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Non-toxic Washing Solutions for Decreasing Myclobutanil, Fenhexamid and Boscalid Residues in Sweet Pepper and Cherry Tomatoes

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Abstract: Four aqueous solutions of 5% sodium carbonate, 5% sodium hypochlorite, 5% glycerol, 5% acetic acid in addition to tap water were evaluated for their removal efficiency of myclobutanil, fenhexamid, boscalid residues from sweet pepper and cherry tomato. The tested crops were treated with the recommended rate of application of myclobutanil, fenhexamid and boscalid. The initial residues of the investigated pesticides before washing treatments in pepper samples were higher than in tomato samples. The tested washing solutions showed similar behaviour in both crops, but the efficiency was slightly different due to the different initial concentration. Tap water was the most effective treatment in reducing myclobutanil residues in pepper and tomato samples but with no significant difference with sodium carbonate, sodium hypochlorite and acetic acid. For fenhexamid, tap water treatment was significantly more efficient in reducing its residues than the other tested washing solutions. The reduction percents of fenhexamid residues by sodium carbonate, sodium hypochlorite and acetic acid were insignificantly different in both crops. Glycerol treatment was significantly the least effective treatment in removal of myclobutanil and fenhexamid residues in both crops. Despite of the seemingly higher efficiency of tap water treatment in removing boscalid residues, statistical analysis showed that there were no significant differences between all tested washing solutions in both crops. The residues of the investigated pesticides after pesticide treatments in both crops were generally higher than the MRL but the tested washing solutions showed significant efficiency in removing the residues to be below than the MRL in most cases.

Key words: myclobutanil, fenhexamid, boscalid, residues, pepper, tomato, washing, reduction and GC/ECD.

INTRODUCTION

Vegetables are prone to many pest attacks (Abou-Arab, 1999). Chemical pesticides provide the effective solution for controlling most pests. Therefore, pesticide residues on the produce are unavoidable, making risk on human health. Egyptian market is still suffering low level of surveillance for food quality check especially in concern of pesticide residues. No set MRL (Maximum Residue Limit) under Egyptian conditions and no market control for legal residues content. All these reasons are putting the Egyptian customer under risk of consuming illegal amounts of pesticides residues. Sweet pepper and cherry tomato are eaten fresh, in salads or in food decoration with no cooking treatments, thus increases the effect on the consumers (Abou-Arab 1999).

Fresh products washing is the main defense line for the blind customer who doesn't know how contaminated his food is. Washing is a major step in most of food processing and household treatments (Radwan *et al* 2005), e.g. cooking, drying, peeling and juicing to allow for effective pesticides decontamination (Cengiz *et al* 2006 and 2007). Different types of non toxic chemicals were reported to enhance the efficiency of washing process e.g. acetic acid (Soliman, 2001) and hydrogen peroxide dilute solutions (Pugliese *et al*, 2004) and (Rawn *et al* 2008). 53% of azinphos-methyl residue was removed from apple fruit with the water wash. Chlorine wash at 50 and 500 ppm removed about 76% and 83% of the pesticide residue, respectively (Ong *et al*., 1996). Myclobutanil is used primarily for powdery mildew (*Uncinula necator*) control in vines and for combined leaf scab and powdery mildew control in apples. Myclobutanil is a moderately toxic

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compound, it inhibits ergosterol biosynthesis (EBI-s) (Athansapolus *et al*, 2003). Fenhexamid is used for control of *Botrytis cinerea*, *Monilia* spp. and related pathogens in grapes, berries, stone fruit, citrus, vegetables and ornamentals. It is one of the new designated (reduced risk pesticides) (Angioni *et al*, 2004). Boscalid is a widely used fungicide for controlling powdery mildew, *Alternaria* spp., *Botrytis* spp., *Sclerotinia* spp. and *Monilia* spp. on a range of fruits and vegetables.

In this paper we are investigating the effect of some non-toxic chemical solutions that can be used on industrial and household levels on washing out certain pesticide residues of different chemical groups, Fig. (1) and posses different physical properties, Table (1), myclobutanil (triazole), fenhexamid (hydroxyanilide) and boscalid (carboxamide). Not much work has been done to investigate the effect of washing on these compounds, so the authors are taking the advantage to explore their behaviour with the tested treatments as well.

Table 1: physical properties of the tested pesticides.

