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# Effect of sub-lethal concentration of *Azadirachta indica* on biology and weight of *Spodoptera litura* on cauliflower under laboratory conditions

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**Abstract**
The larvae were collected from vegetable fields and reared on cauliflower leaves under laboratory conditions to check the mortality and sub-lethal effects of neem oil were comprised of different five concentrations and compared with control (distilled water) on surviving of *Spodoptera litura* on 4th instar larvae at constant temperature of 26±2 ºC and 50-60 RH%. Such doses, 2500, 2000, 1500, 1000 and 500 ppm were dissolved in one litter of water and each treatment was replicated three times. The maximum mortality was observed (50.00±5.77; at 2500 ppm)% and highest larval weight reduction (50.32±0.06; at 2000 ppm), pupal weight was highly reduced (30.43±0.93; at 2500 ppm) with the highest total life period (39.03±0.56; at 2500 ppm) thus, the lowest fertility percentage of female was recorded (56.56±1.74; at 2500 ppm) of *S. litura*. It was concluded that the neem oil was effective by their sub-lethal concentrations however, most preference can be given to which, caused highly sub-lethal effects on the biology of pest.

**Keywords:** Biology, life span, tobacco cutworm, neem oil, cauliflower

## 1. Introduction
The tobacco cutworm, *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae) is a typical cutworm of polyphagous in nature known as a monetarily genuine pest in the Indian subcontinent [1] and it is viewed as one of the significant risk to the present day escalated agribusiness and changing yield designs worldwide [2]. Expanding ecological dangers from substance pesticides and improvement of bug spray resistance in *S. litura* [3] incited the advancement of naturally stable option strategies to control this pest. Among the eco-accommodating strategies being created for administration of Spodoptera spp., natural control and other bio sound methodologies are prevailing [4]. Plants are characteristic compound processing plants, giving the wealthiest wellspring of natural chemicals on earth as well. Approximately, 42 groups of plants convey some therapeutic and insecticidal qualities [5]. The antifeedant and development controlling exercises of PONNEEM, an oil plan containing neem and pungam (Karanj) oils were observed alongside individual neem and karanj oils and Nimbicidine, a business neem-based pesticide against fourth phase of *S. litura* [6].
Plant items have a few uses in pest control [7] however; the neem, *Azadirachta indica* (A. Juss) has been filled in as pest controlling medicinal for a considerable length of time in subcontinent is as yet a well-known practice in remote regions for put away grains to be safest in different parts of the world [8,9]. Azadirachtin is a noteworthy compound of neem with insecticidal properties and has the best consideration as of late [10] be that as it may, a few different mixes like; deacetyl-azadirachtinol, meliantriol, vepol, salannin, sulfur mixes, and so forth do have shifting level of pest hindrance, repellant, against feedant, hostile to ovipositional and development managing properties [11,12]. Advertise requests of low deposits and WTO controls drive agriculturists of Pakistan for sensible pesticide utilized and search for choices. Neem being a local plant and with various bug management properties could be a decent source. The antifeedant and hostile for detaching impact of neem unrefined concentrate were assessed against different lepidopterans and practically identical outcomes were gotten. This pest management has been accounted for to be especially delicate to neem items [13]. Be that as it may, no work has been done yet on neem based items against this pest of cauliflower in Sindh Province of Pakistan.

In the present review research facility based outcomes have been tried in the fields and the adequacy of neem oil has also been checked at different doses on this pest of cauliflower.

## 2. Materials and Methods

### 2.1 Experimental area

The research study was carried out from September to February in Rabi season during, 2015-16. The larvae were collected from Tando Allahyar field conditions for rearing under laboratory conditions at constant temperature of 26±2 ºC and relative humidity 50-60%. Experiments were comprised of different five concentrations and compared with control (distilled water) at Bio control lab at Department of Entomology, Sindh Agriculture University, Tandojam.

