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Mass Rearing of *Spodoptera litura* using a Semi-Synthetic Diet Based on Wheat Germ and Tomato Paste

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ABSTRACT

Spodoptera litura (Hübner) (Lepidoptera: Noctuidae), is the threatening pests of many plants including tomatoes. *In vitro*, mass-rearing of *S. litura* is essential to attain a large number of insects with good quality for bio-ecological studies and biological control programs. In the current study, a modified diet was formulated and assessed against two diets used for mass rearing of *S. litura*, and tomato fruits were taken as a natural diet. Results revealed that development attributes based on mass rearing of *S. litura* on the modified diet (wheat germ + tomato paste) significantly reduced the larval and pupal developmental duration by 30%, while, considerably enhancing eclosion rate, pupation survival rate, and the number of eggs by 20-30% along with the 40-50% reduction in the pupal and larvae mortality rate as compared to the tomato paste diet and chickpea diet. The rearing expenses for 200 larvae on the modified diet (674/ Rs) were 2.5 folds less than the expenses for rearing the same number of larvae on chickpea based diet (1749/Rs.) and almost equal to the tomato paste-based diet (669/Rs.). Therefore, a modified diet based on wheat germ and tomato paste is suitable for mass rearing of *S. litura* for six generations for laboratory and field experimentation.

INTRODUCTION

Fall armyworm also known as the common cutworm, *Spodoptera litura* (Hübner), is a highly mobile, polyphagous, sporadic, and destructive Lepidopterans pest of around 389 plant species worldwide such as cotton, rice, corn, soybean, groundnut, vegetables, etc. (Khan *et al*., 2020). Larvae are primarily leaf feeders and can cause complete defoliation (Yinghua *et al*., 2017), and may cause loss to the crops (Dhir *et al*., 1992). In the last few years, it has become an emerging pest of cotton and vegetable crops in Pakistan (Naeem-Ullah *et al*., 2019). *S. litera* has been categorized as one of the major pests of Solanaceae members including potato and tomato (Bano and Muqarab, 2017; Ahmed *et al*., 2021). Various synthetic insecticides are employed to curb this dangerous pest in Pakistan, however, insect resistance to insecticides is a major concern in crop productivity and is likely to increase with climate change (Bano and Muqarab, 2017).

However, the study of management techniques in the laboratory required mass rearing of healthy insects in substantial numbers (Sørensen *et al*., 2012; Nair *et al*., 2019). The species and quality of the diet are considered the most important factors affecting the fecundity and mass rearing of the insects (Di *et al*., 2021). Different diets consisting of proteins, lipids, phosphorus, and mineral have been used for the development of Lepidoptera species including *S. litura* (Wakil *et al*., 2011; Campos *et al*., 2017; Silva *et al*., 2019). An increasing number of studies are using the artificial diet consisting of canned tomato paste, wheat germ, chickpea powder, soybean, and maize seed along with an artificially prepared vitamin mixture for rearing insects (Wu and Gong, 1997; Wakil *et al*., 2011; Campos *et al*., 2017). Gupta *et al*. (2005) result indicated that a new modified diet (wheat germ, bean flour, chickpea flour, and vitamins) successfully

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sustained the growth and development for more than ten generations of *S. litura*, however, some of the ingredients like chickpea flour and vitamins mixture are considered as economic for mass rearing purposes. In another study, a modified diet containing chickpea, red kidney bean flour, and tomato paste has been found nutritive and suitable for rearing *Helicoverpa armigera* for five generations, while, the study also recommended further improving the recipe of the artificial diets as a larva required longer time to attain the physiological state for molting (Wakil *et al*., 2011). Others have documented, that low-protein diets containing white bean, wheat germ, and soy bean as ideal for higher reproductive rate, weight, pupation rate, and percentage of adult emergence in *H. armigera* (Truzi *et al*., 2019). Likewise, Di *et al*. (2021) reported shorter developmental times and significantly higher fecundity in *S. litura* reared on the artificial diet based on beans, wheat germ, and vitamins. They also emphasized that an artificial diet may not be ideal for mating signals in *S. litura.* So far, longer development times and lower fecundity along with loss of vigor, fitness, and reproductive potential have also been reported due to the rearing of important insects on an artificial diet (Coudron *et al*., 2002). Still, there is a need to formulate an effective, user-friendly artificial diet using naturally available ingredients for the healthier rearing of lepidopterous insects (Nair *et al*., 2019; Di *et al*., 2021).

