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Efficacy of different chemical and non-chemical treatments for management of *Galleria mellonella* in stored combs

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Abstract
Bee keeping has also becoming popular as one of the components in mixed farming systems and source of income for small farmer or landless labourer and taken a shape of hopeful enterprise or business also. But due to attack of natural enemies especially *Galleria mellonella* on honey bees, the beekeeping industries suffers from huge losses. In present study to combat from the pest problems, microbial biopesticide, chemical and non-chemical methods have been tried widely against *G. mellonella* in stored combs. The research was carried out at Research farm, Apiary and Apiculture Laboratory of the Department of Entomology, Chaudhary Charan Singh Haryana Agricultural University, Hisar from June to December of both year 2016 and 2017. Among the different chemical and non-chemical measures tested for management of greater wax moth in stored combs; the most effective was storing stored comb at the low temperature treatment of -15 °C, -10 °C followed by at low temperature of -5°C and 0 °C, Aluminum phosphide fumigation, *Bt* spray, separation of combs with newspapers, neem seed kernel extract (NSKE) spray, formic acid spray, acetic acid spray, hive chamber separation with newspaper, neem oil spray and broadcasting neem leaves powder. The study demonstrated the potentiality of low temperature treatment, Aluminum phosphide fumigation and Bt isolates against *G. mellonella*. Although, study also indicated that Aluminum phosphide fumigation in stored combs targets and destroy all life stages of the moth. Beside this, application of fumigants is economically convenient and requires little knowledge of the pest organism.

Keywords: *Galleria mellonella*, wax moth, stored combs, chemical and non-chemical control

Introduction

Presently beekeeping industry is facing many challenges throughout the world. Among these, a major constraint in beekeeping developmental programmes is the damage caused to the honey bee colonies by various enemies and diseases. Honey bees colonies are attacked by many diseases and pests, which weaken the colonies, absconded the colonies and lower the honey production. The important honey bee insect- pests include Greater wax moth (*Galleria mellonella Linn.*), Lesser wax moth (*Achroia grisella*), Indian meal moth (*Plodia interpunctella*), Mediterranean flour moth (*Ephestia kuhniella*), Fig moth (*Ephestia cautella*), Dried fruit moths (*Vitula serratilinella* and *V. edmansi*), Codling moth (*Carpocapsa pomonella*), Bumble bee moth (*Aphomia sociella*), Death's head hawk moth (*Acherontia styx*), Wingless fly and Small hive beetle. Among these, *Galleria mellonella* L. (Lepidoptera: Pyralidae) is one of the most devastating and economically important pest in the world (Burges 1978 [5]; Chang and Hsieh 1992 [9]) causing considerable damage to combs left unattended by bees. Combs in weak or dead colonies and in storage areas are subject to attack by greater wax moth (Caron 1992) [8].

G. Mellonella is a cosmopolitan pest causing serious economic damage to honey bee combs during storage (Ellis *et al*. 2013) [12]. A normal healthy honey bee colony, however, keeps the wax moth under control by ejecting the larvae but the weakened bee colony with lower bee population is over powered by wax moth infestation destroying the brood combs, ultimately destroying the whole of the bee colony. The pest larval stage feeds on wax and pollen stored in combs of active honey bee colonies (Milam 1970) [17], freshly extracted combs, impure bees wax, slum gum, wax capping and queen rearing materials containing wax in the storage (Williams 1978) [22].

The entire comb is covered with a mass of webbing and fecal matter of the larvae; a condition described as, *Galleriasis*. Later black excreta of the pest can also be noticed entangled in the web. It does not attack adult bees but destruct the combs of a weak colony by chewing the comb, spinning silk lined tunnels through the comb cell walls and over the face of the comb, which prevent the bees to emerge from the sealed comb cells as their body get entangled in silken web, so emerging bees die due to starvation, as they are unable to escape from their silken webbed comb cell.

Possibilities for controlling wax moth including some manipulations in the hive (*i.e.* keeping beehives clean and debris be exploited in three ways, firstly by using parts of the plant in powder form or as crude extracts in water or in organic solvents; secondly as purified extracts (like rotenone and Margosan-O) and finally as a key to synthesize a chemical compound which then could be produced industrially (Downum *et al.* 1993) [11]. Other treatments to stored combs such as, physical, biological and chemical methods can also be used against wax moth (Caron 1992) [8]. Although, the use of the chemicals is somewhat easy and effective, some precautions of safety of environment and contamination of bee products are need to be considered. In addition, some of these materials appear to be ineffective against eggs of the wax moth (Fraser 1997) [13]. In most cases these chemical measures may not be safe due to their pesticidal residue problems, left behind in the hive or combs. A very little information is available on the management of greater wax moth by the use of non-chemical methods and botanicals so, Efforts are being made in different parts of the world to replace existing fumigation based on chemical control methods against *G. Mellonella*, with non-chemical methods and environment friendly treatments those would afford long term protection against the pest.

