Influence of abiotic & biotic factors on the life cycle of *Spodoptera litura* Fabricius, 1775. (Lepidoptera: Noctuidae)

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Executive summary of Ph.D. Thesis in the Field of Zoology for the Degree of Doctor of Philosophy

The Maharaja Sayajirao University of Baroda

September, 2020

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1 Introduction

1.1 *Spodoptera litura*

Spodoptera litura Fabricius, 1775. (Lepidoptera: Noctuidae) is one of the important Lepidopteran noctuid insect pests having high economical importance. It was previously known as *Prodenia litura* and first reported in 1775 by Fabricius. *Spodoptera litura*, commonly known as tobacco caterpillar, has many names as common cutworm, tobacco cutworm, cluster caterpillar, grey streaked moth, cotton leaf worm. The *Spodoptera litura* moths are found primarily active during night as it comes under family Noctuidae. Due to its high mobility, female oviposition capacity on a wide range of host plants promotes survival of *S. litura* individual over a broad range of environmental conditions. *S. litura* is an ubiquitous, polyphagous & voracious lepidopteran pest that feeds on 112 cultivated crops all over the world and on about 60 species from India (Bragard et al., 2019). It is a generalist polyphagus insect pest feeding on more than 290 species of host plants belonging to 99 families (Wu et al., 2004). It has been reported attacking cauliflower, mash/black gram, moong, sunflower, *arvi*, castor and cotton (Kumar et al., 2013). The larva is polyphagus and feeds on mungbean, soybean and various vegetables recorded as hosts (Xue et al., 2010). Fand *et al* (2012) found castor, okra and sunflower suitable equally as food plants while groundnut was less suitable. Ahuja, D.B. & Noor, 1991 reported castor as the most suitable host plant, followed by cabbage, tomato, chili, groundnut, moth bean, green gram, slender pigweed, carpet weed and sesame. It has been identified as a pest of vegetables particularly of family Brassicaceae (Khaliq et al., 2014).

1.2 Influence of biotic and abiotic factors

Abiotic factors such as temperature, humidity, light, and soil can influence a species' ability to survive. Every species is able to survive within a range of each of these factors. This range is called the species' tolerance range. Near the upper and lower limits of the tolerance range, individuals experience stress. This will reduce their health and their rate of growth and reproduction. Within a species' tolerance range is an optimal range, within which the species is best adapted. The largest and healthiest populations of a species will occur when conditions are within the optimal range. Each species has a tolerance range for every abiotic factor. Some species have wide tolerance ranges, while others have much narrower ranges. Species with broad tolerance ranges will tend to be widely distributed and may easily invade other ecosystems. For example, buckthorn, a small tree native to Europe, has become widespread over much of southern and central Ontario due to its broad tolerance range. Conversely, the showy lady's-slipper orchid has a narrow tolerance range. It is found only in specific types of wetlands (Fand et al., 2012).

Anthropogenic and natural environmental variations are voraciously affecting the arthropods with the passage of time. Certain factors like thermal affect is changing the status of pest by suppressing or stimulating genetic potential, rate of fecundity and mortality and range of hosts (Finlay-Doney M et al., 2012). Variable wavelength of white light specifically for red and far red light when absorbed by photosynthetic system as an unambiguous signal of proximity of hearers causing a good developmental responses like shade avoidance responses (Ruberti et al., 2012). Survival rate of young ones of *Cnaphalocrosis medinalis* (Rice leaf folder) was affected at 35 °C even adults emerging from these pupae could not laid eggs. In some biological control agents particularly *C. lividipennis* (egg predator) showed a positive response to predation and decrease the handling time (Holland JM et al., 2014).

S. litura is totally polyphagous (Holloway, 1989). The host range of each species covers over 40 families, among the main crop species attacked by *S. litura* in the tropics are cotton, flax, groundnuts, jute, , maize, rice, soybean, tea, tobacco & vegetables.

