DETERMINATION OF RESIDUAL BROMOPROPYLATE AND ITS DEGRADATION PRODUCT IN HONEY AND WAX AGAINST VARROA INFESTATION IN BEES

A. Nasim and T. Mahmood*

Department of Chemistry, Government College of Science, Lahore, Pakistan
*352-E, Punjab Housing Society, Defence Road, Lahore Cantt, Pakistan

ABSTRACT: A mite infestation control product bromopropylate was used over a 2 year period (1990 - 1992) to control *Varroa jacobsoni* infestation by means of colony treatment in autumn. Treatments were given to 12 hives located in the hilly areas of Islamabad and Murree. The residual levels of Bromopropylate and its degradation product were measured in the honey and wax. The samples were taken from each hive in March, May, July and August in the year following treatment. Chemical Analysis via gas chromatography showed that bromopropylate persisted in wax samples, bromopropylate were present in honey samples.

Key words: Bromopropylate, *Varroa jacobsoni*, Folbex VA

INTRODUCTION

The parasitic mite Varroa is a harmful pest of the honeybee races. Up to now most beekeepers have applied acaricides to control this mite in their colonies. However, the use of acaricides has two major disadvantages: 1) contamination of honeybee products, 2) the occurence of mite resistance. The modern concepts to control the mite use integrated strategies and low environmental impact acaricides as essential oils and organicic acids (Lodesani *et al*., 1990). Various anti-varroatosis products are available but Bromopropylate has advantage of being officially authorized drug available and its common use in Europe. Contamination of bee products with pesticides has been widely documented for many years (Bogdanov *et al*., 1998, Jimenez *et al*., 2005 and Chauzat and Faucon, 2007).

The present study was conducted to determine the therapeutic aspects of Bromopropylate, an anti-varroatosis compound, and also for detection of residues of Bromopropylate in honey and wax from the same colonies.

MATERIALS AND METHODS

Twelve diseased colonies (Mites infestation) of *Apis mellifera* (Honey Bee) comprising of seven bees frame per colony were isolated from two localities in the hilly areas of Islamabad and Murree and were treated with commercially available anti-varroatosis product (Folbex VA) from 1990 to 1992. The Folbox VA used and the administration methods are summarized in Table -1. The colonies used in the tests were homogeneous, as regards the number of bees and stocks of food. Care was taken during the last treatment period to ensure that there was an almost total absence of brood.

Acaricide Treatment: Bromopropylate was used in its commercially available form, Folbex-VA (Ciba-Geigy). The treatment was carried out in the latter half of August, four times each after five days interval. The treatment comprises of fumigation of the bees using one fumigant strip per colony. Each bee colony, having two boxes and seven frames, was treated with one Folbex strip of 370 mg. Treatment was done in autumn because in winter honey bees did not go out from hives and lived in packed form. Extra feed was given to honey bees before the start of fumigation. Treatment was given in the evening when the bees have ceased flying and there was no adult in the brood chamber.

Sample Collection: The Honey and Wax samples were taken from at least 2 combs in each colony, one from the center and the other from the side. A part of each comb was uncapped with a knife and the Honey extracted from the cells with a syringe and placed in test tube.

To remove any impurities, especially particles of Wax, the Honey was centrifuged at 3000 RPM for 10 minutes. The wax taken from the caps which had previously been removed was placed in feeders on the Hive comb covers so that the bees could clean off any remaining Honey during the following 24 hours. Samples were taken at 4 periods during the active season:

- 1st Sampling on 9, May 1991
- 2nd Sampling on 16, May 1991
- 3rd Sampling on 21, July 1991
- 4th Sampling on 11, August 1991

Laboratory Analysis: Initially the method for honey was perfected and then was modified as needed for use with wax.

