Pertanika J. Trop. Agric. Sci. 17(3): 145-148 (1994)

ISSN: 0126-6128

The Effect of Dosage and Storage Time on the Formation of Bound Residues in Paddy, Milled Rice and Maize

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Keywords: malathion, dose, storage time, grains, bound residues

ABSTRAK

Padi, beras dan jagung yang dirawat dengan malathion berlabel membentuk sisabaki ^{14}C terikat. Setelah disimpan selama 3 bulan, amoun sisabaki terikat yang dihasilkan dalam padi, beras dan jagung yang dirawat dengan 10μg/g malathion masing-masing adalah 10.8, 6.5 dan 13.3% daripada dos yang digunakan. Setelah 6 bulan, amaunnya ialah 14.2, 4 dan 17.7% masing-masing. Peratusan sisabaki terikat yang dihasilkan tidak meningkat apabila dos rawatan malathion dinaikkan kepada 50μg/g. Walau bagaimanapun jumlah sebenar sisabaki terikat yang dihasilkan meningkat lebih dari 4 kali ganda dalam semua bijiran yang diuji.

ABSTRACT

Stored unmilled rice (paddy), milled rice and maize treated with radiolabelled malathion formed bound ^{14}C residues. After 3 months of storage, the bound residues in the grains treated with 10μg/g malathion accounted for 10.8, 6.5 and 13.3% of the applied dose in paddy, rice and maize respectively. After 6 months, the corresponding values of bound residues were 14.2, 4 and 17.7% respectively. Increasing the dosage of malathion to 50μg/g does not significantly increase the percentage of the bound residues formed. However, the absolute amount increased by more than 4-fold in all the grains tested.

INTRODUCTION

Organophosphorus insecticides are widely used in agriculture. The toxicity of this class of insecticide is due mainly to its inhibitory effect on the enzyme cholinesterase (Eto 1974). Malathion is still being used in the local granaries to control storage pests although its usage has been reduced. Spraying of malathion in the local godown is normally conducted using a thermal fogger or mist blower. The milled or unmilled rice (paddy) is stored in jute sacks or polyethylene bags. It has been shown that malathion applied to bagged milled rice results in penetration of malathion through the jute sacks into the rice/paddy grains (Arshad and Salehudin 1988). Due to the insecticide's low mammalian toxicity, in some countries malathion is mixed directly with the grains (Zayed *et al.* 1992). It has been shown that insecticides in general form non-extractable residues in grains (Khan 1982; Kovacs 1986). The chemical nature of the non-extractable malathion residues in grains and its biovailability to mammals is generally unknown. Therefore, information on the amount and nature of bound pesticide residues on the grains under controlled conditions is important in managing their use.

The present study reports on the formation of bound malathion residues in paddy (unmilled rice), milled rice and maize. The effects of insecticide dosage and storage time on the formation of bound residues are also investigated.

MATERIALS AND METHODS

Chemicals

^{14}C-malathion (o,o-dimethyl S,1,2-diethoxy carbonyl,1,2-^{14}C ethyl phosphorodithioate) (1,2-ethyl-^{14}C label) with specific activity of 112 mCi/mg was purchased from Amersham Corporation. The insecticide was approximately 98% pure as determined by thin-layer chromatography. Technical non-labelled malathion of 99.5% purity was obtained from the American Cyanamid Company.

Treatment of Samples

Freshly harvested paddy and milled rice, variety MR 71, were obtained from a godown in Malacca. Maize was obtained from the University farm. All the grains were obtained from crops that had not been treated with insecticide.

Samples of 0.5 kg each of milled rice, paddy and maize were treated with 10µg/g equivalent of non-labelled malathion and ^{14}C-malathion of specific activity 4.43 x 10^7 dpm/mg. This was prepared by dissolving 0.89 mg of labelled malathion and 4.11 mg of non-labelled pure malathion in hexane (residue grade). One ml of the insecticide solution was pipetted at a time into the glass jar containing the grains. The jars were then capped and the grains thoroughly mixed by manually shaking and turning the jars end over end. The jars were then uncapped and placed in a fume cupboard to allow the hexane to evaporate completely. Grains were also treated using 50µg/g equivalent of malathion. Portions were removed at 0, 1, 3 and 6 m for analysis. All samples were analysed in triplicate.

Residue Analysis

External Residue (washable residue)

Twenty-five grams of the grain samples were washed with 250 ml of distilled water. The washing was collected and analysed for radioactivity. The process was repeated several times and each washing was counted individually.

Extractable Residue

The washed grains were dried and ground thoroughly using a mortar and pestle. These were then extracted with 400 ml of methanol (residue grade) using a Soxhlet apparatus. Extraction was carried out for 24 h. The methanolic extract was then tested for radioactivity.

Bound Residue

The grains left in the thimble of the Soxhlet apparatus were dried in the oven and then taken for dry combustion in a Harvey Biological Oxidizer to determine the quantity of bound residue.

Total Residue

The levels of the total residue in the grains at the stated time intervals (i.e. 0, 1, 3 and 6 m) were determined by the dry combustion procedure using the Harvey Biological Oxidizer.

