounded apex. The colour of the eggs was white to creamish that turned brown to black just before hatching. The incubation period was of 3-4 days, with a mean time of 3.38 days.

4.1.2 Larva:

The fall armyworm passed through six larval instars. The characteristics and duration of each instar were observed and recorded.

4.1.2.1 First instar:

The first larval instars were very tiny. They completely devoured the egg shells from which they hatched. They had a comparatively large flattened circular black head and a whitish body covered with minute hairs. After feeding on leaves, their body colour changed to greenish white. The mean development time of the first larval instar was 2.82 days and the mean body length was 1.80 mm.

4.1.2.2 Second instar:

The second instar larvae had amber coloured head and a pale white to yellowish coloured body with a tinge of brown on the dorsum. The body also developed faint white dorsal and sub-dorsal lines at this stage. The mean development time of the second larval instar was 2.50 days with the mean body length of 3.50 mm.

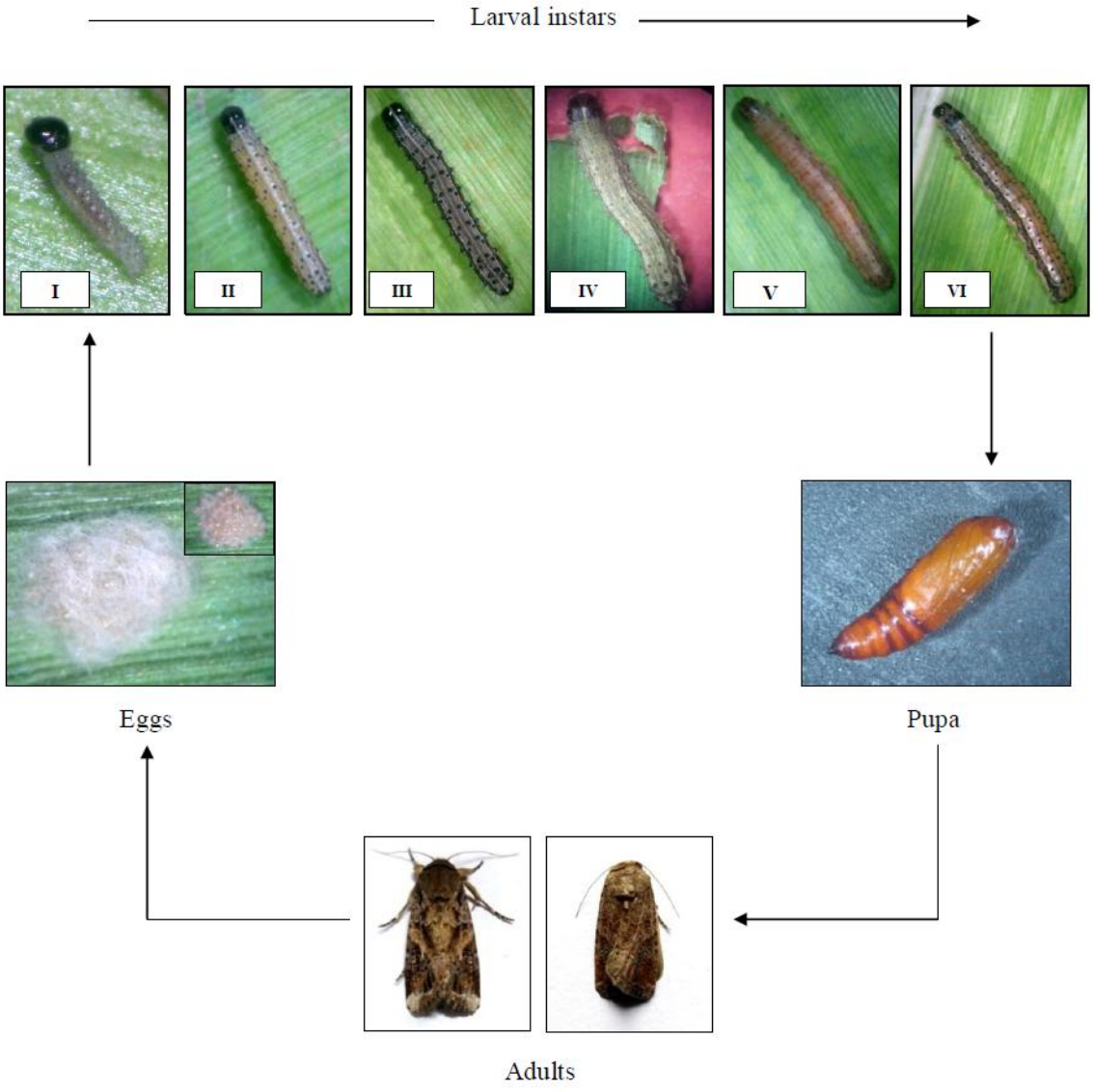


Plate III: Life cycle of fall armyworm

4.1.2.3 Third instar:

The third instar larvae showed an immense change in body colour. The body colour changed from pale white to greenish brown. The larvae were light brown on the dorsum and greenish on the ventral side. The dorsal and sub-dorsal white lines were plainly visible and the black spots became prominent. The mean development time of this instar was 2.10 days with mean body length of 6.20 mm.

4.1.2.4 Fourth instar:

The larvae showed a distinct difference from third to fourth instar in their appearance. Their body colour varied from olive brown to dark brown. The dorsal and sub-dorsal white lines also became conspicuous. The spiracles on prothorax and eighth abdominal segments were elliptical, while the rest were circular. The mean development time of the fourth larval instar was 2.02 days with mean body length of 9.70 mm.

4.1.2.5 Fifth instar:

At this stage, the body attained a grayish brown colour on the dorsum and greenish colour on the ventral and sub-ventral sides. All the spiracles turned elliptical in shape. The mean development time of the fifth larval instar was 2.70 days with mean body length of 16.80 mm.

4.1.2.6 Sixth instar:

The larvae during this stage were most stout and bulged with somewhat cylindrical in shape. Their body was smooth with clear and distinct segmentation. The head was black and slightly bilobed. The colour of the body was grayish brown on the dorsum, while the ventral and sub-ventral sides were greenish mottled with reddish brown colour. The mean development time of this last larval instar was 4.90 days with the mean body length of 33.50 mm.

4.1.2.7 Total larval period:

The mean total larval period was 16.87 days as recorded under laboratory conditions.

4.1.3 Pupa:

The freshly laid pupae of *S. frugiperda* were orange-brown in appearance and changed to dark reddish brown with time. They had a typical cremaster with two spines. The male and female pupae were also distinguishable based on the distance between their genital and anal opening slots. In female pupae, this distance was more than that in male pupae. The mean pupal period was 8.83 days and the mean pupal length was 15.70 mm.

4.1.4 Adult:

The adult of *S. frugiperda* is a small to medium sized moth. Sexual dimorphism was clearly evident; in males the forewings were generally shaded in gray and brown colour, with triangular white spots at the tip and near the center, while in females the forewings were less distinctly marked, ranging from a uniform grayish brown to a fine mottling of gray and brown. The hind wings were iridescent silver-white with a narrow dark border in both sexes.

Morphometric data of the adults reveal that the males were slightly larger than the females. The mean body length (mm), wing length (mm) and wing span (mm) of the male moths were 15.80, 13.70 and 31.70; and of the female moths were 15.00, 13.10 and 30.80, respectively (Table-4).

4.1.4.1 Pre-oviposition, Oviposition and Post-oviposition period:

The mean pre-oviposition period of *S. frugiperda* was 3.39 days. The oviposition took place during the night hours. The mean oviposition time of 2.92 days was recorded. Oviposition period was the shortest among the pre-oviposition, oviposition and post-oviposition periods. The mean post-oviposition period was 6.20 days.

4.1.4.2 Longevity:

The observations reveal that female moths lived more than the male moths. The mean adult longevity of females was 12.20 days while, that of male moths was 9.81 days.

4.1.4.3 Fecundity:

The range of the eggs laid by the female moths varied considerably from 750-2287. However, the average number of eggs laid per female was 1562.

4.1.5 Total life cycle:

Under laboratory conditions, *S. frugiperda* completed its life cycle with a mean duration of 40.24 days.

4.1.6 Sex ratio:

The female: male ratio of 1.14:1, 1.30:1, 1:1 and 1.14:1 was recorded during the study period.

