Effect of Reduced Application Rates of Dicofol and Pirimiphos-methyl on Strawberries and Common Beans on Their Residue Content and Crop Production

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Residues of dicofol and pirimiphos-methyl applied at different rates on strawberry and common beans, respectively, were monitored at different intervals. The rate of crop production of each rate was also studied. High-performance liquid chromatography was employed to detect both compounds and gas chromatography/mass spectrometry analysis was conducted for further confirmation. All tested rates produced residue levels below the respective maximum residue limits (MRL) of dicofol and pirimiphos-methyl. The highest two rates of dicofol (185 and 139 g of active ingredient/feddan) and of pirimiphos-methyl (750 and 562 g of active ingredient/feddan) produced comparable production rates of strawberry and common beans, respectively. Dicofol residues were 0.230 and 0.105 mg/kg, 1 and 2 weeks, respectively, after the application of the highest rate. The highest rate of pirimiphos-methyl produced a residue level of 0.399 mg/kg, 1 week after application.

Keywords: *Pirimiphos-methyl; dicofol; strawberry; common beans; HPLC; GC/MS*

INTRODUCTION

Vegetable production at Ismailia province, located in the north east of Egypt, is a high priority. Growers use pesticides regularly to protect their crops from losses incurred by numerous pests. As a result, on several occasions, exports were turned down because of their high content of pesticides residues. Moreover, several cases of poisoning were unofficially reported among local consumers and attributed to high residue levels in consumed vegetables. In a previous study (Ahmed and Ismail, 1995) up to 25% of strawberries collected at random from local city markets had methomyl residue levels that were much higher than that allowed. Such incidences were ascribed to misuse and negligence of pesticides usage.

Dicofol [4-chloro-α-(4-chlorophenyl)-α-(trichloromethyl)benzenemethanol] is used on strawberry (*Fragaria ananassa* Duch) to control red spider mite (*Tetranychus* sp.). Pirimiphos-methyl [*O*-[2-(diethylamino)-6-methyl-4-pyrimidinyl] *O*,*O*-dimethyl phosphorothioate] is used on common beans (*Phaseolus vulgaris*) to control aphids (*Aphis craccivora*) and white fly (*Bemisia tabaci*). Strawberry and common beans are among the most important export crops. They are grown as annual crops, although strawberry is a perennial plant. Dicofol and pirimiphos-methyl are recommended for preventive application on strawberry and common beans, respectively, provided the application ceases 2 weeks before harvest. However, very often, such safety intervals are not observed as growers fear the risk of poor quality crops. The present study was conducted to investigate the influence of reducing the recommended rates of dicofol and pirimiphos-methyl on crop production and residue level in strawberry and common beans, respectively.

Dicofol residues can be determined by several methods, including gas chromatography (GC) (Zweig and Sherma, 1972) and normal-phase high-performance liquid chromatography (HPLC). However, difficulties regarding thermal degradation are often encountered in GC analysis, and an unstable flow rate of mobile phase poses some problems in normal-phase HPLC analysis. (Mohamed Tawfic, unpublished results). In the present study, a simple, rapid, and sensitive method of dicofol determination based on reversed-phase HPLC is reported. HPLC was also employed to determine pirimiphos-methyl residues in common beans. Residues extracts were further analyzed by GC/mass spectrometry (MS) for confirmation of analytes.

MATERIALS AND METHODS

Field Experiments. Dicofol, formulated as 185 g/kg wettable powder, was applied on strawberries at the manufacturer's recommended rate [185 g of active ingredient (ai)/feddan = 178 g/acre] and two more rates (i.e., 139 and 92 g ai/feddan). Similarly, pirimiphos-methyl, formulated as Actellic [500 g ai/L of emulsifiable concentrate] was applied on common beans. Three rates were tested, the manufacturer's recommended rate (750 g of ai/feddan = 722 g/acre) and two more rates (i.e., 562 and 375 g ai/feddan. A knapsack sprayer, fitted with a single-nozzle boom, was used to spray each tested compound in a water volume of 400 L/feddan.

