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Effect of different temperature regime on biology and food utilization of tobacco leaf eating caterpillar (*Spodoptera litura* F.) on sunflower (*Helianthus annuus* L.) under laboratory conditions

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Abstract
The experiment was conducted at laboratory of Plant Health Clinic, Directorate of Research, Bidhan Chandra Krishi Vishwavidyala (BCKV). The larvae of *Spodoptera litura* were reared at 20, 25 and 30^{0}C with RH 75±5% on sunflower. Basic growth parameters like larval period, length, width, consumption index (C.I.), average digestibility (A.D.), efficiency of conversion of ingested food (E.C.I.) and efficiency of conversion of digested food (E.C.D.) were computed to understand the growth behaviour at the mentioned temperature regimes. At 20^{0}C it took longer time to develop than 25 and 30^{0}C, whereas, maximum length and width were recorded at 30^{0}C in last instar. The larval period was 40.20±2.59 days at 20°C, 23.60±1.52 days at 25°C and 15±1.22 days at 30°C. The pre-pupal and pupal periods were 2.80±0.45 and 6.80±0.45 days at 20°C; 2.20±0.45 and 5.60±0.55 days at 25°C; 1.80±0.45 and 4.80±0.84 days at 30°C. The total developmental period from egg to adult emergence were 57.80±2.78 days at 20°C, 38.60±1.81 days at 25°C and 28.00±2.26 days at 30°C respectively. Food consumption increased with rise in temperature, first instar larvae showed high consumption index, which gradually decreased with larval age. Average digestibility for entire larval life decreased with larval age, but at all temperature regimes fifth and sixth instars digested more leaves than preceding instar. The efficiency of conversion of ingested food and efficiency of conversion of digested food values increased not only with advanced larval instar, but also with rise in temperature.

Keywords: temperature, eating caterpillar, Sunflower, laboratory conditions

1. Introduction
Sunflower (*Helianthus annuus* L.) is one of the imperative edible oilseed crops cultivated in diverse regions of the world in an area of a propos 22.33 million hectares with the total production of about 27.748 million tonnes [5] India is the leading producer of oilseeds in the world and oilseed sector occupies an important position in the agricultural economy of the country. Average seed yield of sunflower (629 kg/ha) in the country is the lowest in the world due to a number of biotic and abiotic production constrains. Among biotic constrains in sunflower production, insect pests and diseases are the major concern. As many as 251 insect and acarine species have been recorded on sunflower at global level [12]. Insect pests of sunflower are different in tropical countries than temperate countries. In India more than 50 insect species have been found to damage the crop at different phenological growth stages of which polyphagous pests tobacco leaf eating caterpillar (*Spodoptera litura* Fabricius), are considered of major economic importance [1]. The larvae of which can defoliate many economically important crops. It is seasonally common in annual and perennial agricultural systems in tropical and temperate region of Asia. This noctuid is often found as part of a complex of lepidopteran and non-lepidopteran foliar feeders. The pest is reported to attack on field crops grown for food and fiber, plantation and forestry crops, as well as certain weed species belonging to 44 families [2]. For taking competent and sustainable management approach of any Lepidopteran pest, knowledge on the larval feeding behaviour and host characteristics are very important. Therefore, with a view to gain knowledge for proper management of *S. litura*, this study was designed to gauge the amount of food consumption, production of excreta and developmental duration of larvae on sunflower in three different temperature regimes.

Considering the exceeding background information in mind, the present investigation was conducted to study the Consumption index, average digestibility, efficacy of conversion of ingested food and Efficacy of conversion of digested food of *S. litura*.

Table 1: Developmental period of tobacco caterpillar *S. litura* (Fab.) feeding on sunflower leaves at 20, 25 and 30^{0}C

Sl. No	Life Stage		20^{0}C(X±SD) (days)	25^{0}C(X±SD) (days)	30^{0}C(X±SD) (days)
1	Egg		4.80±0.45	3.40±0.55	2.60±0.55
2	Larva	1st instar	8.00±0.71	4.20±0.45	4.40±0.89
		2nd instar	7.60±0.89	5.20±0.45	4.00±0.71
		3rd instar	9.00±0.71	6.20±0.84	2.60±0.55
		4th instar	7.20±0.84	3.80±0.45	2.20±0.45
		5th instar	7.00±0.71	4.00±0.71	1.80±0.45
3	Total larval period		40.20±2.59	23.60±1.52	15±1.22
4	Pre-pupal		2.80±0.45	2.20±0.45	1.80±0.45
5	Pupal		6.80±0.45	5.60±0.55	4.80±0.84
6	Adult		5.12±0.45	4.31±0.55	3.80±0.84
7	Total period		57.80±2.78	38.60±1.81	28.00±2.26