4.2 Population dynamics of fall armyworm in maize:

During the course of investigation the data on seasonal incidence of *S. frugiperda* and its associated larval parasitoids was recorded at weekly intervals, which is presented in Table (5).

4.2.1 Seasonal incidence of fall armyworm during *Kharif*, 2019:

The data recorded in Table (5) shows that the fall armyworm infestation initiated in the 3rd week of July (29th SMW) with a mean population of 1.75 larvae/10 plants. The population increased gradually and reached to its peak in the 3rd week of August (33rd SMW) with a mean population of 26.50 larvae/10 plants. At the peak, the mean temperature, mean relative humidity and rainfall were 24.65˚C, 85.00 per cent and 21.85 mm, respectively. Further, the population decreased upto crop maturity and the last incidence was observed during the 38th SMW with a population of 0.30 larvae/10 plants.

The fall armyworm population exhibited a non significant negative correlation with mean atmospheric temperature (r = -0.34); non significant positive correlation with mean relative humidity (r = 0.35) and significant positive correlation with total rainfall (r = 0.68).

The multiple linear regression analysis indicated that all the three weather parameters (mean atmospheric temperature, mean relative humidity and total rainfall) had a joint influence of 46.8 per cent (R^2=0.468) on the fall armyworm population. The regression equation further indicated that the rainfall had a significant positive impact on the population *i.e.* 1 unit increase in rainfall led to 0.774 unit increase in fall armyworm population (Table-7).

4.2.2 Seasonal incidence of larval parasitoids of *S. frugiperda* during *Kharif*, 2019:

The data presented in Table (5) reveal that the parasitoid infestation initiated in the 1st week of August (31st SMW). The population reached its peak in the 3rd week of August with a mean population of 3.75 larvae/10 plants. At the peak parasitization, the mean temperature, mean relative humidity and mean rainfall were 24.65˚C, 85.00 per cent and 21.85 mm, respectively. The parasitoids activity was not observed after 36th SMW.

The parasitoids population exhibited a non significant negative correlation with mean atmospheric temperature (r = -0.47); non significant positive correlation with mean relative humidity (r = 0.29) and significant positive correlation with total rainfall (r = 0.64).

Six hymenopteran parasitoids belonging to three families were recorded during the study period: Braconidae (*Chelonus* sp., *Microplitis* sp. and *Cotesia* sp.), Ichneumonidae (*Campoletis* sp. and other unidentified) and one species of Bethylidae (Plate 4). The parasitization (%) and Effective parasitization (%) were also computed which have been presented in Table (6). It can be observed that the maximum parasitization (14.15 %) was recorded during the 33rd SMW.

The multiple linear regression analysis showed that the total influence of all the weather parameters (mean atmospheric temperature, mean relative humidity and total rainfall) was 44.4 per cent (R^2=0.444) on the parasitoid population (Table-7).

4.2.3 Spatial distribution of fall armyworm on maize during *Kharif*, 2019:

The results on the spatial dispersion pattern of *S. frugiperda* on maize have been presented in Table (8). The values of Dispersion Index (VMR) exceeded unity in all the sampling occasions indicating a contagious or clumped type of distribution. Further, the values of Aggregation Index (K), which measure the degree of aggregation, were less than eight in most cases, suggesting a high degree of aggregation. The values of other parameters *viz.,* Clumping Index (I_{DM}) values were all positive; Mean Crowding Index (X^*) values were greater than Mean Density and Lloyd's Patchiness Index values were greater than one, which confirmed the clumped nature of dispersion of *S. frugiperda*. The mean colony size (C^*) also increased with the increase in number of larvae.

Chelonus sp.

Microplitis sp.

Cotesia sp.

Campoletis sp.

An ichneumonid

A bethylid

Plate IV: Parasitoids of fall armyworm recorded during the study

To find out the type of aggregation involved, *i.e.* whether it was an aggregation of insects in colonies or an aggregation of colonies, Iwao's patchiness regression was fitted over a range of different densities and was computed as $X^* = 0.088+1.137\ \bar{x}$ ($R^2 = 0.987$). The values of α (0.088) and β (1.137), which were equal to zero and more than one, respectively, suggest that the fall armyworm larvae were distributed singly (one larvae per colony), but the colonies were aggregated. The Taylor's Power Law equation was computed as $\log s^2 = -0.013+1.380 \log \bar{x}$ ($R^2 = 0.905$). The value of index of aggregation, b (1.380), was more than unity, also confirmed the aggregate nature of distribution.

4.3 Molecular characterization of fall armyworm:

4.3.1 Morphological identification:

The collected larvae and moths were identified as *S. frugiperda* after studying and examining their morphology and male adult genitalial characters. The grownup larvae were dark brown in colour with granulated cuticular texture all over the body. The dorsal pinacula present on one to eight abdominal segments were large and greater than the diameter of the corresponding spiracles. The dorsal pinacula on the 8^{th} abdominal segment were arranged in a square pattern and the pinacula on the 1^{st} to 7^{th} segment were arranged in a trapezoidal pattern. The male adults were greyish brown, forewings light brown with a reniform spot, small conspicuous white marking at the junction of M3 and CuA1 veins, and a white patch near the apical margin. The female adults were uniformly greyish brown, unlike male moths. The forewings had indistinct pale brown markings and dark grey coloured oval shaped spots along the outer margins. Reniform spot and white patch at apical portion were absent. The male genitalial characters such as broad, almost quadrate valve; short clavus; narrow, elongate costal process; slightly curved ampulla; apically curved, slender and pointed uncus and well developed aedeagus were also observed (Plate 5).

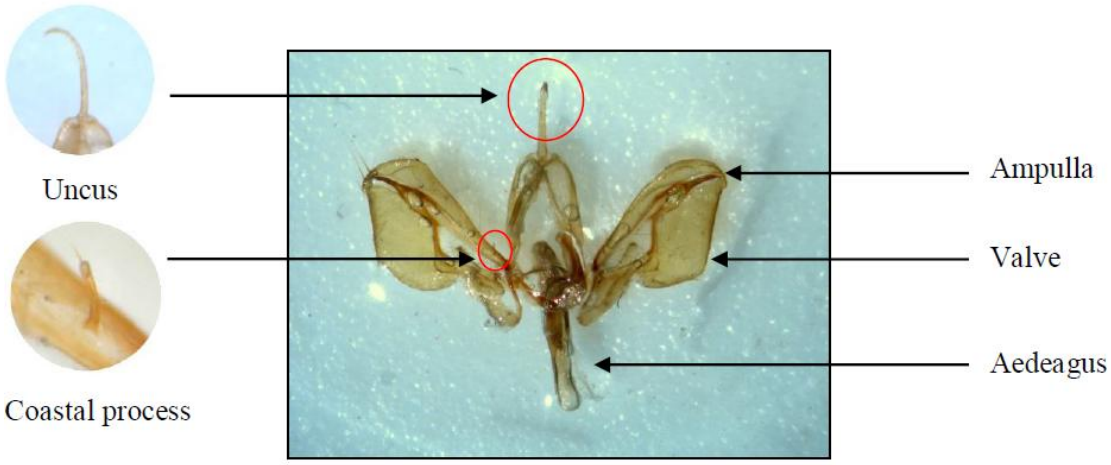
4.3.2 Molecular identification:

The universal primers were used to amplify the target fragment (mitochondrial Cytochrome Oxidase Subunit I (*COI*) gene) of ~650 bp in size from the genomic DNA of collected insects (Plate 6). The search analysis in the BLASTn confirmed the insect species as *S. frugiperda*. The *COI* gene sequence obtained was of 639 bp in size

