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**Full Length Research Paper****Efficacy of Cow Urine against Bacterial Disease, European Foulbrood, in Honey Bee, *Apis mellifera* (L.) Colonies at Different Locations of Uttarakhand-An Eco-Friendly and Novel Approach****Aakash Chand and *Ruchira Tiwari**

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Corresponding Author: Ruchira Tiwari*ABSTRACT**

The effectiveness and feasibility of using an eco-friendly, non-chemical, animal originated product, cow urine have been explored for the first time in preventing and management of infections of European Foulbrood (EFB) disease, in the colonies of honeybee, *Apis mellifera* at various locations of Uttarakhand in the year 2008-2009. The pure cow urine collected from desi cow (used as a spray at 25-100%) and terramycin (125 mg/lit/colony) as food and spray, respectively, have been applied separately on the naturally infected honeybee combs twice at weekly interval. It has been observed that the cow urine spray reduced the disease infection to below detectable limit in 10 and 12 days, respectively, as against terramycin treated infected colonies where only 40-45% recovery in EFB infection was observed. Cow urine treated infected combs not only showed rapid and cent- percent recovery in disease infection but also promotion of growth of brood. The worker bees worked efficiently and cleaned the infected brood area and other bees in the colonies. The present studies revealed that the cow urine can serve as a potential eco- friendly measure for management of EFB in honeybee colonies at different locations of Uttarakhand.

Key words: *Apis mellifera*, Cow urine, European foulbrood disease, Eco-friendly, Honey bee, Uttarakhand**INTRODUCTION**

Honey bees are most useful insects, known not only for the production of honey and other valued hive products (wax, royal jelly, propolis, pollen, bee venom) but also for their more important ecological role as efficient pollinators of flowering plants/trees, which leads to enhanced crop productivity (qualitatively as well as quantitatively) besides contributing to maintenance of plant diversity. The European honey bee, *Apis mellifera* (Linnaeus), an exotic species, has adapted to different climates, plays an important role in crop pollination and yields large amounts of honey and other bee products. This species has made a considerable progress in northern India including Jammu and Kashmir, Himachal Pradesh, Uttar Pradesh, Haryana, Bihar and Punjab (Atwal, 2000). During last 2-3 years, however, microbial brood diseases have been noticed in honey bee colonies in some of the northern states of India.

Diseases of bee are the serious problems which in turn cause economic losses to bee keepers and further hamper the expansion of beekeeping and the bee industry worldwide (Ronan and Petrenes, 1998).

European foulbrood (EFB) is a brood disease caused by the

bacteria, *Melissococcus plutonius* (Shimanuki & Knox, 1997).

It is a gram positive, non-spore forming, rod like bacterium. In *Apis mellifera*, the first incidence of EFB in India was reported in Himachal Pradesh and Dharwad area of Karnatak during 1998 (Singh & Garg, 2000) and in Punjab during 1999 (Gatoria *et al.*, 2000). The disease killed about 25% of the colonies. The clinical signs of the disease are an irregular brood pattern with swollen and twisted larvae with opaque and creamy white to pale yellow guts which finally turns brown to black and dries down to soft scales emits vinegar like odour (Morton and Brown, 1996).

The antibiotics like terramycin, oxytetracycline etc. are mainly used to control EFB are mixed with powdered sugar and fed to bees (Sanjaram & Rana, 2006). But, the development of resistance to terramycin and contamination of honey due to antibiotics has led to a ban on this drug (Miyagi *et al.*, 2000). Requeening was also tried but it was effective only for a short period. Use of pesticides such as residues of organophosphates compounds and organochlorines were found in honey (Khan *et al.*, 2004) and pollen (Chauzet *et al.*, 2006) also causes ill effects on honey bee, bee products and mankind (Dhaliwal & Singh,

2000). To overcome these problems, local beekeepers treated their bee colonies with natural plant products like neem, turmeric, cinnamon (Saville, 2000) etc. as a remedy against this disease. Though, several workers reported the use of cow urine for the control of insect pests of field crops (Kumar *et al*, 1998, Gupta & Rawat, 2004,) and plant diseases (Raja & Kurucheva, 1997 and Wani & Kurucheva, 2004) but the information on the use of animal origin products in honey bee disease management is lacking.

To reduce the ill effects of chemicals on honeybees, it is the need of time to re-evaluate the efficacy of traditional methods to control insect pests and diseases for better production of honey by increasing the resistance power in honeybees. Out of the many non-synthetic insecticidal control methods of diseases, uses of animal originated products are gaining more attention. Keeping these points in mind, the present study was conducted with the objective of having a detailed, scientific information about the impact of cow urine which is an easily available by-product of dairy as well as an eco-friendly material, in different dosages, in comparison to a medicine i.e. antibiotic, terramycin against EFB disease management in honey bees, *A. mellifera* colonies under field conditions at different locations in Uttarakhand.

