Influence of abiotic & biotic factors on the life cycle of *Spodoptera litura* Fabricius, 1775.

(Lepidoptera: Noctuidae)

Synopsis of Ph.D. Thesis
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1 INTRODUCTION

Agro ecosystem is largely governed by interactions between abiotic (temperature, humidity, light, wind, soil etc.) and biotic (host, vegetative biodiversity etc.) components. Insects are powerful and rapid adaptive organisms with high fecundity rate and short life cycle. These factors significantly influence the insects and their population dynamics. In response to these factors insect may prolong their metamorphic stages, survival and rate of multiplication (Khaliq et al., 2014). Global changes are responsible for wide range of anthropogenic and natural environmental variation & these climatic and weather changes not only affect the status of insect pests but also affect their population dynamics, distribution, abundance, intensity and feeding behavior (Ayres & Schneider, 2009). Intensity of change in climatic ecosystem noted by meteorological science has showed a direct and indirect effect on the prey and host relationship, their immune responses and rate of development, their fecundity and various physiological functions (Ayres & Schneider, 2009).

Spodoptera litura Fabricius, 1775. (Lepidoptera: Noctuidae) is a moth also known as cluster caterpillar, cotton leaf worm, tobacco cutworm and tropical armyworm in different parts of the world. It is a polyphagous pest in India, China and Japan of about 290 host plant species belonging to 99 families (Caijun et al., 2004). Economically important crops like cotton, chilli, castor, groundnut, tobacco, pulses etc are attacked by the Pest. In India, *Spodoptera litura* is widespread in almost all the states and inflict significant losses to crops of economic importance like soybean (Choudhary & Shrivastava, 2007), cotton and groundnut. A single larva is reported to cause average pod yield loss of 27.3% in groundnut in per square meter through damage to various parts like leaves, flowers and pods. Since 2002, it has frequently been reported that the larvae of *Spodoptera litura* (Fabricius) are causing widespread damage to soybean crops at several localities in India (Patil et al., 2014). Recent outbreaks of *Spodoptera litura* (Fabricius) on soybean in Kota (Rajasthan state), Marathwada and Vidarbha (Maharashtra state) regions of India have been reported to cause monetary losses to the tune of USD 4.5 crores and USD 22.5 crores respectively (Dhaliwal et al., 2019). Due to prominent climatic changes and the non-judicious use of agrochemicals has increased the problem of pest. It is realized that the inherent resistant power of plant is diminishing day by day. Further noted that there is an urgent need for enhancement of agricultural system productivity due to imminent climate change as agricultural system productivity is going down due to complex problem; insect pests are posing serious threat to agricultural productivity (Selvaraj et al., 2010). Due to the nocturnal nature, moth of *S. litura* becomes active at night and move overnight for oviposition on a wide range of host plants, which promotes or even ensures survival of *S. litura* individual over a broad range of environmental conditions (Selvaraj et al., 2010). It also becomes resistant to many commonly used insecticides, particularly pyrethroids and carbamates, resulting in failure of effective controls (Javar et al., 2013). Out breaks of the pest occurs due to its resistance to insecticides, favorable weather conditions, cyclonic weather and heavy rainfall after a long dry spell. In Punjab, *S. litura* seems to have developed resistance to insecticides, as the pest is not being managed effectively with the commonly recommended insecticides (Kranthi, 2005). The reproductive capacity and migration ability over long distances have made it economically important pest of many agricultural crops, with a wider geographical range throughout Asia, from North Africa to Japan, Australia and New Zealand (Fand et al., 2015). The population of *S. litura* in cabbage crop to be negatively correlated with average and maximum temperature. It is a sporadic pest with a high mobility and reproductive capacity (Holloway, 1989). The caterpillar is widely distributed throughout tropical and temperate regions of Asia, Australia and Pacific islands (Ayyub et al., 2019). Its wide spread and pest status has been attributed to its polyphagy and its ability to undergo both facultative diapause and seasonal migration and also observed three distinct peaks of tobacco caterpillar brood emergence on bean (Peta & Pathipati, 2008). The first peak occurred in the 4th week of January when averages of 823.5 moths per trap was collected. The moth coughed in the trap increased progressively during February with 1143.5 moths per trap indicating a second peak. The next marked increase in the trap catch was in the 4th week of February with 1549.75 moths per trap forming the third brood emergence. *S. litura* started attacking cauliflower initially during mid September and its population touched the peak in second week of October during 2003-04 and during 2004-05, it touched the peak twice 1st in first week of October and 2nd in the 1st week of November also reported that the occurrence of *S. litura* is generally synchronous with the growing period of tobacco, especially during summer, providing plenty of food sources for oviposition and larval feeding (Xue et al., 2010). The seasonal incidence of *S. litura* on banana revealed that the larval population starts to build up during June-July and its minimum infestation on leaves was recorded in the month of August-September (4.50 and 4.78% infestation). The pest population declined in the end of September and disappeared in October (Shukla., *et al* 2011). Its peak incidence was noticed during the first and second fortnights of August during the vegetative growth of capsicum under protected cultivation (Nandini et al 2012). The species is migratory on all continents and is a key pest in all of them (Feng et al., 2005). The pest causes significant damage to different types of tobacco (M et al., 2014). It has become the most destructive pest of cabbage and cauliflower crops in Tamil Nadu (Kumar., 2013). Several factors were identified that cause outbreaks of *S. litura* in northern China. The factors include increasing acreage of tobacco and many preferred crops mainly vegetables which provide abundant food sources, expanding of protected cultivations which provide suitable sites for over wintering, more mild winter and warmer spring, which enables the pest to occur earlier and build up higher populations in the first generation, high temperature and less rainfall in summer and misuse of pesticides that cause resistance to insecticides, less natural enemies and the most potent factor of monoculture (Ramesh babu et al., 2015).