Arrangement of dorsal pinacula on abdominal segments

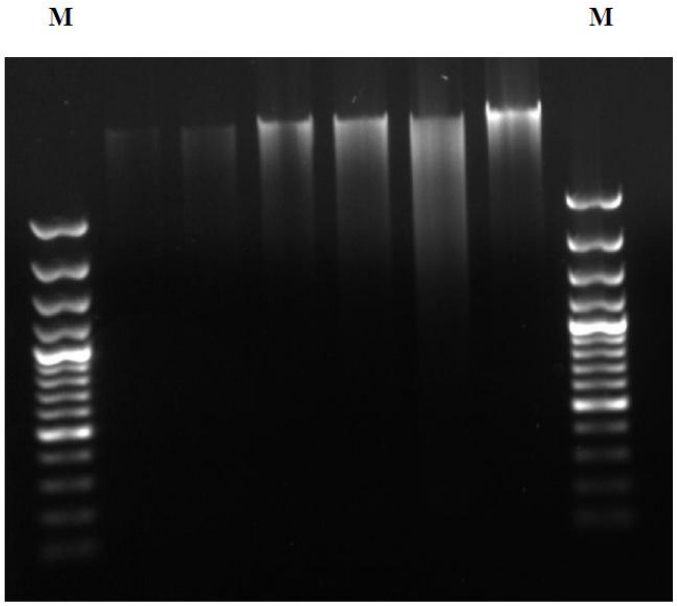
Wings of male moth

Wings of female moth

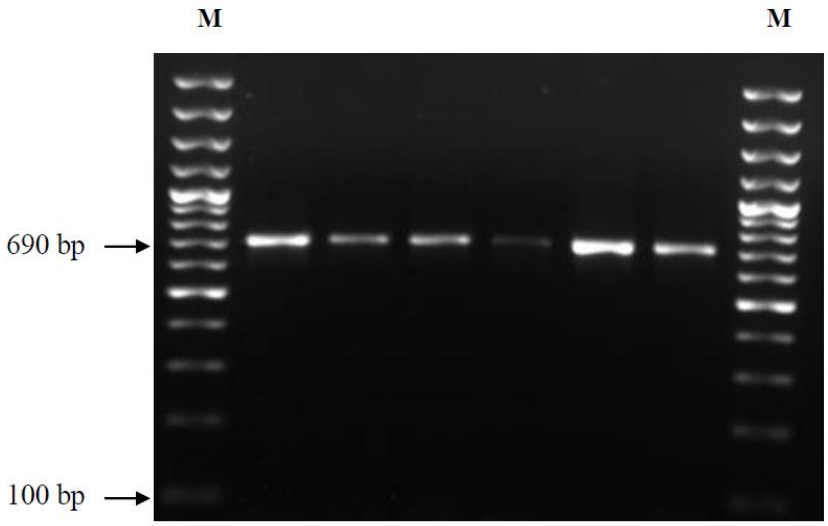


Male genitalia

Plate V: Morphological characters of *S. frugiperda*



Agarose gel electrophoresis analysis of DNA extracted from *S. frugiperda* larvae (M-marker 100 bp ladder)



Agarose gel electrophoresis analysis of purified PCR products of *Cytochrome Oxidase subunit I* (*COI*) of *S. frugiperda* (M-marker 100 bp ladder)

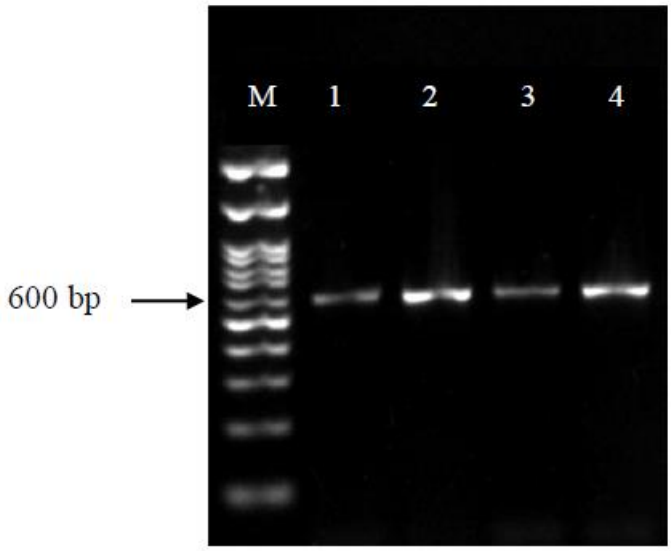
Plate VI: Molecular identification of *S. frugiperda*

and showed 98-100% resemblance with the fall armyworm population from India (Vijayawada: GenBank MH899611 and Tirupati: GenBank MH899610) and other countries (Dominica Republic: GenBank MK3182971, Kenya: GenBank MH190445, South Africa: GenBank MF593258). Similarly, the Barcode of Life Data base identification showed that the present study sequence was 99-100% similar to *S. frugiperda*. The Genbank Accession Number obtained was MN117927 and corresponding sequence was as follows:

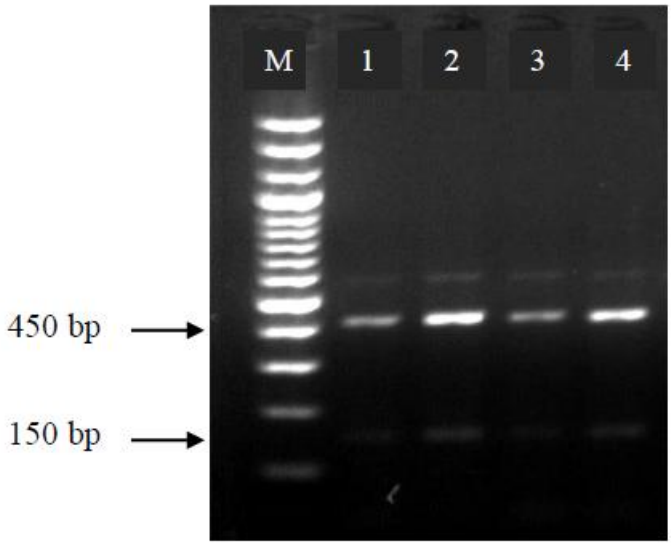
AATAGTAGGTACTTCTTTAAGTTTATTAATTCGAGCTGAATTAGGAACTCCAG
GATCTTTAATTGGAGATGATCAAATTTATAATACTATTGTAACAGCCCATGCT
TTTATTATAATTTTTTTTATAGTTATACCAATTATAATTGGAGGATTTGGAAAT
TGACTTGTACCTTTAATATTAGGAGCTCCTGATATAGCTTTCCCACGTATAAA
TAATATAAGTTTTTGACTTTTACCCCCATCTTTAACTTTATTAATTTCTAGTAG
CATTGTAGAAAATGGAGCAGGAACTGGATGAACAGTTTACCCCCCCCTCTCCT
CTAATATTGCTCATGGTGGTAGTTCAGTAGATTTAGCTATTTTCTCACTTCATT
TAGCTGGAATTTCATCTATTTTAGGAGCTATTAACTTTATTACCACTATTATTA
ATATACGATTAAATAATTTATCATTTGATCAAATACCTTTATTTATTTGAGCTG
TAGGTATTACCGCATTTTTATTATTATTATCTTTACCTGTTTTAGCTGGAGCTA
TTACTATATTACTTACTGATCGAAATCTAAATACATCATTTTTCGATCCTGCAG
GAGGAGGTGATCCTATTCTTTATCAACATTTATTTTGATTTTTTGGTCA

4.3.2 Strain identification:

The strain of the collected fall armyworm larvae was determined using the PCR-RFLP method which is based on the fragment size of 590 bp obtained by PCR amplification of *COI* gene using primers, JM 76 and JM 77 and restriction enzymes, *Msp*I and *Sac*I. When the purified PCR product was digested with restriction enzyme *Msp*I, a single PCR band was observed whereas, when digested with restriction enzyme *Sac*I, two fragments of 450 and 150 bp sizes were observed (Plate 7). The results indicate that, using these mitochondrial markers, the fall armyworm population of Udaipur region was identified to be of “Rice strain”.



