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Challenges of Limiting Pesticide Residues in Fresh Vegetables: The Indian Experience

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Abstract

India is the second largest producer of vegetables after China, and accounts for 13.4% of world production. Surveys carried out by institutions spread throughout the country indicate that 50-70% of vegetables are contaminated with insecticide residues. Rapid, inexpensive and sensitive methods such as pesticide finger printing technique and enzyme-linked immunosorbent assay developed at CFTRI, Mysore, together with GC analysis revealed that in most cases the residue burden was less than the maximum residue limits (MRLs), with some exceptions. Frequently DDT and HCH residues were detected. Evidence suggests that chemical residues in vegetables are due to pick-up from the contaminated soil by plants and migration to edible parts. Bacterial cultures capable of degrading HCH have been isolated at CFTRI and successfully deployed as "Agrocure" to combat HCH residues through accelerated degradation of the chemical in soil. Pseudomonas ptm+ strain developed at CFTRI produced an extracellular surfactant, which was exploited as a cleaning agent "Baxeklen" to dislodge pesticide residues in vegetables. Bioremediation and biocleaning are the redeeming technologies useful to limit pesticide residues in vegetables.

Introduction

India has a wide variety of climate and soils on which a range of vegetable crops can be grown. During the last two decades considerable emphasis has been laid on production of these crops in our country, and vegetable exports have been stepped up (CHADDA, 2000). In vegetable production, India is the second largest producer next only to China with an annual production of 81 million t from 5.12 million ha of land (Table I). However, the development of the export market is hindered by concerns about chemical residues and inadequate monitoring.

Post harvest losses are also a problem, related to lack of adequate harvesting equipment, absence of collection centers in major producing areas, as well as suitable containers, commercial storage plants and cold chains. Considerable losses also occur during transport mainly due to lack of proper packaging and handling technology.

Research on post harvest management of fruits and vegetables is being carried out in a number of laboratories spread all over the country as shown in Table II.

The Pesticide Burden in Vegetables

Pesticide Use Pattern in India

India currently uses about 60 000 t of pesticides, a decline of one-third since 5 years ago. Worldwide, there has been a 44% increase in the use of herbicides over the past decade, with a concomitant reduction in insecticides by 30%. Since insecticides still account for 70% of total pesticide use in India (Table III), it is likely that insecticide residues will continue to be an issue for at least another decade, even if the declining trend in use continues. (KARANTH et al., 1999b)

The Problem

Over the past few years we have been realizing the counterproductive effects of pesticide use, such as pest resistance to farm chemicals. In addition, long persistence of some agrochemicals in the environment sets in a series of undesirable effects through contamination of food and feed. The 30 million non-target bioforms, so far safe in the cradle of nature, are rocked with threat of extinction. Their numbers are reducing. Bioaccumulation of pesticides and biomagnification processes have become the weak links in the food chain.

Notorious Chemicals

Among the pesticides that have acquired notoriety, DDT and BHC (=HCH, =Gammaxane, =Lindane) are particularly important. In India DDT and BHC were the two major chemicals used in agriculture and public health programs. Although now partially banned, they are still very much in use because of their wide spectrum of activity and ready availability at low cost. Our biggest concern is that these molecules are stable in the environment. More than 600 000 t of HCH (Hexacholorocyclohexane) and 270 000 t of DDT have been added to the environment since their respective introductions in 1949 and 1952. It is suspected that most of our water bodies and soils are contaminated with these chemicals or with their degradation products (KRISHNA MURTHY, 1984). DDT persists with a half life of about 10 years, with only minor conversion to p, p' DDT, DDE, TDE, o, p DDT, etc. The uptake and accumulation of DDT and its metabolites in different plants and animal species varies considerably.

Pesticide Monitoring in Market Basket Samples

Vegetables sold in Mysore market are mainly grown in villages around the city and are harvested and sold fresh. As many as 514 samples collected over 4 different seasons were checked for the residue burden, the profile for which is given in Table IV. The data clearly indicate the presence of HCH and DDT residues, the 2 major farm chemicals present in the soil that have migrated in to the vegetables. HCH contamination ranged from 15 – 70% of the samples, while DDT was found in 11 – 28% (SHARADA, 1988). However the concentrations measured varied from traces to about 3ppm (less than the MRL value of 5ppm set by FAO/WHO (1986) and PFA (1954)). Similar surveys were carried out in different states of the country (SETH et al.,1988) and residues of varied pesticides were reported as described in Table V. In Andhra Pradesh, quite high levels of DDT residues were reported, probably due to intensive application of DDT for vector control under the malaria irradiation program (PARMAR & DUREJA, 1990). These results make a strong case for intensifying a pesticide "policing" program through inexpensive methods and the need for control measures to abate pesticide residues in vegetables and other foods.

