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# SYNTHETIC PYRETHROIDES MULTIRESIDUE IN GRAPES FROM SOUTHERN INDIA

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## ABSTRACT
Pyrethroid is a mixture of pyrethrum I and pyrethrum II, extracted from the plant *Chrysanthemum* and is used for ultra low volume applications. Pyrethroids may have a range of toxic effects on humans and as a result, careful control of Maximum Residue Limits (MRL) in foodstuff is warranted. Twenty two samples of grapes were procured from Bangalore City Markets in 2007 with the objective of determining the contamination of α-cypermethrin and fenvalerate residues. The samples were extracted by Multiresidue Method and determined by Gas chromatography using ECD (Electron Capture Detector). Average recoveries ranged from 73.5 to 83.5%. The results showed that all the samples were contaminated with pesticides and urban consumers are at a risk of purchasing fresh fruits because of higher levels of residues, beyond the MRL as defined by the FAO/WHO, and grapes committee. The screening also showed higher concentration of cypermethrin in comparison to fenvalerate residue.

**Key Words:** Cypermethrin, Fenvalerate, Grapes, GC/ECD, Bangalore

## INTRODUCTION
There is increasing pressure in agricultural industry around the world to produce better quality crops in a cost effective manner. Towards this direction programmes to control pests that can potentially cause large-scale economic damage have assumed paramount importance. As a result, pesticides have become now in dependable component of integrated pest control programme. One such class compounds used to control pests are pyrethroids, a mixture of pyrethrum I and pyrethrum II, extracted from the plant *Chrysanthemum* and are used for ultra low volume applications. Pyrethroids are also used in nearly every household as fly spray and they act by interfering with the insect's nervous system. Synthetic pyrethroids, including Allethrin, Cypermetrin, Cyflotrine, Tetramethrin, and Resmetrin act as contact poisons that rapidly penetrate the nervous system. Pyrethroids may have a range of toxic effects on humans and as a result, careful control of Maximum Residue Limits (MRL) in foodstuff is required. However Pyrothroids are amongst the most potent insecticides that have low mammalian toxicity (Elliot, 1977). However, the more recent ones, known as second and third generation pyrethroides carry greater insecticidal property and tend to be more toxic to mammals (Anonymous, 2003). Monitoring of pesticide residues also provides a check on compliance with Good Agricultural Practice (GAP). Many countries in the world have established analytical laboratories to monitor pesticide residues in fruits, vegetables and

other food crops (Dogehim *et al.*, 2001). Analytical methods for pyrethroid insecticides were reviewed (Miyamoto, 1981; Zweig, 1984; Sharma and Cairns, 1993). Further Gas Chromatographic (GC) methods comply multiresidue determination of pyrethroid insecticides in fruits and vegetables and grains (Bolygo, 1983) by using a methyl silicone-coated fused silica capillary column (Nakamura *et al*., 1993). Methods have also been developed that reliably and rapidly detect as many pesticides as possible in a most cost effective manner (Luke *et al*., 1981; Liao *et al*., 1991).