### 2.2 Sub-lethal doses of neem (*Azadirachta indica* A. Juss)

The different doses of neem oil, Azadirachtin such as; 2500, 2000, 1500, 1000 and 500 ppm were dissolved in one litter of water, and control dose was kept only with the use of distilled water. Each treatment concentration was replicated three times. Neem oil solutions were prepared in the bucket and cauliflower leaves were dipped in to each concentration for 10 seconds and kept to dry in shade. Treated food was given to ten larvae of each concentration of 4$^{th}$ instar to feed. After 48 hours normal (untreated) food was provided for surviving of larvae. Treated surviving larvae were kept in plastic bottles in moist soil for pupation, when the adult emerged out they were transferred into emerging cages for egg laying, during that period 40% honey solution was provided for feeding, the solution was changed on an alternate days. The fresh laid eggs were counted and transferred into petri dishes having blotting papers. Fresh food was provided to the newly hatched larvae daily.

The observations were recorded on surviving insects after the treatments with sub-lethal effects of neem oil and recorded the mortality, duration of life cycle, larval growth, pupal weight, pupal period, sex ratio, copulation time, fecundity and fertility as suggested by [13].

### 2.3 Statistical analysis

Finally, the data was analyzed for checking the analyses of variance (ANOVA) and least significant difference (LSD) with the help of SXW software, 8.1, version (USA).

## 3. Results

### 3.1 Effect of neem oil on biology of *S. litura* and larval mortality

The research studies were directed to assess the adequacy of neem oil on the biology of *Spodoptera litura* to examine its rate of larval mortality, larval weight reduction percent, larval period, pupal period, pupal weight, development time, grown-up life span, total life period, sex proportion, sexual intercourse time, fecundity, fertility under laboratory facility conditions. The mortality percentage after 24 hours of *S. litura* was changed significantly (F= 8.30; DF=4, 38, P=0.003) after utilization of neem oil on various doses. At first dose, the most elevated mortality (10.00 at 2500 ppm) percentage was recorded followed by (6.67 at 2000 ppm), (3.33 at 1500 ppm), (3.33 at 1000 ppm) and (0.000 at 500 ppm). At dose two, the most elevated mortality rate following 48 hours was recorded at (23.33 at 2000 ppm) followed by (20.00 at 1500 ppm), (14.33 at 2500 ppm), (10.00 at 100 ppm) and (3.33 at 500 ppm). All the treatments were fundamentally not the same as each other.

The most noteworthy mortality rate following 72 hours was recorded after utilization of at dose three (43.33 at 2500 ppm) followed by (33.33 at 2000 ppm), (26.67 at 1500 ppm), (20.00 at 1000 ppm) and (10.00 at 500 ppm). All treatments were altogether not the same as each other. Along these lines, at dose four, the most extreme mortality rate following 96 hours was recorded after application (50.00 at 2500 ppm) followed by (40.00 at 2000 ppm), (33.33 at 1500 ppm), (20.00 at 1000ppm) and (13.33 at 500ppm). All treatments were discovered altogether not quite the same as each other. Though; the most noteworthy mortality rate following one week was recorded after application at dosage five (50.00 at 2500 ppm) followed by (46.67 at 2000 ppm), (33.33 at 1500 ppm), (23.33 at 1000ppm) and (23.33 at 500 ppm). All treatments at this stage were additionally discovered fundamentally not the same as each other (Table-1).

**Table 1:** Use of neem oil with reduction percentage of *S. litura* larval mortality after 24, 48, 72, 96 hours and one week under laboratory conditions

| Treatment | Dose | Concentration in ppm | (%) Larval mortality Mean + SE |
|---|---|---|---|
| Azadirachtin 24 hours | D1 | 2500 | 10.00±5.77 |
| | D2 | 2000 | 6.67±3.33 |
| | D3 | 1500 | 3.33±3.33 |
| | D4 | 1000 | 3.33±3.33 |
| | D5 | 500 | 0.00±0.00 |
| Azadirachtin 48 hours | D1 | 2500 | 14.33±5.67 |
| | D2 | 2000 | 23.33±3.33 |
| | D3 | 1500 | 20.00±5.77 |
| | D4 | 1000 | 10.00±5.77 |
| | D5 | 500 | 3.33±3.33 |
| Azadirachtin 72 hours | D1 | 2500 | 43.33±3.33 |
| | D2 | 2000 | 33.33±3.33 |
| | D3 | 1500 | 26.67±3.33 |
| | D4 | 1000 | 20.00±0.00 |
| | D5 | 500 | 10.00±0.00 |
| Azadirachtin 96 hours | D1 | 2500 | 50.00±5.77 |
| | D2 | 2000 | 40.00±5.77 |
| | D3 | 1500 | 33.33±3.33 |
| | D4 | 1000 | 20.00±0.00 |
| | D5 | 500 | 13.33±3.33 |
| Azadirachtin one week | D1 | 2500 | 50.00±5.77 |
| | D2 | 2000 | 46.67±5.77 |
| | D3 | 1500 | 33.33±3.33 |
| | D4 | 1000 | 23.33±3.33 |
| | D5 | 500 | 23.33±3.33 |
| Control | Distilled water | 00 | 0.00±0.00 |

