Ecofriendly Management of Greater wax moth *Galleria Mellonella* L. in the Laboratory Conditions

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Abstract

Ecofriendly management of Galleria mellonella L. laboratory experiment was evaluated at Anbil Dharmalingam Agricultural College and Research Institute apiary using the combs of Apis cerana indica located in Tiruchirappalli, Tamil Nadu [(10.7554ºN, 78.6054ºE, 279' (85m) above mean sea level)]. The toxicity of six products Viz., Neem leaf extract 3%, Neem Seed Kernel Extract 5 %, Acorus calamus 5% (vasambu), Bt kurstaki (Halt) (2g/lit), Lime and Sulphur (10% & 25%), Tobacco leaf extract were tested against different instars of Galleria mellonella larvae. Recording the larval mortality and damage of comb in the treated combs. The results revealed that the mean larval mortality was highest in Bt kurstaki (Halt) against second, third and fourth instar (86.66%, 80.00%, 73.33%) respectively, followed by Lime sulphur (73.33%). Whereas, in the fifth instar larvae the mean mortality was highest in Lime sulphur (66.66%).

Keywords: *Galleria mellonella L., Apis cerana indica, Bt kurstaki, Lime sulphur, Wax comb*

1. Introduction

A tropical country like India has an advantage over other countries as it has a rich variety of flora and suitable climate for beekeeping throughout the year. The superfamily Apoidea, which has an estimated 25,000 identified species and 250 genera and 13 families, is thought to be the most significant group of insect pollinators in the order Hymenoptera (Grimaldi and Engel, 2005). The great scope for increasing the bee colonies for honey and wax production and also for pollination of crops. Forging behaviour of honey bees enhance the agricultural productivity

through cross-pollination (Anandhabhairavi *et al*., 2020). *Apis flora*, *Apis cerana*, *Apis dorsata*, *Apis mellifera*, and *Trigona iridipennis* are the five species of honey bees that can be found in India. However, only *Apis cerana*, and *A. mellifera* are reared in hives. In beekeeping, the population of honey bee is influenced by many factors like pest, diseases, parasites, pesticides and the environment.

Among the wax moth pests of the honeybee, the greater wax moth *G.mellonella* (Lepidoptera: Pyralidae) causes the greatest damage, leading to material and financial losses. The greater wax moth is responsible for heavy economic losses reaching up to 60 to 70 per cent to bee keepers in developing countries (Hanumanthaswamy *et al.*, 2009 and Jayapal and Anandhabhairavi 2022).

The larvae often destroy the unprotected combs in storage or in colonies the only feeding stage with the life span of all development stages, builds its silken lined feeding tunnels in the honey comb and feeds on wax, pollen, facies around cocoon of bee larvae. This voracious nature of the larvae leads to the destruction of the honey comb and the subsequent death of weak colonies. Adults do not feed on wax combs (Charriere and Imdorf 1997). In India also, the greater wax moth caused damage to honey bee colonies which results in heavy economic losses to the bee keepers (Kapil and sihag, 1983 and Hanumanthasamy *et al.*, 2009).

Mostly, Physical and chemical methods of management are either inefficient or expensive for the small-scale beekeepers. In addition, most chemical methods were associated with residue problems in honeybee products. While searching for a best alternative, use of bio products will be a good choice for controlling pests and also paves way for ecofriendly pest management. Hence, the present investigation was emphasized on ecofriendly management of *Galleria mellonella* L. under laboratory conditions.

2. Materials and methods:

The laboratory experiment was conducted at Anbil Dharmalingam Agricultural College and Research Institute apiary using the combs of *Apis cerana indica* located in Tiruchirappalli, Tamil Nadu [(10.7554ºN, 78.6054ºE, 279’ (85m) above mean sea level)].

2.1. *Galleria mellonella* culture

The culture of greater wax moth was obtained from already infested combs of ADAC & RI, apiary, Trichy. The larvae were reared on artificial diet and natural comb wax. The culture was maintained in the laboratory incubated at 30°C in Department of Entomology, Anbil Dharmalingam Agricultural College and Research Institute, Tiruchirappalli**.**

2.2. Preparation of the artificial diet and beeswax Artificial diet

2.2.1. Wax moth culture on bee combs

The combs were collected from the *A. cerana indica* which were absconded due to greater wax moth infestation and it was used for wax moth rearing. Paper strips containing eggs laid by fertilized female wax moth were separated and kept along with comb wax at 300 in incubators. The larvae emerged in six to eight days and started feeding on the comb. The culture was confined in incubators at 30° C. Ventilated plastic boxes of size 21 x 8 cm were used for rearing the larvae in different batches.