Table 2: Morphometric parameters of tobacco caterpillar, *S. litura* (Fab.) feeding on sunflower leaves at 20, 25 and 30^{0}C

Stages	Length in cm (X±SD)			Width in cm(X±SD)		
	20^{0}C(X±SD)	25^{0}C(X±SD)	30^{0}C(X±SD)	20^{0}C(X±SD)	25^{0}C(X±SD)	30^{0}C(X±SD)
Egg	0.90±0.06*	0.84±0.05*	0.81±0.08*	0.39±0.05*	0.36±0.04*	0.33±0.05*
1st instar	0.370±0.055	0.32±0.04	0.34±0.04	0.03±0.01	0.03±0.01	0.06±0.01
2nd instar	0.900±0.100	0.83±0.07	0.79±0.07	0.15±0.01	0.13±0.01	0.13±0.01
3rd instar	1.620±0.110	1.44±0.11	1.58±0.08	0.23±0.01	0.18±0.01	0.20±0.01
4th instar	2.220±0.130	2.19±0.22	2.68±0.15	0.29±0.01	0.27±0.01	0.31±0.01
5th instar	3.660±0.270	3.37±0.17	3.90±0.24	0.36±0.01	0.34±0.01	0.41±0.02
6th instar	3.800±0.158	-	-	0.43±0.03	-	-
Pupa	1.720±0.084	1.68±0.08	1.60±0.07	4.50±0.35	4.20±0.27	4.30±0.45
Adult	1.8000±0.70	1.74±0.55	1.71±0.89	4.80±0.286	4.70±0.27	4.70±0.55

*length and width of egg – in Millimeter

Table 3: Consumption and utilization of sunflower leaf by different larval instars of tobacco caterpillar, *S. litura* (Fab.) at 20 ^{0}C, 25 ^{0}C and 30^{0}C

Temp.	Stages	Duration (days)	Ingested (gram)	Excreta (gram)	C.I	A.D	E.C.I	E.C.D
20^{0}C	1 instar	8.00	0.027	0.001	11.990	95.896	1.043	1.087
	2 instar	7.60	0.084	0.004	6.407	94.884	2.054	2.164
	3 instar	9.00	0.161	0.056	1.485	65.217	7.482	11.472
	4 instar	7.20	0.220	0.082	0.779	62.687	17.822	28.430
	5 instar	7.00	1.728	0.237	0.994	86.277	14.377	16.664
	6 instar	2.33	0.543	0.088	0.857	83.811	50.034	59.699
25^{0}C	1 instar	4.20	0.025	0.001	20.993	94.841	1.134	1.196
	2 instar	5.20	0.062	0.004	7.364	94.243	2.612	2.771
	3 instar	6.20	0.104	0.038	1.244	63.256	12.961	20.490
	4 instar	3.80	0.133	0.065	0.839	51.073	31.363	61.409
	5 instar	4.00	2.157	0.369	2.947	82.887	8.482	10.233
30^{0}C	1 instar	3.80	0.017	0.001	15.815	94.591	1.664	1.759
	2 instar	4.00	0.066	0.004	12.024	93.228	2.079	2.230
	3 instar	2.60	0.084	0.033	1.529	60.902	25.162	41.316
	4 instar	2.20	0.172	0.087	1.305	49.104	34.836	70.943
	5 instar	1.80	0.882	0.179	2.486	79.646	22.345	28.055

C.I= consumption index, A.D= approximate digestibility, ECI= Efficacy of conversion of ingested food, ECD= Efficacy of conversion of digested food.

