Effect of Bio-Pesticides on Mortality of Greater Wax Moth *Galleria mellonella* L.

S. Balpande a and A. S. Yadav b*

a Department of Entomology, RVSKVV, College of Agriculture, Gwalior (M.P.) 474002, India.
b Department of Entomology, RVSKVV, Krishi Vigyan Kendra Morena (M.P.) 476001, India.

Authors’ contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/CJAST/2021/v40i3631580

Editor(s):
(1) Dr. Chen Chin Chang, Hunan Women’s University, China.
(2) Ayyagari Ramlal, Indian Agricultural Research Institute, India.
(2) Randy Carlie Pierre-Louis, Haiti.

Reviewers:
Complete Peer review History, details of the editor(s), Reviewers and additional Reviewers are available here: https://www.sdiarticle5.com/review-history/76268

Received 04 October 2021
Accepted 09 December 2021
Published 13 December 2021

ABSTRACT

*Galleria mellonella* larvae feed on wax, pollen, honey and damage the combs with in active bee colonies and storage condition. *Galleria mellonella* larvae can bore tunnel and feed around the midrib base of the wax comb. Larvae produce silk fibers that can trap bee brood cells. Comb is completely covered with webbing and extracted matter of the larvae condition described as “Galleriasis”. The study was carried out at Apiculture Lab of RVSKVV-ZARS-Krishi Vigyan Kendra, Morena (M.P.) during 2019-20. Different three concentration of bio-pesticides viz., *Bacillus thuringiensis* Kurstaki (1, 1.5 and 2%), custard apple seed extract (2, 4 and 6%) and Neem Oil (1, 2 and 3%) were used against the *Galleria mellonella* to assess the effect of larval mortality at 24, 48, 72 and 96 hours after treatment. There was no significant effect showed in all the treatments after 24 hours. The maximum mortality of *Galleria mellonella* larvae was recorded in *Bacillus thuringiensis* Kurstaki 2.0 percent concentration (46.67, 87.08 and 96.67%) after 48, 72 and 96 hours respectively and it was at par with custard apple seed extract 6.0 percent concentration (45.83, 85.0 and 93.75%). Neem oil 3.0 percent concentration expressed 38.33, 75.42 and 87.50 percent larval mortality after 48, 72 and 96 hours respectively. Whereas minimum mortality was noticed in Neem oil 1.0 percent at 48 hours (22.08%), at 72 hours (37.08%) and at 96 hours (56.25%) among the treatments. The higher concentration of all the three bio-pesticides was proved effectiveness against *Galleria mellonella* during investigation.

*Corresponding author: E-mail: ashoksinghrvskvv@gmail.com;
Keywords: Bacillus thuringiensis Kurstaki; custard apple seed extract; Galleria mellonella; neem oil; mortality.

1. INTRODUCTION

Honey bee rearing or scientifically we all so called beekeeping and apiculture. Beekeeping in India dates back to pre-historic era when indigenous methods farthest from scientific beekeeping and approach when in practice. Apiculture occupies an important position in the agriculture sector; it contributes significantly to the gross domestic product (GDP) of the Indian economy as well as the rural economy. In addition to the direct and indirect benefits of beekeeping to humans, there are direct benefit is honey, bee. Bee venom, royal jelly, pollen, propolis, queen bee and medicines row materials and the indirect benefits is improving agricultural productivity due to bee pollination, raising awareness among the people for forest conservation, improving family nutrition and employment generation. Bee keeping industry is currently facing many challenges around the world. Among these, a major obstacle to beekeeping development programme is the increase in the number of enemies and diseases, Bees are attacked by many disease and pests, which cause weakening of the colonies and eventually produce low grade honey. Bees are also attacked by many insects such as the greater wax moth (Galleria mellonella L.) and the small wax moth, (Achroia grisella F.). Two species of wax moth, the greater wax moth and small wax moth are occurs naturally in various region of the world. Wax moth is the most common insect pest. Wax moth caterpillars feed on wax, pollen and honey and damage the combs within active bee colonies and storage conditions, resulting in massive losses worldwide [1,2]. Greater wax moth larvae can bore tunnel and feed around the midrib base of the wax comb. Larvae produce silk fibers that can trap bee brood cells. Comb is completely covered with webbing and excreted matter of the larvae condition described as ”Galleriasis”. In the severe infestation of the wax moth the bee colonies are weak, in strong colonies the number of bees is decreased rapidly and the colonies were completely destroyed by Greater wax moth [3]. Of the two species of wax moth, the Greater wax moth leads to greater losses compared to Lesser wax moth [4]. For the management of greater wax moth best way is use of natural products it’s more sustainable option because of their lower toxicity to the environment and the bee colony.

