Bull. Environ. Contam. Toxicol. (2001) 66:421–426

DOI: 10.1007/s00128-001-0022-1

Bulletin of Environmental Contamination and Toxicology

Contamination of Medicinal Herbs with Organophosphorus Insecticides

M. T. Ahmed, N. Loutfy, Y. Yousef

Plant Protection Department, Faculty of Agriculture, Suez Canal University, Ismailia, Egypt

Received: 9 October 2000/Accepted: 8 February 2001

The demand for medicinal herbs in recent years is significantly increasing. Many people are refraining from using synthetic pharmaceutical drugs and resorting to the more safe, naturally occurring products. This high demand is reflected in the increasing acreage of medicinal and aromatic crops. Consumers of medicinal plants are expecting these herbs to be the products of good agriculture practices, with no residues of pesticides. The presence of high residues of such chemicals can have a detrimental effect on the marketing of these herbs, specially those prepared for exports as regulations in most of the importing countries are very strict. On the other hand, some of these herbs are commonly used by newly born children in Egypt and various parts of the world to treat a varied symptoms that include colic, coughs and irritation.

With their content of pesticides residues, these herbs can inflect some undesirable effects on these children. Various studies have monitored residues of organophosphates on major vegetables (Tawfic Ahmed and Morsy,1991; Tawfic Ahmed et al.1991, Tawfic Ahmed and Ismail 1995; Tawfic Ahmed and Ismail1997). However, studies on the residues of organophosphates on medicinal crops are meager. Tawfic Ahmed et al. (1998), have studied residues of malathion, an organophosphates on some highly demanded medicinal crops, including mint, marjoram and chamomile. Residues were monitored in fresh and dry plants. The study has indicated that it is safe to use malathion up to 2 sprays per season provided the crop is harvested not less than 3 weeks from the last spray. In a similar study, Mikolajewisz et al. (1996) studied the rate of disappearance of the organophosphate fentitrothion in some medicinal plants. Belanger (1989) studied residues of azinophos methyl, cypermethrin, benomyl and chlorothalonil in peppermint and monarda oil.

Correspondence to: M. T. Ahmed

Cypermthein applied at 0.2kg/ha resulted in residue levels of 0.07 ppm in peppermint oil and 0.8 ppm in monarda oil at harvest, Chlorothalonil applied at 2.5 L/ha resulted in 0.44 and 0.77 ppm residues in peppermint oil and monarda oil respectively

In the present study, samples of caraway *Carum carui*, cumin *Cuminum eyminum,* anise *Eimpinella anisum*, cinnamon *Cinnamomum cassia* and ginger, *Zingiber offìcale*, the most consumed medicinal herbs especially by children and newly born babies were collected from local markets and screened for their residue of organophosphosrus insecticides.

MATERIALS AND METHODS

Samples, each of 500 g of each herb were purchased from different retail outlets around Ismailia city and other Suez Canal related regions. Herbs were taken to the laboratory and various portions of each herb were thoroughly mixed. A minimum of three sub samples, each of 10 g were taken from each herb for extraction.

Extraction was conducted using the method reported by Association of Official Analytical Chemists (AOAC) (1995). Each sub sample was mixed with 100 ml of acetone and 50 ml of deionised water in a metal high speed blender. The homogenate was filtered through a Buchner funnel using Whatman no.1 filter paper.The blender jar was further rinsed with extra 50 ml of acetone and the rinse was filtered again on the same funnel, making the total volume of organic solvent 140 ml. Then a 40 ml sample was mixed with 50 ml petroleum ether and 50 ml dichloromethane in a 500 ml separatory funnel and the mixture was shaken vigorously for 3 min. and the phases were allowed to separate. The aqueous lower phase was collected in a measuring cylinder, while the upper organic phase was passed through an anhydrous sodium sulphate bed before evaporation to 5 ml at room temperature in a fume cupboard.

For clean up, a slurry of 10g Florisil in hexane was used to pack a glass column (22 cm x 1.5 cm) and the column was topped with 2 g of anhydrous sodium sulphate. The column was eluted with 30 ml hexane before the extract was loaded on top of the column. Two eluting systems, each of 50 ml were used to elute organophosphates of varied polarity. The first elute was collected with a mixture of hexane, benzene and ethyl acetate (90-9-1-V/V). And the second fraction with a mixture of hexane and ethyl acetate (95-5 V/V). Each fraction was concentrated to 1 ml using a rotary evaporator and kept in a glass vial ready for chromatographic analysis.