Therefore, in the present investigation, a modified diet was prepared by changing a few ingredients in the recommended diet described previously (Ahmad *et al*., 2003; Wu and Gong, 1997; Wakil *et al*., 2011) to develop an economically feasible, simple and nutritious diet for healthier mass rearing of *S. litura*.

MATERIALS AND METHODS

Collection of S. litura

The field population of *S. litura* was collected from leaves and infested fruits of tomatoes from the main tomato farming areas of the province of Punjab, Pakistan. Neonates were reared separately on a chickpea diet, tomato paste diet, and a modified diet. Chickpea diet (Ahmad *et al*., 2003) and tomato paste diet (Wu and Gong, 1997; Wakil *et al*., 2011) were prepared as per published recipes, while the third diet (modified diet) was different in some of the basic ingredients described in tomato paste diet as modified diet contained wheat germ instead of chickpea powder and half amount of tomato paste (Table I).

Rearing procedure

Larvae were reared in 32 wells tray (51 cm long × 26 cm wide × 4 cm high), loaded with 1 mL of each diet separately. The tray was tightly covered with fine muslin cloth kept at 70 ± 5% relative humidity, 25 ± 2°C temperature synchronized with a photoperiod of 14 (L):10 (D) hours. Freshly hatched larvae were shifted on diet from tissue paper strips by using a camel hair brush to avoid abrasive damage. Larvae were brushed gently on a larval diet from tissue paper strips with a camel hair brush to avoid any problems due to fecal matter. Larvae were frequently supervised during the rearing process to avoid the incidence of any disease. After one week, freshly emerged larvae were transferred to individual vials. Pupae appeared after the accomplishment of larval time and were sexed for identification of male or female moths, weighed, and kept individually in plastic vials until adult emergence. The sex of the moth was easily identified by the color of the forewings.

Table I. Ingredients and quantity of different meridic diets used for rearing of *Spodoptera litura.*

Ingredients	Chickpea diet	Tomato paste diet	Modified diet
Agar	13 g	13 g	14.5 g
Chickpea powder	230 g	115 g	0.0 g
Wheat germ	0.0 g	0.0 g	125 g
Tomato paste	0.0 g	115 g	60 g
Ascorbic acid	3.5 mL	3.5 mL	3.5 mL
Sorbic acid	1.2 mL	1.2 mL	1.2 mL
Yeast	37 g	37 g	47 g
Methyl-4 hydroxybenzoate	2.5 g	2.5 g	2.5 g
Vitamin mixture	8.0 g	0.0 g	0.0 g
Distilled water	1000 mL	11000 mL	1000 mL
Linoleic acid	0.0 mL	0.0 mL	1.5 mL
Streptomycin	1.2 g	1.2 g	0.0 g
Corn oil	9.5 mL	0.0 mL	0.0 mL
Sunflower oil	0.0 mL	9.5 mL	0.0 mL

Emerged moths (25 male and 25 female) were placed in a transparent oviposition plastic jar covered with abrasive as nappy liner assisted for egg-laying. A 10% sugar solution placed on a cotton pad was provided as food. Hatching occurred in 1 to 2 weeks after oviposition and eggs that remained unhatched after 5 days were discarded. A brown ring is developed in fertile eggs on the second day and the whole egg turns black on the third day. Eggs were stored at 15 °C for 7-8 days. Next generation was bred through freshly emerged neonates. There were 5 replications of each treatment with 50 neonates per replication.

Table II. Growth and developmental parameters (Mean± SE) of multiple generations of *Spodoptera litura* reared on various diets.