***Msp*I enzyme digested samples; M=molecular marker (100 bp); (1-4 FAW samples)**



***Sac*I enzyme digested samples; M=molecular marker (100 bp); (1-4 FAW samples)**

Plate VII: PCR amplification of *COI* gene of *S. frugiperda* and digested with restriction enzymes, *Msp*I and *Sac*I

Table 2: Biology of *S. frugiperda* on maize in laboratory conditions

S. No.	Life stages	Period of study				Seasonal Mean
		Aug-Sept	Sept-Oct	Oct-Nov	Nov-Dec	
		Mean ±SD	Mean ±SD	Mean ±SD	Mean ±SD	
1.	Incubation period (days)	3.40±0.49	3.33±0.47	3.46±0.50	3.36±0.49	3.38
2.	Larval period (days)	16.46±1.45	16.76±1.54	17.06±1.83	17.23±1.94	16.87
3.	Pupal period (days)	8.36±1.40	8.76±1.10	9.00±0.98	9.20±1.37	8.83
4.	Pre-oviposition period (days)	3.28±0.61	3.30±0.85	3.35±0.49	3.64±0.63	3.39
5.	Oviposition period (days)	2.92±0.68	3.15±0.37	2.50±0.75	3.14±0.66	2.92
6.	Post-oviposition period (days)	6.07±1.38	6.15±0.80	6.25±1.34	6.35±1.08	6.20
7.	Female adult longevity (days)	11.68±2.18	12.35±1.80	12.30±2.25	12.50±1.74	12.20
8.	Male adult longevity (days)	9.50±1.09	9.84±1.42	9.93±1.33	10.00±2.14	9.81
9.	Total life cycle (days)	38.80±2.32	40.66±3.61	40.40±4.47	41.10±3.67	40.24
10.	Eggs/female (No.)	1740±153	1638±109	1629±185	1241±129	1562

Table 3: Duration (days) of larval instars of *S. frugiperda*

Period	I instar	II instar	III instar	IV instar	V instar	VI instar
	Mean±S.D.	Mean±S.D.	Mean±S.D.	Mean±S.D.	Mean±S.D.	Mean±S.D.
Aug-Sept	2.70±0.50	2.40±0.49	2.10±0.30	2.00±0.00	2.60±0.60	4.20±0.49
Sept-Oct	2.90±0.30	2.50±0.50	2.00±0.00	2.10±0.30	2.70±0.50	4.60±0.50
Oct-Nov	2.90±0.30	2.60±0.60	2.00±0.00	2.00±0.00	2.70±0.50	5.30±0.75
Nov-Dec	2.80±0.40	2.50±0.50	2.30±0.45	2.00±0.00	2.80±0.40	5.50±0.50
Mean	2.82	2.50	2.10	2.02	2.70	4.90

Table 4: Morphometric data of *S. frugiperda*

S. No.	Life stages		Mean ± S.D. (mm)
1.	**Larval length**	I instar	1.80±0.15
		II instar	3.50±0.45
		III instar	6.20±0.30
		IV instar	9.70±0.55
		V instar	16.80±1.08
		VI instar	33.50±1.30
2.	**Pupal length**		15.70±1.55
3.	**Adult**		
	Male	Body length	15.80±1.03
		Wing length	13.70±0.85
		Wing span	31.70±2.05
	Female	Body length	15.00±1.22
		Wing length	13.10±0.75
		Wing span	30.80±1.85

Table 5: Influence of key environmental factors on *S. frugiperda*, infesting maize, and its larval parasitoids during *Kharif*, 2019

SMW	Mean Atm. Temp. (˚C)	Mean RH (%)	Total Rainfall (mm)	Mean larvae/ 10 plants	Mean larval parasitoids/10 plants
28	27.90	64.50	0.00	0.00	0.00
29	28.85	66.50	4.94	1.75	0.00
30	28.05	74.50	1.80	5.00	0.00
31	26.95	83.50	2.14	9.00	0.25
32	25.45	83.00	26.02	12.75	1.00
33	24.65	85.00	21.85	26.50	3.75
34	26.65	73.50	9.94	23.00	1.25
35	25.85	86.00	20.14	15.00	0.50
36	27.10	88.50	12.21	8.30	0.25
37	26.80	83.00	5.15	1.30	0.00
38	26.40	75.00	6.02	0.30	0.00
39	25.60	82.50	2.02	0.00	0.00
40	24.40	78.50	9.54	0.00	0.00
Coefficient of correlation (r) for population and mean atmospheric temperature				**-0.34**	**-0.47**
Coefficient of correlation (r) for population and mean relative humidity				**0.35**	**0.29**
Coefficient of correlation (r) for population and total rainfall				**0.68***	**0.64***
Coefficient of correlation (r) between FAW and parasitoids				**0.85***	

*Significant at 5% level, SMW = Standard Meteorological Week

Table 6: Activity of natural larval parasitoids of *S. frugiperda* infesting maize during *Kharif*, 2019

SMW	Mean larvae/ 10 plants	Mean larval parasitoids/10 plants	Parasitization (%)	Effective parasitization (%)
28	0.00	0.00	0.00	0.00
29	1.75	0.00	0.00	0.00
30	5.00	0.00	0.00	0.00
31	9.00	0.25	2.77	2.70
32	12.75	1.00	7.84	7.27
33	26.50	3.75	14.15	12.40
34	23.00	1.25	5.43	5.15
35	15.00	0.50	3.33	3.23
36	8.30	0.25	3.01	2.92
37	1.30	0.00	0.00	0.00

SMW = Standard Meteorological Week

Table 7: Multiple linear regression between abiotic factors and FAW and its parasitoids on maize during *Kharif*, 2019

Insects	Regression equations	R^2 value
Fall armyworm	$Y = -23.417+(0.773)X_1+(0.040)X_2+(0.774^{*})X_3$	**0.468**
Parasitoids	$Y = 5.944+(-0.173)\ X_1+(-0.019)\ X_2+(0.075)\ X_3$	**0.444**

*Significant at 5% level; Y = Dependent variable; X_1 = Mean Atm. Temperature (°C); X_2 = Mean Relative Humidity (%); X_3 = Total Rainfall (mm)

Table 8: Indices of spatial distribution of *S. frugiperda* on maize during *Kharif*, 2019

Dates of Observation	Mean density ($\bar{x}$)	Variance (s^2)	Disp. Index (VMR)	Aggr. Index (K)	Clumping Index (I_{DM})	Mean Crowding Index (X^*)	Llyod's Patchiness Index	Mean colony size (C^*)	Log Mean density	Log Variance
29-Jul	2.00	2.22	1.11	18.18	0.11	2.11	1.06	3.11	0.30	0.34
5-Aug	3.60	5.38	1.49	7.28	0.49	4.09	1.14	5.09	0.55	0.73
12-Aug	4.70	10.46	2.23	3.84	1.23	5.93	1.26	6.93	0.67	1.01
19-Aug	10.50	32.06	3.05	5.11	2.05	12.55	1.20	13.55	1.02	1.50
26-Aug	9.20	16.84	1.83	11.08	0.83	10.03	1.09	11.03	0.96	1.22
2-Sep	5.90	7.66	1.30	19.78	0.30	6.20	1.05	7.20	0.77	0.88
9-Sep	3.30	6.68	2.02	3.22	1.02	4.32	1.31	5.32	0.51	0.82

5. DISCUSSION

The results of the present investigation on **"Bio-ecology of the fall armyworm, *Spodoptera frugiperda* (J. E. Smith) on maize and its molecular characterization"** have been discussed in this chapter after comparing with similar work done by other workers.

5.1 Biology of *S. frugiperda* on maize:

Detailed biology of *S. frugiperda* was studied under laboratory conditions from August to December.

5.1.1 Egg:

Eggs were deposited in egg masses which were either laid in single layer or stacked up in 2- 3 layers and were covered with grayish white scales. Similar pattern of egg laying was observed by Luginbill (1928). The eggs were white to creamish in colour but changed brown to black prior to hatching. The incubation period ranged from 3-4 days with an average of 3.38 days. Manjula *et al.* (2019) observed that the eggs turned reddish brown to black before hatching and took 3-5 days to hatch. Murua and Virla (2004) reported an incubation period of 3.53 days. More or less similar incubation period was also noted by Sharanabassapa *et al.* (2018b) and Venkateswarlu *et al.* (2018). These reports strongly support the present findings.

5.1.2 Larva:

The larvae were found to pass through six instars in the laboratory. Similar observations were made by Pitre and Hogg (1983), Sharanabassapa *et al.* (2018b), Deole and Paul (2018), Manjula *et al.* (2019) and Bhavani *et al.* (2019). The total larval period was of 16.87 days and the different instars took 2.82, 2.50, 2.10, 2.02, 2.70 and 4.90 days respectively for the 1^{st} through 6^{th} instar. The average length measured for each instar (1^{st} to 6^{th}) was 1.80, 3.50, 6.20, 9.70, 16.80 and 33.50 mm respectively. The results of the present finding are in close conformity with the earlier work of Sharanabassapa *et al.* (2018b) who recorded a larval period of 15.9 days and that the six instars lasted for an average duration of 2.60, 2.20, 2.0, 2.0, 2.40 and 4.50 days, respectively. Luginbill (1928) observed the length of each larval instar (first to sixth) which was 1.7, 3.5, 6.4, 10.0, 17.2, and 34.2 mm, respectively. Murua *et al.*

(2008) recorded a larval period of 18.18 days; while, Deole and Paul (2018) observed a larval duration of 14-16 days.