The larvae pupated in cocoons in 35-50 days. The pupae were collected and kept in separate containers with proper aeration for emergence into adult moths. After emergence, the moths were transferred to cages, and were fed with Vitamin E tablet for enhance the oviposition and honey syrup (50%) on cotton buds and were allowed for mating. Zig zag folded paper strips were inserted into the cage to facilitate egg deposition. This culture served as the source to drew larvae and adult required continuously for various experiments.

2.2.2. Wax moth culture on artificial diet

The culture on artificial diet was maintained as standby arrangement and supplement the culture on combs**.**

Preparation of artificial diet

The artificial diet used for rearing larvae was prepared and used as and when needed. The ingredients used in the preparation of artificial diet was composed of corn flour, wheat flour, milk powder, yeast tablets, honey and glycerine (Metwally *et al*., 2012). The ingredients were mixed thoroughly in a container by stirring with hands covered by surgical gloves. Ingredients used for the preparation of artificial diet.

The eggs laid on paper strips were kept on artificial diet and hatched larvae fed on the diet and grew and pupated in the diet.

2.3. Treatment of combs

The undamaged combs were cut into rectangular pieces each weighing 10 g. Then the comb pieces were treated with prepared bio products *viz.,* neem leaf extract 3 %, Neem Seed Kernel Extract 5 %, *Acorus calamus* 5 %, *Bt kurstaki* (Halt) 2.5g /500ml, lime sulphur (lime 10% & sulphur 25%) tobacco leaf extract 10 %, control (water spray) by giving a soaking spray using a hand sprayer. After spraying the comb with above products, the combs were allowed to dry at room temperature. Each treated comb was inoculated with larvae of second, third, fourth, fifth instar at 5 per piece separately and placed in the ventilated plastic boxes (21 x 8).The control consisted of combs applied with water spray. Ten days after larvae release, combs were observed for the wax moth damage. The final weight of comb and larval mortality were recorded (Cantwell and shieh, 1981).The experiments comprised of 7 treatments and 3 replications.

2.4. Preparation of spray solutions

The bio-inoculant PPFM and the antagonistic microorganism *P. fluorescens* were obtained from the Agricultural College and Research Institute, Killikulam. The spray fluids with alum and PPFM were prepared with need based addition of agricultural grade surfactants (Lipstick®). The procedure adopted for the preparation of spray fluid with other materials is detailed below.

2.4.1. Neem Leaf Extract (NLE) 3 %

The 3 % of NLE was obtained by soaking weighed quantity (30 g) of ground neem leaves in the cloth bag for 24 hours and then squeezing the bag for 5 minutes after every hour in 1.0 liter distilled water (Ahmad *et al.,* 2014).

2.4.2. Neem Seed Kernel Extract (NSKE) 5 %

The 5 % NSKE was obtained by soaking weighed quantity (50 g) of neem seed powder in cloth bag for 12 hours and then squeezing the bag for 5 minutes after every hour in 1.0 liter distilled water.

2.4.3. Sweet flag (*Acorus calamus* L.) 5%

A liter of water was boiled and to this 50 g of powered *Acorus calamus* was added and boiled again for half an hour. This liquid was strained through a cloth and used for spraying.

2.4.4. *Bacillus thuringiensis* (Halt) 2g/lit

The Commercial *Bt.* formulation *Bt Kurstaki* (Halt) was obtained from Meenakshi agro foundation Ltd. The spray solution was prepared @ 2.5 gm in 500 ml of water and tested against GWM larvae.

2.4.5. Lime Sulphur (lime 10% & sulphur 25%)

The lime Sulphur is prepared as follows, 2.25 liters of water is boiled first in the tin vessel. To this 200 g quick lime and 550 g of Sulphur are added, the mixture is stirred well and boiled for one hour the resultant liquid is strained and allowed to settle, the clear water settled above is decanted and the thin paste is used for treating the combs.

