Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2017; 5(5): 589-595

Received: 10-07-2017
Accepted: 11-08-2017

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Efficacy of various botanicals and chemicals on ectoparasitic mite, *Varroa destructor* feeding on European honey bee, *Apis mellifera*

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Abstract
The present investigation was carried out to evaluate the chemicals (Formic acid, Oxalic acid and Taufluvalinate) and botanicals (*Artemesia annua*, *Matricaria chamomilla*, *Juglans regia* and mixture *A. annua* + *M. chamomilla* + *J. regia*) under Complete Randomised Block Design (RCBD) for management of *V. destructor* during 2016 at Faculty of Agriculture, Wadura SKUAST-Kashmir. The results revealed that among chemicals, Taufluvalinate (2 strips per colony) was found highly effective against the *V. destructor* mite followed by Formic acid 85% @ 2 ml per colony but in case of botanicals green leaf extract mixture @ 150ml/colony was found highly effective against *Varroa* mite followed by *M. chamomilla.* The order of efficacy of treatments was Taufluvalinate (78.93%) > Formic acid (74.83%) > Oxalic acid (72.16 %) > GLE mixture (67.10 %) > *M. chamomilla* (63.83%) > *A. annua* (61.87%) > *J. regia* (58.34 %). Bio leaf extracts need to be integrated in such a way that there may be no hazard to the bees, moreover further studies on these extracts need to be explored.

Keywords: *V. destuctor*, *A. mellifera*, Botanicals, Management

1. Introduction

Among the insect species, honey bee is the most beneficial as it produces honey, royal jelly, pollen, wax etc. which are well known to possess great value for their use in pharmaceuticals, food products and other industrial products [54]. Honey bees are also playing an important role in bio-diversity. They are known to increase yield of insect pollinated crops to the extent of 10 to 20 times more than the cost of honey produced by them. Bee pollination improve the size, shape, colour, storage capacity and taste of the fruits [5].

The state of Jammu and Kashmir (32°17’ to 37°05’N and 72°40’ to 80°30’E) represents one of the most important beekeeping areas in India. Four agroclimatic zones ranging through low altitude subtropical, intermediate, temperate and cold alpine are experienced in the state [48] which offers great potential for both migratory and non-migratory beekeeping. The migratory system of bee-keeping is more economical than stationary bee-keeping system as it not only helps in boosting income of the individual bee-keeper but also helps in increasing productivity of cross-pollinated crops and generates employment. The commercial bee-keepers practising migratory bee-keeping use Langstroth hives and have 4-5 harvests per year with an average annual yield of approximately 50-60kg per hive) [48]. Current honey production in Kashmir valley was 1.2 thousand tones during the year 2015-2016(3).Since last few years, 60-100 per cent loss to the brood has been reported in some apiaries [43].

Recent catastrophic mass honey bee colony losses are causing overall population declines in the world. This occurrence is drastically threatening the apicultural industry, while causing economic and ecological pressures on agricultural crop production and ecosystem services, respectively. Variety of causal agents have been suggested to explain these colony losses, recent reports point to the spread of honey bee diseases and parasites as an explanation for these mass colony losses [42]. The ectoparasitic mite, *Varroa destructor* is among the core colony losses worldwide and has been responsible for nearly complete eradication of wild and feral honey bee populations in Europe and North America, since it was introduced to this new honey bee host species [27, 33]. This mite feeds on haemolymph of brood and adult bees causing colony disorder, weakness, decreasing brood and deforming immature and mature bees. It also reduces colony ability to pollinate plants [17]. Infested colony may die or migrate resulting in economic loss and honey production [41].

The mite can be found on adult bees, on the brood and in hive debris. In a heavy infestation, pupae may not develop into adult bees. The adults that do emerge may weigh less than healthy bees [49]. The mite weakens the honey bee immunity and increases their susceptibility to other environmental stresses and vectors like lethal honey bee viruses [9].

