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Rearing of *Spodoptera litura* (Fabricius) on Different Artificial Diets and its Parasitization with *Trichogramma chilonis* (Ishii)

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Abstract.- Studies on rearing of *Spodoptera litura* (Fabricius) on different artificial diets *i.e.,* bean based diet (Diet-1) and gram based diet (Diet-2) and its parasitism with *Trichogramma chilonis* (Ishii) were conducted under controlled laboratory conditions of 27±2°C, 65±5% RH and 14:10 L:D during 2013. The results showed that feeding the 1st, 2nd and 3rd generations of the immature stage of *S. litura* on diet-2 resulted in highest % survival rates among all the parameters of immature stages of *S. litura,* lower total larval duration (15.8 days as against 17.2 days of control) and total duration from egg to adult stage (29.6 days as against 31.9 days for diet-1). The mature adult stage of *S. litura* showed almost same trend of better performance of *S. litura* fed on diet-2 in terms of higher oviposition period 5.7 days and higher female fecundity (total number of eggs 1612.3) compared with the oviposition period 5.2 days and female fecundity (total number of eggs 1541.86) recorded for diet-1. No significant difference was observed among the diets in the % parasitism rate of S. *litura* by *T. chilonis*. Increased trend of parasitism coincided with the increased time of exposure. Highest parasitism rate of 91.1% and 89.5 % was observed in the egg cards exposed to *T. chilonis* for 24 h in Diet-2 and Diet-1, respectively, while after 16 h exposure, 90 and 88.6 % was recorded for Diet-2 and Diet-1, respectively. Diet-2 was found to be better compared with Diet-1 for rearing of *S. litura.* Comparatively higher parasitism was observed in 16 and 24 h of exposure time of egg cards of *S. litura* to *T. chilonis*.

Key words: Tobacco cutworm, *Spodoptera litura,* artificial diets, parasitism, *Trichogramma chilonis*

INTRODUCTION

Spodoptera litura (Fabricius) (Lepidoptera: Noctuidae) is a poly-phytophagous insect damaging several vegetables and field crops in many Asian countries including Pakistan (Nadeem *et al.*, 2008; Shu, 1959; Shivayogeshwar, 1991). *S. Litura* is also called as the tobacco cutworm, common cutworm, the cluster caterpillar or tobacco caterpillar. Though it had been a random pest of tobacco for many years, it has been becoming progressively a very important insect pest in the current years (Gao *et al.*, 2004; Qin *et al.*, 2004; Guan and Chen, 1999). *S. litura* is a member of economically important insect pests that infest more than 120 host crop plants and causes serious crop losses (Singh and Jalali, 1997). Due to excessive use of pesticides, it acquired resistance to many commonly used pesticides, particularly carbamates and pyrethroids, resulting in the collapse of effective controls (Kranthi *et al.*, 2002; Wu *et al.*, 1995; Ahmad *et al.*, 2007).

S. litura is a leaf feeding insect of Pakistan (Khan *et al.*, 2011). Host plant survey for two years from 3 different locations at Pakistan, cotton belt exposed 27 plant species belonging to 25 genera of 14 families including vegetables, cultivated crops, weeds, fruits and ornamental plants as host plants for *S. litura* (Ahmad *et al.*, 2013). In Pakistan major host plants on which *S. litura* thrived for maximum period are *Ricinus communis* L., *Gossypium hirsutum* L., *Colocasia esculenta* L., *Brassica oleracae* L., *Sesbania sesban* L. and *Trianthema portulacastrum.* Eggs of *S. litura* were also found on many tree plants. Dependence on major cultivated crops species, *S. litura* needs regular monitoring especially during March to April due to abundance of *S. litura* and early warning for its proper management and control on commercial crops like cotton (Saleem *et al*., 2008).

It is important to rear economically important insect pests in order to study their feeding habits, life history, susceptibility and their resistance to chemical pesticides and bio-control agents *i.e.,* insect viruses (Rezapanah *et al.,* 2008). It is an

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expensive and difficult process to rear insects on artificial diets for the developing countries (Ahmad *et al.*, 1983).

