**SUB-LETHAL EFFECT OF EMAMECTIN BENZOATE ON LIFE HISTORY TRAITS AND RELATIVE FITNESS OF TOBACCO CATERPILLAR, *Spodoptera litura* (LEPIDOPTERA: NOCTUIDAE).**

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**Abstract**

 This experiment was carried out in the laboratory of Department of Entomology, College of Agriculture, Rajendranagar during *kharif*, 2022. The sublethal effects of emamectin benzoate (EB) were investigated on *Spodoptera litura* in field collected population from vegetable crops of Chevella and Maheshwaram mandals, Rangareddy district, Hyderabad under laboratory conditions. Emamectin benzoate (EB) is widely used in the control of lepidopteran pests, but there is sparse information available regarding its sublethal effects on *S. litura*. The LC50 value was 0.018310 and 0.018078 in Chevella and Maheshwaram populations, respectively for 24 hr exposure time. LC25 of the insecticide was used for sublethal effect study in both field populations. The sub-lethal concentrations of the emamectin benzoate resulted in reduction in weight gain of all the stages of *S. litura*, prolonged durations of larvae, pupae and adult stages, increased larval mortality and decreased female survival, reduced pupation, emergence and hatchability rates, as well as fecundity. Further studies on demographic parameters to determine the population dynamics of resistance acquired populations of *S. litura* revealed an increase in mean generation time (T) and a decrease in net reproductive rate (Ro), intrinsic rate of increase (rm), finite rate of natural increase (λ) and reduced relative fitness (Rf) with 0.67 and 0.68 in Chevella and Maheshwaram populations, respectively.

**Keywords:** *Spodoptera litura*, Emamectin benzoate, Life history traits, Net reproduction rate (*R*o), Intrinsic rate of increase (rm), Finite rate of natural increase (λ) and Relative fitness (Rf) with increased Mean generation Time (T).

1. **Introduction**

Vegetable crops play a key role in Indian agriculture because of sizeable portion of the country’s population is vegetarians. India has overall area of 11.37 million hectares with production of 209.14 million metric tonnes of vegetables during 2021-2022 (Indiastat.com), placing it next to China in terms of global production. However, there are many difficulties involved in growing vegetables, most notably insect pests. Estimated losses due to insect pests account for 40 per cent in vegetable production. Among the most common insect pests, lepidopteran species including the tobacco caterpillar, *Spodoptera litura* (Fabricius) causes enormous losses to the crops (Maish, 2019).

Tobacco caterpillar, *Spodoptera litura* (Fabricius) is a persistent polyphagous pest of field and horticultural crops (Shankaramurthy, 2006). It is found throughout Asia, in the eastern section of the planet from North Africa to Japan, Australia and New Zealand (Feaken, 1973). It is a significant pest in India causing damage to a wide range of economically important crops including tobacco, cotton, groundnut, castor, chilli, potato, soybean, cauliflower, cabbage, tomato, beans, sunflower and onion (Tukaram *et al*., 2014) resulting in 26–100% yield loss under field conditions (Dhir *et al*., 1992). *S. litura* is predominantly a defoliator but also consumes the buds, flowers and pods of legumes (Krishnamurthy Rao *et al*., 1983).

Emamectin benzoate is a commonly used insecticide that is essential to the control of agriculturally important lepidopteran pests in many different countries. Its wide range of activity and remarkable effectiveness against this specific insect group are the reasons for its appeal (Yen and Lin, 2004; Ahmad *et al*., 2008; Zhang *et al*., 2014). Avermectin is the chemical group that includes Emamectin benzoate (EB). According to Roditakis *et al*. (2013), this substance functions as a chloride channel activator in insect nervous systems, suppressing muscular contraction and ultimately leading to death. Lepidopteran resistance is notably exhibited by EB (Argentine *et al*. 2002; Parsaeyan *et al*. 2013; El-Sheikh 2015). Noteworthy examples include *Spodoptera frugiperda* (Muraro, 2022; Muraro *et al*., 2021), *Mythimna separata* (Zhao *et al*., 2018; Jie *et al*., 2014) and *S. exigua*, where resistance was noted (Ishtiaq *et al*., 2014; Zhang *et al*., 2014). Applying EB in an integrated pest management (IPM) programme could be viewed as a significant pest control strategy because of its ecological selectivity towards a variety of beneficial arthropods (Lopez *et al*., 2011).

Insect populations that survive exposure to insecticide and other toxicants experience alterations in physiology and/or behaviour at sublethal dosages or concentrations (Desneux *et al*., 2007). Reduced life span, developmental time, fecundity, fertility, alterations in the sex ratio and/or behavioural changes are examples of sublethal consequences (Stark & Banks 2003, Alyokhin *et al.,* 2008; Wang *et al.,* 2008, 2009; Sohrabi *et al*., 2011; Sabre *et al*., 2020). Fitness costs can also be used to compare life cycle features between susceptible and resistant *S. litura* populations produced from field collected insects in order to understand how insecticide resistance has developed over time. Therefore, this study was carried out to investigate the sublethal effects of this insecticide on larval and pupal developmental time, adult longevity, fecundity, hatchability and pupal weight of *S. litura*. This research will provide information for more effective use of EB in management programs for *S. litura* and provide effective pest control strategies.

