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Monitoring of Pesticide Residues in Market Basket Vegetables of Jorhat District of Assam, India.

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ABSTRACT

Five sample of each vegetable in each location per month was collected from three markets of Jorhat districts for three months. Three markets are Jorhat vegetable market, Lichubari vegetable market and Teok vegetable market of Jorhat district. The vegetable species are Tomato, Cabbage, Cauliflower, Brinjal, French bean, Cow pea and Okra. Individual species of samples were mixed and analyzed in triplicate. The samples were analyzed by multi residues method using GC-ECD. The residues of various pesticides in vegetables were investigated and the main group of contaminant was the OCs followed by SPs. The contamination by OC group *viz.,* α-HCH ranged from 0.001 to 0.003 µg g^{-1}**,** β-HCH ranged from 0.001 to 0.003 µg g^{-1}**,** δ-HCH ranged from 0.001 to 0.004 µg g^{-1}**,** alachlor ranged from 0.001 to 0.002 µg g^{-1}**,** aldrin ranged from 0.001 to 0.003 µg g^{-1} , endosulfan-I ranged from 0.001 to 0.003 µg g^{-1}, butachlor ranged from 0.001 to 0.004 µg g^{-1}**,** P,P'-DDE ranged from 0.001 to 0.002 µg g^{-1}, endosulfan-II ranged from 0.001 to 0.002 µg g^{-1}**,** P,P'-DDD ranged from 0.001-0.003µg g^{-1} and P,P'-DDT ranged from 0.001-0.002µg g^{-1} . The contamination by SP group *viz.* fenpropathrin ranged from 0.001 to 0.003µg g^{-1}, λ-cyhalothrin ranged from 0.001 to 0.003µg g^{-1}, α-cypermethrin ranged from 0.001 to 0.002 µg g^{-1} and deltamethrin ranged from 0.001 to 0.003µg g^{-1} **.** The highest contaminated sample is brinjal followed by cauliflower, cabbage and tomato. Among the OC compound the major contaminants were β-HCH followed by δ-HCH , α-HCH, P,P'-DDE and P,P'-DDT.

Key words: GC, ECD, Vegetables, Pesticides, ,Organochlorines, Synthetic pyrethroids.

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1 INTRODUCTION

India is an agrarian country. According to the census of India (2001), the total population of the nation is 1.027 billion in which 110.7 million are farmers, whereas the cultivated cropped area is about 124.07 million ha [1]. The global share of India in vegetable production is about 13.4%. A personal interview carried out among cultivators and local pesticide market showed that about 95% of farmers use synthetic pesticides in vegetable fields to protect their crops from different pests. In the year 2000, the vegetable production in India was 92.8 million tonnes, grown over an area of 6 million hectares, which is about 3% of the gross cropped area of the country. Potato is the most important vegetable crop in India as it occupies 20% of vegetable area and contributes 27% to the total vegetable production. Nevertheless, vegetable production has been diversifying gradually [2]. In the world, India occupies first position in the production of cauliflower, brinjal and peas, second in onion and third in cabbage [3]

Vegetables form an important component of human diet. They are however, infested by various insect pests like aphids, jasssids, diamond moths, caterpillars, etc. Among the vegetables, brinjal, cauliflower, tomato and okra etc. are some very common vegetables cultivated, throughout the country but all are badly affected by insect-pest and diseases. Brinjal (*Solanum melongena*L.) suffers heavily at fruiting stage due to attack of shoot and fruit borers causing 70% damage to the crop and making it totally unfit for human consumption [4],[5]. Cauliflower (*Brassica oleracea*) also an important vegetable crop with an annual production of 3.39 million tones, is heavily attacked by various insects, resulting in severe loss of quality and production [6] , [7] . Okra (*Abelmoschusesculentus* L.) is heavily infested by numerous insect pests for which different insecticides are used [8]. For better yield and quality, pesticides are repeatedly applied by farmers during the entire period of vegetable farming including the fruiting stage. Indiscriminate use of pesticides particularly at fruiting stage and non adoption of safe waiting period leads to accumulation of pesticide residues in consumable vegetables. Since most of the pesticides are toxic in nature, their continuous ingestion by man even in trace amounts, can result in their accumulation in body tissues with serious adverse effects on health [9].