Temperature: Being poikilothermic organisms, the developmental rate in insects is highly contingent on external temperature conditions. Hence, temperature is generally considered the single most significant environmental factor influencing behavior, distribution, development, survival and reproduction in insects (Fand et al., 2015). Knowledge on the temperature-dependent population growth potential of insect pests is highly imperative for understanding their population dynamics and implementing agro-ecoregion specific pest control strategies, especially in the context of predicted global climate warming. The vast majority of studies that infer the effects of temperature on developmental biology of *Spodoptera litura* have been undertaken only under one constant temperature in laboratory (Murata & Tojo, 2002). Few studies that addressed the development of *Spodoptera litura* at a range of constant temperature were concerned with predicting only developmental rates but no emphasis was given to the simulation of variability in development times, mortality and fecundity with temperature changes (Fand et al., 2015). Due to non-linearity in developmental response at temperature extremes, linear models are generally considered poor predictors of insect developmental rates (Shahzad et al., 2014). Yet, the specific effects of associated daily and seasonal temperature extremes on *Spodoptera litura* development are less understood which warrants estimation of the temperature-dependent population growth potential for understanding the impact of climate change on its future incidence and damage activity (Fand et al., 2015).

Humidity: Moisture also plays a critical role in insect development, especially in the desert. Many insect pests encountered in our crops do not require free moisture to survive. They obtain water through their food supply. For instance, leaf miners spend their entire egg and larval stages inside melon or lettuce leaves, extracting water and nutrients from plant tissue. However, relative humidity or lack thereof can influence insect growth and behaviour by affecting the insect's ability to regulate water loss. Low humidity is often detrimental to insect development, but most insects found in desert crops have adapted physiological and behavioural mechanisms to prevent dehydration. Of course, rainfall can be quite deleterious to insects. As a thumb rule, cool, wet extremes in weather are the most detrimental to insects because they can promote disease, slow growth rates and interrupt feeding activities. There is no doubt that weather plays a major role in determining the survival and growth rates of insect populations because of its direct impact on them and on their food supply. These interactions have been studied for many years and are fairly well understood. Unfortunately, predicting insect abundance in particular area is like predicting the weather or vice-versa (Fand et al., 2012).

Host: Insect host plant interaction & relationship is of prime importance, which helps us to know the possible effect of host plants on insect development. It is also observed that food plants and their physical and chemical constituents play a vital role in survival and reproductive potential of insects. The development of insects follows a series of moulting or ecdysis under control condition and the increase in size can be expressed as growth law, which indicates that head capsule of caterpillar increases in width with each moult by a geometrical progression (Vashisth, 2015). Host plant is a key determinant of the establishment, growth, survival and fecundity of herbivorous insects. Though many host plants were reported for *S. litura*, every host does not support the pest in the same way. There have been a number of studies on the biological parameters of *S. litura* on different host plants under different environmental conditions, particularly, in India (Narvekar et al., 2018), Pakistan (Shahzad et al., 2014), Korea (Xue et al., 2010) where *S. litura* has been an important pest on various crops.