2. MATERIALS AND METHODS

The study was conducted in the Apiculture Lab of RVSKV-VZARS- Krishi Vigyan Kendra Morena (M.P.) during 2019-20. The treatments Bacillus thuringiensis var. kurstaki(1, 1.5 and 2%), custard apple seed extract (2, 4 and 6%) and neem oil (1, 2 and 3%) and untreated use as a check.

2.1 Custard Apple Seed Extract

For the preparation of custard apple seed extract the custard apple seeds was collected from fully ripened fruits and washed with water and shade dried, the dried seeds were crushed by using hammer mill and the grinded material was screened through fine mesh sieve. For preparation of 2 percent aqueous custard apple seed extract, the 20 g of seed powder was mixed with 1-liter distilled water and this mixture was soaked overnight. It was then screen through muslin cloth and the volume of the passing liquid (extract) was maintained to 980 milliliters by adding distilled water. Similarly, 4 & 6 per cent aqueous seed extracts were also prepared and the freshly prepared extracts were used for laboratory evaluation.

2.2 Bacillus thuringiensis var. kurstaki

For preparation of desired concentrations, the technical material of Bacillus thuringiensis var. kurstaki 5% WS was collected from the market. Technical material of 1.0, 1.5 and 2.0 ml was mixed with 100, 150 and 200 ml of distilled water for preparation of 1.0, 1.5 and 2.0 percent solution respectively.

2.3 Preparation of Neem oil Solution

The technical grade material of Neem oil 1000 ppm was collected from the market. For preparation of desired 1.0, 2.0 and 3.0 percent solution, 1.0, 2.0 and 3.0 ml of neem oil was mixed with 100, 200 and 300ml of distilled water respectively.

The various concentrations of bio pesticides were prepared in water. The Galleria Mellonella larvae and pupae was collected from stored old combs of Apis mellifera in the apiary of RVSKV-Krishi Vigyan Kendra, Morena for maintain the fresh culture in the laboratory. Galleria Mellonella
male and female was reared in the laboratory and fresh culture of various instar larvae was used in the experimentation. Cut piece of old combs of A. mellifera was dipped in this different type of solutions of respective concentrations for two minutes and shake gently to remove the excess of suspension from the piece comb, treated comb piece was be shade dried. Individual treated comb piece was put in separate jars. The ten numbers of second instar larvae of G. mellonella were released separately into jars containing the treated combs and record the mean mortality of the larva was observed 24, 48, 72 and 96 hours of the various larval stages. The experiment was conducted in completely randomized design (CRD) with ten treatments and four replications. Making the ANOVA after suitable transformations and separated by least significant difference (LSD) at p=0.05 level.

3. RESULTS AND DISCUSSION

The effect of different concentration of bio-pesticides on the larval mortality of Galleria mellonella at 24, 48, 72 and 96 hours were showed in Tables 1, 2 and 3.

3.1 Mortality of Second Instar of Galleria mellonella

The findings of mean mortality of second instar Galleria mellonella revealed that Bacillus thuringiensis Kurstaki 2.0% recorded maximum mortality (63.75%) and it was similar effective with custard apple seed extract 6% (63.12%) and Neem Oil 3% (59.37%) followed by BtK 1.5% (48.75%) and minimum mean mortality was recorded in neem oil 1.0% (36.25%) (Table 3). There was no significant effect showed by all the treatment before 48 hours in second instar larval mortality of Galleria mellonella. Bacillus thuringiensis Kurstaki 2.0% showed significant effect on second instar mortality at 48 hours (95.0%) and 96 hours (97.50%) but at 48 hours custard apple seed extract 6.0% was found most effective (60.0%) among the treatments (Table 1). Neem oil 1.0% was found least effective at 48 hours (35.0%), 72 hours (55.0%) and 96 hours (55.0%) on second instar mortality.

