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Detection of Insecticide Residues on Tomato Fruits Grown in Greenhouses in Khartoum State

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Abstract: The study was carried out to assess the residues of main insecticides used in tomato crops grown in greenhouses in Khartoum State and to investigate the awareness of farmers regarding the hazard of chemical control. A questionnaire was prepared and distributed to farmers of tomato greenhouses to collect data. A total of nine sets of greenhouse tomato fruit samples were collected from Khartoum State for testing. The residues of insecticides were detected and measured using high performance liquid chromatography (HPLC). The results revealed that, all the samples contained lambda cyhalothrin and/or imidacloprid insecticides residues. The LODs (limit of detection) for the imidacloprid and lambda cyhalothrin were found to range between 0.3275 and 0.02818, and the LOQs (limit of quantification) ranged between 0.1087 and 0.09338 mg kg^{-}, respectively. The levels of residues measured in the greenhouse tomato samples were higher than the maximum residue levels (MRLs) for the measured insecticides, except for lambda cyhalothrin in one sample (Sondos Agricultural Project) and imidacloprid (Alafon) in

Keywords: Insecticides; Lambda cyhalothrin; imidacloprid; greenhouse tomato samples

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another sample. Generally, high percentage of greenhouse farmers do not take in consideration the method of application of pesticides especially type of pesticides, time and number of sprays as well as time of harvesting. Most of farmers do not resort to extension service. The study suggested training the farmers, use of organic farming, biological control, consideration of safety period before harvest and using of selective pesticides.

INTRODUCTION

Tomato (*Lycopersicon esculentum* L.), family Solanaceae, is an important food and cash crop specially for low income farmers in the tropics (Abdelrahman, 1994). The origin of tomato is Central and South America, particularly Mexico, from where tomato was transferred to Europe in the 16^{th} century, then to the Old World countries (Andersson and Palsheden ,1998). According to Horticulture Administration Annual report (2003-2010), tomato is the second to onion in scale of production in Sudan. The mean annual production for the last 8 years (2003-2010) was 462250 tons. It is grown in Sudan in almost all parts of the country during the winter months and the rainy season. Greenhouses are designed to be able to grow crops under partially or fully controlled environmental conditions to obtain maximum productivity and quality of fruit and vegetables (Angioni *et al.* 2011and Boobis *et al.* 2008). Greenhouse requirements for optimal tomato production are also favourable for the development of fungal diseases (Conacher and Mes 1993). Insects and mites are usually present in greenhouses, so insecticides are widely used in tomato protection programs.

Chemical control of crop pests is well established in many countries, including Sudan, where insecticide spraying started in the Gezira scheme in 1945 for the control of insects pests of cotton (Coulston and Korte 1975). However, pesticides may have negative effect if they remain on fruit and vegetables, constituting a risk to consumers (Davey *et al*. 1992). Pesticide residues refers to the pesticides that remain on or in food after they are applied to food crops (Dikshit *et al*. 2000), becoming part of food chain. Not all vegetables contain pesticide residues, and where they do occur, they are typically at low levels. The residues of pesticide can include the metabolic degradation products of pesticides (Elbashir *et al*. 2013). Pesticide residues in tomato are monitored with reference to maximum residue limits (MRL) and are based on analysis for residues in tomato samples (Farah and Abdel Rahaman, 1988). With the intensive use of pesticides in greenhouse tomato, residues may be accumulated at levels higher than those permitted by the international MRLs (Ji *et al.* 1998). Intake of active ingredients through food ingestion has been shown to be up to five folds higher than other exposure routes like air inhalation and ingestion of drinking water (Juraske *et al*. 2009 and Luke *et al*.1981).

Imidacloprid is used to control sucking, chewing and soil insects, and fleas on pet animals (Utture *et al*. 2012). Lambda-cyhalothrin has a high activity against a wide range of chewing and sucking insects pests, on various crops and in public health to control vectors (Davey *et al*. 1992, Mathirajan *et al*. 2000). From an environmental and food safety viewpoint, the persistence of lambda-cyhalothrin in animal, soil, water and plants can be problematic (Hill and Inaba, 1991).

