



BIOTOXICITY ASSAY OF NEEM (*Azadirachta indica*) PRODUCTS AND DISTILLERY EFFLUENT ON THE THIRD INSTAR LARVAE OF COCONUT RHINOCEROS BEETLE *Oryctes rhinoceros*

K. MOHAN*¹ AND A. M. PADMANABAN²

¹.PG and Research Department of Zoology, Sri Vasavi College, Erode – 638 316. Tamil Nadu, India.

².Associate Professor, PG and Research Department of Zoology, Sri Vasavi College, Erode – 638 316. Tamil Nadu, India.

ABSTRACT

The coconut Rhinoceros beetle (*Oryctes rhinoceros*) is one of the most damaging insect to coconut palms in India. The experimental study on the growth response of 3rd instar larvae of *Oryctes rhinoceros* infesting the coconut trees. The LC₅₀/96 hours value for the larvae of *Oryctes rhinoceros* were 29.5% for neem cake powder, 24.5% for neem oil and 14.9% for distillery effluent respectively. In the present study, it was observed that the entire larval growth was affected with this bio-pesticides and distillery effluent irrespective of its experimental concentrations. The reduced weight of the larval forms exposed to different concentrations of bio-pesticides and distillery effluent were clear indication of their deterrent activity and they totally affect the larval growth, since no one was undergone pupation. Even mortality occurred at the midst of this experimental duration in higher concentration. The overall data indicated that the distillery effluent caused antifeedent activity ultimately leading to deterrant activity, of larval forms.

KEY WORDS: Rhinoceros beetle, Neem Oil, Neem Cake, Distillery Effluent, 3rd Instar larvae.



K. MOHAN

PG and Research Department of Zoology, Sri Vasavi College,
Erode – 638 316. Tamil Nadu, India.

*Corresponding author

INTRODUCTION

Rhinoceros beetle is a major pest of coconut endemic to coconut growing regions of Asia and was accidentally introduced in to Fiji in early 1953 where it caused devastating damage to coconut palms¹. *Oryctes rhinoceros* is distributed throughout Asia and in western pacific. Bedford² reviewed the historical account of this species in Burma and the pest first appeared in the extreme south of the peninsula. It probably entered from Malaysia during 1895 and worked its way north throughout the coconut growing areas of lower Burma over the following 15 years³. The Rhinoceros beetle (or) Rhino beetle belongs to the subfamily (dynastinae) and is part of the family of scarab beetles (scarabaeidae). Rhinoceros beetles are among the largest of beetles in the world. The Rhinoceros beetle is aptly named because it has horn on its head very much like the rhinoceros does and it reaches 60mm in length. In India damage of inflorescence is also reported in severely infested areas which cause reduction in yield up to 10% level⁴. Ramachandran *et.al.*,⁵ has been reported a loss in yield of 5.5 to 9.1 % due to beetle attack. From artificially pruned leaf damage stimulation studies, it was observed that damage to 50% fronds corresponds to leaf area reduction of 13% and decrease in nut yield by 23% level⁶. Effective control measures have been developed which involve both cultured and biological methods⁷.⁸. A specific baculovirus of *Oryctes* has been successfully established on islands where it has previously been absent, usually reducing beetle populations well below pre-introduction levels^{9, 10}. Female beetles lay about 108 eggs¹¹. They are white brown in color and oval in shape. The eggs are 3 to 4mm long and take 8 to 12 days to hatch. Developmental period of 1st instar larva is 10 to 21 days, 2nd instar larva is 12 to 21 days, 3rd instar larva is 60 to 165 days, pre pupae is 8 to 13 days and pupae is 17 to 28 days^{12, 13}. In India, average adult longevity is about 4 to 7 months and fecundity per female is 108 eggs. Many neem extracts were reported to have pesticidal activity¹⁴. Many extract of neem and their isolated products have been tested as an insecticide. According to Rembold¹⁵ neem

products are effective on growth and metamorphosis of insects. Schmutterer¹⁶ reported that the neem products have adverse effect on endocrine system of bean beetles and may cause sterility in the female insects. Neem products are derived from the neem tree *Azadirachta indica*. Neem pesticide products are usually made by crushing neem seeds, then using water or a solvent such as alcohol to extract the pesticidal constituents. Neem cake is the residual seed meal remaining after extraction of oil from seeds. Neem cake is an excellent organic fertilizer. The high Azadirachtin content in neem cake protects against parasites, nematodes and enriches the soil. Indiscriminate use of pesticides for the eradication of pests causes tremendous changes to the environment and also to other non-target organisms. To prevent such contamination of the environment and pests, phytopesticides are in current use. These phytopesticides are cost effective, ecofriendly, safe and sustainable in the field of agriculture¹⁷. In modern days the application of pesticides, weedicides, herbicides to the agriculture fields for more than the past 50 years which created lot of problems in aquatic and soil system. Hence the present work was undertaken to study the bio-pesticides (neem products) and distillery effluent to control the 3rd instar larvae of *Oryctes rhinoceros*.

