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Efficacy of eco-friendly formulations against honeybee mite, *Varroa destructor* in *Apis mellifera* colonies in Uttarakhand – A novel approach

RUCHIRA TIWARI[1], VAIBHAV MATHUR[2] and MEENA DHAMI[3]

College of Agriculture, G B Pant University of Agriculture and Technology, Pantnagar, Distt. Udham Singh Nagar, Uttarakhand 263 145

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ABSTRACT

Studies to manage the ectoparasitic brood mite, *Varroa destructor* infestation in the colonies of *Apis mellifera* (L.) at Dineshpur, District Udham Singh Nagar in Uttarakhand were conducted applying eco-friendly formulations, i.e. spraying of cow urine (desi breed) @ 25% and 100% and plant decoctions prepared in cow urine and water @ 5% (10-15 ml/hive), leaf powders of neem (*Azardiracta indica* L.), Jatropha (*Jatropha curcas* L.) and ajwain powder (*Trachyspermum ammi* L.) (@ 5g/hive), sulphur powder (@ 100 mg /hive) and thymol powder (@ 500 mg/hive) with two applications in a month revealed that the cow urine 100 per cent, significantly reduced brood mite infestation (77.80%) with highest mean mite fall (50.06%) and highest sealed worker brood area (892.84 cm^2) followed by ajwain powder with reduction in brood mite infestation (70.17%), mite fall (48.40%), sealed worker brood area (740.34 cm^2), after 4 weeks of treatment followed by neem leaf powder and cow urine @ 25 per cent, jatropha leaf powder, thymol and sulphur powder. The plant decoctions prepared in cow urine and water showed average mite fall (25.00% to 28.00%), reduction in brood mite infestation (54.32% to 66.50%) in with decrease in sealed worker brood areas (190.17 to 333.33 cm^2). It has been concluded that the tested eco-friendly formulations cow urine, neem leaf powder, ajwain and jatropha leaf powder were significantly reduced the mite infestation in treated bee colonies with significant increase in sealed worker brood area without adversely affecting the workers and queen activities in comparison to sulphur ,thymol and plant decoctions.

Key words: Ajwain, *Apis mellifera*, Cow urine, Thymol, *Varroa destructor*

The honey bee mite, *Varroa destructor* (Anderson and Trueman 2000) is an ectoparasite of the Asian honey bee, *Apis cerana* (Fabricius) that has became infested *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 (Donz and Guerin 1994) of bees.

Several chemical substances were used successfully to manage 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 fluvalinate 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). Natural products having components with various modes of action might provide effective solution to the problem of varroatosis (Imdorf *et al*. 1999). These natural products such as citric acid was less toxic to varroa than oxalic acid (Milani 2001). Also, grapefruit oil (Elzen *et al*. 2000b), and lemongrass oil (Fathy and Fouly 1995) were used for controlling varroa mites 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).

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

[1] Assistant Professor (e mail: ruchis03@rediffmail.com), Department of Entomology; [2] Field Development Officer (e mail: mathur.vaibhav3@gmail.com), TATA Rallis India Limited, Indore, Madhya Pradesh 452 010; [3] Rural Development Officer (JMGSI), (e mail: meenadhami18@gmail.com), Andhra Bank, Hisar, Haryana 125 001

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 in controlling honeybee mite, *V. destructor* in *A. mellifera* colonies in Uttarakhand.

MATERIALS AND METHODS

The test plants, leaves and seeds of neem (*Azardiracta indica* L.) and Jatropha (*Jatropha curcas* L.) were collected from the University campus and nearby areas of Pantnagar, whereas ajwain seeds [*Trachyspermum ammi* (L) syn. *Carum copticum* L] was purchased from local market and cow urine was collected from desi breed cow.

The experiments were conducted in the honeybee colonies of *A. mellifera* in 3 replications during July-August 2012 at the apiary site, Dineshpur which is situated between 29.05° N latitude and 79.32° E longitude and lies in the foothills of Tarai region of Kumaon Himalayas. 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 infested with mite, *V. destructor*. Use of eco-friendly formulations such as leaf powders of neem and jatropha, seed powder of ajwain and plant decoctions along with thymol powder and sulphur dust were applied separately in infested bee colonies and data were collected. The cow urine and plant extracts (10-15 ml/bee frame) were sprayed with hand sprayer on the bee frames over the bees very gently, whereas plant powders @ 5g/hive and sulphur powder @100 mg/hive was dusted in between the bee frames and thymol powder @ 500 mg/hive was kept on the bottom board in the bee hive. No treatment was given in untreated (control) bee colonies.