3.2 Mortality of Third Instar of Galleria mellonella

Data of mean mortality of third instar Galleria mellonella showed that Bacillus thuringiensis Kurstaki 2.0% (61.88%) and custard apple seed extract 6.0% (61.88%) was found most effective on third instar mortality and it was at par with Neem Oil 3% (56.25%) followed by that Bacillus thuringiensis Kurstaki 1.5% (45.0%) (Table 3). There was no significant effect found in all the treatment before 48 hours in second instar larval mortality of Galleria mellonella. Bacillus thuringiensis Kurstaki 2.0% and custard apple seed extract 6.0% was found most effective at 48 hours (55.0 and 55.0%), 72 hours (90.0 and 92.5%) and 96 hours (97.5 and 95.0%) mortality respectively and it was at par with neem oil 3.0% at 48 hours (45.0%), 72 hours (85.0%) and 96 hours (92.5%). Bacillus thuringiensis Kurstaki 1.0% was least effective at 48 hours (25.0%), 72 hours (52.50%) and 96 hours (55.0%) (Table 1).

3.3 Mortality of Fourth Instar of Galleria mellonella

The observations of mean mortality of fourth instar larvae of Galleria mellonella revealed that Bacillus thuringiensis Kurstaki 2.0% (61.25%) was found most effective and it was similar with custard apple seed extract 6.0% (56.88%) and Neem Oil 3% (55.63%) followed by custard apple seed extract 4.0% (46.25%). Neem oil 1.0% (32.5%) was recorded least effective among the bio-pesticides (Table 3). After 48 hours treatments showed significant effect on fourth instar larval mortality. Bacillus thuringiensis Kurstaki 2.0% was found significantly effective at 48 hours (57.5%), 72 hours (87.5%) and 96 hours (95.0%) and it was at par with custard apple seed extract 6.0% (50.0, 80.0 and 92.5%) and Neem Oil 3.0% (50.0, 77.5 and 90%) at 48, 72 and 96 hours after treatment respectively (Table 1). Bacillus thuringiensis Kurstaki 1.0% was recorded least effective on fourth instar mortality at 48 hours (27.5%), 72 hours (40.0%) and 96 hours (60.0%) among the treatments.

3.4 Mortality of Fifth Instar of Galleria mellonella

The data of mean mortality of fifth instar larvae of Galleria mellonella revealed that Bacillus thuringiensis Kurstaki 2.0% (56.25%) was showed significant effect on fourth instar mortality and it was similar effective with custard apple seed extract 6.0% (55%) and Neem Oil 3.0% (52.5%) followed by that Bacillus thuringiensis Kurstaki 1.5% (37.50%) (Table 3). The neem oil 1.0% (28.13%) was found least effective among the treatments. There was no significant effect recorded in the treatments before 48 hours in fifth instar larval mortality. Bacillus thuringiensis Kurstaki 2.0% (45.0 and...
97.5%) was found most effective on fifth instar larval mortality of *Galleria mellonella* at 48 hours and 96 hours and it was at par with custard apple seed extract 6.0% (42.5% and 92.5%) and Neem Oil 3.0% (35.0% and 90.0%) at 48 and 96 hours respectively. But at 72 hours maximum mortality was recorded in Neem oil 3.0% (82.5%) and it was similar with custard apple seed extract 6.0% (80.0%) and *Bacillus thuringiensis Kurstaki* 2.0% (77.5%) (Table 1).

### 3.5 Mortality of Sixth Instar of *Galleria mellonella*

Data of mean mortality of sixth instar larvae revealed that *Bacillus thuringiensis Kurstaki* 2.0% (53.13%) and custard apple seed extract 6.0% (53.13%) was found similar effective followed by Neem Oil 3.0% (41.25%). Neem oil 1.0% (18.75%) recorded least effective on sixth instar mortality among the treatments (Table 3). Custard apple seed extract 6.0% (35.0%) was found most effective at 48 hours and it was similar with *Bacillus thuringiensis Kurstaki* 2.0% (30.0%) and neem oil 3.0% (25.0%) followed by *Bacillus thuringiensis Kurstaki* 1.5% (22.5%) (Table 2). *Bacillus thuringiensis Kurstaki* 2.0% was found effective on larval mortality of sixth instar at 72 hours (85.0%) and 96 hours (95.0%) and it was also similar effective with Custard apple seed extract 6.0% (80.0% and 92.5%) at 72 hours and 96 hours followed by *Bacillus thuringiensis Kurstaki* 1.5% (60.0%) at 72 hours and neem oil 3.0% (80.0%) at 96 hours.

