EFFECT OF SOFT CHEMICALS AND TOBACCO LEAF-SMOKE TO CONTROL PUNCTURED PUPA DISEASE OF HONEY BEE, *APIS MELLIFERA* L. IN THE DEARTH PERIOD

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Abstract

A study was conducted in the Apiary situated at Amtoli in Gazipur district to investigate the effect of soft chemicals and tobacco leaf-smoke to control punctured pupa disease of honey bee, *Apis mellifera* L. in 2012. Tracheal mite, *Acarapis woodi* was found in the trachea of immature bee and *Tropilaelaps clareae* were found inside the damaged pupal cell, either or both of which are responsible for this disease. Sixteen number of similar strength (4frames/hive) of honey bee hives were selected for the study. Three alternative approaches and an untreated control were utilized to control punctured pupa disease. The treatments were tobacco leaf-smoke, menthol; formic acid and an untreated control (no treatment). The highest egg deposition (20.41%), larval hatching (21.45%), pupae formation (23.95%) and pollen deposition (7.29%) were obtained in tobacco leaf-smoke treated hives and this differed significantly from the untreated control hives and some other treated hives. The highest bee gain (6.00) in terms of occupied frame was also observed in the tobacco leaf-smoke treated hive. The highest mortality of bees (0.79) was observed in the formic acid treated hive. The highest punctured pupa disease (75.42%) was found in the untreated hive compared to treated hives. The highest benefit cost ratio (3.28) was obtained in the tobacco leaf-smoke treated hive. It is found that to control punctured pupa disease and to get higher BCR, the use of tobacco leaf-smoke in dearth period of honey bee could be suggested.

***Key words*: Honey bee, punctured pupa, dearth period, soft chemicals, tobacco leaf-smoke.

INTRODUCTION

Honey bee (*Apis mellifera* L.) colonies are a rich and stable habitat for parasites. Host bees, honey, pollen and wax are present round the year and bees regulate temperature, humidity and carbon dioxide levels within narrow limits (Winston 1987). As a consequence, honey bee colonies are exploited by an array of parasites, pathogens and associated organisms. They act as important selective forces on the evolution of colony structure and function (Gadagkar 1992, Sherman *et al*. 1988).

Mites parasitize honey broods. *Varroa* and *Tropilaelaps* mites feed on honey bee haemolymph, depriving the bee from haemolyrnph and protein (De Jong *et a1*. 1982). The tracheal mite, *Acarapis woodi* lives inside the tracheal system of bee and take their food from the immature bee body. This mite creates severe breathing problem of bees. This effect is usually sub lethal to the individual bee, but infestation by any mite species can kill a bee colony when not controlled. The presence of different mites in honey bee colonies create critical management problem facing by the beekeepers worldwide. However, different soft chemicals and different plant products are used to control different bee mites. Among them oxalic acid, formic acid, thymol, menthol are important because these are not hazardous to bees as well as human beings. Different plant products such as grape leaf smoke, tobacco leaf smoke, neem leaf are also used traditionally to control different bee-attacking mites.

Most of the beekeepers are not conscious about bee health in Bangladesh. Several numbers of diseases and pests are involved to affect bee health throughout the year, especially in dearth period. In Bangladesh context very few literature are available on bee pests and diseases. But, beekeepers often claim that their hive is suffering from different pests or diseases. Even after the dearth period many beekeepers reported that their hive is not functioning well due to unknown pest or diseases. A lot of beekeepers are facing problem of punctured pupae inside the hive. In view of the above fact, the present study was undertaken to identify the causal organism or agent involved in punctured pupa disease, to know the effect of different traditional approaches for the management of punctured pupa disease in the dearth period and to find out the effective management tactics against this disease.