Materials and Methods

Effectiveness of different treatments against *G. Mellonella* in raised stored combs and per cent infested comb area was studied for two years 2016 and 2017 during June to October. The treatment details are given in Table 1. There were 10 treatments. For each treatment, 7 combs (without bees) were stored in hive body (empty chamber) which was placed on bottom board and covered with inner cover. The chamber with frames was made air tight by mud plastering. After, it was covered with a top cover. Observations were recorded on the extent of damaged area in cm^2 at fortnightly interval with three replications in each treatment. The comb area damaged caused by the larvae of *G. mellonella* was recorded using brood measuring frame having wire grid squares, each measuring one inch squares in size. The number of squares covering the infested area completely or partially were counted as damaged and then converted to cm^2 by multiplying with 6.45 (Yadav *et al.* 2012) [24]. The infested comb area was recorded from both sides of each comb. After every treatment, comb area infestation in all the treatments was also recorded fortnightly.

Statistical analysis

All the research data pertaining to management of greater wax moth by different methods were subjected to Analysis of Variance with the OP-STAT system software.

Table 1: Treatment for surface contact up of various chemical and non-chemicals against *G. mellonella* in stored combs

Sr. No.	Treatment	Conc.	Procedure
T_1	Acetic acid spray	80%	Combs was sprayed with acetic acid (80%) solution (20 ml acetic acid per 10 liter of water) and repeated at an interval of fortnight. One hundred ml of solution was sprayed on each comb covering both the side of comb.
T_2	Formic acid spray	85%	Comb was sprayed with formic acid (85%) solution (8ml formic acid per 10 liter of water) and repeated at an interval of fortnight. One hundred ml of solution was sprayed on each comb covering both the side of comb.
T_3	Aluminum phosphide fumigation	1 tablet (3 g)	One tablet of aluminum phosphide (3 gram) tied in muslin cloth was placed on top bar in hive body containing seven combs. The treatment was repeated at an interval of fortnight.
T_4	Low temperature treatment	0, -5 °C, -10 °C and -15 °C	Combs were freezed only once at the initiation of the experiment at 0 °C, -5 °C, -10 °C and -15 °C for 24 h in the deep freezer and then kept airtight in the bee hive chambers. The observations were recorded at fortnightly interval.
T_5	Hive chambers separation with newspaper	One newspaper	Hive bodies with 7 frames in each chamber was stacked separated with newspaper. Each stack had two chambers. Newspapers were changed at an interval of fortnight.
T_6	Separation of combs with newspapers	One frame covered in each newspaper	For the purpose, hive bodies with 7 combs were used and each comb was wrapped in newspapers individually and then stored in hive body. Newspapers were changed at fortnightly interval.
T_7	Neem oil spray	3%	Spray solution of neem oil (3%) was prepared by mixing 30 ml of neem oil in 970 ml of water and sprayed the comb. One hundred ml of solution was sprayed on 7 individual combs covering both the sides of comb. Spray was repeated at fortnightly interval.
T_8	Broadcasting neem leaves powder	40 g	Neem leaves collected from the neem tree were left for drying in shade at room temperature for 15 days. After drying, leaves were ground by a grinder into a powdered form. This dried neem leaf powder was uniformly dusted @ 40g per comb on combs and dusting was repeated at fortnightly interval.
T_9	Neem Seed Kernel (NSKE) spray	5%	For preparing NSKE, mature fallen neem fruit berries were collected. The seeds were put in a plastic drum with full of water for 7 days for removing pulp of the seed. After that, the neem seed kernel were washed with fresh water and under shade dried. The dried kernels were milled into fine powder. 50g of dried neem seeds powder and 100ml of water were put in plastic container and stirred with a piece of wood. The whole mixture was left overnight, then agitated for 10 minutes and filtered through a fine piece of muslin cloth to get fine suspension. Decoction was extracted by pressing the muslin sack (bag) for 10-15 minutes and repeated washings with water and final volume was made 1000 ml by adding water. One hundred ml of NSKE decoction was uniformly sprayed on individual comb, covering both its sides. Spray was repeated at an interval of a fortnight.
T_{10}	*Bt var. kurstaki* spray	5%	Spray suspension was prepared by mixing 5g of *Bt* var. *kurstaki* (Dipel) in one liter of water and 100ml of solution was sprayed on individual comb covering both of its sides. Spray was repeated at fortnightly interval.
T_{11}	Control	No spray	No treatment and even water was not sprayed

Results

Effect of different treatments on *G. mellonella* infestation in raised combs under storage conditions during June to October, 2016

