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PESTICIDE RESIDUE ANALYSIS IN HIVE PRODUCTS OF *APIS CERANA INDICA* F. FROM TAMIL NADU, INDIA

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ABSTRACT

Honey and pollen collected from experimental fields in Kutladampatti village and farmer's field from different locations of Tamil Nadu were analysed for the presence of pesticide residues in modified QuEChERs method which showed the coefficient of determination (R^2) of 0.9939, 0.9919, 0.9869, 0.9803, 0.9981, 0.9918 and 0.9824 for chlorpyrifos, fipronil, lambda cyhalothrin, profenofos, imidacloprid, flubendiamide and thiamethoxam respectively. The method adopted in this experiment resulted in LOQ of 0.0036, 0.0057, 0.0027, 0.0027, 0.0032, 0.0041 and 0.0044 µg/g and LOD of 0.0011, 0.0017, 0.0008, 0.0008, 0.0009, 0.0012 and 0.0013 for chlorpyrifos, fipronil, lambda cyhalothrin, profenophos, imidacloprid, flubendiamide and thiamethoxam respectively. Recovery of the method was recorded as 96.33% for chlorpyrifos spiked with 0.1 µg/ g in honey while fipronil 0.1 µg/ g spiked honey samples recorded the maximum recovery of 102.33%. Lambda cyhalothrin recorded a maximum recovery of 98.67% in honey when spiked with 0.1 µg/ g of pesticide whereas Imidacloprid recorded a maximum recovery of 98.42% in honey when spiked with 0.1 µg/g of pesticide. Profenofos recorded with a maximum recovery of 103.33% in pollen sample spiked with 0.1 µg/ g. Flubendiamide recorded a maximum recovery of 99.67% in honey when spiked with 0.5 µg/ g of pesticide and thiamethoxam recorded 101.67% recovery in 0.1 µg/g spiked honey sample. The modified QuEChERS method recorded reduced matrix effect compared to conventional QuEChERS method. No residue of insecticidal chemicals was found in any of the samples collected from the experimental plots and farmer's holdings as well.

Key words: Chemical pesticides, hive products, honey, pollen, pollinators, residue, QuEChERs method, coefficient of determination, chlorpyrifos, fipronil, lambda cyhalothrin, profenofos, imidacloprid, flubendiamide and thiamethoxam

Honey bees are eusocial insects and are close relatives to wasps and ants. They are found in every continent on earth except Antarctica. They belong to the family Apidae of Hymenopteran order, which have nearly eight well documented species viz., *Apis mellifera* Linnaeus*, Apis cerana indica* Fabricius*, Apis florea* Fabricius, *Apis andreniformis* F. Smith*, Apis koschevnikovi* Enderlein, *Apis dorsata* Fabricius*, Apis nigrocincta* F. Smith*, Apis nuluensis* Tingek, Koeniger and Koeniger and *Apis laboriosa* Smith (Ianson Price and Gruter, 2015). Bees are an important insect pollinator of many plant species. They pick up pollen and visit flowers for better pollination. One third of the crops rely exclusively on insect pollination only. They have a mutualistic relationship with flowering plants and hasten the coevolution. It is estimated that one third of human food supply depends on insect pollination (Jivan, 2013; Bhalchandra et al., 2014; Said et al., 2015).

The Italian bee, *A. mellifera* and the Indian bee, *A. cerana indica* are economically important, domesticated honey bee species (Ianson Price and Gruter, 2015). In India, honey is extracted from *A. dorsata, A. florea* and *Tetragonula iridipennis*. Even Though *A. mellifera* was introduced in India during 1962 to 1968; it was well acclimatized in North Indian condition. In southern part of India *A. cerana indica* is the prevailing bee species. It is also known as Asiatic honey bee or Eastern honey bees or Oriental honey bee (only found in Asia) as it has been distributed in China, Japan, India, Bangladesh, Nepal, Papua, New Guinea and Malaysia (Egelie et al., 2015; Theisen-Jones and Bienefeld, 2016).

Pollen and nectar from plants are the prime food

for bees. Bee bread, a mixture of pollen and honey, is given to young ones with royal jelly. Bee pasturage in the location is essential for better management of the hive and improving the yield (Sodre et al., 2007). Botanical and geographical origins of the honey are studied by pollen analysis. Qualitative analysis of pollen will provide important data for characterization of honey collected by them (Bogdanov and Gallman, 2008; Sodre et al., 2007).