### 3.2 Effect of neem oil on biology of *S. litura* and larval weight

The larval weight reduction percentage following 24 hours of *S. litura* fluctuated non-significantly (F=11.14; DF=4, 89; P=0.002) after utilization of neem oil pesticide. At dosage one, the most astounding larval weight reduction percentage of *S. litura* was recorded (10.33 at 25000 ppm) followed by (8.76 at 2000 ppm), (8.52 at 1000 ppm), (7.28 at 1500 ppm) and (6.04 at 500 ppm). All treatments were significantly not the same as each other. At dosage two, the most larval weight decrease percentage following 48 hours were recorded (26.71 at 2000 ppm) took after (26.41 at 1500 ppm), (19.79 at 1000 ppm), (17.09 at 2500 ppm) and (13.07 at 500 ppm). All insecticides were discovered essentially not quite the same as each other. The most elevated larval weight reduction percentage following 72 hours was recorded after use of dose

three (36.27 at 2000 ppm) followed by (32.61 at 1500 ppm), (32.34 at 500 ppm), (28.61 at 1000 ppm) and (28.49 at 2500 ppm). All treatments were discovered essentially not quite the same as each other. At dosage four, the mortality rate following 96 hours of *S. litura* by all doses is significantly not quite the same as each other. The greatest larval weight reduction percentage was recorded after utilization of neem oil (44.92 at 2000 ppm) followed by (39.49 at 1500 ppm), (30.71 at 1000 ppm), (22.98 at 500 ppm) and (10.16 at 2500 ppm). The most astounding larval weight reduction percentage following one week was recorded after application at dose five (50.32 at 2000 ppm) followed by (43.28 at 1500 ppm), (33.11 at 1000 ppm), (31.81 at 500 ppm) and (5.08 at 2500 ppm). All treatments were significantly unique in relation to each other (Table-2).

**Table 2:** Larval weight reduction percentage of *S. litura* (F.) after 24, 48, 72, 96 hours and one week under laboratory conditions

| Treatment | Dose | Concentration in ppm | (%) Larval weight reduction Mean + SE |
|---|---|---|---|
| Azadirachtin 24 hours | D1 | 2500 | 10.03±0.13 |
| | D2 | 2000 | 8.76±0.07 |
| | D3 | 1500 | 7.28±0.03 |
| | D4 | 1000 | 8.52±0.03 |
| | D5 | 500 | 6.00±0.05 |
| Azadirachtin 48 hours | D1 | 2500 | 17.09±0.01 |
| | D2 | 2000 | 26.71±0.09 |
| | D3 | 1500 | 26.41±0.08 |
| | D4 | 1000 | 19.69±0.02 |
| | D5 | 500 | 13.07±0.02 |
| Azadirachtin 72 hours | D1 | 2500 | 28.49±0.10 |
| | D2 | 2000 | 36.27±0.10 |
| | D3 | 1500 | 32.61±0.02 |
| | D4 | 1000 | 28.61±0.05 |
| | D5 | 500 | 32.34±0.24 |
| Azadirachtin 96 hours | D1 | 2500 | 10.16±0.20 |
| | D2 | 2000 | 44.92±0.07 |
| | D3 | 1500 | 39.49±0.06 |
| | D4 | 1000 | 30.71±0.06 |
| | D5 | 500 | 22.98±0.03 |
| Azadirachtin one week | D1 | 2500 | 5.08±0.26 |
| | D2 | 2000 | 50.32±0.06 |
| | D3 | 1500 | 43.28±0.03 |
| | D4 | 1000 | 33.11±0.10 |
| | D5 | 500 | 31.81±0.04 |
| Control | Distilled water | 00 | 0.00±0.00 |