To fulfill the above aim, the following objectives were undertaken:

1. To study influence of abiotic factors on the life cycle of *Spodoptera litura*
2. To study influence of biotic factors on the life cycle of *Spodoptera litura*
3. To study effect of food preference by *Spodoptera litura*
4. To study effect of different hosts on growth parameters of *Spodoptera litura*

2 Research Methodology

2.1 Collection and multiplication

A colony of *Spodoptera litura* (Fabricius) was collected from agricultural field & reared on artificial diet (Kranthi, 2005) and maintained at constant condition of 27±2°C Temperature & 70±5 % RH. In the laboratory, the larvae were fed with artificial diet placed in plastic containers. Fresh diet was provided every 2-4 days. All the instars of larvae were maintained on artificial diet. Pupae were collected in plastic container & kept 5 pupae in single container. Adults were collected as soon as they emerge; this is to avoid starvation of adults. The adult moths were kept inside containers, covered with black cloth. Male and Female chosen for oviposition cage are such that they are healthy i.e. there is no deformity in them with respect to their development and are freshly emerged. The cloth & surface of container serve as substrate for oviposition. The adult moths were fed with a 10% honey-water solution through cotton swab. Cotton swabs are prepared by using medicated absorbent cotton. The hatched neonates will be allowed to complete their development on artificial diet.

Figure 1: Damage symptoms on castor leaves

Figure 2: Castor field at Padra

Artificial diet preparation methodology:

Ingredient List:

Sr. no.	**Ingredients**	**500ml**
1	Wheat germ	25 gm
2	Chickpea flour	75 gm
3	Sorbic acid	0.75 gm
4	Ascorbic acid	2.50 gm
5	Methyl-p-hydroxy benzoate	2.30 gm
6	Formaldehyde 5%	12 ml
7	Becasule	8 ml
8	Prop ionic acid	1.8 ml
9	Yeast	28 gm
10	Agar agar	10 gm

Procedure for preparing artificial diet:

Half the quantities of distilled water was taken into mixing jar and add propionic acid, formaldehyde and bacasule into distilled water and mix well. Thereafter add ascorbic acid, sorbic acid, methyl p-hydroxybenzoate & mix well. Add wheat germ and chickpea floor, mixed properly by using hand blender. Remaining half quantity of distilled water was taken in pan and placed for heating. When bubbles start coming, yeast is added in pan and dissolved by continuous stirring with the help of spatula. Agar agar was added slowly. After 10 minutes of cooking, this mixture starts boiling. Mixing was done properly by using hand blender. Pour diet in different plastic trays. For storing it was kept in refrigerator at 5°C.