Determination of Radioactivity

Liquid samples (e.g. solvent extracts) were assayed in a Hewlett Packard Liquid Scintillation Counter, Tri-carb Model 460C using a standard cocktail (333 ml Triton X-100, 5.5 g PPO, 100 mg POPOP and 667 toluene). Radioactivity in the solid samples was determined after combustion in the Harvey Biological Oxidizer using the cocktail supplied by R.J.Harvey Instruments Corporation, New Jersey.

RESULTS AND DISCUSSION

The moisture content of the rice grain, paddy and maize was 13.1, 12.3 and 12.5% respectively. This was determined by oven drying the grains at 130°C for 18 h.

Table 1 shows the results of the level of malathion residues in the untreated paddy (unmilled rice). At 0 time, the level of bound residue was 4.7% of the total applied radioactivity. After 3 m, the level rose to 10.8% of the total radioactivity applied. Longer storage time (6 m) resulted in a slight increase in the percentage of bound residues formed. The amount of bound residues formed was 14.2% of the applied dose.

The table also shows the level of malathion residues in the treated milled rice grains determined at various time intervals. The results indicate that very little of the pesticide is bound to the grains. Most of the residues are found in the extractable or washable and the methanolic extracts. After 6 m of storage, the bound residues account for only about 4% of the total residues. Thus longer storage time does not increase the total amount of bound residues formed.

A similar pattern is observed in maize. The amount of external residues obtained is the total of extractable and external residues. The amount of bound residues obtained after 3 m was 13.3%. After 6 m of storage, the level increased slightly to 17.7%.

The low level of bound malathion residues in milled rice which comprised mainly starch and endosperm may be due to the removal of the hull/bran and germ region during the milling process. It has been suggested that bound residues are associated with the hull/bran and germ region of the grains (Arshad and Salehudin 1988).

TABLE 1

Distribution of external, extractable and bound malathion residues in paddy, milled rice and maize treated with 10μg/g malathion as a function of storage time. Results are expressed as percentages of applied dose. Results are the mean of triplicates

Grains		Storage Time (months)			
		0	1	3	6
		Residues (% of the applied dose)			
External	Unmilled rice	65 ± 2.80	52.6 ± 3.68	39.8 ± 0.59	40 ± 2.94
	Milled rice	90 ± 6.37	91 ± 8.49	84.3 ± 3.18	80 ± 4.55
Extractable	Paddy	23.6 ± 2.22	35 ± 2.2	45 ± 3.08	44.7 ± 4.77
	Milled rice	4.0 ± 0.37	4.8 ± 0.64	8.0 ± 0.96	11 ± 1.08
	Maize (external + extractable)	93 ± 5.72	86 ± 58.4	76 ± 3.91	75.8 ± 8.24
Bound	Paddy	4.7 ± 1.2	5.4 ± 0.63	10.8 ± 2.62	14.2 ± 3.12
	Milled rice	1.5 ± 0.72	0.5 ± 0.14	6.5 ± 0.40	4.0 ± 1.10
	Maize	1.4 ± 0.71	5.6 ± 1.87	13.3 ± 2.87	17.7 ± 2.81

Rowlands and Bramhall (1977) reported that malathion residues were found chiefly in the germ and scutellum which probably explains the relatively high amount of bound residues formed in maize.

Table 2 shows that the amount of bound residues formed is dose dependent. The absolute amounts of bound residues formed in paddy, milled rice and maize using 10μg/g labelled malathion were 1.7, 0.69 and 1.33μg/g respectively. The amounts formed when the applied dose was increased to 50μg/g were 5.99, 2.91 and 7.25μg/g respectively. These correspond to 5.4, 4.2 and 5.5 fold increases in the amount of bound residues formed in paddy, milled rice and maize respectively. It appears, therefore, that although the percentage of the applied dose does not increase significantly, the absolute amounts of bound residues formed increased by more than four times. Therefore, it is important that the correct amounts of malathion be used in the control of pests as indiscriminate use may result

TABLE 2

Amount of bound residues formed after 3 months storage as a function of dose applied in unmilled rice, milled rice and maize. Results are the mean of triplicates. Bound residues are formed as described in the text

Grains	Malathion Applied			
	10 μg/g		50 μg/g	
	Bound Residues Formed			
	μg/g	%	μg/g	%
Unmilled rice	1.1 ± 0.49	10.8 ± 2.08	5.99 ± 1.62	12 ± 3.26
Milled rice	0.69 ± 0.26	6.9 ± 2.68	2.91 ± 0.75	5.8 ± 0.82
Maize	1.33 ± 0.12	13.3 ± 1.2	7.25 ± 1.24	14.5 ± 2.49

in the formation of bound residues that may be potentially hazardous to humans.

Although relatively low amounts are bound in milled rice, little is known on its biovailability and hence its potential hazard to humans. Unmilled rice (paddy) is not normally consumed by humans directly. Since very little residues are found in milled rice after treated paddy is milled, bound residues are only significant if the bran is used for consumption. However, the amount of bound residues obtained in maize is substantially higher. Therefore, it is essential that further studies be carried out to determine its biological activity and bioavailability in animals.

ACKNOWLEDGEMENTS

This work was supported in part by a research grant (Contract No.4491/RB) from the International Atomic Energy Agency (IAEA).

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(Received 19 May 1993)