1. **Material and Methods**

**2.1 Test insect**

Tobacco caterpillar, *Spodoptera litura* (Fabricius)

**2.1.1 Collection of susceptible strain of *S. litura***

The initial susceptible population of *S. litura* larvae which were reared under laboratory conditions without exposure to any insecticide for about six generations were obtained from Entomology laboratory of ICRISAT, Patancheru, Hyderabad. The collected population was further reared for four more generations on artificial diet in the laboratory of Department of Entomology, College of Agriculture, Rajendranagar, Hyderabad. The third instar larvae of F10 generation population were then used for bioassay studies.

 **2.1.2 Collection of *S. litura* population from vegetable crop ecosystem of Rangareddy district**

Two major vegetables cultivated mandals *viz.,* Maheshwaram and Chevella were selected and field population of *S. litura* were collected from the vegetable crop ecosystem of these mandals during *Kharif*, 2022. The field collected populations were brought to laboratory and both these populations were reared separately on artificial diet and maintained at 25±2°C temperature and 75±5% relative humidity. The third instar larvae of F1 generation were used for bioassay studies.

**2.2 Insecticide**

Emamectin benzoate 5% SG (Avermectin) was procured from local market

**2.2.1 Preparation of stock solution of insecticides**

The following formula was used to prepare one per cent stock solution of the test insecticides (Naveed, 2005)

 Required concentration (1%)

 Stock solution = x 100\*

 % formulation of test insecticide

\*Quantity of water taken for the preparation of solution

The measured quantity of emamectin benzoate was diluted in distilled water and volume was made up to 100 ml in volumetric flask to get one per cent stock solution. Subsequent serial dilutions were prepared to get five to six concentrations. Prepared insecticide dilutions were stored in refrigerator at 0°C for 24 hours without losing its efficacy. Insecticide was first evaluated at broad concentrations. Narrow range concentrations were explored based on the observed mortality from 20.00 to 80.00% larval mortality. At every stage of the experiment, an untreated control group was maintained for comparison.

 **Table 1 Concentrations of emamectin benzoate used against field population of *S. litura* collected from Chevella and Maheshwaram mandals of Rangareddy district**

|  |  |
| --- | --- |
| **Field populations** | **Concentrations (%)** |
| Chevella and Maheshwaram | 0.011, 0.014, 0.017, 0.020, 0.023, 0.026, 0.029  |

**2.3 Bioassay**

These test concentrations were fixed for determining the median lethal concentration (LC50) and sub-lethal concentration (LC25) against field population of *S. litura* from Chevella and Maheshwaram mandals as mentioned in Table 1. Bioassay studies were conducted with third instar larvae of *S. litura* using standard leaf disc method as described by Johny and Muralirangan (2000). Fresh castor leaves were cut into small leaf discs of 6 cm diameter. These leaf discs were then dipped in test insecticide solution for about 30 seconds. The leaves were air dried by placing them on paper towels and later transferred to petri plates containing moist filter paper to prevent desiccation. The leaf discs dipped in distilled water were considered as control. Ten third instar *S. litura* larvae obtained from the culture maintained from Chevella mandal as well as ten third instar *S. litura* larvae obtained from the culture maintained from Maheshwaram mandal were also tested for the fixed seven different concentrations as mentioned. Based on the mortality data obtained from the bioassay experiment, the LC50 and LC25 values for the assumed resistant population of that particular mandal for that particular test insecticide was calculated through Probit analysis (Finney, 1971) using SPSS software.

* 1. **Life trait parameters of F10 generation susceptible population were compared with sublethal concentration (LC25) of emamectin benzoate treated surviving field population of Chevella and Maheshwaram mandals.**

The studies on life history traits were carried out by two methods *viz*.,

* + 1. Larval feeding method
		2. Adult feeding method

**2.4.1. Larval feeding method**

Newly hatched neonate larvae of F1 generation field collected population of Chevella and Maheshwaram mandals that were reared on artificial diet under laboratory conditions were used for the experiment. The larvae were fed with fresh untreated castor leaves separately for each population until they attained third instar stage. Once they entered third instar, the larvae were reared individually in petri plates containing leaf disc treated with sub lethal concentration (LC25) of emamectin benzoate for 24 hours. The LC25 value that was obtained during preliminary studies for the Chevella population for the emamectin benzoate was used against larvae of Chevella population. Similarly, the LC25 value that was obtained for the Maheshwaram population for the emamectin benzoate during preliminary studies was used against larvae of Maheshwaram population. After 24 hours of feeding on treated leaves, the larvae were fed with fresh untreated castor leaves. Leftover food material was removed and fresh untreated leaves were provided at every 24 hours interval until larvae reached final instar. The final instar larvae were collected and transferred into another jar containing sand for pupation. The pupae thus formed were collected and placed into small plastic jars and covered with muslin cloth for adult emergence. The F10 generation susceptible population that was continuously reared on fresh untreated castor leaves served as control. Three replications each with 50 larvae were set up for a given population.

**2.4.1.1 Observations recorded**

Observations on larval weight, larval period, larval mortality, per cent pupation, pupal weight, pupal period, per cent adult emergence were recorded at 24 hours interval.

**2.4.2 Adult feeding method**

To study the adult longevity and fecundity, a pair of healthy male and female insects emerged from the larvae of emamectin benzoate treated survival population of Chevella and Maheshwaram mandals and susceptible population were used. A pair of adult insects of each population were released into separate plastic jar for mating. The plastic jar was lined with brown paper as a substratum for egg laying. Cotton swab dipped in 10 per cent honey solution was placed inside the jar as food for adults and these swabs were replaced daily. The jar was covered with white muslin fabric and secured in place with a rubber band to prevent escape of the adults. The eggs laid on brown paper and muslin cloth were collected daily and placed in separate jar until the female stopped laying eggs. Ten such pairs of adult insects were used for each population.

