

Residue Determination of Dimethoate in Leafy Vegetables (Spinach) using RP-HPLC

J. BAGYALAKSHMI*, G. KAVITHA AND T.K. RAVI

Sri Ramakrishna Institute of Paramedical Sciences,
College of Pharmacy,
Coimbatore – 641 044.

ABSTRACT

Residues of Dimethoate present in locally available Spinach varieties - Palak : *Beta maritima*, Vendhai : *Trigonella foenumgraecum*, Thandan : *Amarnathus mangostanus* - green and red were determined. The quantification was carried out using RP-HPLC for unprocessed (fresh) and processed (washed, washed and cooked) Spinach samples. The extraction was carried out with benzene. It was found that unprocessed Dimethoate Spinach contains dimethoate residue which is slightly more than the tolerance limit, while the processed sample contains dimethoate residue within the tolerance limit. The washed samples (3 times under ordinary tap water) showed 28-50 % reduction while the cooked samples (boiling for 15 min) shows 58-71 % reduction compared to the unprocessed samples. Hence it can be concluded that in case of greens, it should be washed three times with ordinary water and then cooked (cooked for fifteen minutes) so that the pesticide residue is very much reduced and it will be well within the tolerance limit as specified by EPA (Environmental Protection Agency).

KEYWORDS; Dimethoate, Spinach, Palak, Vendhai, Thandan

INTRODUCTION

Leafy vegetables 'Spinach' is said to be the 'Prince of vegetables'¹. The food value of spinach is very high as it is a source of high grade iron. The composition² of iron in leafy vegetables per 100 g edible portion of Spinach beet (Palak) is 16.2 mg, Spinach- 10.9 mg. The other chemical constituents of spinach are essential amino acids, Vitamin A and ascorbic acid. It is also found to be the daily diet of common people and most of the patients being admitted in the hospital.

Pesticides³⁻⁶ are substances used for preventing, destroying, repelling or mitigating any pest. The agriculture sector dominates the economic scenario in India. The occupation of nearly 60% of the population is predominantly agriculture. Nearly 30% of the food production is lost in India due to insects, pests, plant pathogens, weeds, rodents, birds and in storage. The uses of pesticides have become absolutely essential for increasing food production. India is the third largest consumers of pesticides in the world. Pesticides have become essential to modern agriculture but they are a potential source of chemical degradation of soils. They are also hazardous to the health of domestic animals and human beings if not handled properly. All pesticides interfere with normal metabolic processes in the plant and are classified according to the type of organism they are intended to control, eg. Insecticides to control insects, acaricides to control mites; nematocides to control rats; herbicides to control weeds; molluscides to control snails, slugs etc; fungicides to control fungal or bacterial infections; antibiotics to control bacterial, fungal, viral and mycoplasmal infections. In recent years the role of pesticides in relation to human welfare has been discussed the world over emotionally. Insecticides being toxic in nature, they should be used with extreme caution. If the pesticide content crosses the tolerance limit⁷ specified by FDA it can lead to disastrous effects. From the survey conducted in the local regions of Coimbatore, it was found that 'Rogor' (dimethoate) was the most commonly used pesticides among vegetables and greens.

. The EPA (Environmental Protection Agency) tolerance⁸ level for dimethoate in Spinach fresh and canned is 2 ppm. If this limitation⁹ is crossed it may cause adverse effects like numbness, tingling sensations, in coordination, headache, dizziness, tremor, nausea, abdominal cramps, sweating, blurred vision, difficulty in breathing or respiratory depression and slow heart beat. Very high doses may result in unconsciousness and convulsion or fatality. Hence our project aims at detecting and quantifying, dimethoate present in (unprocessed)

spinach, processed spinach (after washing), processed spinach (after washing and cooking) using RP-HPLC¹. The analysis was carried out in four varieties of spinach – Palak (*Beta maritima*)¹⁰, Vendhai (*Trigonella foenumgraccum*)¹⁰, green and red Thandan – (*Amarntus mangostanus*)¹⁰.

MATERIALS AND METHODS

Dimethoate was purchased from the consolidated Agro Trading Company, Coimbatore. The trade name of dimethoate is 'ROGOR' from Bayer Company. Spinach beet (Palak) and other varieties of spinach, vendhai, red thandan and green thandan were obtained from Thodamuthur field in Coimbatore.

Chromatographic system consist of, Shimadzu-LC 10A HPTLC system, Stationary phase was C₁₈, Phenomenex 5 µ.ODS 250 X 4.60 mm Analytical Column. Pump was LC-10 AT, Detector SPD- 10 A. Isocratic elution method in a mobile phase comprised of water: acetonitrile (60:40% v/v) at a flow rate 1 ml/minute was used. Injection volume was 15 µl, Injected using Rheodyne injector and detection wave length was 221 nm.

For estimation of dimethoate in unprocessed Spinach, 50 g of the samples⁸ were crushed in a mixer separately. From each 50 g, 6 g of the homogenate was extracted with organic solvent (benzene) and then centrifuged for 10 min at 1000 rpm. The organic layer was decolourized by using charcoal and passed through Whatman filter paper. One millilitre was evaporated to dryness under reduced pressure using nitrogen and the residue was reconstituted with 0.5 ml of mobile phase. The samples were injected into the C₁₈ column through a syringe filter.