2. Materials and Methods

The present study was conducted at laboratory of Plant Health Clinic, Directorate of Research, Bidhan Chandra Krishi Vishwavidyala (BCKV) at three different temperature regimes *i.e*., 20, 25 and 30 ^{0}C in the culture room under maintained temperature and relative humidity. The culture of *S. litura* was maintained on sunflower leaves in the laboratory conditions and for this large number of matured larvae were handpicked from the sunflower plants at Center Research Farm of Gayeshpur, during morning hours. The larvae were brought to the laboratory and then reared in a glass jar. The mouth of the jar was covered with a fine muslin cloth tied with rubber band. The matured larvae were fed with fresh sunflower leaves without any infestation for pupation. After a few days, larvae in pre-pupal stage were transferred to another jar containing soil for pupation. On emergence the female (having tuft of hair on tip of abdomen) and male was conformed and a pair was transferred in a cage for mating. The cage was provided with 10% honey solution soaked cotton as food for adults. Female laid eggs not only on lower side of leaves of sunflower plant (raised in pot and kept in cage) but also on polythene bag, in masses covered with hairs. Ten newly hatched larvae were transferred with the help of a soft camel hair brush to the petridishes (5 cm x 1 cm) and were provided with sunflower leaves for feeding. The leaves were changed every day with fresh leaves and faecal matter was collected for dry weight. The larval development was observed daily. After completion of the larval development,

pre-pupal stage went for pupation within the soil and left to record pupal duration.

2.1 Morphological studies of *S. litura* fed on sunflower leaves

The length and breadth of larvae, pupae and adults (5 of each stage) were measured with the help of ocular-stage micrometer for first instar and with millimeter scale for second to last instar. The breadth of different instars was measured at their broadest part of the body region.

2.2 Voracity study of *S. litura* fed on sunflower leaves

In vitro culture, large number of freshly laid egg mass of *S. litura* was collected and brought for studying the efficiency of conversion of ingested leaf of sunflower by *S. litura*. The larvae after hatching were reared on fresh leaves of sunflower collected from the Gayeshpur farm of BCKV. The first instar stage was raised in a common culture within petridish. The larvae were segregated just after the first larval moult and ten larvae were separately reared in eight petridishes, that were provided with known weight of leaves of almost same size and equal quantity of leaves were taken to determine the dry matter content. The larvae were weighed when they freshly moulted and 10 larvae of first instar and 5 of each instar from second to the pre-pupal stage were dried and weighed to determine the dry weight of such larvae. The total quantity of food consumed by the larvae of each instar was determined by subtracting the weight of leftover food after feeding, from the weight of leaves supplied. The faeces of each instar were also carefully collected and were dried to find out the utilization of food as given by [15]. The drying of food and the faecal matters was done in a hot air oven at 100^0C till the weight became constant.

In the expression of results, we used the indices used by [15] and others. The coefficient of digestibility (C.D.) is calculated as:

$$C.D. = \frac{\text{Dry weight of food ingested - Dry weight of excreta}}{\text{Dry weight of food ingested}} \times 100$$

The efficiency of conversion of ingested food to body matter (E.C.I.) is a measure of the overall ability of an animal to grow on a given food. It is calculated as:

$$E.C.I. = \frac{\text{Dry weight gained by animal}}{\text{Dry weight of food ingested}} \times 100$$

The efficiency of conversion of digested food to body matter is calculated as:

$$E.C.D. = \frac{\text{Dry weight gained by animal}}{\text{Dry weight of food ingested – weight of excreta}} \times 100$$

Since larvae on various fresh leaves ate and grew at different rates over variable periods, it was necessary to express all measurements in a form which made possible the comparison of intake and efficiency of utilization. Three components to be considered were amount of food consumed (F), mean weight of insect during feeding period (A) and duration of feeding period in days (T) to determine the consumption index and was calculated as: C.I. = F/TA.

Where - F= Dry weight of food eaten.

T= Duration of feeding period (days).

A= Mean dry weight of larva during feeding period.

3. Result and Discussion

3.1 Developmental periods and morphological variations of tobacco caterpillar (*S. litura*) at different temperature regimes (20, 25 and 30 ^{0}C) on sunflower