### 3.3 Effect of neem oil on different life stages of *S. litura*

The larval time of *S. litura* was found significantly different (F= 991.02; DF=4, 48, P=0.000) after use of neem oil. The most extreme larval time of *S. litura* was recorded in (18.13 at 2500 ppm) followed by (17.23 at 2000 ppm), (16.40 at 1500 ppm), (15.47 at 1000 ppm), (14.70 at 500 ppm) and control (14.23 at 00 ppm). All treatments were significantly not quite the same as each other. The pupal time of *S. litura* fluctuated significantly (F= 292.88; DF=4, 48, P=0.000) after utilization of neem oil. The most elevated pupal time of *S. litura* was recorded in (9.23 at 2500 ppm) followed by (8.70 at 2000 ppm), (7.93 at 1500 ppm), (7.50 at 1000 ppm), (7.10 at 500 ppm) and control (6.80 at 00 ppm). All treatments were significantly not different to each other. The pupal weight of *S. litura* differed significantly (F= 0.97; DF=4, 48, P=0.434) after utilization of neem oil. The pupal weight was profoundly diminished (30.43 at 2500 ppm) followed by (28.93 at 2000 ppm), (27.33 at 1500 ppm), (25.97 at 1000 ppm), (24.80 at 500 ppm) and control (24.03 at 00 ppm). All treatments were altogether unique in relation to each other (F= 27.43; DF=5, 89; P=0.002) (Table-3).

**Table 3:** Larval period, pupal period, pupal weight, development time, adult longevity and total life period of *S. litura* (F.) after 24, 48, 72, 96 hours and one week under laboratory conditions

| Treatment | Dose | Concentration in ppm | (%) Larval weight reduction Mean + SE |
|---|---|---|---|
| Larval period | D1 | 2500 | 18.13±0.84 |
| | D2 | 2000 | 17.23±0.51 |
| | D3 | 1500 | 16.40±0.20 |
| | D4 | 1000 | 15.47±0.16 |
| | D5 | 500 | 14.70±0.16 |
| Control | | | 14.23±0.09 |
| Pupal period | D1 | 2500 | 9.23±0.26 |
| | D2 | 2000 | 8.70±0.20 |
| | D3 | 1500 | 7.93±0.12 |
| | D4 | 1000 | 7.50±0.30 |
| | D5 | 500 | 7.10±0.15 |
| Control | | | 6.80±0.15 |
| Pupal weight | D1 | 2500 | 30.43±0.93 |
| | D2 | 2000 | 28.93±0.63 |
| | D3 | 1500 | 27.33±0.59 |
| | D4 | 1000 | 25.97±0.66 |
| | D5 | 500 | 24.80±0.51 |
| Control | | | 24.03±0.34 |
| Development time | D1 | 2500 | 30.43±0.93 |
| | D2 | 2000 | 28.93±0.63 |
| | D3 | 1500 | 27.33±0.59 |
| | D4 | 1000 | 25.97±0.66 |
| | D5 | 500 | 24.80±0.51 |
| | | | 24.03±0.34 |
| Adult longevity | D1 | 2500 | 8.66±0.31 |
| | D2 | 2000 | 8.93±0.14 |
| | D3 | 1500 | 9.86±0.20 |
| | D4 | 1000 | 10.56±0.33 |
| | D5 | 500 | 11.16±0.20 |
| Control | | | 11.83±0.44 |
| Total life period | D1 | 2500 | 39.03±0.56 |
| | D2 | 2000 | 37.86±0.73 |
| | D3 | 1500 | 37.43±0.17 |
| | D4 | 1000 | 36.53±0.33 |
| | D5 | 500 | 35.96±0.31 |
| Control | Distilled water | 00 | 35.86±0.73 |