V. destructor is generally kept under control by using synthetic acaricides such as amitraz, coumaphos and pyrethroids including fluvalinate and flumethrin [2, 18]. However, the regular use of these insecticides has resulted in the development of resistance in mites in many areas of the world [35, 45] as a result of which, the efficacy of these insecticides for controlling *Varroa* mite has decreased significantly [35]. In recent years, the problem of resistance to acaricides has evolved rapidly. Increased tolerance has been observed to the most widely used synthetic acaricides. In addition, contamination of hive products with pesticide residues has also been reported, especially in honeybee wax [4, 8] and propolis [10, 55]. This situation has resulted in an increased concern about the bee product contamination with synthetics used against *Varroa* [28]. All these problems have negatively affected the beekeeping industry. Therefore, alternative control strategies including the use of natural products such as organic acids, plant extracts and essential oils against *V. destructor* have been developed. In the last decade, several studies throughout the world have confirmed the use of formic acid [50], oxalic acid [38, 39], thymol and eucalyptol [30, 37] in *Varroa* control. Organic acids have a status of generally recognized as safe [11] and essential oils are safe to use as they pose lower health hazards to both humans as well as honeybees [4]. There is an increasing interest in using natural pesticides, derived from plants and microorganisms [32] because they are generally safer than the synthetic insecticides. Keeping in view the serious threat of *Varroa* mite in the beekeeping and several constraints for its control, the present investigation was carried out to develop safe and effective management option for the mite population control.

2. Materials and Methods

The experiment was conducted at apiary of Faculty of Agriculture SKUAST- Kashmir during May-June 2016.Twenty four honeybee *A. mellifera* L. colonies in Langstroth beehives naturally infested by *V. destructor* mites were used for this study.

2.1 Detection methods for *Varroa* infestation

2.1.1 Sugar shaking method

200 adult bees were collected randomly from different colonies and were placed in a wide mouthed mason jar with a lid of 3mm × 3mm mesh screen for mite collection 2-3 tablespoons of powdered sugar were added through the mesh lid and jar was rolled for about 30 seconds so that the bees were thoroughly coated with sugar to collect mites. After that jar was inverted on a muslin cloth for about 20 minutes, mites passed through the mesh screen, while the bees remained behind. The number of dislodged mites were recorded in the experiment [36].

2.1.2 Sticky board method

Screened sticky boards were placed between the hive floor and brood frames. The screen separated the bees from reaching the sticky paper. The dislodged mites fell on the sticky paper through the screen and adhered to it. The sticky board was placed in hive for 24 hours. After 24 hours the sticky board was removed from hive and the dislodged mites were counted.

Table 1: Various chemicals and botanicals used against *V. destructor*

Name of treatment	Concentration used per colony
Formic acid (85%)	2ml
Oxalic acid dihydrate (99%)	50 ml
Taufluvalinate (Apistan strips)	2 strips
GLE of *Artemesia annua*	50ml
GLE of *Matricaria chamomilla*	50ml
GLE of *juglans regia*	50ml
GLE mixture (*A.annua+M. chamomilla+ J. regia*)	50ml
Control	Untreated

Experiment was conducted with eight treatments including control and every treatment was replicated thrice. Pre-treatment mite fall (number) per colony on the sticky paper was recorded before the application of treatments and the post-treatment mite fall on the sticky paper at 7, 14, 21 and 28 days after treatment (DAT). One treatment constituted of three colonies *i.e.* one colony as one replication. Per cent reduction over control and per cent mortality was worked out.

2.2 Methods of application of treatments

Formic acid (85%): 2ml of formic acid were taken in plastic vial plugged with cotton. The vial was placed at the bottom board of hive in between the central frames. Mite drop was evaluated by using sticky boards. Before applying formic acid all the cracks and crevices of hive were closed with mud.

Oxalic acid dihydrate (99%): 2gm of Oxalic acid was mixed with 100 ml of 50 per cent sugar solution. Sugar solution was boiled and cooled before mixing of oxalic acid. With the help of baby sprayer 50 ml solution containing oxalic acid was sprayed on the bars of frames per colony (5ml/frame). Mite drop was recorded on sticky board.

Taufluvalinate (**Apistan strips):** Two Apistan strips per 10 frame colony were hanged in between the frames in the middle of the colony.