The eggs were laid in mass covered with hairs on the lower surface of leaves, muslin cloth and polythene. Eggs were very small, round in shape and yellowish white in color. Freshly laid eggs measured from 0.81 mm. to 0.89 mm. in diameter and 0.33 mm. to 0.39 mm. in width (Table 2). Incubation period was 4.80±0.45 days at 20°C, 3.40±0.55 at 25°C and 2.60±0.55 days at 30°C (Table 73). On hatching, *S. litura* larvae passed through five larval instars (some larva in 20^0C passed through six instars) to become adult. The length of different larval instars increased from 0.315±0.038 to 3.900±0.235 cm and width from 0.025±0.004 to 0.410±0.022 cm starting first instar to fifth instar (Table 2). The larval period was 40.20±2.59 days at 20°C, 23.60±1.52 days at 25°C and 15±1.22 days at 30°C. Time requirement to complete different instars had been presented in table 1. Pupation took place within soil. The length and width of pupa varied from 16.00 to 17.20 mm and 4.70 to 4.80 mm respectively (Table 1). The pre-pupal and pupal periods were 2.80±0.45 and 6.80±0.45 days at 20°C; 2.20±0.45 and 5.60±0.55 days at 25°C; 1.80±0.45 and 4.80±0.84 days at 30°C respectively (Table 1). The total developmental period from egg to adult emergence was averaged to 57.80±2.78 days at 20°C, 38.60±1.81 days at 25°C and 28.00±2.26 days at 30°C (Table 1). The length of the adult varied from 1.71±0.894 to 1.80±0.707 mm and width from 4.70±0.274 to 4.80±0.286 mm (Table 2). The longevity of adult moths was 5.12±0.45, 4.31±0.55 and 3.80±0.84 days at 20, 25 and 30°C respectively (Table 1). Similarly, reported that the shortest duration of the life cycle (26.6 days) was at higher temperature, *i.e*., 34.9^0C and duration increased as the temperature decreases [13]. The present finding was more or less in conformity with that of [14], observed that the length of 1st, 2nd, 3rd, 4th and 5th instar larvae varied from 8.9±0.9 to 10.2±0.8, 13.5±1.0 to 16.3±1.5, 20.5±1.9 to 24.5±0.6, 28.8±1.5 to 32.3±2.8 and 47.5±1.9 to 50.5±1.9 mm, respectively. The breadth of 1st, 2nd, 3rd, 4th and 5th instar larvae were measured 1.3±0.2 to 1.5±0.2, 2.3±0.5 to 2.5±0.5, 3.3±0.5 to 4.0±0.8, 4.8±0.5 to 6.0±0.8 and 7.8±0.5 to 8.3±0.4 mm, respectively. Also, [6] reported larval duration was 23.6-30.4 days at 24^0C, 18.6-22.3 days at 28^0C and 14.5-18.0 days at 32^0C.

3.2 Consumption index (C.I.), average digestibility (A.D.), efficacy of conversion of ingested food (E.C.I.) and Efficacy of conversion of digested food (E.C.D.).

Data presented in table 3, indicated that larvae developing at lower temperature required longer time as compared to higher temperature. At 20^0C larva period was 40.20±2.59 days which reduced with increase in temperature, *i.e*., at 30^0C it took only 15±1.22 days to complete larval period (Table1). The result is in conformity with [13], who reported the shortest duration of the life cycle (26.6 days) at 34.9^0C. During the development, increase in mean dry weight of larvae at various instars also increased with the advancement of larval instar. Dry weight of first instar to last instar larvae increased from 0.0003 to 0.272g at 20^0C; 0.0003 to 0.182g at 25^0C and 0.0003 to 0.197g at 30^0C, respectively. Similar increase in mean dry weight was also recorded for *Spodoptera littoris* on soybean, jute and black gram [11] *Paropsis atomaria* on eucalyptus [3] and *Leptinotersa decemlineata* on potato [4]. However, at the end of last instar, *i.e*., during pre pupal stage, the larvae stopped feeding and their body shrinked, they also defecated. This result not only decreased in body weight but also decreased in length and width of larvae. Generally larvae moulted at five times at all temperature tested except at 20^0C in which three out of five larvae moulted to sixth instar and showed considerable amount of food eaten. *S. littoris* always consume higher amount of food with that increased in larval instar [11]. Similarly in this investigation also as larvae entered into higher phase of development a gradual increase in the amount of food eaten at all temperature was observed. The dry matter ingested by different larval instar at 20^0C, 25^0C and 30^0C varied from 0.017 to 0.027g for 1st instar, 0.062 to 0.084g for 2nd instar, 0.084 to 0.161g for 3rd instar, 0.133 to 0.220g for 4th instar and 0.882 to 2.157g for 5th instar, respectively. However, dry matter ingested by 6th larval instar at 20^0C was 0.543g.

Consumption Index (C.I.): CI explains in a nutshell about the rate at which nutrients enters into digestive system. It can be accepted that feeding is governed by massiveness of food, water content and other physiochemical properties of food material. Therefore, the rate of intake of food is largely a function responsible to feeding. The rate of consumption decreased from first to last instar stages at all the three different temperature regimes (Table 3). But this index gradually increased with the rise in temperature from 20^0C to 25^0C and after that it decreased from 25^0C to 30^0C. At all temperature C.I. was highest during first instar which gradually decreased with the advancement of larval stages. The initial C.I. for 20^0C, 25^0C and 30^0C were 11.99, 20.99 and 15.81. While it decreased to 0.85, 2.94 and 2.48, respectively at latter instars.