MATERIALS AND METHODS

To study about the efficacy of cow urine against the honey bee bacterial disease, EFB at different locations in Uttarakhand (under Tarai and Bhabhar regions) have been surveyed to find out the incidence of EFB in *Apis mellifera* colonies in the year 2008-2010. Under Tarai region, the experiments were conducted at G.B. Pant University of Agriculture and Technology, Pantnagar, Kichha, and Dineshpur whereas in Bhabhar regions, the experiments were done at Haldwani, Khatima, Banbasa and Haridwar at apiaries of different beekeepers.

Field Experiments

The field experiments have been conducted in honey bee, *Apis mellifera* colonies reared in Langstroth size beehive having 5-6 frames strength. The experimental colonies have been naturally infected with European foulbrood (EFB) disease and no hard chemicals have been used in these colonies. The field experiments were conducted in different months of the years 2008-2010 during the appearance of the disease in the colonies of *Apis mellifera* at different locations of Tarai and Bhabhar regions in Uttarakhand. Two sprayings of cow urine 10-15ml (25%, 50%, 75% and 100%) per replication and terramycin sugar syrup (125 mg/lit sugar syrup/colony), respectively, were carried out on the selected and marked highly infected area (100cm²) (taken as 100% infection) in triplicate with the help of plastic sprayer (applied gently on the infected brood) after the interval of 7 days whereas two feedings of terramycin sugar syrup (125mg/lit/colony) were given to the infected colony. Second feeding of terramycin sugar syrup was done after 7 days of first feeding.

The recovery in infection has been calculated by the formula

$$\text{Percent Recovery of Infection} = \frac{\text{No. of recovered cells after spraying}}{\text{Total number of infected cells}} \times 100$$

Observations have been recorded on EFB-infection level on 3,5,7 days after first and second spraying of different treatments. The data subjected to RBD (two factorial) after suitable transformations using programme STPR2.

RESULTS AND DISCUSSION

The experiments on the efficacy of cow urine against bacterial disease, European foulbrood were conducted in the honey bee, *Apis mellifera* colonies in two main regions of Uttarakhand i.e. Tarai region (Pantnagar and Kichha) and Bhabhar region (Haldwani, Banbasa and Haridwar) during 2008-2010. The results of field experiments conducted on the efficacy of cow urine in comparison to standard antibiotic, terramycin against EFB in *Apis mellifera* colonies at Pantnagar during March–April 2008 are presented in Table 1. Among the different treatments, cow urine and terramycin exhibited significant difference before and after spraying, in comparison to control. The data revealed a significant reduction in the per cent infection in treated colonies over control. The per cent infection was markedly lower (45.33%) when the colonies were treated with cow urine 100%. This was followed by cow urine 75% (58.67%) and 50% (average 74%) infection after 3 days of 1st spraying of cow urine whereas level of infection was at par in the treatments with terramycin sugar syrup feeding (86.33%), terramycin sugar syrup spraying (89.33%) and cow urine (25%) (80%). A drastic reduction in per cent infection (19.00%) of EFB was observed in the treatment of 100% cow urine after 7 days of 1st spraying followed by cow urine 75% (average infection 22.3%). The recovery was found to the extent of 100 per cent in 100% cow urine treatment after 3 days of 2nd spraying whereas cent-per cent recovery was observed in treatments with cow urine (75%) after 5 days of 2nd spraying and in cow urine (50%) and (25%) after 7 days of 2nd spraying. On the contrary the average per cent infection was 25.33% and 21.67% in terramycin sugar syrup feeding and terramycin sugar syrup spraying treatments, respectively after 7 days of 2nd application. The control colonies were found infected with the clinical signs of EFB-infection and the infection level increased every day and healthy brood cells were infected. The total recovery of EFB infected bee colonies was observed in cow urine 100% and 75% treatments after 3 and 5 days of 2nd spraying, respectively, followed by cow urine 25% and 50% which showed total recovery after 7 days of second spray. All of the treatments of cow urine and terramycin were found significantly superior to control. The highest mean recovery was 77.34% recorded in cow urine 100% followed by cow urine 75% (70.67%) whereas mean recovery was at par in cow urine 50% (59.17%) and 25% (53.84%) followed by

terramycin sugar syrup feeding and spraying treatments where only 44.17% and 44.51% recovery in EFB infection were recorded, respectively. Interestingly, it was observed that cow urine was harmless (non-toxic) to the brood (eggs, larvae and pupae) and adults bees. It neither impaired the egg laying capacity of queen nor working capacity of workers.

