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#### T Dhar

Associate Professor Regional Research Sub Station (OAZ), Uttar Banga Krishi Viswavidyalaya, Pundibari, Coochbehar, West Bengal, India

#### S Bhattacharya

Visva Bharati University, Santiniketan, Bolpur, Birbhum, West Bengal, India

#### **H** Chatterjee

Visva Bharati University, Santiniketan, Bolpur, Birbhum, West Bengal, India

#### SK Senapati

Uttar Banga Krishi Viswavidyalaya, Pundibari, Coochbehar, West Bengal, India

**PM Bhattacharya** Uttar Banga Krishi Viswavidyalaya, Pundibari, Coochbehar, West Bengal, India

#### P Poddar

Uttar Banga Krishi Viswavidyalaya, Pundibari, Coochbehar, West Bengal, India

TR Ashika

ICAR-National Bureau of Agricultural Insect Resources, Bengaluru, Karnataka, India

#### T Venkatesan

ICAR-National Bureau of Agricultural Insect Resources, Bengaluru, Karnataka, India

Correspondence T Dhar Associate Professor Regional Research Sub Station (OAZ), Uttar Banga Krishi Viswavidyalaya, Pundibari, Coochbehar, West Bengal, India

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### Occurrence of fall armyworm *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) on maize in West Bengal, India and its field life table studies

## T Dhar, S Bhattacharya, H Chatterjee, SK Senapati, PM Bhattacharya, P Poddar, TR Ashika and T Venkatesan

#### Abstract

Fall armyworm (FAW) *Spodoptera frugiperda* (J. E. Smith), an important invasive pest to Indian Sub Continent was found to infest rabi maize in Harischandrapur 2 block of Malda district in West Bengal during the end of November 2018. The infestation was then spread to other five major maize growing blocks of the district. Both rabi and summer maize was infested by the pest. However, the damage was more in summer maize as compared to rabi maize recording maximum of 27.56%. Furthermore, the identity of the pest was confirmed based on the morphological characters and also by amplifying cytochrome oxidase gene I (658 bp) and the DNA barcode was generated for the same. Life table studies of the pest showed maximum mortality at the younger instars having an age specific survivorship curve of type-III. Therefore, effective pest management strategies may be planned when pest is in early larval stages.

Keywords: Spodoptera frugiperda, West Bengal, molecular characterization, life table

#### Introduction

Fall armyworm *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) is an invasive and noxious pest of maize (*Zea mays*), which is native to tropical and subtropical regions of America <sup>[11]</sup>. The pest invaded to West and Central Africa in 2016 <sup>[2]</sup> and then spread to all the countries of sub-Saharan Africa <sup>[3]</sup>. It is now an invasive pest of Indian Sub continent, first reported from Karnataka <sup>[4, 5, 6]</sup> on maize in 2018. Later, its presence was confirmed in Maharashtra, Gujarat and Chhattisgarh <sup>[7, 8, 9]</sup>. It is a polyphagous pest infesting 186 plant species belonging to 42 different families among which Poaceae, Fabaceae, Solanaceae, Asteraceae, Rosaceae, Chenopodiaceae, Brassicaceae and Cyperaceae are important <sup>[10]</sup>. In India, it was reported from maize, sugarcane and sorghum<sup>4</sup>. It causes huge yield loss in maize up to 57.6% to 58% <sup>[11, 12]</sup>.

Fall army worm is a very devastating pest than any other invasive pest due to their polyphagous nature, rapidly dispersing habit across wide geographical areas and persistence throughout the year. The larvae feed on the foliage and also cause direct injury to the cob, though maize plants at all stages of development are damaged. Reports were pouring in from different maize growing parts of Malda district of West Bengal regarding infestations by a new pest during November, 2018 onwards. Accordingly, a survey was conducted to identify the pest, its damage severity and vis-s-vis biology studies both under laboratory and field conditions. The studies on biology were similar to the studies made by different workers <sup>[8]</sup>.

Therefore an attempt has been made to survey on occurrence and intensity of *S. frugiperda* in maize fields of Malda district on both the Rabi and summer crops starting from November, 2018 to 2019. Studies were also conducted on the life table of *S. frugiperda* under laboratory conditions from field collected population for understanding the population dynamics of the same including key mortality factors, natality and mortality percentage and transparent descriptions of the actual properties of the cohort. Above all, being it a first report of *S. frugiperda* infestations on maize in different parts of Malda district in West Bengal, confirmation studies through molecular characterization have been made to authenticate the insect-pest species.