Strawberry. Strawberries (*Fragaria ananassa* Duch cv. Silva) were planted in late September 1994 at intervals of 0.25 m in rows separated by a gap of 0.6 m. Preventive application of dicofol was performed on a monthly basis on plots of five rows that were 7 m in length. Each plot was separated laterally by a guard row of untreated strawberries. Harvest started by January. Ripe fruits were taken for analysis 1, 2, and 3 weeks after the January application of dicofol.

Common Beans. Common beans (*Phaseolus vulgaris* cv. Giza 3) were planted in late October 1994 at intervals of 0.25 m in rows separated by a gap of 0.7 m. Three weeks later, pirimiphos-methyl was applied on plots of five rows that were each 7 m in length. Each plot was separated laterally by a guard row. The application of each rate of pirimiphos-methyl was repeated every other week. The application of pirimiphos-methyl various rates along with control treatment were performed randomly using a completely randomized block design, with an equal number of plots for each treatment. Ripe fruits were taken for analysis at 1, 2, and 3 weeks after the last application. Fruit production was estimated as dry seeds.

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Sampling. Ripe strawberries and common beans (2 kg) were collected from treated and control plots. Representative samples of each crop were taken, chopped, and mixed thoroughly. Three representative analytical subsamples (100 g) for both common beans and strawberries were taken for residue analysis.

Extraction and Cleanup. *Strawberry.* Various methods were tested to extract dicofol from strawberry, including the method reported by Luke *et al.* (1975) and Suzuki *et al.* (1979). However problems regarding the strong color of the extract were encountered. The use of *n*-hexane as recommended by Shafik (1980) was an efficient alternative. Each strawberry subsample (100 g) was successively homogenized with three portions (120−150 mL) of *n*-hexane with a Virtus 23 mechanical homogenizer and eluted through anhydrous sodium sulfate (3 g). Hexane extracts were combined and evaporated to near dryness at room temperature. Dicofol residues were quantitatively transferred with 1 mL of HPLC grade methanol into glass vials that were ready for analysis because no further cleanup was needed.

Common Beans. Extraction was based on the method reported by Al-Samaraiee *et al.* (1988) and Ahmed and Morsy (1991). Each subsample of common beans was homogenized with chloroform (100−120 mL) using a Virtus 23 mechanical homogenizer and eluted through anhydrous sodium sulfate (3−4 g). Chloroform extracts were combined and evaporated to near dryness at room temperature. Pirimiphos-methyl residues were quantitatively transferred in 2 mL of benzene on top of a deactivated Florisil column (8 g). The column was eluted with benzene (30 mL). Benzene was evaporated at room temperature. Residues were quantitatively transferred with 2 mL of HPLC grade methanol into glass vials. The extract was further concentrated to 0.1 mL and was ready for analysis.

To examine the extraction efficiency of both dicofol and pirimiphos-methyl, three samples of strawberries and common beans were spiked with known concentrations of dicofol (0.06−1.2 mg/kg) and pirimiphos-methyl (0.04−1.0 mg/kg) dissolved in methanol. Extraction was performed as described earlier, and the obtained average of recovery rates were 92.5% [standard error of the mean (SEM) 3.4] and 84% (SEM 6.2) for dicofol and pirimiphos-methyl, respectively. Results were not corrected according to recovery rates. The ultraviolet (UV) detection limits at 254 nm were 1.0 and 3 ng for dicofol and pirimiphos-methyl standard solutions, respectively. Detection limits of dicofol and pirimiphos-methyl in the samples were 0.05 and 0.015 mg/kg, respectively.

