

EFFICACY OF COW URINE AND PLANT PRODUCTS AGAINST HONEYBEE MITE, VARROA DESTRUCTOR (ANDERSON AND TRUEMAN) IN APIS MELLIFERA (LINNAEUS) COLONIES IN UTTARAKHAND - A NOVEL APPROACH

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ABSTRACT

The studies to manage the ectoparasitic brood mite, *Varroa destructor* infestation in the bee colonies of *Apis mellifera* (L.) at different locations of Uttarakhand were conducted during the years 2012 and 2013 by applying eco-friendly animal origin and plant products. The complied average data of all three apiaries at Dineshpur, Haldwani and Halduchaur revealed that cow urine (desi breed) @ 100%, significantly reduced brood mite infestation (75.60%) with highest mean mite fall (38.00) and sealed worker brood area (SWBA) (1042.00 cm²) followed by cow urine (jersy breed) @ 100% with reduction brood mite infestation, mite fall and SWBA were (75.27%, 37.33 and 991.36 cm²), ajwain powder @ 5g (74.09%, 35.67 and 988.52 cm²) cow urine (holstein breed) @ 100% (71.57%, 33.00 and 974.34 cm²) and neem leaf powder @ 5g (66.59%, 32.33, 892.32 cm²) in comparison to synthetic chemicals i.e. thymol @ 500mg (58.55%, 29.00 and 464.16 cm²) and formic acid @ 5ml (45.35%, 20.33 and 372.44 cm²) and untreated control where reduction in brood mite infestation was (13.33%) and SWBA was (272.44 cm²) after 4 weeks of treatments. It has been concluded that the tested eco-friendly cow urine and plant products were significantly reduced the mite infestation in treated bee colonies with significant increase in mite fall and sealed worker brood area without adversely affected the workers a queen activities in comparison to synthetic chemicals used in *A. mellifera* colonies. All eco-friendly plant products and cow urine were found significantly superior to untreated control. This was the novel approach to study about the effect of eco-friendly animal origin and plant products as a potential eco- friendly measure for management of mite disease in honeybee colonies in Uttarakhand and which could be suitable alternatives to conventional synthetic chemicals such as thymol, sulphur, apistan, formic acid, dicofol.

INTRODUCTION

The honey bee mite, *Varroa destructor* (Anderson and Trueman, 2000) is an ectoparasite of the Asian honey bee (*Apis cerana*) that has become potentially the main parasite of *Apis mellifera* (L.) in the last few decades (De Jong, 1990). The mite infests the bees by entering into the brood cells before capping and parasitizing honey bee larvae and pupae and its control becomes difficult as majority of the mites stay in the sealed brood for reproduction and are well protected from the chemical treatments (Harold *et al.*, 1989). *Varroa* mites are external honey bee parasites that attack both the adults and the brood. The female mite lays eggs on the pupa, and after eclosion the mites develop into protonymphs and later deutonymphs: all active stages feed on pupal hemolymph of bees (Donz and Guerin, 1994).

Several chemical substances were used successfully to control mites, in recent years, resistance to acaricides has become a major problem in the control of varroa. *Varroa destructor* strains have been reported to be resistant to flouvalinate and flumethrin (Baxter *et al.*, 1998), coumaphos (Spreafico *et al.*, 2001), and to amitraz, (Elzen *et al.*, 2000a). Also, the use of

acaricides should be minimized in beekeeping because of the residues and their breakdown products in honey and wax (Wallner, 1999). The selection pressure for the resistance against natural acaricides is presently low (Milani, 1999). Accumulation in wax does not occur and residues in honey are small and toxicologically not important (Imdorf *et al.*, 1996). The natural products such as citric acid was less toxic to varroa than oxalic acid (Milani, 2001). The efficacy of cow urine against bacterial disease of honeybee, *A. mellifera* was studied under field conditions for the first time by (Tiwari and Mall, 2007, Chand and Tiwari, 2012) and under laboratory conditions by (Chand and Tiwari, 2014). The effect of cow urine on recovery of mite infestation in *A. mellifera* colonies was first time studied by (Tiwari, 2013) in Uttarakhand.

The synthetic chemical thymol (also known as 2-isopropyl-5-methylphenol, IPMP) is a natural monoterpene phenol found in oil of thyme, and extracted from *Thymus vulgaris* (common thyme) and Ajwain (*Trachyspermum ammi*. Syn. *Carum copticum*) a white crystalline substance of a pleasant aromatic odor and strong antiseptic properties. Thymol has been successfully used to control varroa mites and prevent fermentation and the growth of mold in bee colonies, but has

adverse effect on brood, worker bees and queen bee (Hu and Coats, 2008). Therefore, in the present studies, the efficacy of synthetic chemical thymol was compared with naturally occurred thymol in plant product, ajwain seed powder so that synthetic thymol can be replaced by the natural product, ajwain powder.