Average digestibility (A.D.): Comparison of different instars revealed that advanced instars (latter instars) always digested less food for its utilization. It is also clear that during first and second instars there was no marked increase or decrease in A.D. with rise in temperature. But in third, fourth and fifth instars A.D. showed noticeable decreasing trend with rise in temperature. Similar trend during life study of *L. decemlineata*. On the other hand A.D. in fifth larval instar at all temperature increased considerably than the preceding instar, *i.e*., than fourth instar [9]. Also at 20^0C sixth larval instar shows again decreasing trend with advance instar. A.D. value fluctuate from first to last instars was also observed for *Atographa precationis* on *Teraxacum officinale, Luctuca savita, Vigna sinensis, Medicago savita* [7] and *Pseudoplusia includes* on *Glycin max* [8]. But, a gradual decrease in A.D. in successive stages of *L. migratoria* and *S. gregaria* on cabbage. They attributed this change in A.D. to complex digestive physiology of insects [10].

Efficiency of conversion of ingested food (E.C.I.): ECI also varied considerably with rise in temperature. It increased with rise in temperature during first, third and fourth larval phases. Similarly, second larval instar also showed a sharp rise in E.C.I. at 25^0C but at 30^0C it decreased slightly (Table 3). However, the fifth larval instar indicated a decrease in E.C.I. from 20 to 25^0C and recorded lowest among fifth instar after which increased again at 30^0C. On the other hand sixth larval instar at 20^0C shows increasing trend with advance instar. It is really difficult to pin point the comparative E.C.I. value for temperature and instars. This parameter fluctuated during second and fifth larval instar but in other larval instars it showed decreasing trend. Similar larval behaviour was also recorded during early stages of larval development by [17] for *A. preationis* and [8] recorded for *P. includens.*

Efficiency of conversion of digested food (E.C.D.): ECD is affected by the amount of energy devoted for maintenance of physiological functions of the animal. A comparison of E.C.D. reveals that it increased in first, third and fourth instars with increase in temperature. But for second and fifth instars E.C.D. was higher at 25 and 30^0C, respectively. E.C.D. for the sixth larval instar at 20^0C recorded higher than preceding larval stage (Table 3).

It, therefore, appears that not only the rate of feeding and relative utilization of ingested food varied from one species to another, the utilization of food consumed related to the growth of insect was also specific for different stages. The efficiency of utilization of food also varied between the instars of the same species.

The morphmetric characteristics *viz*., the length and breadth of the larvae showed quite logically that these characters registered progressive increase with the progress of instars irrespective of the temperatures at which they were raised. But the intake of food and amount of faecal matter though increased with the developmental stages, *i.e*., larval stages when they were reared under 25 and 30^0C it was so only upto fifth instar and drastically decreased during sixth instar which occurred only when reared at 20^0C. However, the efficacy of conversion of digested food into biomass did not see eye to eye with that of amount of food taken in. At 20^0C though the ingestion of food drastically decreased at sixth instar the conversion of digested food was about four times that of fifth instar. Contrarily, the efficiency of conversion of digested food was highest at fourth instar which was about one sixth during fifth instar when reared at 25^0C while it fed forty percent lower at 30^0C. This shows that conversion of digested food depends on the index of conversion of ingested food.

4. Conclusion

The duration of the egg stage, larval instars and adult duration showed variation during development. At 20^0C it took longer time to develop than 25 and 30^0C. At 20^0C, six larval instars were recorded while at 25 and 30^0C five larval instars were recorded. However, no significant difference was found in length and width at different temperature regimes, though in last instar maximum length and width were recorded at 30^0C. Larvae developing at lower temperature required longer duration compared to higher temperature. First instar larvae

showed high C.I., which gradually decreased with larval age. C.I. gradually increased with rise in temperature. A.D. also varied considerably at different stages of development. A.D. for entire larval life decreased with larval age, but at fifth and sixth instar, at all temperature regimes larvae digested more leaves than preceding instar. The E.C.I. and E.C.D. values increased not only with advanced larval instar, but also with rise in temperature. However, it is really difficult to pin point the comparative E.C.I. and E.C.D. values for temperatures and instars.

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