The objective of this study was to develop a comprehensive temperature & humidity based life cycle model for *S. litura*, which permits prediction of its population growth potential and seasonal dynamics in various host growing in India, and will also aid in forecasting the probable pest aggravation under potential climate warming. Models are in use to predict climatically suitable areas for distribution, abundance and damage activity of pests. It also helps to examine the impact of climate change on future pest status of insects like cotton mealy bug, maize stem borer in economically important crops (Fand et al., 2015). The implications of the present work are multifaceted and include an assumption that any small increases or decrease in average global temperatures and humidity may have measurable effects on *S. litura* performance and thus on the yield losses in different hosts. We contribute to the exploration of the differing developmental responses of *S. litura* to variability in environmental temperatures & humidity. Here, we first establish thermal reaction norms to various constant temperature conditions for generating life cycle data of developmental response in *S. litura*, and use them to test if the developmental effects of temperature & humidity variations predicted from the models established at constant temperatures & humidity are in concordance with those observed in the experiments conducted using daily temperature & humidity fluctuations, and then visualize the potential changes in pest status of *S. litura* due to future temperature & humidity, increase or decrease (Fand et al., 2012).

2 MATERIAL AND METHODS

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 the underside of castor leaves

Figure 2: Sting bug infesting the same host plant.

Figure 3: Castor field at Padra

Figure 4: Maize field at Padra

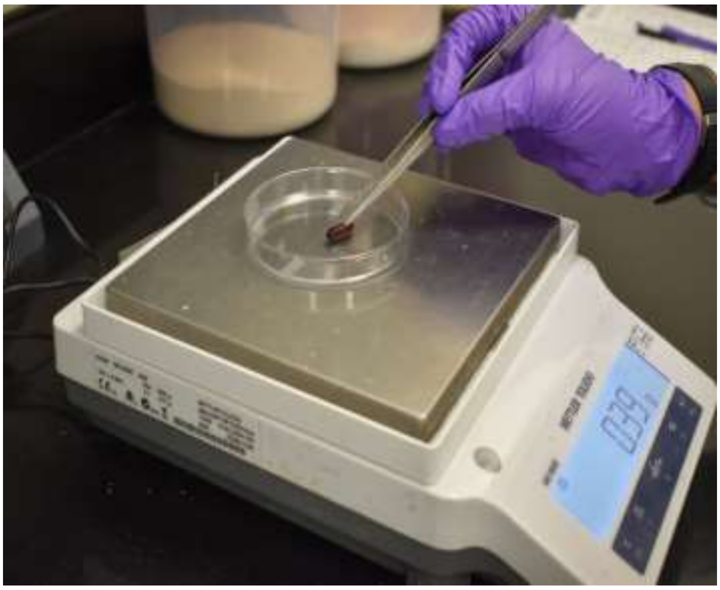


Figure 5: Growth parameters using weighing balance

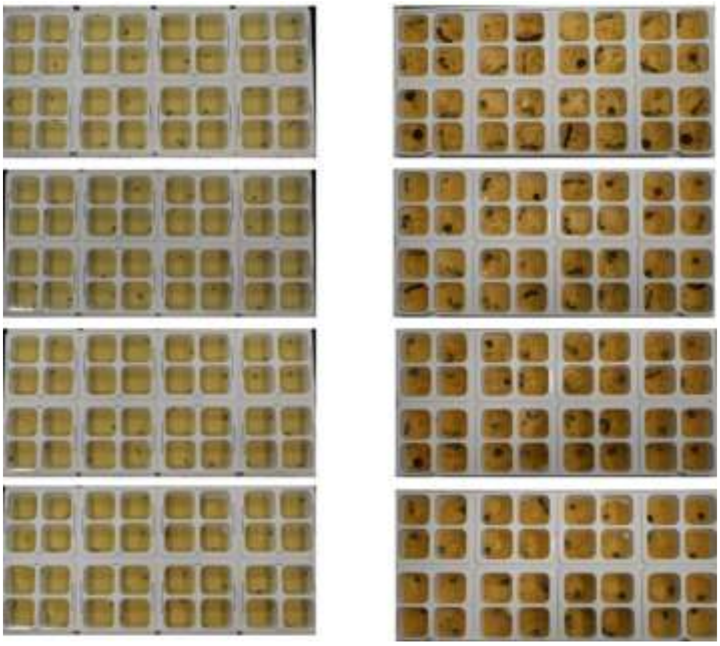


Figure 6: Set-up showing larval rearing

Table 1: List of ingredient used for preparing artificial diet for *S. litura*

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	Becosule	8 ml
8	Propionic acid	1.8 ml
9	Yeast	28 gm
10	Agar agar	10 gm

Procedure for preparing artificial diet:

Distilled water was taken into mixing jar and add propionic acid, formaldehyde and becosule 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.