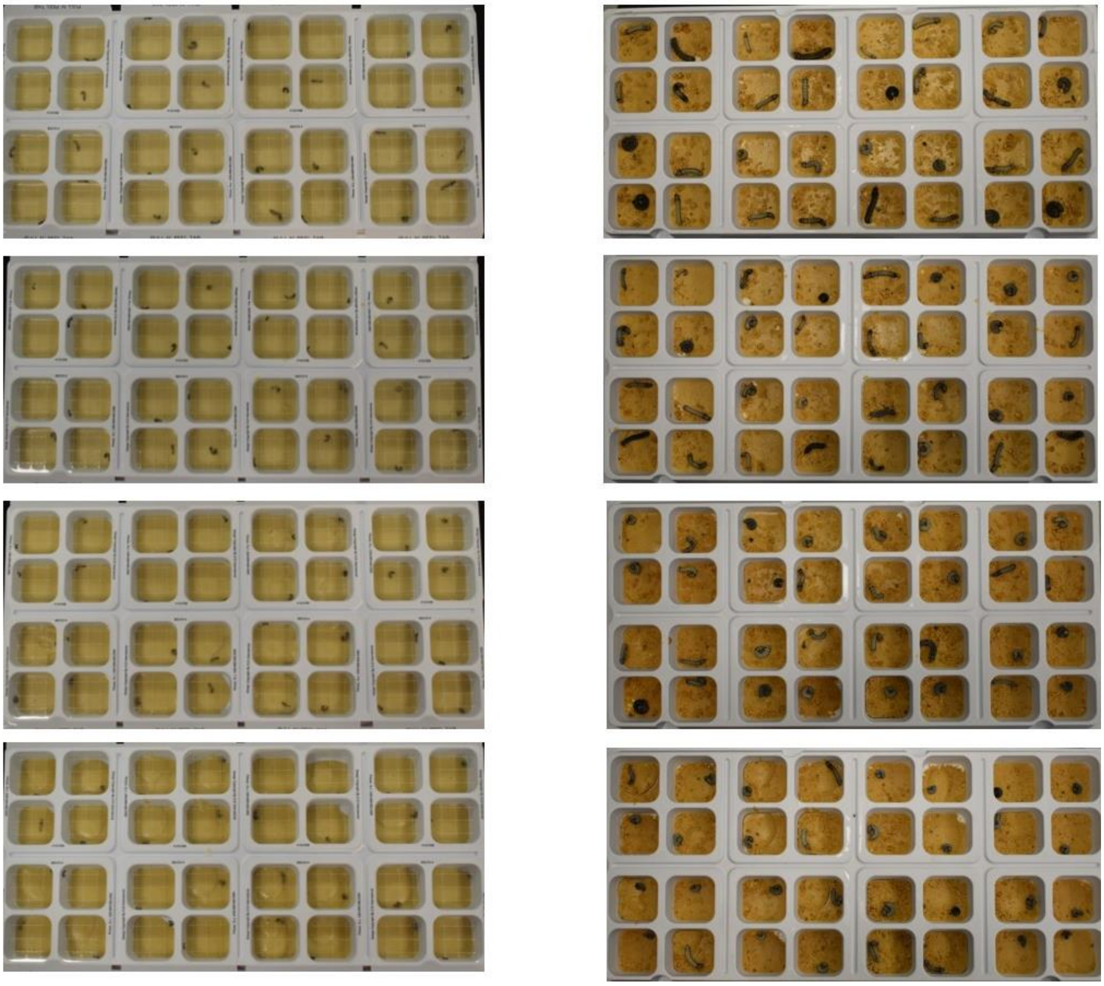


Figure 3: Set-up showing larval rearing

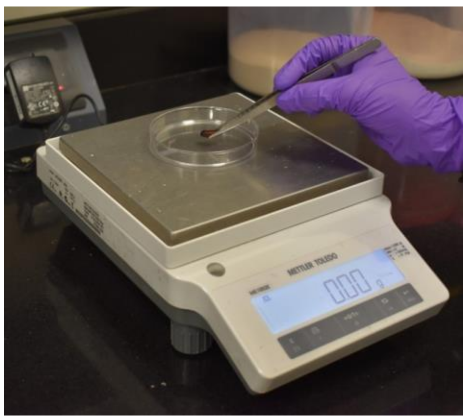


Figure 4: Weighing done for observing growth parameters

2.2 Abiotic experimental design

Abiotic factors like temperature and relative humidity were studied by keeping two different ranges considering optimum range (27±2°C Temp & 70±5 %

RH). First results were obtained for optimum range. When testing temperature all the other factors were kept as normal, this was done to see only impact of that factor which we were looking for. BOD will be utilized to regulate different abiotic factors. In current study decrease in these two parameters were studied i.e. 7°C decrease in temperature & 30% decrease in humidity. The duration of lifecycle was studied by observing whether it prolongs or become shorter due to fluctuation of these factors.

2.3 Biotic experiment design

Newly hatched larvae were selected having age of 0-12 hours old. Plants were grown and leaves collected as and when required from green house. Plastic trays having small cells were used for the experiments. Total of 10 larvae were tested per host. Newly emerged larva was kept singly with fresh leaf in individual cell. Excreta and left behind of leaves were cleaned daily to maintain hygienic condition. Fresh leaves were provided after every 24 hours. All the set up was kept at constant condition i.e. 27±2°C Temperature & 70±5 % RH.

Along with life cycle study another experiment was conducted to see host preference and after feeding, weight gain of 3rd instar larvae. For that four 3rd instar larvae were taken into plastic container individually and provided with pre weighed tomato leaf. Same thing was done with other hosts too. After 48 hours post weight of leaves were recorded. And for larval weight gain study, total of 8 larvae were taken. Before releasing larvae into container having fresh leaf, pre weighing was done for individual larvae and after 48 hours post weight was recorded.

2.4 Statistical analysis and equations

The significance amongst different treatments was studied by different plots made through Minitab 19 software.