#### **Materials and Methods**

#### **Roving Survey and Assessment of Damage**

Roving survey on occurrence of *S. frugiperda* was conducted in maize grown areas under six blocks of Malda district of West Bengal starting from November 2018 to May 2019 both in rabi and summer maize. In each field, five spots were randomly selected each having 4.0 m row length with 3 rows. The number of plants damaged by *S. frugiperda* was counted and percent of infestation was calculated. GPS coordinates of each localities was also taken. Forty localities were surveyed during the period of study. Larvae of the pest were collected for molecular characterization and generation of DNA barcode.

#### **Molecular Identification**

Molecular identification of the pest species was carried out at the division of genomic resources, ICAR-National Bureau of Agricultural Insect Resources, Bengaluru, Karnataka.

DNA was extracted from the larvae using Qiagen D Neasy® kit, following the manufacturer's protocols. The DNA extracts were subjected to polymerase chain reaction (PCR) amplification of a 658bp region near the 5' terminus of the COX1 gene following standard protocol [13]. Primers used forward primer (LCO 1490: 5'were: GGTCAACAAATCATAAAGATATTGG-3'), and reverse primer (HCO 2198: 5'-TAAACTTCAGGGT GACCAAAAAATCA-3'). PCR reactions were carried out in 96-well plates, 50µL reaction volume containing: 5 µL GeNeiTM Tag buffer, 1 µL GeNeiTM 10mM dNTP mix, 2.5  $\mu$ L (20 pmol/ $\mu$ L) forward primer, 2.5  $\mu$ L (20 pmol/ $\mu$ L) reverse primer, 1 µL GeNeiTM Taq DNA polymerase (1 U/µL), 2µL DNA (50 ng/µL), and 36µL sterile water. Thermocycling consisted of an initial denaturation of 94°C for 5 min, followed by 30 cycles of denaturation at 94°C for 1 min, annealing at 46°C for 1 min and extension at 72°C for 1 min. PCR was performed using a C1000<sup>TM</sup> Thermal Cycler. The amplified products were analyzed on a 1.5% agarose gel electrophoresis <sup>[14]</sup>. The amplified products were sent to M/s Chromous Biotech, Bangalore, India, for sequencing. The species was bidirectional sequenced and checked for homology, insertions and deletions, stop codons, and frame shifts by using NCBI BLAST and ORF finder. The sequence was uploaded to GenBank and the Barcode of Life Database (BOLD, http:// www.boldsystems.org).

#### Study of Life Table

The study on life table was conducted by maintaining cultures of *Spodoptera frugiperda* in the laboratory from field collected populations in the maize field of Regional Research Sub Station (OAZ), Uttar Banga Krishi Viswavidyalaya situated at Mathurapur, Malda, West Bengal. The single sex method is adopted for the study of Life Table <sup>[15]</sup>. The agespecific life table was constructed by introducing five pairs of male and female into five wooden cages each measuring (length 34 cm x breadth 30 cm x height 60 cm). The forewing characteristics were used for identifying male and female insects <sup>[6]</sup>. The adults moths were allowed to mate and females to oviposit till they died. The data on total no. of eggs laid were observed along with the survivability of eggs. The mortality rates of 1<sup>st</sup> to 6<sup>th</sup> instar larvae along with the adults were noted on a daily basis and the mortality factors were also ascertained. The life table was thus constructed using the following parameters <sup>[16]</sup>.