It is revealed from the data on the effect of different treatments on *G. Mellonella* infestation in raised combs under storage conditions during June to October, 2016 are presented in Table 2. Indicated that at the time of first observation (15 DAT *i.e.,* day after treatment), the average area damaged per comb (cm^2) ranged from 0.00 in low temperature treatment (-15 °C, -10 °C) to 169.85 in control. The most effective treatment was low temperature treatment at -15°C, -10°C followed by low temperature treatment at -5°C, low temperature treatment at 0°C, Aluminum phosphide fumigation, *Bt* spray, separation of combs with newspapers, NSKE spray, acetic acid spray, formic acid spray, neem oil spray, hive chamber separation with newspaper and broadcasting neem leaves powder, where the comb area damage was 0.00, 0.52, 6.45, 9.68, 21.50, 32.25, 49.45, 58.05, 70.95, 79.55 and 111.8 0cm^2, respectively (Table 2). At this time, the comb area damaged was 169.85 cm^2 in control. At the time of second observation (30 DAT), the average area damaged per comb ranged from 0.00 in low temperature treatment (-15 °C, -10 °C) to 365.50 (cm^2) in control. Again the most effective treatment was low temperature treatment -15 °C, -10 °C followed by low temperature treatment at -5 °C and 0 °C, Aluminum phosphide fumigation, *Bt* spray, NSKE spray, separation of combs with newspapers, acetic acid spray, formic acid spray, hive chamber separation with newspaper, neem oil spray and broadcasting neem leaves powder, where the comb area damage was 0.00, 0.75, 6.99, 9.68, 33.33, 40.85, 45.15, 55.90, 59.13, 94.60, 103.20 and 208.55 cm^2, respectively. At this time, the comb area damaged was 365.50 cm^2 in control. At the time of third observation (45 DAT), the average area damaged per comb (cm^2) ranged from 0.00 in low temperature treatment (-15 °C) to 989.00 in control. The most effective treatment was low temperature treatment (-15 °C) followed by low temperature treatment at -10°C followed by low temperature treatment at -5°C, low temperature treatment (0 °C), Aluminum phosphide fumigation, separation of combs with newspapers, *Bt* spray, formic acid spray, NSKE spray, acetic acid spray, hive chamber separation with newspaper, neem oil spray and broadcasting neem leaves powder, where the comb area damage was 0.00, 0.45, 0.75, 7.53, 10.75, 64.50, 77.40,79.55, 86.00, 94.60, 129.00, 210.70 and 763.25 cm^2, respectively. At this time, the comb area damaged was 989.00 cm^2 in control. At the time of fourth observation (60 DAT), the average area damaged per comb (cm^2) ranged from 0.00 in low temperature treatment (-15 °C) to 1290.00 in control. The most effective treatment was low temperature treatment at -15°C followed by low temperature treatment at -10 °C, -5 °C, low temperature treatment at 0 °C, Aluminum phosphide fumigation, separation of combs with newspapers, *Bt* spray, formic acid spray, NSKE spray, acetic acid spray, neem oil spray, hive chamber separation with newspaper and broadcasting neem leaves powder where, the comb area damage was 0.00, 0.53, 0.88, 8.06, 11.28, 94.60, 129.00, 131.15, 132.23, 144.05, 646.08, 935.25 and 1139.50 cm^2, respectively. At this time, the comb area damaged was 1290.00 cm^2 in control. At the time of fifth observation (75 DAT), the average area damaged per comb ranged from 0.21 in low temperature treatment at -15 °C to 1472.75 cm^2 in control. The most effective treatment was low temperature treatment at -15 °C followed by low temperature treatment at -10 °C, at -5 °C, and at 0 °C, Aluminum phosphide fumigation, *Bt* spray, separation of combs with newspapers, NSKE spray, formic acid spray acetic acid spray, neem oil spray, hive chamber separation with newspaper and broadcasting neem leaves powder, where the comb area damage was 0.21, 0.53, 1.01, 8.60, 12.90, 133.30, 137.60,154.80, 173.07, 187.05, 741.75, 1085.75 and 1247.00 cm^2, respectively. At this time, the comb area damaged was 1472.75 cm^2 in control. At the time of sixth observation (90 DAT), the average area damaged per comb ranged from 0.32 in low temperature treatment (-15 °C) to 1526.50 cm^2 in control. The most effective treatment was low temperature treatment at -15°C followed by low temperature treatment at -10 °C, low temperature treatment at -5°C, low temperature treatment (0 °C), Aluminum phosphide fumigation, *Bt* spray, separation of combs with newspapers, NSKE spray, formic acid spray, acetic acid spray, neem oil spray, hive chamber separation with newspaper and broadcasting neem leaves powder, where the comb area damage was 0.32, 0.54, 1.07, 10.75, 13.97, 139.75,154.80,161.25, 182.75, 199.95, 864.65, 1154.55 and 1552.30 (cm^2), respectively. At this time, the comb area damaged was 1526.50 cm^2 in control.