The results of the experiment conducted on the efficacy of cow urine against EFB disease in *Apis mellifera* colonies in the apiary of Shri Hari Singh at Kichha during August-September 2008 are presented in Table 2. The data showed the effect of different treatments of cow urine and antibiotic terramycin which were found significantly superior to control in recovery of the per cent infection of EFB disease in honey bee colonies. It has been found that 3 days of spraying of cow urine 100%, the per cent infection of EFB was decreased to 45.67% followed by cow urine 75% (78%) in comparison to other treatments which were at par i.e. cow urine 50% (83.67%), cow urine 25% (87.67%), terramycin feeding (82.34%) and terramycin spraying (86.67%), respectively. The EFB infection again went down to 30.67% in cow urine 100% after 5 days of spraying followed by cow urine 75% (60%) and terramycin spraying (69.34%). In the treatments of cow urine 100%, 75% and 50%, the 100 per cent control was observed after 3 days, 5 days and 7 days of 2nd spraying, respectively. A rapid decrease in infection was also observed after 7 days of spraying in the treatments of cow urine 25% (6%) in comparison to terramycin feeding (23.34%) and terramycin spraying (24.67%), respectively. The per cent mean recovery was calculated 77.17% in cow urine 100% followed by 61% in cow urine 75% which was at par with cow urine 25% (53.17%) and cow urine 50% (58.17%) in comparison to terramycin feeding (47.13%) and terramycin spraying (44.34%). The healthy brood area which was free from EFB-infection was however, remained healthy in all the treatments although the spraying of different cow urine treatments were done on the infected area, whereas the healthy brood which was sprayed with cow urine was found healthy and produced fully developed adults.

It has been also observed that an ecto-parasitic mite in the honey bee colonies was controlled indirectly as the workers cleaned the brood area efficiently infested with the mite without killing them. Several mites were found on the bottom board of the hives after spraying cow urine which was not observed in terramycin treatments. It has also been noticed that the queen worked perfectly and laid eggs regularly after the spraying of cow urine in the infected honey bee colonies.

The larvae of wax moth were also found on the bottom board of the hive after spraying cow urine as the worker bees started cleaning the comb very fast and actively and removed all of the infected and infested brood/larvae from the cells and it has been also observed the effect of spraying cow urine on and around the hives in the apiary on wasp attack and robbing amongst the bees. This showed the additional effect of cow urine in comparison to antibiotic terramycin

The data regarding the field experiments conducted on the efficacy of cow urine in the infection/recovery of EFB in *Apis mellifera* colonies of Shri Puran Chand Joshi at Haldwani during April-May, 2009 is presented in Table 3. The data showed the effect of different treatments of cow urine and antibiotic terramycin on the per cent infection of EFB disease in *Apis mellifera* colonies. A significant reduction in the per cent infection was observed in treated colonies than control. The per cent infection was considerably reduced in the treatment of cow urine 100% (54%) followed by cow urine 75% (65%) after 3 days of 1st spraying of cow urine. However, the level of infection was found at par in terramycin sugar syrup feeding treatment (87.33%), terramycin sugar syrup spraying treatment (89%), cow urine (50%), (86.00) and cow urine (25%), (88.33), respectively. A drastic reduction in per cent infection of EFB was observed in the treatment cow urine 100% (7.66%) and (9.33%) respectively in cow urine 100% and cow urine, 75% after 3rd day of 2nd spray in comparison to 35.33%, 42%, 44.66% in cow urine 50%, 25% and terramycin spraying treatments which was again dropped down to 0.0% (cent- per cent control of EFB infection) in both cow urine 100% and 75% after 5 days of 2nd spraying. Cow urine 50% showed 5% infection after 7 days of 2nd spraying whereas 13.66% infection was observed in cow urine 25% which was very low in comparison to terramycin feeding and spraying (31.33% and 26.33%), respectively. The per cent recovery was calculated 73% in cow urine 100% followed by 67.5% in cow urine 75% nevertheless only 50-55% control in EFB infection was recorded in cow urine 50% and 25% treatments whereas 40% control in EFB infection was observed in terramycin feeding and spraying treatments after 14 days of treatment. On the other hand, the broods in control colonies were found badly infected with EFB disease.

The data of field experiments conducted on the effect of cow urine in the recovery of EFB disease infection in *Apis mellifera* colonies of Shri Girish Chandra Joshi at Banbasa during August-September, 2009 is presented in Table 4. All the treatments were found significantly superior to control in the recovery of EFB infection in *A. mellifera* colonies. The infection level of EFB was drastically dropped down to 56.67% in cow urine 100% treatment followed by cow urine 75% (67.67%) after 3 days of 1st spraying. The per cent infection of EFB was observed 1.33% and 6%, respectively, in treatments of cow urine 100% and 75% after 5 days of 2nd spraying followed by cow urine 50% (24.33%) and cow urine 25% (33.33%) which were at par with terramycin feeding (34.33%) and terramycin spraying (36.67%). A total reduction in per cent infection was observed in cow urine 100% and 75% after 7 days of 2nd spraying followed by cow urine 50% (3.67%) and cow urine 25% (18%) in comparison to terramycin feeding and spraying treatments where the reduction in EFB infection was 35% and 23%, respectively. The per cent mean recovery was calculated higher in cow urine 100% (71.67%) followed by cow urine 75% (66.17%), cow urine 50% (54.83%) whereas the per cent recovery on the other hand was

39.67% in terramycin feeding treatment. The mean recovery of EFB infection in cow urine 25% and terramycin spraying treatment was observed to be at par 48.34% and 49%, respectively.