Melissopalynological studies show the bee flora prevailed in the region which differs from region to region and season to season (Datta et al., 2008). Information on bee floral type, density and blooming period determine the honey flow and dearth period in the region (Kumar et al., 2015). Environmental factors viz., temperature, relative humidity, rainfall and wind speed also have influence on the foraging activity and brood development (Abou-Shaara et al., 2012).

Bees when unintentionally or intentionally exposed to excessive pesticide applications change the physiology and behaviour of bees which ultimately weakens the colony (Johnson, 2010; Barganska, 2014). Floral nectars are also found to be contaminated with pesticides which are also the source of bee poisoning (Levot et al., 2016). Nearly 150 pesticides have been detected in hive products and most of them are acaricides. The residue accumulation in the hive products were in increased gradient from honey, pollen and bee wax (Martel et al., 2018; Mullin et al., 2010). In hive products, organochlorine (Blasco et al., 2003), neonicotinoids (Woodcock et al., 2017), organophosphate (Blasco et al., 2003) and other groups (acaricides and fungicides) (Chauzat et al., 2006) were reported. Contaminated honey and pollen consumption will lead to health hazards to both honey bees and humans (Valdovinos-Flores et al., 2017). Pesticide exposed bees will express peculiar symptoms viz., dead bees found in front of hive, proboscis extension, unhooked wings and death of brood and queen (Yang et al., 2008).

MATERIALS AND METHODS

A field experiment was conducted at Kuttladampatti village, Vaadipatti block, Madurai district during 2018 – 2019 to study the pollination efficiency of *A. cerana indica*. The experiment was laid out in Randomized Block Design (RBD) with six treatments viz. T1: Crop caged with bee hives (4/ acre), T2: Hives placed in the field without cage (open condition), T3: Hives kept at 100 m distance from the field boundary, T4: Hives kept at 200 m distance from the field boundary, T5: Crop caged without bees and T6: Open pollination without any hives (control) with four replications. The laboratory study to analyze the presence of pesticide residues in hive products of *A. cerana indica* was carried out at the NADP-NABL laboratory of Department of Agricultural Entomology, Agricultural College and Research Institute, Madurai.

Honey and pollen samples were collected from colonies kept in experimental fields, crop caged with bee hives placed inside the field, crop with hives placed inside the field in open condition (without cages), crop with bee hives placed at 100 m and 200 m distances (T1 – T4) and from colonies in farmer's fields in Kuttladampatti (10.0962196 ^{o}N and 77.9981232 ^{o}E), Thooyaneri (10.0070 ^{o}N and 781808 ^{o}E), Velliankundram (10.0193 ^{o}N and 78.1853 ^{o}E) and T. Aandipatti (10.0598 ^{o}N and 77.9860 ^{o}E) villages of Madurai district, N. Poolampatti (10.6344 ^{o}N and 78.3110 ^{o}E), Elamanam (10.4982 ^{o}N and 78.3102 ^{o}E), Kanjanaickenpatti (10.4874 ^{o}N and 77.6204 ^{o}E) and Karadipatti (9.92911 ^{o}N and 78.0338 ^{o}E) villages of Tiruchirapalli district. Honey samples were collected after honey flow from the hives placed in the field in glass vials and stored at room temperature. Pollen samples were collected by cutting a piece of comb containing stored pollen using a disposable plastic knife and placed in a 1.5 ml eppendorf tube at -20 ^{o}C for storage. Both the samples were collected twice during the experiment, one at approximately 3 months after placement of colonies and the second at 35 days after the first harvest.

Samples were extracted and cleaned-up by QuEChERS method with some modifications for each sample and compared to the conventional QuEChERS method. Five grams of honey was weighed and poured into 50 ml centrifuge tubes to which 7.5 ml water, 10 ml of 1% acetic acid in acetonitrile, 6 g anhydrous Magnesium Sulphate ($MgSO_4$) and 1 g Sodium Chloride (NaCl) were added. It was homogenized immediately by vortexing and centrifuged at 3000 rpm for five minutes. Six ml of the supernatant was transferred into another 15 ml centrifuge tube containing 150 mg of primary secondary amine (PSA) and 450 mg $MgSO_4$ (50 mg of PSA and 150 mg of $MgSO_4$ in conventional QuEChERS method). The mixture was vortexed again and centrifuged at 3000 rpm for five minutes. Finally, 4 ml of supernatant was transferred to a turbovap tube and concentrated to dryness in turbovap LV with a gentle stream of nitrogen at 40 °C until dried (Tong et al., 2016).