- a) X = the pivotal age for the age class in units of time ( interval or days)
- b)  $l_x$  = the number surviving at the beginning
- c)  $D_x$  = the number dying during the age interval x,
- d) K = age specific key mortality, It is a key factor which is primarily responsible for increase or decrease in number from one generation to another and was computed as difference between successive values for 'log lx'. However, the total generation mortality was calculated by adding "K" values of different development stages.
- e)  $L_x$ = the number remain alive in between ages x and x+1 =  $(l_x + l_{x+1})/2$
- f)  $T_x = \text{total number at age x units beyond the age x is calculated as } T_x = L_x + L_{x+1} + L_{x+2} + \dots + L_w \text{ i.e. cumulative sum from bottom}$
- g)  $e_x =$  the expectancy of life remaining for individual age x given by formula ( $T_x/l_x$ )

#### **Results and Discussions**

#### Fall armyworm abundance and infestation level in maize

The fall armyworm incidence in rabi maize was first located in three different localities of Harischandrapur 2 block on 28 November 2018 in 27 to 34 days old crop (Table -1) with an infestation level of  $4.49 \pm 0.81$  % to  $12.85 \pm 0.58$ %. Occurrence of the pest was then observed in other five blocks with an infestation level ranging from  $3.55 \pm 0.91\%$  to 18.24 $\pm$  0.93%. However, the level of infestation was much higher in summer maize ranging from  $8.53 \pm 0.68\%$  to  $27.56 \pm$ 0.65%. Various range of infestation is reported by different workers globally as well as in India [9, 12, 17, 18], which even reaches 95 % plant damage [19]. Most of the literature indicated higher infestation of FAW in summer maize. The percentage of damage observed by Chormule et al., 2018<sup>[7]</sup> on maize is similar to the observation made in the present study during same crop season at similar growth stage (15-35%).

**Table 1:** Incidence of S. frugiperda on rabi and summer maize in Malda district of West Bengal

Block	Village	GPS Coordinates of Crop Field	Сгор	Variety	Date of Survey	Age of Crop (days)	Level of Infestation (%)
Harischandrapur 2	Saranpur	25°28.3180′N 87°52.5220′E	Rabi Maize	P3355	28.11.2018	34	$12.85\pm0.58$
Harischandrapur 2	Islampur	25°22.1810′N 87°46.7520′E	Rabi Maize	P3355	28.11.2018	39	$8.61\pm0.80$
Harischandrapur 2	Dakshin Mukundupur	25°26.3900´N 87°51.8300´E	Rabi Maize	P3355	28.11.2018	27	$4.49\pm0.81$
Harischandrapur 1	Gouripur	25°28.7770´N 87°53.3380´E	Rabi Maize	P3355	28.11.2018	29	$6.03\pm0.96$
Harischandrapur 1	Bairat	25°23.8940′N 87°55.92300′E	Rabi Maize	Yubaraj Gold	28.12.2018	31	6.31 ± 0.81
Chanchal 1	Mulaibari	25°25.2440′N	Rabi	Yubaraj	28.12.2018	47	$18.24 \pm 0.93$