Parameters	Diet	Generations					
		F_1	F_2	F_3	F_4	F_5	F_6
Larval duration (days)	Natural diet	18.39±0.21a	17.73±0.27a	16.89±0.26a	16.56±0.26a	16.33±0.26a	17.56±0.26a
	Tomato diet	16.50±0.09b	15.77±0.09b	15.00±0.04b	14.65±0.04b	14.62±0.04b	15.85±0.04b
	Chickpea diet	14.80±0.17c	14.12±0.17c	13.75±0.28c	13.46±0.24c	13.19±0.28c	14.42±0.28c
	Modified diet	12.98±0.09d	12.58±0.12d	11.94±0.10d	11.61±0.10d	11.38±0.10d	12.61±0.10d
	df	3	3	3	3	3	3
	F	215.1	168.0	110.6	119.76	105.56	106.01
Pupal duration (days)	Natural diet	14.83±0.25a	14.57±0.41a	14.51±0.38a	14.18±0.38a	13.95±0.38a	15.39±0.38a
	Tomato diet	13.65±0.14b	12.94±0.12b	12.43±0.17b	12.10±0.17b	11.88±0.17b	13.29±0.17b
	Chickpea diet	11.60±0.28c	10.91±0.28c	10.32±0.23c	9.98±0.23c	9.72±0.23c	11.17±0.23c
	Modified diet	10.74±0.26c	10.22±0.17c	9.65±0.39c	9.31±0.39c	9.08±0.39c	10.51±0.39c
	df	3	3	3	3	3	3
	F	58.5	52.5	50.8	50.6	50.4	50.8
Adult duration (days)	Natural diet	13.75±0.23a	13.20±0.13a	12.97±0.23a	12.72±0.23a	12.42±0.23a	13.93±0.23a
	Tomato diet	12.30±0.03b	11.86±0.03b	10.93±0.29b	10.70±0.29b	10.38±0.29b	11.92±0.29b
	Chickpea diet	9.88±0.13c	9.31±0.02c	8.93±0.15c	8.72±0.15c	8.39±0.15c	9.93±0.15c
	Modified diet	8.92±0.14d	8.30±0.02d	7.93±0.03d	7.71±0.03d	7.39±0.03d	8.93±0.03d
	df	3	3	3	3	3	3
	F	211.5	103	120.5	121.5	121	121.2
Pupal weight (g)	Natural diet	257±1.87c	249±5.34c	243±1.87c	240±1.87c	236±1.85c	242±1.88c
	Tomato diet	265±4.56c	261±3.21bc	259±4.27bc	257±1.42b	252±1.27bc	259±0.67b
	Chickpea diet	280±2.79b	268±5.87b	263±1.77b	260±0.68b	257±1.77b	262±1.77b
	Modified diet	321±2.51a	315±1.47a	312±7.06a	309±2.03a	305±7.06a	311±1.70a
	df	3	3	3	3	3	3
	F	84.5	45.5	46.5	34.8	61.1	35.5
Number of eggs	Natural diet	186±3.38c	181±2.06d	179±3.10c	175±3.12c	172±3.11c	178±3.10c
	Tomato diet	204±6.32bc	197±2.81c	193±5.99bc	189±5.97bc	185±5.99bc	192±5.99bc
	Chickpea diet	214±8.40ab	207±2.26b	203±5.77ab	199±5.75ab	196±5.78ab	203±5.77ab
	Modified diet	235±2.85a	225±1.22a	222±1.49a	218±1.48a	214±1.49a	221±1.49a
	df	3	3	3	3	3	3
	F	12.0	75.0	16	16.5	17.0	17.8

Mean followed by same letters are not significantly different at P>0.05

For all treatments, larval duration (days after hatching), pupal period (days between larva and adult stages), adult duration (days), pupal weight (mg), egg production (number), eclosion (% egg hatching), larval mortality (%), pupal mortality (%) and total population (%) were counted up to 6 generations.

Statistical analysis

After one-way analysis of variance (ANOVA), the data regarding different investigated attributes like larval duration, pupal period, adult duration, pupal weight, egg production, eclosion, larval mortality, pupal mortality and total population were analyzed through Statistics 8.1 for significance ($P < 0.05$) using the LSD test. The data compared were within generations of similar treatments.

RESULTS

The consequence of a modified diet (D_3) on growth and development for the mass rearing of *S. litura* was compared with the chickpea diet (D_1), tomato paste diet (D_2), and a natural diet (control: D_0) for up to six generations (Table II). Results revealed that development duration in larvae and pupae was significantly ($P \leq 0.05$) less in D_3 as

compared to the other D_0, D_1, and D_2. Pupation consisted of 15-16 days in D_0, 12-13 days with the D_3, followed by 10-11 days with the D_2, and 9-10 days with a D_3. The adult period (7-9 days) also decreased significantly ($P \leq 0.05$) in D_3 in F_1 to F_6 generation, as compared to D_0 (12-14 days) and D_2 and D_3 (10-12 days). Overall, in all six generations, D_3 significantly ($P \leq 0.05$) improved the growth and development period of *S. litura* followed by D_2 and D_1.