Hexane (mobile phase of GC-MS/MS) - 2 ml was added and hexane layer was filtered through a 0.22 micro filter membrane into the autosampler vials of 1.5 ml and stored at -20 °C or introduced into a GC-MS/MS auto sampler for analysis (Calatayud-Vernich et al., 2016).

One gram of pollen sample was weighed and placed in a 50 ml centrifuge tube. Four ml of water was added and shaken to blend the pollen. Glass beads and 10 ml of 1% acetic acid mixture in acetonitrile were added to the blend and vortexed for 2 minutes. The mixture was added with 0.5 g of $MgSO_4$ and 2g of anhydrous sodium Acetate (NaOAc) and vortexed for 2 minute and centrifuged at 3500 rpm for five minutes at -20 °C. Acetonitrile fraction 6 ml was transferred to a 15 ml centrifuge tube containing 150 mg of PSA, 3.75 mg of Graphitized carbon black (GCB) and 450 mg of $MgSO_4$ salt (50 mg of PSA and 150 mg of $MgSO_4$ in conventional QuEChERS method), then tube was vortexed for two minutes and then centrifuged at 3500 rpm for three minutes at -20 °C. Four ml of supernatant was transferred to a turbovap tube and concentrated in turbovap LV with gentle stream of nitrogen at 40 °C until dried. Hexane (mobile phase of GC-MS/MS) 2 ml was added and the hexane layer was filtered through a 0.22 micro filter membrane into the autosampler vials of 1.5 ml and stored at -20 °C or introduced into a GC-MS/MS auto sampler for analysis (Tong et al., 2016).

Profenofos, lambda cyhalothrin, thiomethoxam, fipronil, flubendiamide, imidacloprid and chlorpyriphos were the most commonly used pesticides by the farmers in the study areas where the honey and pollen samples were collected. Hence, the samples collected were tested for the presence of residues of above listed chemicals in honey and pollen collected from the hives kept in the field. The reference standards pesticide residues to be analyzed viz., thiamethoxam (99.7% purity), lamda cyhalothrin (98.7% purity), profenofos (97.6% purity), fipronil (96.7% purity), chlorpyriphos (99.3% purity) were purchased from Sigma Aldrich, Bangalore, India. Primary Secondary Amine (PSA-Bondesil 100 g) and Graphitized carbon block (GCB) were purchased from Agilent Technologies, USA and used for analysis. HPLC grade Acetonitrile, hexane, analytical grade sodium chloride (NaCl), anhydrous sodium acetate, anhydrous magnesium sulphate and acetic acid were also obtained from Merck (Mumbai, India) and utilized.

Stock solution of 1000 ppm of individual pesticides were prepared separately and ide intermediate stock solution of 100 and 10 ppm were prepared by transferring one ml from each pesticide solution to a 10 ml graduated test tube and diluting to volume with hexane. Working standard of individual pesticides (1 ppb to 2 ppm) was prepared by diluting the intermediate stock solution. These working standards were used to find out the retention time of the compounds and for quantitative determination of residues in samples. All the stock and working standard solutions were stored in the refrigerator at -20 °C until further use.

Pesticide residues present in honey, pollen, bee and wax were identified by using gas chromatograph coupled with a mass spectrometer (GC-MS/MS) (GC 2010 plus, GCMS - TQ 8040 SHIMADZU). Sample (1 µl) was injected (spit less mode) through an autosampler (Shimadzu ADL 20S) and an auto injector (AOC 20i) into the capillary column (30m x 0.25 mm, id, 0.25µm) for separating the compound. Constant flow rate of the carrier gas helium was maintained at 1.40 ml/min. Injector temperature of 250 °C, ion-source temperature 230 °C and interface temperature 250 °C were fixed in the system as per the protocol. The oven temperature was programmed from 70 °C for 0 min and then increased to 100 °C @ 10 °C/ min and hold for one min further increased to 220 °C @ 4 °C/ min and hold for four min again the temperature was increased to 280 °C by the rate of 5 °C/ min and hold for 15 min. Mass spectra were taken at 70 eV with a scan interval of 0.5 seconds and fragments from 50 to 600 Da and the system was operating in electron impact mode.