### 3.4 Effect of neem oil on sex ratios of males and female of *S. litura*

The formative time of *S. litura* varied significantly (F= 1352.06; DF=4, 48, P=0.000) after application of neem oil. The most extreme developmental time of *S. litura* was recorded in (30.43 at 2500 ppm) followed by (28.93 at 2000 ppm), (27.33 at 1500 ppm), (25.97 at 1000 ppm), (24.80 at 500 ppm) and control (24.03 at 00 ppm). All treatments were significantly different in relation to each other. The adult longevity of *S. litura* differed altogether (F= 84.69; DF=4, 48, P=0.000) after application. The minimum longevity was recorded (8.66 at 2500 ppm) followed by (8.93 at 2000 ppm), (9.86 at 1500 ppm), (10.56 at 1000 ppm), (11.16 at 500 ppm) and control (11.83 at 00 ppm). All treatments were altogether not the same as each other. The aggregate life time was significantly different (F= 749.78; DF=4, 48, P=0.000) after use of neem oil. The most noteworthy aggregate life time of *S. litura* was recorded (39.03 at 2500 ppm) followed by (37.86 at 2000 ppm), (37.43 at 1500 ppm), (36.53 at 1000

ppm), (35.96 at 500 ppm) and control (35.86 at 00 ppm). All treatments were additionally discovered significantly not the same as each other. Therefore, the sex proportion demonstrates that the male of *S. litura* were more susceptible to these treated neem oil treatments at different doses when compared with the females ones (Table-4), respectively.

**Table 4:** Sex ratio of male and female

| Treatment | Dose (ppm) | Total pupae | Emerged | Not emerged | Male (average) | Female (average) | Sex ratio |
|---|---|---|---|---|---|---|---|
| Azadirachtin | 2500 | 15 | 13 | 2 | 4 | 9 | 4:9 |
| | 2000 | 16 | 14 | 2 | 5 | 9 | 5:9 |
| | 1500 | 20 | 19 | 1 | 6 | 13 | 6:13 |
| | 1000 | 23 | 23 | 0 | 7 | 16 | 7:16 |
| | 500 | 23 | 23 | 0 | 6 | 17 | 6:17 |
| Control | 00 | 30 | 30 | 0 | 10 | 20 | 1:2 |

### 3.5 Effect of neem oil on copulation time, fecundity and fertility of *S. litura*

The intercourse time of *S. litura* fluctuated altogether (F= 10238.0; DF=4, 48, P=0.000) after use of neem oil. The minimum sex time of *S. litura* was recorded (25.16 at 500 ppm) followed by (27.66 at 1000 ppm), (29.83 at 1500 ppm), (33.16 at 2000 ppm), (37.83 at 2500 ppm) and control (57.00 at 00 ppm). All treatments were significantly not the same as each other. The fecundity of females were varied significantly (F= 1482.25; DF=4, 48, P=0.000) after application. The most minimal fecundity of females was recorded (505.00 at 2500 ppm) followed by (535.33 at 2000 ppm), (563.33 at 1500 ppm), (611.00 at 1000 ppm), (631.67 at 500 ppm) and control (1178.00 at 00 ppm). All treatments were discovered fundamentally not the same as each other. The fertility percentage of females varied significantly (F= 1572.42; DF=4, 48, P=0.000). The most minimal fertility percentage of females was recorded (56.56 at 2500 ppm) followed by (66.50 at 2000 ppm), (75.01 at 1500 ppm), (78.26 at 1000 ppm), (82.52 at 500 ppm) and control (93.07 at 00 ppm). All the treatments were non-significantly different to each other (F= 0.21; DF=4, 14, P=0.658), respectively (Table-5).

**Table 5:** Copulation time, fecundity, fertility of *S. litura* (F.) after 24, 48, 72, 96 hours and one week under laboratory conditions

| Treatment | Dose | Concentration in ppm | (%) Larval weight reduction Mean + SE |
|---|---|---|---|
| Copulation time | D1 | 2500 | 37.83±0.16 |
| | D2 | 2000 | 33.16±0.33 |
| | D3 | 1500 | 29.83±0.16 |
| | D4 | 1000 | 27.66±0.16 |
| | D5 | 500 | 25.16±0.16 |
| Control | | | 57.00±0.00 |
| Fecundity | D1 | 2500 | 505.00±1.15 |
| | D2 | 2000 | 536.33±7.31 |
| | D3 | 1500 | 563.33±11.33 |
| | D4 | 1000 | 611.00±8.08 |
| | D5 | 500 | 631.67±11.72 |
| Control | | | 1178.00±50.52 |
| Fertility | D1 | 2500 | 56.56±1.74 |
| | D2 | 2000 | 66.50±1.05 |
| | D3 | 1500 | 75.01±1.59 |
| | D4 | 1000 | 78.26±1.19 |
| | D5 | 500 | 82.52±0.29 |
| Control | Distilled water | 00 | 93.07±5.01 |

