

# Detection and Elimination of Certain Insecticide Residues from Tomatoes and Green Peppers Fruits

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## ABSTRACT

Residues of three common insecticide used lately in greenhouses were determined; pyriproxyfen (insect growth regulator and guvenile hormone), fenitrothion (an organophosphate), and spinosad (a biological insecticide). Degradation of these insecticides was followed on tomatoes and green peppers grown in greenhouses. The residue of each insecticide was also determined after cooling and cooking the fruits after one day of application. Results showed that initial deposits of pyriproxyfen on tomatoes and green peppers were 2.89 and 6.71 mg/kg respectively. Gradually with time the percentage of disappearance reached 84.14% on tomatoes and 88.08% on peppers after 14 days of application. The half life time ( $t_{0.5}$ ) was 5.41 days on both tomatoes and peppers. The results also show that cooling resulted in 15.92 % and 15.35 % loss, while cooking of fruits resulted in 65.4% and 77.35 % loss of pyriproxyfen from tomatoes and peppers, respectively. Regarding fenitrothion, the results showed that the initial deposits on tomatoes and green peppers were 3.48 and 3.37 mg/kg; respectively. Gradually with time the percentage of disappearance reached 97.13% on tomatoes and 98.81% on peppers after 14 days of application. The ( $t_{0.5}$ ) was 3.5 days on tomatoes and 1.5 days on peppers. Cooling process resulted in 9.48% and 25.52 % loss, while cooking reduce 93.1 % and 82.79% of fenitrothion from tomatoes and peppers, respectively. Initial residues of spinosad were 0.52 and 0.23 mg/kg on tomatoes and green peppers. The loss reached 84.62% on tomatoes and 47.83% peppers after 7 days of application. The ( $t_{0.5}$ ) was 3.5 days on tomatoes and 7.5 days on peppers. Cooling process resulted in loss of spinosad residues by 44.23% and 39.13 % while cooking reduce 50% and 56.52% from tomatoes and green peppers, respectively.

**Key words:** Detection, Elimination, Insecticides, residues, Tomatoes, Peppers

## INTRODUCTION

Nowadays world populations increased regularly and need sufficient food for their increment. Vegetable crops presented one of the main components of food so, the demand of vegetables increased. The expected consumption of vegetables of Arab countries will reach 24535 thousand tons by the year of 2010 compared with 20281 thousand ton in year of 2000 ( Ministry of agriculture and water, KSA, 1420H). Production of vegetables in greenhouses are increased in KSA but environment in greenhouses are suitable for

reproduction spreading of pests so, the KSA used about 2000 chemicals most of them are pesticides. Cultivated tomatoes and green peppers are liable to attack by serious insect pests, predominantly chewing and piercing-sucking species which play role as a vector of viral diseases. The control of such insects is mainly relying on the use of chemical pesticides (Prabhaker *et al*, 1998). The extensive use of synthetic organic pesticides for this purpose has inevitably been followed by many problems. One of the most problems is a remaining residue in vegetables and fruits especially with highly stable and persistent insecticides (Emico and Tomoke, 1982; Zidan *et al.*, 1996). It could cause a health hazard to the ultimate consumers, particularly when freshly consumed. Many publications revealed the existence of pesticide residues in various food items (Sullivan 1980; Gartrell *et al* 1985, Salama *et al.*, 1998, El-Bakary *et al.* 1999 and Rosa *et al.*, 2008). Abdel-Gawaad and Shams El-Deen (1989) detected 23 pesticide residues and their degradation products in many foodstuff samples. In addition to the control of application of pesticide, effective ways for removal of pesticide residue on vegetable are in sought as a preventive measure to avoid adverse impacts on human health, for instance many cases of collective pesticide poisoning in China were occasionally reported due to accidentally consumption of pesticides contaminated vegetable (Li, 2002; Deng *et al.*, 2003). Moreover, many technological processes on pesticide decontamination in fruits and vegetables was studies by several researches (Al-Azawi *et al*, 1991; Zidan *et al*, 1996; Schwedler *et al.*, 2000; Sunitha *et al.*, 2007, Jiguo *et al.*, 2007).

