J. Plant Prot. and Path., Mansoura Univ., Vol.7 (12) ,845–849, 2016

Residues of Profenofos with Special Reference to its Removal Trials and Biochemical Effects on Tomato

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ABSTRACT

During June of 2016 tomato plants were sprayed with profenofos (Seliton 72% E.C.) at the rate of 750 cm^3 / feddan (540 g a.i.). Leaves and fruits samples were collected at 2 hrs to 15 days after application and analyzed using GC. Results revealed that, the initial amounts in leaves were much higher than in tomato fruits. A rapid degradation of profenofos residues was noticed in tomato fruits comparing with tomato leaves. The washing of the treated fruits (2 hours) with tap water or 1% of soap, sodium chloride, acetic acid and potassium permanganate reduced indicating the initial deposits (20.53 mg/kg) to 6.11, 9.03, 7.69, 9.87 and 5.06 mg/kg indicating 70.24, 56.02, 62.54, 51.92 and 75.35% dislodge, respectively. Preparation of tomato paste reduced profenofos residues to undetected amounts being 100% removal. The consumable safety time was 3 days after application and this period could be shorted to two hours after spraying if harvested tomato fruits were washed with the above mentioned washing solutions or prepared to tomato paste. Profenofos significantly reduced the mean levels of each N, P, K, Ca, Fe, Mn. As well as the mean levels of total soluble solid (T.S.S.), ascorbic acid, β-carotene, acidity and protein were significantly reduced at 6, 9 and 15 days of spraying. The mean amounts of total soluble sugars and glucose were not significantly affected by profenofos. Dry matter in treated tomato fruits were significantly increased compared with control.

Keywords: profenofos, tomato, residues, home processing, chemical composition

INTRODUCTION

In Egypt, tomato is one of the most important and the largest grown vegetable crops. Egypt is producing 9 million tons of tomatoes annually and considered as the fifth largest producer of tomatoes in the world. Despite the high productivity of tomatoes, Egypt imports tomato paste (sauce) due to the attacked by destructive pest known as *Tuta absoluta* in 2007 and other insect pests. Bekheit (2015). Tomato is known as an important food for its component such as low in fat and calories, free of cholesterol and rich in vitamins A and C, b-carotene, lycopene and potassium Pawar *et al.* (2012).

Foods are protected by using several pesticides. Residues of pesticide in food are associated with severe effects on the human health, so there are many trails to reduce pesticide residues in food using some household processing like washing, peeling, cooking etc Dikshit *et al.* (2003)

Profenofos is a broad spectrum non systemic and foliar organophosphate insecticide. It was developed for control wide range of insect pests that were resistant to chlorpyrifos and other organophosphate insecticide. Jabeen *et al.* (2015). Profenofos is used to control of insects (particularly Lepidoptera) and mites on cotton, maize, sugar beet, soya beans, potatoes, tomato, vegetables, tobacco, and other crops MacBean (2012).

This study aimed to determine:

- The residual behavior of the insecticides profenofos on tomato leaves and fruits and determination of its dissipation rate, half – life value ($T_{½}$) and pre-harvest intervals (PHI).
- Effect of different washing solutions and preparation of tomato paste on profenofos residues in tomato fruits as a removal trails.
- Effect of profenofos residues on some quality parameters and certain essential elements of tomato fruits.

MATERIALS AND METHODS

1- Pesticide selected for this study:

Profenofos (Seliton 72% E.C.), chemical name (IUPAC): *O*-4-bromo-2-chlorophenyl *O*-ethyl *S*-propyl phosphorothioate. The insecticide formulation was obtained from Central Agricultural Pesticides Laboratory, (CAPL), Agricultural Research Center (ARC), Ministry of Agriculture at Giza, Egypt.

2- Field Experiment and sampling

A Field experiment was conducted during June of 2016 (summer plantation) in the Kafr Abo Agwa, Alqenaiat region, Sharkia Governorate, Egypt. The normal agricultural practices were achieved. Mature plants (*Solanum lycopersicum* var yara 410) were sprayed with profenofos 72% E.C. once at the recommended rate of 750 cm^3 / feddan (540 g a.i.); at three plots (6×7 m each) the distance between plots were 2 m and the control plots were left unsprayed. A knapsack-sprayer with one nozzle was used to deliver 200 liters water/ feddan. Samples of tomato leaves and fruits (500 g/ replicate) were taken from three replicates at intervals of 2 hr, 1, 3, 6, 9, 12, and 15 days after applications.