Efficiency of different formulations were measured using the following parameters:

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:

$$\text{Per cent brood infestation} = \frac{\text{No. of cells with mites} \times 100}{\text{Total no. of cells observed}}$$

Reduction (%) in infestation was calculated by using equation given by Henderson and Tilton (1955).

$$\text{Per cent reduction in mite infestation} = 100 \times 1 - \{Ta \times Cb\} / \{Tb \times Ca\}$$

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

Counts of dropped mites were recorded before and after 1 day, 1 week, 2 week, 3 week and 4 week of the application using plastic sheets (51.5 × 36.5 cm) coated with vaseline placed on the hive bottom board.

Sealed worker brood areas (SWBA) were recorded at 15-day intervals using a plastic sheet divided into square inches. Observations were taken for four weeks (before and after treatments) and only two sprayings (done on the frames over the bees and the brood) or dusting (done between the bee frames) of each treatment were given to the mite infested bee frames, where second application was repeated after the 15 days of first application. Statistical

Table 1 Effect of eco-friendly formulations on mite brood infestation in *A. mellifera* colonies during July-August 2012

Treatment	Conc.	Percent mite brood infestation		
		Pre-treatment	Post treatment	Per cent reduction in brood mite
Cow urine (Desi breed)	25%	37.33 (37.66)*	17.33 (24.59)	68.40 68.40
Cow urine (Desi breed)	100%	39.00 (38.71)	9.67 (18.10)	77.80
Neem leaf cow urine extract (NLCUE)	5%	34.67 (36.07)	18.33 (25.34)	54.32
Neem leaf water extract (NLWE)	5%	31.33 (34.03)	12.00 (20.28)	66.50
Jatropha leaf cow urine extract (JLCUE)	5%	32.67 (34.85)	15.67 (23.30)	58.33
Jatropha leaf water extract (JLWE)	5%	35.33 (36.46)	14.67 (22.51)	63.44
Neem seed cow urine extract (NSCUE)	5%	33.67 (35.46)	13.33 (21.40)	64.90
Neem seed water extract (NSWE)	5%	40.00 (39.28)	15.67 (23.30)	65.32
Jatropha seed cow urine extract (JSCUE)	5%	39.00 (38.66)	16.33 (23.82)	62.90
Jatropha seed water extract (JSWE)	5%	33.67 (35.46)	17.33 (24.60)	54.45
Neem leaf powder (NLP)	5 g	31.33 (34.01)	10.67 (19.05)	69.82
Jatropha leaf powder (JLP)	5 g	39.33 (38.33)	18.67 (25.59)	57.90
Ajwain powder (AP)	5 g	33.67 (35.46)	11.33 (19.66)	70.17
Thymol powder (TP)	500 mg	35.33 (36.46)	13.00 (21.14)	67.40
Sulphur powder (SP)	100 mg	39.00 (38.66)	23.77 (29.17)	46.00
Control		33.67 (35.46)	38.00 (38.07)	
Sem±		0.024 (0.011)	0.010 (0.01)	
CD (P=0.01)		0.095 (0.04)	0.040 (0.03)	
Cv		0.12 (0.051)	0.108 (0.07)	

*Figures in the parentheses are angular transformed values.

analysis of the data on the brood mite infestation, mite fall and sealed worker brood area was conducted using Randomized Block Design with suitable transformations.

RESULTS AND DISCUSSION

The data presented in Table 1 clearly showed that cow urine @ 100%, significantly reduced brood mite infestation (77.80%) followed by ajwain powder @ 5g/hive with (70.17%), neem leaf powder @ 5g/hive (69.82%), cow urine @ 25% (68.40%) and thymol powder @ 500 mg/hive (67.40%) after 4 weeks of application of treatment in comparison to plant decoctions where the per cent reduction in mite brood infestation was 66.50% in (NLWE@ 5%) followed by NSWE@5% (65.32 %), NSCUE@ 5% (64.90%), JLWE @5% (63.44%), JSCUE @ 5% (62.90%), JLCUE @5% (58.33%), JSWE (54.45%, JSWE @5% (54.45%) and NLCUE @ 5% (54.32%) in comparison to significantly less reduction in brood mite infestation in sulphur powder @ 100 mg/hive (46.00 %) which was found the least effective among all the treatments for management of mite infestation in *A. mellifera* colonies.

Effect of treatments on mite fall

The number of fallen mites/colony after treatment averaged in the Table 2 showed the highest mean mite fall in cow urine @100% (50.06%) followed by ajwain powder @ 5g (48.40%), neem leaf powder @ 5g (44.40%) and cow urine @25% (41.66%). On the other hand jatropha leaf powder @ 5g/hive and thymol powder @ 500 mg/hive averaged 38.73% and 38.87% of mite fall followed by NLWE @ 5% (30.80%). Among the plant decoctions prepared in cow urine and water, the average mite fall was ranged between 25.00% in NSWE @5% to 28.00% in JSWE @5% with least mean mite fall in JLWE@ 5% (21.80%) followed by sulphur powder @ 100 mg (23.87%) with only 13.27% average mite fall in untreated control.