**2.4.2.1 Observations recorded**

Observations on adult longevity, fecundity and percentage hatchability were recorded at 24 hours interval.

**2.5 The population growth indices**

The population growth indices *viz*., intrinsic rate of increase, net reproductive rate, mean generation time, finite rate of increase and relative fitness for both emamectin benzoate (LC25) treated survival field population of Chevella and Maheshwaram mandals and susceptible population of *S. litura* were worked out using the following formula.

**2.5.1 Net reproduction rate (*R*o)**

Ro = Σlx.mx

Where, lX: the number of surviving individuals at the beginning of age class X mx: age-specific fertility, the number of living females born per female in each interval class.

**2.5.2 Mean generation Time (T)**

T =Σ Xlx.mx / Σ lx mx

**2.5.3 Intrinsic rate of increase (rm)**

rm = Ro/T

where T is the development time from eggs to adult eclosion

**2.5.4 The finite rate of natural increase (λ)**

The number of females per female per day *i.e*., finite rate of increase was determined as:

**λ =** antilog erm

**2.5.5 Relative fitness (Rf)**

Ro of resistant population

**Rf =**

 *R*o of susceptible population

If, Rf > 1- suggest that the fecundity of resistant population was enhanced Rf < 1- suggest that the fecundity of resistant population was reduced

**2.5.6 Data Analysis**

The life history traits of insecticide resistant population of Chevella and Maheshwaram mandals were compared with susceptible population of *S. litura* using one way analysis of variance (ANOVA).

**3. Results and Discussions**

**3.1 Weights of developmental stages of susceptible and survivals of emamectin benzoate (LC25) treated field populations of *S. litura***

The weights of different stages of *S. litura i.e.,* third instar, fourth instar, fifth instar, pre-pupae and pupae of susceptible population in comparison to field collected Chevella and Maheshwaram populations are presented in the Table 2.

**3.1.1 Larval weight during third instar**

The results revealed that field population of Chevella and Maheshwaram treated with emamectin benzoate (LC25) recorded significant lower larval weights of 158.09 mg and 157.76 mg, respectively when compared to susceptible laboratory population which recorded highest larval weight of 186.16 mg (Table 2). However, no significant difference in larval weights of Chevella and Maheshwaram populations was observed.

**3.1.2 Larval weight during fourth instar**

The larval weights of fourth instar increased in all the populations compared to third instar. However, the increase was on par with each other in case of Chevella and Maheshwaram field populations with Chevella population recording 308.84 mg while Maheshwaram population recorded 307.07 mg. The susceptible laboratory population recorded significant highest larval weight of 357.00 mg compared to field populations.

**3.1.3 Larval weight during fifth instar**

Significant difference in mean larval weights was observed between susceptible and field populations that were treated with sublethal concentration (LC25) of emamectin benzoate (LC25) during final instar. Significant lowest larval weight of 527.57 and 528.58 mg were recorded in Chevella and Maheshwaram populations, respectively while the susceptible population recorded significant highest larval weight of 576.01 mg (Table 2).

**3.1.4 Pre pupal weight**

The pre-pupal weight of field collected *S. litura* population was found to be 384.93 mg for the Chevella population and 385.22 mg in the Maheshwaram population when treated with sublethal concentration (LC25) of emamectin benzoate. However, the laboratory susceptible population recorded significant highest pre-pupal weight of 419.51 mg.

**3.1.5 Pupal weight**

Significant reduction in the pupal weight of Chevella (302.93 mg) and Maheshwaram (302.47 mg) populations were observed when treated with sublethal concentration (LC25) of emamectin benzoate compared with that of the susceptible population (330.97 mg) (Table).

The results pertaining to the present study, revealed that the sub-lethal concentrations of an insecticide (emamectin benzoate) for which it has acquired resistance has impact on the larval and pupal weights of *S. litura*. Significant lower larval and pupal weights were observed in emamectin benzoate (LC25) treated field populations than the susceptible population. Significant reduction in the larval weights of emamectin benzoate treated insects when compared to normal susceptible population inspite of adequate food being offered could be explained as, the utilization of the energy derived out of the consumed food by the insecticide treated larval in mitigating the counter effects of insecticide. If higher is the diversion of energy towards breaking down of insecticide molecule through production of enzymes etc. lower is the quantum of energy available for building up of tissues for growth. This could be the probable reason for the reduction of larval weights of field populations of Chevella and Maheshwaram that have acquired resistance. As larval weights were low consequently the pupae formed out of these larvae recorded lower weights. In contrary, the recording of increased weights by susceptible laboratory population could be due to non-diversion of energy for protecting itself from the lethal actions of insecticide as it was not exposed to insecticide. As such the insect population was not habituated to trigger such kind of physiological mechanism as this population was unexposed to insecticides over a long period of time.

Present results are in conformity with the findings of the Ziaee and Sohrabi (2022) who observed significant decrease in the pupal weights of *Tuta absoluta* from Southern Iran that were treated with sub-lethal concentrations (LC10 and LC30) of emamectin benzoate. Similarly, Afzal and Shad (2016) reported reduced crawler and pupal weight gain in emamectin benzoate treated population of *Phenacoccus solenopsis* collected from cotton fields of Pakistan. Zaka *et al*. (2014) reported reduced larval, pre- pupal and pupal weights of *S. litura* that acquired resistance against emamectin benzoate. Similar reduction in larval and pupal weights of *S. litura* was also reported by Abbas *et al*. (2014) and Abbas *et al.* (2012) that acquired resistance against imidacloprid and profenophos, respectively.