2.4.6. Tobacco leaf extracts 10 %

Two liters of water was boiled and to this 200 g of cured tobacco leaf were added and boiled again for half an hour. This liquid was strained through a cloth .To the decoction 65gms of soap is added and boiled till the soap was dissolved .The thin paste thus formed was used for treating the comb.

2.5. Statistical analysis

Statistical analysis for various experiments was done using AGRES-AGDATA software. The data of various results of laboratory experiments were subjected to completely randomized design. The data obtained on the mean number of greater wax moth captured were analyzed after square root (X + 0.5) transformation (Steel and Torrie, 1980).

3. Results and Discussion:

The toxicity of six products *Viz*., Neem leaf extract 3%, Neem Seed Kernel Extract 5 %, *Acorus calamus* 5% (vasambu), *Bt kurstaki* (Halt) (2g/lit), Lime Sulphur, Tobacco leaf extract were tested against different instar larvae of *Galleria mellonella* and recording a larval mortality and damage of comb in the treated combs.

3.1. Effect of different treatments against second instar larvae of *Galleria mellonella*

There were significant differences among the treatments with respect to mean larval mortality. The mean larval mortality was highest in *Bt kurstaki* (Halt) (86.66 per cent) followed

by Lime Sulphur (73.333 per cent), Neem seed kernel extract 5 % (73.33 percent), *Acorus calamus* 5 % (73.33 percent), Tobacco leaf extract (66.66 percent), Neem leaf extract 3% (53.33 percent) and the control no larval mortality was recorded. The reduction of comb weight was highest at 7.51 g in control which was significantly higher than the loss of comb weight in all other treatments (Table 1).

Table 1. Effect of different treatments against second instar larvae of greater wax moth, *Galleria mellonella*

Treatments	Mean final weight of comb (g)	Weight reduction of comb (g)	Larval mortality (%)
T1- Neem leaf extract 3%	7.23	2.77	53.33
T2- Neem seed extract 5 %	7.79	2.20	73.33
T3- *Acorus calamus* 5 %	8.49	1.51	73.33
T4- *Bt Kurstaki* (Halt)	9.30	0.70	86.66
T5- Lime Sulphur	8.64	1.36	73.33
T6- Tobacco leaf extract	7.45	2.55	66.66
T7- Control (water spray)	2.49	7.51	0.00
F value	**	**	**
SEm ±	0.06	0.11	8.08
C.D (P = 0.01)	0.17	0.34	31.43

****** Significant difference

3.2. Effect of different treatments against third instar larvae of *Galleria mellonella*

Mean percent mortality of wax moth larvae showed significant damage among the treatments. The mean larval mortality was highest in *Bt kurstaki* (Halt) at (80 per cent) followed by *A. calamus* 5 % and Lime Sulphur (73.33 percent), Neem seed kernel extract 5 % (66.6 percent), Tobacco leaf extract (53.33 percent), Neem leaf extract 3% (46.66 percent) and the control has no larval mortality was recorded. The loss of weight was higher in control 7.82 GM in control treatment which was significantly higher than the loss of comb weight in all other treatments (Table 2).

Table 2. Effect of different treatments against third instar larvae of greater wax moth, *Galleria mellonella*

Treatments	Mean final wt. of comb (g)	Wt. reduction of comb (g)	Larval mortality (%)
T1- Neem leaf extract 3%	4.86	5.14	46.66
T2- Neem seed extract 5 %	3.71	6.29	66.66
T3- *Acorus calamus* 5 %	4.35	5.65	73.33
T4- *Bt Kurstaki* (Halt)	5.44	4.56	80.00
T5- Lime Sulphur	6.28	3.72	73.33
T6- Tobacco leaf extract	3.64	6.36	53.33
T7- Control (water spray)	2.18	7.82	0.00
F value	**	**	**
SEm ±	0.07	0.05	5.87
C.D (P = 0.01)	0.20	0.14	24.88

****** Significant difference

3.3. Effect of different treatments against fourth instar larvae of *Galleria mellonella*

There were significant differences among the treatments with respect to mean larval mortality. The mean larval mortality was highest in *Bt kurstaki* (Halt) (73.33 per cent), Lime Sulphur (73.33 per cent), Neem seed kernel extract 5 % (60 percent), Tobacco leaf extract (46.66 percent), Neem leaf extract 3% (46.66 percent), *Acorus calamus* 5 % (40 percent) and the control larval mortality was nil. The weight reduction of the comb was highest in control 7.82 gm (Table 3).