Effect of treatments on brood rearing activity

The data presented in Table 2 showed significantly increase in average sealed worker brood area (SWBA) in the bee colonies given cow urine @ 25%, cow urine @100%, neem leaf powder and ajwain powder @ 5g/hive with 723.83, 892.84,678.50 and 740.34 cm^2 respectively after the end of 4 weeks of treatments followed by jatropha leaf powder (615.67cm^2) in comparison to control (334.67 cm^2). It has been clearly noticed that the plant decoctions prepared in cow urine and water were limited the brood rearing activity of bees as the sealed worker brood areas started was ranged from 242.33 cm^2 to 346.28 cm^2 which was drastically reduced to 128 cm^2 to 218.67 cm^2 after four weeks of treatments with average SWBA ranged from 190.17 to 333.33 cm^2 which was significantly least sealed brood area than the brood area calculated for the bee colonies had cow urine, leaf powders of neem , jatropha and ajwain seed powder treatments.

The plant decoctions sprayed on mite infested bee frame gave significantly reduced sealed worker brood areas which was their noticeable adverse effect on brood rearing activities of worker bees, queen stopped egg laying as newly laid eggs were not found indicated that egg laying tendency of queen bee was greatly affected and damaged brood was found in the hives due to direct spray of [plant decoctions on the brood areas but adult mortality was not observed. It has been noticed in these treated colonies that new brood was very less and old brood was damaged which was picked out by the worker bees and thrown out of the colonies. This proved deleterious effects of plant decoctions on bee brood in comparison to cow urine alone and leaf powders of neem, jatropha and ajwain seed powder which gave significantly highest mite fall with increase in recovery in brood mite infestation and sealed worker brood areas.

These results are supported by the findings 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 natural plant products are more recommended than other chemical acaricides to keep the social life of honeybee away from any harmful effect (Dimetry *et al.* 2005). Wang *et al*. (2007) investigated the acaricidal activity of leaf extracts from walnut, *Juglans regia* L., on the mites *Tetranychus cinnabarinus* (Boisduval).

The studies have also been made on the efficacy of cow urine for management of bacterial disease, European foul brood (EFB) caused by *Melisococcus plutonius* (Corrig) at different locations of Uttarakhand (Tiwari and Mall 2007, Aakash and Tiwari 2012). The efficacy of animal origin compounds such as cow urine, cow dung powder and cow dung ash powder and plant product, ajwain powder were found effective against management of mite infestation in *A. mellifera* colonies as different locations of Uttarakhand (Tiwari *et al*. 2014) with significantly increase in sealed worker brood areas with promotion of growth of bee brood.

This was the novel approach to study about the effect of animal origin product, i.e. cow urine , plant products and plant decoctions against mite disease in bee colonies in Uttarakhand. The present studies revealed that the cow urine and plant products such as leaf powders of neem, jatropha and ajwain seed powder 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.

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Table 2 Effect of eco-friendly formulations on mite fall and sealed worker brood area (SWBA) in *A. mellifera* colonies at Dineshpur during July-August 2012