Other growth indexes were studied like larval growth index, pupal growth index and total growth index. For finding larval growth index two parameters were required. Firstly, how many larvae undergo pupation on the basis of which we calculate percent pupation and secondly, we need to know total larval period days. Total larval period was recorded by recording day on which larvae hatches to larvae goes into pupal stage.

Equation 1

$$\text{Larval growth index} = \frac{\%\ \text{Pupation}}{\text{Larval period (days)}}$$

For finding pupal growth index two parameters required that is how many adults emerged from pupae and on the basis of that we calculate percent adult emergence and another we have to have total pupal period days. Pupal period was calculated by day on which pupa formed to day on which adult emergence seen.

Equation 2

$$\text{Pupal growth index} = \frac{\%\ \text{Adult emergence}}{\text{Pupal period (Days)}}$$

For finding total developmental index two parameters required that is how many larvae, pupae and adult survived throughout the life cycle and on the basis of that we calculate percent survival and another we have to have total developmental period in days.

Equation 3

$$\text{Total developmental index} = \frac{\%\ \text{Survival}}{\text{Total developmental period (Days)}}$$

Equation 4

$$\%\ \text{efficiency of ingested food} = [(D-C)/(A-B)] \times 100$$

A=Pre weight of the leaf

B=Post weight of the leaf

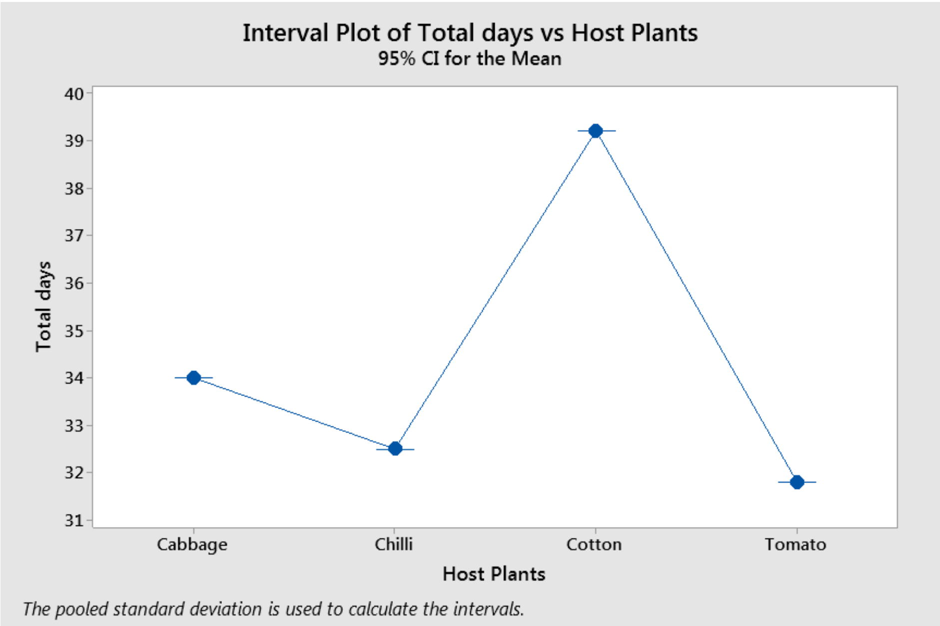
C=Pre weight of larvae

D=Post weight of the larvae

3 Results

3.1 Influence of biotic factors on life cycle of *S. litura*

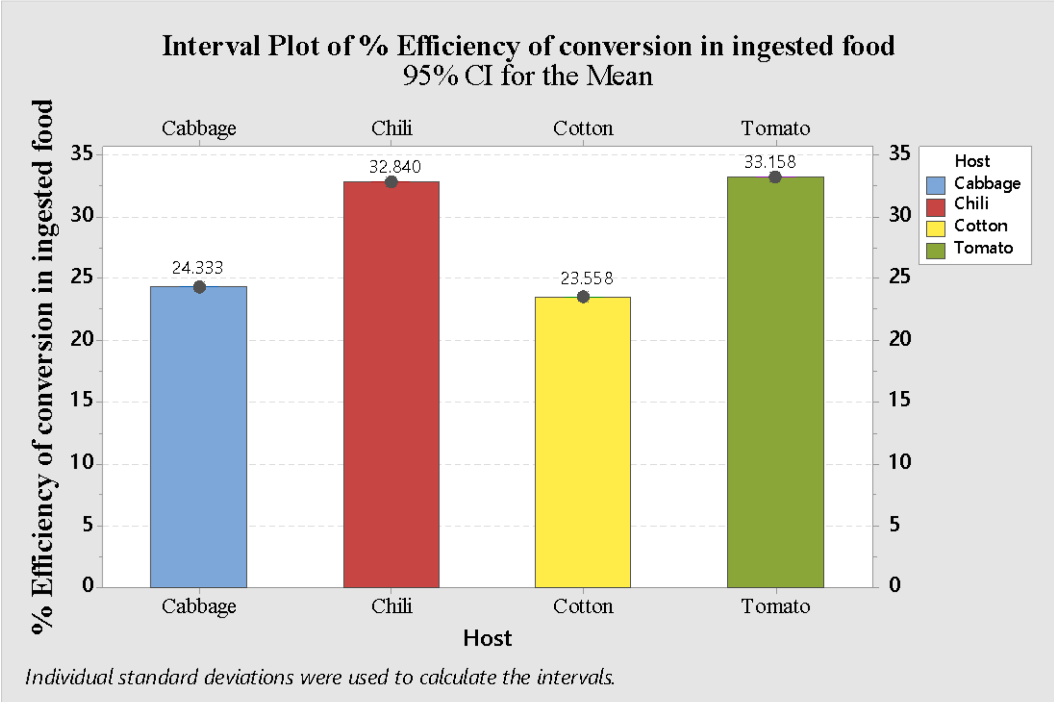
Spodoptera litura is lepidopteran insect and it undergoes complete metamorphosis & having four stages i.e egg, larvae, pupa and adult. Results indicate that growth and development were influenced by nutrition and rearing conditions. Five larval moults were noted while studying host insect relationship. Larval development from first instar to fifth instar was observed in number of days which indicated in table along with pupal and adult period. The developmental period of *S. litura* was significantly affected by host plant tested. Results pertaining to biology of *S. litura* on different hosts revealed that total life cycle period ranged for about 31.80 to 39.20 days.



Graph 1 Lifecycle of *Spodoptera litura* on different host plants

As seen from the table that more feeding was observed in case of tomato followed by chili, cabbage and cotton & same will reflect in percent efficiency of ingested food of different host against *S. litura*.

Highest value of 33.15% was found in case of tomato followed by 32.84, 24.33 & 23.55 in chili, cabbage & cotton respectively.

