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Determination of Residues of Endosulphan and its Metabolites in Spinach

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Endosulphan is non-systemic insecticide and acaricide with contact and stomach action. It is widely used on various vegetable crops. Time period between the last spray on the vegetables and its consumption by the customer is often much shorter than that of the other crops like wheat, rice, pulses, grains etc. Therefore, the studies on residual endosulphan have been carried out on spinach in the climatic conditions of Nagpur city. Residues and metabolites of endosulphan were extracted from plant samples and determined using gas chromatography. Normal dose showed negligible residues but higher doses showed the concentration of residues shifting towards MRL (maximum residual limit) values.

Key Words: Insecticides, Residue analysis, Endosulphan, Spinach, Gas chromatography.

INTRODUCTION

Endosulphan is widely used for the control of sucking, chewing, boring insects and mites on a wide variety of crops. It also controls tsetse flies. It is a non-systemic insecticide and acaricide with contact and stomach action.

Spinach is a very commonly grown vegetable and is available almost throughout the year. Use of endosulphan to control pests in case of spinach is very common. Unlike general food grains, vegetables are plucked and marketed immediately and used by the consumer when these are fresh. This often increases the possibility of reducing the safe waiting period between the last spray and its consumption. Secondly, there is a general feeling among farmers to use more amount of insecticide for better control or to save the crop from insect attack. Many a time the specifications regarding dose are not correctly followed.

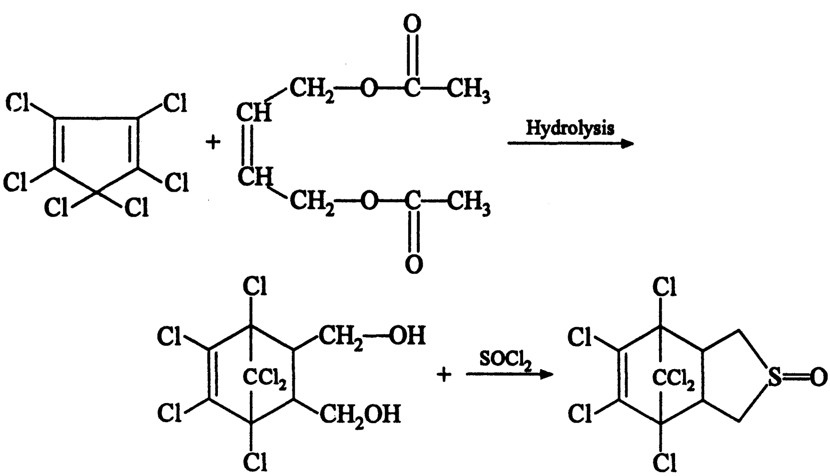
Endosulphan being poisonous, its excess use may pose certain problems of toxic residues left on the crop. Most of the problems associated with residues can be overcome through its judicious use. But indiscriminate use can endanger the health of humans and other domestic animals. Over-exposure to endosulphan is known to cause serious health problems, *e.g.*, decrease in IQ, memory loss and even deformities in newborn. Hence the presence of residues of toxicants in food and fodder is of great concern to everyone.

Endosulphan gets degraded to a considerable extent within 7–8 days. Various factors, *viz.*, temperature, humidity, soil characteristics are responsible for its degradation. In view of this, it was felt useful to study the endosulphan residues in vegetables like spinach in the climatic conditions of Nagpur.

The insecticides used in agricultural practice are widely distributed in the environment and many of them remain there due to their persistence. Movement from area of application to other is also possible[1–3]. This depends on mobility of respective insecticide with respect to plant-soil ecosystem and also various soil characteristics such as organic carbon, pH, water holding capacity, etc.

Endosulphan is a chlorinated cyclodiene with the molecular formula $C_9Cl_6H_6O_3S$. It is commercially available in the form of dust, wettable powder, emulsion concentrate. It is applied at a concentration of 0.2%. It is relatively more stable towards sunlight than alkali attack.

In general, it is produced[4] from hexachlorocyclopentadiene, butane diol and thionyl chloride. The formation is shown below:



EXPERIMENTAL

All the chemicals used during the study were of high purity, *e.g.*, *n*-hexane (HPLC grade E-Merck), sodium sulphate (GR grade E-Merck), floricil, alpha, beta endosulphan, endosulphan sulphate, endosulphan lactone. Standards were of very high purity (Supelco Sigma-Aldrich).