To measure the levels of a large number of pesticides and derived products, in tomato, multiresidue extraction methods and separation techniques using gas chromatography (GC) and liquid chromatography (LC) are required (Luke *et al*. 1975; Coulston and Corte 1975).

The aim of the study was to assess whether the insecticide residues in tomato fruit grown in greenhouses, comply with the allowed MRLs set by the European Union for many of insecticides, as well as to investigate the awareness of farmers regarding the hazard of chemical control.

MATERIAL AND METHODS

2.1 Sampling Tomato samples were collected randomly from nine greenhouses during the growing season of 2012/2013 in Khartoum State. The representative samples of tomato fruits were taken from three different locations: Khartoum (Taiba Hasanab (1), Jabal aolia (2), Sondos Agriculture Project (3), Khartoum North (Abu Halima (4), Omdom (5), Alafon (6) and Omdurman (Markheyat (7), Jamoea (8) and Ahamda (9). From each location fruits of 1kg sample of tomato fruit were collected randomly and placed into polyethylene bags, labelled and immediately taken to the pesticides laboratory for analysis. The samples from each area were finely chopped using a pre-cleaned knife and mixed thoroughly to homogenise the samples prior to extraction.

2.2 Questionnaire

A questionnaire of 14 questions was prepared and distributed to tomato greenhouse farmers. Ten farmers were questioned while they were working in their greenhouses. The questions included: types of pesticides used, farmer's knowledge of pesticides, intervals between spraying, recommended doses, signs in treated greenhouses, use of short duration pesticides, levels of spraying per season, safety periods, MRLs and other points.

2.3 Chemical and reagents

Analytical standards of imidacloprid and lambda-cyhalothrin (99.9% pure) were obtained from the Plant Protection Directory (Ministry of Agriculture, Bahri, Sudan). The standards were stored in a freezer at -10°C. Standard solutions of 2 $mg.ml^{-1}$ of these insecticides were made by dissolving 20 mg from each of the analytical standards in 10 ml from the mobile phase (7:3 water: methanol). All solvents used were of analytical grade or similar quality. The solvents used (acetone (C_3H_60), dichloromethane (CH_2C_{l2}) and N-hexane (C_6 H_6)) were HPLC grade (Scharlau, Spain). The toluene used (C_6H_5-CH_3) was analar grade. Other reagents such as anhydrous (Na_2SO_4), Florisil® (60-100) mesh and sodium chloride (NaCl) were purchased in Khartoum, Sudan.

2.4 Extraction

Extraction was done according to the methods of Specht and Winkleman (1980) and Pang *et al.* (1999). Forty grams per sample were blended with 5 ml water and 100 ml acetone in a high speed chemical resistant blender (National Analytical Corporation, Mumbai, India) for two minutes. The extract was collected in an Erlenmeyer flask and filtered through a fast rate filter paper (Whatman no. 1) in a Buchner funnel. The Erlenmeyer flask was rinsed with a little water and cleaned with acetone and the extract was filtered. The combined filtrates were collected in an Erlenmeyer flask for partitioning.

Extracts from each sample were put into a 500 ml separation funnel. Fifty ml of dichloromethane and 10 ml of saturated NaCl solution were added. The mixtures were carefully shaken for 2 minutes (with an open top to reduce pressure), and left to stand for 10 minutes to allow separation of layers. The organic layer was collected and then re-extracted with 50 ml dichloromethane. The combined extracts of dichloromethane were filtered through cotton wool and mixed with 25g of anhydrous Na_2SO_4, which was added to improve the extraction of polar pesticides and for its moisture absorbing ability. The products were then collected in 500 ml round-bottom flasks. Extracts were again re-filtered through cotton wool and a 3 cm layer of anhydrous Na_2SO_4 in a separation funnel. The solvent was removed to dryness by a rotary evaporator (Buchi, Postfach, Switzerland) operating under vacuum at a temperature of 40°C. Dried extracts were dissolved in 10 ml of hexane and kept in closed vials at -10°C for clean-up and insecticide residue analysis.