Some additional effects of cow urine were also observed by the beekeeper during the experiment. It has been observed that ectoparasitic mites fallen down on the bottom board of the treated hives and reduction in stinging tendency of bees while handling the colonies. The attack of wasps and robbing amongst the bees were also reduced.

The results of the field experiments conducted on the efficacy of cow urine against EFB infection in *Apis mellifera* colonies of Shri Surendra Tomar at Dhanauri, Haridwar during March-April 2009 are summarized in Table 5. The data revealed that the spraying of cow urine 100% in the infected colonies showed significant reduction in the per cent infection to 52% after 3 days of 1st spraying followed by cow urine 75% (66.67%) whereas 70-80% reduction in infection was observed in other treatments of cow urine and terramycin. A drastic reduction in EFB infection was recorded again in cow urine 100% after 3 days of 2nd spraying (9.33%) followed by cow urine 75% (17%) whereas 25-45% reduction in EFB infection was recorded in other treatments. A cent- per cent reduction in EFB infection was observed after 5 days and 7 days of 2nd spraying in cow urine 100% and cow urine 50% treatments whereas reduction in infection was recorded 1.33% in cow urine 50% and 14.67% in cow urine 25% after 7 days of 2nd spraying. On the other hand, 38.33% and 33.67% reduction in EFB infection was recorded in terramycin feeding and spraying treatments after 7 days of 2nd spraying. The mean per cent recovery was 74.0% in cow urine 100% and 66.67% in cow urine 75% treatments followed by 57.17 and 53.84% in cow urine 50% and terramycin spraying. In terramycin feeding and cow urine 25% treatments, however, the mean recovery in EFB infection was recorded 41.17% and 47.17%, respectively.

Observations after spraying cow urine in honey bee colonies at different locations of Tarai and Bhabhar regions in Uttarakhand

- Rapid and cent- per cent recovery in disease infection and promotion in brood growth. The brood became healthy and the worker bees worked efficiently
- The workers started working more efficiently and they took out unhealthy larvae from the infected cells and cleaned the brood area and other bees in the colonies, then the queen bee laid eggs in these cells.
- No adverse effect have been observed on the healthy brood (eggs, larvae, pupae), newly hatched eggs, egg laying capacity of queen and worker activities
- Due to the regular spraying of cow urine in the colonies at the interval of 10-15 days, re-occurrence of disease was also not observed
- Some additional positive effects of spraying of cow

urine were protection of colonies from robbing, wasp attack, mite control and marked reduction in the stinging capacity of worker bees.

- The contamination of honeybee hive products and smell of cow urine in honey have not been reported by any beekeeper.

It was revealed that cow urine can serve as a potential eco-friendly measure for management of EFB disease in honey bees colonies at different locations under different climatic conditions in Uttarakhand.

DISCUSSION AND CONCLUSION

In the era of environmental awareness, more emphasis is given to the natural insecticides as they are biodegradable and less harmful to the environment and human beings. Beekeeping is an essential component of agriculture today for its essential role in crop pollination. Yet, beekeeping has been sustaining heavy losses through pesticides as they frequently kill bees, reduce colony strength and contaminate hive products. Therefore, it is the need of time to re-evaluate the efficacy of traditional methods to control insect pests and diseases of honey bees organically for better production of honey by increasing the resistance power in honeybees and by making the surroundings eco-friendly. Out of the many non-synthetic insecticidal control methods, uses of animal originated products are gaining more attention throughout the world.

As being an organic state of India, Uttarakhand, has tremendous scope of development of cow urine based formulations as people of rural areas are having cattle (mainly cows) for their additional source of income which certainly would reduce the pressure on the use of chemicals and antibiotic medicines in honey bee colonies for disease management. While this sounds a little unconventional for many, it could be a major step in helping to increase the honeybee population. Traditional methods of pest control will also be get promoted by this eco-friendly approach. The present studies were made with consideration of economic status of the rural community of the organic state of Uttarakhand. The studies have great applied value as it is economically viable and self-sustainable.