Different larval stages of *Spodoptera litura* (Fabricius)

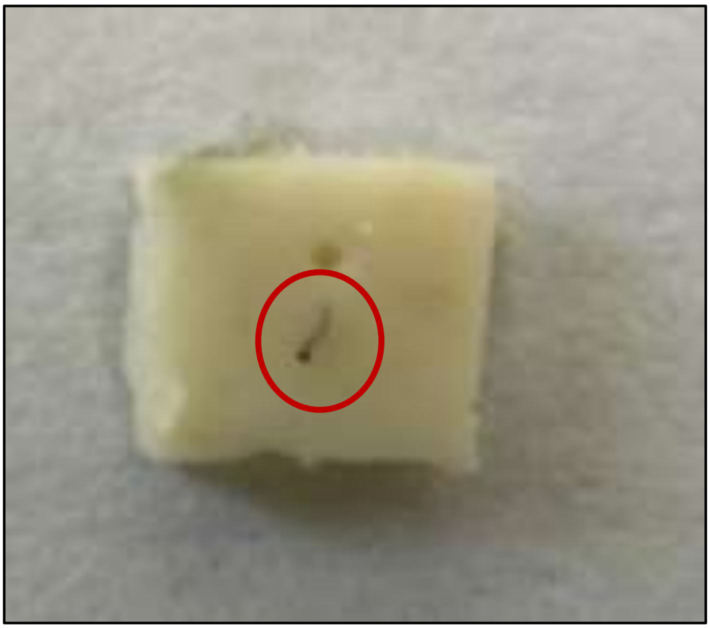


Figure 7: Neonate Larvae

Figure 8: 1st instar Larvae

Figure 9: 3rd Instar Larvae

Figure 10 : 5^{th} Instar Larvae

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 Data Analysis

For data analyses following formula were used. From these observations, the growth and developmental index were calculated as follows:

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

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

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

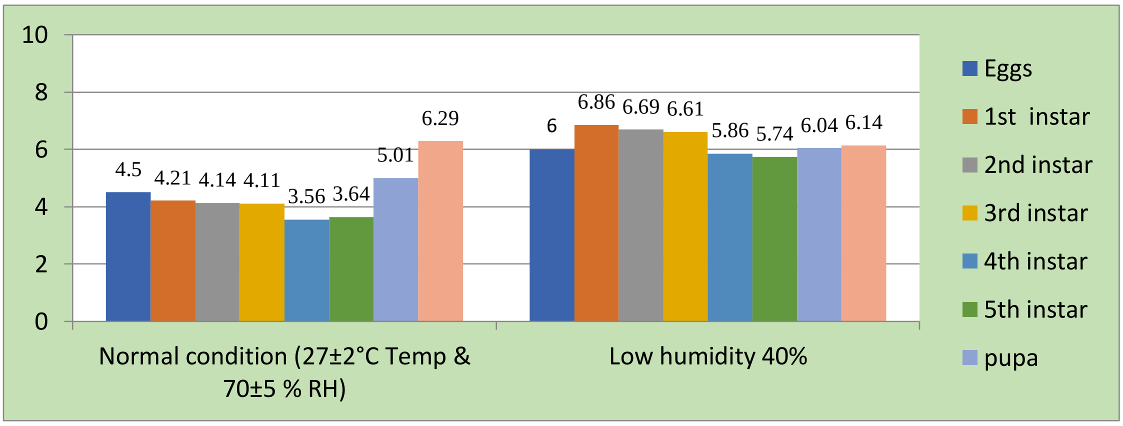
3 Results

3.1 Abiotic factor

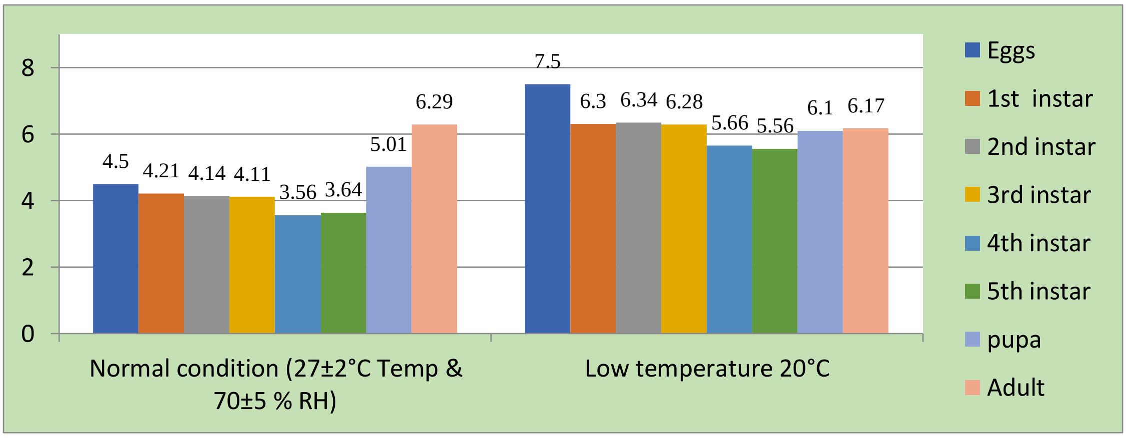
Influence of these abiotic factors were studied by observing life cycle of *Spodoptera litura* i.e. total number of days were recorded to complete one generation. For that influence of temperature and humidity were observed on 100 larvae. This study and data shown in the table 1 indicates, days taken 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 1. So we can see clear difference among parameters i.e. temperature and humidity on life cycle of *Spodoptera litura.* As Seen from the graphs that days will be prolonged when we decrease temperature & humidity.

Table2: Days to complete one generation at different environmental conditions

Conditions	Eggs	Instar					pupa	Adult	Total days
		1st	2nd	3rd	4th	5th			
Normal condition (27±2°C Temp & 70±5 % RH)	4.5	4.21	4.14	4.11	3.56	3.64	5.01	6.29	35.46
Low humidity 40%	6	6.86	6.69	6.61	5.86	5.74	6.04	6.14	49.94
Low temperature 20°C	7.5	6.3	6.34	6.28	5.66	5.56	6.1	6.17	49.91



Graph 1. Effect of low humidity on life cycle of *Spodoptera litura*



Graph 2. Effect of low temperature on life cycle of *Spodoptera litura*

Table 3: The biological attributes recorded on normal conditions were compared with other condition.

Condition	Larval growth index	Pupal growth index	Total developmental index
Normal condition (27±2°C Temp & 70±5 % RH)	5.41	20.00	3.13
Low humidity 40%	3.57	16.67	2.22
Low temperature 20°C	3.57	16.67	2.11

3.2 Biotic factor

Larval development from one instar to another instar was observed in number of days which indicates that *Spodoptera litura* takes as much as of 31.80 days when fed with tomato leaves, 32.50 days when fed with chilli leaves, 34.00 days if we fed with cabbage leaves and 39.20 days when fed with cotton leaves.

Table 4: Lifecycle of *Spodoptera litura* on different host plants.

Host Plants	Larval period (Days)	Pupal period (Days)	Adult period (Days)	Total days
Tomato	19.40	6.20	6.20	31.80
Chilli	19.80	6.30	6.40	32.50
Cabbage	21.40	6.40	6.20	34.00
Cotton	26.00	6.80	6.40	39.20

In case of feeding preference clear response was observed by seeing on consumption of leaf. Leaf was pre weighed and given to larvae and after 48 hours post weight difference recorded. Highest consumption was observed on tomato leaf i.e. 0.475g followed by chilli leaf 0.423g, cabbage leaf 0.375g and cotton leaf 0.260g.

Larval growth in terms of weight gain on different host was observed after 48 hours of feeding. Maximum gain in weight was recorded when larvae fed with tomato leaf followed by chilli, cabbage and cotton leaves. Weight gain was 0.157g, 0.138g, 0.091 and 0.061g when fed with tomato, chilli, cabbage and cotton leaves respectively.

4 Discussion

4.1 Abiotic factor

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). 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.

4.2 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*. 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*. Favorable 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 (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.It can also be helpful for farmers to see when insect pest can invade the crop.

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