2.4.1 Clean-up

Sample clean-up followed the methods of Specht and Winkleman (1980) and Pang *et al.* (1999). Sample clean-up was done using a solid phase extraction (SPE) column containing Florisil® and anhydrous Na_2SO_4. The column was first rinsed with a few ml of hexane. Extracts from each sample were added as soon as the hexane dried in the top of the Florisil® layer and was then eluted by 200 ml of toluene: acetone in a 19:1 mixture. The elutes were concentrated to dryness by a rotary evaporator (Buchi, Postfach, Switzerland). The dry powder was dissolved in 10 ml of water: methanol (7:3) and then was transferred to a 10 ml volumetric flask and stored at –10°C for subsequent residue analyses by HPLC.

2.5 Residue analyses

A Shimadzu (Kyoto, Japan) CLASS-VP, Version 5.22 high performance liquid chromatography (HPLC) device with a UV/visible detector was used for identification and quantification of insecticides. Separation was performed on a Luna C18 column. The instrument system consisted of LC-10 ADvp binary pump, DGU- 14 A online degasser, SPD-M10-Avp Luna absorbance detector, Sil-10 ADvp auto injector, CTO-10 ASvp column oven fitted with Shim- Pack VP-ODS (150 mm x 4.6 mm 10μm) column and a similar pre- column (4mm x 4mm i.d) were used for the separation. Samples were auto-injected. The detector was connected to the computer for data processing. The working condition of the HPLC was a binary gradient, with the mobile phase being acetonitrile: water (70:30), the flow rate was 1 ml min^{-1}, injection volume was 10 µL and the wavelength of the UV/Visible detector was fixed at 210 nm for the residual analysis of two insecticides, imidacloprid and lambda-cyhalothrin.

A total of 5 minutes per sample was necessary to assay the insecticides. Four concentrations (2, 10, 16 and 24 ppm) of the analytical standard solution of the insecticides were prepared in mobile phase solution and injected under the same instrument condition. The response was used for the construction of standard curves (Fig. 1, 2, 3, 4, 5 and 6). Insecticides residues were identified by comparing the retention time and calculated peak area. The limit of detection (LOD) and limit of quantification (LOQ) of the method (sensitivity) for imidacloprid and lambda cyhalothrin were determined from the signal-to-signal ratio using the equations:

LOD = 3*SD (standard deviation) of intercept/slope
LOQ = 10*SD (standard deviation) of intercept/slope

RESULTS AND DISCUSSION

3.1 Questionnaire

A simple questionnaire was distributed to ten greenhouse farmers. All farmers were using pesticides to control insects and disease pests. The majority of farmers (80%) were using pesticides not specific for greenhouse crops, only 40% of them were using recommended doses, 80% were using short half-life pesticides, 40% were harvesting their crops before the safety period, they ignored pre-harvest intervals of pesticides and 30% of them did not put signs in treated greenhouses. 40%

of the farmers did not know about the maximum residue levels (MRLs) and only 60% of the interviewed farmers using protective clothing when applying pesticides. Tomatoes fruit grown in greenhouses were sprayed with pesticides every 4-6 days from the first week after seed germination to the last picking. These findings are in agreement with the report of Abdelrahman (1994) who found that tomato crops were sprayed with pesticides every 3-6 days from the first week of seed germination to the last picking.