At the time of seventh observation (105 DAT), the average area damaged per comb ranged from 0.32 cm^2 in low temperature treatment at -15 °C to 1599.60 cm^2 in control. The most effective treatment was low temperature treatment at -15 °C followed by low temperature treatment at -10 °C, low temperature treatment at -5 °C, low temperature treatment (0 °C), Aluminumphosphide fumigation, *Bt* spray, NSKE spray, separation of combs with newspapers, formic acid spray, acetic acid spray, hive chamber separation with newspaper, neem oil spray and broadcasting neem leaves powder, where the comb area damage was 0.32, 0.75, 1.61, 10.75, 17.20, 154.80, 169.85, 180.60, 197.80, 219.30, 1285.70 and1599.60 cm^2, respectively. At this time, the comb area damaged was 1599.60 cm^2 in control. At the time of eighth observation (120 DAT), the average area damaged per comb (cm^2) ranged from 0.54 in low temperature treatment (-15 °C) to 1599.60 in control. The most effective treatment was low temperature treatment at -15 °C followed by low temperature treatment at -10 °C followed by low temperature treatment at -5 °C, low temperature treatment 0 °C, Aluminum phosphide fumigation, *Bt* spray, NSKE spray, separation of combs with newspapers, formic acid spray, acetic acid spray, hive chamber separation with newspaper, neem oil spray and broadcasting neem leaves powder, where the comb area damage was 0.54, 1.07, 2.15, 11.82, 19.35, 167.70, 176.30,187.05, 1290.00 and1599.60 cm^2, respectively. At this time, the comb area damaged was 1599.60 cm^2 in control. At the time of ninth observation (135 DAT), the average area damaged per comb ranged from 1.07 (cm^2) in low temperature treatment at -15°C to 1599.60 in control. The most effective treatment was low temperature treatment at -15 °C followed by low temperature treatment at -10 °C followed by low temperature treatment at -5 °C, low temperature treatment at 0 °C, Aluminum phosphide fumigation, *Bt* spray, separation of combs with newspapers, NSKE spray, formic acid spray, acetic acid spray, neem oil spray, hive chamber separation with newspaper and broadcasting neem leaves powder, where the comb area damage was 1.07, 1.61, 3.23, 11.83,

19.35, 180.60, 197.80, 322.50, 377.55, 1502.85 and 1599.60 cm^2, respectively. At this time, the comb area damaged was 1599.60 cm^2 in control. At the time of last observation (150 DAT) i.e. end of the experiment, the average area damaged per comb ranged from 1.08 in low temperature treatment at -15 °C to 1599.60 cm^2 in control. The most effective treatment was low temperature treatment at -15 °C followed by low temperature treatment at -10 °C, low temperature treatment -5 °C, low temperature treatment 0 °C, Aluminum phosphide fumigation, *Bt* spray, separation of combs with newspapers, NSKE spray, formic acid spray, acetic acid spray, neem oil spray, hive chamber separation with newspaper and broadcasting neem leaves powder, where the comb area damage was 1.08, 1.61, 4.30,12.90, 23.65, 197.80, 208.55, 240.80, 346.15, 369.80 1586.70, 1599.60 and 1599.60 cm^2, respectively. At this time, the comb area damaged was 1599.60 cm^2 in control.

Table 2: Effect of different treatments on comb area infestation by *Galleria mellonella* infestation in raised combs under different storage conditions during June to October, 2016

Sr. No.	Treatments	Average area infested per comb (cm^2)										Mean
		15 DAT	30 DAT	45 DAT	60 DAT	75 DAT	90 DAT	105 DAT	120 DAT	135 DAT	150 DAT	
T_1	Acetic acid spray	49.45	55.90	94.60	144.05	187.05	199.95	219.30	247.25	337.55	369.80	190.49
T_2	Formic acid spray	58.05	59.13	79.55	131.15	173.07	182.75	197.80	225.75	322.50	346.15	177.59
T_3	Aluminumphosphide fumigation	9.68	9.68	10.75	11.28	12.90	13.97	17.20	18.28	19.35	23.65	14.67
T_4	Low temperature treatment 0 °C	6.45	6.99	7.53	8.06	8.60	10.75	10.75	11.82	11.83	12.90	9.57
	Low temperature treatment -5 °C	0.52	0.75	0.79	0.88	1.01	1.07	1.61	2.15	3.23	4.30	1.63
	Low temperature treatment -10 °C	0.00	0.00	0.45	0.53	0.53	0.54	0.75	1.07	1.61	1.61	0.71
	Low temperature treatment -15 °C	0.00	0.00	0.00	0.00	0.21	0.32	0.32	0.54	1.07	1.08	0.35
T_5	Hive chambers Separation with newspaper	79.55	94.60	129.00	935.25	1085.75	1154.55	1231.95	1290.00	1502.85	1599.60	910.31
T_6	Separation of combs with newspapers	32.25	45.15	64.50	94.60	137.60	154.80	180.60	187.05	197.80	208.55	130.29
T_7	Neem oil spray	70.95	103.20	210.70	646.08	741.75	864.25	1285.70	1343.75	1464.15	1586.70	831.72
T_8	Broadcasting neem leaves powder	111.80	208.55	763.25	1139.50	1247.00	1552.30	1599.60	1599.60	1599.60	1599.62	1142.09
T_9	NSKE spray	38.70	40.85	86.00	132.23	154.80	161.25	169.85	176.30	223.60	240.80	142.44
T_{10}	*Bt var. kurstaki* spray	21.50	33.33	77.40	129.00	133.30	139.75	154.80	167.70	180.60	197.80	123.52
T_{11}	Control	169.85	365.50	989.00	1290.00	1472.75	1526.50	1599.60	1599.60	1599.60	1599.60	1221.21
	Mean	46.34	73.12	179.54	333.04	382.59	425.91	476.42	490.78	533.24	556.24	
	Factor	CD				SE(d)				SE(m)		
	Treatment	5.20				2.64				1.86		
	Time	4.40				2.23				1.58		
	Treatment × Time	16.45				8.40				5.91		