According to the survey conducted during the year 2006-2007 of different locations in Uttarakhand it has been observed that beekeepers had a severe loss of their honey bee colonies due to menace of EFB disease and mite diseases. This problem might have also occurred due to injudicious use of certain chemicals like sulphur, formic acid, oxalic acid for mite control and antibiotic, terramycin for EFB control, without considering about the doses resulted the ill effects of these chemicals on the brood, and the adult bees. Due to the excessive use of these chemicals, the mite and EFB disease causing organism probably developed resistance, moreover, these chemicals and antibiotic medicines appeared to have prime role in the contamination of hive products.

Considering all these problems in the management of honeybee diseases, novel approach of using a natural by-product of dairy appeared to be promising to manage EFB infection during different months of the years according to the natural appearance of the disease in honey bees colonies at different locations in Uttarakhand. Similar results were obtained by Tiwari and Mall, (2007) when they sprayed 25-100% cow urine in the EFB infected honey bee colonies at Pantnagar only, during March-May 2006. They observed cent-per cent recovery of EFB infection within 10-12 days of application of cow urine whereas in terramycin treatments only 40-50% recovery was observed in EFB infected colonies. Cow urine was used in a very little quantity which was being fanned out by the bees so, there was no any problem of high moisture level inside the hive.

None of the workers have tested the efficacy of cow urine against EFB in honey bees under agroclimatic conditions of Uttarakhand and throughout the world. Though, several workers have done experiments on cow urine to find out its efficacy against insects (Purwar, J.P. 2001, unpublished data, Sadawarte and Sarode, 1997 and Chaman Lal and Verma, 2006) and microbes (Gupta, 1989, Alonso *et al.*, 1994 and Kumar and Sehgal, 1998).

Fugro (2000) conducted experiment in Konkan region Maharashtra to test efficacy of cow urine to control leaf curl and die back disease (caused by *Colletotrichum capsici*) of chilli and found cow urine as the promising treatment. They found highest sclerotia removal (92.33%-93.53%) from 20 and 30 % cow urine with rubbing and with significantly high seed germination and seedling growth was recorded in cow urine treated seeds. Sapre and Verma (2006) on the other hand reported that the cow urine retarded the growth of fungi viz. *Rhizoctonia bataticola* (*Macrophomina phaseolina*), *Sclerotium rolfsii* and *Fusarium solani* f. sp. *Glycine*.

Thus, it may be concluded that the use of cow urine is safe, eco-friendly, animal origin product, readily available, almost free of cost to farmers. It can not only be effectively used for control EFB disease but also indirect control of mite diseases in honey bees colonies. Further research is, however, needed to assess the feasibility and effectiveness of this easily available, cheap and eco-friendly natural product, cow urine against the control of EFB and other disease outbreaks and for its better use in the field of honey bee disease management.

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Table-1 Effect of cow urine and terramycin on infection/recovery of EFB disease in honey bee, *Apis mellifera* colonies in an apiary at Pant nagar (Tarai region) Uttrakhand during March-April 2008.

| Treatment | % Infection before spraying | I Spraying (%Infection) | | | II Spraying (%Infection) | | | Mean | Recovery (%) Range Mean |
|--|-----------------------------|-------------------------|------------------|------------------|--------------------------|------------------|------------------|------------------|----------------------------|
| | | 3DAS | 5DAS | 7DAS | 3DAS | 5DAS | 7DAS | | |
| T ₁ Cow urine (25%) | 100 | 80.00 (63.45)* | 77.66 (61.88) | 66.33 (54.58) | 36.33 (36.99) | 12.33 (20.35) | 0.00 (0.00) | 45.44 (39.54) | 20.00-87.67 53.84 |
| T ₂ Cow urine (50%) | 100 | 74.00 (59.43) | 60.00 (50.77) | 48.33 (44.04) | 14.67 (22.22) | 7.67 (15.93) | 0.00 (0.00) | 34.11 (32.07) | 26.00-92.34 59.17 |
| T ₃ Cow urine (75%) | 100 | 58.67 (49.99) | 43.66 (41.34) | 22.33 (28.08) | 4.33 (11.51) | 0.00 (0.00) | 0.00 (0.00) | 21.50 (21.82) | 41.33-100 70.67 |
| T ₄ Cow urine (100%) | 100 | 45.33 (42.31) | 32.33 (34.56) | 19.00 (25.77) | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | 16.11 (17.11) | 54.67-100 77.34 |
| T ₅ Terramycin sugar syrup (125mg/lt/colony) feeding | 100 | 86.33 (68.56) | 78.66 (62.56) | 65.66 (54.18) | 47.67 (43.66) | 36.66 (37.04) | 25.33 (30.16) | 56.67 (49.36) | 13.67-74.67 44.17 |
| T ₆ Terramycin sugar syrup (125mg/lt/colony) spraying | 100 | 89.33 (71.15) | 71.00 (57.46) | 62.33 (52.21) | 47.00 (43.26) | 34.33 (35.81) | 21.67 (27.73) | 54.28 (47.94) | 10.67-78.34 44.51 |
| T ₇ Control | 100 | 100 (90.00) | 100 (90.00) | 100 (90.00) | 100 (90.00) | 100 90.00 | 100 (90.00) | 100 (90.00) | 0.0 |
| Mean | | 76.23 (63.56) | 66.19 (56.94) | 54.85 (49.84) | 35.71 (35.38) | 27.23 (28.45) | 21.00 (21.13) | 46.87 (42.55) | |