MATERIALS AND METHODS

COLLECTION OF LARVAE

The test organism chosen for the present investigation was the 3rd instar larvae of *Oryctes rhinoceros* were collected in different places such as Suriyampalayam, Lakkapuram, Pudhuvalasu and Veppadai. The larvae were collected from decaying, semi decaying cattle manure and composed organic manure. The size and weight of the larvae were more or less equal (10-20g).

CHARACTERISTIC OF LARVAE OF *Oryctes rhinoceros*

The newly hatched larva was creamy white and measures about 10-15 mm in length and 2-4 mm in width. They feed on decaying

organic matter. The Mean body length and width are 23-32 mm and 7.71 mm respectively for the 3rd instar. The 3rd instar larvae were stout, sub cylindrical "C" shaped and dirty white in colour. They have short pairs of thoracic legs; soft fleshy body without abdominal legs, the head was dark brown with strong mandibles. The 3rd instar larvae have robust mouth parts and initially attack tough causing significant damage and creating varied geometrical forms of galleries in it layers.

THE EXPERIMENTAL PRODUCTS (NEEM CAKE POWDER, NEEM OIL AND DISTILLERY EFFLUENT)

The neem cake powder was collected from the Sathya neem oil mill in Chithode a place nearby Erode city, Tamil Nadu, India. The neem cake powder consist two type of nature. The powder had with oil and other one was without oil content. In this work the collected neem cake powder was without oil. The total amount of neem cake powder was 50 kg. The neem oil was collected from the Sathya neem oil mill in Chithode. The total amount of neem oil used was 5 liters. The distillery effluent was collected from the distillery factory at Periya Puliur, Erode District. The distillery Effluent consists of dirty odour and black in colour. The total amount of distillery effluent used was 5 liters and was diluted in 1:1 ratio.

PREPARATION OF SUBSTRATUM

Well decomposed organic matter (paddy straw and soil) was used as the substratum. The decomposed organic matter was filled in experimental wooden boxes (L=50cm; B=50cm; H=50cm). The experimental boxes were filled with 5kg of decomposed organic mixture along with each bio-pesticide such as neem cake powder, neem oil and distillery effluent and one box was maintained with organic mixture only to serve as control. Each wooden box was labeled as A, B, C&D. For LC₅₀ Experiment and Sub lethal experimental boxes were labeled as E, F, and G&H for the exposure period of 50 days.

EXPERIMENTAL SETUP

The mortality/survival of the experimental and control were recorded. The concentrations at which 50% mortality / survival occurred was

taken as the median lethal concentrations (LC₅₀) for various exposure periods. Ten larvae were introduced in each experimental setup along with the control. The experimental setups were maintained in the laboratory at room temperature 28±4°C. Many preliminary trials were done for the selection of the specific concentrations and doses. After such preliminary experiments wide range of concentrations (5% to 35%) was determined. The narrow ranges of concentration were utilized to find LC₅₀. Based on this value, sub lethal concentrations of bio-pesticides (neem cake powder, neem oil and distillery effluent) were to be taken into consideration for this experiment. The rate of average weight of larvae and the growth rate (weight gain / loss) of larvae were conducted by using the sub lethal concentrations ranging from 1.5% to 3.0% for the exposure period of 50 days. At every 10th day, the total weight of larvae in each experimental setup including the control one was verified and the values were tabulated for further analyses. Further observations were made on the morphological, behavioural changes and also the rate of growth of larva. The pupation of the larvae was also observed.