Each value represent mean of three observations
* DAT= Days after first treatment

Effect of different treatments on combs infestation by *G. Mellonella* in raised combs under different storage conditions during June to October, 2017

Data on the effect of different treatments on *G. mellonella* infestation in raised combs under different storage conditions during June to October, 2017 are presented in Table 3. It is revealed from the data that at the time of first observation (15 DAT), the average area damaged per comb ranged from 0.00 cm^2 in low temperature treatment at -15 °C, -10 °C to 170.38 cm^2 in control. The most effective treatment was low temperature treatment at -15 °C, -10 °C followed by low temperature treatment at -5 °C, low temperature treatment (0 °C), Aluminum phosphide fumigation, *Bt* spray, separation of combs with newspapers, NSKE spray, acetic acid spray, formic acid spray, neem oil spray, hive chamber separation with newspaper and broadcasting neem leaves powder, where the comb area damage (cm^2) was 0.00, 0.51, 6.45, 8.60, 22.03, 32.78, 39.77, 51.60, 59.66, 72.02, 80.62 and 170.38 cm^2, respectively. At this time, the comb area damaged was 170.38 cm^2 in control. At the time of second observation (30 DAT), the average area damaged per comb (cm^2) ranged from 0.00 in low temperature treatment at -15 °C cm^2 to 371.95 cm^2 in control. The most effective treatment was low temperature treatment at -15°C, followed by low temperature treatment at -10 °C, low temperature treatment at -5 °C, low temperature treatment at 0 °C, Aluminum phosphide fumigation, *Bt* spray, separation of combs with newspapers, NSKE spray, acetic acid spray, formic acid spray, hive chamber separation with newspaper, neem oil spray and broadcasting neem leaves powder where the comb area damage was 0.00, 0.43, 0.52, 6.99, 9.14, 38.16, 51.68, 52.68, 56.98, 60.74, 96.75, 108.58 and 218.76 cm^2, respectively. At this time, the comb area damaged was 371.95 cm^2 in control. At the time of third observation (45 DAT), the average area damaged per comb ranged from 0.00(cm^2) in low temperature treatment at -15 °C to 1010.50 cm^2 in control. The most effective treatment was low temperature treatment at -15 °C followed by low temperature treatment at -10 °C, low temperature treatment at -5 °C, low temperature treatment 0 °C, Aluminum phosphide fumigation, separation of combs with newspapers, formic acid spray, *Bt* spray, NSKE spray, acetic acid spray, hive chamber separation with newspaper, neem oil spray and broadcasting neem leaves powder where the comb area damage (cm^2) was 0.00, 0.43, 0.75, 7.53, 10.21, 65.04, 81.70, 87.61, 89.69, 95.14, 131.15, 217.15 and 786.90 cm^2, respectively. At this time, the comb area damaged was 1010.50 cm^2 in control. At the time of fourth observation (60 DAT), the average area damaged per comb ranged from 0.00 in low temperature treatment at -15 °C to 1294.30 in control. The most effective treatment was low temperature treatment at -15 °C followed by low temperature treatment at -10 °C, low temperature treatment at -5 °C, low temperature treatment at 0 °C, Aluminum phosphide fumigation, separation of combs with