*Figures in parentheses are angular transformed values

C.D. values (Factorial RBD)

Sem
 Treatments(a) : 0.85(0.57)
 Days (b) : 0.92(0.61)
 (axb) : 2.26(1.50)
 Cv : 8.36(6.12)

CD at 1%
 3.18(2.12)
 3.44(2.29)
 8.43(5.60)

CD at 5%
 2.40(1.60)
 2.59(1.73)
 6.36(4.23)

Table-2 Effect of cow urine and terramycin on infection/ recovery of EFB disease in honey bee, *Apis mellifera* colonies at Kichha (Tarai region) of Uttarakhand during August- September, 2008

| Treatment | % Infection before spraying | I Spraying (%Infection) | | | II Spraying (%Infection) | | | Mean | Recovery(%) | |
|---|-----------------------------|-------------------------|------------------|------------------|--------------------------|------------------|------------------|------------------|--------------|-------|
| | | 3DAS | 5DAS | 7DAS | 3DAS | 5DAS | 7DAS | | Range | Mean |
| T ₁ Cow urine (25%) | 100 | 87.67 (69.46)* | 77.33 (61.66) | 60.33 (51.02) | 39.00 (38.65) | 17.33 (24.26) | 6.00 (11.62) | 47.94 (42.78) | 12.33-94 | 53.17 |
| T ₂ Cow urine (50%) | 100 | 83.67 (66.32) | 77.33 (61.63) | 71.00 (57.46) | 16.00 (23.37) | 6.00 (14.09) | 0.00 (0.00) | 42.33 (37.15) | 16.33-100 | 58.17 |
| T ₃ Cow urine (75%) | 100 | 78.00 (62.09) | 60.00 (50.82) | 47.00 (43.27) | 3.33 (8.19) | 0.00 (0.00) | 0.00 (0.00) | 31.39 (27.39) | 22.00-100 | 61.00 |
| T ₄ Cow urine (100%) | 100 | 45.67 (42.51) | 30.67 (33.47) | 19.33 (26.08) | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | 15.94 (17.01) | 54.33-100 | 77.17 |
| T ₅ Terramycin sugar syrup (125mg/lt/colony) feeding | 100 | 82.33 (65.44) | 78.00 (62.09) | 63.33 (52.83) | 49.00 (44.42) | 40.00 (39.23) | 23.34 (28.71) | 56.00 (48.79) | 17.66-76.66 | 47.16 |
| T ₆ Terramycin sugar syrup (125 mg/lt/colony) spraying | 100 | 86.67 (68.73) | 69.33 (56.40) | 60.00 (50.85) | 44.00 (41.52) | 29.33 (32.78) | 24.67 (29.75) | 52.33 (46.67) | 13.33- 75.33 | 44.34 |
| T ₇ Control | 100 | 100 (90.00) | 100 (90.00) | 100 (90.00) | 100 (90.00) | 100 (90.00) | 100 (90.00) | 100 (90.00) | 0.0 | |
| Mean | | 80.57 (66.37) | 70.38 (59.44) | 60.14 (53.07) | 35.90 (35.16) | 27.52 (28.62) | 22.00 (22.87) | 49.42 (44.26) | | |

*Figures in parentheses are angular transformed values

C.D. values (Factorial RBD)

| | Sem | CD at 1% | CD at 5% |
|---------------|------------|--------------|-------------|
| Treatments(a) | 1.06(0.81) | 3.95(3.01) | 2.98(2.27) |
| Days (b) | 1.14(0.87) | 4.27 (3.25) | 3.22(2.45) |
| (axb) | 2.80(2.13) | 10.46 (7.96) | 7.89 (6.00) |
| Cv | 9.83(8.35) | | |

