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Pesticide Residue Analysis in Mango (*mangifera indica*) Samples From Chittoor District, Andhra Pradesh, India

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ABSTRACT

The present study was aimed to identify fifteen commonly used pesticides in commercial mango fruit samples. The analytical screening was performed by GC-ECD/FPD. The pesticide residues in fruit samples were determined and confirmed by their retention time. The retention time of pesticides was compared with that of the reference standard according to standard guidelines. The data showed that among fifteen mango samples tested, five samples were found to contain the following pesticides: 0.024 µg g^{-1} chlorpyrifos (MRL~0.05 µg g^{-1}), 0.007 µg g^{-1} profenofos (MRL~0.05 µg g^{-1}), 0.013 µg g^{-1} hexaconazole (MRL~0.5 µg g^{-1}), 0.0012 µg g^{-1} dimethoate (MRL~1 µg g^{-1}) and 0.589 µg g^{-1} acephate (MRL~2 µg g^{-1}), respectively. The residue levels were found to be within the permissible limits of these pesticides in fruit samples. The results are part of preliminary screening of the presence of pesticide levels in mango samples and the study shows that the levels are within the maximum permissible limits.

KEYWORDS: Gas Chromatography ECD/FPD, Mangoes, Pesticides, QuEChERS method

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INTRODUCTION

Mango (*Mangifera indica*) belongs to the family of *Anacardiaceae* and is one of the most popular consuming delicious seasonal fruits in the world and it is known as 'King of Fruits because of its high nutritive values (vitamins, minerals, antiaging factors, antioxidant and anti cancer property)[1]. If we look at Indian scenario, Andhra Pradesh stands first in mango production in India[2,3,4] and also highest pesticide consuming state[5] because of modern and intensive agricultural practices. Though pesticides are used to enhance the agricultural productivity of crops by protecting from the pests however they are also affecting non-target organisms, thereby causing deleterious effects to the ecosystem and creating a tremendous loss to the biodiversity and human beings[6]. Majority of pesticides (organic /inorganic toxic substances) are seeping into the environment and contaminating our necessary basic needs like air, water, food etc.,[7, 8] causing neuro-degenerative diseases to human beings[9, 10] and other possible health effects including respiratory problems such as asthma[11], chronic obstructive pulmonary disease (COPD) and lung cancer[12, 13, 14, 15], hypersensitivity and also linked with fetal development[16]. Although, there are several reports available on pesticide residues in food samples, however for the first time we focused study on the largest mango producing region in Andhra Pradesh, India. In view of the above, the major objective of the present study was to evaluate pesticide residues in mango (Totapuri) sample obtained from different regions of Chittoor district, Andhra Pradesh, India.

MATERIALS AND METHODS

Pesticide Standards and Stock solutions

Analytical reference standards (Dichlorvos 98.8%, Dicofol 99.3%, phosphamidon 94.8%, Hexaconazole 94.8%, Acephate 98.8%, Dimethoate 99.7%, Monocrotophos 99.9%, Deltametrin 95.2%, Malathion 99.1%, Chlorpyrifos 99.7%, Profenofos 95.0%, Cypermethrin 97.8%, and Phosalone 95.2%, Propiconazole 99.1%, Tebuconazole 99.3%) were purchased from Sigma Aldrich (USA). Stock solutions (500 ppm) were prepared by dissolving in N-Hexane.

Sample collection

Fresh mangoes (Tothapuri) from 15 different adjoining villages of Chittoor District, Andhra Pradesh, India were collected. Mango Samples were brought to the laboratory and analyzed.

Sample Preparations

The collected samples were homogenized by a blended homogenizer. The samples were then extracted as per the QuEChERS protocol as reported by Anastassiades *et al.*[17]. Briefly, an amount of

15 g of homogenized samples mixed with 30 ml of acetonitrile followed by sonication for 3 min. To this 3 g of activated NaCl was added and shaken gently. The extract was centrifuged for 3 min at 3000 rpm. A 16 ml of the upper layer was transferred into a 50 ml centrifuge tubes which contained 9 g anhydrous Na_2SO_4, shaked for 1 min and kept for sedimentation. The 9 ml upper layer was allowed for clean up with 0.4 g PSA and 1.2 g of $MgSO_4$ into 15 ml centrifuge tube. The resultant extract was shaken for 1 min and centrifuged at 3000 rpm. The supernatant (2 ml) was collected and evaporated to dryness. The residue was reconstituted with 1 ml of hexane for the analysis. An aliquot of 1 µl extract was injected into GC with ECD and FPD.