Field Experiments

A field experiment was carried out in Nagpur city within the premises of Visvesvaraya National Institute of Technology during the period of May–June 2002. Spinach was grown in four blocks of 1 m^2 area each. During the studies, the normal dose (0.2%) and excess dose, *i.e.*, 5 times of normal dose (1%) was applied on a separate spinach block. The first application was done on the 15th day after the emergence of crop, while the second application was done after 20 days of first application. The plant samples were taken out when the plant was fully grown (after 25 days of emergence).

Extraction of Residues from Plant Samples

Sufficient quantities of plant samples were plucked out and air dried at room temperature. The roots and leaves were separately crushed to paste and 20 g of crushed samples were taken and subjected to extraction separately using *n*-hexane in a soxlet extractor for 6 h. The resultant extract was passed through sodium sulphate and floricil column pre-activated by heating at 120°C for 10 h. The column was eluted using same solvent. The extract was concentrated and made up to 0.5 mL before chromatographic analysis. Efficiency of extraction with spiking the samples with 0.1 microgram of endosulphan was carried out separately on the root and leaf samples and found to be 90%.

Analysis and Estimation

The residues of endosulphan and its metabolites were determined on Shimadzu 14B model equipped with ^{63}Ni ECD detector and microprocessor based integrator chromatopac 2 UC-R6A.

The operating conditions were as follows:

Initial temperature	–	60°C for 1 min
Programme rate	–	10°C/min
Final temperature	–	300°C for 1 min
Injection volume	–	1 microlitre
Injector temperature	–	250°C
Detector temperature	–	300°C

RESULTS AND DISCUSSIONS

Residues of endosulphan and its metabolites were found to be present in plants, *viz.*, leaves and roots showing absorption and translocation in plants.

TABLE-1
VARIOUS ENDOSULPHAN METABOLITES IN SPINACH

Compound	Retention time (min)	Normal dose of endosulphan (0.2%)		Five times of normal dose of endosulphan 1%	
		Leaves (ng/g)	Roots (ng/g)	Leaves (ng/g)	Roots (ng/g)
α-endosulphan	7.3	0.23	18.78	11.9	20.96
β-endosulphan	11.8	ND	3.12	6.99	0.25
Endosulphan sulfate	3.9	0.40	7.98	11.5	7.66
Endosulphan lactone	7.9	0.48	0.31	0.4	0.50
α-HCH	2.8	0.22	4.73	7.3	5.22
γ-HCH	3.3	0.12	3.51	2.1	3.52

In addition to α-, β-endosulphan and its metabolites, some residues of α-, γ-HCH were also found to be present. It may be due to pre-application of HCH in soil and its persistent nature.

A larger proportion of α-HCH and γ-HCH isomers was found in roots and leaves which suggested the effect of technical grade HCH which contains large amount of α and γ-isomers and also isomerization of γ-HCH to α-HCH[5, 6]. Higher amount of endosulphan sulfate in roots can be attributed to its metabolism in plants during translocation from the leaves to roots as reported by Beard and Ware[7]. In this study α-endosulphan was found to be present in higher amount as compared to β-endosuophan. This can be attributed to several factors, *viz.*, use of technical grade endosulphan (70% α, 30% β), less persistence of β-endosulphan (half life 218 was for α-Endosulphan, 187 was for β-endosulphan at 20°C and pH 7) and conversion of endosulphan sulphate to α-endosulphan by plants[8]. The half life for the conversion of α-endosulphan to β-endosulphan is 60 days which is greater than time period between application of endosulphan and sampling in this study. Hence the possibility of conversion of α-endosulphan to β-endosulphan is negligible.

Overall, the extent of residues in normal dose has not shown higher quantities that could cause concern. However, excess dose (5 times) showed the presence of residue values shifting towards MRL value. In spite of application of higher dose of insecticide, the residues have not crossed the MRL. This may be mostly due to the use of organic manure for the proper growth of plants and relatively high temperature conditions in Nagpur city. Other metabolities were found to be present as per given in Table-1.

It can be concluded that a prevalent shorter span between the plucking of crop and its consumption by customer invites the attention for careful usage of prescribed dose in addition to strict maintenance of waiting period between last spray and its use.

The above precautions become more important for the spinach grown on the land which is frequently loaded with insecticides for other crops as it could cause accumulation in soil. Declining of organic matter in the soil (which is responsible for adsorption of insecticide in soil) and increased use of inorganic fertilizers may cause absorption of these toxicants through the roots.

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