The pupal weight was also significantly ($P \leq 0.05$) different amongst different diets in six generations. Larvae fed on D_3 had significantly highest pupal weight (300-320 g) up to six generations as compared to D_0 (242-257 g), D_1 (262-280 g), and D_2 (252-259 g). Diet nature significantly ($P \leq 0.05$) altered egg production as the highest numbers of eggs (218-234) in F_1 till F_6 were recorded in D_3 when compared with 200-214 eggs in D_2 and D_1, while 172-186 eggs in T_0 (Table II).

A significantly ($P \leq 0.05$) greater eclosion rate (>90%) was noticed in insects fed on a D_3 as compared to 70-80%, 80-85%, and 80-90% in D_0, D_1, and D_2, respectively (Fig. 1). By the 3rd and 4th generations pupal and larval mortality rates were not significantly different between diets. However, statistically ($P \leq 0.05$) less mortality rate (larval and pupal) was recorded in insects fed on D_3 at the 5th and 6th generations, as compared to D_0, D_1, and D_2 (Figs. 2 and 3). Likewise, % pupation was significantly ($P \leq 0.05$) greater (87-94%) in D_3 as compared to D_o (63-75%) and the remaining two diets (70-85%) (Fig. 4).

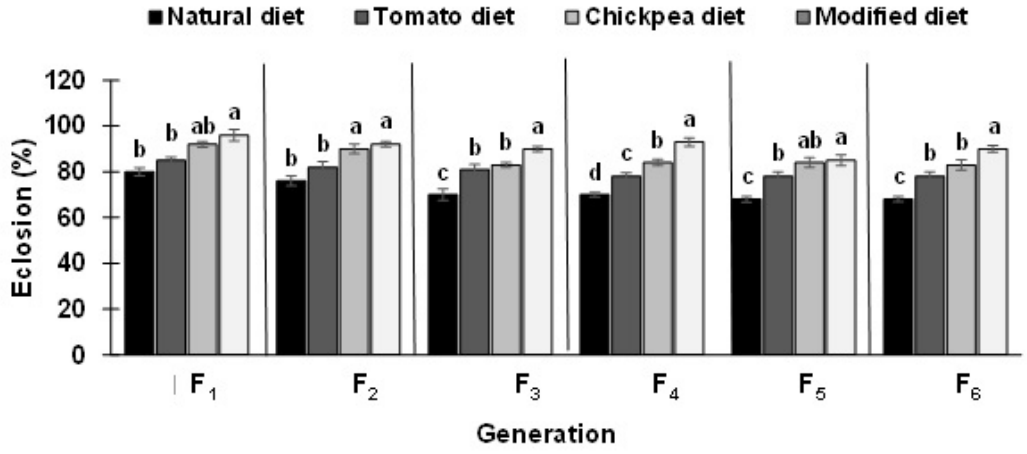

Fig. 1. Eclosion (percent egg hatching) of *Spodoptera litura* reared on different diets. Error bars indicate the mean of replicates. Values with different letters show a significant difference ($P \leq 0.05$) within generations of similar treatments as determined by the LSD test.

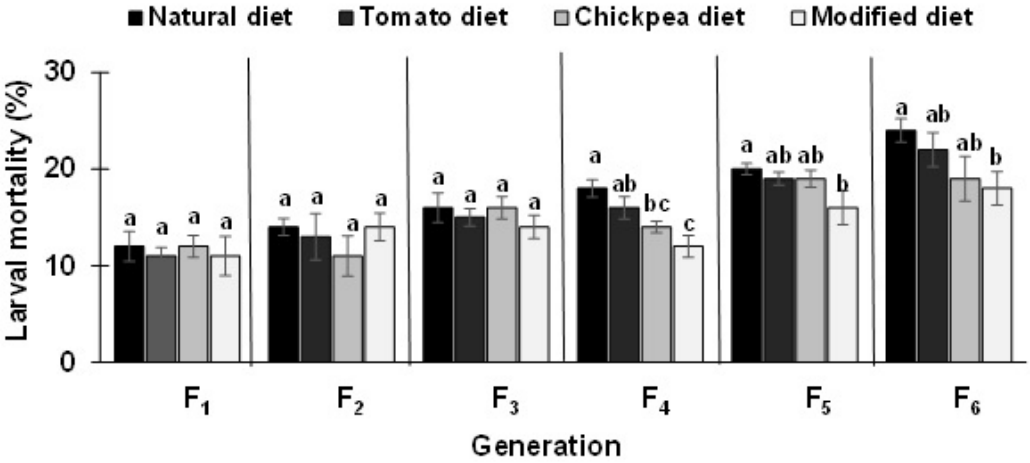

Fig. 2. Larval mortality of *Spodoptera litura* reared on different diets. Error bars indicate the mean of replicates. Values with different letters show a significant difference ($P \leq 0.05$) within generations of similar treatments as determined by the LSD test.