[		88°3 0040 F	Maiza	Gold			
Chacnal 1	Kaligram	25°23.8320'N	Rabi	Yubaraj	28 12 2018	37	10 99 + 0 88
Chanchal 1	Kaligram	88°2.5170′E 25°23.7370′N	Maize Rabi	Gold Yubaraj	28 12 2018	41	16.48 + 0.67
	Kangrann	88°1.2900′E 25°22.5340′N	Maize Rabi	Gold DKC	20.12.2018	41	10.48 ± 0.07
Chanchal 2	Nehalpur	87°59.0490′E	Maize	9081	28.12.2018	46	$13.84 \pm 0.80$
Chanchal 2	Khanpur	25°17.2990′N 88°5.4890′E	Rabi Maize	DKC 9081	28.12.2018	27	$5.99\pm0.74$
Chanchal 2	Gopalpur	25°19.4950′N 87°59.4580′E	Rabi Maize	DKC 9081	28.12.2018	20	$4.50\pm0.76$
Ratua 1	Darbasini	25°11.6730′N 87°47.7460′E	Rabi Maize	DKC 9081	28.11.2018	31	$8.42\pm0.62$
Ratua 1	Bhaluara	25°14.3160′N 87°55.1810′E	Rabi Maize	P3355	28.11.2018	36	$11.69\pm0.74$
Ratua 1	Bhaluara	25°14.3320′N 87°55.1870′E	Rabi Maize	P3355	28.11.2018	26	$7.21\pm0.57$
Ratua 1	Bhaluara	25°14.3840′N 87°55.2650′E	Rabi Maize	P3355	28.11.2018	28	6.18 ± 0.49
Ratua 1	Jhawabari	25°11.9080′N 87°59.1720′E	Rabi Maize	DKC 9081	28.12.2018	49	$16.25\pm0.82$
Ratua 1	Lockrigola	25°11.4890′N 87°58.8990′E	Rabi Maize	DKC 9081	28.12.2018	45	$11.24\pm0.65$
Ratua 1	Matiyari	25°12.0480′N 87°58.5460′E	Rabi Maize	DKC 9081	28.12.2018	32	$9.33 \pm 0.58$
Manikchak	Shekhpura	25°5.9130´N 87°53.8760´E	Rabi Maize	DKC 9081	28.11.2018	28	$5.15\pm0.86$
Manikchak	Ugritola	25°4.2020´N 87°54.0670´E	Rabi Maize	DKC 9081	25.01.2018	32	$7.12\pm0.74$
Manikchak	Mathurapur	25°6.1510′N 87°53.7360′E	Rabi Maize	P3355	25.01.2018	30	3.55 ± 0.91
Manikchak	Lalbathani	25°8.3000′N 87°56.1390′E	Summer Maize	DKC 9081	02.05.2019	47	$27.56 \pm 0.65$
Manikchak	Nurpur	25°9.2310′N 87°57.2450′E	Summer Maize	Badsha 22	02.05.2019	38	$26.55\pm0.92$
Manikchak	Naryanpur	25°4.3420´N 87°53.3850´E	Summer Maize	Badsha 22	18.05.2019	42	$24.60\pm0.93$
Manikchak	Naryanpur	25°4.2170′N 87°53.3590′E	Summer Maize	DKC 9144	18.05.2019	31	$17.03\pm0.74$
Manikchak	Naryanpur	25°4.1980´N 87°53.36300´E	Summer Maize	DKC 9081	18.05.2019	41	$21.41\pm0.72$
Manikchak	Naryanpur	25°4.1880´N 87°53.3670´E	Summer Maize	DKC 9081	18.05.2019	32	$19.04\pm0.55$
Manikchak	Naryanpur	25°4.3330´N 87°53.4000´E	Summer Maize	Badsha 22	18.05.2019	26	$15.43\pm0.81$
Manikchak	Naryanpur	25°4.31900´N 87°53.4380´E	Summer Maize	DKC 9144	18.05.2019	22	$8.53 \pm 0.68$
Manikchak	Naryanpur	25°4.2610´N 87°53.4020´E	Summer Maize	Badsha 22	18.05.2019	26	$10.96\pm0.78$
Manikchak	Naryanpur	25°4.2730´N 87°53.4670´E	Summer Maize	Badsha 22	18.05.2019	31	$18.29\pm0.70$
Manikchak	Naryanpur	25°4.2810´N 87°53.4590´E	Summer Maize	Badsha 22	18.05.2019	27	$15.77\pm0.84$
Manikchak	Dhanrajgram	25°7.1170´N 87°54.3040´E	Summer Maize	Badsha 22	18.05.2019	32	$18.16 \pm 0.81$
Manikchak	Bhutni	25°6.8170´N 87°52.1940´E	Summer Maize	Local	22.05.2019	34	$21.79\pm0.80$
Manikchak	Bhutni	25°6.9030´N 87°52.1630´E	Summer Maize	Local	22.05.2019	26	14.15 ± 0.79
Manikchak	Bhutni	25°6.9080´N 87°52.1600´E	Summer Maize	Local	22.05.2019	31	$17.05 \pm 0.59$
Manikchak	Shekhpura	25°5.6110′N 87°54.1880′E	Summer Maize	Badsha 22	22.05.2019	26	$16.25 \pm 0.78$
Manikchak	Shekhpura	25°5.6560′N 87°54.1670′E	Summer Maize	DKC 9144	22.05.2019	32	$16.76\pm0.48$
Manikchak	Shekhpura	25°5.6310′N 87°54.1310′E	Summer Maize	Badsha 22	22.05.2019	28	$13.09 \pm 0.77$
Manikchak	Mathurapur	25°6.2080′N 87°53.7290′E	Summer Maize	DKC 9144	10.06.2019	28	$14.95\pm0.67$