Table-3 Effect of cow urine and terramycin on infection/recovery of EFB disease in honey bee, *Apis mellifera* colonies in an apiary of Shri Puran Chand Joshi , Haldwani (Tarai region) Uttrakhand during April- May 2009

| Treatment | % Infection before spraying | I Spraying (%Infection) | | | II Spraying (%Infection) | | | Mean | Recovery(%) | |
|---|-----------------------------|-------------------------|------------------|------------------|--------------------------|------------------|------------------|------------------|-------------|-------|
| | | 3DAS | 5DAS | 7DAS | 3DAS | 5DAS | 7DAS | | Range | Mean |
| T ₁ Cow urine (25%) | 100 | 88.33 (70.05)* | 80.33 (63.70) | 68.66 (55.98) | 42.00 (40.33) | 30.00 (33.10) | 13.67 (21.32) | 53.83 (47.42) | 11.67-86.34 | 49.10 |
| T ₂ Cow urine (50%) | 100 | 86.0 (69.88) | 62.00 (51.95) | 46.66 (43.08) | 35.33 (36.42) | 25.33 (30.16) | 5.00 (9.75) | 43.39 (40.20) | 14.00-95.00 | 54.50 |
| T ₃ Cow urine (75%) | 100 | 65.00 (53.79) | 48.66 (44.24) | 19.33 (25.43) | 9.33 (12.93) | 0.00 (0.00) | 0.00 (0.00) | 23.72 (22.73) | 35.00-100 | 67.50 |
| T ₄ Cow urine (100%) | 100 | 54.00 (47.31) | 39.33 (38.75) | 15.33 (22.99) | 7.66 (15.93) | 0.00 (0.00) | 0.00 (0.00) | 19.39 (20.83) | 46.00-100 | 73.00 |
| T ₅ Terramycin sugar syrup (125 mg/lt/colony) feeding | 100 | 87.33 (69.66) | 85.00 (68.00) | 66.33 (54.60) | 50.00 (45.00) | 37.33 (37.63) | 31.33 (34.00) | 59.56 (51.48) | 12.67-68.67 | 40.67 |
| T ₆ Terramycin sugar syrup (125 mg/lt/colony) spraying | 100 | 89.00 (71.12) | 75.00 (60.12) | 54.33 (47.51) | 44.67 (41.93) | 38.67 (38.42) | 26.33 (30.80) | 54.67 (48.31) | 11-73.67 | 42.34 |
| T ₇ Control | 100 | 100 (90.00) | 100 (90.00) | 100 (90.00) | 100 (90.00) | 100 (90.00) | 100 (90.00) | 100 (90.00) | | 0.0 |
| Mean | | 81.38 (67.40) | 70.04 (59.54) | 52.95 (48.51) | 41.28 (40.36) | 33.05 (32.76) | 25.19 (26.56) | 50.65 (45.85) | | |

*Figures in parentheses are angular transformed values
C.D. values (Factorial RBD)

| | | | |
|---------------|--------------|---------------|-------------|
| Treatments(a) | Sem | CD at 1% | CD at 5% |
| Days (b) | 1.28(1.02) | 4.79(3.81) | 3.61(2.88) |
| (axb) | 1.38(1.10) | 5.17 (4.12) | 3.90 (3.11) |
| Cv | 3.40(2.70) | 12.68 (10.09) | 9.56 (7.61) |
| | 11.62(10.22) | | |

Table-4 Effect of cow urine and terramycin on infection/ recovery of EFB disease in honey bee, *Apis mellifera* colonies at Banbasa, Bhabhar region of Uttarakhand during August to September, 2008

| Treatment | % Infection before spraying | I Spraying (%Infection) | | | II Spraying (%Infection) | | | Mean | Recovery(%) Range Mean |
|--|-----------------------------|-------------------------|------------------|------------------|--------------------------|------------------|------------------|------------------|---------------------------|
| | | 3DAS | 5DAS | 7DAS | 3DAS | 5DAS | 7DAS | | |
| T ₁ Cow urine (25%) | 100 | 85.33 (67.79)* | 78.33 (62.27) | 67.33 (55.30) | 47.67 (43.66) | 33.33 (35.01) | 18.00 (24.49) | 55.00 (48.09) | 14.67-82.00 48.34 |
| T ₂ Cow urine (50%) | 100 | 86.67 (68.73) | 66.67 (54.91) | 46.33 (42.89) | 36.33 (37.04) | 24.33 (29.32) | 3.67 (10.10) | 44.00 (40.50) | 13.33-96.33 54.83 |
| T ₃ Cow urine (75%) | 100 | 67.67 (55.51) | 53.00 (46.74) | 25.00 (29.44) | 16.00 (22.88) | 6.00 (11.48) | 0.00 (0.00) | 27.94 (27.68) | 32.33-100 66.17 |
| T ₄ Cow urine (100%) | 100 | 56.67 (48.86) | 46.00 (42.68) | 28.00 (31.78) | 12.67 (20.67) | 1.33 (5.42) | 0.00 (0.00) | 24.11 (24.90) | 43.33-100 71.67 |
| T ₅ Terramycin sugar syrup (125 mg/l/colony) feeding | 100 | 85.67 (67.93) | 81.33 (64.41) | 66.00 (54.52) | 43.00 (40.97) | 34.33 (35.82) | 35.00 (36.19) | 57.56 (49.97) | 14.33-65.00 39.67 |
| T ₆ Terramycin sugar syrup (125 mg/l/colony) spraying | 100 | 79.00 (62.88) | 72.67 (58.67) | 51.00 (45.57) | 43.33 (41.74) | 36.67 (37.25) | 23.00 (28.61) | 51.11 (45.79) | 21.00-77.00 49.00 |
| T ₇ Control | 100 | 100 (90.00) | 100 (90.00) | 100 (90.00) | 100 (90.00) | 100 (90.00) | 100 (90.00) | 100 (90.00) | 0.0 |
| Mean | | 80.14 (65.96) | 71.14 (59.96) | 54.81 (49.93) | 42.86 (42.42) | 33.71 (34.90) | 25.67 (27.06) | 51.39 (46.70) | |

