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***Research Article***

# TOXICITY OF NEEM AGAINST DIFFERENT LARVAL INSTARS OF *SPODOPTERA LITURA*

***Sudha Summarwar**
*Department of Zoology S.D. Government College Beawar (Rajasthan)*
*M.D.S. University Ajmer India*
**Author for Correspondence*

## ABSTRACT
The Present investigation was carried out to ascertain toxicity of Neem against different larval instars of *Spodoptera litura*. For this purpose, freshly emerged 3rd, 4th, 5th, and 6th instar larvae of uniform size and age were collected from the mass culture maintained in the laboratory. Each instar larvae were treated separately with the leaf and seed extracts of *Azadirachta indica*. Highest larvicidal activity was shown by seed extract of *A.indica* followed by leaf extracts of *A.indica*. Direct toxicity of plant is probably due to the presence of complex mixture of active compounds like alkaloids, flavenoids and terpenoids etc. Maximum mortality occurred during metamorphosis and moultings. This is because the poorly sclerotised cuticle of larvae fails to resist the penetration of the extracts in the body which results in death.

***Keywords:*** *Larval Instars, Spodoptera Litura, Toxicity, Neem*

## INTRODUCTION
Several plant species of family Meliaceae possessing terpenoids have been reported to show insecticidal properties (Simmonds *et al.,* 1990). *Azadirachta indica,* have been reported earlier to possess insecticidal, growth regulatory and behaviour disrupting properties (Govindhari *et al.,* 1996). Siddiqui (1986) extracted three bitter compounds from neem oil and named them as nimbin, nimbinin and nimbidin. A complex secondary metabolite, azadirachtin isolated from seeds of *A.indica* has been reported to affect several physiological processes of insects and also have direct toxic effects on different tissues (Schluter *et al.,* 1985, Nasirudin and Mordue (Luntz) 1993). Larvicidal and pupicidal effects are dose-dependant and at higher concentrations mortality induced was high. Similar dose dependant effects were reported by Martinez and Van Emden (2001).

On most crops, damage arises from extensive feeding by larvae, leading to complete stripping of the plants. Larvae are leaf eaters but sometimes act as a cutworm with crop seedlings. If heavy feeding on a young plant occurs, it may lead to stunted development and fruit may be small or late to develop (USDA, 2005). *Spodoptera litura* feeds on the underside of leaves causing feeding scars and skeletonization of leaves.

Early larval stages remain together radiating out from the egg mass. However, later stages are solitary. Initially there are numerous small feeding points, which eventually spread over the entire leaf. Because of this pest's feeding activities, holes and bare sections are later found on leaves, young stalks, bolls, and buds. Keeping the facts in view the preset study was conducted at Toxicity of Neem against different larval instars of *Spodoptera litura.*

## MATERIALS AND METHODS
### *Method*
*Larvicidal Action:* Freshly emerged 3rd, 4th, 5th, and 6th instar larvae of uniform size and age were collected from the mass culture maintained in the laboratory. Each instar larvae were treated separately with the leaf and seed extracts of *Azadirachta indica*.

*Method of Treatment:* 1. Leaf application method
2. Topical application method

*Leaf Application Method:* The oral or systemic toxicity of the plant extracts was investigated against 3rd, 4th, 5th, and 6th instar larvae through no-choice bioassay using leaf discs (6 cm in diameter) prepared from

cabbage leaves. Test formulation was prepared by dissolving the extract in distilled water and adding Tween-80 as an emulsifier.

For treatment, each leaf disc was dipped for 1 minute in the extract solution at each concentration, air dried to evaporate solvent and then placed in a plastic container. A moist filter paper was kept below the leaf disc to prevent it from drying. The plastic container was covered with fine muslin cloth held with rubber band. Three replicates each with 10 larvae were maintained for each treatment. Larval mortality was observed after 48 hours of treatment and percent mortality was corrected by Abbott's formula (1925).

*Topical Application:* Larvae were collected from rearing stock and were kept in ventilated plastic containers (20 cm diameter and 8 cm in height) for the bioassay. For topical application, 2 ul of the solvent extract was applied topically on each larva with the help of a micropipette. After treatment the larvae were released in the plastic container containing cabbage leaves. Three replicates were run for each concentration per solvent extract and controls treated with solvent were kept in each experiment. Ten larvae were treated in each replicate. Larval mortality was observed after 48 hours of treatment. Percent mortality was calculated and corrected using Abbott's formula (Abbott, 1925). The correction was done only when the death in control groups was between 5-20%.

$$\text{Abbott's corrected mortality} = \frac{\%\ \text{mortality in control} \ - \ \%\ \text{mortality in treated}}{100 - \%\text{mortality in control}}$$

## RESULTS AND DISCUSSION

### *Result*

*Larvicidal Action: Experiments* with 1st and 2nd instar larvae of *Spodoptera litura* were not conducted due to their smaller size, soft body and high susceptibility. The 3rd, 4th, 5th and 6th instar larvae being most destructive were selected for the study and were treated with different concentrations of the leaf extracts and seed extract of *Azadirachta indica*.