Interpretation on mass spectrum GC-MS was done by referring to the database of National Institute Standard and Technology (NIST 14) and PESTEI_3. The spectrum of the unknown component was compared with the spectrum of the known components stored in the library. The name, molecular weight and structure of the components of the test materials were ascertained in the standard chromatograms obtained.

Various concentrations of respective standards of the identified pesticide compounds were fed into the GC-MS system by following the same procedure as done for the samples and calibration curves were developed for the identified pesticides. Concentrations of pesticide in the samples were quantified by using the calibration curves.

RESULTS AND DISCUSSION

Pesticides residues in honey and pollen

Honey and pollen samples collected from the

experimental field and farmer's bitter gourd fields from different locations of Tiruchirapalli and Madurai districts were analyzed for the presence of pesticide residues. Efficiency of analytical methods followed was accessed by the following test.

Linearity

Calibration curve was developed by feeding five different concentrations of the following pesticides viz., chlorpyrifos, fipronil, lambda cyhalothrin, profenofos, imidacloprid, flubendiamide and thiamethoxam ranging from 1 to 50 µg/g. Coefficient of determination (R^2) was found from the calibration curve as 0.9939, 0.9919, 0.9869, 0.9803, 0.9981, 0.9918 and 0.9824 for the above said pesticides respectively (Table 1).

Sensitivity of the method

Sensitivity of the method was determined by the limit of detection (LOD) and limit of quantification (LOQ). The method adopted in this experiment resulted in LOQ of 0.0036, 0.0057, 0.0027, 0.0027, 0.0032, 0.0041 and 0.0044 µg/ g and LOD of 0.0011, 0.0017, 0.0008, 0.0008, 0.0009, 0.0012 and 0.0013 for chlorpyrifos, fipronil, lambda cyhalothrin, profenophos, imidacloprid, flubendiamide and thiamethoxam respectively (Table 1).

Recovery (%) and matrix effect

Recovery of the pesticides was found by spiking the samples with 0.1, 0.25 and 0.5 µg/ g of respective pesticide standards. Chlorpyrifos resulted in 96.33% of recovery in honey samples which spiked with 0.1 µg/ g. Minimum per cent recovery (89.00%) was observed in pollen samples spiked with 0.5 µg/ g of chlorpyrifos (Table 2).

Thiamethoxam recorded maximum (101.67%) recovery in 0.1 µg/g spiked honey samples and minimum (89.00%) in pollen samples spiked with 0.5µg/ g. A maximum recovery of 98.42% in honey samples spiked with 0.1µg/ g of and minimum (90.56%) in pollen samples spiked with 0.5 µg/ g of imidacloprid and in flubendamide maximum recovery was recorded when spiked with 0.5 µg/ g of standard and minimum recovery was recorded when spiked with 0.5 µg/ g of standard (95.33) in pollen samples.

Lambda cyhalothrin recorded maximum recovery (%) (98.99) in honey spiked sample with 0.5 µg/ g of pesticide and minimum (95.67) in pollen spiked sample with 0.25 µg/ g. Profenofos recorded maximum recovery in pollen sample (103.33%) in pollen sample spiked with 0.1 µg/ g and minimum (97.33%) with 0.25 µg/ g in the honey sample. Fipronil 0.1 µg/ g spiked honey samples recorded maximum recovery of 102.33% and minimum of 90.33% when the pollen samples spiked with 0.1 µg/ g of fipronil (Table 2).

Matrix effect found for the modified QuEChERS method was maximum (-6.8%) in the honey matrix with lambda cyhalothrin. In pollen samples the matrix effect of -10.27% was observed in chlopyrifos. The modified QuEChERS method recorded reduced matrix effect compared to conventional QuEChERS method (Table 3).

Pesticide residues in samples

Different matrixes viz., honey and pollen collected from the experimental field and different locations (farmers' fields) were subjected to modified QuEChERS method of multi-residue pesticide extraction and clean-up process and identification and estimation were done by using GC-MS/MS. The samples revealed no residues of the above mentioned standard pesticides viz., chlorpyrifos, fipronil, lambda cyhalothrin, profenophos, imidacloprid, flubendiamide and thiamethoxam.