*Figures in parentheses are angular transformed values
C.D. values (Factorial RBD)

| | | | |
|---------------|---------------|--------------|--------------|
| Sem | | CD at 1% | CD at 5% |
| Treatments(a) | : 1.40(0.99) | 5.24(3.68) | 3.95(2.78) |
| Days (b) | : 1.51(1.07) | 5.67 (3.97) | 4.27(3.00) |
| (axb) | : 3.72(2.61) | 13.87 (9.74) | 10.46 (7.34) |
| Cv | : 12.54(9.68) | | |

Table-5 Effect of cow urine and terramycin on infection/recovery of EFB disease in honey bee, *Apis mellifera* colonies in an apiary of Shri Surendra Tomar, Dhanauri, Haridwar (Bhabhar region) Uttrakhand during March-April 2009.

| Treatment | % Infection before spraying | I Spraying (%Infection) | | | II Spraying (%Infection) | | | Mean | Recovery (%) Range Mean |
|---|-----------------------------|-------------------------|------------------|------------------|--------------------------|------------------|------------------|------------------|----------------------------|
| | | 3DAS | 5DAS | 7DAS | 3DAS | 5DAS | 7DAS | | |
| T ₁ Cow urine (25%) | 100 | 91.00 (72.67)* | 81.67 (64.68) | 72.67 (58.50) | 45.33 (42.30) | 30.67 (33.36) | 14.67 (22.22) | 56.00 (48.96) | 9.0-85.39 47.17 |
| T ₂ Cow urine (50%) | 100 | 84.33 (66.71) | 71.33 (57.63) | 48.67 (44.23) | 27.67 (31.65) | 18.67 (25.25) | 1.33 (5.42) | 42.00 (38.48) | 15.67-98.67 57.17 |
| T ₃ Cow urine (75%) | 100 | 66.67 (54.76) | 56.00 (48.50) | 33.00 (34.85) | 17.00 (23.84) | 7.33 (12.80) | 0.00 (0.00) | 30.00 (29.13) | 33.33-100.0 66.67 |
| T ₄ Cow urine (100%) | 100 | 52.00 (46.16) | 34.33 (35.82) | 20.67 (26.92) | 9.33 (17.77) | 0.00 (0.00) | 0.00 (0.00) | 19.38 (21.11) | 48.0-100.0 74.00 |
| T ₅ Terramycin sugar syrup (250 mg/lt /colony) feeding | 100 | 86.33 (68.74) | 78.33 (62.73) | 64.33 (53.46) | 49.67 (44.81) | 38.33 (38.22) | 31.33 (33.98) | 58.10 (50.32) | 13.67-68.67 41.17 |
| T ₆ Terramycin sugar syrup (250 mg/lt/colony) spraying | 100 | 77.00 (61.50) | 67.00 (54.99) | 58.33 (49.83) | 48.33 (44.04) | 33.67 (35.41) | 25.33 (30.19) | 51.61 (45.99) | 33.0-74.67 53.84 |
| T ₇ Control | 100 | 100 (90.00) | 100 (90.00) | 100 (90.00) | 100 (90.00) | 100 (90.00) | 100 (90.00) | 100 (90.00) | 0.0-0.0 |
| Mean | | 79.62 (65.79) | 69.81 (59.19) | 56.81 (51.11) | 42.48 (42.06) | 32.67 (33.58) | 24.67 (25.97) | 51.00 (46.28) | |

*Figures in parentheses are angular transformed values

C.D. values (Factorial RBD)

| | | |
|-----------------------------|--------------|--------------|
| Sem | CD at 1% | CD at 5% |
| Treatments (a) : 1.37(0.96) | 5.11(3.58) | 3.86(2.70) |
| Days (b) : 1.48(1.04) | 5.53 (3.87) | 4.17(2.92) |
| (axb) : 3.63(2.54) | 13.54 (9.48) | 10.21 (7.15) |
| Cv : 12.33(9.51) | | |