### 3.6 Mortality of Seventh Instar of *Galleria mellonella*

The mean data of seventh instar larval mortality showed that *Bacillus thuringiensis Kurstaki* 2.0% (56.25%) was found most effective and it was at par with custard apple seed extract 6.0% (55.0%) followed by Neem Oil 3.0% (52.50%) (Table 3). There was no significant effect recorded in the treatments before 48 hours. *Bacillus thuringiensis Kurstaki* 2.0% was recorded significantly effective on seventh instar larval mortality at 48 hours (37.5%), at 72 hours (87.5%) and at 96 hours (97.5%) (Table 2). *Bacillus thuringiensis Kurstaki* 2.0% was showed similar results on seventh instar mortality with custard apple seed extract 6.0% (32.5%), custard apple seed extract 4.0% (27.5%), ). *Bacillus thuringiensis Kurstaki* 1.5% (25.0%) and neem oil 3.0% (25.0%) at 48 hours and also with custard apple seed extract 6.0% at 72 hours (85.0%) and 96 hours (95.0%). Neem oil 1.0% was found least effective at 48 hours and 72 hours among the treatments.

### 3.7 Overall Mortality of *Galleria mellonella*

The data of overall mortality of different instar of *Galleria mellonella* revealed that before 48 hours there was no significant effect found in all the treatments (Table 2). The maximum overall mortality was recorded in *Bacillus thuringiensis Kurstaki* 2.0% at 48 hours (46.67%), at 72 hours (87.08%) and at 96 hours (96.67%), it was similar effective with custard apple seed extract 6.0% (45.83%, 85.0% and 93.75%) at 48, 72 and 96 hours respectively. Neem oil 3.0% was also proved effectiveness against overall mortality of *Galleria mellonella* larvae, it was 38.33% mortality at 48 hours, 75.42% at 72 hours and 87.50% at 96 hours. Neem oil 1.0% was found least effective on overall mortality among the treatments at 48, 72 and 96 hours.

The present findings supports from the findings of Viraktamath et al., [5], Gowda and Roopa [6] and Burges [7] who reported that small larvae of greater wax moth were more susceptible to Bt than the older larvae. Verma [8], MC Killup and Brown [9], Cantwell and Shieh, [10] Izhar-ul-Haq et al., [11], Swamy et al., [12] and Kapil and Sihag [4] also studied the effect of *Bt* as a bio-control agent against *G. mellonella* and effective to controlling *G. mellonella* without any adverse effect on the honey bees. Goodwin [13] and Molin et al. [14] also found adequate control of *G. mellonella* by using B 401 (a formulation of Bt spores and crystals) for a longer period. Verma [8] also witnessed a similar efficacy of Dipel on *G. mellonella* larvae in *Apis cerana indica* colonies. The data recorded on effect of different concentration of bio-pesticides on the overall mortality of greater wax moth larvae after 48, 72 and 96 hours of treatments application showed significant effect on overall larval mortality, maximum mortality was recorded in *B. thuringiensis var. kurstaki* @ 2% con. and it was at par with custard apple seed extract @ 6% con. followed by neem oil @ 3% con. Viraktamath et al. [5], Kuusik et al. [15] and Swamy et al., [12] are support the above findings. The different concentrations of the custard apple seed extract was used for management of other lepidopteron pest at field and laboratory levels but there is no work and review found on greater wax moth management. Custard apple seed extract was showing significant effect on the all-larval stage of greater wax moth management at laboratory level in present studies.
Table 1. Effect of bio-pesticides on mortality of Greater wax moth (II, III, IV and V instar larval stage)

