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RESEARCH ARTICLE

Detection of Carbamates in Honeybees and Bee-products of Karnataka State.

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Abstract

Honeybee species like *A. cerana*, *A. dorsata* and *A. florea* are well distributed in Karnataka state. Monitoring of pesticides by honeybees and their products as biological indicators is of great importance because of their toxicity to human beings and potential hazard. The monitoring of pesticide residues in honey helps to assess the potential risk to consumers' health, providing information on the pesticides used in the field crops. The indiscriminate use of pesticides in the agro ecosystems is the major environmental health concern. In the present investigation, 253 samples of honeybees, honey, pollen, brood and wax were collected in different zones of Karnataka during 2010-2013. The concentration of three pesticides like carbaryl carbofuran and methomyl belonging to carbamate group was determined through HPLC. Twenty samples were contaminated of which carbofuran and methomyl were detected in 16 and four samples respectively. Carbamates have one of the highest acute toxicities to humans among pesticides widely used on agri-horticultural crops due to its activity as a cholinesterase inhibitor and neurotoxicity. The study revealed that the agro and forest ecosystems of Karnataka has not been contaminated with high degree of pesticide residues as indicated by the bees and bee products except very low concentration in few areas of the state. There is an urgent need to monitor bee pollinator health as they are indispensible organism in the ecosystem and contributing for increased crop production and food security.

Introduction

Pesticides constitute an important input in modern agriculture and are used worldwide in the control of several pests to protect crops in order to increase agricultural productivity. However, uncontrolled application may cause the contamination of environment, animal species and human beings (Al-Waili et al., 2012). The excessive use of pesticides has become hazardous to the beneficial insects and environment (Johansen, 1979; Abrol, 2000). Among all useful insects the organism most widely affected by pesticide toxicity is the honeybee (Durdane, 2004).

Honeybee species like *Apis cerana*, *Apis dorsata* and *Apis florea* are distributed in almost all districts of Karnataka state. Bees collect nectar from flowers and convert it into honey, which is a natural sweet food having high nutritional and medicinal properties. Rapidly changing agricultural practices and decline in wild flora have caused an increasingly dependence of honeybees on various cultivated crops for forage. A pesticide having toxic properties when applied in an incorrect way affects the non-target organisms like honeybees and also may result in the contamination of terrestrial habitats. Honeybees are considered as efficient pollinators in the terrestrial ecosystem. Monitoring of pesticides by honeybees and their products as biological indicators is of great importance because of their toxicity to human beings and potential hazard (Dobsikova, 2003). Pesticides residues have been shown to cause genetic mutation and cellular degradation. In addition to the public health problems, the presence of pesticides in bee products decreases its quality (Chauzat et al., 2006; Al-Waili et al., 2012).

Honeybees and bee products as tool for monitoring environmental pollution has been studied in many parts of the world such as Portugal and Spain (Blasco et al., 2003); Italy (Ghini et al., 2004); France (Chauzat et al., 2006, 2007, 2009); and Turkey (Yavuz et al., 2010). Honeybees are free flying organisms which are known to measure the environmental contamination of almost all environmental sectors like soil, vegetation, water and air, providing numerous indicators through foraging for each season. The effectiveness of the insect as an ecological detector is founded upon several ethological features such as high rate of reproduction, great mobility, large flying range and numerous flower inspections per day (Chauzat et al., 2006).

Honey is usually consumed by most of the people especially by children and ill persons due to its high nutritional and medicinal properties (Yavuz et al., 2010). Quality of honey is directly related to its floral origin and its region of production (Koc et al., 2008). There are three main purposes for monitoring bee products: consumer health protection, International commercial competition and better product quality (Chauzat et al., 2006). Pesticide residues in the environment and on-food have multi-dimensional and complex features (Durdane, 2004).