In the same context, 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 natural products are gaining more attention. The present study therefore, deals with the impact of natural product such as animal origin product, cow urine and plant products *i.e.* ajwain, neem and jatropha powders in controlling honeybee mite, *V. destructor* in *Apis mellifera* colonies in Uttarakhand.

MATERIALS AND METHODS

Field experiments on mite infestation in honeybee colonies at different locations in Uttarakhand

The experiments were conducted in the honeybee colonies of *Apis mellifera* at the apiary sites at Dineshpur, Halduchaur and Haldwani in 3 replications during the years 2012 and 2013. The honey bee colonies used in the studies were with young prolific queens which were free from diseases. The bee colonies were of equal strength (5-6 bee frames) in respect of brood, hive stores and adult bee population in Langstroth hives. The experimental colonies were naturally infected with mite, *Varroa destructor*. Use of eco-friendly animal origin and plant products along with synthetic chemicals *i.e.* thymol powder and formic acid were applied separately in infested bee colonies twice in a month after 15 days interval and data were collected.

Collection of cow urine and plant material

Cow urine was collected from desi, jersey and holstein breed cows and used as a spray @ 100%. The test plants, leaves and seeds of neem, (*Azadiracta indica, L.*) and Jatropha (*Jatropha curcas L.*) were collected from the University campus and nearby areas of Pantnagar, whereas, Ajwain (*Trachyspermum ammi. Syn. Carum copticum*) was purchased from local market at Pantnagar, Uttarakhand.

Mode of application of treatments

1. Spraying of cow urine 10-15 ml/bee frame @ 100% was conducted by using plastic sprayer (Flit pump). 2. Leaf and seed powders of neem, jatropha and ajwain @ 5g/hive were dusted in between the frames. 3. Thymol powder @ 500 mg/hive was kept on the bottom board. 4. Formic acid 85% @ 5ml/colony in a vial with cotton plug was kept on the bottom board in the bee colonies. 5. Control –no chemical agent was used

Efficiency of different eco-friendly products was measured using the following parameters

Infestation per cent - Extent of damage was worked out by recording data on per cent brood infestation, which was determined by randomly opening of 50 brood capping per

colony for the presence of mites (different stages). The percent brood infestation was calculated by the formula according to (Tiwari and Mall,2011)

Per cent brood Infestation = (No. of cells with mites / Total no. of cells observed) X 100

Evaluation of the tested materials and techniques was based on the efficiency (%) was evaluated according to (Henderson and Tilton, 1955) equation:

% Reduction of infestation = $100 \times 1 - \{Ta \times Cb\} / \{Tb \times Ca\}$

Where:

T = % infestation of treated mites and C = % infestation of untreated mites (a = after; b= before treatment).

Mite fall - Counts of dropped mites were recorded before and after one month of the application using plastic sheets (51.5 x 36.5 cm) coated with vaseline placed on the hive bottom board and collective mean data was taken for all three apiary sites. (Abd El-Halim, 2006).

Brood measurement - Sealed worker brood areas (SWBA) were recorded after one month of application of the treatments using a plastic sheet divided into square inches, converted cm² when multiplied by 2.54 according to (Abd El-Halim, 2006). Statistical analysis of the data on the brood mite infestation, mite fall and sealed worker brood area was conducted using Randomized Block Design using programme STPR3. Data collected was statistically analyzed after square root transformation. Treatment means were compared at 5% probability levels by LSD test (Snedecor and Cochran, 1967).

RESULTS AND DISCUSSION

Effect of cow urine and plant products on mite fall in *A. mellifera* colonies

The number of fallen mites/ colony before and after treatments at three apiary sites *i.e.* Dineshpur, Halduchaur and Haldwani in Uttarakhand is averaged in the (Table 1) showed the highest mean mite fall in cow urine (desi breed) @ 100% (38.00) followed by cow urine (jersey breed) @ 100% (37.33) and cow urine (holstein breed) 100% (33.00), ajwain powder @ 5g (35.67), neem leaf powder @ 5g (32.33), neem seed powder @ 5g (30.33), jatropha leaf powder @ 5g (26.00), jatropha seed powder @ 5g (20.67) in comparison to synthetic chemicals, thymol @ 500mg (29.00) and formic acid @ 5ml (13.33) with mean mite fall in untreated control (10.27).