| Treatment                          | Percent mortality of different larval instar of *Galleria mellonella* | 24 hr. | 48 hr. | 72 hr. | 96 hr. | 24 hr. | 48 hr. | 72 hr. | 96 hr. | 24 hr. | 48 hr. | 72 hr. | 96 hr. | 24 hr. | 48 hr. | 72 hr. | 96 hr. |
|------------------------------------|-----------------------------------------------------------------------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|
| T1- Bacillus thuringiensis var. kurstaki @ 1.0 % | (0.00)*                                                             | 27.50 | 50.00 | 72.50 | 5.00  | 25.00 | 52.50 | 55.00 | 0.00  | 27.5  | 40.00 | 60.00 | 0.00  | 25.00 | 37.50 | 55.00 |
| T2- Bacillus thuringiensis var. kurstaki @ 1.5 % | (9.10)                                                              | 42.13 | 50.77 | 69.30 | 9.10  | 39.23 | 45.00 | 69.30 | 9.10  | 40.69 | 47.87 | 60.00 | 9.10  | 37.76 | 46.43 | 63.44 |
| T3- Bacillus thuringiensis var. extract @ 2.0 % | (0.00)                                                              | 37.76 | 49.31 | 58.37 | 0.00  | 40.69 | 49.31 | 53.73 | 0.00  | 36.27 | 46.43 | 60.00 | 0.00  | 30.00 | 40.69 | 55.24 |
| T4- Custard apple seed extract @ 4.0 % | (0.00)                                                             | 45.00 | 52.24 | 61.68 | 0.00  | 42.13 | 49.31 | 61.68 | 0.00  | 37.76 | 53.73 | 65.27 | 9.10  | 33.21 | 43.57 | 60.00 |
| T5- Custard apple seed extract @ 6.0 % | (12.92)                                                            | 50.77 | 74.11 | 77.08 | 12.92 | 47.87 | 74.11 | 77.08 | 12.92 | 45.00 | 63.44 | 74.11 | 12.92 | 40.69 | 63.44 | 74.11 |
| T7- Neem oil @ 1.0 %               | (0.00)                                                             | 35.00 | 55.00 | 55.00 | 0.00  | 27.50 | 45.00 | 70.00 | 0.00  | 25.00 | 42.50 | 62.50 | 0.00  | 27.50 | 32.50 | 52.50 |
| T8- Neem oil @ 1.5 %               | (0.00)                                                             | 42.50 | 67.50 | 70.00 | 0.00  | 40.00 | 57.50 | 70.00 | 2.50  | 30.00 | 55.00 | 75.00 | 2.50  | 25.00 | 50.00 | 72.50 |
| T9- Neem oil @ 2.0 %               | (0.00)                                                             | 40.69 | 47.87 | 55.24 | 9.10  | 39.23 | 49.31 | 60.00 | 9.10  | 33.21 | 47.87 | 60.00 | 9.10  | 30.00 | 45.00 | 58.37 |
| T10- Untreated check               | (9.10)                                                             | 45.00 | 71.57 | 77.08 | 9.10  | 42.13 | 67.21 | 74.11 | 12.92 | 45.00 | 61.68 | 71.57 | 9.10  | 36.27 | 65.27 | 71.57 |
| SE±                               | 3.36                                                              | 3.01  | 4.41  | 4.27  | 4.31  | 2.78  | 4.29  | 4.36  | 3.56  | 2.81  | 3.45  | 4.03  | 3.76  | 2.95  | 3.2   | 4.06  |
| CD                                | NS                                                                | 8.68  | 12.73 | 12.33 | NS    | 8.04  | 12.41 | 12.61 | NS    | 8.13  | 9.96  | 11.65 | NS    | 8.54  | 9.24  | 11.75 |

*Figures in parenthesis are arcsine transformed value, NS=Non-Significant*
Table 2. Effect of bio-pesticides on mortality of Greater wax moth (VI and VII instar larval stage)