## MATERIALS AND METHODS

Pesticide standards were obtained from all India network project on pesticide residues, division of Agricultural Chemicals, Indian Agricultural Research Institute, New Delhi. The solvents, Anhydrous Sodium Sulfate and activated graphitized charcoal were of Analytical Grade procured from Merck, Fisher Scientific and Sigma Company. A Gas Chromatograph (Shimadzu 2010, Japan) equipped with $Ni^{63}$ Electron Capture Detector was used for analysis. The capillary column of 30 m ×0.25 mm ID with film thickness 0.25µm was used. Column temperature was set at $260^0$ C and detector temperature at $300^0$C with a nitrogen flow rate of 3 ml/min. Fresh grape samples were purchased from two main City Markets (K.R. and Yeshwantpur Markets) and 25 g samples was extracted in a blender with 100 ml solvent mixture (n-hexane: acetone (1+1) and 25 g Sodium Sulfate at high speed for 1.5 min, filtered and re-extracted twice with additional 70 ml aliquot of solvent mixture. The extract was transferred to a 500 ml separating funnel, 200 ml of 2 percent sodium sulfate was added to the separating funnel and shaken vigorously for 2 min. The phases were allowed to separate and the supernatant layer of n-hexane was transferred to a 250 ml round bottom flask. The extract was passed through 4 cm anhydrous sodium sulfate in 250mm×20mm glass column. Dry extract was collected in a conical flask column was washed with 10 ml n-hexane. The combined extract was concentrated on rotary vacuum evaporator. For column clean up 5 ml n-hexane extract was transferred on to a 400 mm × 25mm id glass column containing 10 g florosil, 8 g silica gel, 0.3 g activated charcoal and 2 cm layer of anhydrous sodium sulfate that had been slurry packed with n-hexane. The solution was allowed to pass through the column until the liquid level reached the top of the column and vacuum evaporator. The residues were dissolved in 5 ml HPLC grade n-hexane. From the dissolved residues, eluted with 200 ml of 9:1 hexane: acetone, concentrated to dryness under reduced pressure at 40˚ C on a rotary 1µl was injected to gas chromatograph and peak areas were compared with those obtained from similar injections of standards, (Baker *et al*., 1982; Anonymous, 2001; Anonymous, 2007; Iffat *et al*., 2007 and Mukherjee *et al*., 2007). In fortification or recovery studies, 1ml pesticide of desired concentration (0.1, 0.01µg/ml) was added to 25 g of grapes in a blender jar. Resulting samples were mixed and allowed to stand for 20 min before extraction. Two replicates at each fortification level were prepared. The spike samples were then prepared in the same manner and concentrations were calculated by measuring peaks from extracted current profiles and comparing with those obtained from matrix-matched standard concentrations similar to that of regular samples (Harry *et al*., 1993; Fong *et al*., 1999; Michel *et a*l., 2003 and Anna *et al*., 2004).

## RESULTS AND DISCUSSION

### Recovery studies

GC method was used for analysis of insecticide residues in grapes. Before analysis the efficiency of the method was evaluated as recovery experiment by spiking samples with 2 concentrations of insecticides. For pyrethroid 25 g, well homogenized sample was spiked with known amount of standard insecticide each at 2 levels. For each spiking level, two replicates were extracted with n-hexane and cleaned up on Florosil column and analyzed by GC using ECD. Blank extracting solvent with no added pesticide was also processed in a similar manner. Average recoveries ranged from 73.5 to 83.5% (Table 1). The results confirmed that the analysis by GC/ECD gave almost good recoveries with low reproducibility indicating good performance of extraction, clean up and chromatographic parameters.

Table1. Fortification levels, percent recoveries in grape

| pesticides | Fortification level mg/kg | Recovery % | Average Recovery % |
|---|---|---|---|
| α-Cypermethrin | 0.1<br>0.01 | 83, 64<br>89, 78 | 73.5<br>83.5 |
| Fenvalerate | 0.1<br>0.01 | 56, 92<br>81, 72 | 74<br>76.5 |

### Pesticide residues in grapes

Fresh grape samples were procured from markets and analyzed to assess the residue levels of synthetic pyrethroid insecticides. The samples were extracted, cleaned-up and were analyzed using gas chromatographic techniques equipped with Electron Capture Detector. Since maximum residue limit (MRL) has been fixed for pesticide in grapes, the results are compared with established MRL (Anonymous, 2005; Anonymous, 2006a).

Table 2: Pesticide residues in grapes samples (pyrethroid) mg/kg

| Sample No. | Pesticides | Residue level(mg/kg | MRL(mg/kg |
|---|---|---|---|
| 22 | α-cypermetrin | 0.003-0.051 | 0.50 |
| 22 | Fenvalerate | 0.001-0.231 | 0.02 |

It is observed that all the samples are contaminated with pesticides and the frequency of residue is both below and above the prescribed MRL (Table2 and Fig 1). α -cypermethrin ranged 0.003- 0.386 mg/kg with an average of 0.078 mg/kg. The Fenvalerate varied between 0.002- 0.231 with an average of 0.042 mg/kg. The screening also showed higher concentration of cypermethrin in comparison to fenvalerate. However, only 2 of 22 (9%) samples contained cypermethrin nearer to MRL whereas 8 out of 22 (36.3%) samples contained fenvalerate above MRL (Anonymous, 2006).