RESULTS AND DISCUSSION

In the present investigation the beetles were subjected to various concentrations of neem cake, neem oil and distillery effluents. The percentage of mortality of the larva *Oryctes rhinoceros* in different concentrations of Neem cake powder, neem oil and distillery effluent the calculation of LC₅₀ for 96 hours. 29.5% of the neem cake powder kills 50% of the larvae at 96 hours (Table 1 a and Fig 1 a). 24.5% of the neem oil kills 50% of the larvae at 96 hours (table 1 b and Fig. 1 b). 14.9% of the distillery effluent kills 50% of the larvae at 96 hours (table 1 c and Fig. 1 c). It has been observed that the distillery effluent is highly toxic to kill the 3rd instar larvae. Observations of weight changes of larvae in different concentrations of neem cake powder, neem oil and distillery effluent are presented in Tables 2 a, 2 b and 2 c & Fig 2 a, 2 b and 2 c. Observations on the loss / gain of weight of larva with different concentrations of neem cake powder, neem oil and distillery effluent in Table 3 a, 3 b and 3 c.

According to Thomson¹⁸ neem products which could be applied for this experimental *Oryctes rhinoceros*. The neem cake powder was found to influence the growth rate, which could be due to its antifeedent effect and subsequently a deterrent effect. The weight loss could be observed for the larval forms, exposed to different concentrations. The higher concentrations, particularly 2.5% and 3.0 % were found to be more effective. It would perhaps be due to its antifeedent effect, since the neem cake powder is found to be unpalatable for the larvae. The experimental larvae were very lethargic and inactive or immobile when compared to that of control. They further displayed some other morphological variations such as paleness, uneven distribution of pigmentation on the body and more even many parts of the epidermis were found to have the areas of detachment as on indication of alteration on the moulting cycle. When neem cake powder

was applied, the body of the larvae absorbs the neem. These neem active ingredients soon after their entry into larvae body, act as "Ecdysone blockers", which lock the production and release of vital hormones required for moulting. When the neem components especially Azadirachtin enter into the body of larvae, the activity of ecdysone is suppressed and the larva fails to moult, remains in the larval stage and ultimately dies. The results involved in distillery effluent indicate that the individual reared in sub lethal concentration of distillery effluent failed to report positive growth progressive reduction in body weight was observed during the test period. So it is evidenced from the present study that minimum concentration of distillery effluent causes damage to the body of the larvae. Finally concluded, the distillery effluent is highly toxic to the Rhinoceros beetles when compared with Neem cake powder and Neem oil.

Table 1 (a)
The percentage of mortality of the larva *Oryctes rhinoceros* in different Concentrations of Neem cake powder (NCP) and the calculation of LC₅₀ for 96 hours.

| Concentration of NCP in Percentage | No. of grub exposed | Exposure Time and Mortality | | | | Percentage of mortality at 96 hours |
|------------------------------------|---------------------|-----------------------------|----------|----------|----------|-------------------------------------|
| | | 24 hours | 48 hours | 72 hours | 96 hours | |
| 29.0 | 10 | 0 | 0 | 0 | 1 | 10% |
| 29.5 | 10 | 0 | 2 | 3 | 5 | 50% |
| 30.0 | 10 | 2 | 3 | 4 | 6 | 60% |
| 30.5 | 10 | 3 | 4 | 6 | 8 | 80% |
| 31.0 | 10 | 4 | 5 | 6 | 9 | 90% |

Table 1 (b)
The percentage of mortality of the larva *Oysters rhinoceroses* different Concentrations of Neem Oil (NO) and the calculation of LC₅₀ for 96 hours.

| Concentration of NO in Percentage | No. of grub exposed | Exposure Time and Mortality | | | | Percentage of Mortality at 96 hours |
|-----------------------------------|---------------------|-----------------------------|----------|----------|----------|-------------------------------------|
| | | 24 hours | 48 hours | 72 hours | 96 hours | |
| 23.0 | 10 | 0 | 0 | 0 | 0 | 0% |
| 23.5 | 10 | 0 | 0 | 0 | 1 | 10% |
| 24.0 | 10 | 1 | 1 | 2 | 3 | 30% |
| 24.5 | 10 | 3 | 4 | 4 | 5 | 50% |
| 25.0 | 10 | 3 | 4 | 6 | 7 | 70% |

Table 1(c)
The percentage of mortality of the larva *Oysters rhinoceros* in different Concentrations of Distillery Effluent (DE) and the calculation of LC₅₀ for 96 hours.

| Concentration of DE in Percentage | No. of grub exposed | Exposure Time and Mortality | | | | Percentage of Mortality at 96 hours |
|-----------------------------------|---------------------|-----------------------------|----------|----------|----------|-------------------------------------|
| | | 24 hours | 48 hours | 72 hours | 96 hours | |
| 14.7 | 10 | 0 | 0 | 0 | 0 | 0% |
| 14.8 | 10 | 0 | 0 | 1 | 2 | 20% |
| 14.9 | 10 | 2 | 3 | 4 | 5 | 50% |
| 15.0 | 10 | 3 | 3 | 4 | 6 | 60% |
| 15.1 | 10 | 4 | 4 | 5 | 7 | 70% |