5.1.3 Pupa:

The mean pupal period was of 8.83 days and the mean pupal length was of 15.70 mm. The results of the present investigation are corroborated by the findings of Pitre and Hogg (1983), who recorded a pupal period of 8-9 days and pupal length of 14-18 mm. Similar observations on pupal period have been recorded by Murua *et al.* (2008), Silva *et al.* (2017), Bhavani *et al.* (2019), Malo and Hore (2019) and Manjula *et al.* (2019).

5.1.4 Adult:

5.1.4.1 Pre-oviposition, Oviposition and Post-oviposition period:

In the present investigation, the mean pre-oviposition, oviposition and post-oviposition period were 3.39, 2.92 and 6.20 days, respectively. Oviposition period was the shortest amongst the three. Sharanabassapa *et al.* (2018b) recorded the pre-oviposition, oviposition and post oviposition period ranging between 3-4, 2-3 and 4-5 days, respectively.

5.1.4.2 Longevity:

The mean longevity of female adults was 12.20 days; while, that of the male adults was 9.81 days indicating that female moths lived longer than the male moths. Similar results were obtained by Sharanabassapa *et al.* (2018b), who recorded female longevity of 9-12 days and male longevity of 7-9 days, and Bhavani *et al.* (2019), who recorded female longevity of 10 days and male longevity of 8 days. However, Murua *et al.* (2008) recorded a longer duration of male moths (16.6 days) than the female moths (12.7 days).

5.1.4.3 Fecundity:

The average number of eggs laid per female was 1562. Certain moths even laid more than 2000 eggs in captivity indicating an enormous egg production capacity of female moths. The findings of the present investigation tally with the earlier work of Capinera (2014), who recorded an average fecundity of about 1500 eggs per female. Sharanabassapa *et al.* (2018b) recorded the fecundity ranging from 839-1169 with an average of 1064 eggs per female.

5.1.5 Total life span:

Under laboratory conditions, the average life span of *S. frugiperda* was of 40.24 days. The results of the present finding are in close conformity with the earlier work of Murua and Virla (2004) who recorded the total life span of 36.80 days. Sharanabassapa *et al.* (2018b) recorded the total life cycle of 32-46 days; whereas, Bhavani *et al.,* (2019) observed the life cycle of 32-36 days for female individuals and 30-34 days for male individuals.

5.1.6 Sex ratio:

The female: male ratio of 1.14:1, 1.30:1, 1:1 and 1.14:1 was recorded during the study period which, for majority of the times, was clearly a female-biased sex ratio. Murua *et al.* (2008) also observed a female-biased ratio of 1.2:1. Silva *et al.* (2017) observed a sex ratio of 1:1 during their study.

5.2 Population dynamics of *S. frugiperda* in maize:

Seasonal incidence of *S. frugiperda* and its associated larval parasitoids was recorded at weekly intervals, during *Kharif*, 2019.

5.2.1 Seasonal incidence of *S. frugiperda*:

The population of *S. frugiperda* was active throughout the growing stage. The infestation started in the 3rd week of July (29th SMW) with an initial intensity of 1.75 larvae/10 plants. The peak in the pest population was observed during the 3rd week of August (33rd SMW) with a mean population of 26.50 larvae/10 plants (Fig. 1). The findings of the present investigation are in close conformity with the earlier work of Visalakshi *et al.* (2019) who recorded the maximum infestation in the 2nd week of August. Dhar *et al.* (2019) also observed the first appearance of *S. frugiperda* in 20-22 days old crop. Bhavani *et al.* (2019) noticed the incidence in 20-60 days old crop. Chormule *et al.* (2019) reported that the pest incidence was initiated in 3rd week of August. However, Venkateswarlu *et al.* (2018) observed the first incidence in the first week of October (40th SMW) which reached maximum in the last week of October.

Simple correlation coefficient (r) was calculated between fall armyworm population and abiotic factors. Among which the larval population showed a negative non significant correlation with mean atmospheric temperature (r = -0.34) as there was no significant variation in the temperature throughout the season; whereas, there

was a positive but non significant correlation with the mean relative humidity (r = 0.35).

The mean rainfall during the crop season was congenial for the larval development on account of which there existed a significant positive correlation (r = 0.68) with the larval population. The findings of the present investigation corroborate with the results of Murua *et al.* (2006) who reported that the fall armyworm population had a significant and positive correlation with rainfall. Fonseca-Medrano *et al.* (2019) reported that the pest exhibited a significant and positive correlation with minimum temperature and relative humidity, but negative correlation with maximum temperature; whereas, non significant and positive correlation with rainfall. Kumar *et al.* (2020) reported a positive correlation between fall armyworm population and maximum temperature, and a negative correlation with minimum temperature, relative humidity and rainfall.

5.2.2 Seasonal incidence of larval parasitoids of *S. frugiperda*:

The initial occurrence of associated larval parasitoids was noted in the 1st week of August (31st SMW) that continued up to 2nd week of September (36th SMW). The peak parasitization was observed in the 3rd week of August (33rd SMW), similar to that observed for *S. frugiperda* (Fig. 1). In the present study, as a density-dependent activity, the population of larval parasitoids happened to increase with an increase in the availability of fall armyworm larvae.

As for the influence of the abiotic factors of the environment, the parasitoids evinced a non significant negative correlation with mean atmospheric temperature (r = -0.47); non significant positive correlation with mean relative humidity (r = 0.29) and significant positive correlation with rainfall (r = 0.64). Castro *et al.* (1988) reported that the parasitization in fall armyworm larvae increased with an increase in rainfall. Murua *et al.* (2006) reported that the parasitoid population was positively and significantly affected by temperature.

During the crop season, six hymenopteran larval parasitoids were recorded out of which, the parasitoids identified included *Chelonus* sp., *Cotesia* sp., *Microplitis* sp., *Campoletis chlorideae* Uchida and two unidentified species one each of Ichneumonidae and Braconidae. The results of the present findings are similar to that reported by Magali *et al.* (2015), who found *Chelonus* sp.; Shylesha *et al.* (2018),