3- Extraction, clean up and residue determination

As mentioned by Mollhof (1975) representative fruit samples of tomato (100 g) and leaf samples (25 g) were extracted which adapted to use methanol instead of acetone as a solvent for the extraction of profenofos residues. Samples were cut into small pieces in a warring blender. A constant volume of distilled methanol 150 ml was used for extraction. The sample was blended for three minutes at high speed then filtered through a dry pad of cotton into a graduated cylinder. A known volume of the filtrate (100 ml) was taken and partitioned successively with 100, 50 and 50 ml of methylene chloride in a 500 ml separatory funnel after adding 40 ml of saturated sodium chloride solution. The combined methylene chloride phase was dried by filtration on filter paper No. 1 and anhydrous sodium sulfate then evaporated to dryness on a rotary evaporator at 40 °C. The dry extract was then subjected to the clean up procedure suggested by Mills *et al.* (1972) using florisil chromatograph column [40 cm×18 mm (i.d.) glass column] filled with 10 g of activated florisil (60-100 mesh) and topped with 2 g anhydrous sodium sulfate and compacted thoroughly. The column was pre washed using 50 ml petroleum ether. The sample extract was dissolved in 10 ml. of the same solvent and transferred to the column then eluted with 200 ml of the eluent (50% petroleum ether: 50% diethyl ether). The eluent was evaporated to dryness by a rotary evaporator at 40 °C and stored in the freezer until residue analysis.

Agilent Technologies 7890A gas chromatograph equipped with flame photometric detector (FPD) operated in the phosphorus mode (526 nm filter) was used for the determination of profenofos residues. The column was HP-5 (30 m×0.32 mm×0. 25 µm film thickness). Injector temperature was 250 °C. Detector temperature was 250 °C. Column temperature was 230 °C for profenofos. Gases flow rates were 60, 30 and 30 ml/min for nitrogen, hydrogen and air, respectively. Under the previous conditions, profenofos showed a retention time of 3.79 min and a good chromatographic separation was obtained Hegazy *et al.* (2006).

To estimate the effectiveness of the used extraction, clean-up and final determination method, three samples from each fruit and leaves were spiked with recognized concentration (5, 1, 0.5 mg/kg) of the active ingredient profenofos standard solution. Extraction, clean-up and detection methods were performed as described before, and the average recovery rates were 93.47% for leaves and 90.25% for fruits. Results were corrected according to the mean of recovery.

4- Effect of profenofos residues on some quality parameters and trace elements

To study the effect of profenofos residues on some quality parameters and trace elements of treated and untreated tomato fruits were taken at 9, 12 and 15 days after application. Quality parameters included total soluble sugars, glucose, acidity, total soluble solid, ascorbic acid, β-carotene, protein and dry matter. While the trace essential elements were N, P, K, Fe, Mn, Ca and Zn were also determined.

Nitrogen, potassium and phosphorus were determined by the method of Evenhuis and waard (1980). Calcium, manganese, iron and zinc were determined by atomic adsorption spectroscopy Jackson (1967). Total soluble sugars and glucose were determined colorimetrically using the picric acid method as described by Dubois *et al.* (1956). Total soluble solid were estimated using a refractometer. Acidity, ascorbic acid, protein and dry matter were determined according to the methods of Association of official Analytical Chemists (AOAC)(1984). β-carotene was determined by the method of Ben-Amotza and Avron (1983).

5-Removal of profenofos residues from contaminated fruits

Removal tests were done after 2 hr from treated tomato fruits either with different washing solutions or preparation tomato paste to reduce profenofos residues. The fruit samples were divided into two parts the first part was divided to 5 sub-samples and each of them was soaked in a jar filled with any of the following solutions (tap water, soap 1%, $KMnO_4$1%, NaCl 1%, and CH_3COOH 1%) for 2 min. The washed samples were allowed to dry. The second part was crushed into small pieces in a warring blender. The juice was concentrated at 100^0C until form paste with the addition of 2.5% NaCl. Ismail *et al.* (1993). The washed fruits and paste were analysis as described before.

6- Statistical Analysis:

Statistical significance of the data was determined by using the analysis of variance with L.S.D method at the probability of 0.05 Steel and Torrie (1980). The rate of degradation (K) and Half-life (t½) were obtained according to the equation of Gomaa and Belal (1975).