The present investigation aimed to determine the insecticide residues in indoor treated tomatoes and peppers fruits and the role of cooling and cooking processes in elimination of these residues. The half lives of insecticides residues were also estimated.

## MATERIALS AND METHODS

### Chemicals:

The following insecticides were purchased locally and used for the field trails at the recommended rate, Admiral (pyriproxyfen, 10% EC, at rate of 0.75 ml/l. Sumitomo Co.); Sumithion (fenitrothion, 50% EC, at

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Received December 3, 2008, Accepted December 17, 2008

rate of 0.75 ml/l. Sumitomo Co.) and Tracer (spinosad, 48 % SC, at rate of 0.25 ml/l. Dow Agrosiences Co).

### Field Trials:

The experiment was carried out at Zed pen Rashed El-Tamemy farm, El-Kharge, Riyadh, KSA. Four greenhouses were used, half of each is cultivated with tomatoes and the other half with green peppers. Each half was divided to three replicates. One of the greenhouses was used as control. Cultural practices were applied as recommended for commercial production of tomatoes and peppers. Insecticide applications were done one time at the recommended rates to control the chewing and piercing-sucking insects on indoor tomatoes and green peppers plants. A Hydraulic sprayer was used to apply the insecticides.

### Residue analysis:

#### Sampling:

Fruit samples (1 kg, each) of non treated and treated fruits were taken from each treatment at different intervals after treatment (zero, 1,3, 5, 7, 9 and 14 days) in case of pyriproxyfen and fenitrothion, while after, zero, 1,3, 5 and 7 days in the case of spinosad. Each fruit sample were divided into three parts. Insecticide residues were directly determined in first part. The second part was stored at 4°C for 24 h, then residues determined. Third part of samples was cooked at 100°C for 20 min. in presence of 20 ml of water.

### Extraction and Clean up:

#### 1) Pyriproxyfen and fenitrothion

Extraction and cleaned-up were done according to the method adopted by Bullock (1984) and El-Sarar (1996). Fruit samples were cut into small pieces and three sub samples (100g of each) were blended and 10 gm of resultant was extracted with 70 ml acetonitrile /distilled water (80:20) for 3 min. The whole extract decanted through a glass wool plug in a glass funnel containing 20 gm anhydrous sodium sulfate. The filtrate was concentrated using rotary evaporator. The filtrate was shaken vigorously with acetonitrile in a separatory funnel. The aqueous layer was taken and another 50 ml acetonitrile was added and shaken again for 2 min. The two organic layers were taken and concentrated using rotary evaporator and dissolved in hexane (2ml).

Activated silica (SI-HF) (20 gm activated at 130°C for 16 hours) was added to 300 mm X 25mm chromatographic column in small portions, while tapping the column. A layer of anhydrous sodium sulfate was added to the top of silica. The column, pre wet by allowing 20 ml methylene chloride then 20 ml of hexane. Samples extract was transferred to the column. A total of 30 ml of the methylene chloride 100% was used as eluent for pyriproxyfen and fenitrothion. The

eluent was concentrated to dryness and dissolved in 1 ml of methanol.

#### 2) spinosad

Extraction and cleaned-up were done according to the method adopted by Yeh *et al.* (1997) with some modification. The chromatographic column was packed with (C18-HF). A total of 30 ml of the (10 ml acetone then 20 ml, triethyl amine 2%) was used as eluent for spinosad. The eluent was concentrated to dryness and dissolved in 1 ml of acetonitrile and filtrated using (0.45  $\mu$ m, filter).