Apparatus and Chromatography. *High-Performance Liquid Chromatography.* Reversed-phase HPLC was performed with a Beckman 342 liquid chromatograph equipped with two pumps (model 112), a solvent programmer (model 340), an injector (model 210), and a fixed wave length UV detector (model 160). A Nova Pack C18 analytical column (10 cm × 8 mm i.d.; Water Chromatography) was used. Water and chromatography grade solvents were filtered through membrane filters (pore size, 0.45 m) and deaerated in an ultrasonic bath before use. The mobile phase used to elute dicofol was a mixture of methanol and water (9:1, v/v). A methanol:water mixture (8:2, v/v) was also used to separate pirimiphos-methyl. The best wavelength for the separate determination of dicofol and pirimiphos-methyl was 254 nm according to their UV spectra. Peak areas were measured with a Spectra Physics Data jet computing integrator. Under these conditions, the retention time of dicofol was 6.7 min and the retention time of pirimiphos-methyl was 8.6 min. Standard curves for each insecticide were constructed by plotting peak areas against concentrations (external standard method). Good linearity was observed over a 400-fold range (5 ng−2 mg).

Gas Chromatography−Mass Spectrometry. A Hewlett-Packard gas chromatography (model 5890+) coupled with a model 5972 quadrupole mass spectrometer equipped with a HP pesticide library data system was used for identification and confirmation of dicofol and pirimiphos-methyl. A HP MS-5 capillary column (30 m × 0.25 mm i.d.) was used. GC operating conditions were as follows: splitless injection injector temperature, 225 °C; detector temperature, 280 °C; and helium flow rate, 0.8 mL/min. The temperature program used was 150 °C for 0 min, and 20 °C/min to 270 °C for 15 min. Standard solutions containing dicofol and pirimiphos-methyl were analyzed with a scan range from *m/z* 50 to 550 under full-scan conditions and characteristic ions with those of standards. The criteria observed for both compounds identification were the co-elution of all characteristic ions must be within ±0.02 min of the retention time window, and the relative abundance of selective masses must agree within 20%, as in the mass chromatogram of the standard.

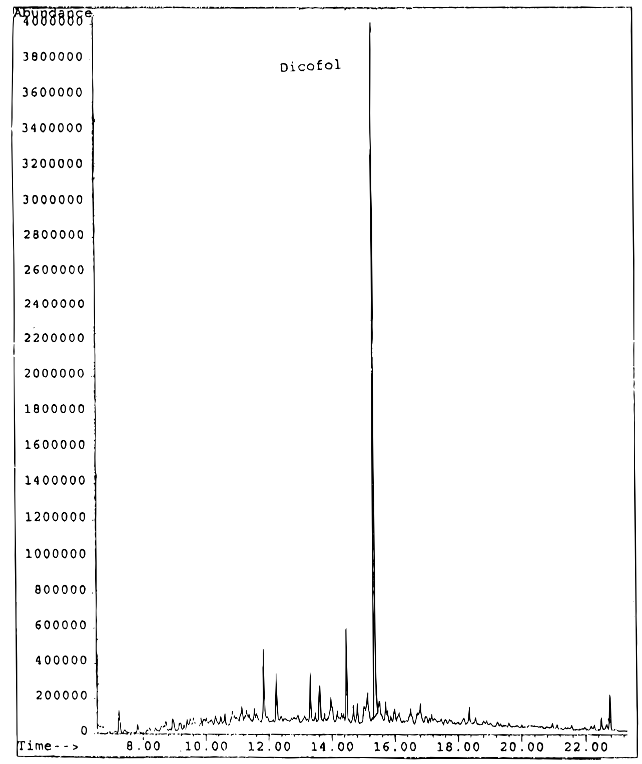

Figure 1. GC/MS chromatogram for dicofol residues in a strawberry sample 2 weeks after pesticide application.

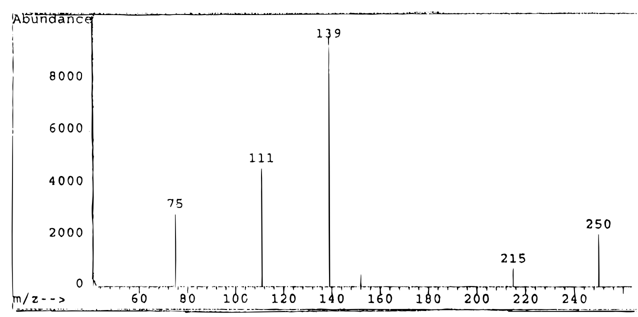

Figure 2. Mass spectrum (EI) of dicofol separated from a strawberry sample 2 weeks after pesticide application.