A Rapid Method for Pesticide Residue Detection

A sensitive one-step method has been developed at CFTRI for the ultra-rapid *in situ* detection of organocholorine insecticide residues in vegetables and fruits (KARANTH et al., 1982b). The "Finger Printing Technique" enables the detection of DDT, BHC and Endosulfan in vegetables through the development of green, prussian blue and yellow colors respectively in the sunlight; by gently pressing the vegetable tissues on filter paper impregnated with an o-tolidine solution. This method is simple and sensitive (sensitivity 0.3 to 10ppm). The entire procedure takes less than one minute and does not require any equipment, clean-up procedure or technical personnel. Finger printing techniques have already been proven to be useful tools for assessing on the spot the quality of the farm produce.

Recent studies have revealed that the finger printing technique has wider applications. It is also useful in locating other chlorine containing chemicals such as synthetic pyrethroids, carbamates and organophosphates in vegetable and fruit tissues. The method is reliable and reproducible, as the identity of the insecticide residues in vegetables was confirmed by TLC method (KARANTH et al., 1983b).

ELISA for Pesticides - a Unique Method

Enzyme Linked Immuno Sorbent Assay (ELISA), based on an antibody-antigen reaction, has become very useful and popular for the detection of environmentally harmful chemicals such as pesticides. Pesticide immuno assays have, in the past two decades, made much progress and appear to be an appropriate technique for the detection of trace quantities of residues usually present in vegetables and fruits (SKERRITT et al., 1998). The method involves particular antigen antibody reactions and hence is sensitive and specific. It is rapid and could be a particularly good method of choice when a large number of samples have to be analyzed. CFTRI was the first institute in India to develop ELISA for pesticides under a joint collaborative international research project with CSIRO (Commonwealth Scientific and Industrial Research Organisation), Canberra from 1993 to 1996 funded by the Australian Centre for International Agricultural Research (ACIAR) (Project No. 9309). Prior to that there was no research on the development of the ELISA method for pesticide residue analysis in India.

Principle

Immunoassays apply antibodies that have been prepared in rabbits, mice or sheep to a particular pesticide or family of pesticides (KARANTH et al., 1999a). The pesticide molecules are too small to elicit an immune response, and so must be conjugated to a large carrier molecule, usually a protein. Bovine serum albumin, ovalbumin, human serum albumin, keyhole limpet haemocyanin are some of the frequently used immunogenic carrier proteins. The pesticide or a precursor or a metabolite having a functional group such as -OH, -SH, -COOH or $-NH_2$ is useful for conjugation with the carrier. However, frequently a derivative of the pesticide, possessing such a functionality must first be synthesized before conjugation can be effected. If an analogue is used, it must retain all the characteristic features of the pesticide, but contain a new chemical group in its structure that can act as a handle (spacer arm) for coupling to the protein.

The pesticide moiety along with the spacer arm is referred to as hapten. The spacer arm not only helps to attach the analyte to the protein, but also helps keep the hapten away from the

protein surface and make it freely available to interact with the antibody. Following immunization with the hapten protein conjugate, antibodies are produced by the animal's immune system. Successful antibody production strongly depends on the immune response of the animal, characteristics of the conjugate and the route and mode of immunization. The immune response consists of an initial primary response followed by secondary response. Antibodies of secondary response are usually of a higher magnitude of affinity than the primary response. Antibodies are collected at this time by obtaining the serum, and they are characterized for selectivity, sensitivity and suitability for the immunoassay.

Assay Format for Competitive Direct ELISA

In this assay, the analyte specific antibodies are immobilized into the walls of the microassay plate or tube. The sample to be analyzed (100 μl) along with the fixed amount of enzyme labeled analyte (100 μl) are added to the antibody coated plate. The sample is incubated, during which time the enzyme-labeled and -unlabeled analyte in the test sample compete for binding sites on the antibodies. Afterwards the unreacted material is washed and the amount of enzyme substrate that forms a chromatic product is estimated by calorimetric method. The amount of color developed is inversely proportional to the amount of unlabeled analyte in the original sample (KARANTH et al., 1998b). The IC_{50} values are at the ppb levels as shown in Table VI.