### Determination

Determination of pyriproxyfen and fenitrothion was carried out by gas liquid chromatography ( Agilent, model 6890 and Agilent, model 5973) equipped with NPD and MSD detectors). The program of chemstation was used to control GC devices. The conditions were: column (HP5MS, 30m X 0.25mm), Temperature (90-250)°C at rate of 20 °C /min. till 250 °C, remaining 5 min at this temperature, injector 250°C and detector(NPD), 300 °C. MSD detector was used to confirm the presence of insecticides using (SIM selected ion monitoring mode at 136, 226 and 277 m/z) system.

High performance liquid chromatography (HPLC) (Waters, model) was used for determining the residues of spinosad. Separation column (Nova pak) (150mm X 3.9 mm) packed with C18. Solvent system was (acetonitril: methanol: ammonium acetate 2%) at ratio of (42%: 42% :16%), respectively. 15  $\mu$ l of samples was injected for 15 min. Retention time  $t_R$  was 8 min for spinosyn-A and 12 min for spinosyn-B. The peaks of those compounds was detected by UV at wavelength of 250 nm. The residue analysis was calculated according to the method of Goodspeed and Chestnut 1991. The average rates of recovery of each insecticide were determined and the quantification of the residues was corrected according to the rate of recovery. The data was subjected to statistical analysis (Snedecor and Cochran 1967).

## RESULTS AND DISCUSSION

### Remaining residues of pyriproxyfen, fenitrothion and spinosad from tomato fruits.

The remaining residues of pyriproxyfen, fenitrothion and spinosad from tomato fruits that cultivated under indoor conditions were determined at different intervals. The residue of each insecticide was also determined after cooling and cooking the fruits after one day of application. The results showed that the initial deposits of pyriproxyfen and fenitrothion on tomatoes were 2.89 and 3.48 mg/kg, respectively. Gradually with time the percentage of disappearance reached 84.14% and 97.13% after 14 days of application. Regarding, spinosad, the results showed that the initial deposits on

tomatoes was 0.52 mg/kg and gradually with time the percentage of disappearance reached 84.62% after 7 days of application.

The results also showed that cooling of tomato after one day of application resulted in loss of pyriproxyfen from 2.89 to 2.43 mg/kg (15.92%) while the loss reached 65.4% after cooking process. Regarding, fenitrothion the loss because of cooling was from 3.48 to 3.15 mg/kg (9.48%) while it decreased from 3.48 to 0.24 mg/kg (82.79%) because of cooking. In case of spinosad, cooling process decreased the residue of spinosad from 0.52 to 0.29 mg/kg (44.23%). On the other hand cooking of tomato fruits that has spinosad residues resulted in 50% decrease of initial deposits (Table 2).

The half life as a kinetic parameter of decay of insecticide residues was deduced from the logarithmic linear relationship between insecticide residues versus the time. The half lives that reflect the degradation behavior of the insecticides under the indoor condition showed that pyriproxyfen was the most persistent insecticide ( $t_{0.5}$ ) = 5.41 days while it was 3.5 days for both fenitrothion and spinosad (Table 1).

### Remaining residues of pyriproxyfen, fenitrothion and spinosad from green peppers fruits.

The results showed that the initial deposits of pyriproxyfen and fenitrothion on green peppers was 6.71 and 3.37 mg/kg, respectively. Gradually with time the percentage of disappearance reached 88.08% and 98.81% after 14 days of application. The  $t_{0.5}$  were 5.41 days and 1.5 days. The results also show that cooling process of green peppers after one day of application resulted in loss of pyriproxyfen from 6.71 to 5.68 mg/kg (15.35%). On the other hand, cooking of green peppers after the same time period resulted in elimination of residues from 6.71 to 1.52 mg/kg (77.35%). Cooling process of green peppers that treated with fenitrothion resulted in elimination of about 25.52% while cooking process eliminate about 82.79%.