| Treatment                     | Percent mortality of different larval instar of *Galleria mellonella* | Overall Per cent Mortality |
|-------------------------------|------------------------------------------------------------------------|-----------------------------|
|                               | VI Instar                                                              | VII Instar                  |                               |
|                               | 24 hr. | 48 hr. | 72 hr. | 96 hr. | 24 hr. | 48 hr. | 72 hr. | 96 hr. | 24 hr. | 48 hr. | 72 hr. | 96 hr. |
| T1- Bacillus thuringiensis var. kurstaki @ 1.0 % | 0.00  | (0.00)* | 12.50  | 40.00  | 55.00  | 0.00  | 17.50  | 40.00  | 60.00  | 0.83   | 22.50  | 43.93  | 59.58 |
|                               | 24 hr. | 96 hr. | 24 hr. | 96 hr. | 24 hr. | 96 hr. | 24 hr. | 96 hr. | 24 hr. | 96 hr. | 24 hr. | 96 hr. |
| T2- Bacillus thuringiensis var. kurstaki @ 1.5 % | 2.50  | (9.10) | 22.50  | 60.00  | 77.50  | 0.00  | 25.00  | 62.50  | 75.00  | 2.08   | 35.42  | 56.67  | 80.42 |
| T3- Bacillus thuringiensis var. kurstaki @ 2.0 % | 2.50  | (9.10) | 30.00  | 85.00  | 95.00  | 0.00  | 37.50  | 87.50  | 97.50  | 4.17   | 46.67  | 87.08  | 96.67 |
| T4- Custard apple seed extract @ 2.0 % | 0.00  | (0.00) | 15.00  | 27.50  | 35.00  | 0.00  | 12.50  | 30.00  | 40.00  | 0.00   | 27.92  | 44.59  | 59.17 |
| T5- Custard apple seed extract @ 4.0 % | 0.00  | (0.00) | 22.50  | 35.00  | 52.50  | 0.00  | 27.50  | 45.00  | 55.00  | 0.42   | 35.42  | 52.08  | 70.00 |
| T6- Custard apple seed extract @ 6.0 % | 5.00  | (12.92)| 35.00  | 80.00  | 92.50  | 0.00  | 32.50  | 85.00  | 95.00  | 4.17   | 45.83  | 85.00  | 93.75 |
| T7- Neem oil @ 1.0 %          | 0.00  | (0.00) | 5.00   | 25.00  | 45.00  | 0.00  | 12.50  | 22.50  | 52.50  | 0.00   | 22.08  | 37.08  | 56.25 |
| T8- Neem oil @ 1.5 %          | 2.50  | (9.10) | 15.00  | 37.50  | 60.00  | 0.00  | 25.00  | 60.00  | 77.50  | 2.08   | 28.75  | 48.33  | 68.33 |
| T9- Neem oil @ 2.0 %          | 2.50  | (9.10) | 25.00  | 57.50  | 80.00  | 0.00  | 25.00  | 60.00  | 77.50  | 2.50   | 38.33  | 75.42  | 87.50 |
| T10- Untreated check          | 0.00  | (9.10) | 30.00  | 49.31  | 63.44  | 0.00  | 30.00  | 50.77  | 61.68  | (9.1)  | (38.25) | (60.28) | (69.3) |
|                               | 0.00  | (0.00) | 0.00   | 0.00   | 0.00   | (0.00) | 0.00   | 0.00   | 0.00   | (5.24) | (9.1)  | (9.83) | (10.52) |
| SEms                          | 3.36  | 2.68   | 2.29   | 2.98   | 1.46   | 2.79  | 2.45   | 3.09   | 2.85   | 1.38   | 1.16   | 1.48   |
| CD                            | NS    | 7.75   | 6.61   | 8.62   | NS     | 8.06  | 7.07   | 8.91   | NS     | 3.99   | 3.35   | 4.27   |

(*) Figures in parenthesis are arcsine transformed value, NS=Non-Significant
Table 3. Efficacy of different concentration of bio-pesticides on mortality of Greater wax moth

| Treatment                   | Mean mortality of different larval instar of Galleria mellonella (%) | Over all mean larval mortality |
|-----------------------------|----------------------------------------------------------------------|-------------------------------|
|                             | I         | II        | III        | IV        | V         | VI         |                                                      |
| T1- Bacillus thuringiensis var. kurstaki @ 1.0 % | 37.50     | 34.38     | 31.88      | 29.38     | 26.88     | 29.38      | 31.56                                                 |
| T2- Bacillus thuringiensis var. kurstaki @ 1.5 % | 48.75     | 45.00     | 43.75      | 43.13     | 40.63     | 43.13      | 43.65                                                 |
| T3- Bacillus thuringiensis var. kurstaki @ 2.0 % | 52.98     | 51.87     | 51.5       | 48.59     | 46.79     | 48.59      | 49.98                                                 |
| T4- Custard apple seed extract @ 2.0 % | 41.88     | 41.25     | 40.63      | 33.75     | 19.38     | 33.75      | 32.92                                                 |
| T5- Custard apple seed extract @ 4.0 % | 47.50     | 45.00     | 46.25      | 38.75     | 27.50     | 38.75      | 39.48                                                 |
| T6- Custard apple seed extract @ 6.0 % | 63.13     | 61.88     | 56.88      | 55.00     | 53.13     | 55.00      | 57.19                                                 |
| T7- Neem oil @ 1.0 %        | 36.25     | 35.63     | 32.50      | 28.13     | 18.75     | 28.13      | 28.85                                                 |
| T8- Neem oil @ 1.5 %        | 41.25     | 43.75     | 40.63      | 37.50     | 28.75     | 37.50      | 36.88                                                 |
| T9- Neem oil @ 2.0 %        | 59.38     | 56.25     | 55.63      | 52.50     | 41.25     | 52.50      | 50.94                                                 |
| T10- Untreated check        | 5.00      | 4.38      | 3.13       | 1.88      | 0.00      | 1.88       | 2.40                                                  |