So far, different artificial diets have been developed and proposed for the maintenance and consistent rearing of economically important insect pests mostly lepidopterous pests (Cohen *et al*., 2001; Ahmed *et al*., 1998; Castane and Zapata, 2005). Though there is some success in efforts to rear succeeding generations of these insects entirely on an artificial diet, in many cases there is loss of both fitness and reproductive potential, which causes longer developmental period and lower fecundity rate (Coudron *et al*., 2002). As a result, the cost-saving ratio is diminished. For those who work on life and fecundity tables of insects, it is important to know on what type of diet the insect has been reared. This factor affects the accuracy of biological characteristics of the insect, *e.g*. growth, viability, reproduction and population density, and consequently the accuracy of the life table (Bellows *et al.,* 1992). Moreover, the shifting emphasis in insect control, using biological entities such as natural enemies predators, parasitoids and insect pathogens (bacteria, viruses and fungi), has increased the demand for constant reliable sources of such insects.

A number of studies have been conducted on the biological parameters of *S. litura* on different artificial diets in the Asian countries under different environmental conditions (Guan and Chen 1999; Ahmad *et al.,* 2007; Zhu *et al.*, 2000; Qin *et al., 2004;* Zhu *et al.,* 2005). However, none of them had reared *S. litura* on the bean based and gram based diets.

Trichogramma spp. are small tiny wasps and egg parasitoids of Lepidoptera including *S. litura*. Nearly 18 different species of *Trichogramma* are being mass reared to control the pests of tobacco, sugarcane, sugar beet, rice cotton, vegetables and pine in almost 16 countries (Knustan, 2000). Studies with *T. chilonis* in more than 50 countries have been carried out and commercial releases are performed successfully in nearly 32 million hectares every year. So *Trichogramma* are one of the most important groups of Bio-control agents for the containment of many lepidopterous pests (Smith, 1996; Saljoqi and Yu-rong, 2004). Parasitism behavior is a significant indicator of performance of parasitoid and its successful reproduction. Therefore, it is required in controlling the pest population to demonstrate the capacity of these natural enemies, so as to use them in biological control programs against the pest. Such an attempt has made in the present study. The specie *Trichogramma chilonis* (Ishii) was used in this research work to study its parasitization potential against *S. litura*.

Keeping in view all the above mentioned facts and figures, the present research work was conducted to study the mass rearing of *S. litura* fed on two different artificial diets (bean based and gram based diets) and to determine the best artificial diets on the basis of its different pre-mature and adult stage biological parameters. Also to study the efficacy of *T. chilonis* as a bio-control agent against *S. litura*, fed on bean based and gram based diets.

MATERIALS AND METHODS

Complete Randomized Design (CRD) was used in all the experimental work. Also there were three replications in all the experiments.

General materials and methods

The main requirements for these experiments were the living material of *S. litura*, the two prepared diets *i.e.*, bean based diet (Diet-1) and gram based diet (Diet-2) and the living material of *T. chilonis* and the eggs of its host *S. litura*. In the primary phase the availability of the parasitoids and its host culture were ensured by rearing them in the labs. This rearing was separated in two phases. In the primary phase rearing of *S. litura* was conducted. In the secondary phase culture of *T. chilonis* was maintained separately on the eggs of *S. litura* fed on these Diets. All experimental work was conducted under controlled laboratory conditions at 27±2°C, 65±5% RH and 14:10 L:D.

Preparation of diets

Two artificial diets for mass rearing of *S. litura* from the 1st instar to adult stage were developed. The diet ingredients consisted of kidney bean flour (200g), yeast powder (30g), ascorbic acid (3.5g), methyl-p-hydroxybenzoate (2g), sorbic acid

(1g), formaldehyde solution (2.5ml), agar (14g) and 500ml distilled water. For the 2nd diet the bean flour was replaced by chickpea powder while rest of the ingredients was kept the same just like in the 1st diet. The dry ingredients of the diet was weighed carefully, and kept in separate containers. The wet ingredients were measured, and kept in separate containers. The entire quantity of agar was suspended and brought to a boil. The total quantity of bean powder/gram flour was added to the boiled agar. Then, all the dry and wet ingredients were added to the mixture. The prepared diets were then poured into the desired number of sterilized plastic boxes, and allowed to cool and harden (Shorey and Hale, 1965)

Rearing procedure of S. litura

A colony of *S. litura* was raised from two pairs of adults collected from field at NARC Research and Education Center, and was transferred to the laboratory. Each pair was kept in a separate bucket with a nappy liners pasted at the walls of buckets for egg laying. The mouth of buckets was kept closed with a muslin cloth. The adults were fed on a piece of cotton soaked in sucrose solution. The eggs laid were collected and kept in Petri dishes for hatching. As the larvae emerged, they were reared on the two diets *i.e.,* bean based diet (Diet-1) and gram based diet (Diet-2) for testing the effectiveness of the diets (Ahmed *et al*., 2007).