#### Nature of Damage

The incidence of fall armyworm was more severe on young crops and started infesting the crop at the age of 20-22 days. Neonate larvae fed the leaves by scrapping of chlorophyll, which led to silvery transparent membrane in the initial stage ultimately resulting in white elongated patches (Fig 1 and 2). Later instars created 'window pane' on leaves leaving moist saw dust like frass near funnel and upper leaves (Fig 3 and 4). Mature larvae is characterized by white inverted 'Y' shaped capsule on head and distinct four black spots on 8<sup>th</sup> abdominal segment (Fig 5). It is mostly found to feed in the whorls of young plants (Fig 6) and severe damage observed between V<sub>12</sub> to VT growth stages <sup>[3]</sup> of the crop i.e. 42 to 56 days after planting. The findings are similar to other workers <sup>[2, 9]</sup>, where it was observed that fall armyworm are capable of damaging all growth stages of maize, however, damage is more severe in vegetative stage.



Fig 1: Initial Symptom of damage of Scrapping of chlorophyll created by neonate larvae



Fig 2: Symptom of white elongated patches created by the neonate larvae



Fig 3: Creation of 'Window Pane' by matured larvae



Fig 4: Moist saw dust-like frass in funnel



Fig 5: S. frugiperda Larvae



Fig 6: S. frugiperda Larvae feeds in whorls of young leave

#### Molecular characterization and DNA barcoding of FAW

The high quality DNA was obtained from the larvae eventually resulted into a PCR product. The sequence showed 100% similarity to *S. frugiperda* through BLAST analysis with other populations of *S. frugiperda* (MH881529, MH881530, MH881531, MH881532, MH881533, MK913645, MK913646, MK913647 and MK913648) from NCBI Genbank. The sequence was submitted to Genbank through banklt and obtained the accession number MN117908. and the DNA Barcode was further generated through bold system (Barcode of Life Database) and represented in Figure 7.



Fig 7: COI Gene sequencing and DNA barcoding of Spodoptera frugiperda (Malda Population)

#### Life Table studies of *S. frugiperda*

The mortality and its factors was observed (Table 2) on different stages of development of S. frugiperda like egg, early larval stages, late larval stages and pupal stages of S. frugiperda. The highest mortality of S. frugiperda was recorded in early larval stages (1<sup>st</sup> to 3<sup>rd</sup> larval instars) recording 82.7 % followed by late larval stages i.e. 4th to 6th instars (62.26 %). However, the mortality in egg stage and pupal stage were recorded 14.64% and 6.08% respectively. Mortality in the egg stage was mainly due to sterility and unhatchability. The early instars recorded increased mortality due to dispersal, parasitism (Apantales spp) and infection by bacterial (Bacillus thuringiensis), viral (nuclear polyhedrosis virus) and fungal entomopathogens (Nomuraea rileyi and Beauveria bassiana). During the late larval stages especially in the 6<sup>th</sup> instar cannibalism was noticed on other larvae or the newly formed pupae even when food was not a limiting factor. There were other unknown causes which lead to increased mortality rates in the early and late larval stages.

Many reports suggested that a number of natural enemies and entomopathogens are found to infest the larvae of fall armyworm among which *Trichogramma spp*, *Telenomus spp*, *Campoletis chloridae*, Apantales *spp* and *Nomuraea rileyi* are very common in the field. This may pave the way to introduce bio-intensive pest management programme against fall armyworm <sup>[6, 7]</sup>.

Table 3 depicts the trend index value (I) to be positive which indicates that the population of *S. frugiperda* would be much higher in the ensuing generations. Each egg in first generation will contribute 67% increase in egg production in the next generation.

The survivorship curve denotes a type III curve (Figure 8) that indicates all late stages of the insects to be good survivors. This indicates that the 4<sup>th</sup> to 6<sup>th</sup> instars larvae are the major destructor of the crop. The patterns of survivorship observed indicated that the immature stages like egg and 1<sup>st</sup> to 3<sup>rd</sup> instars larvae are vulnerable to management practices.