*Leaf Extract:*

*Third Instar Larvae:* Leaf extract of *Azadirachta indica* showed significant larval mortality at all the concentrations tested ranging from 0.1 to 2.0% (Table 1). Highest mortality of 100 percent was observed at 2% concentration when larvae were treated by topical application method. At same concentration, 94.66 percent mortality was recorded in larvae treated by leaf-disc application method. At 0.1% extract mortality recorded was 27.00 and 33.33 percent in larvae treated by leaf-disc and topical application experiments respectively. In control, mortality observed was 4.5 and 4.45 percent in leaf-disc and topical treatment experiments respectively.

*Fourth Instar Larvae:* Maximum mortality of 88.00 and 90.66 percent was observed in larvae treated at 2% by leaf-disc and topical application methods respectively (Table 1). At 0.1% concentration larval mortality observed was 17.33 and 29.30 percent in leaf-disc treatment and topical application methods respectively. Mortality in control experiments was recorded as 3.35 and 3.53 percent in leaf-disc and topical application experiments respectively.

*Fifth Instar Larvae:* Fifth instar larvae were observed to be slightly less susceptible in comparison to earlier instars. At 2% concentration, mortality of 86.66 and 87.33 percent was recorded when larvae were treated by leaf-disc application and topical application methods respectively (Table 1). Minimum mortality observed was 15.33 and 22.66 percent at the concentration of 0.1% in leaf-disc and topical application methods respectively. Control mortality observed was 3.35 percent in both leaf-disc and topical application experiments respectively.

*Sixth Instar Larvae:* Percent larval mortality recorded was 85.33 and 78.65 at the concentration of 2% when treated by topical and leaf-disc application methods respectively (Table 1). At 0.1% concentration mortality observed was 21.33 and 12.33% in topical treatment and leaf-disc treatments respectively. Mortality recorded in control was 2.05 and 2.15 percent in topical application and leaf-disc treatment experiments respectively.

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**Table 1: Toxicity of *Azadirachta Indica* Leaf Extract against Different Larval Instars of *Spodoptera Litura***

| Doses in % | Percent Mortality During Larval Instars | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | III Instar Larvae | | | | IV Instar Larvae | | | | V Instar Larvae | | | | VI Instar Larvae | | | |
| | Leaf-Disc Application | | Topical Application | | Leaf-Disc Application | | Topical Application | | Leaf-Disc Application | | Topical Application | | Leaf-Disc Application | | Topical Application | |
| | Percent Mortality | Corrected Mortality | Percent Mortality | Corrected Mortality | Percent Mortality | Corrected Mortality | Percent Mortality | Corrected Mortality | Percent Mortality | Corrected Mortality | Percent Mortality | Corrected Mortality | Percent Mortality | Corrected Mortality | Percent Mortality | Corrected Mortality |
| 0.1 | 27 | 23.56 | 33.33 | 30.22 | 17.33 | 14.46 | 29.3 | 26.71 | 15.33 | 12.2 | 22.66 | 19.85 | 12.33 | 10.44 | 21.33 | 19.68 |
| 0.5 | 38 | 35.07 | 46.66 | 44.17 | 31 | 28.6 | 41.29 | 39.14 | 31 | 28.45 | 38.66 | 36.43 | 28.66 | 27.12 | 34.66 | 33.29 |
| 1 | 50 | 47.64 | 59.33 | 57.43 | 47 | 45.16 | 50.6 | 48.79 | 45 | 42.96 | 46.6 | 44.66 | 41 | 39.73 | 44 | 42.82 |
| 1.5 | 70.66 | 69.27 | 80 | 79.06 | 68.66 | 67.57 | 68 | 66.82 | 61.33 | 59.9 | 67.58 | 66.4 | 57.3 | 56.38 | 64.33 | 63.58 |
| 2 | 94.66 | 94.4 | 100 | 100 | 88 | 87.58 | 90.66 | 90.31 | 86.66 | 86.66 | 87.33 | 86.87 | 78.65 | 78.19 | 85.33 | 85.02 |
| Control | 4.5 | | 4.45 | | 3.35 | | 3.53 | | 3.35 | | 3.35 | | 2.1 | | 2.05 | |
| F-Value | 117.25 | | 213.45 | | 128.33 | | 125.66 | | 109.66 | | 224.44 | | 214 | | 99.55 | |
| CV at 5% | 3.66 | | 3.61 | | 2.88 | | 3.55 | | 3.61 | | 3.61 | | 3.34 | | 3.61 | |