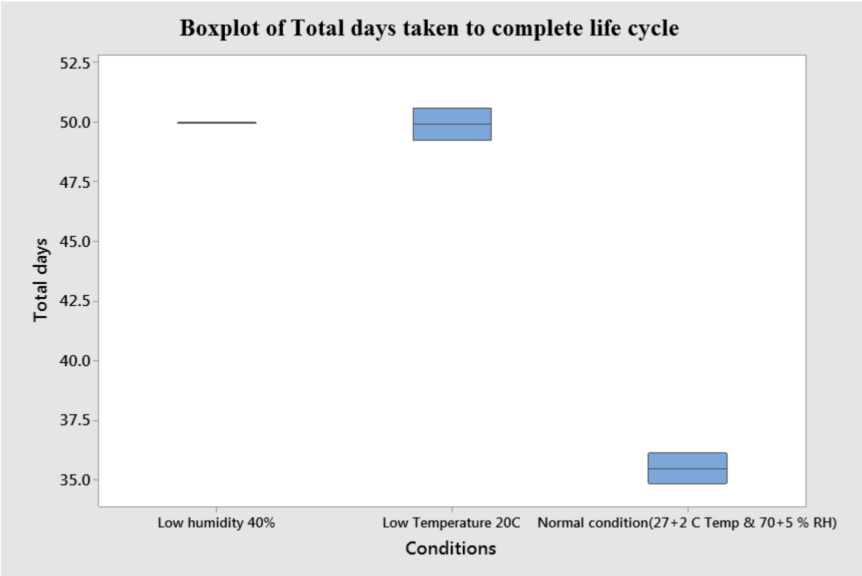


Graph 2 Percent efficiency of ingested food by *S. litura* on host plants

3.2 Influence of abiotic factors on life cycle of *S. litura*

As insects are poikilothermic organism so they cannot withstand adverse environmental conditions. The current study focuses on impact of different abiotic factors like temperature and humidity on life cycle of *S. litura*. Influence of these abiotic factors were studied by observing life cycle of *Spodoptera litura* i.e. total number of days were recorded to complete one generation. Total 50 larvae were taken for this study and data shown in the table 1 indicates, days taken to complete one generation. When optimum condition provided than it takes 31-35 days to complete one generation but when we provide decreased temperature it will take 47-49 days to complete one generation. Same with the case when we decrease humidity, it will take 48-50 days to complete one generation. Day wise data were given in table 6 & 7. Normal condition was taken as 27+2°C Temp & 70+5 % RH and 40% humidity & 20ºC temperature taken as experimental conditions.

The temperature & humidity within the evaluation data point had a large impact on the development times of *S. litura* life stages. There were increases in immature development times were observed in case of both temperature and relative humidity.



Graph 3 Total days to complete the life cycle of *S. litura*

Different growth index were calculated like larval growth index, pupal growth index and total developmental index by considering developmental period. For Larval growth index percent pupation and total larval development period were considered (Equation 1). Pupal growth index consist of percent adult emergence and total pupal period (Equation 2). For total developmental period percent survived and total developmental period taken into consideration (Equation 3).

Table 1 Biological attributes recorded on Normal & experimental conditions

Condition	Larval growth index	Pupal growth index	Total developmental index
Normal condition	5.08	19.96	2.82
Low humidity	3.14	16.56	2.00
Low temperature	3.32	16.39	2.00

Data on larval, pupal & total development were recorded. From these observations, the growth and developmental index were calculated and shown in table 1.

4 Key findings

- *Spodoptera litura* is a ubiquitous, polyphagous& voracious lepidopteran pest that feeds on large range of host plants & so attracted many researchers like me to know more about this insect pest.
- Outbreak of the pest occurs due to its resistance to insecticides, favorable weather conditions and heavy rainfall after a long dry spell.
- Castor was grown in Padra, region of Gujarat, regularly and *Spodoptera litura* is one of the major pest and becomes a collection site for the research work
- During the field visit many insect pest were also observed like Jssid, Sting bug & *Spodoptera litura*. Other pests which were not observed but pest of castor include, red hairy caterpillar (*Amsactamoorei* Butler), semilooper (*Achoeajanata* L.) and shoot and capsule borer (*Conogethespunctiferalis* Guen.).
- Important task during the study was to maintain culture of *Spodoptera litura* throughout the year without any failure.
- For culturing very well established method i.e. rearing of *S. litura* on artificial diet was followed (Kranthi, 2005). Diet proves to be easy and accurate method of rearing insects.
- Precautions are taken during rearing of *S. litura* while handling specially the to avoid any fungal or any other pathogenic infection
- When food was not there cannibalism recorded among larvae and due to that utmost care should be given while transferring the larvae to new/fresh food.
- Pupae were taken on regular basis from rearing tray and transferred to plastic container because if left unnoticed the adult will emerge inside rearing the cell and its wing can be stuck by artificial diet.
- Always healthy adults were chosen for the oviposition pot preparation because if not selected properly it will hamper fecundity. The adults having undisturbed scales on wings & active ones were selected which gives more egg masses.
- Neonates and 1st instar larval handling should be done in a very proper way to avoid injury to them as they are very small and delicate.
- Different factors plays significant role in influencing the normal behaviour of the insect. As insect shows response to these factors in some or the other way.