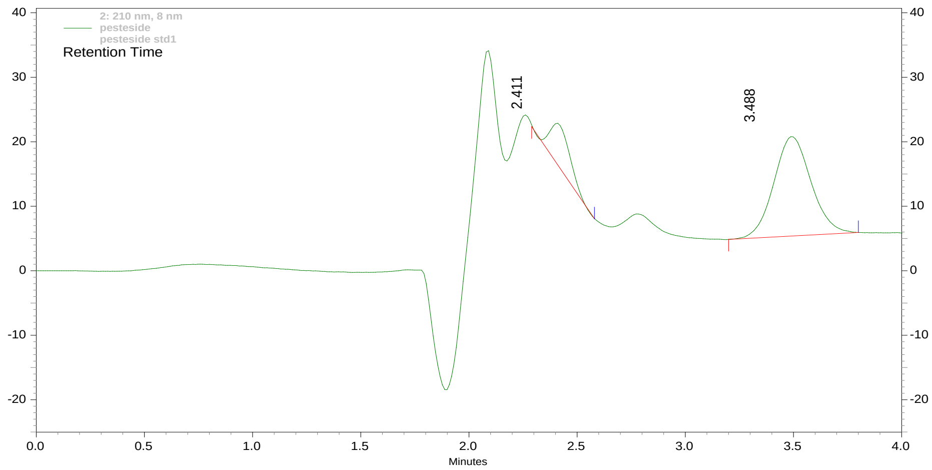

Fig. 1**:** Chromatogram of lambda cyhalothrin and imidacloprid standards (2 ppm) by HPLC

Insecticide name	Retention time	Area	Standard concentration	Unit
imidacloprid	2.411	39270	2.000	ppm
Lambda cyhalothrin	3.488	184193	2.000	ppm

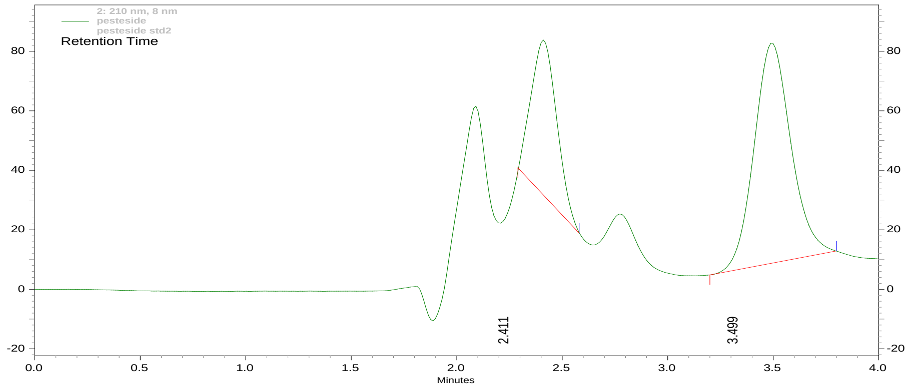

Fig. 2: Chromatogram of lambda cyhalothrin and imidacloprid standards (10 ppm) by HPLC

Pesticide name	Retention time	Area	Standard concentration	Unit
imidacloprid	2.411	434595	10.000	ppm
Lambda cyhalothrin	3.499	895607	10.000	ppm

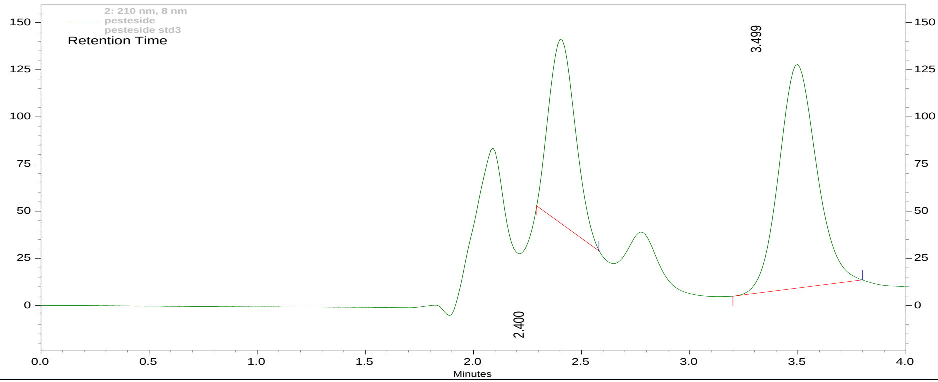

Fig. 3: Chromatogram of lambda cyhalothrin and imidacloprid standards (16 ppm) by HPLC

Insecticide name	Retention time	Area	Standard concentration	Unit
imidacloprid	2.400	824506	16.000	ppm
Lambda cyhalothrin	3.499	1432822	16.000	ppm