RESULTS AND DISCUSSION

Dicofol. The extraction procedure of dicofol described in this work needed no further cleanup of plant extract, hence, offering a more economical method with less labor and time consumption. Co-extractives peaks were eluted much earlier than dicofol which had a retention time of ∼6.7 min.

The GC/MS chromatogram of dicofol residues in a strawberry sample harvested 2 weeks after application is shown in Figure 1. The electron-impact mass spectrum of the same sample is shown in Figure 2. Residues of dicofol applied at three different rates are shown in

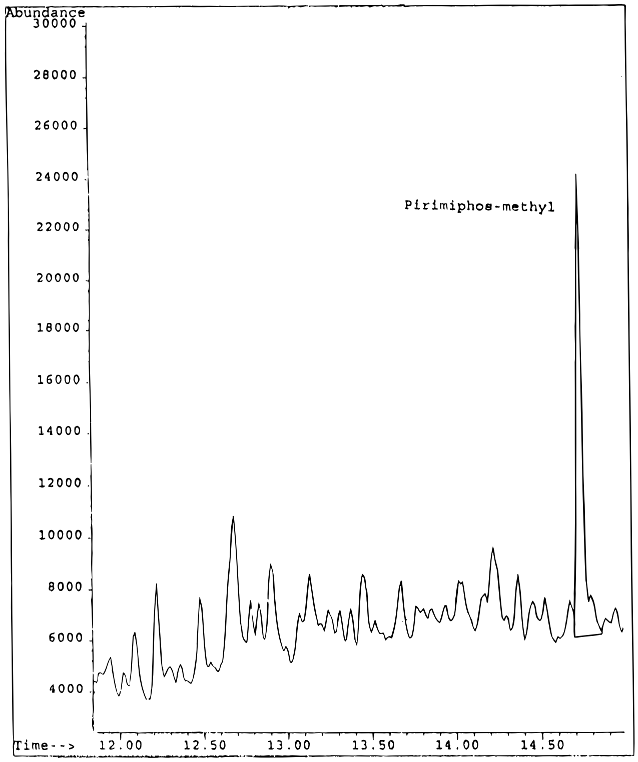

Figure 3. GC/MS chromatogram for pirimiphos-methyl residues in a common beans sample 2 weeks after pesticide application.

Table 1. Residues of Dicofol Applied at Different Rates on Strawberries

rate applied (g of ai/feddan)	residues detected (mg/kg) at different intervals after application[a] 1 week	2 weeks	3 weeks
185	0.230 ± 0.012	0.105 ± 0.005	nd
139	0.201 ± 0.008	0.075 ± 0.003	nd
92	0.137 ± 0.007	0.055 ± 0.002	nd

[a] Results are expressed as mean ± SD for three determinations; the detection limit of dicofol in the sample was 0.05 mg/kg; nd = not detected = <0.05 mg/kg.

Table 2. Effect of Different Rates of Application of Dicofol on Crop Production of Strawberries[a]

rate applied (g of ai/feddan)	production (kg/feddan) mean ± SD	% increase[b]
185	11 433 ± 571[a]	83.63
139	10 908 ± 654[a]	75.20
92	8 294 ± 331[b]	33.21
untreated	6 226 ± 313[c]	

[a] Means with the same letter are not significantly different at the 5% level of least significant difference. [b] In comparison with untreated plots.

Table 1. The three tested rates showed no detectable level 3 weeks after application. However, detectable levels were monitored 2 weeks after application. The highest two rates produced were 0.23 and 0.201 mg/kg, 1 week after application. Such results are probably attributed to differences in the posture of treated plants and/or surface nature that could allow for differential retention of sprayed solution. Previous reports have emphasized the importance of crop shape in residue burden (Ahmed et al., 1992).