MATERIAL AND METHODS

The present study on the management of punctured pupa disease of *Apis mellifera* L. in the dearth period of the year was conducted at Amtoli, Gazipur district from June, 2011 to August, 2012. Sugar syrup and soybean flour were used as nectar and pollen substitutes, respectively. Twenty five samples were collected from five different apiary and the samples were examined thoroughly under a compound microscope to find out different causal organisms or agents of punctured pupa disease of honey bee. Sixteen uniform hives of the same species were selected. Each hive consisted of four frames (two brood frames + two occupied/built frames) and a feeder frame to study the effects of soft chemicals and tobacco leaf smoke on the honey bee, (*A. mellifera*) colonies. All the frames were considered for data collection. Tobacco leaf smoke (1 leaf/4 hive/smoking) and two soft chemicals, viz. formic acid (10 ml formic acid/4hives/week) and menthol (5 g/hive) were considered as three different treatments and an untreated control (without any management practice) was chosen also for the study. The experiment was done in a Randomized Complete Block Design (RCBD). Pollen supplement, nectar and pollen substitute, nectar substitute (treatments), pollen substitute, smoking with tobacco leaves, application of menthol, application of formic acid were prepared for the study. The percentage data calculated by following formula:

$$\text{Percentage (\%) of eggs in the hive} = \frac{\text{Area covered by eggs (cm)}}{\text{Total occupied area (cm)}} \times 100$$

$$\text{Percentage (\%) of larvae in the hive} = \frac{\text{Area covered by larvae (cm)}}{\text{Total occupied area (cm)}} \times 100$$

$$\text{Percentage (\%) of pupae in the hive} = \frac{\text{Area covered by pupae (cm)}}{\text{Total occupied area (cm)}} \times 100$$

$$\text{Percentage (\%) of pollen in the hive} = \frac{\text{Area covered by pollen (cm)}}{\text{Total occupied area (cm)}} \times 100$$

$$\text{Percentage (\%) of punctured pupa in the hive} = \frac{\text{Area covered by punctured pupa (cm)}}{\text{Total occupied area (cm)}} \times 100$$

Benefit cost ratio (BCR) for each treatment was calculated by following formula:

$$\text{Benefit cost ratio (BCR)} = \frac{\text{Adjusted net return}}{\text{Total management cost}}$$

The adjusted net return was determined by following formula:

Adjusted net return = Net return in treated hive − Management cost in control hive

The data for each parameter was analyzed statistically to find out the variation among the treatments. The percent data were transformed into square root transformation. The analysis of variance for different parameters was done using MSTAT-C software. The means were separated by Least Significance Difference (LSD) Test.

RESULTS AND DISCUSSION

Identification of causal agents/organisms for punctured pupal disease

Three types of parasitic mite species, viz. *Acarapis woodi*, *Varroa destructor* and *Tropilaelaps clareae* found in the punctured pupal cells identified as causal agents for the punctured pupa disease in *Apis mellifera* (Table 1).

Table 1. Organismspresent in the infested cells of *A. mellifera* hive and their symptoms of attack observed in different locations during the study period (2011).

Sample collection	Organisms in the infested cell (No.)	Type of infested cell	Symptoms
Gazipur (Amtoli)	*Acarapis woodi* (5), *Tropilaelaps clareae* (4)	Worker cell, Drone cell	Punctured pupal cell, Juicy body fluid, No bad smell
Gazipur (Vadun)	*Acarapis woodi* (5), *Varroa destructor* (1) *Tropilaelaps clareae* (4)	Worker cell, Drone cell	Punctured pupal cell, No bad smell
Gazipur (Dherasrom)	*Acarapis woodi* (5) *Tropilaelaps clareae* (3)	Worker cell	Punctured pupal cell, No bad smell
Tangail	*Acarapis woodi* (5) *Tropilaelaps clareae* (5)	Worker cell	Punctured pupal cell, Juicy body fluid, No bad smell
Shatkhira	*Acarapis woodi* (5) *Tropilaelaps clareae* (5)	Worker cell	Punctured pupal cell, Juicy body fluid, No bad smell

Among the parasitic species, *Acarapis woodi* was observed in every damaged pupal cell containing frame (total 25). Most of the frame (total 21) containing damaged cells were also infested by *T. clareae* mite. Only one sample was found with *Varroa destructor*. In the healthy cells no mite was found inside the cell or even inside the trachea of pupae. In every case the infested cells were found punctured and no bad smell was omitted. Juicy body fluid of pupa was observed in the samples of two locations. When the bodies of dead pupa were picked up by a needle from the cell, most of the pupal dead bodies of pupae were found detached from the abdomen and thoracic segment. It was evident that the disease was caused by either tracheal mite, *A. woodi* or the mite, *T. clareae* or their combination. In most cases the pupal cells of worker were found in the damaged condition which not occurred if attacked only by *Varroa* as *Varroa* mite mostly prefers drone cells. Hosamani *et al.* (2006) and other researchers reported 50 per cent brood mortality in *A. mellifera* due to *T. clareae* infestation.