Residue analysis

Residue analysis was performed on Shimadzu GC-2010 equipped with ECD and FPD detectors. The capillary column (EB-5) with a stationary phase of 5% phenyl and 95% dimethylpolysiloxane (0.25 mm film thickness).The injector port temperature and detector temperatures were set at 250^0C and 280^0C, respectively. Nitrogen was selected as a carrier gas with a flow rate of 30 ml min^{-1}, hydrogen was used as a makeup gas with a flow rate of 30 ml min^{-1} and zero air 60 ml min^{-1}. The oven temperature program was set initially at 240^0C with a hold of 3 min, then increase up to 280 at 5^0C min^{-1}, hold for 3 min, then increased up to 320 ^{0}C at 5 ^{0}C min^{-1} with a hold time of 14 min. The split ratio was set at 1:10, the total run time was 40 min.

Method validation

The validation of the analytical method was performed with respect to the construction of linearity, limit of detection (LOD) and limit of quantification (LOQ). Linearity was established by constructing a calibration graph at five different concentrations in the range of 0.1-1 ppm for all pesticides. The limit of detection (LOD) and limit of quantitation (LOQ) were calculated statistically from the calibration curve and linear regression analysis.

RESULTS AND DISCUSSION

Mango samples from 15 different locations were analyzed for the presence of pesticides residue by GC-ECD/FPD. The peak identification was done by comparing the retention time of standard pesticides with that of samples peaks[17,18] that illustrates acephate (RT-10.5 min), chlorpyrifos (RT-17.3 min), dimethoate (RT-14.4 min) hexaconazole (RT- 6.1 min) profenofos (RT-20.4 min) with retention times. The calibration graph was constructed for all the pesticides in the range of 0.1-1 ppm and was found to be linear with an acceptable regression coefficient in the range

of 0.980-0.995. The R^2 values for acephate hexaconazole, chlorpyrifos, profenofos, dimethoate was 0.985, 0.985, 0.995, 0.989, and 0.995, respectively as shown in Fig.1.

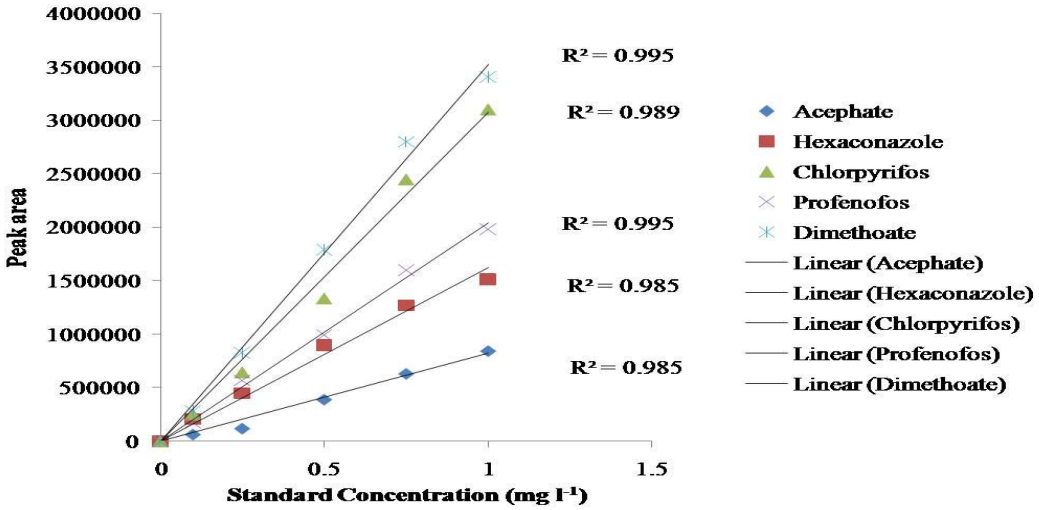

Figure1. Linearity calibration curve for pesticides (Acephate, Hexaconazole, Chlorpyrifos, Profenofos, Dimethoate)

Furthermore, the results of validation parameters LOD (0.04 mg l^{-1}, 0.03 mg l^{-1}, 0.04mg l^{-1}, 0.08 mg l^{-1}, 0.05 mg l^{-1}) and LOQ (0.12 mg l^{-1}, 0.11 mg l^{-1}, 0.12 mg l^{-1}, 0.26 mg l^{-1}, 0.15 mg l^{-1}) for acephate, chlorpyrifos, dimethoate, hexaconazole, profenofos were depicted in Table 1.

