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**Biology of Armyworm, *Spodoptera litura* and Effect of *Beauveria bassiana* against Third Instar Larvae Under Laboratory**

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**ABSTRACT**

Armyworm, *Spodoptera litura* is a destructive and polyphagous pest of several crops *i.e.* agricultural and horticultural throughout the world. The present research was performed to check the biology and efficacy of *Beauveria bassiana* on larvae and pupae of pests. Eggs were laid on the lower surface of cabbage leaves and on the wall of the containers in cluster form. The colour of newly laid eggs was pale white, later changed to grey or dark near to hatching. The average developmental parameters of 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup> and 5<sup>th</sup> larval instar were 2.43±0.43, 2.65±0.80, 3.21±0.54, 2.43±0.56 and 2.33±0.86, respectively. The colour of pre-pupa and pupa was lighter reddish and pale yellowish, respectively. The average mean pre-pupal and pupal periods were 0.63±0.21 and 7.00±0.76, respectively. The larval length was 2.6, 2.5 and 2.8 cm in 2.4×10<sup>4</sup>, 2.4×10<sup>5</sup> and 2.4×10<sup>6</sup> spore/ml, respectively. The highest and lowest pupation percentage was 87.2% and 40.6%, respectively in 2.4×10<sup>4</sup> spore/ml and control. The pupal weight was 181.2 and 202.3 mg in 2.4×10<sup>5</sup> and 2.4×10<sup>6</sup> spore/ml, respectively. The pupal duration was 8.3, 8.1, and 8.4 days in 2.4×10<sup>4</sup>, 2.4×10<sup>5</sup>, and 2.4×10<sup>6</sup> spore/ml, respectively. The current study will be proved the best strategy in integrated pest management strategy (IPM) in the country.

**INTRODUCTION**

Armyworm, *Spodoptera litura* is a polyphagous pest of many crops like agricultural and horticultural crops throughout the world especially in India, China and Pakistan. This pest is widely spread in tropical, subtropical and temperate areas of the world (Saleem *et al.*, 2008; Shah *et al.*, 2019). Approximately more than a hundred plant species have reported its host plants (Mallikarjuna *et al.*, 2004; Ahmad *et al.*, 2007). The major hosts of pests are sorghum, rice, cabbage, okra, cauliflower, tomato, cotton and brinjal, etc. (Murtaza *et al.*, 2019; Ramzan *et al.*, 2019; 2020).

This pest is invading other host plants and rapidly spreading to other areas of the

world in form of different genotypes. All parts of the plants or hosts like stem, leaves, shoots and capsule are attacked by all larval instars of the pest which ultimately reduces the crop production and causes economic crop losses. It has been reported that pests can cause 35-55% crop losses during a high attack in favorable climatic conditions and the availability of host plants.

The different control strategies have been tested to control this sporadic pest in the world including cultural, physical, mechanical, chemical, biological and botanicals. There is a need to get rid of this pest by using a control method alternative to chemicals because the pest has developed insecticides resistance against various chemicals. By keeping in view, the importance of alternative methods, the current study was conducted.

## MATERIALS AND METHODS

### Collection of Moths and Rearing Method:

Moths of Armyworm, *Spodoptera litura* were collected from light traps installed in the fields and moths were brought to Rearing laboratory for rearing purposes. Moths were shifted into plastic containers of 1.5 L and the cotton swab was soaked into a 10% sugar solution, given to moths for feeding. A tissue paper was hung inside the containers for egg-laying purposes. Eggs were collected from tissue paper and placed individually into petri dishes for hatching. Larvae were placed individually into petri dishes with cabbage leaves for feeding. On daily basis, new cabbage leaves were given to larvae and old ones were removed from reared culture until larvae reached pupation. The pupated larvae were kept separated from non-pupated larvae even adults emerged. Adults were identified on six bases and one pair (M: F) was placed into a separate container to continue the culture and make the culture pure from all chemical hazards. The third instar larvae of third generations were used to perform the bioassay study. All insect parameters like egg, larva, pupa and adult were recorded during the whole study period. The rearing was carried out at  $26.00 \pm 5.00$  °C temperature and 60% relative humidity (RH).