Percentage of egg, larva, pupa, pollen deposition and number of occupied frame of A. mellifera influenced by different type of management approaches

Different approaches to control punctured pupa disease in the hive has significant effect on egg laying, larval and papal development and pollen deposition inside the hive. Therefore, bee populations were directly affected by different types of control strategy.

Table 2. Effect of management approaches against punctured pupa disease and its impact on egg deposition, larval hatching, pupae formation, pollen deposition per hive and number of frames occupied by honey bee, *A. mellifera* L.

Treatments	Egg/hive (%)	Larva/hive (%)	Pupa/hive (%)	Pollen/hive (%)	No. of occupied frame
Tobacco leaf-smoke	20.41 (26.84) a	21.45 (27.55) a	23.95 (29.29)a	7.29 (15.63)a	4.75 a
Menthol	19.58 (26.24) a	20.41 (26.86) a	21.45 (27.58)ab	7.29 (15.63)a	4.21 b
Formic acid	16.25 (23.76) b	17.91 (24.99) a	18.54 (25.49)b	5.21 (13.12) b	4.00 b
Untreated control	12.96 (21.00) c	12.71 (20.85) b	10.83 (19.13)c	3.13 (10.15) c	3.63 c
CV(%)	12.46	15.09	14.34	8.27	10.17
Level of significance	*	*	**	**	**

Mean followed by uncommon letters differed significantly from each other by LSD.

The effect of different management approaches to control punctured pupa symptom on honey bee queen’s ability to lay eggs was evaluated during the experimental period (Table 2). The highest percentage of egg (20.41%) was observed during the dearth period in the hives treated with tobacco leaf smoke which was statistically similar with the result where menthol was provided, but differed significantly where formic acid was applied.

The lowest percentage of egg deposition (12.96%) was noticed in the untreated control hives treatment where no management approach was attempted. All the treatments differed significantly with that of untreated control in terms of egg deposition percentage. Significantly the highest egg deposition per hive (20.41) was recorded in the hives treated with tobacco leaf-smoke which was followed by those of menthol treated hives (19.58%) and formic acid treated ones (16.25%). The former two were statistically identical (Table 2).

Significantly the highest percentage of larval hatching per hive (21.45%) was found in the colony treated with the tobacco leaf-smoke which was followed by menthol treated hive

(20.41), formic acid treated hive (17.91%) having no significant difference among them but they were statistically differed from those of the untreated control hives (12.71%) (Table 2).

The highest percentage of pupa formation per hive (23.95%) was observed in the tobacco leaf smoke applied hives which is significantly different from the untreated control hives in which the lowest percentage of pupation was 10.83%. The hives treated with menthol showed the second highest rate of pupation (21.45%) which was not significantly different from those of the hives treated with tobacco leaf smoke (23.95%) and formic acid treated hives (18.54%) (Table 2).

Significantly the highest rate of pollen deposition (7.29%) was observed in the hives treated with tobacco leaf smoke and menthol treated hives (7.29%) which was followed by the hive treated with formic acid (5.21%) which was significantly lower than those of the former two. Significantly lowest rate of pollen deposition (3.13%) was obtained in the untreated hives. Significantly the highest number of frame occupied (4.75) by the bees of the hive treated with tobacco leaf smoke which was followed by menthol treated hive (4.21), formic acid treated hive (4.00) with no significant difference between the latter two. Significantly the lowest number of frames occupied (3.63) by the bees of the untreated control hives (Table 2).