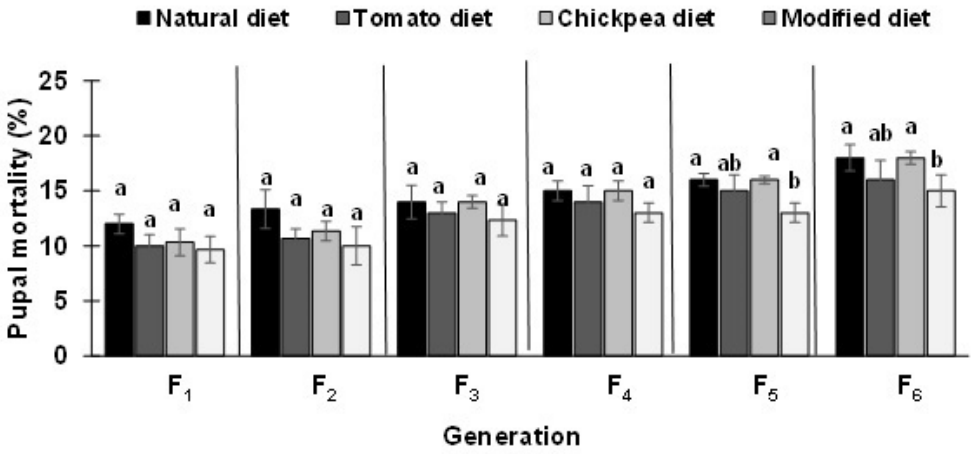

Fig. 3. Pupal mortality of *Spodoptera litura* reared on different diets. Error bars indicate the mean of replicates. Values with different letters show a significant difference ($P \leq 0.05$) within generations of similar treatments as determined by the LSD test.

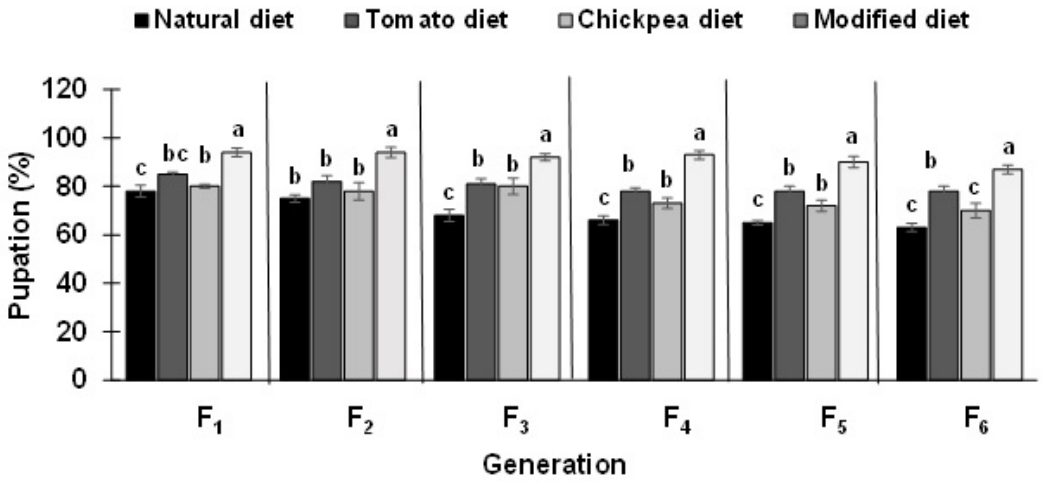

Fig. 4. Percentage pupation of *Spodoptera litura* reared on different diets. Error bars indicate the mean of replicates. Values with different letters show a significant difference ($P \leq 0.05$) within generations of similar treatments as determined by the LSD test.