newspapers, *Bt* spray, NSKE spray, formic acid spray, acetic acid spray, neem oil spray, hive chamber separation with newspaper and broadcasting neem leaves powder, where the comb area damage was 0.00, 0.43, 0.86, 8.06, 10.96, 95.68, 126.85, 134.38, 141.90, 146.20, 685.85, 956.75 and 1143.80 cm^2, respectively. At this time, the comb area damaged was1294.30 cm^2 in control. At the time of fifth observation (75 DAT), the average area damaged per comb (cm^2) ranged from 0.21 in low temperature treatment (-15 °C) to 1472.75 in control. The most effective treatment was low temperature treatment (-15 °C), followed by low temperature treatment at -10 °C followed by low temperature treatment at -5 °C, low temperature treatment (0°C), Aluminum phosphide fumigation, *Bt* spray, separation of combs with newspapers, NSKE spray, formic acid spray, acetic acid spray, neem oil spray, hive chamber separation with newspaper and broadcasting neem leaves powder where the comb area damage was 0.32, 0.43, 0.96, 9.13, 12.90, 135.45, 141.90, 163.40, 178.45, 191.35, 789.05, 1107.25 and 1272.80 (cm^2), respectively. At this time, the comb area damaged was 1599.60 cm^2 in control. At the time of sixth observation (90 DAT), the average area damaged per comb (cm^2) ranged from 0.32 in low temperature treatment at -15 °C to 1599.60 in control. The most effective treatment was in low temperature treatment at -15 °C followed by low temperature treatment at -10 °C, low temperature treatment at -5°C, low temperature treatment (0 °C), Aluminum phosphide fumigation, *Bt* spray, separation of combs with newspapers, NSKE spray, formic acid spray, acetic acid spray, neem oil spray, hive chamber separation with newspaper and broadcasting neem leaves powder where the comb area damage was 0.32, 0.43, 1.03, 10.75, 13.43, 144.05, 156.95, 169.85, 189.20, 234.35, 894.40, 1176.05 and 1599.60 (cm^2), respectively. At this time, the comb area damaged was 1599.60 cm^2 in control. At the time of seventh observation (105 DAT), the average area damaged per comb ranged from 0.52 in low temperature treatment (-15 °C) to 1599.60 cm^2 in control. The most effective treatment was low temperature treatment at -15 °C followed by low temperature treatment at -10 °C, low temperature treatment at -5 °C, low temperature treatment at 0 °C, Aluminum phosphide fumigation, *Bt* spray, NSKE spray, separation of combs with newspapers, formic acid spray, acetic acid spray, hive chamber separation with newspaper, neem oil spray, and broadcasting neem leaves powder where the comb area damage was 0.52, 0.52, 1.55, 10.75, 17.73, 153.72, 180.60, 182.75, 201.12, 258.00, 1244.85, 1296.45 and 1599.60 cm^2, respectively. At this time, the comb area damaged was 1599.60 cm^2 in control. At the time of eighth observation (120 DAT), the average area damaged per comb ranged from 0.54 in low temperature treatment at -15 °C to 1599.60 cm^2 in control. The most effective treatment was low temperature treatment at -15 °C followed by low temperature treatment at -10°C, low temperature treatment at -5 °C, low temperature treatment 0 °C, Aluminum phosphide fumigation, *Bt* spray, separation of combs with newspapers, NSKE spray, formic acid spray, acetic acid spray, hive chamber separation with newspaper, neem oil spray and broadcasting neem leaves powder, where the comb area damage was 0.54, 1.07, 2.04, 11.29, 18.81, 160.17, 191.35, 225.75, 227.90, 267.35, 1298.60, 1353.35 and 1599.60 cm^2, respectively. At this time, the comb area damaged was 1599.60 cm^2 in control. At the time of ninth observation (135 DAT), the average area damaged per comb ranged from 0.86 in low temperature treatment at -15 °C to 1599.60 cm^2 in control. The most effective treatment was low temperature treatment at -15 °C, followed by low temperature treatment at -10 °C, low temperature treatment at -5 °C, low temperature treatment (0 °C), Aluminum phosphide fumigation, *Bt* spray, separation of combs with newspapers, NSKE spray, formic acid.

Table 3: Effect of different treatments on *Galleria mellonella* infestation in raised combs under storage conditions during June to October, 2017