### Fungal Source and Bioassay:

*B. bassiana* was obtained from the University of Agriculture Faisalabad. Four fungus concentrations such as ( $1 \times 10^4$ ,  $1 \times 10^5$ ,  $1 \times 10^6$ ,  $1 \times 10^7$  and  $1 \times 10^8$  spores/ml) were prepared using Neubauer's improved hemocytometer. Third instar larvae of 3<sup>rd</sup> generations were randomly collected from the pure culture and used in the study. There were three replications and each replication consisted of ten larvae. The fungus suspension was sprayed on the larvae for 15s and larvae were kept into petri dishes containing fresh cabbage leaves for feeding. The new and fresh leaves were provided to larvae on daily basis even larvae died or reached pupation. The distilled water was applied to larvae as controls treatment. The larval mortality rate was recorded on daily basis.

## RESULTS AND DISCUSSION

### Biology of *S. litura*:

#### Eggs:

Eggs were laid by females on the lower surface of cabbage leaves and on the wall of the containers in groups or cluster form. The colour of newly laid eggs was pale white which later changed to grey or dark near to hatching. Kumar and Bhattacharya (2019) and Ramzan *et al.*, (2021) had reported similar findings of eggs. Some researchers have recorded the pale green colour of newly laid eggs which changed into the whitish green after a few hours of hatching. Brown hair or scales were observed on the egg cluster. The average mean embryonic period was  $2.75 \pm 0.10$ . The description of eggs can be seen in

Table 1. The hatching percentage can be varied according to climatic factors. Shukla et al., (2000) had reported 87.33 to 97.33 hatching percent at  $24.96 \pm 3.03$  °C temperature and  $58.04 \pm 7.71$  RH. Other researchers have also reported almost similar findings as recorded in the current study (Rabari *et al.*, 2018; Ramaiah and Maheshwari, 2018).

#### Larvae:

Five larval instars of *S. litura* were observed during the current study. The average developmental parameters of first, second, third, fourth and fifth larval instar were  $2.43 \pm 0.43$ ,  $2.65 \pm 0.80$ ,  $3.21 \pm 0.54$ ,  $2.43 \pm 0.56$  and  $2.33 \pm 0.86$ , respectively. Approximately 10-17 days were the total larval life period while Ramzan *et al.* (2021) had reported 11-16 days on cabbage. Our findings are almost similar to early researchers (Shekhawat and Maheshwari, 2018). Tuan *et al.* (2015) had investigated similar results. They investigated that the average total larval period is  $14.3 \pm 0.2$  days and 14.54 days recorded by Yadav (2020) while 15.55 days recorded by Shahout *et al.* (2011).

**Table 1.** Developmental parameters of *Spodoptera litura* under laboratory conditions.

Parameters	Mean± SE	Range (days)
Embryonic period	2.75±0.10	2-4
First instar	2.43±0.43	2-3
Second instar	2.65±0.80	2-3
Third instar	3.21±0.54	3-4
Fourth instar	2.43±0.56	2-3
Fifth instar	2.33±0.86	2-3
Total larval period (first to fifth)	13.60±3.80	10-17
Pre-pupal period	0.63±0.21	0.70-1.10
Pupal period	7.00±0.76	6-9
Male longevity	6.34±0.55	5-8
Female longevity	7.99±1.33	5-10
Total life cycle (egg to adult)	32.23±1.21	30-36