The results on the residues of spinosad showed that the initial deposits on green peppers was 0.23 mg/kg. Gradually with time the percentage of disappearance reached 47.83% on peppers after 7 days of application. The half life time ( $t_{0.5}$ ) was 7.5 days. The results also show that cooling process resulted in elimination of about 39.13% from peppers while cooking process eliminate about 56.52% (Tables 3& 4).

**Table 1. Residues and percent of loss of pyriproxyfen, fenitrothion and spinosad from tomato fruits under indoor conditions at different intervals**

Sampling time intervals (Days)	Insecticides					
	Pyriproxyfen		Fenitrothion		Spinosad	
	Residues* (mg/kg)	Loss (%)	Residues (mg/kg)	Loss (%)	Residues (mg/kg)	Loss (%)
Zero-time #	2.89 ± 0.25 a **	--	3.48 ± 0.50 a	--	0.52 ± 0.24 a	--
1	2.36 ± 0.28 ab	18.34	3.36 ± 1.97 a	3.45	0.49 ± 0.05 a	5.77
3	1.84 ± 0.16 bc	36.21	1.63 ± 0.98 b	53.16	0.27 ± 0.06 b	48.08
5	1.68 ± 0.35 c	42.07	1.58 ± 1.14 b	54.60	0.16 ± 0.03bc	69.23
7	1.39 ± 0.31 cd	52.07	0.80 ± 0.21 bc	77.01	0.08 ± 0.08 c	84.62
9	0.85 ± 0.35 dh	70.59	0.29 ± 0.04 bc	91.67	--	--
14	0.46 ± 0.07h	84.08	0.10 ± 0.03c	97.13	--	--
LSD <sub>0.05</sub>	0.56		1.43		0.19	
Half life period	5.41 days		3.5 days		3.5 days	

# an hour after application

\* Residues were the average of three replicates

\*\* same letters mean no significant difference.

**Table 2. Effect of cooling and cooking processes of tomato fruits on residues of pyriproxyfen, fenitrothion and spinosad**

Processes	Insecticides					
	Pyriproxyfen		Fenitrothion		Spinosad	
	Residues* (mg/kg)	elimination (%)	Residues (mg/kg)	elimination (%)	Residues (mg/kg)	elimination (%)
Control #	2.89	--	3.48	--	0.52	--
Cooling	2.43	15.92	3.15	9.48	0.29	44.23
Cooking	1.09	65.40	0.24	93.10	0.26	50.00
LSD <sub>0.05</sub>	0.56		1.43		0.19	

# sample without cooling or cooking taken after 24 hr from insecticide application

\* Residues were the average of three replicates

**Table 3. Residues and percent of loss of pyriproxyfen, fenitrothion and spinosad from green peppers fruits under indoor conditions at different intervals**

Sampling time intervals (Days)	Insecticides					
	Pyriproxyfen		Fenitrothion		Spinosad	
	Residues* (mg/kg)	Loss (%)	Residues (mg/kg)	Loss (%)	Residues (mg/kg)	Loss (%)
Zero-time #	6.71 ± 1.73 a**	--	3.37 ± 1.03 a	--	0.23 ± 0.09 a	--
1	5.35 ± 1.14 ab	20.27	2.06 ± 1.07b	38.87	0.19 ± 0.03 ab	17.39
3	4.61 ± 0.24 bc	31.30	0.64 ± 0.77 c	81.01	0.19 ± 0.05ab	17.39
5	3.76 ± 0.72 cd	43.96	0.19 ± 0.16c	94.36	0.14 ± 0.02 ab	39.13
7	3.39 ± 0.32 cd	49.48	0.10 ± 0.21 c	97.03	0.12 ± 0.02 b	47.83
9	2.36 ± 0.34 dh	64.83	0.07 ± 0.04c	97.92	--	--
14	0.80 ± 0.11f	88.08	0.04 ± 0.02c	98.81	--	--
LSD <sub>0.05</sub>	1.41		1.08		0.10	
Half life period	5.41 days		1.5 days		7.5 days	

# an hour after application

\* Residues were the average of three replicates

\*\* same letters mean no significant difference.