Table 2(a)

Observation of average weight of Larva of *Oryctes rhinoceros* Exposed to Different sub lethal concentration of Neem cake Powder at different duration.

| Sl.No | Duration in Days | Concentration of the Medium | | | | |
|-------|------------------|-----------------------------|--------------|--------------|--------------|--------------|
| | | Control | 1.5 % NCP gm | 2.0 % NCP gm | 2.5 % NCP gm | 3.0 % NCP gm |
| 1 | Initial | 15 | 15 | 14 | 13 | 13 |
| 2 | 10 | 17 | 14 | 13 | 12 | 11 |
| 3 | 20 | 18 | 13 | 12 | 11 | 10 |
| 4 | 30 | 20 | 12 | 12 | 11 | 10 |
| 5 | 40 | 21 | 11 | 10 | 9 | 9 |
| 6 | 50 | 22 | 10 | 9 | 8 | 7 |

Table 2(b)

Observation of average weight of Larva of *Oryctes rhinoceros* Exposed to Different sub lethal concentration of Neem Oil at different duration.

| Sl.No | Duration in Days | Concentration of the Medium | | | | |
|-------|------------------|-----------------------------|-------------|-----------|-------------|-------------|
| | | Control | 1.5 % NO ml | 2 % NO ml | 2.5 % NO ml | 3.0 % NO ml |
| 1 | Initial | 20 | 18 | 17 | 18 | 16 |
| 2 | 10 | 22 | 16 | 15 | 14 | 12 |
| 3 | 20 | 23 | 14 | 12 | 12 | 10 |
| 4 | 30 | 25 | 12 | 11 | 11 | 9 |
| 5 | 40 | 27 | 11 | 10 | 10 | 8 |
| 6 | 50 | 28 | 10 | 9 | 8 | 7 |

Table 2(c)

Observation of average weight of Larva of *Oryctes rhinoceros* Exposed to Different sub lethal concentration of Distillery Effluent at different duration.

| Sl.No | Duration in Days | Concentration of the Medium | | | | |
|-------|------------------|-----------------------------|-------------|-------------|-------------|-------------|
| | | Control | 1.5 % DE ml | 2.0 % DE ml | 2.5 % DE ml | 3.0 % DE ml |
| 1 | Initial | 18 | 17 | 15 | 14 | 13 |
| 2 | 10 | 19 | 16 | 14 | 13 | 12 |
| 3 | 20 | 21 | 15 | 12 | 11 | 10 |
| 4 | 30 | 22 | 14 | 11 | 10 | 9 |
| 5 | 40 | 24 | 13 | 10 | 9 | 8 |
| 6 | 50 | 25 | 12 | 9 | 6 | 5 |

Table 3(a)

Growth rate (weight gain/loss) of larva of *Oryctes rhinoceros* exposed in different concentration of Neem Cake Powder

| Sl.No | Duration in Days | Concentration of the Medium | | | | |
|-------|------------------|-----------------------------|--------------|--------------|-------------|--------------|
| | | Control | 1.5 % NCP gm | 2.0 % NCP gm | 2.5 % NCPgm | 3.0 % NCP gm |
| 1 | 10 | +2 (+13.3) | -1(-6.7) | -1(-7.1) | -1(-7.7) | -2(-15.4) |
| 2 | 20 | +3(+20.0) | -2(-13.3) | -2(-14.3) | -2(-15.4) | -3(-23.1) |
| 3 | 30 | +5(+33.3) | -3(-20.0) | -2(-14.3) | -2(-15.4) | -3(-23.1) |
| 4 | 40 | +6(+40.0) | -4(-26.7) | -4(-28.6) | -4(-30.8) | -4(-30.8) |
| 5 | 50 | +7(+46.7) | -5(-33.3) | -5(-35.7) | -5(-38.5) | -6(-46.2) |

Table 3(b)