Sr. No	Treatments	Average area infested per comb (cm^2)										Mean
		15 DAT	30 DAT	45 DAT	60 DAT	75 DAT	90 DAT	105 DAT	120 DAT	135 DAT	150 DAT	
T_1	Acetic acid spray	51.60	56.98	95.14	146.20	191.35	234.35	258.00	267.35	402.05	449.35	215.37
T_2	Formic acid spray	59.66	60.74	81.70	141.90	178.45	189.20	202.12	227.90	339.70	354.75	183.61
T_3	Aluminumphosphide fumigation	8.60	9.14	10.21	10.96	12.90	13.43	17.73	18.81	19.89	23.11	14.47
T_4	Low temperature treatment 0 °C	6.45	6.99	7.53	8.06	9.13	10.75	10.75	11.29	11.82	11.83	9.46
	Low temperature treatment -5 °C	0.51	0.52	0.75	0.86	0.96	1.03	1.55	2.04	3.16	3.76	1.51
	Low temperature treatment -10 °C	0.00	0.43	0.43	0.43	0.43	0.43	0.52	1.07	1.51	1.61	0.68
	Low temperature treatment -15 °C	0.00	0.00	0.00	0.00	0.32	0.32	0.54	0.54	0.86	1.08	0.36
T_5	Hive chambers Separation with newspaper	80.62	96.75	131.15	956.75	1107.25	1176.05	1244.85	1298.60	1524.35	1599.60	921.59
T_6	Separation of combs with newspapers	32.78	51.60	65.04	95.68	141.90	156.95	182.75	191.35	219.30	236.50	137.38
T_7	Neem oil spray	72.02	108.58	217.15	685.85	789.05	894.40	1296.45	1353.35	1472.75	1595.30	848.49
T_8	Broadcasting neem leaves powder	126.85	218.76	786.90	1143.80	1272.80	1566.60	1599.60	1599.60	1599.60	1599.60	1151.41
T_9	NSKE spray	39.77	52.68	89.69	134.38	163.40	169.85	180.60	225.75	237.62	238.65	153.23
T_{10}	*Bt var. kurstaki* spray	22.03	38.16	87.61	126.85	135.45	144.05	153.72	160.17	172.00	198.88	123.89
T_{11}	Control	170.38	371.95	1010.50	1294.30	1599.60	1599.60	1599.60	1599.60	1599.62	1599.60	1244.47
Mean		47.94	76.66	184.55	339.00	400.21	439.78	482.05	496.95	543.15	565.25	

Factor	CD	SE(d)	SE(m)
Treatment	4.21	2.14	1.51
Time	3.55	1.81	1.28
Treatment × Time	13.31	6.77	4.78

Each value represent mean of three observations
* DAT= Days after first treatment

Spray, acetic acid spray, neem oil spray, hive chamber separation with newspaper and broadcasting neem leaves powder where the comb area damage was 0.86, 1.51,3.16,11.82, 19.89, 172.00, 219.30, 237.62, 339.70, 402.05, 1472.75, 1524.35 and 1599.60 cm^2, respectively. At this time, the comb area damaged was 1599.60 cm^2 in

control. At the time of last observation (150 DAT) *i.e.*, end of the experiment, the average area damaged per comb ranged from 1.08 in low temperature treatment at -15 °C to 1599.60 cm^2 in control. The most effective treatment was low temperature treatment at -15 °C, followed by low temperature treatment at -10 °C, low temperature treatment at -5 °C, low temperature treatment at 0 °C, Aluminum phosphide fumigation, *Bt* spray, separation of combs with newspapers, NSKE spray, formic acid spray, acetic acid spray, neem oil spray, hive chamber separation with newspaper and broadcasting neem leaves powder where the comb area damage was 1.08, 1.61, 3.76, 11.83, 23.11, 198.88, 236.50, 238.65, 354.75, 449.35, 1595.30, 1599.60 and 1599.60 cm^2, respectively. At this time, the comb area damaged was 1599.60 cm^2 in control. From the above observations, it can be concluded that the order of effectiveness of treatment was the low temperature treatment (-15 °C), followed by low temperature treatment at -10 °C followed by low temperature treatment at -5 °C, low temperature treatment (0 °C), Aluminum phosphide fumigation, *Bt* spray, separation of combs with newspapers, NSKE spray, formic acid spray, acetic acid spray, neem oil spray, hive chamber separation with newspaper and broadcasting neem leaves powder.

Overall all mean area (cm^2) damage and % comb infestations for both the year

Data in the table 4 indicated that the lowest comb area damage (cm^2) and comb area infestation (%) was observed in case of low temperature treatment at -15 °C followed by low temperature treatment at -10 °C followed by low temperature treatment at -5 °C, low temperature treatment at 0 °C, Aluminum phosphide fumigation, *Bt* spray, NSKE spray, separation of combs with newspapers, formic acid spray, acetic acid spray, hive chamber separation with newspaper, neem oil spray, and broadcasting neem leaves powder while maximum comb area infestation was highest in control during both the years. Thus, it was clear from the present findings that wax moth infestation could be managed effectively in the month of September and special care should be taken as the activity of wax moth was found at its peak. Furthermore, the findings on the effectiveness of different storage treatment of raised combs on wax moth infestation revealed that raised combs when stored in polythene sheet with fumigation would be less prone to wax moth damage. Although, application of non-chemical method was, however, considered by far the cheapest and safest method.