Culture maintenance of S. litura

To maintain the culture the larvae of *S. litura* were regularly fed on the prepared diets and the adult stages were fed with sucrose solution for the timely availability for studying its different biological parameters and then later on for the supply of its eggs for the parasitoid *T. chilonis* rearing and its utilization as bio-control agent *S. litura* (Ahmed *et al*., 2007).

Culture maintenance of T. chilonis

T. chilonis culture was maintained separately on the eggs of *S. litura* fed on the bean based diet and gram based diet. Rearing procedure was the same as used by Saljoqi and He-Yurong (2004) and Saljoqi *et al*. (2012).

Effect of bean and gram based diets on S. litura

Developmental duration of immature stages and survival rate

The experiments were conducted to study the biological parameters of immature stages of *S. litura* fed on the bean based diet and gram based diet were conducted up to 3 generations.

Fresh eggs were collected from the culture of *S. litura* and kept in Petri dishes for hatching. Total replications were 3 and each replication contained 4 batches of eggs of *S. litura*. All immature stages data was recorded in days. When the eggs hatched, the diets quantity present in plastic boxes was infested with the newly emerged larvae. Three replications for each diet were taken and each replication contained 50 counted number of larvae. The plastic boxes were kept closed. Each day the diets were provided on need basis and as the size of the larvae changed, these were shifted to large containers. This procedure was followed up to three generation at the same laboratory conditions for each diet. The data was recorded on the biological parameters of immature stages of *S. litura* included Incubation period, Instars durations (1st, 2nd, 3rd, 4th, 5th and 6th instars), Pre-pupal period, pupal period, number of pupae survived and total duration from egg to adult emergence.

Longevity and reproductive capacity of adults

The studies were conducted on the biological parameters of the adult of *S. litura* fed its larval stage on the bean based and gram based diets under controlled laboratory conditions. Freshly emerged, one day old adults of *S. litura* were collected from the stock culture. 6 male and female were paired and kept in separate buckets. The sides of the buckets were pasted with nappy liners as an oviposition medium. Petri dishes were kept at the bottom of the buckets in which cotton soaked in sucrose solution (10%). The diets were provided daily on need basis. The experiment was replicated 3 times. The insects were observed daily for pre-oviposition, oviposition and post oviposition period in each replicate. During oviposition period, the eggs were collected from nappy liners pasted on the sides of the buckets daily up to 6 days. The eggs were counted and kept in Petri dishes for hatching under growth chamber. Upon hatching the larvae were fed the same diets

for further generations and calculation of biological parameters (Asim *et al*., 2013). The data was recorded on the pre-oviposition period, oviposition period, post-ovipostion period, fecundity and adult longevity.

Efficacy of T. chilonis *as a bio-control agent*

Fresh batches of eggs of *S. litura* fed on bean based and gram based diets *were* collected and 100 eggs were left on each batch, the remaining was removed with needle. These batches were then pasted with fine gum film on the hard paper strips for the preparation of egg cards. These egg cards were exposed for 1 h to UV-sterilization treatment. The experimental design was CRD having 7 treatments with four replications. All these cards were exposed to adult parasitoids of *T. chilonis* for 1, 4, 8, 12, 16, 20 and 24 h.

Data was recorded on the percent parasitization. The adults of *T. chilonis* were fed with honey solution (10%). The developing parasitoids had reached the pupal stage in 5-6 days after oviposition date and the parasitized eggs, which had blackened were easily recognized (Saljoqi and He-Yurong, 2004). The percent parasitization was calculated by using the following formula.

$$\text{Percent parasitization} = \frac{\text{Total No of black eggs} \times 100}{\text{Total number of eggs}}$$

RESULTS AND DISCUSSION

The data was recorded on the biological parameters, including both immature and mature adult stage of *S. litura* fed on the said diets, up to 3 generations. Almost same trend of the collected data was observed in 1st, 2nd and 3rd generation of *S. litura.* Therefore, only the average mean data regarding the overall 3 generations have been presented.