Several of reports on the presence of pesticide residue in fruits and vegetables are available. Some recent studies include those by Kumari *et al.,* (2006) with residues of Cypermethrin and Fenvalerate ranging 0.045–0.064 µgg-1, 0.046–0.067 µgg-1 respectively in grapes. The recovery of SP at the spiking levels of 0.25 µgg-1, ranged from 75–98 %. An analysis of 821 routine samples of 71 different types of fruits and vegetables and their processed products residue reported Cypermethrin (0.045–0.064 µgg-1) and fenvalerate (0.046–0.067 µgg-1) in six samples of grapes (anonymous, 2006b). A monitoring programme of pesticide residues in fruit and vegetables from Danish market indicated residues in 54% of the samples of fruit and 13% of vegetables. Residues above the MRL were found in 4% of all samples of fruit and in 1% of vegetables (Anderson, 2001). A simple and rapid liquid chromatographic (LC) method also proved the occurrence of 9 pyrethroid insecticides (biphenthrin, cypermethrin, fenpropathrin, fenvalerate, flucythrinate, methothrin, permethrin, py-115, and tetramethrin) in fruits and vegetables. (Pang *et al*., 1995). Residues of pyrethroids (fenvalerate and cypermethrin in vegetarian and non-vegetarian foods collected from various hotels brought to light residue level of cypermethrin and fenvalerate to be BDL to 0.00 and BDL to 0.001 mg/kg respectively (Reddy *et al*., 2000).

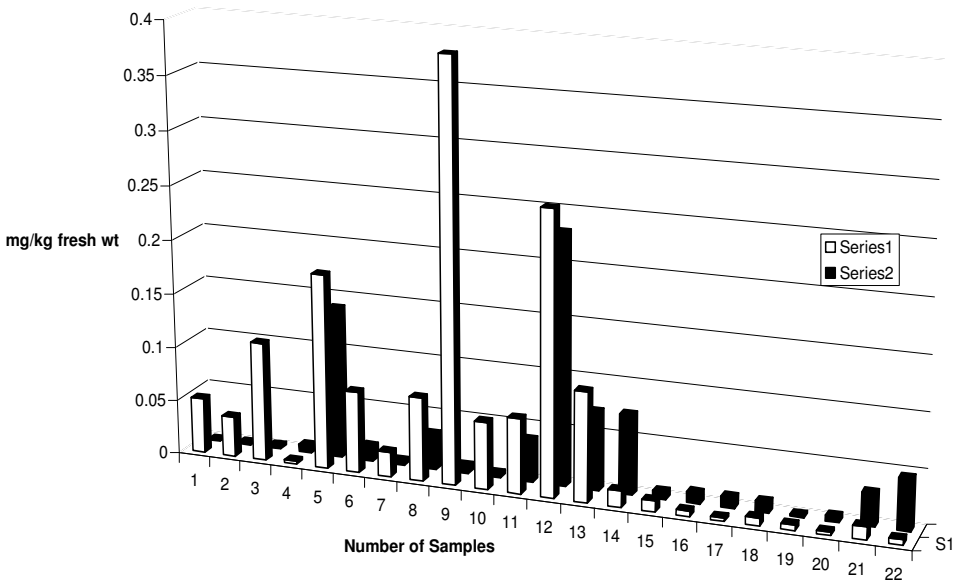


**Figure 1. alfa-cypermethrin and fenvalerate residues in grapes from Bangalore City Markets**

The results of survey indicated that despite majority of growers using agrichemicals in a responsible way residue levels in grapes are higher than MRL and could pose health problems as this popular fruit in consumed regularly by the population. Presence of SP residues in fruits is also an indication of change in usage pattern of insecticides in India where shift has taken place from OC to the easily degradable groups of these insecticides in the last decade. The present findings are in good agreement with these of survey

conducted in India, Pakistan and Egypt (Masud, 1992; Dogheim *et al.,* 2001; Gyana *et al*., 2007; Mukherjee *et al*., 2007). A number of chemicals are in use today in the agricultural sector and they are considered essential to modern agriculture. Contamination of fruits and vegetables with residues may result from treatment as well as conditions such as improper use of pesticide from preceding treatments in the soil and cross contamination. Prevention of health risks including toxicological risks due to food intake is central in food safety policy (Anonymous, 2005). Climatic conditions in Karnataka state are warm, and delicate fruits and vegetables crops are susceptible to pest infestation. However, as prolonged use of pesticides promotes pest resistance, each time higher doses are applied. Against this background, pesticides it is expected that should be used in accordance with good agriculture practices. To avoid residue problems, the rotation of pesticides used to combat the pest is a much recommended practice (Anonymous. 1988). Worldwide surveys on pesticide residue in foodstuff undertaken from the time to time have confirmed the residue levels beyond the permitted standards. The routine monitoring and reporting of data as in the present case would however go a long way in either modifying or amending the standards for different continents or sub continent, or countries whenever are need arises. Besides it also helps in educating farmers and to create public awareness.

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