SEm± 2.08  1.89  1.80  1.97  1.17  1.97  0.76
CD  6.01  5.46  5.21  5.71  3.39  5.71  2.18

(*) Figures in parenthesis are arcsine transformed value
4. CONCLUSION

Different concentration of bio-pesticides incorporated in the study revealed that the treatment Bacillus thuringiensis Kurstaki 2.0% and custard apple seed extract 6.0% results in the superior to control the infestation of Galleria mellonella as compared to other treatments. Treatments Bacillus thuringiensis Kurstaki 2.0% and custard apple seed extract 6.0% concentration were proved effectiveness on overall larval mortality at 24, 48, 72 and 96 hours. Both treatments were found similar effective on different instar larval mortality of Galleria mellonella during the investigation. Bacillus thuringiensis Kurstaki 2.0% and custard apple seed extract 6.0% concentration can be used for control of Galleria mellonella commercial beekeeping.

FINANCIAL SUPPORT

All the financial support and assistance received the course of this investigation have been acknowledge by the scholars.

ACKNOWLEDGEMENT

The authors are acknowledge Head Department of Entomology, College of Agriculture Gwalior, Associate Director Research ZARS, Morena and Senior Scientist & Heads Krishi Vigyan Kendra, Morena for their support and encouragement.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Williams JL. Insects: Lepidoptera (moths). Honey bee pests, predators, and diseases. 1997;3:119-142.
2. Milam VG. Moth pests of honeybee combs. Glean. Bee Culture. 1970;68:824–428.
3. Swamy HBC, Rajagopal D, Kenchaddi. Seasonal incidence of greater wax moth, Galleria mellonella in Indian honeybee colonies. Indian bee J. 2005;67(4):176-186.
4. Kapil RP, Sihag RC. Wax moth and its control. Indian Bee J. 1983;45(2-3):47-49.
5. Viraktamath S, Basalingappa S, Lingappa. Efficacy of commercial formulations of Bacillus thuringiensis against the larvae of the greater wax moth, Galleria mellonella. Indian Bee J. 2005; 67:72-77.
6. Gowda GD, Roopa AN. Effect of Bt protein on larval and adults of Indian honey bee, Apis cerana. Proceedings of 6th Asian Apiculture Association International Conf. Bangalore, India, 24th Feb-1st March. 2001;57(2):36-41.
7. Burges HD. Control of wax moths: Physical, Chemical and Biological Methods. Bee World. 1978;59(4):129-138.
8. Verma SK. Studies on the control of greater wax moth, G. mellonella in Apis cerana colonies with biological insecticide, Dipel. Indian Bee J. 1995;57:121-123.
9. Mc Killiup, Brown DG. Evaluation of formulation of Bacillus thuringiensis against greater wax moth in stored honey combs. Aus. J. Exp. Agri. 1991;31(5):709-711.
10. Cantwell GE, Shieh TA. Certan- A new bacterial insecticide against greater wax moth, Galleria mellonella L. Am. Bee J. 1981;121:424-426,430-431.
11. Izhari-Ul-Haq M, Saleem M, Ahmed S. Effect of neem (Azadirachta indica) seed extracts against greater wax moth (Galleria mellonella L.) larvae. Pak. J. of Ento. 2008;30:137-140.
12. Swamy HBC, Rajagopal D, Gowda BLV. Management of greater wax moth (Galleria mellonella: Pyralidae; Lepidoptera). Asian Bee J. 2003;5(1&2):207-212.
13. Goodwin WD. A unique method for the prevention and amelioration of greater wax moth infestations in honey combs and wax foundations. South African Bee J. 1985;2:36-41.
14. Molin MJL, Perez GF, Gomez PA. Comparative study of the activity of 3 substances (Sulphur dioxide, Comesan L.S. and B 401) in the control of wax moth, Galleria mellonella L. in honey combs. Vida Apicola. 1987;26:23-31.
muscular and respiratory activity patterns in yellow meal worm (*Tenebrio molitor*) and Greater wax moth (*G. mellonella*) pupae caused by some plant extracts, Juvenile hormone analogues and a pyrethroid. Eesti Teaduste Akadeemia Toimetied Biologia. 1993;42: 94-107.