#### Pupa and Adult:

The mature larvae converted into C- shape before changing into pupation. The colour of pre-pupa was lighter reddish or dark brown while pupa was pale yellowish. The pre-pupa has lasted about 0.75-1.10 days while Narevkar *et al.* (2018) reported that pre-pupa lasted for 2 days. The average mean pre-pupal and pupal periods were  $0.63 \pm 0.21$  and  $7.00 \pm 0.76$ , respectively. The total pupal period on cabbage was 4-8 days which is similar to Shakya *et al.* (2015) who reported 7-9 days. The description of pupae is given in Table 2. Kumar and Bhattacharya (2019) had conducted a study to determine the life cycle of *S. litura* on various host plants including cabbage, tomato, gram and cauliflower. They reported that the total life cycle (Egg-Adult) was 35.23 days. Larvae of pest can consume more leaves of cabbage as compared to other tested host plants like tomato, grams and cauliflower. The adults of the pest were yellowish-brown in colour with black compound eyes. The forewings and hindwings of the moth were brown and silvery-white in colour, respectively. The size of females was greater than the males. It was recorded that female was long-lived as compared to male. Ravi *et al.* (2015) had suggested a similar conclusion about adult longevity.

**Table 2.** Pre-oviposition, oviposition periods, fecundity, and percentage of egg hatching of *S. litura*

Parameters	Mean± SE	Range (days)
Pre-ovipositional period	3.13±0.77	2.5-3.75
Ovipositional period	4±0.63	3.5-4.5
Fecundity (no. of eggs laid by single female)	890.5±16.13	889-900
Percentage of hatching	90.44±6.43	93-95.5

**Bioassay Study:**

The larval length was 2.6, 2.5 and 2.8 cm in  $2.4 \times 10^4$ ,  $2.4 \times 10^5$  and  $2.4 \times 10^6$  spore/ml, respectively. The highest and lowest pupation percentage was 87.2% and 40.6%, respectively in  $2.4 \times 10^4$  spore/ml and control (Table 3).

**Table 3.** Impact of *B. bassiana* on development of larvae

Treatments		Parameters			
		Larval length (cm)	Larval weight (mg)	Larval duration (days)	Pupation (%)
<i>B. bassiana</i> spore/ml	$2.4 \times 10^4$	2.6a	358.1ab	7.5bc	40.6a
	$2.4 \times 10^5$	2.5a	354.2ab	7.6bc	42.8a
	$2.4 \times 10^6$	2.8a	361.7b	7.1a	47.1a
Control	Distilled water	3.10c	378.1c	7.7d	87.2b
CD (P = 0.05)		0.2	27.2	0.27	20.1

Note: Values with different letters in each column differ significantly (5%) by LSD

Pupal growth significantly differed with concentration to spores. The pupal weight and length were 176.2 gm and 1.3 cm, respectively in  $2.4 \times 10^4$  spore/ml while deformed pupae were also recorded during the study. Not many variations in pupal length were recorded in different concentrations. The pupal length was 1.3, 1.5, and 1.3 cm in  $2.4 \times 10^4$ ,  $2.4 \times 10^5$ , and  $2.4 \times 10^6$ , respectively while 1.7 cm control (Table 4). The current study findings are in line with the previous studies (Ramzan et al., 2019). The pupal weight was 181.2 and 202.3 mg in  $2.4 \times 10^5$  and  $2.4 \times 10^6$  spore/ml, respectively. The pupal duration was 8.3, 8.1, and 8.4 days in  $2.4 \times 10^4$ ,  $2.4 \times 10^5$ , and  $2.4 \times 10^6$  spore/ml, respectively as against the untreated (4.1 days).

**Table 4.** Impact of *B. bassiana* on development of pupae

Treatments		Parameters			Adult emergence	
		Pupal length (cm)	Pupal weight (mg)	Pupal duration (days)	Healthy	Deformed
<i>B. bassiana</i> spore/ml	$2.4 \times 10^4$	1.3b	176.2b	8.3b	10.2b	84.5ab
	$2.4 \times 10^5$	1.5a	181.2b	8.1b	32.3c	60.4c
	$2.4 \times 10^6$	1.3a	202.3c	8.4b	24.6c	67.3bc
Control	Water	1.7c	207.3c	8.6b	73.0d	18.2d
CD (P = 0.05)		0.06	21.8	4.1	21.2	20.3

Note: Values with different letters in each column differ significantly (5%) by LSD.

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