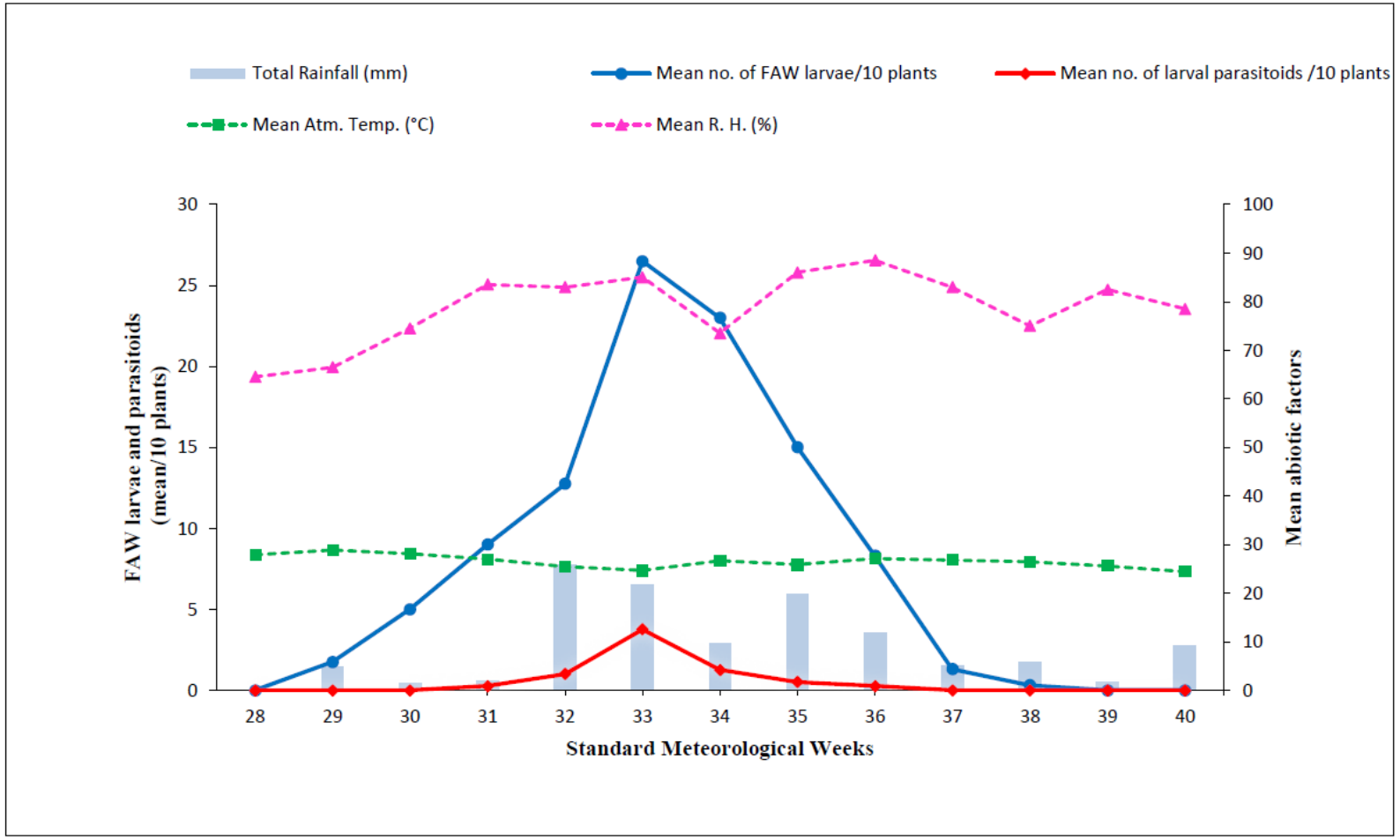


Fig. 1: Population dynamics of the fall armyworm on Pratap Makka maize during *Kharif*, 2019

Chormule *et al.* (2019) and Sharanabassapa *et al.* (2020), who found *Campoletis* sp., and Sisay *et al.* (2019), who found *Cotesia* sp. parasitizing *S. frugiperda* during their study.

The results show a significant abundance and diversity of the fall armyworm parasitoids and a maximum parasitization of 14.15%, which indicate that they can be exploited to implement conservation biological control for managing this pest in near future.

5.2.3 Spatial distribution of *S. frugiperda* on maize:

In the present investigation, *S. frugiperda* was found to follow clumped or aggregated distribution as evinced by various dispersion indices (VMR was greater than one, k values were positive and mostly less than eight, I_{DM} values were positive, X^* values were greater than Mean density and Lloyd's Patchiness Index values were greater than one). The values of Dispersion Index imply that *S. frugiperda* followed clumped dispersion throughout the study period and the maximum dispersion was observed during the 3rd week of August (Fig. 2). Iwao's patchiness regression was computed as $X^* = 0.088+1.137\ \bar{x}$ (Fig. 3) which indicate that the fall armyworm larvae were distributed singly (one larvae per colony) and the colonies were aggregated. Taylor's Power Law was computed as $\log s^2 = -0.013+1.380 \log \bar{x}$ (Fig. 4), where the value of b (1.380) also confirmed the clumped distribution of *S. frugiperda*. The aggregation may be due to either some environmental heterogeneity or due to the fact that the females lay eggs in masses.

The results of the present investigation are in agreement with the findings of Farias *et al.* (2001a), Fernandes *et al.* (2002), Farias *et al.* (2008) and Rios *et al.* (2014), who also observed clumped distribution of fall armyworm. However, contrary results were also reported by Farias *et al.* (2001b), Melo *et al.* (2006) and Hernandez-Mendoza *et al.* (2008), who observed random nature of distribution.

5.3 Molecular characterization of *S. frugiperda*:

In the present investigation, the collected larvae and moths were first morphologically identified by studying their various characters like arrangement of dorsal pinacula on abdominal segments of larvae, wing markings in adults and male adult genitalia. The characters observed during the study were similar to those reported by Sharanabasappa *et al.* (2018b), Shylesha *et al.* (2018), Venkateswarlu

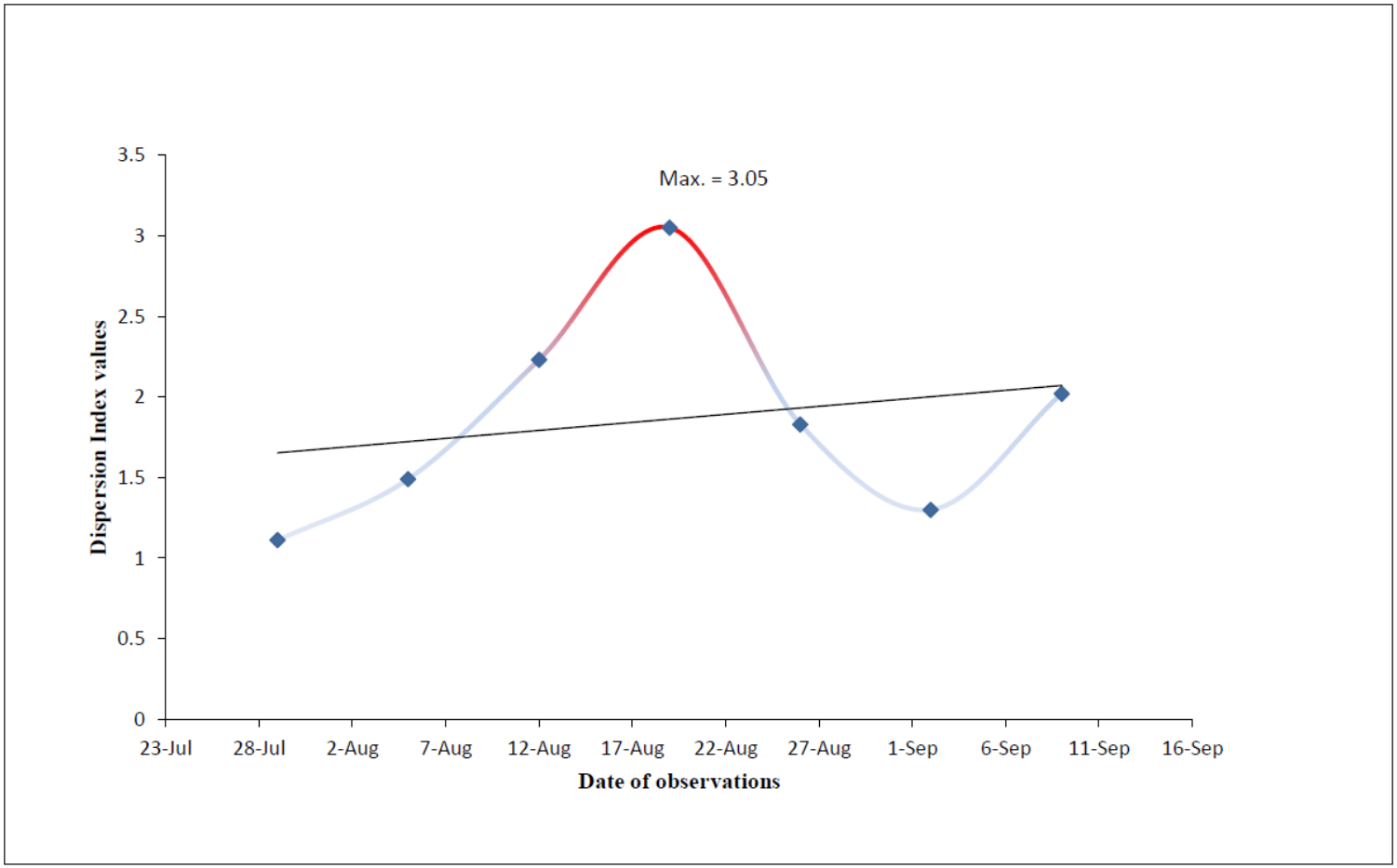


Fig. 2: FAW larval dispersion with maximum dispersion index during third week of August, 2019