Table 2: Life-table for S. frugiperda under laboratory conditions	from t	field	collected	population
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Age interval (x)	Number alive at the beginning of <i>x</i>	Number dying during x	Factor responsible for <i>Dx</i>	<i>Dx</i> as % of <i>lx</i>	Survival within <i>x</i>	Log of l <sub>x</sub>	K - value	$(l_{x} + l_{x+1})/2$	$L_{x} + L$ $x+1 + L_{x+2} + L_{x+2} + L_{w}$	(Tx/ lx)
Х	lx	D <sub>x</sub>	$D_x f$	100qx	Sx	log x		Lx	T <sub>x</sub>	ex
Egg	1448	212	Sterility & unhatchibility	14.64	0.853	3.16	0.07	1342	5181	3.57
1 <sup>st</sup> instar (N <sub>1</sub> )	1236	274	dispersal	22.16	0.778	3.09	0.11	1099	3839	3.10
2 <sup>nd</sup> instar	962	232	Bacterial, viral and	24.11	0.758	2.98	0.12	846	2740	3.95
3 <sup>rd</sup> instar	730	266	fungal infections,	36.43	0.635	2.86	0.20	597	1894	2.59
4 <sup>th</sup> instar	464	117	parasitoids,	25.21	0.747	2.66	0.12	405.5	1297	2.79
5 <sup>th</sup> instar	347	75	unknown factors	21.61	0.783	2.54	0.11	309.5	891.5	2.56
6 <sup>th</sup> instar	272	42	Cannibalism	15.44	0.845	2.43	0.07	251	582	2.13
Pupal stage	230	14	Deformed pupae	6.08	0.939	2.36		223	331	1.43
Adult emerged	216 of which 109 ♀ & 107 ♂	Sex ratio:- Male : Female = 1: 0.98		$Total K = k_E + k_{L1} + k_{L2}$ $+ k_{L3} + k_{L4} + k_{L5} + k_{L6}$		= 0.80		108	108	0.5

• K = age specific key mortality

• X = the pivotal age for the age class in units of time (days)

•  $l_x$  = the number surviving at the beginning

•  $D_x$  = the number dying during the age interval x,

•  $e_x$  = the expectancy of life remaining for individual age x given

by formula  $(T_x / l_x)$ 

•  $L_x$ = the number remain alive in between ages x and x+1 =  $(l_x + l_{x+1})/2$ 

 $T_x$  = total number at age x units beyond the age x is calculated as  $T_x$  =  $L_x + L_{x+1} + L_{x+2} + \dots + L_w$  i.e. cumulative sum from bottom

Seasonal reproductive rate	No. of females emerged/ total no. of eggs observed in first generation	7.39 %
Mean fecundity of the cohort	Average no. of eggs produced by a female x no. of females. (Total no. of eggs laid) (where average fecundity is 915)	97,905
Trend index (I)	No. of eggs produced by female cohort/ No. of eggs started life in first generation.	67.61
Generation survival	Total no. of males and females observed/ No. of first instar observed in first generation.	0.174



Fig 8: Survivorship Curve of S. frugiperda

There are limited reports on construction of life table for *S. frugiperda*. Similar trend was found in other study, where abiotic factors and predation had a greater effect on egg and early larval mortality. More than 95% of the mortality was recorded due to predation. Mortality in early larval population could not be replaceable as compared to egg mortality. Therefore, control measures in early larval stage may be more effective in reducing generational survival <sup>[20]</sup>. The late instars larvae of fall armyworm feeding on late whorl stage of the crop are difficult to be controlled by application of pesticide as penetration of pesticides were obstructed by the larval excreta (saw dust like frass) present in the whorl of maize plant. Therefore, it is easy to control 1<sup>st</sup> to 3<sup>rd</sup> instars larvae in early growth stage of the crop by application of pesticides <sup>[3]</sup>.

#### Conclusion

The report reflects that fall armyworm has appeared in northern part of West Bengal in November, 2018 and gradually infested both rabi and summer maize in Malda district of the state within seven to twelve months after invasion in Karnataka state. It indicates the enormous dispersal capability of the pest to reach eastern part of the country from southern part by covering more than 1000 miles within seven months. The possible expansion of the pest to entire maize growing areas of eastern gangetic plains including India, Bangladesh and Nepal is now posing a serious threat of economic loss to the small and marginal farmers of the region. Therefore, effective management strategies are required to control the pest menace in near future. The egg and 1st to 3rd instars larvae of S. frugiperda may effectively be controlled either by bio-intensive pest management strategies like conservation and inundative release of natural enemies along with application of biopesticides or need based application of chemical pesticide as per CIBRC label claims at the early crop growth stages to avoid economical loss of the crop and the environmental hazards also.

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