Effect eco-friendly formulations on brood mite infestation

The compiled data for two years (2012-2013) at all three apiary sites is presented in (Table-1) showed that cow urine (desi breed) @ 100% significantly reduced brood mite infestation (75.60%) from 24.67 per cent before application to 08.67 per cent after 4 weeks of treatment followed by cow urine (holstein breed) (75.27%), ajwain powder (74.09%), cow urine (jersey breed) (71.57%) whereas on the other hand, reduction in brood mite infestation in jatropha leaf powder, neem leaf powder, neem seed powder and jatropha seed powder treated bee colonies were 68.11%, 66.59%, 66.10% and 64.29%, respectively, in comparison to synthetic chemical treated colonies such as thymol showed only (58.55%) and

formic acid (45.35%) reduction in brood mite infestation after 4 weeks of treatment and was found the least effective among the treatments to control mite infestation in *A. mellifera* colonies at different locations in Uttarakhand.

Effect of treatments on brood rearing activity

The data presented in (Table 1) showed significantly increase in average sealed worker brood area (cm²) (SWBA) in the bee colonies given sprays of cow urine (desi breed) @ 100% (1042.46 cm²) followed by cow urine (jersy breed) @ 100% (991.36 cm²), ajwain powder @ 5 g (988.52 cm²), cow urine (holstein breed) @ 100% (974.34 cm²) and neem leaf powder @ 5 g (892.32 cm²) after of 4 weeks of treatments followed by jatropa leaf powder @ 5g (632.54cm²), seed powders of neem and jatropa @ 5g (609.00 cm² and 586.56 cm², respectively, in comparison to significantly very less sealed worker brood area was calculated in synthetic chemical, thymol (464.16 cm²), formic acid (372.44 cm²) and untreated control (334.67 cm²).

The decrease in SWBA in case of synthetic chemicals, thymol and formic acid may be due to their noticeable adverse effect on brood rearing, queen loss, queen egg laying behavior and damaged brood. It has been noticed in these treated colonies that old brood was damaged and new brood was very less which was picked out by the worker bees. The newly laid eggs were not found indicated that egg laying tendency of queen bee was greatly affected. Due to which the strength of adult worker bees declined and finally the colonies collapsed after two months of applications of these formulations on the other hand cow urine of all three desi, jersy and holstein cow breeds, leaf and seed powders of ajwain, neem, jaropha were found significantly superior in reduction in mite brood infestation, highest mite fall and healthy normal and highest sealed worker brood areas with more honey and pollen stores.

In recent years, formic acid was candidate to control varroa mite. However, application of formic acid is limited due to persistence in honey, lack of applicability in areas with temperatures above 32°C (due to rapid evaporation), damage to the brood (when converted to the larval stage, pupae) is of limited use (Calderon *et al.*, 2000). Other alternative controls

including more natural substances like the essential oil, thymol, can result in build up of residues in wax over the years of treatments and become toxic to the bees (Floris *et al.*, 2004).

The effect of many plant products such as essential oils and extracts as acaricides in protecting varroa mite infestation has been studied, and it has been shown that this mite is susceptible to the some plant derived materials. Most of these extracts cause the mite to release from the bees, but do not cause mite mortality, and are therefore of limited value. These results are supported by the finding of (Shoreit and Hussein, 1994) who found that, the maximum mean number of dead mites was noticed after the first treatment with coriander extract in both of winter or spring feeding. Grapefruit (*Citrus sp.*) was found to cause rapid knockdown of varroa after infested bees were exposed to smoke of burning dried leaves (Eischen and Wilson, 1997). The control of varroa mite using naturally plant products are more recommended than other chemical acaricides to keep the social life of honeybee away from any harmful effect. It has been also found that neem oil spray killed 90 to 94% of varroa mites but thymol oil spray reduced varroa mites to 79% in honeybee colonies (Dimetry *et al.*, 2005).

Abd El-Wahab and Ebada, (2006) studied the toxicity of *Citrus aurantium L.* (sour Orange), *Cymbopogon flexuosus* (lemon grass) and Citronella volatile oils against varroa mite and found that the mean percentage of varroa infestation on the worker brood and adult workers of honey bee reduced to 100% after the fourth week of treatment. These results demonstrated that tested plant extracts and resin can be suitable alternatives to conventional chemical materials such as apistan and apivar.