Matrix Clean-Up

Pesticide residues in vegetables are usually present in low concentrations. Hence the residues need to be partitioned to organic solvents before analysis. During the process of extraction, some constituents of the vegetables also get extracted into the solvent and often interfere with immunoassay. This is called “Matrix effect”. We have developed methods for removal of matrices from the extract. The matrix clean-up method varies according to the commodity and the pesticide. A universal clean-up procedure for different classes of foods including vegetables has been developed and described in Table VII.

Practice

ELISA methods can be considered as appropriate techniques for developing countries like India, as they are simple (little training needed), cheap to perform and do not require expensive equipment. Immunoassays can be formatted as compact test kits which can be designed for either laboratory or field study. Laboratory kits usually use microwell ELISA plates, which enable simultaneous analysis of 1 to 96 samples at a time, hence with a very low cost per test.

Prototype Laboratory Kit
Indigenous laboratory prototype kits based on ELISA techniques have been developed at CFTRI for pesticides such as Endosulfan, DDT, Methyl and Ethyl parathion and Carbendazim and are ready for commercialization.

Challenges of Limiting Pesticide Residues in Vegetables

Intensive studies carried out at CFTRI revealed that the source of pesticide contamination of vegetables is mainly soil on which the vegetables are grown. Several challenging approaches have been made to limit the migration of pesticides into vegetables. Some of the salient achievements are described below.

Selective Food Production in Pesticide-Contaminated Soil

Plants' differential ability to pick up pesticide residues from the soil has been exploited to identify "pesticide-insensitive" crops. Studies with HCH-treated soil (10 ppm) has indicated that knol-khol and carrot are not sensitive to soil residues, as shown in Table VIII. Growth and yield of these crops and nutrient quality of the tubers are not decreased (Table IX). GLC analysis of the residue in edible parts suggested a presence of under 3 ppm, the tolerance limit established for human consumption (Table X). Hence, carrot and knol-khol can be identified as "choice plants" (KARANTH et al, 1982a, SRIMATHI et al., 1983).

Knol-khol and carrot can be grown as selective rotation crops in HCH-contaminated horticultural and agricultural soil, or can be grown to reclaim the heavily polluted soils such as those present in coffee plantations in India (KARANTH et al, 1983a).

Pesticide Residue Abatement Using a "Catch Plant"

Mini field plot experiments showed that coriander and chili plants have the ability to absorb most of the HCH residues from the soil and prevent leaching to ground water. These are hence designated as "Catch Plants" useful for abating HCH residues from soil and irrigation water. Laboratory studies have revealed that the chili plant has a well developed biochemical mechanism to deal with this alien chemical and hence the good quality fruits formed are devoid of residues. Chili cultivation in HCH contaminated soils has dual advantages of: (a) removing pesticide from the contaminated soil (Table X), and (b) decontaminating it through degradation in the vegetative plant body. Hence cultivation of chili could form a suitable "redeeming technology" in agricultural and horticultural practice (KARANTH et al, 1982a, 1983a).

Microbial Degradation of Pesticides in Soil and Prevention of Residue Contamination of Vegetables

Apart from using the plants for limiting entry of pesticide residues into vegetables as described above, the potential of microorganisms to degrade and remove pesticides from agro-horticultural soils through bioremediation has also been successfully attempted in our Institute (KARANTH, 1992, DORIS et al., 1990).

Agrocure for HCH Degradation

A microbial preparation has been developed to degrade HCH in the soil. A Pseudomonas strain Ptm^+ was isolated from soil treated with HCH and shown to degrade HCH (KARANTH & DEO, 1993, 1995, 1997; KARANTH & ANU, 1994). This strain was cultivated in a mineral medium in the laboratory and then the culture was poured into a mixture of neutral lignite powder and neutral coir pith taken in a sterile polythene pouch. After thorough mixing, the preparation was "cured" in polythene pouches by incubating at ambient temperature ($25 \pm 2^{o}C$). The resultant product, "Agrocure", has a 90-day shelf life and contains about 3 billion cells per gram of the product.

Pot culture experiments with tomato and groundnut have proved that augmenting the soil with this bio-powder accelerated HCH degradation in the soil (Table XI) (KARANTH, 1992). At the same time, application of Agrocure minimized the uptake of the insecticide by crops from the soil due to reduced availability as a result of degradation. Decrease in the residue level by increased rate of degradation led to a cascade of beneficial effects. The bio-augmentation thus protected the soil enzyme activity (KARANTH et al., 1984), promoted the growth of plants, improved the nutrient quality of the vegetables and removed the phytotoxic effect of HCH (Table XII) (DORIS et al, 1990).