**3.2 Duration of different stages of susceptible and survivals of emamectin benzoate (LC25) treated field populations of *S. litura***

The duration of larvae, pupae and adult longevity of susceptible and emamectin benzoate treated field population of Chevella and Maheshwaram are given in Table 3.

**3.2.1 Larval duration**

The mean larval period of emamectin benzoate (LC25) treated field population of both Chevella (13.87 days) and Maheshwaram (13.91 days) recorded significant increase compared to susceptible population (12.73 days). However, there is no significant difference in larval period of Chevella and Maheshwaram populations was observed (Table 3).

**3.2.2 Pupal duration**

Significant longest mean pupal period of 8.17 days was recorded in emamectin benzoate (LC25) treated Chevella population, which was significantly on par with Maheshwaram population with the mean pupal period of 8.17 days. However, the susceptible population recorded significant shortest mean pupal duration of 7.59 days compared to field populations.

The field populations of both Chevella and Maheshwaram treated with sub- lethal concentrations (LC25) of emamectin benzoate exhibited significant prolonged larval and pupal duration compared to the susceptible population. As larva is the only feeding stage in the life cycle of a lepidopteran, it tries to feed maximum quantity of food in a specified duration as per its life cycle and assimilates it and then transforms itself into pupa. However, when a larva which has acquired resistance against a specific insecticide and if it is treated with sub lethal concentrations of that insecticide which is not lethal to kill it but might reduce its feeding ability due to behavioural resistant and will certainly interferes with general digestive physiology of the larva. As descried early the larva diverts certain quantum of energy in breaking down of insecticide molecule that result in the short fall of gaining minimum quantum of energy to transfer itself to pupa. To acquire that minimum quantity of energy the larva has to feed little more quantity of food for little longer duration of time. Apart from that, the interference of the insecticide on the juvenile hormone and ecdysone production as well as their activity also cause delayed larval moults which ultimately increases larval and pupal durations in resistance acquired insects.

The findings of present investigation are in accordance with the findings of Ziaee and Sohrabi (2022) who reported significant extension of larval period in *T. absoluta* when exposed to LC30 of emamectin benzoate. Similarly, extension of larval and pupal periods was also reported by Taleh *et al.* (2022) when *T. absoluta* was exposed to emamectin benzoate. Zaka *et al*. (2014) reported prolonged larval and pupal duration in emamectin-selected strain of *S. litura*. Similarly, increase in larval and pupal durations was observed by Ishtiaq *et al*. (2014) in *S. exigua*. Parsaeyan *et al.* (2013) found extended larval and pupal developmental periods in *Helicoverpa armigera* when exposed to the LC30 of emamectin benzoate.

**3.2.3 Female adult longevity**

Significant longest female adult longevity was recorded in emamectin benzoate (LC25) treated field populations of both Chevella and Maheshwaram compared to the susceptible population. Chevella and Maheshwaram population recorded female adult longevity of 9.54 and 9.47 days, respectively. Significantly shortest adult longevity of 7.40 days was observed in susceptible population. (Table 3).

The female adult lepidopteran life span in general is longer than males so as to enable its eggs to get fertilized by the sperms received from the male and further to oviposit the fertilized eggs. However, the increased female adult longevity of insects that acquired resistance and were treated with sub-lethal concentrations could be attributed to the interference of the insecticide molecule on reproductive physiology resulting in delayed fertilization of acquired sperms or delayed oviposition of fertilized eggs. These results are in conformity with the findings of Liu *et al*. (2022) who found significant longer female lifespan of *S. frugiperda* selected population when treated with emamectin benzoate at LC20. Mokbel and Huesien (2020) reported prolonged female longevity in *Spodoptera littoralis* when treated with LC5 and LC15 doses of emamectin benzoate. Afzal and Shad (2016) reported prolonged female longevity in emamectin benzoate treated population of *P. solenopsis* compared to susceptible population.

**3.3 Percentage of larval mortality, pupation, adult emergence and hatchability of susceptible and survivals of emamectin benzoate (LC25) treated field population of *S. litura***

The results on impact of sublethal concentration (LC25) of emamectin benzoate on per cent larval mortality, pupation, adult emergence and hatchability of *S. litura* in field populations collected from Chevella and Maheshwaram were presented in Table 4

 **3.3.1 Larval mortality**

The results revealed significant highest larval mortality of 8.00 per cent in emamectin benzoate (LC25) treated field population of Maheshwaram. A slightly lower but significantly on par larval mortality of 6.75 was observed in Chevella population. The untreated susceptible population recorded no mortality as the population was maintained under laboratory conditions and was significantly different from both the field populations.

The per cent mortality recorded in the insecticide treated populations was found to be in the range of 6.75 to 8.00 which in fact is low because of application of sublethal concentrations of insecticides and existence of resistance in the populations against emamectin benzoate. Though the population of both the locations have acquired resistance against emamectin benzoate, the existence of variability among individuals of same population and development of few weak individuals in the population due to genetic segregation could be the probable reasons for mortality of that weak individuals. These findings are in accordance with the findings of Afzal and Shad (2016) who recorded lower per cent mortality of *P. solenopsis* crawler population treated with sublethal concentrations of emamectin benzoate. Rehan and Freed (2015) reported higher larval mortality of susceptible *S. litura* larva exposed to methoxyfenozide when compared to resistant population. Ishtiaq *et al*. (2014) recorded decreased larval survival per cent, indicating high mortality in susceptible population of *S. exigua* when exposed to emamectin benzoate. Similar observation of low larval mortality was also recorded by Abbas *et al*. (2014) and Abbas *et al.* (2012) in the field populations of *S. litura* that acquired resistance to imidacloprid and profenophos, respectively compared to the susceptible population.