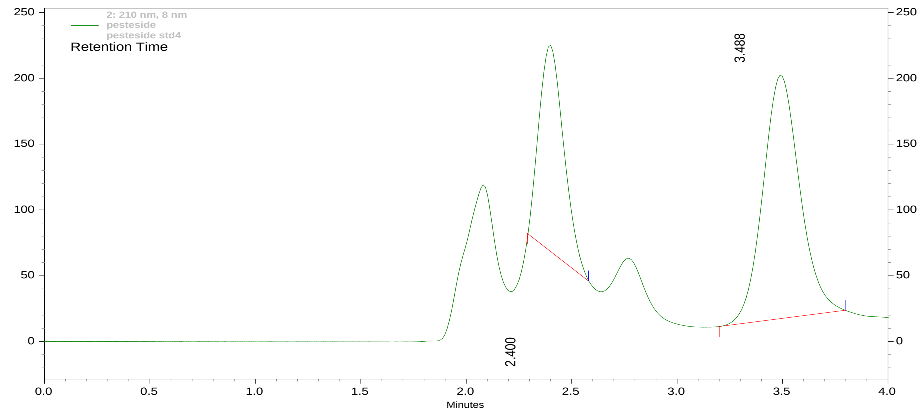

Fig. 4: Chromatogram of lambda cyhalothrin and imidacloprid standards (24 ppm) by HPLC

Insecticide name	Retention time	Area	Standard concentration	Unit
Imidacloprid	2.400	1272589	24.000	ppm
Lambda cyhalothrin	3.488	2237058	24.000	ppm