Non-conventional Methods for Pesticide Degradation
The HCH scavenger P. tralucida produced an extra-cellular surfactant which increased the dispersibility of HCH almost 250-fold. This is the first ever report anywhere in the world on the production of a surfactant specific to solid organochlorine pesticides (ANU & KARANTH, 1991).

There is sufficient evidence to suggest that the surfactant has varied and diverse roles: dispersion of HCH in the medium, increasing the surface area of HCH, enhancing cell aggregation and aiding in scavenging. Thus, the bio-emulsifier is involved in the overall decontamination and degradation of HCH. Exploitation of such secondary metabolites of micro-organisms has great promise in the abatement of pesticide residue in the environment (KARANTH & DEO, 1998a).

Development of a Biosurfactant to Remove HCH Residues from the Contaminated Surfaces
"Baxeklen", a biosurfactant produced by an HCH-degrading bacterial strain, is useful in dislodging HCH residues from vegetables (Table XIII), for pesticide container cleaning, in washing of pesticide mixing tanks in the pesticide manufacturing industry and also in preparing pesticide EC (emulsifiable concentrate) formulations. This is again a redeeming technology in the management of objectionable lipophilic molecules posing occupational health hazard.

Future Directions

The pioneering work conducted at CFTRI , as described above, indicates that there is an urgency to monitor our water and soil resources for pesticides and develop redeeming technologies to mitigate residues so as to limit the pesticide entry into the vegetables and ensure safe food production. We are aware that our research contribution is just a tip of the iceberg. However, we have definitely shown that the challenges to abate chemical pollutants such as insecticides can find solutions by the deployment of plants and microbes. A collaborative and joint venture including different research and development organizations, industries and governmental agencies is needed to strengthen the voyage towards a cleaner environment. Only such multi-institutional, mega-projects can help to offer a package deal to meet the challenges of limiting pesticide residues in vegetables to the benefit of the common man and of conserving nature in all its grandeur.

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Table I. Current scenario of fresh vegetables in India

Vegetable production	81 million t
Global share	13.4%
Area under cultivation	5 120 000 ha
Potato production	25 million t
Value of fresh vegetables and fruits exported	US$ 5.5 billion

Table II. Research institutes in India on post-harvest management of vegetables

North India	**South India**
IARI, New Delhi	IIHR, Bangalore
HAU, Hissar	APAU, Hyderabad
Central Institute of post harvest technology – Ludhiana	TNAU, Periakulam
East India	**West India**
BUCKVV, Kalyani, West Bengal	BARC, Mumbai
RAU, Pusa	KKV, Dapoli
ICAR Lab, Shillong	MPKVV, Rahuri
	Post-Harvest Technological Management Institute, Pune

Acronyms: IARI (Indian Agriculture Research Institute);HAU (Haryana Agricultural University); IIHR (Indian Institute of Horticultural Research); APAU (Andra Pradesh Agricultural University); TNAU (Tamil Nadu Agricultural University); BUCKVV (Bidhan Chandra Krishi Viswa Vidyaly); RAU (Rajendra Argicultural University); ICAR (Indian Council of Agricultural Research); BARC (Babha Atomic Research Centre); KKV (Konkan Krishi Vidyapeth); MPKVV (Mahatma Phule Krishi Vishwa Vidyalaya)

Table III. Production of technical grade pesticides in India (%)

	1994-95	Current status
Insecticides	83.7	69.3
Fungicides	06.8	14.3
Herbicides	06.9	13.8
Rodenticides	00.6	00.4
Fumigants	02.0	02.2
Total	100.0	100.0

Total production 1994-1995 = 88 950 t; Total current production = 59 847 t

Table IV. Extent of pesticide contamination in vegetables in Mysore City (%)

	Nil	HCH	DDT	HCH + DDT
Tomato	0	72	14	14
Egg plant	44	15	25	16
Chili	0	57	28	15
Peas	40	39	11	0
Cow pea	14	70	12	4

Total number of samples tested over 4 seasons: 514

Table V. Pesticide residues (ppm) in market samples of fruits and vegetables

Sampling site	Pesticide detected	Range of residues
Andhra Pradesh	DDT	Traces to 35.0
Punjab	HCH	Traces to 6.0
Karnataka	Aldrin	Traces to 2.0
Haryana	Endosulfan	Traces to 6.0
Maharashtra	Endrin	Traces
Delhi	Heptachlor	Traces
Veg. oils Delhi & Punjab	DDT, HCH	Tr. to 25.7 , Tr. to 0.8