Botanicals: Foliage of botanicals were collected and oven dried separately. Dried leaves were ground into fine powder with a HYP-50 crusher. The powder was filtered through a 50 mm mesh, sealed in plastic bags and was held at room temperature (27 ± 3Ċ and 40% RH) over night. For the extractions, 1kg of fine powder was mixed with 1 litre of water and was kept at room temperature for three days. After three days green leaf extract solution was passed through a muslin cloth for collection of pure extract. Green leaf extracts of *A.annua, M. chamomilla, J. regia* and green leaf extract mixture (*A.annua + M.chamomilla + J. regia*) were taken at 5% concentration (50ml/colony). All the extracts were sprayed with baby sprayer directly on bee frames.

2.3 Mite mortality

To check the mite mortality, mite collection trays with sticky white formica sheet covered by a screen to avoid the bees to come in contact with the sticky sheet were kept in the bottom board of each hive. Dead *Varroa* mites fallen on the bottom board trays were collected and counted in all tested colonies at an interval of 7, 14, 21 and 28 days of post treatment. Per cent mortality of mites in bee colonies was calculated by using formula [44].

$$\text{Per cent mite mortality} = \frac{\text{Mite mortality in treatment}}{\text{Mite mortality in treatment} + \text{mite mortality in control}} \times 100$$

$$\text{Per cent reduction over control} = \frac{\text{No. of dead mites in treatment} - \text{No. of dead mites in control}}{\text{No. of dead mites in treatment}} \times 100$$

2.4 Statistical analysis

Data generated from the experiments was statistically analysed using the Statistica-AG Software licensed to Faculty of Agriculture, SKUAST-K, Wadura.

3. Results and Discussion

The efficacy of experimental chemicals and botanicals against infestation with *V. destructor* mites is shown in Table 2 and 3. Results revealed that among different treatments used, Taufluvalinate (Apistan, 2 strips per 10 frame colony) followed by formic acid 85% @ 2ml per colony through plastic vials, oxalic acid 99 % @ 50 ml per colony, green leaf extract (GLE) mixture@ 50ml per colony, GLE of *Matricaria chamomilla* @50ml per colony, GLE of *Artemesia annua* @ 50 ml per colony and GLE of *Juglans regia* @ 50 ml per colony were found highly effective in suppressing the *Varroa* mite population as compared to control. The order of efficacy was Taufluvalinate (78.93 per cent) > Formic acid (74.83 per cent) > Oxalic acid (72.16 per cent) > GLE mixture (67.10 per cent) > *M. chamomilla* (63.83 per cent) > *A. annua* (61.87 per cent) > *J. regia* (58.34 per cent). Moreover, the results indicated that the highest number of mite drop on sticky paper per colony was observed in first week of treatments which gradually declined in second, third and fourth week of treatments.

A very large number of chemicals have been used to control *Varroa* mite both "hard and soft" chemicals. The "hard" chemicals include products such as Apistan (fluvalinate), perizin (coumaphos), Bayvarol (flumethrin) and Apivar (amitraz), which give effective mite control in the order of 97-100 per cent [25, 56]. In the present studies, Fluvalinate (Apistan, 2 strips / 10 frame colony) was found highly effective against the *V. destructor* by providing mite drop of 51.00 and per cent mortality of 78.93 after four weeks. A reduction in mite drop over control to the tune of 73.69 per cent was observed [22]. achieved nearly 100 per cent efficacy with Fluvalinate in susceptible mite populations. "Soft chemicals" such as formic aid and oxalic acid do not give the same degree of control [1, 30]. Repeated use of "hard" varroacides results in the development of resistance in the *Varroa* mites against the product. *Varroa* populations have been even shown resistance to fluvalinate in many parts of Europe [51] and Israel [26, 7]. documented first that mites apparently showed resistance to Apistan in the United States. To overcome problem of resistance, the alternative chemical treatments such as organic acids and botanicals have gained great attention in recent years. Certainly, all these *Varroa* treatments are cheaper but at the same time less effective [30]. The most commonly used alternative compound is formic acid used in many formulations and devices for *Varroa* treatment. The efficacy of Formic acid ranges from 29.6 per cent [6] to more than 90 per cent [19] depending on doses, modalities of application and experimental or environmental conditions. In the present studies, formic acid 85 per cent through plastic vials (Vaporization) @ 2 ml/colony gave an effective mite control (mite drop) up to the extent of 40.58 and per cent mortality of 74.83 after four weeks of the treatment. A reduction in mite drop over control to the tune of 66.94 per cent was observed. Present findings are in line with [13] who achieved 94 per cent efficacy when treating colonies with 300 ml of 65 per cent formic acid in a slow release evaporator. Formic acid is a fumigant that kills *Varroa* by respiratory inhibition [29]. Formic acid has a strong acaricidal effect [52] has the advantage of killing mites on adult bees as well as in sealed brood [34]. *Varroa* control with formic acid carrying efficacy range of 26-77 per cent is very temperature dependent [1, 23]. Mite mortality of 56 per cent was reported by using formic acid in an autumn treatment [12].