Standardization of GC method: Gas chromatographic method application was validated by experiments using recovery test for the active ingredient. The known

concentration of bromopropylate was added to the untreated samples of wax and honey and recovery percentages were calculated by comparing the sample readings with that of the standard solution of bromopropylate The external standard method was used for quantitative calculations (Table 02). The reagents Acetone, n-Hexane, Methanol, Bromopropylate, 4,4-dibromobenzophenone were used.

Preparation of Honey Sample: Five gm Honey treated with bromopropylate was blended overnight with a mixture of Water-Acetone (2:8) using Magnetic Stirrer. The blended sample was successively shaked four times with a mixture of water n-Hexane and methanol (5:7.5:7.5). The combined extract was centrifuged and separated by a Separating Funnel. The upper layer was concentrated in a rotary vacuum evaporator and finally dried by nitrogen current. The dried residue was dissolved in n-Hexane-Methanol (1:1) mixture and subjected to gas chromatography.

Preparation of Wax Sample: Five gm of wax sample was allowed to blend with 20 cm^3 of a water-acetone mixture (1:1) for overnight. Impurities were removed by filtering and centrifuging, in similar manner as described above for the Honey. The extracts were concentrated in the rotary evaporator, dried in nitrogen current and dissolved in 05 cm^3 n-Hexane and subjected to gas chromatography.

Detection: This was performed by gas chromatography using GC 14-A with an ECD detector and borosilicate glass column 10% OV-101 on gas Chrom Q under the following conditions:

- Column temperature = 270°C
- Injector temperature = 280°C
- Detector temperature = 300°C
- Carrier gas = Nitrogen 99.99% pure
- Gas Flow Rate = 40 cm^3/minute

Procedure: Equal volume (5 ml) of standard solution and honey samples solution were separately injected into the column. The responses for the bromopropylate and dibromobenzophenone were recorded. Concentration of BP and BBP in samples was calculated by external standard method (Formica, 1984).

RESULTS AND DISCUSSION

The effect of bromopropylate on the degree of infestation and honey yield in treated and untreated colonies is given in Table-3. The data indicated that the bromopropylate was effective against Varroa disease and as a result treated colonies yielded better.

The residual pesticides in samples, collected from the bee colonies treated with 4 fumigant strips of Folbex VA in 1990, were determined by GC and HPLC. The feed contained 0.01-0.08 mg bromopropylate/kg and 0.01-0.05 mg 4, 4-dibromobenzophenone/kg. The wax samples collected from apiaries at Islamabad had 9-23 mg bromopropylate /kg and 16-29 mg 4,4-dibromobenzophenone/kg, whereas, wax samples from Murree had 10-25 mg/kg bromopropylate and 18-30 mg/kg dibromobenzophenone.

The honey samples from Islamabad was found to contain 0.008-0.08 mg/kg bromopropylate and 0.003-0.02 mg/kg 4,4-dibromobenzophenone and from Murree had 0.004-0.08 mg/kg bromopropylate and 0.002-0.01 mg/kg dibromobenzophenone. This showed that the residual concentration were thus substantially higher in the wax than in the honey. The results of Hansen and Petersen (1988) were quite in agreement with the results of the present investigations.

The results from colonies treated with Folbex VA, for over two consecutive years, treated only once and those not treated at all are reported in table-4. The wax samples from the treated bee colonies had 34-102 mg/kg bromopropylate and 10-28 mg/kg 4,4-dibromobenzophenone. The amount of residue in honey was low as compared to wax. It had 0.006-0.09 mg/kg bromopropylate and 0.008-0.09 mg/kg 4,4-dibromobenzophenone. One of the four treated colonies died during the winter. Results showed that active ingredient as well as its residue remain in honey and wax even after 6 months (Table- 05) and these observations are inline with previous work of Lodesani *et al.*, 1992, Barbina-Taccheo *et al.*, 1988 who also observed that active ingredient continued to be present in brood chamber honey even a number of months after Treatment. They also observed that the concentration