Vegetables consume 14% of the total pesticides used in India, in which, the share of different types of pesticides in Indian agriculture market shows that organophosphorus (50%) ranked first, followed by pyrethroids (19%), organochlorines (18%), carbamates (4%) and biopesticides (1%)

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[10]. Pesticide application is a necessary step for coping with the pest related problems and therefore, it is very important to assess their residues in vegetables. Many countries have established regular monitoring programs for quantitative determination of residues in food products [11] as pesticide residues above the maximum tolerance limits (MRL) at harvest time are a subject of great concern both globally and nationally. Surveys carried out by institutions spread throughout the country indicate that 50 to 70% of vegetables are contaminated with insecticide residues [12].

Pyrethroids are synthetic derivatives of pyrethrins, the natural insecticides that are produced by certain species of *Chrysanthemum*. Pyrethroids of greatest interest to water quality include cypermethrin and fenvalerate. Pyrethroids are extremely toxic to aquatic organisms, with lethal concentration (LC50) values less than 1.0 ppb. They are applied in urban areas primarily for structural pest control, in agricultural areas and in the home as pet sprays and shampoos. Some of the new pyrethroids such as cypermethrin, which is used in much lower amounts, could be up to 20 times more toxic than permethrin [13]. The primary transport pathway for pyrethroids is receding waters from agricultural and urban applications through runoff. Pyrethroids are persistent compounds and feebly soluble in water [14]. Several recent monitoring studies in California have reported synthetic pyrethroid contamination of both surface waters and sediments [15], [16], [17] . Assam is a state where huge vegetables are produced in different vegetable growing pockets. These vegetables are transported to the various region of Assam including North Eastern States for millions of people. So a huge population is depends on the vegetables grown in various vegetable growing pockets of Assam which needs monitoring of pesticides residue. Hence, the present study was undertaken with the objective to monitor/quantify the residue levels of some most frequently used pesticides on vegetables in Jorhat districts of Assam so that contamination in vegetables due to pesticides can be removed or reduced.

2 MATERIAL AND METHODS

Five sample of each vegetable in each location per month was collected from three markets of Jorhat districts for three months. Three markets are Jorhat vegetable market, Lichubari vegetable market and Teok vegetable market of Jorhat district. The vegetable species are

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Tomato, Cabbage, Cauliflower, Brinjal, French bean, Cow pea and Okra. Individual species of samples were mixed and analyzed in triplicate. These mixed samples were grind with the help of an electrical grinder. From this blended samples 100g were taken, mixed with 200ml of acetone and grinded in the electrical grinder for 2 min at high speed. After 2 min these samples were transferred to a Buchner funnel containing filter paper fitted with suction bottle and filtration was completed within less than 1 min. From this extract, transferred an aliquot of 80 ml to 1 litre separatory funnel. To this aliquot 200 ml mixture of hexane: dichloromethane (1:1, v/v) were added and shaken vigorously for 1 min. The lower aqueous phase was then transferred to another 1 litre separatory funnel. The organic phase of the first separatory funnel was dried by passing through approximately 1.5" sodium sulfate supported on pre-washed cotton in 4" funnel. To the separatory funnel containing aqueous phase, 10mL saturated sodium chloride solution was added and shaken vigorously for 30 sec. To this, 100 mL dichloromethane were added, shaken vigorously and lower organic phase was dried by passing through the same sodium sulfate used for drying the organic extract of the first separatory funnel. Repeated the extraction once more with 100 mL dichloromethane and dried as above. Sodium sulfate was rinsed with about 50 mL dichloromethane and the extract was concentrated by using vacuum rotary evaporator. Concentration step was repeated in the presence of hexane to remove all traces of dichloromethane, and the final volume (3ml) was made in n-hexane.

The sample was then cleaned by column chromatography. The column of 22mm inner diameter was packed by placing solvent washed cotton plug at the bottom of column above which 2g of cleaned dried anhydrous sodium sulphate was placed. Then 4g activated florosil were placed slowly on the uniformly leveled clean anhydrous sodium sulphate. The florosil was leveled properly by shaking and above it again 2g clean anhydrous sodium sulphate was placed and leveled properly. The column was pre-wetted by pouring 50ml of double distilled n-hexane and the lower knob of column was remain open so that the solvent can elute drop by drop. Then the whole extract was placed drop wise in the column and eluted by using 50ml of 50% dichloromethane:1.5% acetonitrile: 48.5% hexane elution mixture. The eluant was collected and dried in rotary evaporator and the final volume was made 1 ml by using n-hexane which was injected to GC [18].