Table 3. Effect of different treatments against fourth instar larvae of greater wax moth, *Galleria mellonella*

Treatments	Mean final wt. of comb (g)	Wt. reduction of comb (g)	Larval mortality (%)
T1- Neem leaf extract 3%	8.59	1.41	46.66
T2- Neem seed extract 5 %	9.14	0.86	60.00
T3- *Acorus calamus* 5 %	8.79	1.21	40.00
T4- *Bt Kurstaki* (Halt)	9.26	0.74	73.33
T5- Lime Sulphur	9.58	0.42	73.33
T6- Tobacco leaf extract	8.81	1.19	46.66
T7- Control (water spray)	1.44	7.82	0.00
F value	**	**	**
SEm ±	0.02	0.08	12.07
C.D (P = 0.01)	0.08	0.25	48.34

****** Significant difference

3.4. Effect of different treatments against fifth instar larvae of *Galleria mellonella*

There were significant differences among the treatments with respect to mean larval mortality. The mean larval mortality was highest in Lime sulphur (66.66 per cent), Neem seed kernel extract 5 % (46.66 percent), Tobacco leaf extract (40 percent), Neem leaf extract 3% (33.33 percent), *Acorus calamus* 5 % (33.3 percent), *Bt kurstaki* (Halt) (26.67 per cent) and zero larval mortality and weight reduction of the comb (9.9 gm) was highest was recorded in control (Table 4).

Table 4. Effect of different treatments against fifth instar larvae of greater wax moth, *Galleria mellonella*

Treatments	Mean final wt. of comb (g)	Wt. reduction of comb (g)	Larval mortality (%)
T1- Neem leaf extract 3%	1.47	8.5	33.33
T2- Neem seed extract 5 %	2.33	7.7	46.66
T3- *Acorus calamus* 5 %	2.05	8.0	33.33
T4- *Bt Kurstaki* (Halt)	1.14	8.9	26.66
T5- Lime Sulphur	2.42	7.6	66.66
T6- Tobacco leaf extract	2.77	7.2	40.00
T7- Control (water spray)	0.04	9.9	0.00
F value	**	**	**
SEm ±	0.13	0.06	4.18
C.D (P = 0.01)	0.40	0.19	15.54

****** Significant difference

The present results agree with those of Vishwas (2006) who reported that the effect of Bt formulation was highly detrimental to first instar larvae of greater wax moth causing 100 per cent mortality. This study revealed with the increase in instar there was significant reduction in the effect of Bt formulation against the larvae. Viraktamath *et al.* (2005) have come to a similar conclusion that the toxicity of Bt depends upon larval age, temperature and crystal content of the bacterial preparation. Babarinde *et al.,* (2010) observed that treating top bars of hive with lime was effective against apicultural pests but did not harm honey bees. Treating cracks and crevices of hive with lime sulphur paste protected the colony up to two months from wax moth infestation (Shylesha, 1987).

The mortality of the larvae caused by neem derivatives was comparatively lower than other treatments. Hanumanthaswamy (2009) also reported less effectiveness of neem seed kernel extract. The results are dissimilar with Viraktamath and Basalingappa (2000) who observed 92.3 per cent mortality of *G. mellonella* larvae treated with neem derivatives. The mortality was high with seed extract of neem (*Azadirachta indica*) from 84.81 ± 2.7 to 93.65 ±3.25 per cent at different concentrations (Surendra *et al.,* 2010).

4. Conclusion

For beekeepers and honey bee colonies all over the world, in the country as a whole, and in the study region in particular, wax moths continue to be a troublesome source of issues. The quantity of investigations into wax moth control has dramatically decreased recently. There have been no recommendations referring to relevant backdrops for emerging nations that are seeking to produce organic hive products. This may be primarily because wax moths are regarded as a secondary pest of bee colonies and have a significant economic impact on rural beekeepers in those developing nations. The uses of these plant products are not causing any damage to the honeybee. Hence, these products can be used in control of Lepidopteron pest, greater wax moth, *Galleria mellonella.* These natural plant products are more economical when compared to other chemicals and also they would cause no health hazards to honey consumers. Beekeepers can handle these products very easily.