Effect of diet-1 and 2 on S. litura

Duration of immature stages and survival rate

A significantly low mean total larval duration (17.2 days) and total immature duration (31.9 days) (from egg to adult stage) was recorded in the immature stages of *S. litura* fed on Diet-2 compared with 15.8 days and 29.6 days total larval duration and total immature duration of *s. litura* fed on Deit-1, respectively (Table I). Also significantly higher percent survival rate of *S. litura* was recorded among all the parameters of immature stages of *S. litura* fed on Diet-2 as compared with the Diet-1. All other parameters including incubation period, 1st to 6th instars periods, pre-pupal and pupal durations were found non significantly different from one another regarding the impact of diets.

The results of the present work were in conformity with Ahmad *et al.* (1998) who worked on rearing of *S. litura* on gram based diet. They recorded total larval duration 17.8 days, pre-pupal duration 2.8 days, pupal duration 8.5 days and total immature duration 31.2 days, while Bhattacharya *et al.* (2005) recorded 17.2 days total larval duration, pre-pupal duration 2.7 days, pupal duration 8.3 days and 30.8 days total immature duration. Assemi *et al.* (2012) and Gupta *et al.* (2005) worked on rearing of *Helicoverpa armigera* (Hubner) on bean based diet. They recorded 18.3, 3.2, 7.8 and 30.7 days total larval duration, pre-pupal period, pupal period and total immature duration, respectively of *S. frugiperda.*

Duration of adult stages

Data regarding the developmental duration of mature stage of *S. litura* showed that Diet-2 was found to be the best by recording longer oviposition period (5.7 days), longer adult longevity (9.1 days) and higher fecundity (1612.3 total number of eggs) as compared with *S. litura* fed *on* diet-1 where 5.2 days, 8.7 days and 1541.86 total number of eggs was recorded (Table II). No significant effect of diets was observed in case of pre-oviposition and post-oviposition period of *S. litura*. The findings of the present work are having the same trend reported by Chenchiah and Battacharya *et al.* (2009). They worked on the suitability of artificial diets for rearing of *S. litura* on the gram based diet. They recorded 5.6 days oviposition period, 8.4 days adult longevity and 1551.2 numbers of eggs (total fecundity). Battacharya *et al.* (2005) worked on rearing of *S. litura* fed on bean based diet and reported the same trend as recorded in the present study *i.e.*, 5.3 days, 9 days and 1434.6 eggs as ovipoistion period, adult longevity and total fecundity, respectively.

Table I.- Mean developmental durations ± S.E of *Spodoptera litura* (Fabricus) fed on bean based diet (Diet-1) and gram based diet (Diet-2) (Means of three generations data).

Developmental stages	Diet 1		Diet 2		LSD (α 0.05)	F value
	Developmental durations (days)	% survival	Developmental durations (days)	% survival		
Incubation period	3.6±0.02a		3.5±0.03a		0.12	0.53*
1st instar	1.6±0.02a	82.2b	1.4±0.03b	90.4a	1.0	12.5*
2nd instar	3.2±0.02a	74.6b	2.9±0.03a	87a	0.72	0.41*
3rd instar	2.8±0.02a	75.6b	2.5±0.03a	91.2a	1.0	2.53*
4th instar	3.4±0.02a	91b	3.2±0.03a	98.4a	0.12	4.76*
5th instar	3.2±0.02a	96.4b	2.9±0.03a	97.1a	0.96	0.18*
6th instar	3.2±0.02a	96.1b	3.0±0.03a	97.4a	0.96	0.19*
Total larval duration	17.2±0.29a	38.8b	15.8±0.29b	67.3a	0.85	0.74*
Pre-pupal period	3.0±0.12a	97.3a	2.6±0.14a	94.8b	0.61	1.95*
Pupal period	8.2±0.13a	90.8b	7.7±0.12a	93.3a	0.91	7.31*
Total duration from egg to adult stage	31.9±0.04a	36.6b	29.6±0.04b	64.6a	0.77	1.47*

Mean(s) followed by the same letter (s) (row wise) are non significantly different*= significant p<0.05 (using Bartlett's Test)

Table II.- Mean developmental durations (days) of mature stages ± S.E of *S. litura* fed its larval stages with bean based (Diet-1) and gram based diet (Diet-2) (Means of three generations data).