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GC analysis: Samples were analyzed by using Shimadzu Gas Chromatograph (GC-2010) equipped with "Ni electron capture detector (ECD) and capillary column (DB-1, 30mxID-0.25mm) with AOC-20i auto injector. Operating conditions were: Injector temperature:280^{0}C and Detector Temperature:300^{0}C oven temperature programmed at 170^{0}C (hold for 5 minute) - 220^{0}C (hold for 10 minute and increased @1.5^{0}C)-280^{0}C (hold for 7.0 minute and increased @4.0^{0}C).The nitrogen used as carrier gas with flow rate 7.7 ml per minute with split less ratio.

Reference standard of 21 pesticide mixture was obtained from M/S sigma Aldrich, USA. The list of pesticide along with retention time (Rt) are given in the table1.

Table: 1 List of Organochlorine and Synthetic Pyrothroids pesticide along with retention time (Rt)

Peaks	Pesticide	Rt(Minute)	Recovery (%)	LOQ(ng/kg)
Organochlorines (OCs)				
1	α-HCH	9.02	83.08	0.02
2	β-HCH	10.08	84.00	0.02
3	δ-HCH	11.28	82.23	0.05
4	Alachlor	15.89	90.45	0.01
5	Aldrin	18.48	86.25	0.05
6	Endosulfan-I	24.69	88.03	0.05
7	Butchlor	25.77	92.00	0.01
8	P,P'-DDE	27.38	91.08	0.03
9	Endosulfan-II	27.85	93.09	0.05
10	P,P'-DDD	31.21	87.05	0.02
11	P,P'-DDT	35.41	94.04	0.01
12	Dicofol	41.93	87.30	0.03
Synthetic pyrethroids (SPs)				
13	Fenpropathrin	43.14	88.09	0.02
14	λ-Cyhalothrin	51.41	94.40	0.01
15	Permethrin-I	55.20	87.32	0.06

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16	Permethrin-II	55.91	88.90	0.03
17	β-Cyfluthrin	58.99	96.03	0.01
18	α-Cypermethrin	60.18	89.09	0.04
19	Fenvalerate-I	62.85	90.01	0.03
20	Fenvalerate-II	63.63	84.04	0.02
21	Delamethrin	65.64	92.08	0.03

3 RESULTS AND DISCUSSION

Recovery experiments were carried out with the entire representatives group. The recoveries from vegetable samples fortified at the level of 0.1 and 0.01mg/kg varied from 80.23-96.03 per cent for all Organochlorine and Synthetic pyrethroids pesticides.

Table 2: Pesticide residues (μg g^{-1}) in tissues of vegetable sample collected from different markets of Jorhat districts of Assam during the month of November,2010.

S. No.	Pesticides	Tomato	Cabbage	Cauliflower	Brinjal	French bean	Cow pea	Okra
1	α-HCH	BDL	0.003	BDL	0.003	BDL	0.002	0.001
2	β-HCH	0.001	BDL	0.001	BDL	0.002	BDL	0.002
3	δ-HCH	BDL	0.001	0.003	0.001	0.001	0.001	BDL
4	Alachlor	BDL	BDL	BDL	BDL	BDL	BDL	0.001
5	Aldrin	BDL	0.002	BDL	0.003	BDL	BDL	BDL
6	Endosulfan-I	0.002	BDL	BDL	BDL	0.001	BDL	BDL
7	Butchlor	BDL	BDL	0.001	BDL	BDL	BDL	BDL
8	P,P'-DDE	BDL	BDL	BDL	BDL	BDL	0.001	BDL
9	Endosulfan-II	BDL	BDL	BDL	0.002	BDL	BDL	0.002
10	P,P'-DDD	0.001	BDL	BDL	BDL	BDL	0.003	BDL
11	P,P'-DDT	BDL	BDL	0.001	BDL	BDL	BDL	BDL
12	Dicofol	BDL	BDL	BDL	BDL	BDL	BDL	BDL