Bee colony or pollinator losses are mainly by man-made unpleasant environment viz., habitat destruction, use of agrochemicals and climate changes (Pettis et al., 2012). Bees are frequently exposed to the pesticides

Table 1. Standard curve parameters, LOD and LOQ

S.No.	Pesticide name	Upto 50 ppb	R^2 Values	LOD (mg kg^{-1})	LOQ (mg kg^{-1})
1.	Chlorpyrifos	y = 790.9x - 528.67	0.9939	0.0011	0.0036
2.	Fipronil	y = 146.25x + 193.18	0.9919	0.0017	0.0057
3.	Lambda cyhalothrin	y = 263.97x - 284.12	0.9869	0.0008	0.0027
4.	Profenofos	y = 189.54x + 466.75	0.9803	0.0008	0.0027
5.	Thiamethoxam	y = 63.094x + 140.1	0.9824	0.0013	0.0044
6.	Imidacloprid	y=328.7x -478.9	0.9981	0.0009	0.0032
7.	Flubendiamide	y=296.0x -431.5	0.9918	0.0012	0.0041

Table 2. Recovery (%) of detected pesticides in honey and pollen

Pesticides	Spiking level (µg/ g)	Honey*	Pollen*
Chlorpyrifos	0.1	96.33	91.33
	0.25	95.89	89.67
	0.5	92.67	89.00
Lambda cyhalothrin	0.1	98.67	96.33
	0.25	96. 00	95.67
	0.5	98.99	96.33
Thiamethoxam	0.1	101.67	94.33
	0.25	98.00	92.33
	0.5	98.06	89.00
Imidacloprid	0.1	98.42	94.63
	0.25	97.33	93.52
	0.5	91.02	90.56
Flubendiamide	0.1	95.57	96.33
	0.25	98.22	97.83
	0.5	99.67	95.33
Profenofos	0.1	99.67	103.33
	0.25	97.33	99.00
	0.5	100.79	97.67
Fipronil	0.1	102.33	96.33
	0.25	98.67	95.67
	0.5	99.33	93.33

*Values are mean of three replications

Table 3. Comparison of conventional and modified QuEChERS method by recovery (%) and matrix effect in honey and pollen

Pesticides	Recovery (%)				Matrix effect (%)			
	Honey*		Pollen*		Honey*		Pollen*	
	Conventional	Modified	Conventional	Modified	Conventional	Modified	Conventional	Modified
Chlorpyrifos	92.33	94.96	89.25	98.11	-8.25	-6.74	-3.86	-10.27
Fipronil	99.67	100.11	91.25	94.66	-1.25	-0.73	-5.36	-7.24
Lambda cyhalothrin	96.33	98.83	95.12	99.10	-7.69	-6.8	-2.18	-2.45
Profenofos	97.27	99.26	98.12	95.66	-4.68	-3.84	-5.09	-2.68
Thiamethoxam	98.36	99.24	87.36	98.22	-4.25	-3.47	-13.54	-1.78
Flubendiamide	97.56	99.67	91.23	95.66	-4.35	-3.62	-6.23	-5.24
Imidacloprid	95.67	98.33	88.76	94.36	-4.53	-3.73	-7.53	-3.28

*Values are mean of three replications

in different ways viz., foraging on pesticide applied crops, direct exposure to the pesticide during spraying activity, pesticide drift, hive management practices etc. (Abrol, 2009; Simon-Delso et al., 2017). Pesticide poisoning to bee occur by contact action or by oral or sometimes through olfaction action. It will lead to death of the forager, brood and queen and further leads to abnormalities in the behaviour and physiology (Levot et al., 2015; Williams et al., 2015). Hence the pesticide level estimation is of greater importance.

The efficiency of the analytical method is assessed by working out linearity, sensitivity and recovery (accuracy). In the present study, the recovery ranged from 89.00% to 96.33% in chlorpyrifos, 93.33% to 102.33% in fipronil, 95.67% to 98.99% in lambda cyhalothrin, 97.33% to 103.33% in profenofos, 95.33 to 99.67% in flubendiamide, 90.56 to 98.42% in imidacloprid and 89.00% to 101.67% in thiamethoxam. The method adopted in this experiment resulted in good LOQ and LOD values for chlorpyrifos (0.0036

and 0.0011 µg/ g), fipronil (0.0057 and 0.0017 µg/ g), lambda cyhalothrin and profenofos (0.0027 and 0.0008 µg/ g), flubendiamide (0.0041 and 0.0012 µg/ g), imidacloprid (0.0032 and 0.0009 µg/ g) and thiamethoxam (0.0044 and 0.0013 µg/ g). The methods and findings are comparable with the work of Blasco et al., (2003). They adopted methods for extraction and quantification of pesticide residues in honey samples resulting in the presence of organochlorine, carbamate and organophosphorus pesticide residues which had recovery of 73 to 98% in spiked samples and 0.003 to 0.1 mg kg^{-1} as limits of quantification.