Pesticide belongs to different chemical classes but major ones are organochlorines, organophosphates, carbamates and pyrethroids. Carbamates are comparatively less toxic but effective as insecticides, fungicides, herbicides and nematocides and have been used since the beginning of 1960s (Koc et al., 2008). Carbamate pesticides exert their toxic action by inhibiting the enzyme acetylcholinesterase (AChE) and this enzyme is responsible for the hydrolysis of acetylcholine (ACh), a neurotransmitter that conducts nerve impulses across neuromuscular junctions in the nervous system. Inhibition of this enzyme causes accumulation of Ach leading to generalised cholinergic action resulting in rapid paralysis, respiratory failure and death (Podolska and Napierska, 2006). AChE inhibition by organophosphates are generally irreversible while inhibition by carbamates is reversible, hence, carbamates are less toxic than organophosphates and the persistent organochlorines (Ogah and Coker, 2012). During the last few years, following the general trend of using what nature is directly offering, bee products got an increasing importance as essential natural resources in promoting healthy food and a new therapy absolutely free from the side effects of the chemical medicines. However, the tough market competition on honey imposes extra guarantee conditions that can be ensured only complying to high quality conditions. Thus, honey should not contain toxic substances in an amount, which may constitute a hazard to health. There is tremendous scope in the present study, as the proposed work aims to determine the level of pesticide residues in the honeybees and hive products.

Carbofuran (2,3-dihydro-2,2-dimethylbenzofuran- 7-yl methylcarbamate) is a broad-spectrum N-methyl carbamate, a systemic insecticide and nematicide registered for control of soil and foliar pests on a variety of field, fruit and vegetable crops. As a result of its widespread use, carbofuran has been detected in ground, surface and rain waters, in soils, air, foods and wildlife (EPA 2006). It was first registered in the United States in 1969, through an agreement between EPA and the technical registrant in 1991; granular carbofuran has been limited to the sale of 2,500 lbs of active ingredient per year in the U.S. since 1994, for use only on certain crops. Today granular carbofuran is limited to use on spinach grown for seed, pine seedlings, bananas (in Hawaii only), and cucurbits only. Carbofuran is classified as a restricted use pesticide (EPA 2006). Carbamates have one of the highest acute toxicities to humans among pesticides widely used on agri-horticultural crops due to its activity as a cholinesterase inhibitor and neurotoxicity. The increasing use of carbamate pesticides poses a risk to apiculture and human environment (Koc et al., 2008).

Methomyl also was first registered in the United States in October, 1968. All Methomyl products, except the 1% bait formulations, are classified as restricted use pesticides. Methomyl photolyzes quickly in water but more slowly in soils. It is moderately stable to aerobic soil metabolism but degrades more rapidly under anaerobic conditions. Risks to aquatic invertebrates from exposure to methomyl are likely to occur wherever methomyl is used. Accumulation of methomyl from repeated applications contributes to the chronic risks (EPA 1998).

The aim of this study was the application of honeybees and bee products like honey, pollen and wax as biological indicators of detecting carbamate pesticides. The occurrence and distribution of three carbamate pesticides namely carbofuran, carbaryl and methomyl in bee and bee products in Karnataka from 2010 to 2013 has been presented here. In the present investigation, 253 samples of honeybees, honey, pollen, brood and wax were collected for determination of pesticide residues.

Materials and Methods

Samples of honeybees and their products like honey, pollen, wax and brood were collected directly from the domesticated bee hives, *A. cerana* and wild bee, *A. dorsata*, from different locations of Karnataka, India (19° N,11° S,78° 48′E, 74° 20′ W). The samples were then analyzed for the presence of carbamates like carbaryl, carbofuran and methomyl. The honey samples collected were stored in glass bottles under normal room temperature and the samples of honeybees, brood, pollen and wax were stored in vials at -20°C until analyzed.

Five grams of honey samples were blended with 50 mL of 35% acetone and extracted thrice with 20 mL methylene chloride using liquid-liquid partitioning. The organic phase was pooled together and finally concentrated using rotary evaporator at 40°C.