Treatment	Conc.	Mean no. mite fall after period given 2							Per cent sealed worker brood area (SWBA) (cm^2)			
		Pre-treatment	1 day	1 week	2 week	3 week	4 week	Mean mite fall	Pre-treatment	After 2 weeks	After 4 weeks	Mean SWBA (cm^2)
Cow urine (Desi breed)	25%	18.33 (25.34)*	28.33 (32.15)	39.67 (39.03)	49.33 (44.61)	46.33 (42.88)	44.67 (41.93)	41.66 (40.19)	310.58 (17.63)#	572.33 (19.38)	875.3 (29.59)	723.83
Cow urine (Desi breed)	100%	19 (25.85)	36.33 (37.06)	48.33 (44.03)	52.33 (46.32)	55 (47.88)	58.33 (49.79)	50.06 (45.03)	322.34 (17.96)	627.00 (23.61)	1058 (31.57)	892.84
Neem leaf cow urine extract (NLCUE)	5%	13.33 (21.41)	28.67 (32.36)	27 (31.31)	31.67 (34.24)	33.33 (35.26)	22.33 (28.19)	28.6 (32.33)	297.06 (17.24)	247.67 (25.03)	128 (11.27)	212.84
Neem leaf water extract (NLWE)	5%	19.67 (26.32)	32 (34.46)	38.33 (38.24)	33.00 (35.07)	29.67 (32.99)	21.00 (27.28)	30.8 (33.71)	277.34 (16.66)	320.33 (15.72)	180.33 (13.61)	250.33
Jatropha leaf cow urine extract (JLCUE)	5%	23 (28.66)	28.67 (32.36)	33.67 (35.46)	32.00 (34.46)	23.67 (29.10)	18.00 (25.11)	27.2 (31.44)	346.28 (18.62)	309.67 (17.99)	173.67 (13.25)	291.67
Jatropha leaf water extract (JLWE)	5%	14.67 (22.51)	23.33 (28.87)	20.67 (27.03)	28.33 (32.15)	19.00 (25.85)	17.67 (24.84)	21.80 (27.84)	325.35 (18.13)	373.33 (17.55)	193.33 (14.04)	333.33
Neem seed cow urine extract (NSCUE)	5%	28.33 (32.15)	33.67 (35.46)	27.00 (31.31)	30.33 (33.41)	28.33 (32.15)	13.67 (21.69)	26.6 (31.05)	242.33 (25.63)	293.00 (17.12)	168.67 (12.87)	197.84
Neem seed water extract (N SWE)	5%	23.33 (28.88)	26.33 (30.87)	24.00 (29.34)	29.00 (32.59)	25.67 (30.43)	20.00 (27.28)	25.00 (30.01)	263.67 (16.95)	282.33 (16.89)	162.00 (14.71)	190.17
Jatropha seed cow urine extract (JSCUE)	5%	21.67 (27.73)	38.33 (38.25)	27.67 (31.73)	34.67 (36.06)	26.67 (31.08)	19.33 (26.07)	27.33 (31.51)	242.67 (15.65)	308.67 (17.50)	218.67 (14.73)	263.67
Jatropha seed water extract (JSWE)	5%	22.67 (28.42)	34.67 (38.06)	29.33 (32.74)	34.67 (35.46)	23.00 (28.67)	18.33 (25.34)	28.00 (31.96)	271.33 (16.51)	324.33 (18.09)	214.00 (29.58)	269.17
Neem leaf powder (NLP)	5g	22.33 (28.19)	36.67 (37.26)	40.33 (39.41)	47.00 (43.29)	45.33 (42.31)	52.67 (46.52)	44.40 (41.79)	314.67 (17.75)	482.33 (22.01)	874.67 (27.49)	678.5
Jatropha leaf powder (JLP)	5g	17.33 (24.60)	26.00 (30.67)	39.00 (38.65)	38.33 (38.24)	48.33 (44.03)	42.00 (40.40)	38.73 (38.48)	294.00 (17.16)	473 (21.79)	758.33 (31.22)	615.67
Ajwain powder (AP)	5g	22.33 (28.20)	36.33 (37.06)	46.67 (43.08)	48.67 (44.23)	51.00 (45.58)	57.33 (49.21)	48.40 (44.08)	347.68 (18.65)	509.00 (22.49)	971.67 (29.96)	740.34
Thymol powder (TP)	500 mg	14.67 (22.51)	32.67 (34.85)	40.67 (39.61)	46.00 (42.71)	38.33 (38.24)	36.67 (37.26)	38.87 (38.56)	335.67 (18.34)	492.33 (22.28)	898.00 (29.96)	585.67
Sulphur powder (SP)	100 mg	21.67 (27.73)	23.00 (28.67)	29.33 (32.78)	26.00 (30.67)	21.67 (27.73)	19.33 (26.07)	23.87 (29.23)	285.37 (16.90)	373.33 (19.39)	408.67 (20.20)	391.00
Control		17.33 (24.60)	15.33 (23.05)	12.00 (20.28)	16.67 (24.08)	13.00 (21.15)	9.33 (18.73)	13.27 (21.35)	363.03 (19.06)	312.67 (17.78)	356.67 (18.89)	334.67
SE m±		0.08	0.012 (0.12)	0.016 (0.01)	0.019 (0.06)	0.011 (0.004)	0.006 (0.008)	0 .005 (0.009)	0.646 (0.018)	4.32 (0.095)	1.717 (0.052)	
CD (P = 0.01)		0.031	0.046 (0.04)	0.062 (0.04)	0.075 (0.03)	0.04 (0.01)	0.025 (0.03)	0.024 (0.03)	1.686 (0.018)	16.803 (0.839)	6.679 (0.203)	

*Figures in the parentheses are angular transformed values. #Figures in the parentheses are square root transformed values with adding 0.5 in original value

Cow urine based Formulations for Honeybee disease management and Crop Protection in Uttarakhand. We extend thanks to Director Experimentation Station, Dean Agriculture and Head, Department of Entomology, College of Agriculture, Govind Ballabh Pant University of Agriculture and Technology, Pantnagar for providing

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