From results it is evident that tobacco leaf smokes treatment provided better colony development compared to other treatment approaches. It is found that the colonies of untreated control resulted the weakest colonies with lower population as their occupied frames were reduced from the initial number (4 frames/hive). Higher performances of the hives treated with tobacco leaf smoke was resulted due to the killing of more parasitic mites which provided proper brood development compared to other treatment approaches. The present findings were similar to those of Eischen and Vergara (2004), though they recommended not using smoke in the hives.

Weekly rate of egg deposition during the experimental period

The highest percentage of eggs (25%) laid by honey bee queen was observed on the 5th week in the hives treated with menthol (Fig. 1). Similar pattern was also observed in the

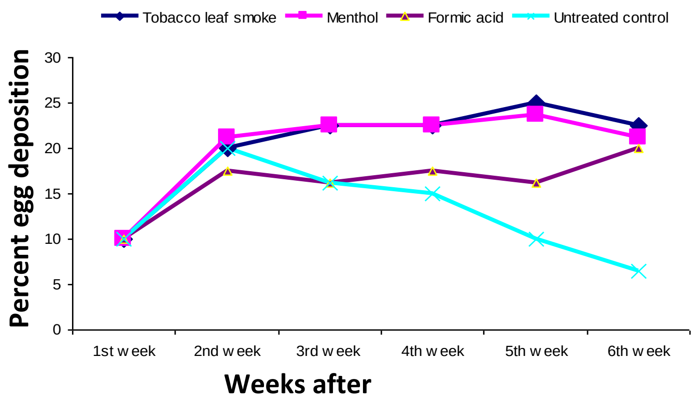

Fig. 1. Weekly egg laying performance of honey bee queen after the application of different control approaches during the dearth period 2012.

hives treated with tobacco leaf smoke, but showing lower egg deposition compared to those of menthol treatment except those of the 3rd and 4th week where equal egg deposition was evident.

The hives treated with formic acid showed fluctuating results and always had lower egg deposition compared to those of menthol and tobacco leaf smoke treated hives. At 6th week egg laying increased in the hives treated with formic acid. Decreasing trend of egg laying rate was observed in the hive where no management approaches were practiced (untreated control) and it reached at the lowest level (6.5%) in the 6th week of the study period (Fig. 1).

Weekly larval hatching after treatment during the study period

The highest percentage of larvae (25%) of honey bee hatched form the egg on the 4th and 5th week after the application of tobacco leaf smoke in the hive (Fig. 2). In menthol treated hive the highest larval hatching (25%) was obtained in the 6th week of treatment application which was higher than the other two treatments. Formic acid treated hive showed an increased pattern of larval hatching but always lower than the hive treated with methanol and tobacco leaf smoke but higher than that of untreated control (Fig. 2). Decreasing trend of larval hatching was observed in the untreated control hives and it reached to the lowest (10%) on the 6th week of the study period.

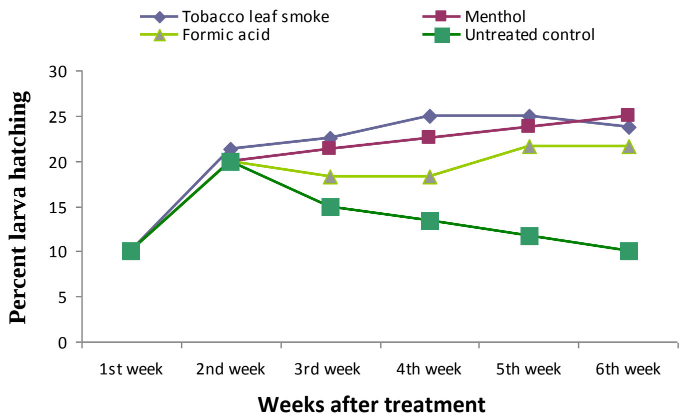

Fig. 2. Weekly differences of larval percentage of honey bee inside the hive after the application of different control approaches during dearth period 2012.

Weekly formation of pupae after treatment during the study period

The highest percentage of pupae (33.3%) of honey bee was formed on the 6th week after treatment in the hive smoked with tobacco leaf (Fig. 3). The second highest pupae formation (30.0%) was found on the 6th week after treatment in the hive applied with menthol. Hive treated with formic acid showed a stable percentage of pupae formation in the dearth period but remained lower compared to tobacco leaf smoke and menthol. The lowest pupation was observed in the hive of untreated control on the 5th week of application of treatment compared to all other treated hives. A declining trend of pupation was evident in untreated control hives (Fig. 3).

