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Beet armyworm *Spodoptera exigua* (Hubner): A newly emerging pest of Egyptian clover in Punjab

Arshdeep K. Gill*, Ramesh Arora and Vikas Jindal
Punjab Agricultural University, Ludhiana-141004, India
*Corresponding author e-mail: arsh.pau@gmail.com
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Abstract
Studies on population dynamics of *Thysanoplusia oricalcea, Helicoverpa armigera* and *Spodoptera exigua* were carried out on berseem. All the three lepidopterans were prevelant throughout the season but *H. armigera* population was negligible during Feburary-March and increased in April. Mean larval population of *T. oricalcea*, *S. exigua* and *H. armigera* was 2.33, 0.88 and 1.13 larvae/m^2 at PAU, Ludhiana and 0.83, 3.71 and 0.92 larvae/m^2 at RRS, Gurdaspur. Investigation on biological characteristics along with molecular phylogeny of beet armyworm *S. exigua* were undertaken keeping in view presence of damaging population of pest on the berseem at two locations (Ludhiana and Gurdaspur) in Punjab. The study revealed that the average egg, larval, pupal, adult male and adult female period were 2.4 ± 0.55, 18.6 ± 1.7, 8.4 ± 1.14, 6.8 ± 0.8 and 7.6 ± 0.9 days, respectively. The average life cycle was completed in 29.4 ± 1.5 days and average fecundity was 241.2 ± 30 eggs per female. On the basis of mitochondrial sequences, Ludhiana population of *S. exigua* was almost similar to all The Netherlands populations and one of the German populations.

Keywords: Armyworm, Berseem, Biology, Climate change, Cox I

Introduction

Egyptian clover (berseem) is the most popular annual forage legume crop cultivated in north, northwest and central India. *H. armigera* is the key pest of Egyptian clover seed crop in northern India and losses up to 70-80 per cent in seed yield have been reported (Dhaliwal and Sidhu, 1990; Arora *et al*., 2011). In addition to *H. armigera*, other pests infesting Egyptian clover include Bihar hairy caterpillar, *Spilosoma obliqua* and cabbage semilooper, *Thysanoplusia oricalcea* which account for 30-80 per cent and 25-50 per cent losses, respectively (Arora, 2012). In present studies, another lepidopteran pest *Spodoptera exigua* (Hübner) (Noctuinae: Noctuidae: Lepidoptera) was recorded damaging the berseem fodder crop at two locations in Punjab. Beet armyworm (*S. exigua*) is a polyphagous pest reported from more than 170 plant species over 30 families (Pogue, 2006) with a worldwide distribution (Zheng *et al*., 2011). In India, *S. exigua* have been reported from onion, garlic, groundnut, chilli, pearl millet, black gram, green gram, pea, dhaincha, brinjal, maize and safflower. Further, Singh and Sethi (1993) reported it on sugarbeet from Jalandhar (Punjab). Moreover, it is not enlisted among insect-pests damaging Egyptian clover crop in India (Saxena, 2002; Arora, 2012). In the present study the population dynamics, biology and morphometric alongwith molecular phylogeny of the pest was carried out to devise suitable management strategies.

Materials and Methods

A plot of 0.4 ha of berseem genotype BL10 was sown on December 5, 2012 at PAU, Ludhiana and on December 10, 2012 at RRS, Gurdaspur by broadcasting method. All the recommended agronomic practices were followed uniformly for raising the crop (Anonymous, 2012).The first cutting of both these crops for fodder were taken in the first fortnight of Feburary, 2013. After this regular cuttings for fodder were taken at 30-35 day intervals. The population of various pests was recorded from two quadrats of 1m^2 each selected randomly from each plot at both locations and the observations were recorded at fortnightly intervals during Feburary- April, 2013.

Biology of S. exigua: The larvae were collected from berseem fields at university seed farm, Ladhowal and reared on berseem leaves at of 25±2°C and 70±5% RH. The pre pupae were transferred to jars with 5 cm layer of moist sand at the bottom to prevent desiccation**.** The adults were paired and transferred to oviposition chamber. A diet swab was dipped in a solution of the adult diet (10 g sucrose + 2 ml ABCDE multivitamin mixture + 200 mg methyl-4-hydoxy benzoate in 1 ml ethanol and 10 ml distilled water) was hung inside oviposition

chamber. Various developmental period of egg, larvae, pupae and adults were recorded. Fecundity of S. *exigua* was also worked out. Observations were recorded daily for incubation period.

***Molecular characterization*:** The total genomic DNA was isolated from individual representative larva of *S. exigua* using a modified CTAB method (Cubero *et al*., 1999). The dried DNA pellet was dissolved in 100 µl of TE buffer containing DNase free pancreatic RNase A (10 µg per ml) and stored at -20°C until used. The quality of isolated DNA was determined by horizontal agarose (0.7 per cent containing ethidium bromide @1 µg per ml) gel electrophoresis in 1x TAE buffer. The DNA bands were visualized and photographed under a UV transilluminator in 'Ultra Cam Gel Documentation system.