Table: 01: Folbex va used and method of administration

Treatment	Folbex VA
Active ingredient	Bromopropylate
Method of administration	Fumigation
Number of treatment	4
Intervals	4
Dose	370 mg/colony
Period of study	August 1990 - August 1992

Table: 02 Recovery test

Product	***Amount added of Bromopropylate ppm***	***Standard % ± ppm***
Honey	0.004	
	0.020	92.5% ±2.5
	0.010	
Wax	0.20	
	0.10	83% ±4.0
	0.04	

Table: 3 Effect of bromopropylate on the degree of infestation and honey yield from treated and control colonies

	Degree of Infestation %			
	Prior to Treatment	After Treatment 3 Week	After Treatment 17 Week	Honey Yield (Kg)
Bromopropylate	7.0	0.0	1.0	7.0
	12.0	0.0	0.0	7.0
	23.0	0.0	3.2	5.0
	19.0	0.0	0.0	8.0
	24.0	0.0	3.0	3.0
	17.0	0.0	2.0	7.0
Average	17.0	0.0	1.5	6.2
	17.0	0.0	4.3	0.0
	13.0	35.0	52.0	0.0
	20.0	28.0	68.0	0.0
Control	4.0	9.0	45.0	0.0
	4.0	7.0	82.0	0.0
	35.0	39.0	36.0	0.0
	8.0	11.0	92.0	0.0
Average	14.4	18.4	54.2	0.0

Table 4: Residual pesticides in honey and wax in July 1992 following treatments carried out in autumn 12/08/90 and 12/08/91 (using 4 fumigation strips on each occasions).

Bee colony	No. of Times box treated	BY GC				BY HPLC			
		Honey		Wax		Honey		Wax	
		BP (ppm)	BBP (ppm)	BP (ppm)	BBP (ppm)	BP (ppm)	BBP (ppm)	BP (ppm)	BBP (ppm)
	2	0.090	0.040	41.0	17.0	0.1	N.D.	41.0	18.0
1	1	0.040	0.020	34.0	10.0	N.D.	N.D.	35.0	10.0
	0	0.006	0.008	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
	2	0.090	0.080	102.0	28.0	0.1	N.D.	100.0	27.0
2	1	0.050	0.050	60.0	20.0	N.D.	N.D.	62.0	21.0
	0	0.006	0.010	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
	2	0.090	0.090	42.0	19.0	0.1	0.1	42.0	21.0
3	1	0.040	0.060	37.0	14.0	N.D.	N.D.	37.0	15.0
	0	0.008	0.008	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.

BP= Bromopropylate
BBP= Dibromobenzophenone
N.D. = Not Detected

Table: 05 Residual Concentration of BP and BBP in Honey and Wax at different Time Intervals. After Folbex-VA Treatment on 12-08-90.

	March 91		May 91		July 91		August 91	
Product	**BP ***	**BBP ****	**BP ***	**BBP ****	**BP ***	**BBP ****	**BP ***	**BBP ****
	ppm		ppm		ppm		Ppm	
Honey	0.06	0.04	0.05	0.06	0.04	0.08	0.04	0.08
	0.07	0.05	0.06	0.06	0.05	0.08	0.04	0.08
Wax	28	0.08	26	0.08	24.0	0.08	22	0.08
	27	0.09	25	0.08	24.0	0.08	23	0.08

*****Bromopropylate, ****** 4,4-Dibromobenzophenone

curve for this matrix when compared with that for wax suggested the hypothesis that the active ingredient in the wax was transferred to the honey. The gradual and continuous active ingredient decay in bees was probably due to the progressive turnover of adult bees during the active season.

Conclusions: The chemical analysis of wax demonstrated that bromopropylate persisted in almost all the samples. We consider that future research projects should examine the negative implications of this, especially as regards the consumption of honey in the comb, the use of this wax in the cosmetics and pharmaceutical industries, and its reuse in the bee keeping field.

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