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13	Fenpropathrin	BDL	BDL	0.003	BDL	BDL	BDL	BDL
14	λ-cyhalothrin	BDL	BDL	BDL	BDL	BDL	BDL	BDL
15	Permethrin-I	0.001	BDL	BDL	BDL	BDL	BDL	BDL
16	Permethrin-II	BDL	BDL	BDL	BDL	BDL	BDL	BDL
17	β-cyfluthrin	BDL	BDL	BDL	BDL	BDL	BDL	BDL
18	α-cypermethrin	BDL	0.002	BDL	BDL	BDL	BDL	BDL
19	Fenvalerate-I	BDL	BDL	BDL	BDL	BDL	BDL	BDL
20	Fenvalerate-II	BDL	BDL	BDL	BDL	BDL	BDL	BDL
21	Delamethrin	BDL	BDL	BDL	0.001	BDL	BDL	BDL
Total		**0.004**	**0.006**	**0.005**	**0.006**	**0.004**	**0.006**	**0.006**

Table 3: Pesticide residues (μg g^{-1}) in tissues of vegetable sample collected from different markets of Jorhat districts of Assam during the month of December,2010.

S. No.	Pesticides	Tomato	Cabbage	Cauliflower	Brinjal	French bean	Cow pea	Okra
1	α-HCH	0.002	BDL	0.001	BDL	0.002	BDL	0.001
2	β-HCH	0.001	0.002	BDL	0.001	BDL	0.003	BDL
3	δ-HCH	BDL	0.001	0.003	BDL	0.004	0.001	0.001
4	Alachlor	0.001	BDL	BDL	0.002	BDL	BDL	BDL
5	Aldrin	BDL	BDL	BDL	BDL	BDL	BDL	0.001
6	Endosulfan-I	0.003	BDL	0.001	BDL	BDL	BDL	BDL
7	Butchlor	BDL	BDL	BDL	0.004	BDL	BDL	BDL
8	P,P'-DDE	BDL	0.002	BDL	BDL	0.001	BDL	BDL
9	Endosulfan-II	BDL	BDL	0.001	BDL	BDL	BDL	BDL
10	P,P'-DDD	BDL	BDL	BDL	BDL	BDL	0.001	BDL

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11	P,P'-DDT	BDL	0.002	BDL	BDL	BDL	BDL	BDL
12	Dicofol	BDL	BDL	BDL	BDL	BDL	BDL	BDL
13	Fenpropathrin	BDL	BDL	BDL	BDL	0.001	BDL	BDL
14	λ-cyhalothrin	BDL	BDL	BDL	0.002	BDL	BDL	BDL
15	Permethrin-I	BDL	BDL	BDL	BDL	BDL	BDL	BDL
16	Permethrin-II	BDL	BDL	BDL	BDL	BDL	BDL	BDL
17	β-cyfluthrin	BDL	BDL	BDL	BDL	BDL	BDL	BDL
18	α-cypermethrin	BDL	BDL	0.001	0.001	BDL	BDL	BDL
19	Fenvalerate-I	BDL	BDL	BDL	BDL	BDL	BDL	BDL
20	Fenvalerate-II	BDL	BDL	BDL	BDL	BDL	BDL	BDL
21	Delamethrin	BDL	BDL	BDL	BDL	BDL	BDL	BDL
Total		**0.0026**	**0.0026**	**0.0027**	**0.0022**	**0.0036**	**0.0014**	**0.0023**

Table 4: Pesticide residues (μg g^{-1}) in tissues of vegetable sample collected from different markets of Jorhat districts of Assam during the month of January,2011.

S. No.	Pesticides	Tomato	Cabbage	Cauliflower	Brinjal	French bean	Cow pea	Okra
1	α-HCH	BDL	0.001	0.002	BDL	0.001	0.002	BDL
2	β-HCH	0.002	BDL	0.001	0.002	0.003	BDL	0.001
3	δ-HCH	BDL	0.003	BDL	0.001	BDL	0.001	BDL
4	Alachlor	0.002	BDL	BDL	BDL	BDL	BDL	BDL
5	Aldrin	BDL	BDL	BDL	BDL	0.001	BDL	BDL
6	Endosulfan-I	0.001	BDL	BDL	0.001	BDL	BDL	BDL
7	Butchlor	BDL	BDL	BDL	0.001	BDL	BDL	0.001
8	P,P'-DDE	BDL	BDL	0.001	BDL	BDL	BDL	BDL