For estimation of dimethoate in processed spinach (after washing), Samples were washed with ordinary tap water for three times. They were crushed in a mixer and the extraction procedure followed was same as the unprocessed spinach .For estimation of dimethoate in processed spinach beet (after washing and cooking), 50 g samples were washed three times under ordinary tap water and cooking process was carried out by boiling the samples in 15 ml of water for 15 min. Each 50 g were subjected to this heat treatment separately under same conditions. They were cooled, crushed and same extraction procedure⁸ was followed as mentioned above.

Dimethoate (0.5 ml) was pipetted into 250 ml standard flask and made up to the volume with mobile phase. The concentration is 710 ppm. Serial concentrations of working standards were prepared and made up with mobile phase. The estimation of dimethoate carried out in varieties of spinach (both unprocessed and processed) are as mentioned in Table 1.

RESULTS AND DISCUSSION

From the results obtained, it was found that the concentration of dimethoate residues found in unprocessed samples were above EPA tolerance level (2 ppm) in case of Palak and Vendhai spinach whereas red thandan and green thandan spinach were within the tolerance limit. After kitchen processing of the same samples, the dimethoate residue was found to reduce below the EPA tolerance. Hence it can be concluded that in case of greens, it should be washed three times with ordinary water and then cooked (cooked for 15 min) so that the pesticide residue is very much reduced and it will be well within the tolerance limit as specified by EPA.

REFERENCES

- [1] Caleras, P., Diana, P., Meloni, M. and Pirist, M., J. Agri. Food Chem., Residue determination of some insecticides and fungicides on grapes by reversed-phase high-performance liquid chromatography, 1982, 30, 569.
- [2] Singh, G., Kawatra, A. and Sehgal, S., Nutritional composition of selected green leafy vegetables, herbs and carrots . **Plant food for human nutrition**, 2001, 56, 359.
- [3] Beck, E.W., and Johnson, J.C. Getz, M.E., Skinner, and F.B., Dawsey, L.H., Woodham, D.W. and Derbyshire, J.C., **J. Econ. Ent.**, 1968, 61, 605.
- [4] Wepplo , P .,Imidazolinone herbicides: Synthesis and novel chemistry **Pesticide Science**, 1990, 29,293.
- [5] Stephen, G.N.A., Ahmad, N and Newell, G., Dimethoate spray residues in strawberries **Pesticide Science**,1985, 16, 143.
- [6] Young, W.L., Neil., D.W., and Westcott, V., Gas chromatographic quantitative analysis and persistence of dimethoate and dimethoxon residues on and in wheat plants **J. Agri Food Chem.**, 1981, 29, 860.
- [7] Cabras, P., Meloni, M., Perra, M. and Pirisi, F.M. **J. Chromatogr.**, 1979 b, 180, 184.
- [8] Elkins, E.R., Farrow, R.P Lamb, F.C., Cook, R.W., Kawai, M., and Kimball, T.R., **J. Agri. Food Chem.**, Removal of DDT, malathion, and carbaryl from green beans by commercial and home preparative procedures, 1968, 16, 962.
- [9] Edson, E.F., Jones, K.H. and Watson, W.A. Safety of dimethoate insecticide. **Br. Med. J.**, 1967, 4, 554.
- [10] Nadkarni, K.M., Eds., In; K.M. Nadkarni's Indian Materia Medica, 3rd Edn., **Popular prakashan Pvt. Ltd. Bombay**, 1954, 89, 197, 1240.

TABLE I : DETERMINATION OF DIMETHOATE RESIDUE IN UNPROCESSED AND PROCESSED LEAFY VEGETABLES (SPINACH) USING RP-HPLC

Leafy vegetables	Concentration of dimethoate residue		
	Unprocessed (ppm)	Processed (washed) (ppm)	Processed (washed and cooked) (ppm)s
Palak	2.54	1.63	0.73
Vendhai	2.43	1.27	0.82
Red Thandan	1.59	0.78	0.53
Green Thandan	1.79	1.25	0.74

Above EPA tolerance level (2 ppm) Values are the results of 4 different lots (mean \pm S.E.M.) and the samples on each occasion in duplicate.

TABLE II : COMPARISION OF REDUCTION OF DIMETHOATE RESIDUE IN PROCESSED AND UNPROCESSED LEAFY VEGETABLES

Leafy vegetables	Concentration of dimethoate in unprocessed leafy vegetables (ppm)	Percentage reduction of dimethoate residue in processed leafy vegetables compared to unprocessed	
		Processd (Washed)	Processd (Washed and Cooked)
Palak	2.54	35.83	71.26
Vendhai	2.43	47.70	66.26
Red Thandan	1.59	50.94	66.67
Green Thandan	1.79	30.18	60.89

Above EPA tolerance level (2 ppm) Values are the results of 4 different lots (mean \pm S.E.M.) and the samples on each occasion in duplicate.