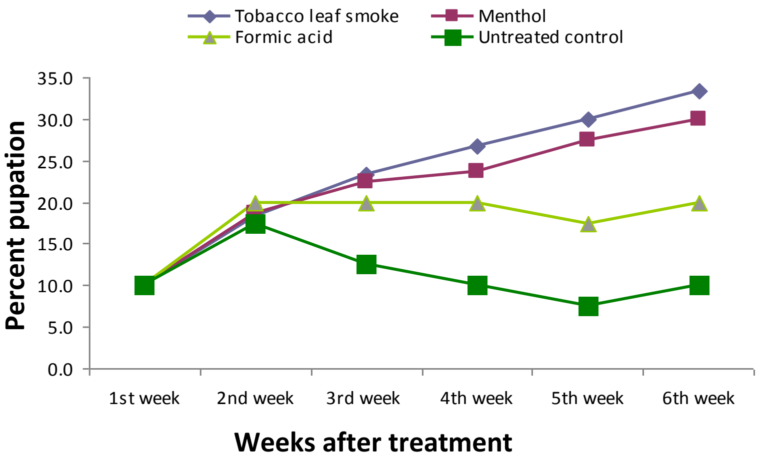

Fig. 3. Weekly differences of pupal percentage of honey bee inside the hive after the application of different control approaches during dearth period 2012.

Pollen deposition as influenced by different management approaches

The pollen deposition by worker bees inside the hive was observed during the study period. The pollen deposition percentage was the highest (10%) in the hives treated with tobacco leaf smoke at 3rd and 4th week of application (Fig. 4). Menthol treated hive also showed the highest percentage of pollen deposition at the 6th week of treatment. Fluctuating trend was observed in the hives treated with formic acid. The lowest pollen deposition percentage (1.7%) was observed in the hives of untreated control at the 5th week of application.

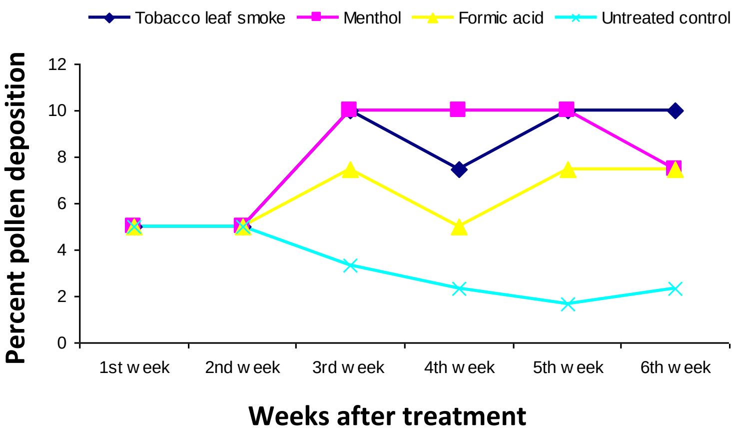

Fig. 4. Weekly pollen deposition from initial deposit (5.0%) as influenced by different management approaches applied during the dearth period (2012).

It is found that all the management actions have effects on the bee health where tobacco leaf-smoke showed better results than any other treatments. It is due to the killing effect or repelling effect on bee parasitic mites. Nectar flows increased the working efficiency of worker bee and enhance auto grooming. Some scientists found significant difference on brood development in menthol treated colonies along with other treatments which are similar to the present result (Prof. Halim Khan, 2006: personal communication). They concluded that parasitic mite was killed by volatile oil menthol along with other treatments. Westcott and

Winston (1999) found that colonies treated with formic acid reduced brood areas compared to control colonies, which could potentially reduce colony strength and this was more or less similar to the results of present investigation.