RESULTS AND DISCUSSION

Data in table (1) revealed that the initial deposits of profenofos in/on tomato fruits and leaves two hours after application were 20.53 and 58.41 mg/kg, correspondingly. A rapid degradation of profenofos residues was noticed in tomato fruits one day after spraying recording 28.35% of the initial amounts comparing with 19.52% in case of tomato leaves. Results in the same table showed that the first 6 days were critical, showing high dissipation rates from tomato fruits and leaves, being 83.26% and 66.55%, respectively. At the end of experiment (15 days) tomato fruits and leaves contained 0.20 and 2.02 mg/kg recording 98.97% and 96.54% loss of the initial deposits of the tested insecticide profenofos. The rate of loss was higher in fruits as comparison with leaves. These differences in the loss of the initial deposits in leaves and fruits may be reflecting the titer of metabolizing enzyme. Also, it is obvious that the initial deposits were grater in leaves compared to fruits. This finding is due to the effect of nature of the recipient surface (i.e., morphological and chemical aspects) on retention of residues, also leaves of tomato have a large surface per weight units in comparison to fruits. Similar results were obtained by Shalaby *et al.* (1998); Soliman (1998); Radwan *et al.* (2005); Romeh *et al.* (2009); shiboob (2012); Cherukuri *et al.* (2015) Alen *et al.* (2016); Ali *et al.* (2016); and Ramadan *et al.* (2016) studying on the residues of profenofos and other insecticide residues in tomato and same vegetable crops.

Table 1. Residues of profenofos detected in tomato leaves and fruits at different intervals.

Days after treatment	leaves			fruits		
	Residues (mg/kg)	Loss %	Persistence %	Residues (mg/kg)	Loss %	Persistence %
0	58.41	—	100	20. 53	—	100
1	47.01	19.52	80.48	14.71	28.35	81.19
3	35.24	39.67	60.33	8.66	55.25	44.75
6	19.54	66.55	33.45	3.24	83.26	16.74
9	11.04	81.10	18.90	1.22	93.69	6.31
12	7.11	87.83	12.17	0.48	97.52	2.48
15	2.02	96.54	3.46	0.2	98.97	1.03
K	0.198			0.303		
$t_{½}$	3.49 days			2.28 days		
Liner equation	y = -0.086x + 1.766			y = -0.134x + 1.312		

K = degradation rate, $t_{½}$= half – life

Profenofos residues and its removal percentages as affected with dissimilar washing solvents and processing treatments on contaminated tomato fruits collected two hours after spraying are giving in Table (2). Data showed that the residue of profenofos on unwashed (raw) tomato fruits determined after 2 hours of spraying was 20.53 mg/kg. The washing of the treated fruits with tap water reduced this amount to 6.11 mg/kg recording 70.24% removal. While solutions 1% of soap, sodium chloride, acetic acid and potassium permanganate reduced the initial amounts in tomato fruits to 9.03, 7.69, 9.87 and 5.06 mg/kg, with corresponding removal percentages of 56.02%, 62.54%, 51.92% and 75.35%, respectively. Data also show that preparation of tomato paste reduced profenofos residues to undetected amounts being 100% removal. Several investigators pointed out that washing process resulted in removing major amounts of profenofos, as well as other insecticide residues present on the surface of many vegetables, fruits and field crops and suggested that different processing operations can be effectively applied on fruits and vegetables to minimize the risk of pesticides on human health Shiboob (2012); Shiboob *et al.* (2014); Vemuri *et al*, (2014); Sheikh *et al.* (2015) and Andrade *et al.* (2015)

The differences between the tested washing solutions on the removal of profenofos residues from treated tomato fruits may be depended on the phyesico-chemical properties of the insecticide profenofos, such as its solubility in water (28 mg/l), readily miscible with most organic solvents, relatively stable under neutral and slightly acidic conditions and unstable under alkaline conditions MacBean (2012). Also, the removal percentages of pesticide from vegetables and fruits with washing affected by the washing time, the temperature of washing water and initial concentration of pesticide (Youssef *et al.* 1995). Kumari (2008) observed that washing was effective in dislodging the residues, however, it depend on a number of factors like location of residues, age of residues, water solubility, temperature and type of washing.