Growth rate (weight gain/loss) of larva of *Oryctes rhinoceros* exposed in different concentration of Neem Oil

| Sl.No | Duration in Days | Concentration of the Medium | | | | |
|-------|------------------|-----------------------------|-------------|-------------|-------------|-------------|
| | | Control | 1.5 % NO ml | 2.0 % NO ml | 2.5 % NO ml | 3.0 % NO ml |
| 1 | 10 | +2(+10.0) | -2(-11.1) | -2(-11.8) | -4(-22.2) | -4(-25.0) |
| 2 | 20 | +3(+15.0) | -4(-22.2) | -5(-29.4) | -6(-33.3) | -6(-37.5) |
| 3 | 30 | +5(+25.0) | -6(-33.3) | -6(-35.3) | -7(-38.9) | -7(-43.8) |
| 4 | 40 | +7(+35.0) | -7(-38.8) | -7(-41.2) | -8(-44.4) | -8(-50.0) |
| 5 | 50 | +8(+40.0) | -8(-44.4) | -8(-47.1) | -10(-55.5) | -9(-56.3) |

Table 3(c)
Growth rate (weight gain/loss) of larva of *Oryctes rhinoceros* exposed in different concentration of Distillery Effluent

| Sl.No | Duration in Days | Concentration of the Medium | | | | |
|-------|------------------|-----------------------------|-------------|-------------|-------------|-------------|
| | | Control | 1.5 % DE ml | 2.0 % DE ml | 2.5 % DE ml | 3.0 % DE ml |
| 1 | 10 | +1(+5.6) | -1(-5.9) | -1(-6.7) | -1(-7.1) | -1(-7.7) |
| 2 | 20 | +3(+16.7) | -2(-11.8) | -3(-20.0) | -3(-21.4) | -3(-23.1) |
| 3 | 30 | +4(+22.2) | -3(-17.6) | -4(-26.7) | -4(-28.6) | -4(-30.8) |
| 4 | 40 | +6(+33.3) | -4(-23.5) | -5(-33.3) | -5(-35.7) | -5(-38.5) |
| 5 | 50 | +7(+38.9) | -5(-29.4) | -6(-40.0) | -8(-57.1) | -8(-61.5) |

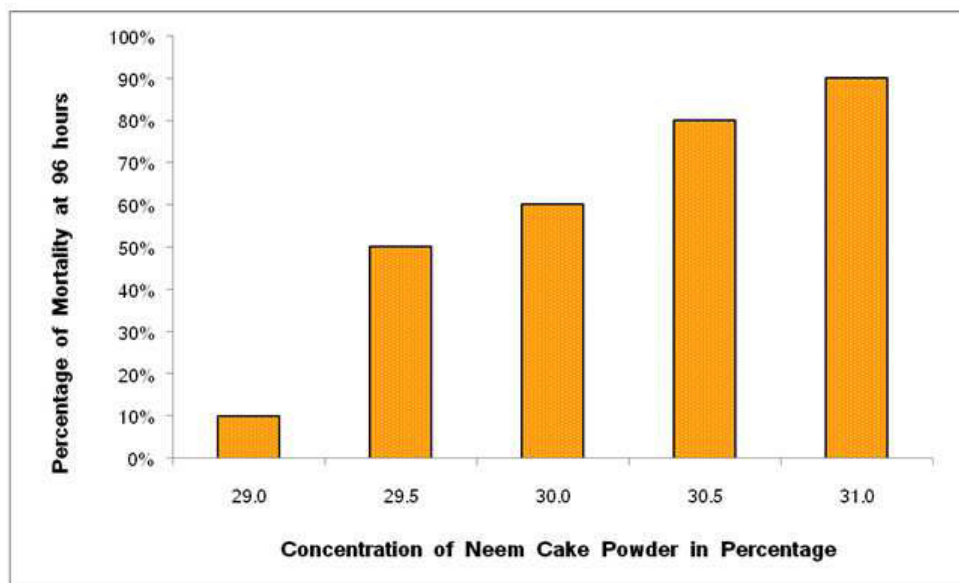


Figure 1 (a)

The percentage of mortality of the larva *Oryctes rhinoceros* different Concentration of Neem cake powder and approximate calculation of LC_{50} for different durations