Three grams of honeybees/brood samples were homogenized with 20 mL acetone, followed by filtering through a layer of celite 545. 50 mL of 1% ammonium chloride and 2% orthophosphoric acid were added and allowed to stand for 30 minutes and filtered. The sample was diluted to 100 mL with 2% sodium chloride and extracted twice with 50 mL methylene chloride. The combined organic phase was evaporated to dryness in a rotary evaporator at 35°C and the residue was redissolved in 2 ml of acetone (Ghini et al., 2004). Florisil column was prepared using 11mm column packed with 10 cm of activated florisil placed between 1cm of anhydrous sodium sulphate. The column was pre-conditioned with 20 ml of hexane followed by loading of the extracts of honey, honeybees and brood respectively and eluted with 100 ml each of 6%, 15% and 50% hexane in methylene chloride. The eluates were collected and evaporated to dryness in a rotary evaporator at 40°C, further the residue was re-dissolved in 1 mL of methanol for HPLC injections.

Two grams of beewax were dissolved in 40 mL of hexane by ultrasound shaking and gentle heating at 40°C. The sample was centrifuged for 15 minutes at 3500 rpm after cooling. The supernatant fraction was collected and concentrated followed by addition of 6 mL of hexane and 20 mL of hexane and acetonitrile (1: 9). The sample solution was extracted twice and the acetonitrile phase was collected, pooled and concentrated using rotary evaporator. The extract was cleaned using florisil column, conditioned by successive elutions of 6 mL methanol, 6 mL water and 6 mL acetonitrile. The extract was then eluted with 15 mL of acetonitrile and water (5: 1) (Chauzat and Faucon, 2007). The solution was dried in a rotary evaporator and the residue was dissolved in 1 mL of methanol for HPLC injections. One gram of pollen sample was centrifuged for 4 minutes at 3500 rpm with 50 mL acetone, petroleum ether and dichloromethane in a ratio of 1:2:2. The liquid phase was decanted, evaporated and dried in a rotary evaporator. The residue was dissolved in 8 mL of cyclohexane and ethyl acetate mixture in a ratio of 1:1 and cleaned on a gel permeation chromatograph. (Skerl et al., 2009).

The methanol extracts of the samples were analyzed using HPLC-20AD with detector UV-Vis filter with column C-18 (250 mm× 466 mm×5 µl). The mobile phase for the three pesticides carbaryl, carbofuran and methomyl was acetonitrile : water (50:50). The detector wavelength was 220 nm and the injection volume was 20 µl with a flow rate of 0.8 ml/min for carbamates.

Results and Discussion

In the present investigation 253 samples of honeybees and hive product such as honey, pollen, brood and wax were collected throughout Karnataka. The type of sample matrix collected and the pesticides detected are shown in Table 2. Twenty samples were found to be contaminated with carbamates of which carbofuran and methomyl were detected in 16 and 4 samples respectively with concentration of carbofuran ranging from 0.137µg/kg to 6.3 mg/kg and that of methomyl ranging from 0.05 mg/kg to 3.64 mg/kg (Table 3).

Table 1: List of the Pesticides analyzed.

Pesticides	Pesticide Group	Toxicity	Purpose of Use	Retention Time(min)	LOD (ppb)	LOQ (ppm)
Carbaryl	Carbamates	Highly Toxic	Insecticides	7.170	50	10
Carbofuran	Carbamates	Highly Toxic	Insecticides	7.026	100	10
Methomyl	Carbamates	Minimal & Hazardous	Insecticides	10.060	100	10

LOD: Limit of detection (in ppb); LOQ: Limit of quantification (in ppm)