DISCUSSION

This research work was conducted to assess the effect of a new diet on the larval duration (days), pupal period (days), adult duration (days), pupal weight (mg), egg production (number), eclosion (% egg hatching), larval mortality (%), pupal mortality (%) and total population (%) of *S. litura* for consecutive six generations to check its suitability for insect mass rearing (Nair *et al*., 2019). The modified diet (D_3) differed from the published diets in eliminating the use of ingredients such as chickpea powder and vitamin mixture. Instead, wheat germ and linoleic acid were added in addition to tomato paste, ascorbic acid, sorbic acid, yeast, and methyl-4 hydroxybenzoate. The results depicted that larvae reared on a modified diet had higher growth and egg production along with a shorter developmental period up to six generations than on a natural diet, tomato paste, and chickpea diet. Wheat germ is lighter in carbs, high in carbohydrates, and has more thiamin, riboflavin, niacin, pantothenic acid, and vitamin B_6 than the chickpea diet. Moreover, wheat germ is similar to chickpeas for protein. It seems that a D_3 may be the most suitable for growth and biomass development in *S. litura* due to the presence of a balanced amount of prerequisite food ingredients as compared to D_1 or D_2 (Di *et al*., 2021). Over and above, D_3 also promoted pupation, pupal weight, egg production, and eclosion rate, as compared to the D_0, D_1, or D_2 diet probably due to the consequences of the

nutritional quality of diet, which may alter the duration of larval phases during development of larvae (Pinto *et al*., 2019). The highest average pupation (F_1 to $F_{6=}$ 92%) obtained through a modified diet (D_3) was comparable to the previous study of Wakil *et al*. (2011), who obtained 95% of pupation of *H. armigera* reared on a chickpea modified diet. The results were also comparable with 79% pupation of *H. armigera* obtained by rearing on the tapaioca-based diet (granular tapioca, chickpea powder, ascorbic acid, sorbic acid, yeast, methyl-4-hydroxybenzoate, vitamin mixture, and formaldehyde).

Table III. Economic analysis of cost on each diet used for rearing of *S. litura*.

Diet ingredients	Cost of ingredients (Rs.)	Chickpea diet (Rs.)	Tomato paste diet (Rs.)	Modified diet (Rs.)
Agar	11000/kg	143	143	160
Chickpea powder	80/kg	18.40	9.20	0.00
Wheat germ	100/kg	0.00	0.00	12.50
Tomato paste	100/kg	0.00	11.50	6.00
Ascorbic acid	5500/kg	19.25	19.25	19.25
Sorbic acid	3500/kg	4.20	4.20	4.20
Yeast	9400/kg	348	348	442
Methyl-4 hydroxybenzoate	9600/kg	24.00	24.00	24.00
Vitamin mixture	3000/25ml	960.00	0.00	0.00
Linoleic acid	500/kg	0.00	0.00	0.75
Streptomycin	5000/25g	230	230	0.00
Corn oil	150/kg	1.43	0.00	0.00
Sunflower oil	150/kg	0.00	1.43	0.00
Total price per kg of diet	-	Rs.1749	Rs. 791	Rs. 668

Economic analysis indicated (Pakistani Rs.) that about 200/kg larvae can be reared successfully in D_3 (Rs. 668), which was three times less than the chickpea diet, while Rs.100 less than the tomato paste diet (Table III). The estimated total cost for the production of one pupa was Rs. 3.34 in D_3, Rs. 3.95, in D_2, and Rs. 58.74 in D_1, respectively. The increased cost of rearing using a chickpea diet was due to the addition of some of the expensive ingredients like vitamin mixture and streptomycin, which are not needed in the new diet. Tomato paste contains frequent amounts of vitamins, which can serve their part in the vigorous growth of larvae. The new diet exhibited significant prospective to be used as an adequate and nutrient-rich diet for the rearing of numerous other economically important lepidopterous insects in the laboratory. Further perfections for the formulation are feasible, and this would likely augment the cost-effective use of the diet for mass rearing of *S. litura*.

CONCLUSION

The modified diet with few modifications yielded a greater pupation rate, pupal weight, egg production, and eclosion rate, as compared to the natural diet, chickpea diet, and tomato paste diet. The modified di*et al*so reduced the duration for larval, pupal and, adult, along with a low rate of larval and pupal mortality. Therefore, it was concluded new modified diet mediated with fewer and cheap ingredients (wheat germ along with tomato paste) is a simple diet that can be successfully used for mass rearing of *S. litura*.

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Statement of conflicts of interest

The authors have declared no conflict of interest.

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