**Table 4. Effect of cooling and cooking processes of green peppers fruits on residues of pyriproxyfen, fenitrothion and spinosad**

Processe	Insecticides					
	Pyriproxyfen		Fenitrothion		Spinosad	
	Residues* (mg/kg)	elimination (%)	Residues (mg/kg)	elimination (%)	Residues (mg/kg)	elimination (%)
Control #	6.71	--	3.37	--	0.23	--
Cooling	5.68	15.35	2.51	25.52	0.14	39.13
Cooking	1.52	77.35	0.58	82.79	0.10	56.52
LSD <sub>0.05</sub>	1.41		1.08		0.10	

# sample without cooling or cooking taken after 24 hr from insecticide application

\* Residues were the average of three replicates

The degradation of the most common insecticide residues that used lately in greenhouses, pyriproxyfen (insect growth regulator and guvenile hormone), fenitrothion (an organophosphate), and spinosad (a biological insecticide) reflect that initial deposits of pyriproxyfen on tomatoes and green peppers were 2.89 and 6.71 mg/kg, respectively. The results indicate that the residues after two weeks of application are considered high and above the maximum residue levels (MRL) which is 0.1 for pyriproxyfen. While it was less than the MRL (0.5 mg/kg) for fenitrothion on tomatoes and peppers after 14 days. Jiguo *et al.*, (2007), reported that pesticides concentrations in vegetable were higher than the national Maximum Residue Limit (MRL). Also, Rosa *et al.*, 2008 reported that pesticide residues were detected in 84% of the total samples (63 from 75 samples) and pesticide concentrations were higher than MRL in 18 samples. For spinosad, the residue levels were within the permissible range set by FAO. It has been also noticed that the disappearance of spinosad was slower on peppers than on tomatoes. Cooling of the fruits had little effect in the disappearance of the residues of the three insecticides on both fruits, while cooking had a noticeable effect on the disappearance of

the residues, although the disappearance of pyriproxyfen was more on peppers than on tomatoes and the disappearance of fenitrothion was more on tomatoes than on peppers. The variation of the removed percentage between the insecticides might depend on the physicochemical properties of insecticides which organize, the absorption, penetration or binding insecticide with the fruit surface. The results in line with that reported by Calumpang *et al* (1988). They found that washing cucumbers reduced ethylene bis dithiocarbamate (EBDC) levels by 46% and 50%. Moreover the results in agreement with that reported by Al-Samariee *et al*, 1987 and Al-Sarar, 1996. They mentioned that pirimiphos-methyl is rapidly degraded on cucumber fruits. They claimed that the quick decay might due to the temperature, humidity inside the protected house and the higher water contents of the cucumber fruits. In addition, Abdalla *et al*, 1993, reported that 83.71% of the pirimiphos-methyl residues diminished after 24 hour from the tomato fruits under the field conditions and that might due to the weather, metabolism and the high vapor pressure of pirimiphos-methyl.

The results in agreement with that reported by Sayed *et al*, 1977; Khan *et al*, 1985 and Al-Khalaf *et al* 1992. They mentioned that the time required to reach the acceptable residue limits is 3-6 days for the organophosphorus compounds. Moreover, Hegazy *et al*, 1989, reported that the  $t_{0.5}$  was 3 days for pirimiphos-methyl on tomato under the indoor conditions. In addition Al-Sarar, 1996, reported that the rate of decay of cypermethrin residues on tomato fruits was higher than that on the cucumber fruits. Where, the  $t_{0.5}$  of cypermethrin was 0.88 and 2.95 day on tomato and cucumber fruits, respectively. In the meantime he reported that the  $t_{0.5}$  of pirimiphos-methyl was 2.31 day on tomato fruits.