A Hewlett Packard gas chromatograph, model 5890, equipped with a nitrogen phosphorus detector was used in this study. A capillary column DB-1701, 25m.x 0.32 mm, film thickness, 0.25 μm was used to separate organophosphates. Splitless injections of a volume of 2μl was performed throughout. Chromatographic conditions were as follows:
Injection port temperature 225 °C, detector temperature 280 °C and the initial temperature of the column was 90 °C for 2 min. with two ramps. In the first ramp temperature was raised at a rate of 20 °C / min. up to 150 °C / min., while the second ramp had a rate of increase of 6 °C / min. up to a temperature of 270 °C / min. which was held for 15 min. Nitrogen was used as a carrier gas at a flow rate of 2.5 ml / min.

A standard solution containing 15 organophosphorus insecticides dissolved in acetone was injected several times to ascertain the average retention time of each. Herb extracts were injected and their content of organophosphate residues were identified according to their retention times. A standard solution of each identified insecticide was prepared and injected to establish the relationship between peak areas and concentrations. Good linearity was obtained in the range of 100 folds, (0.001- 0.1 ng). The concentrations of organophosphates in herb extracts were determined using the external standard method.

GC/MS analysis was conducted to confirm insecticide residues detected in various samples. A Hewlett Packard gas chromatograph, model (5890+) coupled with a model 5972 mass spectrometer, equipped with a HP pesticides library data system was used for identification and confirmation of organophosphates.
A HP MS-5 capillary column (30 m x 0.25 mm i.d.) was used. GC operating conditions were as follows : splitless injections; injector temperature, 225 ^{O}C; detector temperature ,280 ^{O}C; helium carrier gas flow rate, 1 mL / min. The temperature program was 150 ^{O}C for 0 min, 20 ^{O}C/min to 270 ^{O}C for 15 min.
Standard solutions containing malathion, dimethoate, chlorpyrifos and profenofos were analyzed with a scan range from *m / z* 50 to 550 under full scan conditions and characteristic ions with those of the standards. The criteria observed for all compounds identification were co- elution of all characteristic ions within ± 0.02 min., and agreement of retention timeswindows and relative abundance of selected masses within 20 %. Determination was performed by comparing the peak area of a single primary ion obtained by external standardization. Each of the screened herbs was fortified with the three insecticides, one at the time. Fortification was made at three levels, so that the concentration of each

insecticide would be equivalent to 0.01, 0.1, and 0.5 mg kg^{-1}. Three replicates of each fortified plant samples were made. Malathion recovery obtained from fortified samples had an average of 86 %, 83 %, 85%, 89% and 83% from cumin, anise, caraway, ginger and cinnamon respectively. Meanwhile, dimethoate recovery had an average of 79%, 81%, 80%, 81%, and 85% from the same plants respectively. Chloropyrifos, had an average recovery rates of 82%, 86%, 78%, 79%, and 80% respectively. While profenofos had an average recovery rates of 77%, 78%, 81%, 83%, and 79% respectively. Results were not corrected according to recovery rate.

RESULTS AND DISCUSSION

Residues of the organophosphorus insecticides, malathion, dimethoate, chlorpyrifos and profenofos were detected in samples of medicinal herbs collected at random from different retailer outlets. The retention times, and the least detectable concentrations of the analyzed compounds are shown in Table 1. In the present study, the least detectable concentration is that concentration giving a signal 3 times than that of the blank signal at the retention time of the particular insecticide when 2 µl blank extract was injected. Detected organophosphates were further confirmed by GC/ MS analysis. Results indicated that cumin, anise and cinnamon had some detectable concentrations of at least one organophosphate, while, ginger and caraway had no detectable concentrations of any organophosphate. It is probable that the bitter taste and the strong smell of ginger and caraway have repelled insects attacks, thus no pesticides were applied. The present study indicated that malathion was the insecticide detected the most.