**3.3.2 Per cent pupation**

Significant lowest mean per cent pupation of 86.96 was observed in Maheshwaram population which was found significantly on par with Chevella population (87.84 %). While, significantly highest per cent pupation was recorded in susceptible population (97.16). Significant but slight reduction in pupation of field populations compared to susceptible untreated laboratory population could be attributed to the development of weak individuals that could sustain life during larval period until they were consuming food and breaking down the insecticide molecule. The energy levels might be insufficient in the weak individuals to tolerate the impact of insecticide and drive them for pupation. Apart from, this change of form from larval to pupa requires activity of ecdysone and synthesis of chitin which might have hampered due to interference of the traces of the treated insecticide.

These findings were in line with the findings of Liu *et al*. (2022) who reported significantly lower pupation rate in emamectin benzoate selected population of *S. frugiperda* at LC20 compared to control. Ishtiaq *et al*. (2014) reported reduced pupation rate in emamectin benzoate selected population of *S. exigua* compared to laboratory susceptible population. In contradiction to the results obtained, Jia *et al*. (2009) reported that the per cent pupation in tebufenozide resistant population was higher than the susceptible population of *S. exigua.*

**3.3.3 Per cent adult emergence**

The per cent adult emergence was found to be significantly low in Chevella population (83.33) and Maheshwaram population (82.50) when compared to untreated susceptible population (91.30%). The application of sublethal concentrations of emamectin benzoate slightly reduced the per cent adult emergence of field populations that acquired resistance. The reason for reduction in the per cent adult emergence could be due to the death of insecticide treated larva after pupation. The transformation of adult from pupa is a complex process where pupa tries to utilize the stored energy. The reason for death of pupa can be attributed to the presence of traces of unmetabolized insecticide if any that was deposited in the fat tissues of larva, which might have been processed during adult formation resulting in the death of pupa.

These findings are in conformity with the findings of Liu *et al*. (2022) who found significantly lower per cent adult emergence in emamectin benzoate selected population at LC10 and LC20 of *S. frugiperda* compared to control. Afzal and Shad (2016) reported reduced per cent adult emergence of *P. solenopsis* in emamectin benzoate selected population compared to unselected population. Rehan and Freed (2015) reported less per cent adult emergence in methoxyfenozide selected population of *S. litura* when compared to the unselected field population and susceptible population.

**3.3.4 Per cent hatchability**

The per cent hatchability of eggs laid by the Chevella and Maheshwaram populations were found to be 72.41 and 72.36 per cent, respectively which was significantly low compared to that of the susceptible population (84.44%) (Table 4). However, there was no significant difference between the populations of the locations *viz.,* Chevella and Maheshwaram population.

The significant reduction in the per cent hatchability of eggs that were laid by the adults which were exposed to insecticide during their larval stage can be attributed to the impact of insecticide on reproductive physiology of female insect. The female larval insects store huge quantity of energy in the fat bodies to make them utilize for egg production at a later stage *i.e.,* during adult stage. Majority of insecticides being lipophilic gain entry into fat bodies escaping metabolism by enzymes. The traces of these insecticides might have killed the embryo during embryogenesis resulting in reduced per cent hatchability of eggs.

These findings are in accordance with the findings of Liu *et al*. (2022) who recorded significant lower hatching rate of *S. frugiperda* eggs that were laid by adults which were exposed to sublethal concentrations (LC10 and LC20) of emamectin benzoate. Similarly, Afzal and Shad (2016) reported reduced per cent hatchability in emamectin benzoate selected population of *P. solenopsis* compared to unselected population. Zaka *et al*. (2014) revealed that the per cent hatchability was higher in susceptible population of *S. litura* when compared to the resistant population when exposed to emamectin benzoate*.* Parsaeyan *et al.* (2013) found that the larvae of *H. armigera* when exposed to emamectin benzoate exhibited decreased egg hatching rate compared to control.

**3.4 Female per cent and fecundity (No. of eggs laid/ female) of susceptible and survivals of emamectin benzoate (LC25) treated field populations of *S. litura***

The results pertaining to impact of sublethal concentrations of emamectin benzoate (LC25) on per cent female formation and fecundity (No. of eggs laid/ female) of *S. litura* in susceptible and field populations were presented in Table 5.

**3.4.1 Female per cent**

The present study recorded significant reduction in female formation percentage in both Chevella (51.43) and Maheshwaram (55.88) populations compared to susceptible population which recorded significant higher female per cent of 59.52.

In general, the sex ratio in nature though described as 1:1 as a whole, but the number of females actually produced are little higher in number compared to males in insects. This is evident by the results wherein the susceptible population recorded 59.52 per cent female survival. However, the reduction in female per cent formation in field populations that were treated with insecticides could be attributed to the variation in general metabolism of males and females. The female insects require little additional quantity of food compared to male insects which shall be processed into energy and utilized for egg production at a later stage. Therefore, the female insects divert less quantity of energy for toxin metabolism compared to males. This variation in resources (energy) partitioning could be the primary attribute for death of few weak females exposed to insecticide compared to that females which are unexposed.