Observation of number of occupied frame during the experimental period

Up to third week all the treatments have similar number of occupied frame (Fig. 5). On the 6th week after application the highest (6.0) number of occupied frame was recorded in the hives treated with tobacco leaf-smoke which was higher than any other treatments. The second highest number of occupied frame (5.0) was obtained in the hive treated with menthol. Formic acid treated hive showed stable number of occupied frame (4.0) which was higher than that of the untreated control hive (3.0) and this was lower than any other treated hives.

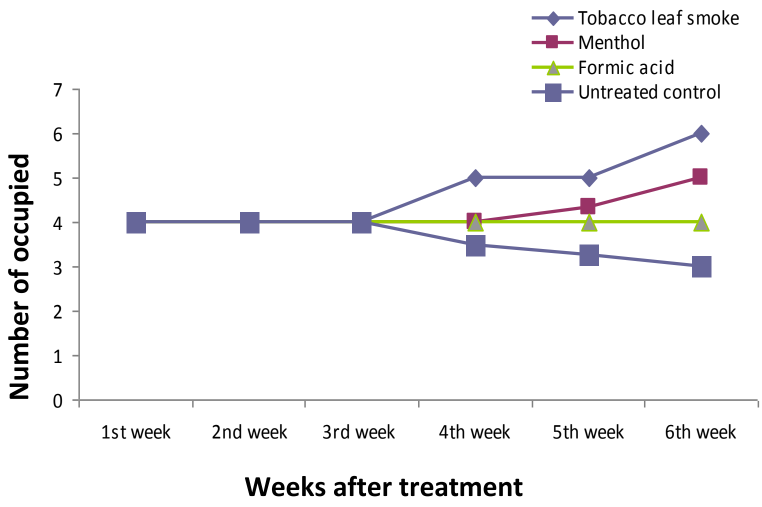

Fig. 5. Weekly occupation of frame from initial number (4.0) as influenced by different management approaches applied during dearth period (2012).

The results showed that brood development was better in the hives treated with any of the treatment approaches showing higher number of frames occupied. The present result was contradictory to the results of some other researchers (Cox *et al*. 1989 and Ellis *et al*. 2001). The authors observed reduced brood development in menthol treated hives. It might be due to the ecological difference and treated in peak honey flow season. In Bangladesh context punctured pupa symptom was not generally observed in the peak honey flow season due to bees' activity to clean the hive and natural nectar increases the immunity of bees compared to artificial feeding. The results of the present study is fully supported by the work of Eischen (1987) who stated that heavily infested colonies lost significantly more bees than the moderately infested group.

Mortality of bees inside the hive in different treatment during the study period

The highest mean mortality (0.79) was observed in the hives treated with formic acid during the study (Fig. 6). The lowest bee mortality (0.13) was noticed in the hives treated with tobacco leaf-smoke. The second highest of bee mortality was found in the hives treated with menthol (0.63) which was higher than the hives treated with tobacco leaf-smoke and the hives of the untreated control.

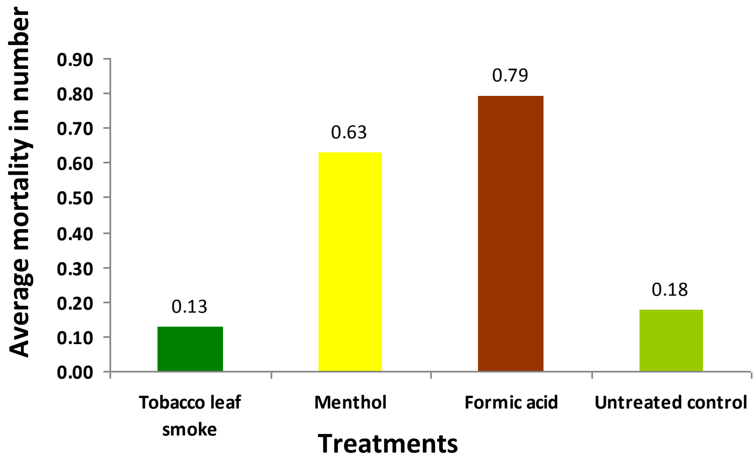

Fig. 6. Average bee mortality inside the hive of honey bee after the application of different control approaches during dearth period 2012.