## 4. Discussion

The larvae were taken from vegetable field cultivated area close to the research laboratory, which were raised on cauliflower leaves under lab conditions to improve the further research experiments. The neem oil treatment created mortality in larval stage that lessened the pupal weight and sex ratio, sexual intercourse time, fecundity and fertility when compared with the control. The research findings were in agreement with the [14] who found its control that has depended predominantly on use of different insecticides sprays however; the unpredictable utilization of different insecticide sprays has brought about the improvement of high level of imperviousness to chlorinated hydrocarbons, cyclodienes, organophosphates, carbamates and engineered pyrethroids. *S. litura* is the main Lepidopterous pest and second horticultural pest that created invulnerability to pest sprays in India. Spodoptera sp. positions the main 20 most safe pest species [15] that created immunity to numerous pest sprays [16, 17].

In this research study, the biology of *S. litura* (F.) influenced by the effectiveness of neem oil, Azadirachtin in which, the larval mortality and weight reduction percentage was recorded following at 24, 48, 72, 96 hours and one week as well. All outcomes were depended over different doses, the most extreme mortality and larval weight reduction percentage was recorded in higher dosages when contrasted with lower once following 24 and 48 hours. The mortality, and in addition larval weight decrease, did not record in charge but rather their larval weight expanded. The pupal weight of regarded hatchlings additionally reduced when compared with control. The length of the life cycle as larval and pupal periods and developmental time expanded by the utilization of neem oil when compared with control, the males' were discovered more vulnerable when compared with females. The copulation time, fecundity and fertility were additionally diminished when compared with control. Due to sub-lethal, effects of insecticides, the term of life cycle expanded, pupal weight, copulation time, adult longevity weight pick up (nourishment utilization) reduced when compared with control trial [18]. The trials led on sub-lethal reported by [19] that the both span and advancement of hatchlings and pupae of *S. exigua* were delayed and larval, pupal weight diminished, and pupation rate, fecundity and fertility were reduced when compared with the control.

The insects were influenced both adversely and emphatically by the utilization of neem oil as indicated by [20] who examined on the sub-lethal impacts of carbaryl, monocrotophos, and endosulfan on *S. litura* (F). The leaves were treated with sub-lethal concentrations of this bio pesticide. Further, the research study was led by [21,22] who described that the sub-lethal concentrations of indoxacarb on *P. xylostella* were additionally successful under lab conditions though; in this research, every one of the parameters were likewise influenced by the use of neem oil with various concentrations when compared with un treated trial. Subsequently, the another research described by [6] who described that among every one of the insectides, PONNEEM

recorded the most extreme antifeedant action (88.6%) at 0.6% with neem and karanj singular insecticide stretched out larval length when compared with control. Like this, in this research finding the pupal weight and fecundity were decreased and pupal period was significantly expanded. It is additionally suggested that neem oil ought to be utilized as a part of modification with different mixes against *S. litura* (F.) to maintain a strategic distance from resistance component. A similar investigation ought to be rehashed under field conditions so far. More insect development controllers (insect sprays) ought to be tried for their sub-lethal consequences for biology of *S. litura* with the neem oil for its better control measures.

## 5. Conclusion

In our review, the biology of *S. litura* (F.) decidedly influenced by the near proficiency of neem oil brought about mortality, lessening in larval and pupal weight, expanded span of life cycle as larval and pupal periods; formative time and total life period, diminished lovemaking time, fecundity and fertility. From the outcomes specified above research findings it is concluded that the neem oil was effective by their sub-lethal concentrations on *S. litura* (F.). Because of ecological safety that could be viewed as appropriate for IPM program as a result of bio pesticide, which is not successful to the effective to the beneficial insects, animals and individuals to human beings. So there is dire need to investigate the sub-lethal effects after generations of this pest under laboratory and field conditions.

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