These findings are on par with the findings of Rehan and Freed (2015) who reported less female per cent in methoxyfenozide selected population of *S. litura* among the total adults emerged when compared to the female per cent in unselected field population and susceptible population. Similar reduction in per cent female formation was also reported by Ishtiaq *et al*. (2014) in *S. exigua* resistance population that were exposed to emamectin benzoate when compared to susceptible population*.* Zaka *et al*. (2014) also reported less female per cent in emamectin benzoate selected population of *S. litura* compared to unselected field population and susceptible population.

**3.4.2 Fecundity**

The female insects that survived sublethal concentrations (LC25) of emamectin benzoate produced significant lowest number of eggs of 588.23 (Maheshwaram population) and 592.85 (Chevella population), respectively. Whereas, significant highest number of eggs (892.85) were produced by untreated susceptible population.

The reduction in the number of eggs by field populations compared to susceptible population can be attributed to the diversion of energy by the relatively strong female insects to metabolize the insecticide. During the process of metabolizing the insecticides huge quantum of energy might have been shelled out by these insects for production of various enzymes such as Mixed function oxidases, Glutathione -S- transferases and Esterases etc. The energy shelled out by the insect for its self-defence at the cost of reproduction.

The results of the present investigation are in accordance with the results of Ziaee and Sohrabi (2022) who reported significant reduction of fecundity in *T. absoluta* when exposed to LC10 and LC30 value of emamectin benzoate when compared to susceptible population. Zaka *et al*. (2014) reported decreased no. of eggs per female in emamectin selected population compared to susceptible and unselected population of *S. litura*. Ishtiaq *et al*. (2014) found reduced no. of eggs per female in the emamectin benzoate resistance population of *S. exigua* when compared to susceptible population*.* Parsaeyan *et al.* (2013) found that the larvae of *H. armigera* when exposed to emamectin benzoate exhibited decreased fecundity compared to the control.

In the present study, Chevella and Maheshwaram population of *S. litura* that were exposed to the sublethal (LC25) concentrations of emamectin benzoate were found to show reduced weight gain in larval and pupal stage*,* prolonged larval and pupal durations followed by increased adult longevity, increased larval mortality, reduced female per cent, per cent pupation, per cent adult emergence, per cent hatchability and fecundity. The overall negative effect on all the life history parameters of insecticide selected population *S. litura* may be attributed to the energy distribution among fitness cost and resistance. Mokbel and Huesien (2020) reported the negative sublethal effects of emamectin benzoate on general life history parameters was due to availability of reduced resources. Xu *et al.* (2016) reported that many insecticides reduced the nutrition efficiency and increased larval energy consumption in detoxification processes of those insecticides. Zaka *et al*. (2014) reported sub lethal effects on life history traits of *S. litura* against emamectin benzoate due to development of resistance. Lai and Li (2011) reported that longer larval periods have been interpreted as the result of increased energy consumption during detoxification processes of insecticides. Several workers have reported the negative impact of insecticides on life trait parameters of major insect pests including *S. litura* (Abbas *et al*., 2012), *H. armigera* (Lixia *et al.,* 2011), *P. xylostella* (Jun *et al*., 2017; Gope et al., 2022), *T. absoluta* (Taleh *et al*., 2022; Ziaee and Sohrabi, 2022). Rimoldi *et al* (2012) indicated that sublethal effects could also contribute to the the reduction of pest population levels.

**3.5 Population growth parameters of susceptible and survivals of emamectin benzoate (LC25) treated field populations of *S. litura***

The population growth parameters of *S. litura viz*., Net reproduction rate (*R*o), Mean generation Time (T), Intrinsic rate of increase (rm), Finite rate of natural increase (λ) and Relative fitness (Rf) of susceptible and emamectin benzoate survival population of Chevella and Maheshwaram are presented in Table 6.

**3.5.1 Net reproduction rate (*R*o)**

Significant lower net reproduction rate (*R*o) of 90.16 and 90.38 were observed in Chevella and Maheshwaram populations, respectively, when compared to susceptible population which recorded highest *R*o value of 135.10.

**3.5.2 Mean generation Time (T)**

Chevella populations recorded significant highest mean generation time of 43.14 days followed by Maheshwaram population (36.00 days) and mean generation time of these two populations were found significantly on par with each other. Significant lowest mean generation time of 30.18 days was recorded in susceptible population.

**3.5.3 Intrinsic rate of increase (rm)**

The results indicated in Table 6 showed significant lowest intrinsic rate of increase in Chevella population (0.32) which was significantly different from Maheshwaram population (0.39). Significant highest intrinsic rate of increase was observed in susceptible population (0.65) compared to resistant populations.

**3.5.4 The finite rate of natural increase (λ)**

Finite rate of natural increase was significantly less in Chevella population (23.83) followed by the Maheshwaram (30.99) population compared to that of the susceptible population which recorded significant highest finite rate of natural increase (81.28).

**3.5.5 Relative fitness (Rf)**

Considering the relative fitness of susceptible populations as a reference (Rf=1.00), the relative fitness for the Chevella and Maheshwaram populations were found to be 0.67 and 0.68, respectively. Decrease in the relative fitness of both Chevella and Maheshwaram population were observed when compared to susceptible population.