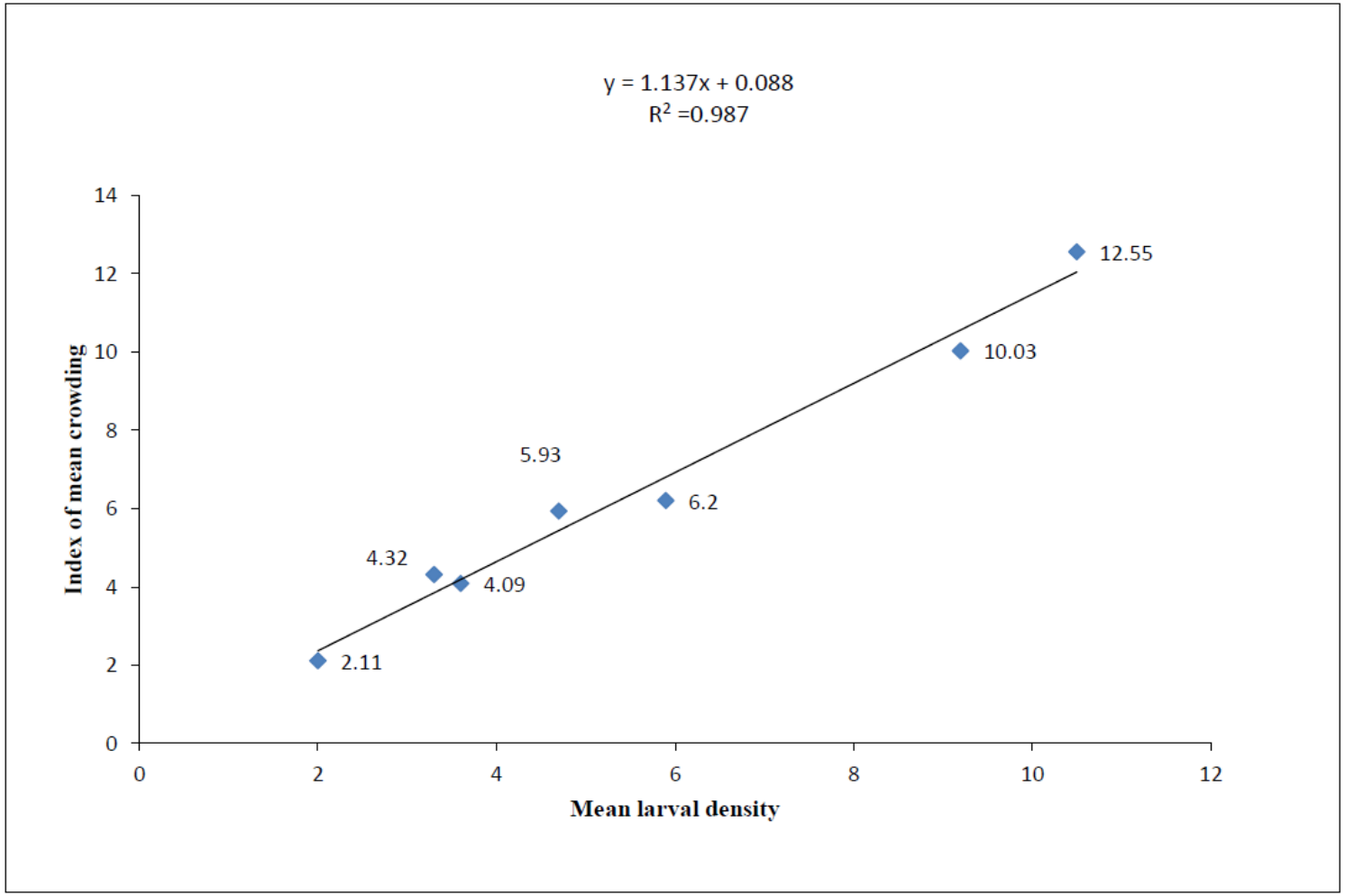


Fig. 3: Iwao's regression for mean FAW larval density per plant and index of mean crowding

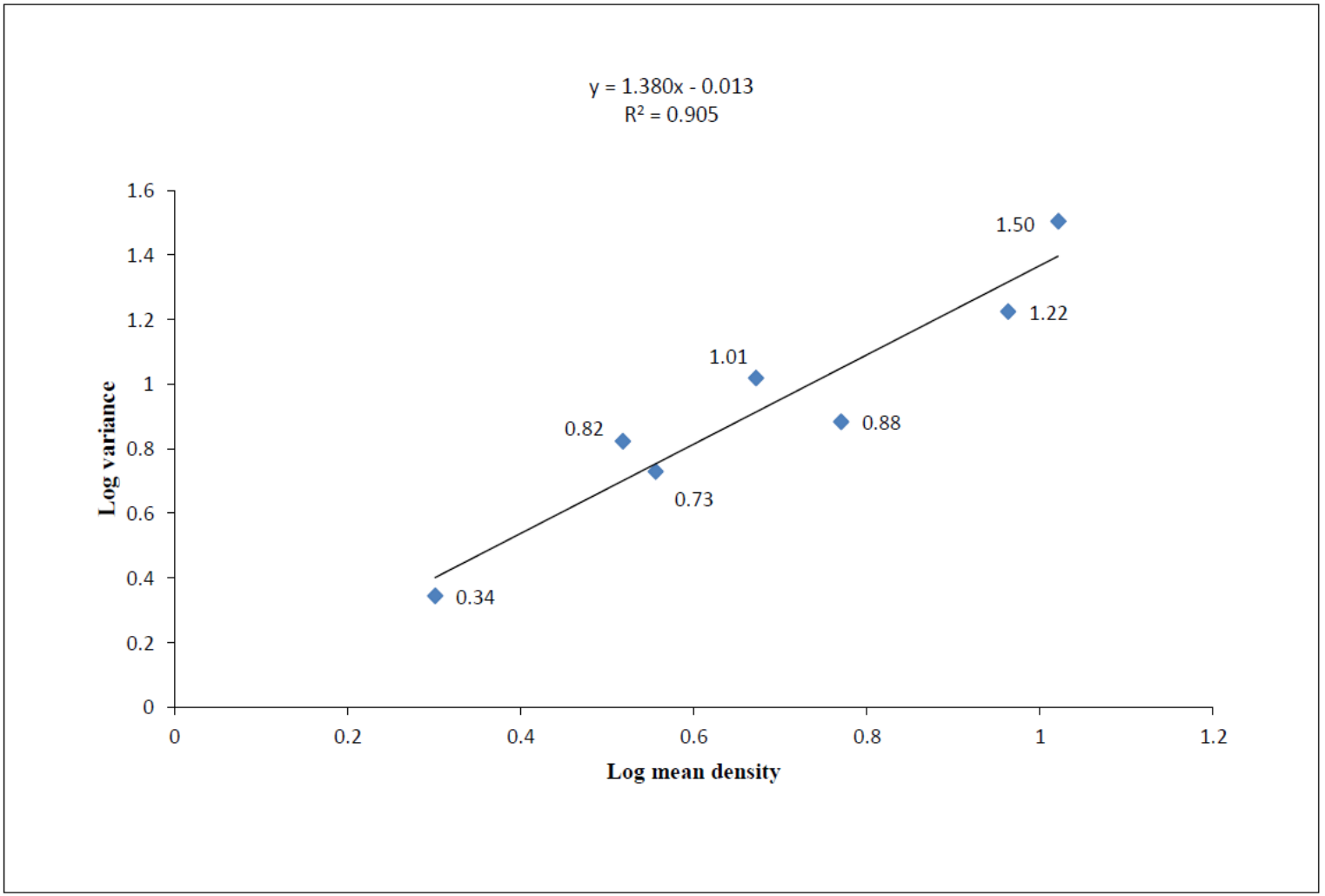


Fig. 4: Taylor's Power Law – relationship between log variance and log FAW larval density

et al. (2018), Babu *et al.* (2019) and Bhavani *et al.* (2019). The identity of the insect species was further confirmed using molecular identification techniques where universal primers were used to amplify the mitochondrial *COI* gene of ~650 bp size from the genomic DNA of collected insects. The *COI* gene sequence was of 639 bp size and the corresponding Genbank Accession Number was MN117927. Similar *COI* gene fragments were also reported by Shylesha *et al.* (2018) and Mahadeva Swamy *et al.* (2018).

The study revealed that the collected fall armyworm larvae belonged to Rice strain. The strain identification was done using PCR-RFLP marker analysis using primers, JM 76 and JM 77 and restriction enzymes, *Msp*I and *Sac*I. The PCR-RFLP products of the *COI* gene were cut into two fragments of 450 and 150 bp size on digestion with restriction enzyme *Sac*I, but not by *Msp*I which is an indication of rice strain. The findings of the present investigation corroborate the findings of Levy *et al.* (2002), Nagoshi *et al.* (2006) and Juarez *et al.* (2012).