Compound	Water solubility	K_{ow} logP
Myclobutanil	142 mg/l (25 °C)	2.94 (pH 7-8, 25 ºC)
Fenhexamid	20 mg/l (pH 5-7, 20 °C)	3.51 (pH 7, 20 °C)
Boscalid	4.6 mg/l (20 °C)	2.96

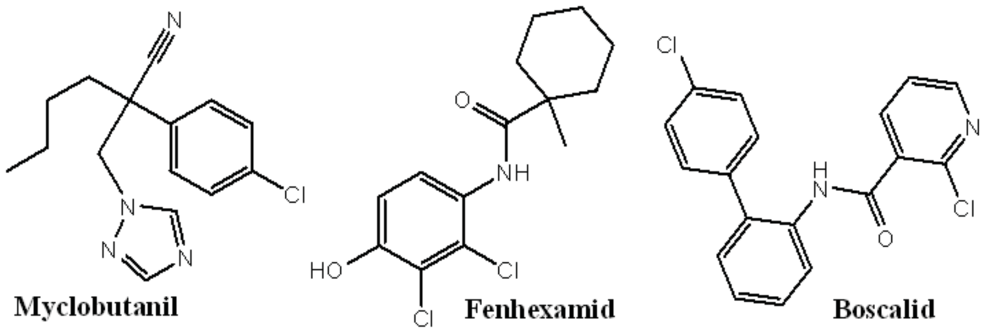

Fig 1: Chemical structure of the tested pesticides.

MATERIALS AND METHODS

1. Chemicals:

1.1. Pesticide Reference Material:

Myclobutanil, 2-(4-chlorophenyl)-2-(1*H*-1,2,4-triazol-1-ylmethyl)hexanenitrile), fenhexamid, (2',3'-dichloro-4'-hydroxy-1-methylcyclohexane carboxanilide) and boscalid, (2-chloro-*N*-(4'-chlorobiphenyl-2-yl)nicotinamide) pure materials up to 98% were obtained from Sigma-Aldrich.

1.2. Pesticide Formulations:

Systhane 12.5% EC (Dow AgroSciences) of myclobutanil Teldore 50% SC fenhexamid (Bayer CropScience) and Bellis 25.2% WP of boscalid (BASF) were all purchased from the local market.

1.2. Reagents:

Sodium carbonate, acetic acid and glycerol were obtained from EL-Nasr Chemicals Company, Cairo, Egypt and commercial Sodium hypochlorite 5% (Clorox) was obtained from local market. Acetonitrile 99% purity was purchased from **Riedel**-de Haën, Uk. Sodium chloride and anhydrous sodium sulfate (activated at 250 °C overnight prior analysis) were obtained from El-Nasr Chemicals Company.

2. Chromatography:

Analysis were carried out on an Agilent GC 6890N network GC system, equipped with microECD detector and Chemstation software. compounds were separated on HP-5 (J&W Scientific, Folsom, CA USA) fused-silica capillary columns 30 meters length, 0.25 mm internal diameter and 0.25 microns film thickness. Auto injector 5973 Agilent technologies 7683B series.

2.1. Chromatographic Conditions:

A multi residue method was implemented under the following conditions:

The injector was in the splitless mode at 240 C. carrier gas N2 was used with 3 ml per min. oven temperature program as follows 220 C for 2 min then to 260 in 3 C / min rate and hold for 10 min. Retention times for the tested compounds were 5.23, 7.26 and 15.17 for myclobutanil, fenhexamid and boscalid, respectively

2.2. Calibration Curves:

Stock solutions (100 ppm) of myclobutanil, fenhexamid and boscalid were prepared in toluene. Matrix matched calibration standard at the concentration of 0.05, 0.1, 0.5 and 1 mg/kg were prepared. Each concentration was injected under the mentioned chromatographic conditions twice. The peak area was plotted against each concentration and the calibration curve for each pesticide was established. Values of r^2 were 0.995, 0.989 and 0.991 for myclobutanil fenhexamid and boscalid, respectively.

3. Recovery Study:

Untreated pepper and cherry tomato were spiked with the tested pesticides at 0.50 mg/kg and extracted using QuEChERS method stands for (Quick, Easy, Cheap, Effective, Rugged and Safe). Each recovery was replicated three times. The recovery percents reached (76, 95 and 112 % in tomato; 73, 91 and 108% in pepper for myclobutanil, fenhexamid and boscalid, respectively.

4. Sample Treatment:

4.1. Pesticide Treatment:

Sweet pepper and cherry tomato samples were obtained from a cooperative farm, the samples were not sprayed previously with the tested compounds and the analysis showed no trace of the tested compounds.

Entire pepper and cherry tomato fruits were immersed in the pesticides mixture of Systhane, Teldor and Bellis for 3 min. treated vegetable units were taken out and left to dry out for 24 hours. Three replicates, 250 g each, were taken to determine the initial residue level and the rest was used for the washing treatments.

4.2. Washing Treatments:

The washing treatments consisted of immersing the units of the treated and pepper tomato in the aqueous washing solution for 3 min, followed by spraying for 15 seconds with tap water with gentle rotation by hand. The assayed washing solutions were the following: tap water, 5% aqueous sodium carbonate, 5% aqueous Sodium hypochlorite, 5% aqueous glycerol and 5% aqueous acetic acid.