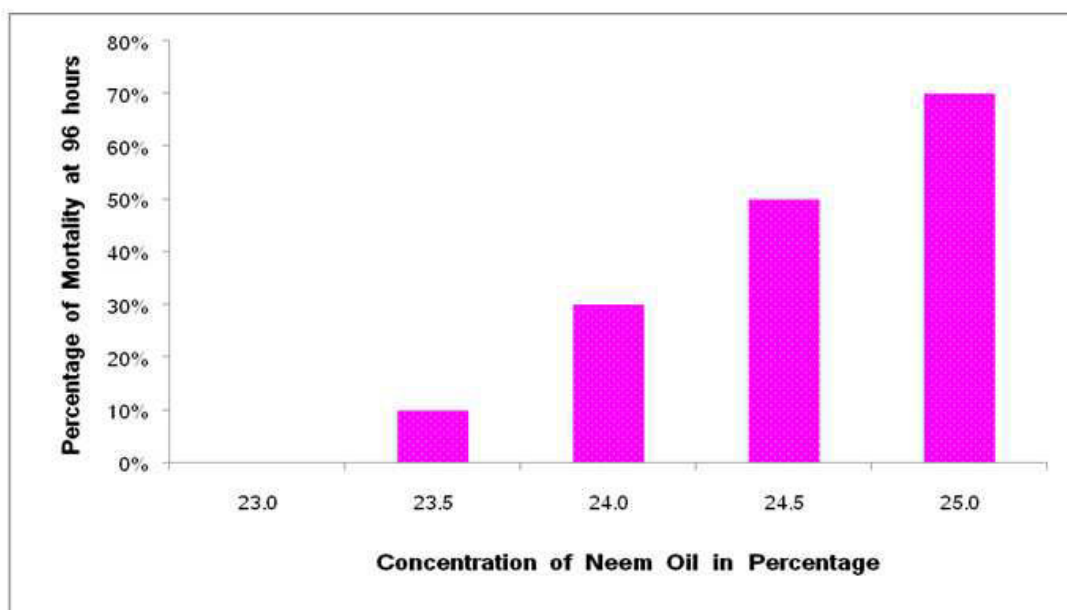


Figure 1 (b)

The percentage of mortality of the larva *Oryctes rhinoceros* different Concentration of Neem oil and approximate calculation of LC_{50} for different durations.

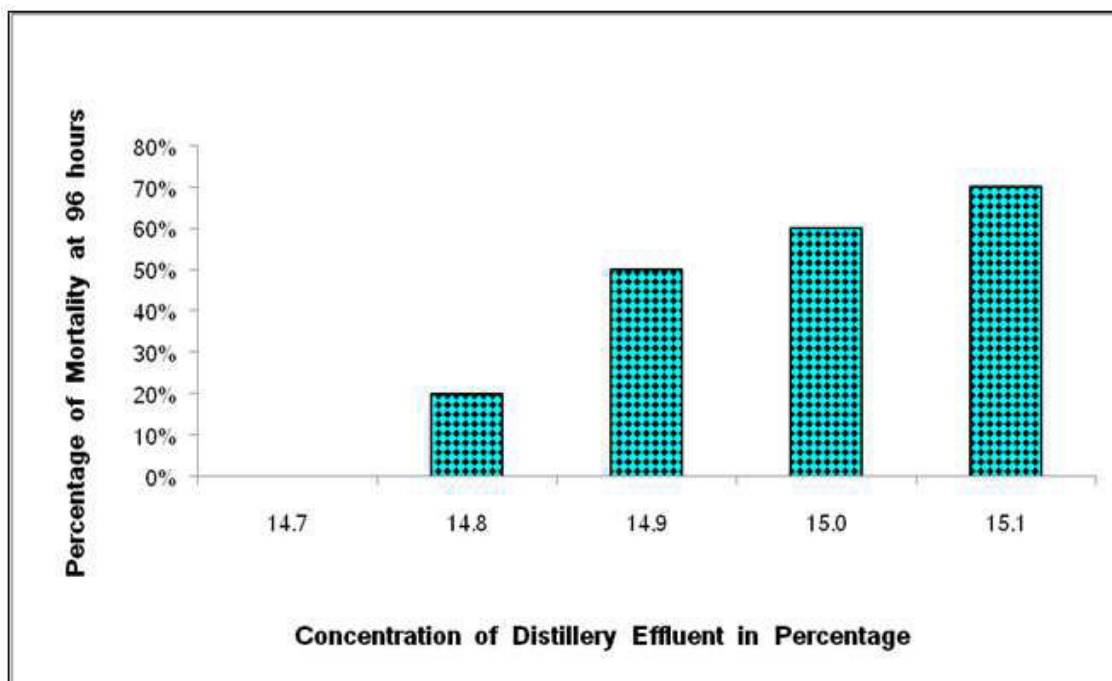


Figure 1 (c)

the percentage of mortality of the larva Oryctes rhinoceros different Concentration of Neem cake powder and approximate calculation of LC₅₀ for different durations