The results of the present study show an inverse association of the strain and the host plant, *i.e* the fall armyworm larvae sampled from maize fields belonged to rice strain rather than corn strain. Similar results were found by Georgen *et al.* (2016) who reported that the fall armyworm population feeding on maize in Africa was predominantly rice strain. Similar findings were reported by Srinivasan *et al.* (2018) in Nigeria and Tanzania. Mahadeva Swamy *et al.* (2018) also reported the prevalence of rice strain in six states of India. The present investigation gives a preliminary indication of presence of rice strain of *S. frugiperda* in the maize fields of Udaipur region and this information can be useful in successfully managing fall armyworm in coming times.

6. SUMMARY

The present investigations on **"Bio-ecology of the fall armyworm, *Spodoptera frugiperda* (J. E. Smith) on maize and its molecular characterization"** were carried out at Agricultural Research Station, Banswara; Department of Entomology and the Instructional farm, Rajasthan College of Agriculture, MPUAT, Udaipur during *Kharif*, 2019.

During the study, the fall armyworm completed its life cycle in a mean duration of 40.24 days. The incubation period ranged from 3-4 days with a mean time of 3.38 days. The larvae passed through six instars that developed in a mean duration of 2.82, 2.50, 2.10, 2.02, 2.70 and 4.90 days, respectively. The mean total larval period was of 16.87 days. The mean body length of the six larval instars was 1.80, 3.50, 6.20, 9.70, 16.80 and 33.50 mm, respectively. The mean pupal period was 8.83 days and the mean pupal length was 15.70 mm. The mean pre-oviposition, oviposition and post-oviposition period were of 3.39, 2.92 and 6.20 days, respectively. The mean longevity of the female moths was 12.20 days, while that of the male moths was 9.81 days. The fecundity ranged from 750-2287 with an average of 1562 eggs per female.

The fall armyworm population initiated in the third week of July (29th SMW) and remained active throughout the growing stage and reached to its peak in the third week of August (33rd SMW). The pest showed a non significant negative correlation with mean atmospheric temperature; non significant positive correlation with mean relative humidity and significant positive correlation with total rainfall.

The infestation of larval parasitoids of fall armyworm initiated in the first week of August (31st SMW) and reached to its peak in the third week of August (33rd SMW). The infestation was observed upto 36th SMW. The parasitoids population evinced a non significant negative correlation with mean atmospheric temperature; non significant positive correlation with mean relative humidity and significant positive correlation with total rainfall. During the study, six hymenopteran larval parasitoids were recorded: *Chelonus* sp., *Cotesia* sp., *Microplitis* sp., *Campoletis chlorideae* Uchida and two unidentified species one each of Ichneumonidae and Braconidae. The maximum parasitization (14.15 %) of *S. frugiperda* by its larval parasitoids was recorded during the 33rd SMW.

The fall armyworm population in maize followed clumped or aggregated distribution as evinced by various dispersion indices. The aggregation was of larval colonies, where each colony was composed of a single larva.

The identity of the fall armyworm larvae, collected from the maize fields, was confirmed using morphological and molecular studies, prior to their strain analysis. The strain was determined by PCR-RFLP marker analysis, using primers JM 76 and JM 77 and restriction enzymes *Msp*I and *Sac*I, which revealed that the sampled fall armyworm larvae belonged to Rice strain.

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Bio-ecology of the fall armyworm, *Spodoptera frugiperda* (J. E. Smith) on maize and its molecular characterization

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ABSTRACT

The present investigations on "Bio-ecology of the fall armyworm, *Spodoptera frugiperda* (J. E. Smith) on maize and its molecular characterization" was carried out at Agricultural Research Station, Banswara, Department of Entomology and the Instructional farm, Rajasthan College of Agriculture, MPUAT, Udaipur during *Kharif*, 2019.

The studies on the biology of fall armyworm on maize revealed that the average fecundity of this pest was 1562 eggs/female and the incubation period was 3.38 days. The larvae developed through six instars that took 2.82, 2.50, 2.10, 2.02, 2.70 and 4.90 days respectively for the 1st through 6th instar. The total larval period was 16.87 days. The pupal period lasted for 8.83 days. The pre-oviposition, oviposition and post-oviposition period were 3.39, 2.92 and 6.20 days, respectively. The longevity of female adults was 12.20 days; while, that of the male adults was 9.81 days. The total time taken to complete the life cycle was 40.24 days. The morphometric data *viz.*, larval body length; pupal length and adult body length and wing span were measured.

The population of fall armyworm and its larval parasitoids reached to their peaks in the 3rd week of August (33rd SMW) and both showed a significant positive correlation (r = 0.68), (r = 0.64) with total rainfall, respectively. During the study, a total of 6 hymenopteran larval parasitoids comprising 3 species of Braconidae, 2 species of Ichneumonidae and 1 species of Bethylidae were recorded. The fall armyworm population in maize followed aggregated pattern of distribution.

The studies on molecular characterization of fall armyworm larvae collected from maize fields revealed that they belonged to "Rice strain".

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मक्का में फॉल आर्मीवर्म, *Spodoptera frugiperda* (J. E. Smith) की जैव-पारिस्थितिकी एवं इसका आणविक निरूपण

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अनुक्षेपण

खरीफ 2019 के दौरान, कृषि अनुसन्धान केंद्र, बांसवाडा एवं राजस्थान कृषि महाविद्यालय, एम.पी.यू.ए.टी., उदयपुर के प्रशिक्षणात्मक प्रक्षेत्र एवं कीट विज्ञान विभाग में मक्का में फॉल आर्मीवर्म, *Spodoptera frugiperda* (J. E. Smith) की जैव-पारिस्थितिकी एवं इसका आणविक निरूपण पर अध्ययन किया गया।

मक्का पर फॉल आर्मीवर्म की जैविकी के अध्ययन में यह पाया गया कि इस कीट की औसतन प्रजनन क्षमता 1562 अंडें/मादा तथा ऊष्मायन अवधि 3.38 दिन थी। डिम्भक 6 अवस्थाओं के माध्यम से क्रमशः 2.82, 2.50, 2.10, 2.02, 2.70 एवं 4.90 दिन की अवधि में विकसित हुए। कुल डिम्भक अवधि 16.87 दिन पायी गई। कोशित अवधि 8.83 दिन पायी गई। पूर्व–अंडनिक्षेपण, अंडनिक्षेपण तथा अंडनिक्षेपण–उपरांत की अवधि क्रमशः 3.39, 2.92 एवं 6.20 दिन अभिलेखित की गई। मादा व्यस्क 12.20 दिन तक जीवित रहीं, जबकि नर व्यस्क 9.81 दिन तक जीवित रहे। कुल जीवन काल 40.24 दिन का पाया गया। आकृति संबंधी आंकड़ो में डिम्भक की लम्बाई, प्यूपा की लम्बाई तथा व्यस्क के शरीर की लम्बाई व पंख विस्तार को मापा गया ।

खरीफ 2019 के दौरान, फॉल आर्मीवर्म व इसके डिम्भक परजीव्याभों की अधिकतम समष्टि अगस्त के तीसरे सप्ताह (33वें मानक मौसमी सप्ताह) में देखी गई तथा दोनों का ही क्रमशः ($r = 0.68$), ($r = 0.64$) कुल वर्षा के साथ सार्थक और सकारात्मक सहसंबंध देखा गया। अध्ययन के दौरान, Hymenoptera गण के 6 परजीव्याभ अभिलिखित किये गए जिसमे Braconidae कुल की तीन प्रजातियां, Ichneumonidae कुल की 2 प्रजातियां एवं Bethylidae कुल की एक प्रजाति शामिल है। मक्का में फॉल आर्मीवर्म समष्टि सम्मुचयित स्थानिक वितरण में पायी गई।

मक्का की फसल से लिए गए फॉल आर्मीवर्म डिम्भकों के आणविक निरूपण पर अध्ययन में यह पाया गया कि वे ''राईस स्ट्रेन'' के थे।

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Analysed Document:	Deepika Kalyan_Entomology_2018-0206-019_MSc Ag_08_2020.pdf (D78113162)
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