Oxalic acid is another alternative chemical that has been used for *Varroa* control and has recorded greatest success of mite fall up to 95 per cent [1, 38, 46]. demonstrated that aerosol treatments with 3 per cent oxalic acid on bees infested with adult mites induced 97.3 per cent mite mortality similarly highest efficacy of 99.5 per cent in broodless colonies has been reported. In the present studies, oxalic acid 0.2 per cent @ 50 ml/colony showed mite drop of 35.16 after four weeks with per cent mortality of 72.16 and per cent reduction over control up to 61.85. The results obtained are in line with the findings of [24] who showed that trickling 30 ml of 3.2 per cent oxalic acid solution is significantly more effective (92.2 per cent efficacious) than trickling 60 ml of 1.6 per cent oxalic acid solution (68.3 per cent efficacious). Further it was observed that concentration of oxalic acid is critical for high efficacy rather than the total amount of oxalic acid applied to a colony. Similarly, [14, 31] observed that a single spray treatment using a 3.0 per cent oxalic acid sugar water solution (1:1 by weight) and doses of 3-4 ml per comb side provided efficacies of 97.3 to 98.8 per cent.

Botanical extracts obtained from different plant species have shown broad spectrum of acaricidal activity against *Varroa* [16, 29]. Satisfactory results have been reported from the application of homemade plant extracts such as thymol, sage, garlic oil, walnut extract etc [15, 47, 53]. In present investigation, green leaf extract mixture (*A. annua* + *M. chamomilla* + *J. regia*) @ 50 ml / colony showed highest per cent mite mortality of 67.10 with mite drop of 28.16 after four weeks and lowest per cent mortality of 58.34 was observed in case of *Juglans regia* @ 50ml/ colony with mite drop of 19.41 after four weeks. Laboratory studies reported smoke from burning leaves of walnut knock down 70 to 90 per cent of the exposed mites [20, 21]. The percentage of efficacy and control of *Varroa* mite by green leaf extracts presented in this work seems to be low in comparison to those obtained from synthetic acaricides commonly used to control *Varroa* but the results are still promising.

From the results and discussion it may be concluded that Taufluvalinate was found most effective followed by Formic acid against the *V. destructor* among chemicals and green leaf extract mixture (*A. annua* + *M. chamomilla* + *J. regia*) among botanicals in reducing the mite population as compared to control. Fluvalinate (Apistan, 2 strips / 10 frame colony) was found highly effective against the *V. destructor* by providing mite drop of 51.00 and per cent mortality of 78.93 after four weeks. A reduction in mite drop over control to the tune of