Mature stages	Bean based (Diet-1)	Gram based (Diet-2)	LSD (α 0.05)	F value
Pre-oviposition period	2.16±0.06a	1.9±0.07a	0.14	2.57*
Oviposition period	5.2±0.02b	5.7±0.03a	0.37	16*
Post oviposition period	1.3±0.05a	1.4±0.06a	0.82	0.54*
Female fecundity (eggs)	1541.86±0.3b	1612.3±0.9a	0.88	0.56*
Adult longevity	8.7±0.2b	9.1±0.10a	0.69	11.3*

Means followed by the same letter (s) (row wise) are non significantly Different*= significant at p<0.05 (using Bartlett's Test)

Table III.- Percent parasitization of eggs of *Spodoptera litura* (Fabricus), fed on bean based (Diet-1) and gram based (Diet-2) by *Trichogramma chilonis* (Ishii) at different exposure time (Hours) of egg cards ± S.E.

Exposure time (Hours)	Percent parasitization	
	Bean based diet (Diet-1)	Gram based diet (Diet-2)
1	59.8±0.07f	58.3±0.06f
2	67.8±0.40e	69.5±0.50e
4	71.0±0.40d	72±0.50d
8	77.6±0.05c	76.10±0.06c
12	79.70±0.07b	81.1±0.07b
16	88.6±0.04a	90±0.05a
24	89.5±0.50a	91.1±0.40a
Mean values	**76.2±0.01a**	**76.8±0.01a**

Means followed by same numbers (s) column wise are non significantly different*= significant at p<0.05 (using Bartlett's Test)

Percent parasitization of S. litura *(Table-III)*

Over all no significant effect of diets was observed on the mean percent parasitization of eggs cards of *S. litura* fed on Diet-1 and Diet-2 by *T. chilonis*. Mean percent parasitization of 76.2±0.01 was recorded for Diet-1 and 76.8±0.01 was recorded for Diet-2. However a significant effect of the exposure time was observed in both diets. According to the results the total percent parasitization of eggs of egg cards from the withdraw cards at 1, 2, 4, 8, 12, 16 and 24 h for Diet-1 was 59.8, 67.8, 71.0, 77.6, 79.7, 88.6 and 89.5, respectively, while that for Diet-2 % parasitization from the egg cards was 58.3, 69.5, 72, 76.1, 81.1, 90 and 91.1, respectively. These results are in a compliance with Puneeth and Vijayan (2013). There results have shown almost same trend by recording 88.2% parasitization for the 24 hexposure time of egg cards. Sultan *et al.* (2013)

reported 91.4% parsitization by exposing the egg cards of *S. cereallela* for 24 h to *T, chilonis*, which are almost similar with the present findings.

The present findings revealed that mass rearing of *S. litura* on artificial diets can be easily conducted in the controlled laboratory conditions. Further it also showed that Gram based diet (Diet-2) was more suitable for rearing of *S. litura* as compared to the bean based diet (Diet-1). One of the possible reasons for the maximum survival of *S. litura* is may be because that it is a major pest of gram as compared to kidney bean crop and another possible reason is that kidney bean plant has the ability to do trypsin inhibition (an enzyme usually present in the digestive tract of *S. litura*) in the *S. litura* and disturb its damage causing potential. It is also found that at 16 and 24 h of exposure of the *S. litura* egg cards, the recorded rate of parasitization of *T. chilonis* was maximum which revealed that with the increase in the exposure time, increase in the percent parasitization rate was recorded, while the percent parasitization rate was totally independent of the diets provided to the host, *S. litura.*

CONCLUSIONS AND RECOMMENDATIONS

Gram based diet (Diet-2) was found to be the best as compared with the Bean based diet (Diet-1) for rearing of *S. litura*. Maximum % survival was recorded among all the parameters of immature stages of *S. litura* fed on Diet-2 as compared with the Diet-1. The total immature duration and total larval duration was found lower on Diet-2 than Diet-1. Highest Longevity and fecundity rate was recorded on Diet-2 as compared to Diet-1.

The effect of time was observed on the total % parasitization of eggs of *S. litura* by *T. chilonis*. Maximum parasitization was recorded on the egg cards withdrawn after 16 and 24 h of exposure as compared with all other time of exposures. So 16 and 24 h were recommended enough in the present study for the comparatively higher percent parasitization of *T. chilonis* on *S. litura* eggs. Host fed with different diets *i.e.,* Diet-1 and Diet-2 had no significant effect on the parsitization of *T. chilonis.*

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