Table 4: Overall all mean area (cm^2) damage and % comb infestations for both the year

Sr. No.	Treatments	Mean of comb area damage (cm^2)		
		Mean 2016	Mean 2017	Overall mean
T_1	Acetic acid spray	190.49	215.37	202.93
T_2	Formic acid spray	177.59	183.61	180.6
T_3	Aluminumphosphide fumigation	14.67	14.47	14.57
T_4	Low temperature treatment 0°C	9.57	9.46	9.515
	Low temperature treatment -5°C	1.63	1.51	1.57
	Low temperature treatment -10°C	0.71	0.68	0.695
	Low temperature treatment -15°C	0.35	0.36	0.355
T_5	hive chambers Separation with newspapers	910.31	921.59	915.95
T_6	Separation of combs with newspapers	130.29	137.38	133.835
T_7	Neem oil spray	831.72	848.49	840.105
T_8	Broadcasting neem leaves powder	1142.09	1151.41	1146.75
T_9	NSKE spray	142.44	153.23	147.835
T_{10}	*Bt var. kurstaki* spray	123.52	123.89	123.705
T_{11}	Control	1221.21	1244.47	1232.84

Each value represent mean of five observations

Discussion

From the above observations, it was found that the order of effectiveness of treatment was the low temperature treatment at -15 °C, followed by low temperature treatment at -10 °C, low temperature treatment at -5 °C, low temperature treatment (0 °C), Aluminum phosphide fumigation, *Bt* spray, separation of combs with newspapers, NSKE spray, formic acid spray, acetic acid spray, neem oil spray, hive chamber separation with newspaper and broadcasting neem leaves powder. The study of Babarinde *et al.* (2013) [3] also reported that treatment containing Aluminum phosphide tablet (3 g) was incorporated as chemical method of control and the treated honeycomb samples were stored for two month while, Goodman (1990) [14] found best result for comb retained freshness post two months storage with the hermetic storage and Aluminum phosphide treatment. The result are insame line with the study of Kumari and Jha (2013) [15] where minimum infestation (160.60 cm^2) was in case when the comb frames were stored in the polythene sheet with fumigation. Munshimbwe *et al.* (2011) [18] suggested that an 80 per cent solution of acetic acid could be used on top-bars to control wax moths in stored comb frames. The findings are in agreement with the Bhopale *et al.* (2013) [4] as minimum cell damage was observed in *Bt* kurstaki (Halt) which was on par with that of Pongamia oil followed by *Bt* local strain-1 and neem oil (12 to 20% cell damage). Dried neem leaf and *Bt* local strain-2 performed poorly with 32.22 to 37% cell damage, respectively. The comb damage in control was 90 per cent, which was significantly higher than the damage recorded in all other treatments Charriere and Imdorf (2004) [26]. Zhou (2016) [25] determined the optimal cryopreservation (at low temperature) method for eradication of the greater wax moth from honey bee combs. This study showed that all larvae, pupae and adults died after they were treated for one hour at -15 to -20°C low temperature. The results are also in close propinquity with the Swamy (2000) [19]. None of the treatments could protect comb sufficiently except the refrigerated condition. The present findings are also in line with that of Williams (1997) [23] who reported that phosphine controlled wax moth in empty combs. Atwal and Sharma (1967) [2] and Verma *et al.*

(1997) [20] reported that the use of sulphur and cleaning of beehives assisted in reducing wax moth population. Kwadha *et al.* (2017) [16] infested combs could also be exposed to cold rooms or refrigerator equipment such as home freezers set at -7 °C to -15 °C for 2-4.5 hours. These techniques are advantageous, since growth and development of GWM is dependent on environmental factors such as temperature. Almost complete loss (1599.60 cm^2) of stored combs was reported 90 days after first treatment, indicating total ineffectiveness of spreading dried out neem leaves powder (Yadav *et al.* 2012) [24]. Effectiveness of sulphur fumigation on *G. mellonella* collobrated the finding of Ahmed *et al.* (1993) [1] and Calderone (2000) [6] who advocated that observed that sulphur fumigation could be used for controlling wax moth. These results are in line with those reported earlier by Cantwell and Shieh (1981) [7] that Certan, a *Bacillus thuringiensis* preparation provided excellent wax moth control on stored combs. Vishwas and Gowda (2006) [21] also reported that 500 ppm of *Bacillus thuringiensis kurstaki* UAS strains (Dipel and Halt) resulted in 80, 63.33 and 63.33 per cent mortality of 3rd instar larvae of *G. Mellonella*, also reported that Neem oil spray resulted in significantly higher overall mean damage to stored combs (625.31 cm^2) as compared with Karanj oil spray (473.2 cm^2).

Conclusion

From the study, it is concluded that the most effective treatment low temperature treatment (-15 °C, -10 °C) followed by low temperature treatment (-5 °C and 0 °C), Aluminum phosphide fumigation, *Bt* spray, separation of combs with newspapers, NSKE spray, formic acid spray, Acetic acid spray, neem oil spray, hive chamber separation with newspaper and broadcasting neem leaf powder. Although, there is no better indemnity against the attack of the greater wax moth than to observe the apiary and stored combs and follow management practices that keep away the moth.

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