73.69 per cent was observed. Repeated use of Taufluvalinate however, results in the development of resistance in the *Varroa* mites. As formic acid is a natural constituent of honey, hence it is environmentally safe to use formic acid as an acaricide in *A. mellifera* L. colonies. In the present study, formic acid 85 per cent through plastic vials (Vaporization) @ 2 ml/colony gave an effective mite control (mite drop) up to the extent of 40.58 and per cent mortality of 74.83 after four weeks of the treatment. A reduction in mite drop over control to the tune of 66.94 per cent was observed. The Green leaf extract mixture can be an alternative to formic acid, a chemical treatment widely being used in *A. mellifera* colonies against *Varroa* mite. The use of green leaf extract mixture (*A. annua+ J.regia+ M. chamomilla*) may fit well into Integrated Pest Management(IPM) Programme for alternative use with other control measures for the management of *Varroa* mite and other pests in honey bee colonies as they enhance chance for colony survival and ensure residue free hive products. Still more studies are required to be performed under laboratory and field conditions on the *Varroa* control options. These studies should be done on a wide scale and under different ecological conditions. All possible factors impacting treatment efficacy should be considered during studies (*e.g. Varroa* mite population and brood rearing activity). Thus, using per cent of dead *Varroa* after treatment application is advisable to be used as a standard indicator of treatment efficacy for any study on *Varroa* control.

4. Acknowledgement
Authors are highly thankful to Division of Entomology, SKUAST- Kashmir for providing necessary facilities to carry out this research work.

5. Conclusion
Integration of botanicals is urgent need in the development of overall scenario of apiculture. Present studies depicted the facts that there is lot of scope in studying the botanicals up to the biochemical level and bioactive extracts involved in the mite control need to be studied in future.

Table 2: Effect of various chemicals and plant extracts on *V. destructor* infesting *A. mellifera*

Treatment	Dead mites before treatment (Number)/ colony	Dead mites after treatment (Number)/colony				Mean	Per cent reduction over control
		7 DAT	14 DAT	21 DAT	28 DAT		
Formic acid (2ml of 85%)	21.67	48.33 (44.02)	44.67 (41.92)	39.00 (38.62)	30.33 (33.41)	40.58 (39.5)	66.94
Oxalic acid (2g of 99%)	19.00	43.67 41.34)	35.00 (36.25)	34.00 (35.65)	28.00 (31.94)	35.16 (36.3)	61.85
Taufluvalinate (Apistan strips)	23.33	60.00 (50.75)	55.67 (48.23)	48.33 (44.02)	40.00 (39.21)	51.00 (45.55)	73.69
GLE (5%) of *Artemesia annua*	12.67	30.00 (33.19)	24.00 (29.31)	20.33 (26.79)	15.33 (23.02)	22.41 (22.08)	40.15
GLE (5%) of *Matricaria chamomilla*	20.00	30.00 (33.19)	28.67 (32.35)	20.00 (26.56)	18.33 (25.33)	24.25 (29.35)	44.67
GLE (5%) of *Juglans regia*	16.67	26.00 (30.64)	22.00 (27.95)	17.00 (24.33)	12.67 (20.83)	19.41 (25.94)	30.90
GLE Mixture (*A. annua+ M. chamomilla+ J.regia*)	13.00	37.33 (37.64)	31.00 (33.81)	23.33 (28.86)	21.00 (27.26)	28.16 (31.90)	52.37
Control	11.67	13.33 (21.66)	11.67 (19.93)	14.33 (22.23)	14.00 (21.95)	13.41 (21.44)	-
Mean		36.12 (36.56)	31.58 (33.72)	27.04 (30.88)	22.45 (27.87)		
CD ((P≤ 0.05) Treatments=0.65; Days= 0.46; Treatment× Days= 1.31							

*Values with in parenthesis are arc sine transformed values

Table 9: Efficacy of various treatments on the basis of percent mortality of *V. destructor* infesting *A. mellifera*