The MRL of dicofol on strawberry fruit is 1 mg/kg (CCPR, 1985). In view of the present results, residues of the three tested application rates of dicofol were much below that of MRL, and consumption of treated fruits seems to be safe 1 week after application. The effect of applying different rates of dicofol on strawberry production is shown in Table 2. The use of any of the tested application rates resulted in significant increase in crop yield compared with the yield of untreated control plots. However, production rates of both the recommended rates (i.e., 185 g/feddan and the next lower rate of 139 g/feddan) were not significantly different, indicating an equal efficiency of combat pest pressure.

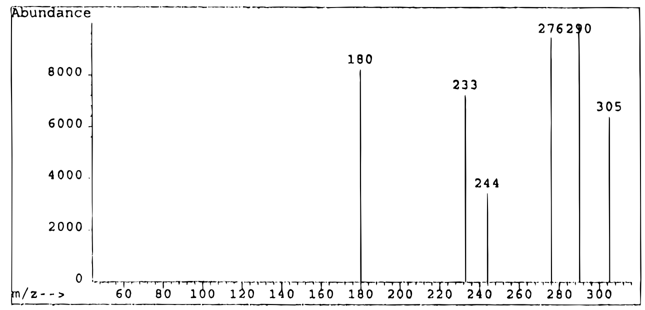

Figure 4. Mass spectrum (EI) of pirimiphos-methyl separated from a common beans sample 2 weeks after pesticide application.

Table 3. Residues of Pirmiphos-methyl Applied at Different Rates on Common Beans

rate applied (g of ai/feddan)	residues detected (mg/kg) at different intervals after application[a] 1 week	2 weeks	3 weeks
750	0.399 ± 0.016	0.127 ± 0.005	nd
562	0.0445 ± 0.002	0.0375 ± 0.002	nd
375	0.017 ± 0.001	nd	nd

[a] Results are expressed as mean + SD for three determinations; the detection limit of pirmiphos-methyl in the sample was 0.015 mg/kg; nd = not detected = <0.015 mg/kg.

Table 4. Effect of Different Rates of Application of Pirmiphos-methyl on Crop Production of Common Beans as Dry Seeds

rate applied (g of ai/feddan)	production (kg/feddan) mean + SD[a]	% increase[b]
750	480 ± 32.6[a]	53.85
562	465 ± 43.2[a]	49.04
375	396 ± 31.7[b]	26.9
untreated	312 ± 18.7[c]	

[a] Means with the same letter are not significantly different on the 5% level of least significant difference. [b] In comparison with untreated plots.

Pirimiphos-methyl. The GC/MS chromatogram of pirimiphos-methyl in a common bean sample extract, 2 weeks after application is shown in Figure 3. The electron-impact mass spectrum of the same sample is shown in Figure 4. Residues of pirimiphos-methyl applied at three different rates are shown in Table 3. The recommended rate and the next lower one reached an undetectable residue level 3 weeks after application, but the lowest rate was not detected 2 weeks after application. The MRL of pirimiphos-methyl on common beans is 0.5 mg/kg, as set forth by the CCPR (1985). In the present study, residues remaining 1 week after application of the recommended rate (750 g/feddan) are just marginally below that of MRL. Growers tend to harvest export crops earlier than the usual time, so observing the 2-week safety period suggested by local regulatory bodies is hardly heeded. As a result, the likelihood of turning down pirimiphos-methyl treated shipment exists. On the other hand, our results indicated that the application of recommended rate (750 g) and the next rate (562 g) produced comparable production rates (Table 4).

In view of the present results, it is suggested that reducing the recommended rate of pirimiphos-methyl by 25% has no significant effect on crop production. However, residues remaining on common beans would be far below the MRL. Similarly, the reduction of recommended rate of dicofol by 25% has no significant effect on strawberry crop production. Considering such results, the present study would favor the reduction of dicofol and pirimiphos-methyl recommended rates on strawberry and common beans by 25%.

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