The results of the present study showed increase in mean generation time (T) and reduction in net reproductive rate (Ro), intrinsic rate of increase (rm), finite rate of natural increase (λ) and relative fitness (Rf) compared to susceptible population. These findings are in conformity with the findings of Taleh *et al.* (2022) who reported increased mean generation time (T) and decreased net reproduction rate (*R*o), intrinsic rate of increase (rm), finite rate of natural increase (λ) and relative fitness (Rf) in emamectin benzoate treated population compared to control in *T. absoluta.* Mokbel and Huesien (2020) reported that the treatments with LC5 and LC15 showed prolonged mean generation time (T) and reduced intrinsic rate of increase (rm), finite rate of natural increase (λ), gross reproductive rate, net reproductive rate (Ro) and relative fitness (Rf) compared to control population of *S. littoralis*. Zaka *et al.* (2014) reported that emamectin-selected strain in *S. litura* when compared to the susceptible strain showed decreased net reproductive rate (R0) and relative fitness (Rf). Ishtiaq *et al.* (2014) reported reduced overall fitness in emamectin benzoate selected (Ema- SEL) population than the laboratory susceptible (Lab-PK) population of *S. exigua*. Similarly, Jia *et al*. (2009) reported decrease in relative fitness and intrinsic rate of population growth compared to susceptible population of *S. exigua.*

The results showed decrease in overall fitness of emamectin benzoate selected field population from Chevella and Maheshwaram population compared to susceptible population. It may be caused due to increased tolerance in the populations exposed to insecticides, distribution of resources among maintaining fitness and resistance. These findings were explained by Taleh *et al.* (2022) who reported sublethal concentration of emamectin benzoate in *T. absoluta* affected life table parameters and consequently resulted in reduction of population growth. Further, the declining number of survivals with limited resources could not manage well, which in turn reduced the population and number of generations per year. Ziaee and Sohrabi (2022) reported that the population dynamics of *T. absoluta* were negatively influenced by sublethal effects of emamectin benzoate by affecting their life history parameters and population growth rate. As the population tried to maintains fitness along with low- moderate resistance against sublethal effect of insecticides at the cost of reduction of population growth gradually over years. Mokbel and Huesien (2020) found that relative fitness, fecundity, gross reproductive rate (GRR), intrinsic rates of growth (r), finite rate (λ) and net reproduction rates (R0) were all lower in emamectin benzoate treated larvae of *S. littoralis* than that of the control group. They also reported that negative sublethal effects of emamectin benzoate could interfere with the histological effects of parental generation which would subsequently affect the reproductive characteristics of offspring generation reducing its fertility parameters and reducing the population count under check. Zaka *et al*. (2014) reported that resistance to emamectin benzoate caused a significant decline in the majority of the life history parameters in the *S. litura* population and indicated the existence of a trade-off in the allocation of resources between resistance and fitness costs. In another study, Parsaeyan *et al.* (2013) indicated that the majority of the life history traits of an insect under investigation were adversely impacted by emamectin benzoate. They further, suggested that sub-lethal effects of an insecticide (emamectin benzoate) are crucial from a practical standpoint because it will result in development of only fewer progeny which probably further contribute to a reduction in the field insect population. In addition, the combination of lethal and sublethal effects of insecticides like emamectin benzoate will probably have a deleterious impact on population dynamics of insect in long run*.* Fitness costs associated with insecticide resistance occur where the development of resistance to an insecticide is accompanied by high energetic cost or other significant disadvantages that diminish the insect's fitness compared with its susceptible counterparts in the population (Kliot and Ghanim 2012). Decrease of relative fitness associated with insecticide resistance has been demonstrated for many insects including *S. litura* (Rehan and Freed, 2015), *S. exigua* (Jia *et al*., 2009; Ishtiaq *et al*., 2014) and *P. xylostella* (Cao and Han 2006; Sun *et al*., 2012; Jun *et al.,* 2017), *Phenacoccus solenopsis* (Afzal and Shad, 2016).

1. **Summary and Conclusions**

Tobacco caterpillar, *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae) is a polyphagous and potentially destructive insect pest that causes significant damage to economically important crops such as cotton, legumes, vegetables and others.

Studies pertaining to sub lethal effects of emamectin benzoate on life history parameters of *S. litura* against F1 generation field populations revealed that significant lower larval weight during third instar (158.09 and 157.76 mg), fourth instar (308.84 and 307.07 mg) and fifth instar (527.57 and 528.58 mg) were recorded in Chevella and Maheshwaram populations, respectively as compared to that of susceptible population which exhibited significantly higher third, fourth and fifth instar larval weight of 186.16, 357.00 and 576.01 mg, respectively. Significant lower pupal weight of 302.93 mg and 302.47 mg were observed in Chevella and Maheshwaram populations compared with that of the susceptible population (330.97 mg). Prolonged larval and pupal duration were observed in Chevella (13.87 and 8.17 days, respectively) and Maheshwaram (13.91 and 8.17 days, respectively) population of *S. litura* as compared with that of the susceptible population (12.73 and 7.59 days, respectively). Increased larval mortality was observed in emamectin benzoate treated population from Chevella (6.75%) and Maheshwaram (8.00%) compared to susceptible population which recorded zero per cent larval mortality. Per cent pupation (87.84 and 86.96%, respectively) per cent adult emergence (83.33 and 82.50%, respectively), per cent hatchability (72.41 and 72.36%, respectively) and female per cent (51.43 and 55.88%, respectively) were significantly reduced in Chevella and Maheshwaram populations compared to susceptible population (97.16, 91.30, 84.44 and 59.52%, respectively). Similarly, fecundity of treated populations from Chevella (592.85 eggs) and Maheshwaram (588.23 eggs) was reduced compared to susceptible population (892.85 eggs). Sub lethal concentrations of emamectin benzoate showed negative impact on biological parameters of *S. litura* that might be due distribution of resources between the resistance and fitness cost of
*S. litura.*

Population growth parameters revealed that reduced net reproduction rate (*R*o), finite rate of natural increase (λ), intrinsic rate of increase (rm) in *S. litura* population from Chevella (90.16, 23.83 and 0.32, respectively) and Maheshwaram (90.38, 30.99 and 0.39, respectively) which differed significantly from the susceptible population (135.10, 81.28 and 0.65, respectively). Mean generation time (T) was prolonged in Chevella (43.14 days) and Maheshwaram population (36.00 days) than susceptible population (30.18 days). The overall relative fitness (Rf) was reduced both in Chevella (0.67) and Maheshwaram (0.68) population.