Results and Discussion

***Population dynamics of lepidopteran larvae*:** Based on six fortnightly observations, the mean larval population of *T. oricalcea*, *S. exigua* and *H. armigera* was 2.33, 0.88 and 1.13 larvae/m^2 at PAU, Ludhiana and 0.83, 3.71 and 0.92 larvae/m^2 at RRS, Gurdaspur (Table 1). Among various pests, *S. exigua* recorded maximum population (2.21 larvae/m^2) followed by *T. oricalcea* (1.58 larvae/m^2) and *H. armigera* (1.20 larvae/m^2). Among two locations Gurdaspur recorded higher incidence of *S. exigua* (3.71 larvae/m^2) when compared to Ludhiana (0.88 larvae/m^2). The latter location recorded higher population of *T. oricalcea* (2.33 larvae/m^2) and *H. armigera* (1.13 larvae/m^2) when compared with Gurdaspur (0.83 larvae/m^2 and 0.92 larvae/m^2, respectively). All the three lepidopterans were prevelant throughout the season but *H. armigera* population was negligible during Feburary-March and increased in April. The cuttings of fodder were taken on Feburary and March 25 and 29 at Ludhiana and on Feburary 26 and March 29 at Gurdaspur, caused a steep decline in population of all the lepidopterans as most of the larvae were picked up by the birds.

But *S. exigua* population resurged rapidly as compared to the other two lepidopterans. The appearance of *S. exigua* on berseem in such higher numbers could be due to changing climate. The major climate change occurring in the state is that the minimum temperature during winters has been rising slowly by 0.06º C per annum during the last 30 years (Kaur *et al*., 2013). It is known that *S. exigua* does not undergo hibernation during winter and is favoured by mild winter temperatures as it has a lower development threshold of 12.2º C (Hogg and Guttierrez, 1980).

***Biology and morphometry*:** The eggs were spherical with longitudinal lines and were laid in clusters, mostly on upper side of leaves in field. The incubation period ranged from 2-3 days with an average of 2.4 ± 0.55 days. Afify *et al*. (1970) stated that this parameter was between 2 to 3 days and 2.8 to 3.1 days, respectively but Azidah and Sofian-Azirun (2006) and Sivapragasam (2001) found the incubation period of *S. exigua* to be 3 days but mostly depended on temperature. Percent hatchability of eggs was high and varied from 75.8 to 84.3 per cent. The mean hatchability of eggs was 80.2 per cent.

Newly hatched larvae were light green with black head. Larvae fed on green tissue of leaf leaving behind parchment like membrane. In the present studies, total larval period varied from 16 to 20 days with an average of 18.6 ± 1.7 days. Full grown larvae measured 26.20 ± 2.21 mm and weighed 298.8 ± 17.6 mg. Results were also similar to those of findings of Azidah and Sofian-Azirun (2006). However, Berdegue *et al*. (1998) have reported that the larval period of *S. exigua* was within 16 days on shallot, 10 to 24 days on cotton and 21.3 days on common weed (*Chenopodium murale*) and 16.5 days on celery, respectively. Pupation took place in a thin, loose coccon and pupa was light brown which became reddish brown later on. The pupal stage on an average continued for 8.4 ± 1.14 days. It ranged from 7 to 10 days. Pupal period varied from 4 to 10 days in summer and 6 to 20

Table 1. Population dynamics of lepidopteran pests of Egyptian clover fodder crop at two locations during 2012-13

Observation date	Mean larval population/m^2 Ludhiana			Observation date	Mean larval population/m^2 Gurdaspur		
	To Mean±SD	Se Mean±SD	Ha Mean±SD		To Mean±SD	Se Mean±SD	Ha Mean±SD
5th Feb	3.00±0.82	0.50±0.58	0.50±0.58	10th Feb	0.50±0.58	2.75±0.50	0.25±0.50
20th Feb **Cutting**	3.25±0.50	0.75±0.50	1.25±0.50	25th Feb **Cutting**	1.25±0.50	3.75±0.50	1.25±0.50
8th Mar	1.00±0.82	1.00±0.82	0.25±0.50	28th Mar	1.25±0.50	5.25±1.26	1.50±0.58
24th Mar **Cutting**	3.75±0.96	1.25±0.50	1.25±0.50	26th Mar **Cutting**	0.50±0.58	2.50±0.58	0.50±0.58
10th Apr	0.25±0.50	0.50±0.58	0.50±0.58	13th Apr	1.25±0.50	5.75±0.96	1.50±0.58
25th Apr	2.75±0.96	1.25±0.50	3.00±0.82	29th Apr	0.25±0.50	2.25±0.96	0.50±0.58
Mean	**2.33**	**0.88**	**1.13**	**Mean**	**0.83**	**3.71**	**0.92**

To: *Thysanoplusia oricalcea,* **Se**: *Spodoptera exigua*, **Ha:** *Helicoverpa armigera*

days in winter, 7.5 days on celery (Berdegue *et al*., 1998) and 9.02 to 10.21 days on shallot and lady's finger respectively (Azidah and Sofian- Azirun, 2006).