Table 2. Effect of different washing solutions on profenofos residues contaminated tomato fruits.

treatments	Residues (mg/kg)	% loss (removal)
Unwashed fruits	20. 53	—
Washing solutions		
Water	6.11	70.24
Soap 1%	9.03	56.02
sodium chloride 1%	7.69	62.54
acetic acid 1%	9.87	51.92
potassium permanganate 1%	5.06	75.35
Tomato paste	UND	100

UND = undetectable amounts

The residue tolerance for profenofos in tomato fruits was 10 mg/kg as published Codex Allimentarius Committees (CAC/PR) (2008). Comparing this level with amounts of residues of the tested insecticide found on and in unwashed tomato fruits after 3 days of spraying (Table, 1), data show that unwashed tomato fruit 3 days after spraying contained lower amounts of profenofos and thus could be used with apparent safe for human consumption after this period. The waiting period between spraying and harvesting (PHI) tomato fruits treated with profenofos with 540 g a.i./feddan could be shorted to two hours after spraying if harvested tomato fruits were washed with the above mentioned washing solutions or prepared to tomato paste.

Results of analysis of some biochemical components in unwashed and treated tomato fruits after 6, 9 and 15 days of spraying time are shown in Tables (3 and 4).

Data in Table (3) shows that the tested insecticide profenofos significantly reduced the mean levels of each N, P, K, Ca, Fe, Mn, however, throughout the tested experimental period (6, 9 and 15 days after profenofos spraying) compared with control, while the level of Zn was not affected by application of profenofos Shalaby *et al.* (1991); shalaby and Eisa (1992) and Salem *et al.* (2011). This finding may be due to profenofos reduced the ability of tomato plants to absorb these elements from soil.

Table 3. Effect of profenofos residues on trace elements of tomato plants.

elements	Days after spraying	Untreated fruits	Treated fruits
N %	6	1.940 a	1.845 b
	9	1.740 a	1.405 b
	15	1.560 a	1.460 b
	means	1.746 a	1.570 b
P %	6	0.651 a	0.594 b
	9	0.743 a	0.536 b
	15	0.615 a	0.594 b
	Means	0.669 a	0.608 b
K %	6	2.220 a	2.100 b
	9	2.035 a	1.865 b
	15	2.005 a	1.810 b
	Means	2.086 a	1.925 b
Fe	6	46.050 a	31.205 b
	9	32.435 b	36.156 a
	15	33.220 a	34.275 a
	Means	37.235 a	33.878 b
Mn	6	34.160 a	25.240 b
	9	27.100 a	24.810 b
	15	28.325 a	25.070 b
	Means	29.861 a	25.040 b
Ca	6	0.561 a	0.464 b
	9	0.546 a	0.482 b
	15	0.475 a	0.389 b
	Means	0.527 a	0.445 b
Zn	6	18.290 a	17.150 b
	9	16.540 a	16.845 a
	15	13.845 b	15.470 a
	Means	16.225 a	16.488 a

In each raw values followed by the same letter are not significantly different at $P \leq 0.05$

Concerning the effect of profenofos on internal quality parameters of tomato fruits, data in Table (4) show that the mean level of dry matter in treated tomato fruits were, however, significantly increased comparing with untreated fruits (control). On the other hand the mean levels of total soluble solids, ascorbic acid, β-carotene, acidity and protein were significantly reduced as compared with the tomato fruits control.

several authors reported that some pesticide effected the chemical components of some plants after used Othman *et al.* (1985); Radwan (1988); Habiba *et al.* (1992); Ismail *et al.* (1993); Radwan *et al.* (1995) and Radwan *et al.* (2004).

Data also show that, no significant difference was observed between the mean values of total soluble sugars and glucose in treated and untreated tomato fruits.

The decreasing mean value of T.S.S during the experimental period (15 days) could be due to the inhibition effect of profenofos on the enzymes which hydrolyzed the complex insoluble compounds, such as starch, to simple soluble compounds like sugars, which are the major T.S.S components.

The increasing of the dry matter mean values in treated tomato fruits when compared with untreated one may be due to concentrating fruit juice because of higher water loss by transpiration and higher respiration rates resulting in accumulation of different solutes in cell vacuoles Ibrahim and Gad (2015).

The decreasing of protein level in treated tomato fruits was related with levels of N and K i.e. positive correlation was noticed. It is known that potassium element enhanced the conversion of amino acids to protein.

