Pak J Sci Ind Res 2001 45(4) 246-249

FUNGICIDE RESIDUES IN APPLE AND CITRUS FRUITS AFTER POST HARVEST TREATMENT

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(Received 2 December 2000; accepted 3 May 2001)

Thiabendazole and benomyl fungicides after post harvest treatment, were monitored in samples of apples and citrus fruits collected from the main fruit markets of Karachi during the year 1998-99 and 1999-2000. A total of 150 samples (75 samples of each commodity) were screened out of which 82 samples were found to contain either of the two studied fungicides. Only in 8 samples, Codex exceeded maximum residue limits (MRL's) while 74 samples contained residues well within permissible limits.

Key words: Fungicide residues, Apples, Citrus fruits.

Introduction

With increasing insistence in recent years by importing countries on food shipments being free from pest infestation, periodical pesticide monitoring of agricultural commodities and other food commodities have become absolutely essential. Fruits are abundantly grown in Pakistan and are exported in modest quantities to different countries. In order to ensure quality, it is necessary that they should be free from any pest infestion. For this purpose fruits were sprayed with different pesticides during growth and storage. While pesticides are essential for enhancing agricultural production, their misuse poses a serious threat to human health and the environment.

Egan and Weston (1977) discussed the role of surveys in monitoring pesticide residues in specific foods with particular reference to food surveys in the United Kingdom. Pre and post harvest control of fungal diseases of fruits and vegetables is a major economic issue and a huge investment of resources is required to minimize losses and to preserve quality. Agrochemicals play a major role in food protection and quality preservation. Therefore, thorough monitoring of fungicide residues is crucial for proper assessment of human exposure to fungicides through food. Systemic agrochemicals, with fungitoxic activity, such as benomyl, thiphanate-methyl, carbendazim and thiabendazole are widely used for pre and post harvest protection of crops (Pesticide Manual 1991; Roy *et al* 1997; Broglia *et al* 1999).

Thiabendazole (TBZ) and benomyl are benzimidazole fungicides having systemic properties in plants. The two compounds are best quantitated by liquid chromatography (LC). Numerous LC methods have been developed that use either ultraviolet or fluorescent detection with partition and/or column cleanup (Hiemstra Joosten and Kok 1995, Bushway *et al* 1995, Fucci Cairavol and Mazza 1995; Ogawa *et al* 1998). Methods based on gas chromatography, thin-layer chromatography and enzyme immunoassay have also been reported (Oishi *et al* 1994, Anastassiades and Schwack 1998; Fytianos *et al* 1998). In continuation of our pesticides monitoring programmes in fruits and vegetables, present studies were conducted on screening of two fungicides commonly used for protection of fruits during storage, i.e., thiabendazole and benomyl. Work was carried out in two phases i.e. (i) development of a workable analytical methodology in fruits spiked with known amounts of the fungicides and (ii) monitoring of these fungicides in fruit samples collected from the main selling points of Karachi.

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Materials and Methods

Apparatus. High Performance Liquid Chromatograph (HPLC) Model No.SPD-10A Schimadzu.

Operating conditions. *Light Source*. Deuterium Lamp with changeable wavelength, wavelength=223 nm; Pressure: 2000 PSI; Column C-18 (ODS)-15 cmx6.0 mm i.e., stainless steel; Injection volume: 20ul; Mobile phase: Methanol, Water (1:3); Flow Rate: 0.5 ml min^{-1}. Dynac Centrifuge.

Reagents. Methanol, HPLC Grade; n-Hexane, analytical grade; sodium hydroxide; deionized water; activated charcoal (Merck), Volac disposable pipettes for mini-column, florisil 60-100 us mesh BDH.

Sampling. Seventy five samples each of apples and citrus fruits were purchased from local fruit markets of Karachi from 1998 to 1999. One kg sample of each commodity was procured in accordance with the standard procedure (FAO/WHO 1982).

Samples preparation. A food processor was used to homogenize apple and citrus samples. After homogenisation of each

sample, sodium hydroxide (1M) was added to adjust the pH to 5.5, mixed well and allowed the sample to stand for 10 minutes and then proceeded for analyses.

Fortification. Prior to monitoring studies, the efficiency of analytical methodology was evaluated in model experiments in which homogenized samples of apple and orange were fortified with known quantities of each studied fungicide separately and allowed to stand for six hours. These samples along with a control sample were then carried through the following procedures and finally analyzed for percent recovery by high performance liquid chromatography.

Extraction. Primary evaluation of different solvents was made for extraction of fungicides but petroleum-ether, acetone (1:1) mixture was found to be the best for extraction. 150 ml of extraction mixture was added to 50 gms of each homogenized sample and transferred to a blender jar, blended for 3 minutes and the blended mixture was then centrifuged for 5 minutes at 4000 rpm. The upper layer of organic phase was transferred to a measuring cylinder, its volume was noted and the residue was discarded. The extract was concentrated down to aproxi-mately 2ml in a rotary vacuum evaporator at 40°C.

Clean-up. A mixture of activated charcoal and Florisil (1:10) was used for cleanup step to remove coloured compounds and other impurities from the sample extract. A Volac disposable glass pasteur pipette was plugged with cotton, a small quantity of anhydrous sodium sulphate was poured into it and then a mixture of 0.5gm activated charcoal and Florisil was added with continuous tapping. Little amount of sodium sulphate was added to the column so as to form a bed on top of the column. The column was prewashed with a mixture of petroleum ether and acetone (4:1). After washing, the sample extract was poured and first 2 to 5 drops were discarded. Then the eluate was collected in a vial of 5 ml. Each eluate was exaporated to dryness at room temperature and then taken up in 2 ml of methanol for analysis by HPLC.