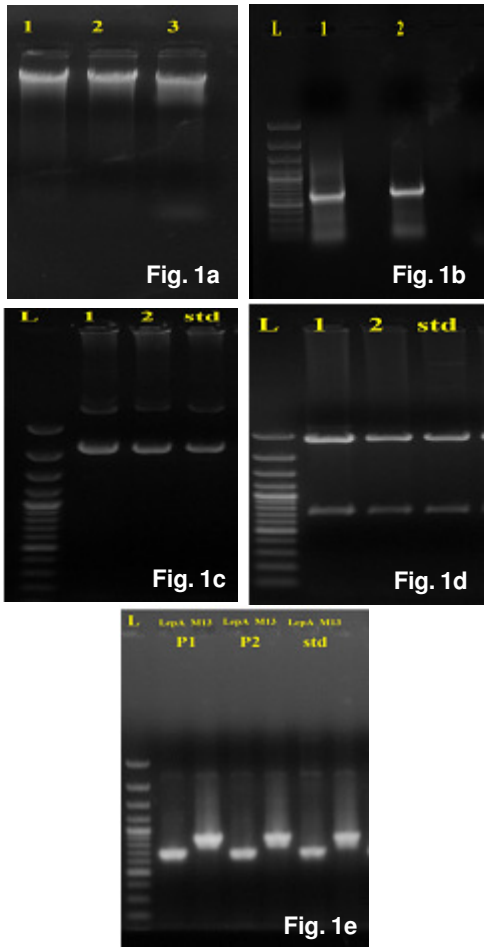

Fig 1. Molecular characterization of *S. exigua*. 1a- DNA Isolation, 1b- Specific PCR with LepA, 1c- Plasmid DNA isolation, 1d- Restriction of plasmid DNA and 1e- PCR using plamid DNA with LepA and M13

Adult longevity ranged from 6 to 8 and 7 to 9 days in case of males and females, respectively. Average adult longevity was 6.8 ± 0.8 and 7.6 ± 0.9 days in case of males and females, respectively. Total life cycle ranged from 27 to 31 days. On an average life cycle was completed in 29.4 ± 1.5 days. Number of eggs laid per female ranged from 198 to 280 eggs per female with an average fecundity of 241.2 ± 30 eggs per female. Our results on the fecundity deviate from the findings of Abdullah *et al*. (2000) who reported it to be 472.5 eggs on soybean leaf and 282 to 1307 eggs per female respectively, however the differences may be due to differences in host plant, location and period of study.

***Phylogenetic analysis and evolutionary relationship*:** The mitochondrial specific primers resulted in amplification and cloning of a single amplicons (Fig. 1). The true identity of the insert sequences in the recombinant plasmid was confirmed by PCR amplification of cloned DNA by M13 universal primers which resulted in amplification of H" 648 bp DNA fragment. The sequence is identified based upon the derived Taxonomy report generated on the basis of maximum sequence homology/ query coverage in database from other species (Table 2) and submitted in Barcode of life and is available under bold id: AAA6644, sequence page: LEPIN067-14. Indian populations of *S. exigua* based on mt COI sequences forms two clusters (Fig. 2), one of Delhi populations and second cluster composed two sub clusters one of Ludhiana and other of Coimbatore and Bangalore populations. Cluster I differs from cluster II by molecular divergence of 0.7%. Two sub clusters of second cluster differ by 0.4% divergence.

Table 2. Sequence producing maximum alignments with Cox I region of *S. exigua* collected from Ludhiana infesting Egyptian clover with 99% query coverage, maximum score and lowest e- value (0.00)

Description	Max Score	Total Score	Query cover	E Value	Identity	Accession
S. exigua voucher BC ZSM Lep 27643 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	1214	1214	99%	0.0	100%	JF415658.1
S. exigua mitochondrion, complete genome	1203	1203	99%	0.0	99%	JX316220.1
S. exigua voucher TNAUMESI-9 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial	1203	1203	99%	0.0	99%	JQ064572.1
S. exigua voucher BC ZSM Lep 31746 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	1203	1203	99%	0.0	99%	HM914242.1
S. exigua voucher Sex cytochrome c oxidase subunit I (COI) gene, partial cds; mitochondrial	1203	1203	99%	0.0	99%	EU779856.1
S. exigua strain SeL1 cytochrome oxidase subunit I gene, partial cds; mitochondrial	1197	1197	99%	0.0	99%	KJ940205.1

Fig 2. Dendrogram showing genetic divergence between Indian populations of *S. exigua*

The appearance of *S. exigua* as a regular or even a sporadic pest in Punjab may challenge the success of existing IPM technology in berseem. At present no chemical control measures are recommended on fodder crop of berseem (Dhaliwal, 1998; Arora, 2012). The presence of *S. exigua* in large numbers may alter this situation. It is not certain that avian predators will prefer these larvae as food. Even if they do feed on *S. exigua,* it may not result in effective control as avian predators are known to be less effective at high population density of lepidopteran insect pests (Glen, 2004). So additional suitable cultural or chemical control measures may have to be developed/ adopted for management of *S. exigua* on fodder crop of berseem.

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