Tomato fruits are a good source of ascorbic acid (vitamin C) which is a very important nutrient, being essential e.g. for the synthesis of collagen. Ascorbic acid is also a natural antioxidant used in food stuff formulators in order to prevent browning and discoloring, and to enhance shelf life Castro *et al.* (2004). The loss in ascorbic acid content in tomato fruits treated with profenofos might be attributed to the rapid conversion of L-ascorbic acid into dihydro- ascorbic acid in the presence of L- ascorbic acid oxidase Hussien *et al,* (1998) and Gad (2008).

Table 4. Effect of profenofos residues on some quality parameters of tomato plants.

Quality Parameters	Days after spraying	Untreated fruits	Treated fruits
Total soluble sugar	6	4.075 a	3.598 b
	9	2.900 a	3.215 a
	15	3.655 a	3.26 a
	Means	3.543a	3.357 a
Glucose	6	21.725 a	20.490 b
	9	18.505 b	19.860 a
	15	20.310 a	20.160 b
	Means	20.18 a	20.17 a
Acidity	6	2.265 b	2.320 a
	9	2.470 b	2.520 a
	15	2.560 a	2.485 b
	Means	2.431 b	2.441 a
Total soluble solid (T.S.S.)	6	6.825 b	7.950 a
	9	8.160 a	6.955 b
	15	9.040 a	8.530 b
	Means	8.008 a	7.811 b
Ascorbic acid	6	15.870 a	11.250 b
	9	10.555 a	8.610 b
	15	10.180 a	9.840 b
	Means	12.201a	9.900 b
β-carotene	6	5.360 a	3.605 b
	9	3.460 a	3.695 a
	15	3.930 a	3.765 a
	Means	4.250 a	3.688 b
Dry matter	6	20.710 a	19.830 b
	9	18.780 b	20.166 a
	15	19.765 a	20.055 a
	Means	19.751 b	20.017 a
Protein	6	12.120 a	11.525 b
	9	10.870 a	8.775 b
	15	9.745 a	9.120 b
	Means	10.911a	9.808 b

In each raw values followed by the same letter are not significantly different at P ≤ 0.05

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متبقيات البروفينوفوس مع محاولات إزالتة وتأثيراته البيوكيميائية فى الطماطم

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تم رش نباتات الطماطم خلال يونيو ٢٠١٦ بمبيد البروفينوفوس بمعدل ٧٥٠سم[٣] / للفدان واستخدام جهاز GC لتقدير متبقى المبيد خلال ساعتين الى ١٥ يوم بعد الرش. وأوضحت النتائج أن كمية المترسب الأولى فى الأوراق كانت أعلى من الثمار. ومعدل التحطم كان أسرع فى الثمار عن الأوراق. حدث نقص فى كمية المترسب الأولى ٢٠.٥٣ ملجم /كجم لثمار الطماطم المعاملة بعد ساعتين من الرش نتيجة الغسيل بالماء وكل من الصابون وكلوريد الصوديوم وحمض الأسيتك وبرمنجنات البوتاسيوم وذلك بتركيز ١% وصلت إلى ٦.١١ ، ٩.٠٣ ، ٧.٦٩ ، ٩.٨٧ و ٥.٠٦ ملجم /كجم مسجلة نسبة إزالة تصل إلى ٧٠.٢٤ ، ٥٦.٠٢ ، ٦٢.٥٤ ، ٥١.٩٢ و ٧٥.٣٥% وذلك على الترتيب. أما تحضير معجون الطماطم (الصلصة) أدى إلى إزالة متبقى المبيد بنسبة ١٠٠%. ويمكن استهلاك الثمار بآمان بعد ٣ أيام من المعاملة ويمكن تقصير هذه المدة إلى ساعتين بعد المعاملة إذا تم غسيل الثمار الملوثة بمواد الغسيل سابقة الذكر. وكان لمتبقى البروفينوفوس خلال ٦ ، ٩ و ١٥ يوم من المعاملة تأثير معنوى فى إنخفاض النيتروجين ، الفوسفور ، البوتاسيوم ، الكالسيوم , الحديد ، المنجنيز وإنخفاض معنوى لبعض صفات الجودة مثل المواد الصلبة الذائبة الكلية وحمض الأسكوربيك (فيتامين C) والبيتا كروتين والحموضة والبروتين وكان التأثير غير معنوى على كل من السكريات الكلية والجلوكوز وزيادة معنوية فى الوزن الجاف مقارنة بالكنترول.