The studies have also been made on the efficacy of cow urine in bee disease management (Tiwari and Mall, 2007 and Chand and Tiwari, 2012) showed rapid recovery in bacterial disease infection of bees within 10-14 days of cow urine spray with promotion of growth of bee brood. Other beneficial effects of cow urine on robbing, aggressiveness, egg laying and adult activities were also observed. 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. It has also been

Table 1: Effect of cow urine and plant products on mite fall, per cent reduction in brood mite infestation and sealed worker brood area, cm² (SWBA) in *A. mellifera* colonies in Uttarakhand during 2012 and 2013 (Average data of three apiary sites in Uttarakhand)

Treatments	Conc.	Average mite fall		Per cent mite brood infestation		Reduction in brood mite(%)	Sealed Worker brood area (cm ²) SWBA
		before treatment	after 4 weeks	Pre treatment	Post treatment		
Cow urine (Desi breed)	100%	13.33 (3.71)*	38.00_(6.20)	24.67 (5.01)	08.67(3.02)	75.60	1042.46(32.63)
Cow urine(Jersy Breed)	100%	15.67(4.01)	37.33(6.14)	29.00(5.43)	10.33(3.29)	75.27	991.36(31.49)
Cow urine (Holstein Breed)	100%	12.00(3.53)	33.00(5.78)	27.67 (5.30)	11.33(3.43)	71.57	974.34(31.22)
Neem Leaf Powder (NLP)	5g	11.33(3.43)	32.33 (5.14)	26.33(5.17)	12.67(3.62)	66.59	892.32(29.88)
Neem Seed Powder	5g	15.00(3.93)	30.33(5.55)	28.00(5.33)	13.67(3.76)	66.10	609.00(24.68)
Jatropa Leaf Powder (JLP)	5g	10.33 (3.29)	26.00(5.14)	24.67(5.01)	11.33(3.43)	68.11	632.54(25.16)
Jatropa Seed Powder	5g	16.67(4.14)	20.67(4.60)	23.33 (4.88)	12.00(3.53)	64.29	586.56(24.22)
Ajwain Powder (AP)	5g	09.33(3.13)	35.67 (6.01)	25.00(5.05)	09.33(3.13)	74.09	988.52(31.44)
Thymol Powder	5g	13.00(3.67)	29.00(5.43)	22.33(4.77)	13.33(3.71)	58.55	464.16(21.55)
Formic acid	5ml	14.33(3.84)	13.33(3.71)	20.33(4.56)	16.00(4.06)	45.35	372.44(19.31)
Control	-	17.33 (4.22)	10.27(3.28)	28.00(5.33)	40.33(6.38)		334.67(18.30)
SEm ±		0.41(0.13)	0.13(0.16)	0.12(0.20)	0.44(0.14)		0.63(0.54)
CD 5%		0.12(0.37)	0.39(0.46)	0.37(0.59)	0.13(0.42)		0.33(0.16)
CV		0.52(0.58)	0.82(0.51)	0.86(0.69)	0.53(0.65)		0.57(0.36)

*Figures in the parentheses are square root transformed values (after addition 0.5)

noticed that the queen worked perfectly and laid eggs regularly after the spraying of cow urine in the infected honey bee colonies.

In the present studies, cow urine (collected from cow breeds, desi, jersey and holstein) @ 100% and plant products *i.e.* seed and leaf powders of neem, jatropha and ajwain @ 5g / bee colony showed the highest reduction in the mite brood infestation, increase in mite fall and sealed worker brood area without affecting the working tendency of worker bees, egg laying capacity of queen bee and newly laid eggs, brood and the bees.

Among the natural plant products, ajwain powder in comparison to synthetic thymol (a crystalline phenol occurring naturally in *Thymus vulgaris*) may be also be used in place of chemicals like sulphur, formic acid, thymol for mite disease management in honeybee colonies.

Meena *et al*, 2013 observed the efficacy of plant products such as tobacco, onion and neem seed kernel extract @ 5% and cow urine @ 50 litre/ha against mustard aphid, *Lipaphis erysimi* (Kalt.) and their safety to natural enemies and pollinators. Similar studies were conducted by Alexander *et al*, 2012 under laboratory conditions to know the comparative bio-efficacy of chemical and indigenous products such as neem leaf and seed extracts in cow urine against the larvae of diamond back moth, *Plutella xylostella* (Linn) on Cabbage. All the treatments were found significantly superior over control.

This is the novel approach to study about the effect of animal origin product, cow urine and plant products against mite disease in bee colonies in Uttarakhand. The present studies revealed that the cow urine and plant products can serve as a potential eco- friendly measure for management of mite diseases in honeybee colonies and can be suitable alternatives to conventional chemical materials such as thymol, formic acid, sulphur etc. and are safe, eco-friendly, readily available, almost free of cost to farmers and have long term effect without having any adverse effect on bees and hive products in Uttarakhand. Further studies are surely required to come to the final conclusions regarding the role of cow urine and use of natural thymol containing plant product, ajwain and neem and jatropha powders on the management of honeybee diseases in honey bee colonies at different locations of Uttarakhand.

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