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9	Endosulfan-II	BDL	BDL	BDL	BDL	BDL	BDL	BDL
10	P,P'-DDD	BDL	BDL	BDL	BDL	BDL	BDL	BDL
11	P,P'-DDT	BDL	BDL	BDL	BDL	BDL	BDL	BDL
12	Dicofol	BDL	BDL	BDL	BDL	BDL	BDL	BDL
13	Fenpropathrin	BDL	BDL	BDL	0.001	BDL	BDL	BDL
14	λ-cyhalothrin	BDL	BDL	BDL	BDL	BDL	BDL	0.003
15	Permethrin-I	BDL	BDL	BDL	BDL	BDL	BDL	BDL
16	Permethrin-II	BDL	BDL	BDL	BDL	BDL	BDL	BDL
17	β-cyfluthrin	BDL	BDL	BDL	BDL	BDL	BDL	BDL
18	α-cypermethrin	BDL	0.002	BDL	BDL	BDL	BDL	BDL
19	Fenvalerate-I	BDL	BDL	BDL	BDL	BDL	BDL	BDL
20	Fenvalerate-II	BDL	BDL	BDL	BDL	BDL	BDL	BDL
21	Delamethrin	BDL	BDL	0.003	BDL	BDL	BDL	BDL
Total		**0.0027**	**0.0033**	**0.0035**	**0.0026**	**0.0037**	**0.0016**	**0.0025**

Average of three replicates, BDL: Below Determination Level

Table 5: Month wise number of contamination of samples by pesticides

Samples	Nevemver,2010	December,2010	January,2011	Total
Tomato	4	4	3	11
Cabbage	4	4	3	11
Cauliflower	5	5	4	14
Brinjal	5	5	5	15
French bean	3	4	3	10
Cow pea	4	3	2	9
Okra	4	3	3	10

The present study was undertaken to determine the concentration of different pesticides residues in market basket vegetables of Jorhat district. Pesticides are known to be present in

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vegetables due to extensive use of corresponding pesticides in interfiled cultivation. The residues of various pesticides in vegetables are presented in table 2, 3 and 4.The results revealed that the main group of contaminant is the OCs followed by SPs. However, the degree of contamination of later group is negligible. The contamination by OC group *viz.,* α-HCH ranged from 0.001 to 0.003 µg g^{-1}**,** β-HCH ranged from 0.001 to 0.003 µg g^{-1}**,** δ-HCH ranged from 0.001 to 0.004 µg g^{-1}**,** alachlor ranged from 0.001 to 0.002 µg g^{-1}**,** aldrin ranged from 0.001 to 0.003 µg g^{-1} , endosulfan-I ranged from 0.001 to 0.003 µg g^{-1}, butachlor ranged from 0.001 to 0.004 µg g^{-1}**,** P,P'-DDE ranged from 0.001 to 0.002 µg g^{-1}, endosulfan-II ranged from 0.001 to 0.002 µg g^{-1}**,** P,P'-DDD ranged from 0.001-0.003µg g^{-1} and P,P'-DDT ranged from 0.001-0.002µg g^{-1} . The contamination by SP group *viz.* fenpropathrin ranged from 0.001 to 0.003µg g^{-1}, λ-cyhalothrin ranged from 0.001 to 0.003µg g^{-1}, α-cypermethrin ranged from 0.001 to 0.002 µg g^{-1} and deltamethrin ranged from 0.001 to 0.003µg g^{-1}**.** The degree of contamination of individual sample during the three months is almost same. The highest contaminated sample is brinjal followed by cauliflower, cabbage and tomato. Similar results are also reported by Charan et al. [19] in farm gate vegetables. Among the OC compound the major contaminants were β-HCH followed by δ-HCH, α-HCH, P,P'-DDE and P,P'-DDT. All the samples were contaminated by one or more pesticides.

The result of study reveals that all the samples were contaminated with different pesticide residues. Of course no contaminated samples were exceeded the maximum residual limit (MRL) values as per the FAO/WHO [20].Therefore, periodic monitoring of market basket vegetables must be carried out to know the prevailing scenario of pesticide contamination of vegetables grown in Jorhat district.

4 CONCLUSION

The present research will not only serve as reference document but also helpful in taking necessary and timely preventive measure to mitigate such problems. The investigation reveals that the consumption of vegetable is safe from consumer's point of view as residues of all the pesticides were far below their MRLs, though the vegetable eater is not so safe for which safety measures have to be adopted to protect the future generation.

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