Table VI. Sensitivity of ELISA test developed at CFTRI for some pesticides

	IC50 Values (ppb)
Endosulfan	03 to 25
DDT	0.3 to 10
Methyl Parathion	01 to 05
Ethyl Parathion	0.2 to 10
Carbendazim	3 to 10

Table VII. Universal clean-up procedures for different classes of foods

	Endosulfan	DDT	DDE	MP	EP
High moisture /Low fat	a. Cauliflower a. Cabbage a. Blue grapes a. Green grapes a. Tomato c. Spinach	a. Cauliflower a. Cabbage b. Blue grapes b. Green grapes a. Tomato a. Spinach	a. Cauliflower a. Cabbage b. Blue grapes b. Green grapes	a. Cauliflower a. Cabbage b. Blue grapes a. Green grapes a. Tomato a. Spinach	a. Cauliflower a. Cabbage b. Blue grapes a. Green grapes c. Spinach
Low moisture /Low fat	d. Paddy Rice d. Basmati rice b. Button mushroom c. Oyster mushroom	d. Paddy Rice d. Basmati rice b. Button mushroom c. Oyster mushroom	d. Paddy Rice d. Basmati rice b. Button mushroom c. Oyster mushroom	d. Paddy Rice d. Basmati rice b. Button mushroom c. Oyster mushroom	d. Paddy Rice d. Basmati rice b. Button mushroom c. Oyster mushroom
High moisture /High fat		a. Milk f. Butter	a. Milk f. Butter	a. Milk f. Butter	a. Milk f. Butter
Low moisture /High fat	e. Cottonseed			e. Cottonseed	e. Cottonseed
Colored foods	c. Tea c. Coffee	c. Tea c. Coffee	c. Tea c. Coffee	c. Tea c. Coffee	c. Tea c. Coffee

a: Dilution of the extracts; b: Change of extractants; c: Modified Tsumura's method; d: One step C18 column clean-up method; e: Sulfonation; f: Alcoholic ethanol treatment

Table VIII. Effect of soil treatment with HCH on vegetable growth, yield and residue

	Growth (cm)		Yield		Residue	
	0 ppm	10 ppm	0 ppm	10 ppm	0 ppm	10 ppm
Khol-Khol	22.1	21.2	2.4	2.6	-	0.04
Carrot	20.6	21.6	2.2	2.6	-	0.10

Table IX. Effect of soil treatment with HCH on vegetable nutrient quality

	Protein (% dry wt.)	Nitrogen (ppm dry wt.)	Phosphorus (ppm dry wt.)	Fe 2+ (ppm dry wt.)
Knol-Khol Soil – HCH	6.31	1.01	0.38	79
Knol-Khol Soil + HCH	6.70	1.07	0.38	80
Carrot Soil – HCH	4.94	0.78	0.45	272
Carrot Soil + HCH	5.56	0.87	0.54	305

Table X. Balance sheet of residues (ppm) in selected vegetables

	Soil	Whole plant	Edible tissue
Khol-Khol	2.0	0.04	0.05
Carrot	1.5	0.10	0.60
Coriander	0.5	0.50	0.50
Chili	0.8	0.04	1.60

Soil treatment with 10 ppm HCH.

Table XI. Accelerated degradation of HCH is soil with application of Agrocure (residual HCH in soil - ppm)

Dose applied	Without Agrocure		With Agrocure		Extra HCH degraded (μg/g)		Accelerated degradation (%)	
	30 days	90 days	30 days	90 days	30 days	90 days	30 days	90 days
50 ppm	24.8	1.4	15.7	1.1	9.1	0.3	36.7	21.4
100 ppm	57.4	2.1	37.2	1.5	20.1	0.6	35.1	28.6

Table XII. Protective effect of Agrocure

	Enzyme activity FORMAZAN (mg/10g/24h)		Groundnut (60 days)	
	Day 4	Day 20	Growth (cm)	N content (%)
Soil	2.21	2.8	63.9	5.2
Soil + HCH	0.09	0.21	46.0	2.4
Soil + HCH + Agrocure	3.45	4.0	67.0	8.0

Table XIII. Efficacy of Baxeklen in the removal of HCH from vegetables

	Residue Burden μg/100g	**Residue removed μg/100g**	**% removal**
Radish	26.2	22.8	87.0
Carrot	90.7	67.0	73.0
Lady's finger	38.4	27.5	71.6
Tomato	10.4	9.1	87.5