- These limiting factors are any factor that places an upper limit on the size of a population. Limiting factors may be biotic, such as the availability of food, host or abiotic, such as temperature, humidity & access to water.
- Present study emphasizes on influence of these factors on lifecycle of *S. litura*.
- In field conditions also insects respond to different abiotic & biotic factors by going into hibernation or diapauses. They slow down or faster the rate of development to cope up with these conditions.
- In the current study two abiotic factors temperature and humidity were studied.
- Selection of these parameters done on the basis of insect response seen against these fluctuating conditions in literature survey
- The data point selected for temperature and humidity were not studied earlier so curiously we want to know how *S. litura* behave to these data points.
- For studying these parameters were studied life cycle of *S. litura* on 20°C temperature and 40% humidity which denote experimental conditions and at the same time 27 ± 2°C and 70 + 5 % R.H was normal/optimum condition.
- Influences of these experimental conditions were observed in life cycle of *S. litura*.
- At normal condition (27 ± 2°C and 70 + 5 % R.H) the larval period observed was 19.66 days. At 20°C temperature larval period prolonged to 30.14 days & at 40% humidity larval period reaches to 31.76 days.
- If we talk about pupal period same type of data was observed that days were prolonged. At normal condition (27 ± 2°C and 70 + 5 % R.H) the pupal period observed was 5.01 days. At 20°C temperature larval period prolonged to 6.1 days & at 40% humidity larval period reaches to 6.04 days.
- Overall if we see total days taken for the life cycle to complete it takes 35.46 days in case of normal condition (27 ± 2°C and 70 + 5 % R.H), 49.91 days in case of 20°C temperature & 49.94 days as in case of 40% humidity.
- Different growth indexes also studied like larval growth index, pupal growth index and total developmental index. This also indicates there was influence of these abiotic factors on growth and development of the *S. litura*.
- At normal condition larval growth index was 5.08 where as it reduces to 3.32 & 3.14 in case of 20°C temperature & 40% humidity respectively.

- Pupal growth index also shows similar results and shows 19.96 pupal growth index at normal condition & 16.39 & 16.56 at 20°C temperature & 40% humidity respectively.
- Total developmental index also calculated to see overall development & it shows 2.82 growth index in case of normal condition & 2.00 & 2.00 at 20°C temperature & 40% humidity respectively.
- These figures clearly indicate influence of these abiotic factors on life cycle of *S. litura.*
- Host plant relationship also plays significant role in growth and development of insect. Insects are having wide host range but we divide into primary & secondary host because insect has selection and attraction from one to another.
- Study mainly focuses on four different hosts which were highly preferred by the *Spodoptera litura* & we want to see if four host present at the same time what will be the preference.
- Four host selected for the study were: Tomato (*Solanumly copersicum* – Solanaceae), Chili (*Capsicum annuum* – Solanaceae), Cabbage (*Brassica oleracea* - Brassicaceae) and Cotton (*Gossypium* – Malvaceae).
- Selection of the host was based on the literature survey and economical importance of the host. Data on life cycle study of *S. litura* on these hosts under laboratory condition was not studied so we decided to take these four host plants.
- Hatched larvae were taken directly on different host plants to see how many days it takes to reach next stage. When larvae feed on tomato leaves it takes 19.40 days followed by 19.80, 21.40, 26.00 days when fed with chili, cabbage and cotton leaves.
- More time was observed in case of cotton fed larvae so it indicates less preference as compared to other hosts.
- But in case of pupal and adult period no significant difference was observed among different hosts. Pupal period was 6.20, 6.30, 6.40 and 6.80 when larvae fed with tomato, chili, cabbage and cotton leaves. Adult period was 6.20, 6.40, 6.20 and 6.40 when larvae fed with tomato, chili, cabbage and cotton leaves. This shows only leaf feeding stage i.e. larval stage was more influenced by the different hosts.

- If we see total days of life cycle when fed with tomato, chili, cabbage and cotton were 31.80, 32.50, 34.00 and 39.00 days respectively.
- So days overall days taken to complete different stage was taken when larvae fed with cotton leaves.
- Other parameters also checked on 3rd instar larvae like leaf consumption and larval weight gain which also reflect that the tomato leaf was preferred more followed by chili, cabbage and cotton leaves. 0.475g, 0.423g, 0.375g and 0.260g consumption was observed within 48 hours of feeding on tomato, chili, cabbage and cotton respectively.
- Highest weight gain in 3rd instar larvae was observed after 48 hours when larvae fed with tomato leaf i.e. 0.157g followed by 0.138g, 0.091g, 0.061g when fed with chili, cabbage and cotton leaves respectively.
- So we can say that consumption of leaves and weight gain was positively correlated with each others.
- Different growth indexes were also studied like larval growth index, pupal growth index and total developmental index when larvae were fed with different hosts.
- Data indicates that all the three growth indexes having higher values when larvae fed with tomato leaves followed by chili, cabbage and cotton leaves.
- From the life cycle, leaf consumption, larval weight gain and different growth indexes shows tomato as most preferred host among other host like chili, cabbage and cotton tested.
- So we can conclude from our study that abiotic factor (Temperature & Humidity) and biotic factor (Host) probably exert more influence upon tobacco cutworm, *Spodoptera litura*, life cycle and developmental growth rates.