5. Sample Extraction:

The QuEChERS method was used with some modification for the sample extraction and clean-up, 10 g of homogenized sample was weighed into a Teflon 50 ml centrifuge tube and 10 ml 1% acetic acid in acetonitrile was added. The tubes were hand shaken for 1 min before adding 5 g of activated anhydrous sodium sulfate and 1 g sodium chloride and hand shaken again for another 1 min. the tubes was centrifuged at 4000 RPM for 5 min then 1 ml aliquot of the acetonitrile extract were transferred to 2 ml dispersive SPE clean up tube containing 25 mg PSA and 150 mg sodium sulfate anhydrous and shaken for 0.5 min then centrifuged at 4000 RPM for 5 min then 0.5 ml was taken in a vial for GC determination.

***6. Statistical Analysis*:**

Analysis of variance (ANOVA) test and means separation test, Least Significant Differences (LSD) were performed for the obtained results at the significance level (0.05) using SAS software (1996).

RESULTS AND DISCUSSION

Data in Table (2) showed that the initial residues in pepper before washing treatment of the tested pesticides were 2.09, 2.29 and 0.64 $mgkg^{-1}$ for myclobutanil, fenhexamid and boscalid, respectively. The reduction rates (Table 3) of myclobutanil residues in pepper after washing treatments were 35.75, 24.13, 27.43, 17.30 and 29.74 for tap water, sodium carbonate, sodium hypochlorite, glycerol and acetic acid, respectively. However, fenhexamid residues were reduced by 53.76, 41.37, 34.89, 19.87 and 29.42 % for tap water, sodium carbonate, sodium hypochlorite, glycerol and acetic acid, respectively. In general all washing treatments showed high reduction percents of boscalid residues reached 65.47% (tap water), 64.0% (sodium carbonate), 52.33%

(sodium hypochlorite), 45.44% (glycerol) and 53.06% for acetic acid.

The statistical analysis revealed that, glycerol treatment was significantly the least effective treatment in reducing myclobutanil residues. No significant difference was observed in the reduction percent of myclobutanil residues treated by tap water, sodium carbonate, sodium hypochlorite and acetic acid. For fenhexamid, tap water treatment was significantly more efficient in reducing its residues than the other tested washing solutions.

Table 2: Initial deposit and MRL values for the tested pesticides in pepper and tomato, according to EU legislations.

Compound	Initial residues in pepper	MRL pepper $mgkg^{-1}$	Initial residues in tomato	MRL tomato $mgkg^{-1}$
Myclobutanil	2.09	0.5	1.08	0.3
Fenhexamid	2.29	2	1.59	1
Boscalid	0.64	2	0.59	1

Furthermore, sodium carbonate, sodium hypochlorite and acetic acid were insignificantly different. Glycerol treatment was significantly the least effective treatment in removal of fenhexamid residues. Despite of the seemingly higher efficiency of tap water treatment in removing boscalid residues, statistical analysis showed that there were no significant differences between all tested washing solutions.

Table 3: pesticides residues remained and reduction percent in pepper after treatment.

	Myclobutanil			Fenhexamid			Boscalid		
	Conc. found	Reduction %	RSD	Conc. found	Reduction %	RSD	Conc. found	Reduction %	RSD
Tap water	1.34	35.75	11.78	1.06	53.76	11.95	0.22	65.47	10.31
Sodium carbonate	1.58	24.13	11.31	1.34	41.37	12.75	0.23	64.0	9.17
Acetic acid	1.47	29.74	22.52	1.62	29.42	40.65	0.30	53.06	22.15
Sodium hypochlorite	1.52	27.43	3.45	1.49	34.89	5.55	0.31	52.33	8.90
Glycerol	1.73	17.30	14.46	1.83	19.87	17.37	0.35	45.44	14.38

The initial residues before washing treatments in tomato samples (Table 2) were 1.08, 1.59 and 0.59 mg/kg for myclobutanil, fenhexamid and boscalid, respectively. Data in Table (4) revealed that reduction percents, in tomato, of myclobutanil residues were 30.04, 27.58, 29.18, 13.33 and 28.07% for tap water, sodium carbonate, sodium hypochlorite, glycerol and acetic acid, respectively. However, fenhexamid residues were removed by 49.9% (tap water), 52.72% (sodium carbonate), 47.65% (sodium hypochlorite), 28.10% (glycerol) and 53.44% (acetic acid). The decontamination percents of boscalid residues were 41.25, 51.42, 52.53, 43.37 and 48.93% for tap water, sodium carbonate, sodium hypochlorite, glycerol and acetic acid, respectively.

Table 4: pesticides residues remained and reduction percent in tomato after treatment.