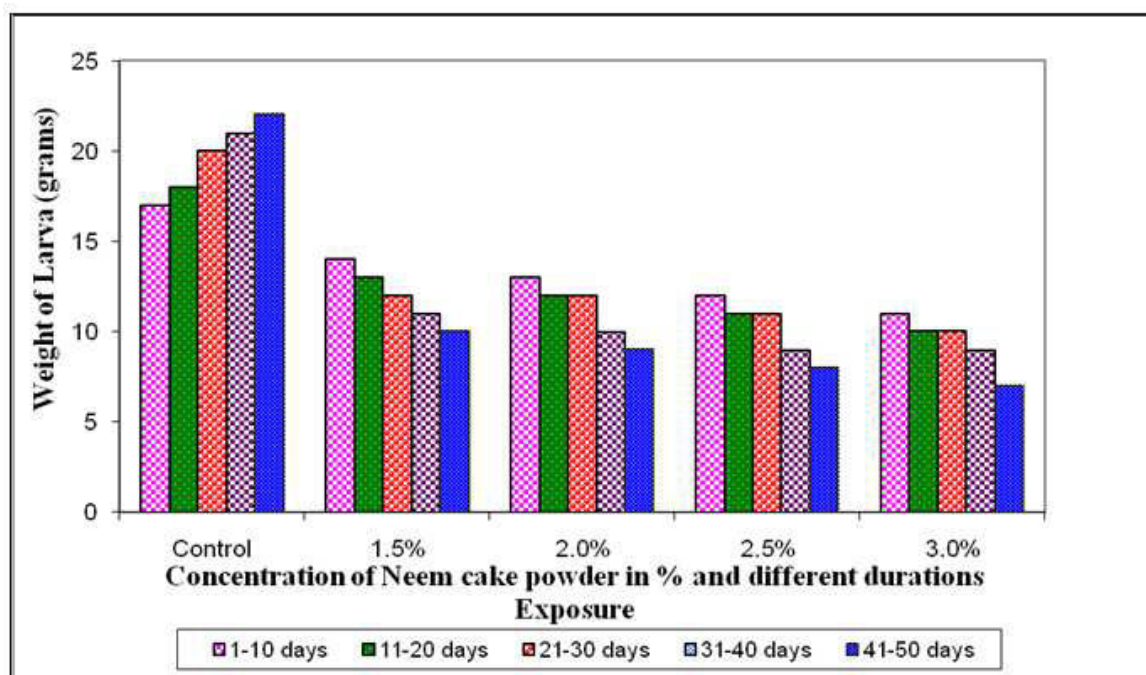


Figure 2 (a)

Observation of average weight of Larva of Oryctes rhinoceros of different duration Concentrations of Neem cake powder and approximate calculation of LC₅₀ for different durations.