Treatments	Percent mortality				Mean
	7 DAT	14 DAT	21 DAT	28 DAT	
Formic acid	78.42 (62.30)	79.34 (62.95)	73.12 (58.75)	68.44 (55.80)	74.83 (59.95)
Oxalic acid	76.61 (61.07)	75.04 (60.02)	70.33 (56.97)	66.67 (54.73)	72.16 (58.2)
Apistan strips(Fluvalinate)	81.81 (64.75)	82.71 (65.42)	77.12 (61.40)	74.10 (59.38)	78.93 (62.74)
GLE of *Artemesia annua*	69.30 (56.34)	67.35 (55.15)	58.61 (49.94)	52.23 (46.26)	61.87 (51.92)
GLE of *Matricaria chamomilla*	69.29 (56.33)	71.10 (57.48)	58.24 (49.72)	56.69 (46.26)	63.83 (53.09)
GLE of *Juglans regia*	66.17 (54.41)	65.45 (53.50)	54.24 (47.41)	47.53 (43.56)	58.34 (49.84)
GLE mixture (Artemesia+Matricaria+Juglans)	73.74 (59.15)	72.72 (58.50)	61.92 (51.87)	60.02 (50.75)	67.10 (55.07)
Control	-	-	-	-	
Mean	73.62 (59.19)	73.38 (59.07)	64.80 (53.72)	60.81 (51.33)	
CD ((P≤ 0.05) Treatments= 0.84; Days=0.63 ; Treatment× Days=1.68					

*Values with in parenthesis are arc sine transformed values

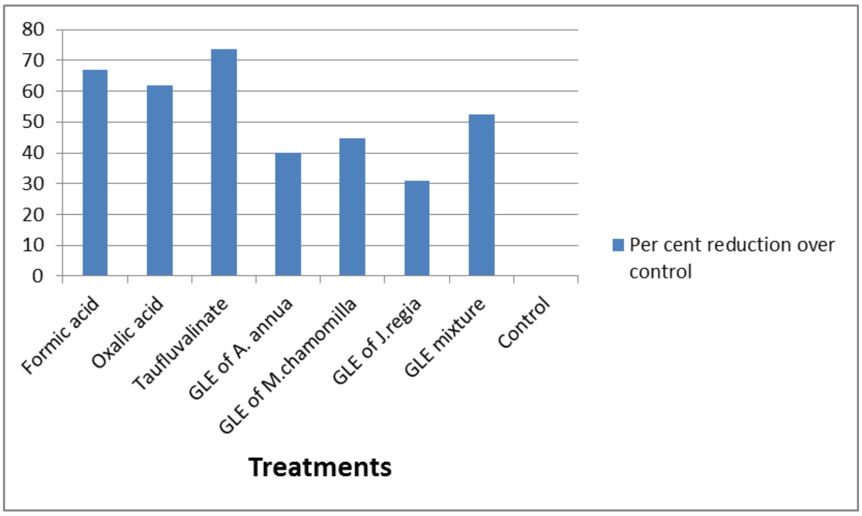

Fig 1. Per cent reduction of ectoparasitic mite, *V. destructor* over control

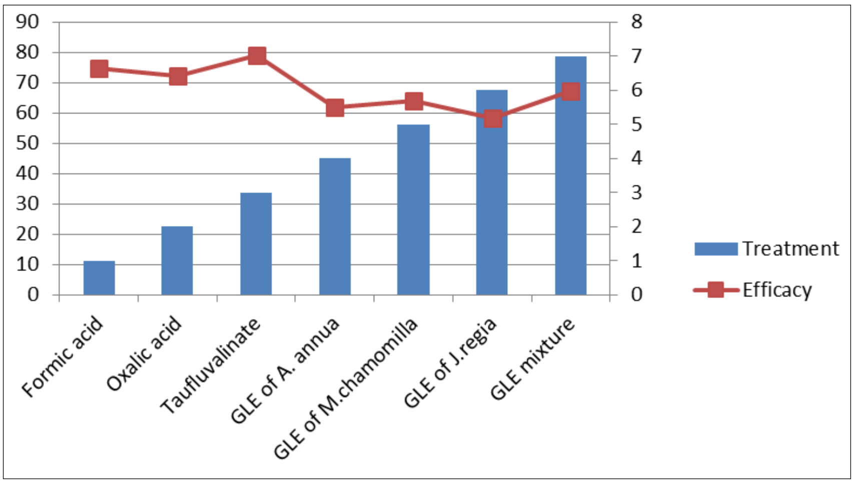

Fig 2. Efficacy of chemicals and botanicals against *V. destructor*

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