5 Conclusions

5.1 Abiotic factors

Our results are in larger agreement with those reported by Rao et al., (1989), who also did not get *S. litura* oviposition at constant high temperatures of 35°C and 37°C, however, only deviation that existed for low temperature of 15°C, where they reported egg laying. The studies by Rao et al., (1989) on the developmental effects of constant and alternating temperatures on *S. litura* addressed only the development rates and estimation of thermal constants. However, they did not consider the temperature-dependent immature mortality, adult senescence and female fecundity which are considered highly important in understanding pest population dynamic (Wagner et al., 1984). Rest of the studies that deal with estimating *S. litura* life table parameters were conducted using only single constant temperature (Hashmat M, 1977). From the results we can see the influence of abiotic factors like temperature and humidity on lifecycle of *Spodoptera litura*. When we reduce temperature and humidity the lifecycle was prolonged. This study can be helpful in predicting seasonal distribution of insect pests depending on the weather conditions. Also can be helpful for farmers to see when insect pest can invade the crop. So by this we can reduce the use of pesticides by correlating abiotic factors with pest of different crops.

5.2 Biotic factors

The results from this present study revealed the effect of different host plant on the growth and development of S. *litura*. Generally, shorter developmental times, higher reproduction rates, and low mortality of insects on a host indicate greater suitability of a host plant (Bale et al., 2002). This result on pupal duration was little similar with previous study reported by Shahout et al., (2011) where pupal duration was about 8.43 days when larva fed on soybean. Favetti et al., (2015) also revealed that pupal duration of *S. litura* when larva fed on soybean cultivar ranged from 10.5-11.2 days for female and 11.2-11.8 days for male, indicated longer than pupal duration in this present study. The biological parameters of *S. litura* including life cycle duration, larval weight, and the number of larval instars were studied towards assessing the suitability of hosts for the larvae of *S. litura*. The results were compared with similar parameters reported from other host plants especially castor, a plant that has been considered as the most suitable host for *S. litura*. Favourable results were obtained for all biological parameters of *S. litura* when the larvae were fed on tomato leaves. Moreover, the insect pest went through a normal life cycle without impaired morphological or during the process testing. From the results we can see the influence of biotic factors like host has influence on lifecycle of *Spodoptera litura*. When we change the host the days to complete life cycle will also changes.

6 Recommendations

6.1 Abiotic factors

Every organism particularly insects in arthropods respond to every deviation from normal environmental conditions. Against high or low thermal thresh hold or fluctuating humidity and varied wavelength of light stimulate the inset to respond in a plenty of way. It can affect their ovulation, rate of fecundity, development, survival, multiplication and various immune and genetic responses. In biotic stresses certain plant characters (anti-xenosis, anti-biosis), nutritional modifications, variation in flora (landscape diversity, cover crops) and insect crowding influence insect multiplication, emergence and migration. From the results we can see the influence of abiotic factors like temperature and humidity on lifecycle of *Spodoptera litura* (Fand et al., 2015). So this type of study can be helpful in predicting seasonal distribution of insect pests depending on the weather parameters. Other researcher also can see influence of these types of parameters on other economical important insect pests.

6.2 Biotic factors

The biological parameters of S. *litura* including life cycle duration, larval weight, and the number of larval instars were studied towards assessing the suitability of hosts for the larvae of *S. litura* in the current study. From the results we can see the influence of biotic factors like host has influence on lifecycle of *Spodoptera litura*. When we change the host the days to complete life cycle will also changes (R. A. Patil et al., 2015). This study can be helpful in predicting seasonal distribution of insect pests depending on the host and also predict pest pressure if similar crops are sown in adjoin fields. Can also be helpful for farmers to see when insect pest can invade the crop. So such studies taken in future by other researcher to see influence of other biotic factors like crowding, competition for host, etc.

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