Pesticide residue methods developed in Egypt also had 84.20 to 120.30% of recovery and 0.001-0.168 mg/ kg of limits of detection (LOD) (Eissa et al., 2014). The LOD of chlorpyrifos and profenofos was 0.14 and 5.4 ng/ g and 0.31 and 2.3 ng/ g respectively in honey samples and respective recovery of 92.4% and 109.6% (Al Naggar et al., 2015). New modified multi-residue method developed for pollen samples had a range of recovery between 60% and 136% with less than 30% relative standard deviations (RSDs) (Tong et al., 2016). Honey samples collected from the insectary of an Agricultural college contained residues less than permitted level and hence safe for human consumption according to Hemalatha et al., 2018.

Another method of analysis obtained 69.4% to 91.8% of recovery and correlation coefficient was 0.97 for neonicotinoids (Al Naggar et al., 2015). The coefficient of determination observed in this study showed 0.9939, 0.9919, 0.9869, 0.9803, 0.9981, 0.9824 and 0.9824 for chlorpyrifos, fipronil, lambda cyhalothrin, profenofos, flubendiamide, imidacloprid and thiamethoxam respectively. Residue analysis in honey and hive matrices are difficult due to the complexity of the matrix (Orso et al., 2014).

The matrix effect of honey and pollen are presented in Table 3. Linearity range and LOD and LOQ of chlorpyrifos, profenofos and thiamethoxam obtained in our result were similar to this data of linearity of 0.9995, 0.9935 and 0.9975 for chlorpyrifos, profenofos and thiamethoxam respectively and LOD and LOQ of 0.0638 and 0.1914; 0.0189 and 0.0568; 0.0028 and 0.0084 ng/ g for chlorpyrifos, profenofos and thiamethoxam (Tong et al., 2016). The residue studies conducted by (Orso et al., 2014) are in line with the present investigation that the provides viz., fipronil and lambda cyhalothrin had an LOD and LOQ of 6, 20 µg/kg and linearity of 0.9873 and 0.9532, respectively.

The matrix effect of the modified QuEChERS method over conventional QuEChERS method showed a maximum difference of 2.11% in the matrix of chlorpyrifos and minimum (0.42%) difference were found in pollen samples of profenofos. This shows the modified QuEChERS enhance the pesticide extraction from hive matrices than conventional QuEChERS method. This variation is due to the increased level of PSA (150 mg) and $MgSO_4$ (150 mg) in modified methods which will remove the sugars, fatty acids etc.

Our analysis for pesticide residues in honey and pollen revealed no detectable (ND) level of pesticide residues in both the matrices. The result obtained is in close accordance to that of Hemalatha et al. (2018) who reported very low levels of pesticide residue when analyzed by the QuEChERS method in honey samples collected from Madurai area. This is also in line with (Renvall, 1977) who reported Swedish honey samples are free from pesticide residues. Beck (1983) also reported that Danish honey samples are safer (without residues of pesticides) to consumers while the imported honey samples detected 0.005 mg/kg of OP pesticides. But our results are against the reports of (Ruiz-Toledo et al., 2018). They confirmed the presence of organochlorine pesticide residue in honey and pollen samples from apiaries of *A. mellifera*.

Al Naggar et al. (2015) reported that pollen samples collected from the hives had the highest concentration of OP compounds, which is against our findings. When the orchards of dandelion and apple were sprayed with deltamethrin and mancozeb the nectar was polluted with its residues. Residual remains of imidacloprid and its metabolites were found to be present below detection limit (10 mg/ kg) in the nectar of sunflower (Ambolet et al., 1999). Gregore and Bozi (2004) reported that pollen samples acquired from sunflower had imidacloprid residues at 3.9 µg/ kg.

From the above statements we conclude that usage of organic pesticides is relatively safer when compared with synthetic chemical insecticides for insect pest control in the bitter gourd ecosystem. This will also help in conserving solitary as well as managed bee colonies in farmer's holdings. This in turn improves the farmer's health and income from increased crop yield, cost spent on purchasing synthetic chemical insecticides and additional income from honey harvest and other by products from honey bees. Thus, residue free produce (vegetables) will be made available to the world.

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