Table 1. Retention times* and least detectable concentrations of monitored organophosphates

Pesticide	Retention time / min	Detection limit mg/kg
Dimethoate	13.7	0.005
Malathion	14.5	0.005
Chlorpyrifos	16.2	0.005
Profenfos	20.4	0.002

* Average of three injections

Table 2. Residues* of organophosphates detected in some medicinal herbs collected from local markets around Ismailia, Egypt (mg/kg)

Pesticide	Cumin	Anise	Ginger	Caraway	Cinnamon
Malathion	0.40	0.007	n.d	n.d.	0.027
Dimethoate	n,d.	n.d.	n.d	n.d.	0.006
Chloropyrifos	0.010	n.d.	n.d	n.d.	n.d.
Profenofos	0.37	n.d.	n.d	n.d.	n.d.

* Average of three replicates
n.d. not detected

Residues of malathion were detected in cumin, anise and cinnamon. In Egypt, as in many other countries malathion, if any, is the only recommended insecticides on medicinal plants.
This would probably explain the relatively high incidence of malathion residues in the collected sample

Malathion residues in cumin were comparatively higher than residues detected in anise and cinnamon but still below the maximum residue limits suggested for vegetable crops by the Codex Alimentarius Committee for pesticides residues (1985). But , considering that cumin is usually sold dry some few weeks or probably months after harvesting, it is rather possible that fresh supplies could have even higher residues. Meanwhile residues of malathion detected in anise and cinnamon are too small and not likely to be a cause of major concern.

The present study has indicated the presence of some profenofos residues in cumin.. Codex Alimentarius Committee for pesticides (1985) has set no limits for pesticides residues in herbal plants, however, the maximum residue limits set for profenofos in vegetables is 0.2 mg kg -1.

The presence of such high residue level of profenofos is rather alarming, bearing in mind the toxicity of profenofos and the influence these residues may impact, specially if consumed by someone who would use cumin as a treatment for some particular health problem.

Herbs screened in this study are locally grown and also imported from other countries. The presence of pesticide residues, other than malation as revealed in the present study would indicate that some growers do not abide with the recommendations suggested by regulatory bodies. Some of the detected insecticides as dimethoate is not even recommended on

edible crops in many countries, including Egypt, because of its systemic properties that pose some potential risk to consumers.

International organizations such as Food and Agriculture Organization and the World Health Organization of the United Nations have not included medicinal plants in their lists for guidelines on pesticides residues. Such lack of information in the Joint FAO/ WHO Codex Alimentarius Committee's recommendation is another major factors that exacerbates an already serious problem.

At the present time there is a veritable increase in the popularity for herbal plants. Considering the special health conditions of some of those who use these herbs; and the presence of strong consensus in many circles that foster the use of herbs as a sound alternative to synthetic pharmaceutical compounds in particular cases; the need for strict guidelines and more concern in international regulatory bodies seems quite evident.

Acknowledgment. The present work was sponsored by a research grant from the Regional Council of Agricultural Research and Extension, Ministry of Agriculture, Egypt.

REFERENCES

Association of Official Analytical Chemists (1995) Official methods of analysis 16th Ed.,10.1.02, method 985.22

Belanger A.(1989) Determination of residues of some pesticides on some horticulture crops. Acta Hort 249: 67-73

Codex Committee on Pesticides Residues (1985) Pesticides limits in foodstuff FAO/ WHO Food Standard Program

Milkolajewicz M, Gnusowski B, Fioda GI. (1996) Field application of fentrithion on some medicinal plants and its rate of disappearacne. Herba Polonia 42:104-111

Tawfic Ahmed M, Morsy MM. (1991) Residues of profenofos on some methyl on lettuce and endive, the influence of variety. Deutsche Lebensmittel- Rundchau 88:150-151

Tawfic Ahmed M, Ismail SM. (1996) Effect of reduced application rates of dicofol and pirmiphos methyl on strawberries and common beans on their residue content and crop production. J Agric Food Chem 44:2694-2697

Tawfic Ahmed M, Ismail SM, Mosleh YY. (1998) Determination of malathion residues in some medicinal plants by liquid chromatography with gas chromatography / mass spectroscopic confirmation. J Assoc Off Anal Chem. 81: 1026-1029