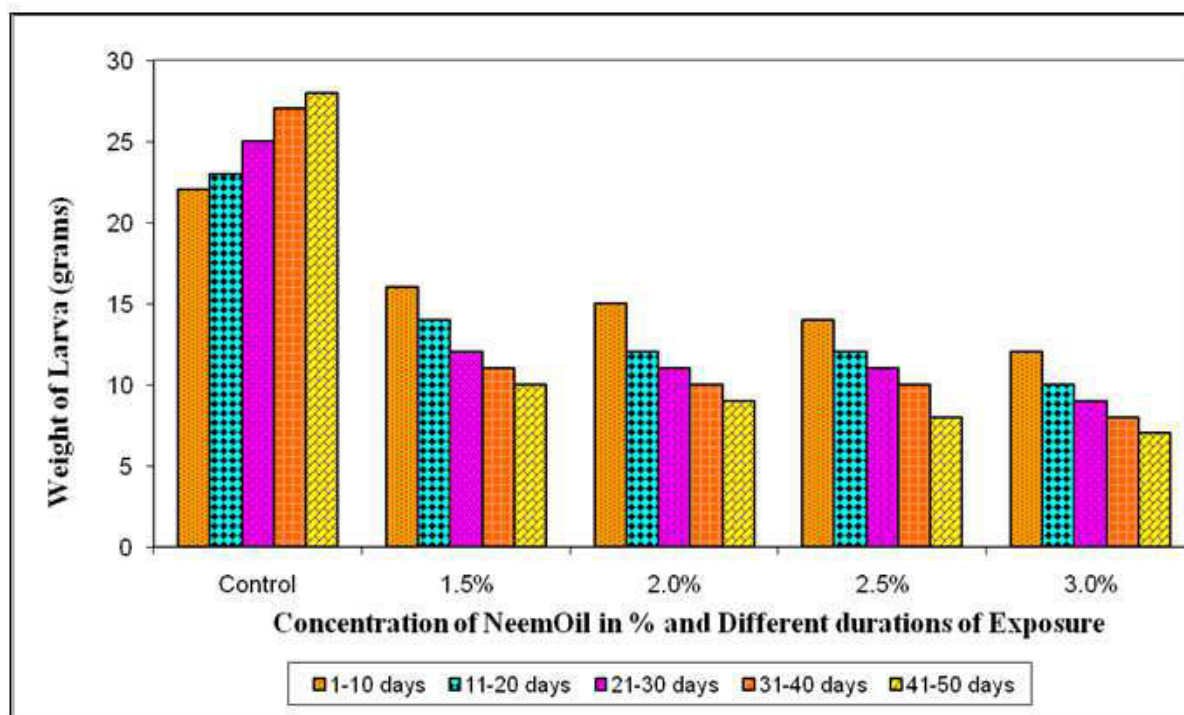


Figure 2 (b)

Observation of average weight of Larva of *Oryctes rhinoceros* of different Concentrations of Neem oil and approximate calculation of LC_{50} for different durations.

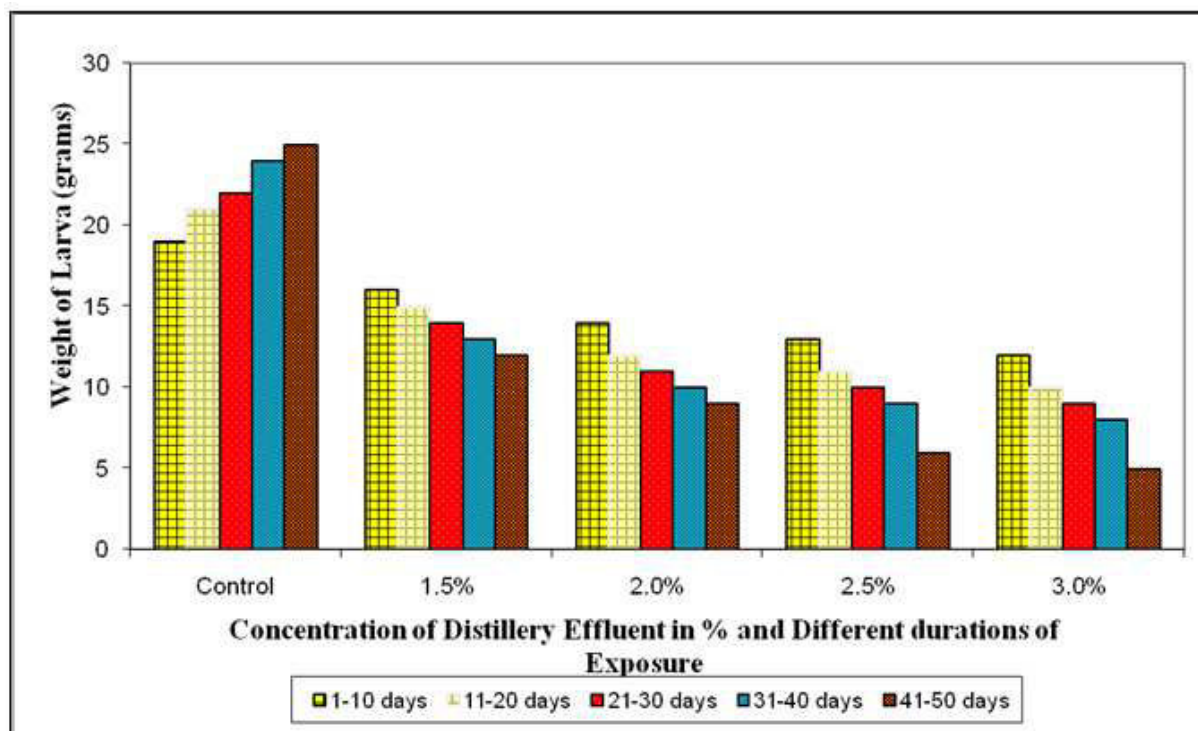


Figure 2 (c)

Observation of average weight of Larva of *Oryctes rhinoceros* of different Concentrations of Distillery effluent and approximate calculation of LC_{50} for different durations.

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