Table 1: Retention time of pesticides along with LOD, LOQ and their concentrations insample against MRLs

Name of the pesticide	Retention Time(min)	LO(mg l^{-1})	LOQ(mg l^{-1})	Concentratin of Residues (µg g^{-1})	MRL (µg g^{-1})
Acephate (98.8%)	10.5	0.04	0.12	0.589	2
Chlorpyrifos (99.7%)	17.3	0.03	0.11	0.024	0.05
Dimethoate (99.7%)	14.4	0.04	0.12	0.0012	1
Hexaconazole (94.8%)	6.1	0.08	0.26	0.013	0.5
Profenofos (95.0%)	20.4	0.05	0.15	0.007	0.05

Out of fifteen analyzed mango samples, only five samples were contaminated with pesticides. The pesticides detected in the mango samples were as follows: Hexaconazole, Acephate, Chlorpyrifos in sample 1, Acephate, Chlorpyrifos, Profenofos in sample 2, Dimethoate, Acephate, Chlorpyrifos in Sample 5 and Chlorpyrifos in samples 3 and 4 (Fig. 2). The other samples analyzed were not found to contain any pesticides at the analyzed detection limits. The variability in identification levels of pesticide residues in analyzed samples may be attributed to the usage of pesticides and different climatic/growing conditions[19].