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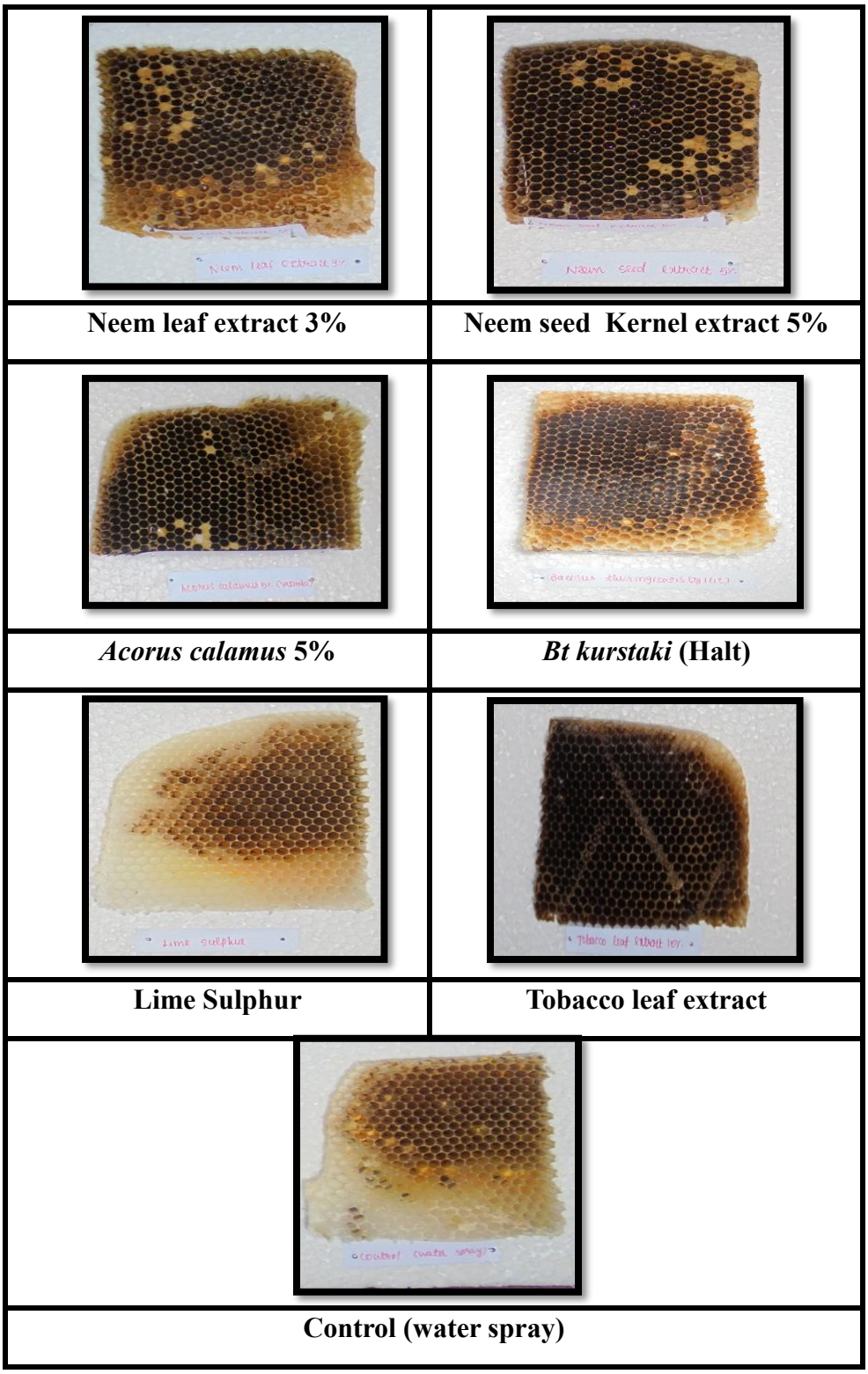
Plate 1. Combs treated with different types of materials against larvae of *G.mellonella* under laboratory condition



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