**Table 2.** **Weights of developmental stages of susceptible and emamectin benzoate (LC25) treated field populations of *S. litura***

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Stage** | **Third instar larvae (mg)****(Mean ± SEM)** | **Fourth instar larvae (mg)****(Mean ± SEM)** | **Fifth instar larvae (mg)****(Mean ± SEM)** | **Prepupae (mg)****(Mean ± SEM)** | **Pupae (mg)****(Mean ± SEM)** |
| **Susceptible population** | 186.16 ± 1.13b | 357.00 ± 2.50b | 576.01 ± 1.58b | 419.51 ± 1.52b | 330.97 ± 1.51b |
| **Chevella population** | 158.09 ± 0.81a | 308.84 ± 2.20a | 527.57 ± 1.69a | 384.93 ± 1.19a | 302.93 ± 2.45a |
| **Maheshwaram****population** | 157.76 ± 0.82a | 307.07 ± 2.23a | 528.58 ± 1.68a | 385.22 ± 1.18a | 302.47 ± 2.51a |
| **C.D** | 2.47 | 2.66 | 2.13 | 1.67 | 2.24 |

\*Superscript letters indicated by similar alphabets has no significant difference (p<0.05) and values indicated by sequential alphabets has

 significant difference

**Table 3. Duration of different stages of susceptible and emamectin benzoate (LC25) treated field populations of *S. litura***

|  |  |  |  |
| --- | --- | --- | --- |
| **Parameters** | **Larval period (Days)****(Mean ± SEM)** | **Pupal period (Days)****(Mean ± SEM)** | **Female adult longevity (Days)****(Mean ± SEM)** |
| **Susceptible population** | 12.73 ± 0.27b | 7.59 ± 0.22 b | 7.40 ± 0.07 b |
| **Chevella population** | 13.87 ± 0.48 a | 8.17 ± 0.44 a | 9.54 ± 0.08 a |
| **Maheshwaram population** | 13.91 ± 0.10 a | 8.17 ± 0.13 a | 9.47 ± 0.09a |
| **C.D** | 0.65 | 0.46 | 0.71 |

\*Superscript letters indicated by similar alphabets has no significant difference (p<0.05) and values indicated by sequential alphabets has significant difference

**Table 4. Percentage of larval mortality, pupation, adult emergence and hatchability of susceptible and emamectin benzoate (LC25) treated field populations of *S. litura***

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Parameters** | **Larval mortality** **(%)** | **Pupation** **(%)** | **Adult emergence (%)** | **Hatchability** **(%)** |
| **Susceptible population** | 0.00 ± 0.00a | 97.16 ± 1.08b | 91.30 ± 1.25b | 84.44 ± 2.65b |
| **Chevella population**  | 6.75 ± 0.44b | 87.84 ± 1.14a | 83.33 ± 1.84a | 72.41 ± 2.81a |
| **Maheshwaram population** | 8.00 ± 0.58c | 86.96 ± 0.95a | 82.50 ± 1.22a | 72.36 ± 0.09a |
| **C.D** | 0.28 | 1.24 | 1.16 | 1.43 |

\*Superscript letters indicated by similar alphabets has no significant difference (p<0.05) and values indicated by sequential alphabets has

 significant difference

**Table 5. Female per cent and fecundity (No. of eggs laid/ female) of susceptible and emamectin benzoate (LC25) treated field populations of *S. litura***

|  |  |  |
| --- | --- | --- |
| **Parameters** | **Female Per cent**  **(%)** | **Fecundity** **(No. of eggs laid/ female)** |
| **Susceptible population** | 59.52 ± 0.57a | 892.85 ± 0.07c |
| **Chevella population** | 51.43 ± 0.33b | 592.85 ± 0.09b |
| **Maheshwaram population** | 55.88 ± 0.12c | 588.23 ± 0.09a |
| **C.D** | 1.33 | 1.73 |

\*Superscript letters indicated by similar alphabets has no significant difference (p<0.05) and values indicated by sequential alphabets has significant difference

**Table 6. Net reproduction rate (*R*o), Mean generation Time (T), Intrinsic rate of increase (rm), Finite rate of natural increase (λ)**

 **and Relative fitness of susceptible and emamectin benzoate (LC25) treated field populations of *S. litura***

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Parameters** | **Net reproduction rate (*R*o)** | **Mean generation Time (T)** | **Intrinsic rate of increase****(rm)** |  **Finite rate****of natural increase (λ)** | **Relative fitness****(Rf)** |
| **Susceptible population** | 135.1b | 30.18a | 0.65c | 81.28c | 1.000b |
| **Chevella population** | 90.16a | 43.14c | 0.32a | 23.83a | 0.667a |
| **Maheshwaram population** | 90.38a | 36.00b | 0.39b | 30.99b | 0.668a |
| **C.D** | 1.37 | 1.11 | 0.04 | 0.76 | 0.08 |

 \*Superscript letters indicated by similar alphabets has no significant difference (p<0.05) and values indicated by sequential alphabets has

 significant difference

 \*Relative fitness (Rf)= R0 of field population/ R0 of susceptible population

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