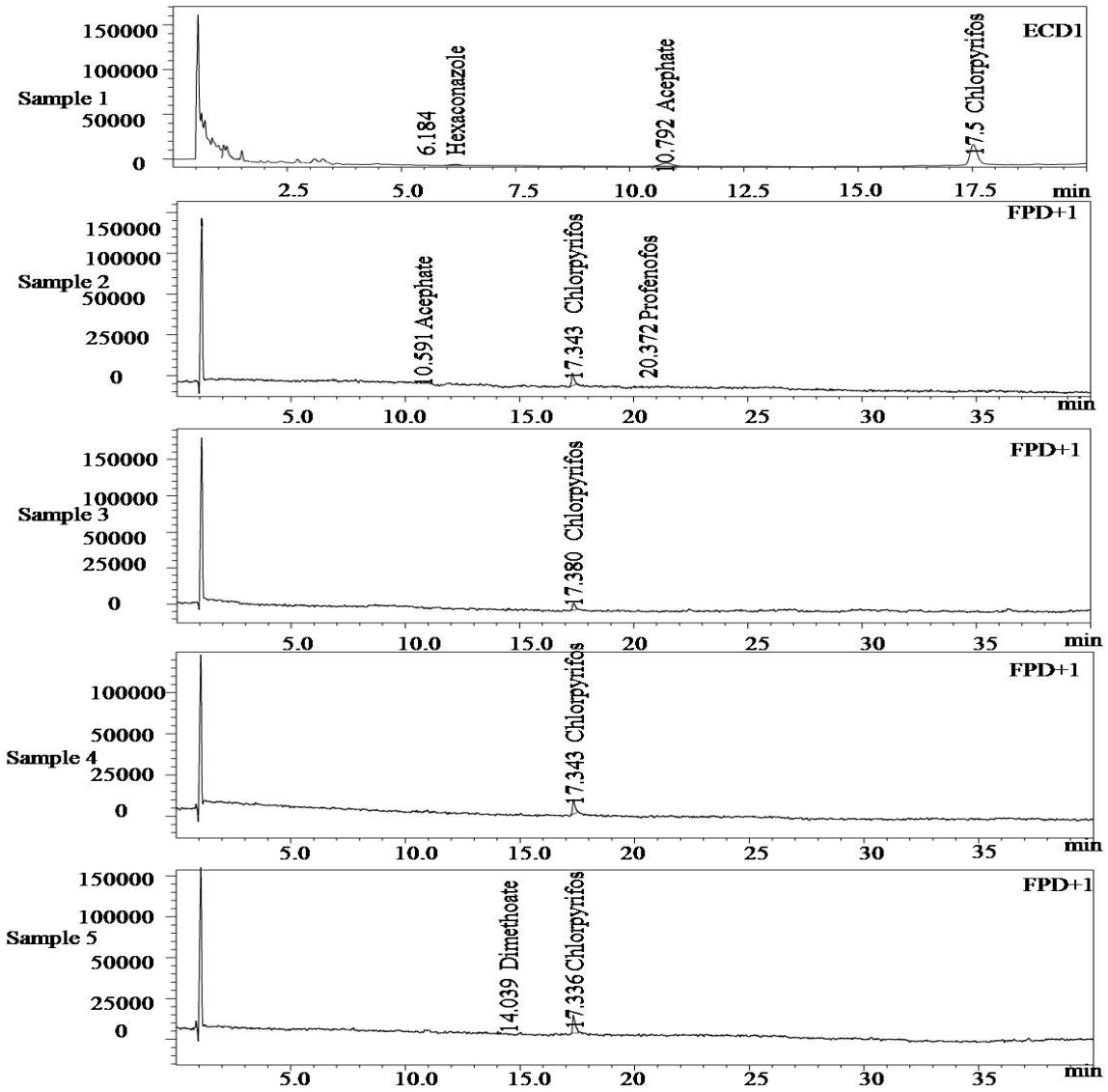

Figure 2. GC ECD/ FPD Chromatograms for mango samples showing their retention time

Despite the number of studies available for monitoring the levels of pesticide residue, a study was undertaken to assess the residues of commonly used pesticides, which provides the baseline information for future policy in chemical usage of sample collected. Published reports on pesticide residue analysis in fruits (ber, grapes, and guava) have shown that the residues of the samples were found below its respected MRLs[20]. In similar lines in the present findings, out of 15 pesticides, only 5 pesticides were identified within the permissible limits and one concentration value of analysed pesticides samples (1-5) against its MRLs (~2 µg g^{-1}, ~0.05 µg g^{-1}, ~1 µg g^{-1}, ~0.5 µg g^{-1} ~0.05 µg g^{-1}, respectively) established from Japan Food Chemical Research Foundation 2010[21] is shown in Table1. This might be due to several factors influencing the fruit such as rain falls[22], the time lag

between pesticide spray, harvesting, storage and transportation[23]. Secondly, most of the farmers might have enough knowledge about the good agricultural practices. Whereas chlorpyrifos was detected in five samples within the concentration range of 0.017 µg g^{-1} – 0.036 ug g^{-1} (0.034 µg g^{-1}, 0.024 µg g^{-1}, 0.027 µg g^{-1}, 0.017 µg g^{-1}, 0.036 µg g^{-1}), and found below the permissible levels. In this manner, one can assume that there is no apparent risk of the health of consumers, but though they are in permissible limits, previous studies states that continuous exposure to extremely low levels of chlorpyrifos during gestation or early pregnancy may cause detrimental effects on critical periods of development, which has been well documented[24]. Particularly it was found to be associated with low birth weight[25] followed by increased body fat in children[26] and finally leads to obesity, cluster of metabolic diseases much later in life[27, 28]. The results further revealed that the traces of other two pesticides like acephate (0.589 µg g^{-1}, 0.002 µg g^{-1}) and profenofos (0.007 µg g^{-1}) were found, which are considered as unapproved pesticides in fruits according to Food Safety and Standards Authority of India (FSSAI) (2014-15 annual report of the Ministry's Department of Agriculture) [29] and people are deliberately bypassing the environmental and health concerns by using these untested pesticides. The fungicide hexaconazole (0.013 µg g^{-1}) was found below detectable levels. According to Liang *et al.*[30], hexaconazole exposure might result in thyroid endocrine toxicity and even low concentrations of hexaconazole may affect the development or lead to malformation of Zebrafish larvae. Improper use of hexaconazole may affect the target organism also by reducing the net photosynthesis of plant[31]. Recently, hexaconazole has emerged as a causative agent for oxidative stress in plants[32] and ultimately it affects the cellular growth in plants[33]. Dimethoate, a widely used organophosphate insecticide, was also detected 0.0012 µg g^{-1} in one of the mango samples. There was experimental evidence that other than chlorpyrifos, dimethoate at low levels is also associated with lowered acetylcholinesterase in the brain of rodents[34]. Few studies have observed that prolonged exposure to low level pesticides can cause harmful health impact on thirteen organ systems[35] in human beings. Hence, the present findings indicate that despite a high usage and occurrence of pesticide residues in fruits, it might not be considered as a serious health concern as it was found below permissible limits. However, it is suggested that continuous evaluation of pesticide residues in mango fruits, and further research on changes of metabolic constituents of fruits may throw light on pesticide usage and its impact on fruit quality.

CONCLUSION

Based on the series of samples analyzed in the present study, <50% of the mango samples were contaminated with pesticides and were below permissible limits. These levels of contamination

might vary according to different agricultural practices. However, further experiments need to be conducted to ensure consistent results.

CONFLICT OF INTERESTS

Declared none

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