**Table 2: Toxicity of *Azadirachta Indica* Seed Extract against Different Larval Instars of *Spodoptera Litura***

| Doses in % | Percent Mortality During Larval Instars | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | III Instar Larvae | | | | IV Instar Larvae | | | | V Instar Larvae | | | | VI Instar Larvae | | | |
| | Leaf-Disc Application | | Topical Application | | Leaf-Disc Application | | Topical Application | | Leaf-Disc Application | | Topical Application | | Leaf-Disc Application | | Topical Application | |
| | Percent Mortality | Corrected Mortality | Percent Mortality | Corrected Mortality | Percent Mortality | Corrected Mortality | Percent Mortality | Corrected Mortality | Percent Mortality | Corrected Mortality | Percent Mortality | Corrected Mortality | Percent Mortality | Corrected Mortality | Percent Mortality | Corrected Mortality |
| 0.1 | 37.33 | 34.37 | 41.33 | 38.59 | 33.33 | 30.91 | 35.35 | 33.1 | 30.65 | 28.39 | 30.66 | 28.47 | 17.38 | 15.52 | 26.65 | 25.11 |
| 0.5 | 50.65 | 48.32 | 50.65 | 48.35 | 46.65 | 44.71 | 47.66 | 45.84 | 45.3 | 43.52 | 40 | 38.11 | 37.4 | 35.99 | 34 | 32.61 |
| 1 | 62.68 | 60.92 | 69.33 | 67.9 | 58.59 | 57.08 | 62.66 | 61.36 | 54.66 | 53.18 | 54.68 | 53.25 | 50.59 | 49.47 | 44 | 42.82 |
| 1.5 | 82.59 | 81.76 | 86.66 | 86.03 | 74.66 | 73.74 | 78.66 | 77.92 | 70.59 | 69.63 | 77.33 | 76.61 | 58.66 | 57.73 | 73.38 | 72.82 |
| 2 | 100 | 100 | 100 | 100 | 97.33 | 97.23 | 97.33 | 97.23 | 93.33 | 93.11 | 94.66 | 94.49 | 90.66 | 90.44 | 93.33 | 93.19 |
| Control | 4.5 | - | 4.45 | | 3.50 | | 3.35 | | 3.15 | | 3.05 | | 2.2 | | 2.05 | |
| F-Value | 176.14 | | 117.94 | | 425 | | 108.59 | | 173.24 | | 550 | | 176.14 | | 215.11 | |
| CV at 5% | 3.61 | | 3.34 | | 3.61 | | 3.61 | | 3.61 | | 3.61 | | 3.61 | | 3.61 | |

*Seed Extract*

*Third Instar Larvae:* A maximum mortality of 100 percent was observed when larvae were treated by topical and leaf-disc application methods at 2% concentration (Table 2). At 0.1% extract mortality recorded was 41.33 and 37.33 percent in larvae treated by topical and leaf-disc application methods repectively. In control 4.45 and 4.50 percent mortality was observed in topical and leaf-disc applications respectively.

*Fourth Instar Larvae:* Percent mortality at 2% concentration was recorded as 97.33 when larvae were treated by both leaf-disc and topical treatments (Table 2). Mortality at 0.1% concentration was 35.35 and 33.33 percent in larvae treated by topical and leaf-disc applications respectively. Larval mortality in control experiments was 3.35 and 3.50 in topical and leaf-disc experiments respectively.

*Fifth Instar Larvae:* 94.66 and 93.33 percent mortality was observed at highest concentration of 2% when larvae were treated by topical and leaf-disc methods respectively (Table 2). At 0.1% concentration mortality recorded was 30.66 and 30.65 percent in larvae treated by topical and leaf-disc application methods respectively. Control mortality was 3.05 and 3.15 in topical and leaf-disc treatments respectively.

*Sixth Instar Larvae:* Maximum mortality of 93.33 and 90.66 percent was recorded at 2% concentration in larvae treated by topical and leaf-disc treatments respectively (Table 2). At 0.1% mortality induced was minimum of 26.65 and 17.38 percent in topical and leaf-disc treatments respectively. In control percent mortality recorded was 2.05 and 2.20 in topical and leaf-disc treatments respectively.

***Discussion***

From the results, it is evident that the plant tested possessed significant larvicidal properties and caused high mortality in larvae of *Spodoptera litura.* Mortality in larvae and pupae may be due to general toxicity of the chemical compounds present in the plant extracts. Highest larvicidal activity was shown by seed extract of *A.indica* followed by leaf extracts of *A.indica*. Direct toxicity of plant is probably due to the presence of complex mixture of active compounds like alkaloids, flavenoids and terpenoids etc. Maximum mortality occurred during metamorphosis and moultings. This is because the poorly sclerotised cuticle of larvae fails to resist the penetration of the extracts in the body which results in death.

According to Park *et al.,* (2000), the toxicity of most of the plant extracts could be attributed to the presence of certain toxic compounds, like alkaloids, flavenoids, tannins, terpenoids, phenols, steroids etc, which interfere with the normal metabolism and metamorphosis. Eliman *et al.,* (2009) suggested that compounds present in plants may individually or collectively contribute to produce larvicidal, pupicidal, adult emergence inhibition and other bioactivities against insects.

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