From the results it was found that formic acid and menthol had more effect on bee mortality than tobacco leaf smoke. Beekeepers regularly use coconut fiber smoke when they inspect hive, and therefore, bees are in general resistant to smoke, but might have repelling effect. De Guzman *et al*. (1999) also found less drone production in the hive treated with formic acid. Otis and Scott-Dupree (1992) also reported an increased mortality of colonies infested with tracheal mites in absence of any management action.

Mean punctured pupa cell inside the hive treated with different management approaches

The highest percentage of punctured pupa (75.42%) was recorded in the hives of untreated control and the lowest punctured pupa percentage (21.29%) was obtained in the hive treated with menthol. The second highest percent of punctured pupa (25.63%) was recorded in the hive treated with formic acid (Fig. 7). However, the hives of untreated control showed the higher percent punctured pupa in compared to any other treatment approach.

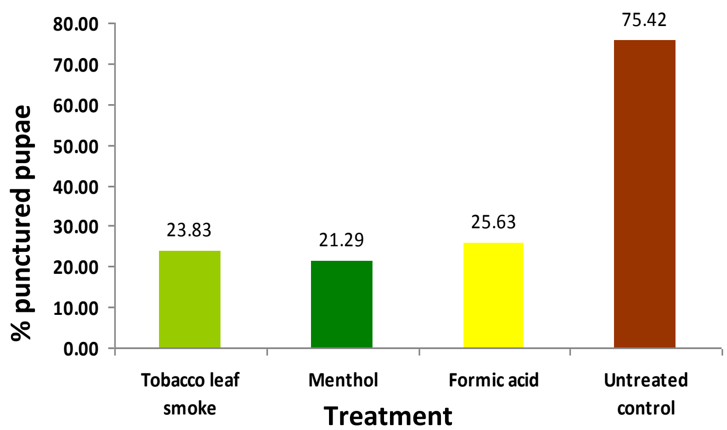

Fig. 7. Average percent punctured pupa inside the hive of honey bee after the application of different control approaches during dearth period 2012.

The results evidenced that colony without treatment (control) showed more punctured pupa which had more mite infestation. However, all the treatment approaches reduced punctured pupa, i.e. reduced mite infestation level compared to untreated control. Eischen (1987) also described that both the moderately and heavily mite infested groups of bees had less brood than no mite infested bees. Hosamani *et al.* (2006) also found 50 per cent brood mortality in *A. mellifera* due to *T. clareae* infestation. Their result-patterns are harmonious to the findings of the present investigation.

Economic analysis of the treatments

The highest adjusted net return (Tk. 920) per hive was obtained from the hive treated with tobacco leaf smoke followed by menthol treated hive (Tk. 530). The lowest adjusted net return (Tk. 160) was obtained from formic acid treated hive (Table 3). Accordingly the highest (3.28) benefit cost ratio (BCR) was achieved in the hives treated with tobacco leaf smoke and the second highest BCR (1.96) was in the hives treated with menthol and the lowest BCR (0.66) was calculated in the hives treated with formic acid. The cause of this lowest BCR was due to comparatively lower bee growth and development, more death of bees and higher cost of management. The highest BCR in the tobacco leaf smoke treated hives was due to lower management cost, higher bee yield (occupied frame/hive) and lower number of death of bees along with higher number of frames occupied.

Table 3. Economic analysis and benefit cost ratio (BCR) of various management approaches against punctured pupa disease in the dearth period (2012).

Treatment	Management cost (Tk)/hive	Occupied frame/hive	Gross return/hive (Tk)	Net return (Tk)	Adjusted net return (Tk)	BCR
Tobacco leaf-smoke	280.00	6.00	2400.00	2120.00	920.00	3.28
Menthol	270.00	5.00	2000.00	1730.00	530.00	1.96
Formic acid	240.00	4.00	1600.00	1360.00	160.00	0.66
Untreated control	----	3.00	1200.00	1200.00	-	-

The result obtained in the investigation showed that all the management approaches against punctured pupae diseases seem to be effective, but management with tobacco leaf-smoke to control punctured pupa disease may be suggested as the cheapest approach in the dearth period.

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