HPLC determination. Determination by HPLC was carried out with C-18 (ODS) column using a mixture of methanol and deionized water (5:1) at the rate of 0.5 ml minute^{-1} as mobile phase. Detection of benomyl and TBZ was made with a variable wave length of 223 nm. The retention time of benomyl and TBZ were 7.064 and 7.966 minutes, respectively. The minimum limits of detection for benomyl and TBZ were 100ng 20µl.

20µl aliquots of control and fortified cleanup extracts were injected into the HPLC column and the amount of benomyl and TBZ in fortified sample extract was calculated by comparing its peak areas with that of the standard. Control sample processed in a similar manner did not show any interferring peak that might be attributed to benomyl and TBZ. Percent recovery of the two fungicides is presented in Table 1.

Monitoring studies. One hundred and fifty samples of post treated apple and citrus fruits (seventy five samples of each commodity) were purchased from local fruit markets in Karachi. Each sample was sub-divided into three sub-samples of equal size and subjected to extraction, cleanup, identification and determination according to procedures described above.

Results and Discussion

Studies were carried out in two phases namely, (1) development of analytical methodology involving extraction, cleanup and HPLC determination and (2) sceening of studied fungicides in fruits.

The developed analytical methodology for TBZ and Benomyl is economical, versatile, sensitive and reproducible. The extraction procedure employed in the study has also been successfully used for the extraction of organophosphate and synthetic pyrethroid pesticides in vegetables and fruits. The cleanup procedure employs a mini-column and uses the least possible quantities of solvents and other chemicals. It is inexpensive compared to usual practice of using large columns with greater quantities of materials. Similarly HPLC method is quite reliable and sensitive. The HPLC operating parameters used were suitable for quantifying residues of the two fungicides studied. The linearity of response was confirmed by injecting different concentrations of analytical grade fungicides into HPLC column and noting their peaks. Prior to the actual monitoring programmes, the accuracy and precision of the developed methodology was tested in model experiments by adding known amounts of each fungicide to untreated fresh apple and orange samples and employing the above described analytical procedures.

Table 1
Percent recoveries of Thiabendazole and Benomyl from fortified apple and orange samples

Commodity	Fortification level ppm (µg/g)	Recovery* % ± SE	
		Benomyl	Thiabendazole
Apple	0.1	N.D**	89.72±0.82
	0.5	N.D	90.37±0.75
	1.0	88.79±0.41	96.78±0.84
Orange	0.1	N.D	91.78±0.35
	0.5	N.D	94.42±0.58
	1.0	93.68±0.38	98.26±0.49

*Each value is the average of three replicates. **N.D= Not detected.

Table 2
Benzimidazole fungicide residue in apple and citrus fruits

Name of commodity	No. of samples analyzed	Thiabendazole				Benomyl			
		No. of samples contaminated	Qty found μg kg^{-1}	MRL μg kg^{-1}	MRL exceeded in samples	No. of samples contaminated	Qty. found μg kg^{-1}	MRL μg kg^{-1}	MRL exceeded in samples
Apple									
Amri	15	02	0.66-1.32	10	-	04	0.22-34.13	05	02
Golden	16	06	0.09-9.07	"	-	03	1.09-6.37	"	01
Mashhadi	15	04	0.03-1.92	"	-	05	Traces-6.7	"	02
Summer	15	04	0.05-5.33	"	-	02	2.15-5.57	"	01
White Kulu	14	02	1.32-2.57	"	-	02	Traces-4.1	"	-
Citrus									
Grape fruit	22	06	0.13-2.71	10	-	09	3.6-8.57	10	-
Kino	26	08	0.26-4.28	"	-	07	0.21-5.66	"	-
Orange	27	08	0.07-4.64	"	-	10	0.13-13.06	"	02

Total samples, 150; Contaminated samples, 82; % samples contaminated, 53.66; % of samples exceeding MRL, 5.33.

Over-all average recoveries for TBZ and benomyl were greater than and equal to 89% respectively at three fortification levels and in both matrices tested (Table 1).

Entire apple including its peel and pulp, and with juice in case of ctirus fruits were analyzed for fungicide residues because true measure of human dietary exposure must be made from analysis of edible portions.

A comparative picture of analytical data for two years apple and citrus fruit samples are given in Table 2. The studied benzimidazole fungicide residues were found in 53.66% during two years survey, out of which 5.33% samples had residues greater than the maximum residue limits (MRL).

Amongst the TBZ, 18 out of 75 apple samples and 22 out of 75 of citrus samples were found contaminated and did not exceed the MRL. Benomyl residues were confirmed in 16 out of 75 apple samples and 6 out of 16 samples were found to contain residue higher than the FAO/WHO recommended MRL (Table 2). Extremely high value of 34.13 ppm was present in a sample of apple (Amri variety). In case of citrus fruits 26 out of 75 were found contaminated and only in two orange samples the MRL was exceeded. In the rest of the contaminated fruit samples, TBZ and benomyl fungicide residues were within permissible limits.

Conclusion

The conclusive results obtained in this investigation highlight the seriousness of the problem and it should be tackled prudently. Pesticides should be applied strictly in accordance with "good agricultural practice". Shelf life of each pesticide after post harvest treatment of fruits/vegetables should be determined and the commodity should be brought to market only after the residues have come down to permissible levels. For this purpose, pesticide monitoring at this stage must be made mandatory.

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