	Myclobutanil			Fenhexamid			Boscalid		
	Conc. found	Reduction %	RSD	Conc. found	Reduction %	RSD	Conc. found	Reduction %	RSD
Tap water	0.75	30.04	4.75	0.79	49.99	7.68	0.35	41.25	17.68
Sodium carbonate	0.78	27.58	3.80	0.75	52.72	29.48	0.29	51.42	8.87
Acetic acid	0.77	28.07	24.41	0.74	53.44	24.27	0.30	48.93	4.99
Sodium hypochlorite	0.76	29.18	16.92	0.83	47.65	22.01	0.28	52.53	8.19
Glycerol	0.93	13.33	36.18	1.14	28.10	38.64	0.33	43.37	14.16

The statistical analysis of the obtained data showed that myclobutanil residues were efficiently removed by tap water treatments but with insignificant differences with the other examined washing solutions. For fenhexamid, glycerol treatment was significantly the lowest treatment in reducing the residues than the other tested solutions. On the other hand, tap water, sodium carbonate, sodium hypochlorite and acetic acid showed slight differences in removal of fenhexamid residues. Nevertheless, all tested solutions showed good elimination of boscalid residues with insignificant differences.

Examination of the obtained data clearly indicated that the initial residues of myclobutanil, fenhexamid and boscalid in pepper samples were higher than in tomato samples. This could be attributed to that the surface area in pepper is higher than in tomato, (Hanafi *et al* 2010) found that boscalid initial deposit in spring onion was approximately 7 times higher than in green beans, as the surface area in spring onion is much higher than in green beans.

Tap water treatment was the most effective washing solution in reducing myclobutanil residues in both tomato and pepper samples; this finding is due to high water solubility of myclobutanil (142 mg/l) (The pesticides manual). Cabras *et al*. (1999) reported that home washing with water removed about 50% of imazalil residues and 90% of thiabendazole's from oranges. On the other hand, acetic acid and sodium carbonate treatments did not show better reduction percents of myclobutanil residues than tap water in both crops.

Also fenhexamid residues were significantly removed by tap water in pepper and tomato samples, this finding is in harmony with Christensen, *et al* (2003) finding, that the average reduction of fenhexamid residues by rinsing process was 34% in strawberry fruits. Although the water solubility of myclobutanil is 7 times higher than the water solubility of fenhexamid, the reduction percents of myclobutanil residues by water treatments is lower than it appeared for fenhexamid residues in both crops. An explanation for this finding might be that myclobutanil, the most water soluble (142 mg/l) (Table 1), is a systemic pesticide and it is transported to the inner parts of the crops so it is not easily subjected to the removal process while fenhexamid (water solubility 20 mg/l) (Table 1) is not systemic one and mainly expected to be present on the plant surface so it is more subjected to washing. Christensen, *et al* (2003) found that the rinsing process in strawberry showed that tolylfluanid, (not systemic) and the least soluble in water, was reduced the most, while pyrimethanil, (systemic) and the most soluble in water, was reduced the least and this finding is might be attributed to the systemic/ non-systemic properties of the pesticides.

Sodium hypochlorite solution showed similar effect of tap water treatment in reducing myclobutanil and fenhexamid residues in tomato and pepper. This is because of the actual concentration of sodium hypochlorite solution was very diluted (0.25% in water) also this result is in harmony with (Pugliese *et al*, 2004) who reported that sodium hypochlorite solution was not more effective than tap water in removing the tested pesticides from nectarines samples. Furthermore, glycerol solution showed the lesser reduction percents than the other treatments of myclobutanil and fenhexamid in tomato and pepper samples. This is due to the viscosity of glycerol solution (Pugliese *et al*, 2004).

However, all examined washing solutions showed, in general, similar reduction percents of boscalid residues in both crops. This might be due to the type of boscalid formulation. Boscalid is formulated as wettable powder which means that the residue is in the form of very fine particles on the fruits surface after pesticides treatment, these fine particles will be easily removed by any washing solution. Cabras *et al* (1998) reported that diazinon, bitertanol, iprodione, phosalone, and procymidone were adsorbed during treatment on the dusts which located upon plum fruits surfaces and washing removed both the dust and the adsorbed residues. Angioni *et al* (2004) mentioned that if at the time of the treatment some dust from soil is present on fruit surface, pesticides will settle on it. Since the dust is easily removed by water, the effectiveness of residue removal form strawberry fruits will depend on the amount of dust on the fruit surface.

Conclosion:

In conclusion, the residues of the investigated pesticides after pesticide treatments in both crops were generally higher than the MRL but the tested washing solutions showed significant efficiency in removing the residues to be below than the recommended MRL in most cases and longer soaking time might help in the other cases when reduction didn't reach below MRL. Soliman (2001) demonstrated that washing with tap water and/or other solutions such as sodium chloride and acetic acid solution effectively reduces organo-chlorine and organo-phosphorus pesticides from the surface of potatoes.

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