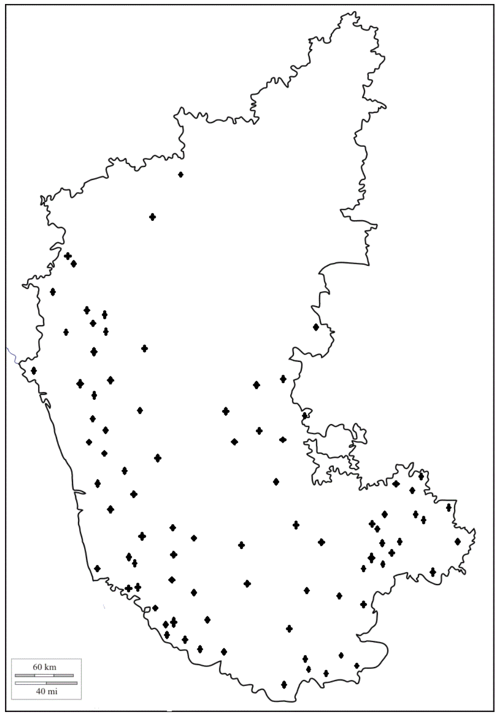

Fig. 1: Sample collection locations in Karnataka State.

Table 1: Details of the type of sample matrix and pesticides detected.

Type of sample	Collected	Detected	Pesticides detected		
			Carbofuran	Carbaryl	Methomyl
Honeybees	29	3	3	ND	ND
Honey	136	3	1	ND	2
Pollen	40	7	5	ND	2
Brood	14	2	2	ND	ND
Wax	34	5	5	ND	ND

ND: Not Detected.

Table 2: Concentration of pesticide residues in the collected samples.

Pesticides	Number of positive sample (frequency)	Residue concentration	
		Min. (mg/Kg)	Max. (mg/Kg)
Carboryl	0	-	-
Carbofuran	16	0.000137	6.3
Methomyl	4	0.05	3.64

Five pollen and wax samples each were contaminated with carbofuran. Pollen sample from Yellapura exhibited high carbofuran contamination (3.7 mg/kg) and wax sample from Shivpura contained very high (6.3 mg/kg) carbofuran in the analysed samples. Carbofuran was also reported in the pollen samples from France with an average value of 14.0µg/kg (Chauzat et al., 2006). Carbofuran was detected in two brood and three honeybee samples in the present study having concentration ranging from 8.55µg/kg to 4.77 mg/kg and 2.16µg/kg to 1.18 mg/kg respectively, similar detection was also found in honeybee samples in the province of Bologna, Italy with concentration ranging from 0.009 to 0.669 mg/kg (Ghini et al., 2004). Only one honey sample was found to be contaminated with carbofuran with high concentration of 6.2 mg/kg. Carbofuran was also reported in the honey samples from Spain and Portuguese at concentration ranging from 0.02 to 0.645mg/kg and 0.01 to 0.11mg/kg respectively (Blasco et al., 2003). In the present study Methomyl was detected in four samples, two honey and two pollen samples and its concentration ranged from 0.05mg/kg to 3.64 mg/kg.

Poisoning of bee pollinators is a serious adverse effect of insecticide use, which leads to a decrease in the insect populations, reduction of honey yields, crop production, presence of insecticide residues in food and ultimately to a significant loss of a beekeeper’s income (Chauzat et al., 2006; Al-Waili et al., 2012).

Systemic introduction of pesticides into nectar and pollen may have direct consequences for honey bee health and ultimately lead to pesticide contamination of honey-containing food. The effects of pesticides on human health are harmful based on the toxicity of the chemical and the length and magnitude of exposure. Aberrantly, farmers and their families have the greatest exposure to agricultural pesticides. Since children are most susceptible and sensitive to pesticides the chemicals have the ability to bioaccumulate and biomagnify and can bio concentrate in the body over time. Effect of exposure to pesticides ranges from mild skin irritation to birth defects, tumors, genetic changes, blood and nerve disorders, endocrine disruption and even coma or death (Al-Waili et al., 2012).

Thus, the study revealed that the agro and forest ecosystems of Karnataka has not been contaminated with high degree of carbofuran and methomyl residues as indicated by the bees and bee products except very low concentration in few areas of the state. Although there is an urgent need to monitor pesticide contamination in the ecosystem in order to protect the bee pollinators as they are indispensible organisms contributing for increased crop production and food security through pollination.

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