The present investigation suggests that, the less persistent and more save insecticides should be used under the indoor conditions to control insect pests on vegetables. Avoidance of insecticide application at the fruit stage. The harvest of fruits should be after adequate time from the last application and that depends on the type of insecticides. Management of the chemical control and integrates few methods or disciplines under the umbrella of IPM may be useful to control the serious pest and reduce the environmental and health hazards.

#### ACKNOWLEDGEMENT

The authors would like to thank King Abdull Aziz City for Sciences and Technology for financial support of research No. AT-11-132 Permission No. 67715/60

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## الملخص العربي

### كشف وإزالة متبقيات مبيدات معينة من ثمار الطماطم والفلفل الحلو

سليمان بن عبد الكريم بن علي المحميد، علاء صلاح الدين كامل، ضيف الله بن هادي الراجحي

من على ثمار كل من الطماطم والفلفل على التوالي. بلغت المتبقيات الأولية المقدرة من مبيد الفينيتروثيون على ثمار الطماطم والفلفل 3.48 و 3.37 مجم/كجم على التوالي. وكانت النسبة المئوية لفقد مبيد الفينيتروثيون من ثمار الطماطم 97.13% ومن ثمار الفلفل 98.81%، وكان زمن اختفاء نصف الكمية ( $t_{0.5}$ ) من ثمار الطماطم 3.5 يوم ومن ثمار الفلفل 1.5 يوم. انخفضت كمية المتبقي من المبيد نتيجة عملية التبريد بنسبة 9.48% و 25.52% بينما عملية الطبخ أدت إلى خفض كمية المتبقي 93.1% و 82.79% من ثمار كل من الطماطم والفلفل على التوالي. أما بالنسبة لمبيد السبينوساد فإن قيم المتبقيات الأولية بلغت 0.52، 0.23 مجم/كجم على كل من ثمار الطماطم والفلفل. ووصل الفقد إلى 84.62% و 48.83% على كل من الطماطم والفلفل بعد 7 أيام. وكان زمن اختفاء نصف الكمية ( $t_{0.5}$ ) 3.5 يوم في حالة ثمار الطماطم و 7.5 يوم في حالة ثمار الفلفل وانخفض المتبقي بنسبة 44.23%، 39.13% نتيجة عملية التبريد بينما كان الخفض نتيجة عملية الطبخ 50% و 56.52% في حالة ثمار كل من الطماطم والفلفل على التوالي.

تم تقدير المتبقيات لثلاثة من المبيدات الحشرية الأكثر استخداماً وشيوعاً في البيوت المحمية في الآونة الأخيرة وهي البيريروكسفين (منظم النمو والمحاكي لهرمون الشباب) والفينيتروثيون (من المبيدات الفسفورية العضوية) والسبينوساد (من المبيدات الحيوية الواعدة) وتم تتبع تحطم تلك المبيدات على ثمار محصولي الطماطم والفلفل، كما تم تقدير مستويات المبيدات بعد تبريد وطبخ الخضروات المذكورة بعد يوم واحد من الرش.

وأظهرت الدراسة أن المتبقيات الأولية من مبيد البيريروكسفين على ثمار الطماطم والفلفل الأخضر وصلت إلى 2.89، 6.71 مجم/كجم على التوالي. تدريجياً بمرور الزمن وصلت النسبة المئوية لفقد المبيد إلى 84.14% على ثمار الطماطم وإلى 88.08% على الفلفل بعد 14 يوماً من التطبيق، كان زمن اختفاء نصف كمية المتبقي من المبيد البيريروكسفين ( $t_{0.5}$ ) على ثمار الطماطم والفلفل الأخضر هو 5.14 يوم. وأوضحت الدراسة أن عملية التبريد أدت إلى إزالة 15.92% من متبقي المبيد من ثمار الطماطم أما بالنسبة لثمار الفلفل فحدث إزالة 15.35%. أما عملية الطبخ فأدت إلى تقليل كمية المتبقي من مبيد بييريروكسفين بنسبة 65.4%، 77.35%