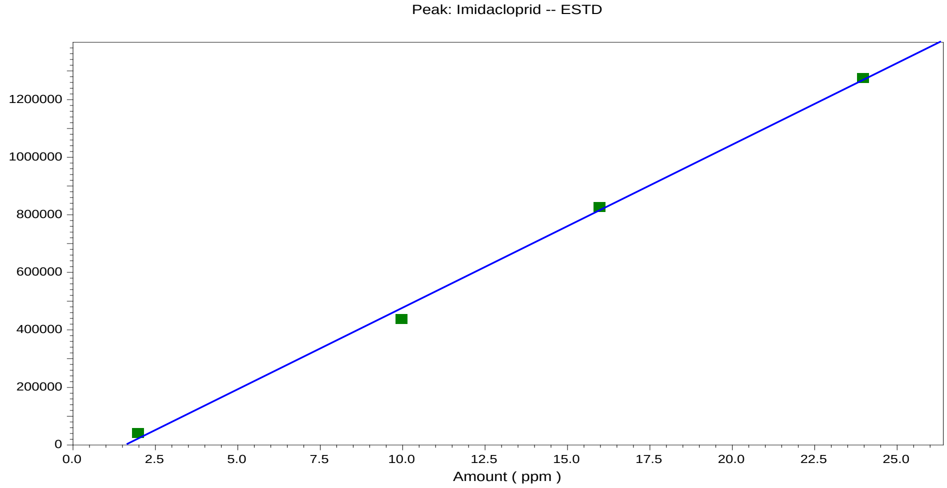

Fig. 5: Calibration curve of imidacloprid standards

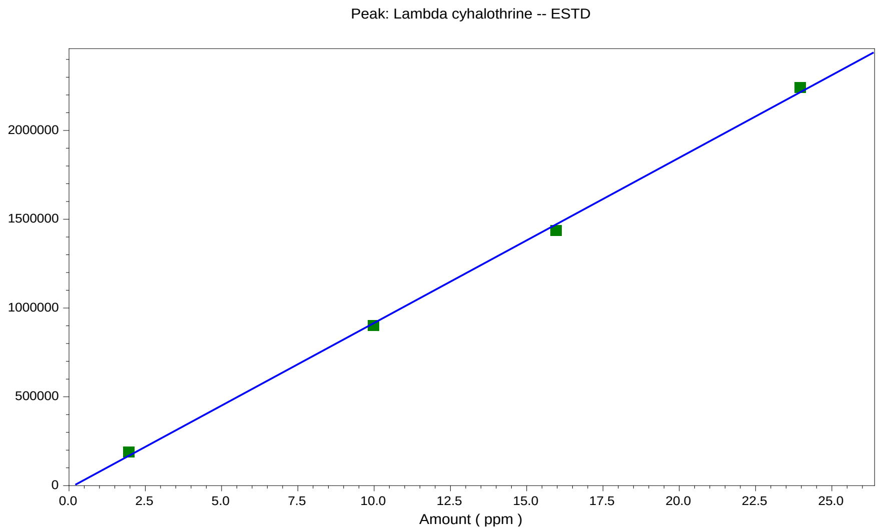

Fig. 6: Calibration curve of lambda cyhalothrin standards

3.2 Residue analysis

A total of nine greenhouse tomato fruit samples collected from Khartoum State were analyzed. High performance liquid chromatography (HPLC) was used to analyse all samples for the presence of two pesticides in greenhouse tomato fruit samples in the present study. The LODs (four replicates) for the imidacloprid and lambda cyhalothrin were calculated and ranged between 0.3275 and 0.02818, and the LOQs were ranged between 0.1087 and 0.09338 mg kg^{-1}, respectively (Table 1).

All the samples were contaminated with lambda cyhalothrin and/or imidacloprid pesticides residues. Samples obtained from the Khartoum State (Sample 1, 2 and 3) contained imidacloprid residue in Taiba Hasanab at level of 1.53 mg.kg^{-1}. lambda cyhalothrin and imidacloprid residues in Jabal aolia were at levels of 0.37 and 0.71 mg.kg^{-1}, and 0.01 and 0.859 mg.kg^{-1} in Sondos Agriculture Project. In tomatoes from the Khartoum North (Abu Halima, Omdom, Alafon) lambda cyhalothrin degradation residues were found at levels of 0.12, 0.16, and 15 and imidacloprid at levels of 1.53, 2.6 and 0.47 mg.kg^{-1}, respectively. Two of the tomato samples from the Omdurman (Markheyat and Ahamda) were contaminated with lambda cyhalothrin at levels of 0.11 and 0.13 mg.kg^{-1}, respectively. In Jamoea and Ahamda imidacloprid residues were at levels of 1.17 and 3.56 mg.kg^{-1}, respectively. The highest concentration of pesticide residue was 3.56 mg.kg^{-1}of imidacloprid found in Ahamda collected from the Omdurman (Table 2). The positive residue values measured in the greenhouse tomato samples were all higher than the maximum residue levels established by either Codex Alimentarius (FAO/WHO, 2009) for the two insecticides, except for lambda cyhalothrin in Sondos Agriculture Project and imidacloprid in Alafon (Table 1).

These findings are similar to those of Elbashir *et al*. (2013) who found that the pyrethroid residues levels in tomato fruit exceeded the MRL. The high levels of insecticide residues in tomato fruit in Sudan raise questions over the safety of food commodities sold across the country. The presence foregoing insecticides on tomatoes indicates misuse of pesticides by Sudanese farmers. These results also are similar to other studies on residues in tomato fruit but the levels of the residues found were higher than those reported by others (Jayakrishnan *et al.* 2005; Tahany *et al.* 2011; Chauhan and Kumari, 2012; Elbashir, 2013).

Table 1: Retention time, LOD and LOQ for lambda cyhalothrin and imidacloprid screened in greenhouse tomato fruit samples by HPLC

Pesticide names	Retention time (min)	peak Area	LOD (mg.kg^{-1})	LOQ (mg.kg^{-1})	MRL (mg.kg^{-1})
lambda cyhalothrin	3.499	1187420	0.00142	0.02818	0.1
imidacloprid	2.411	642740	0.00331	0.3275	0.5

LOD; limit of detection, LOQ; limit of quantification, MRL; maximum residue level

Table 2: Concentrations of Lambda cyhalothrin and imidacloprid residues in greenhouse tomato fruit samples collected from different locations in Khartoum State, Sudan

Location and sample number	Mean of pesticide residues (mg.kg-1)	
	Lambda cyhalothrin	Imidacloprid
Khartoum (Taiba Hasanab)	ND	1.1
Khartoum (Jabal aolia)	0.37	0.71
Khartoum (Sondos Agriculture Project)	0.01	0.85
Khartoum North (Abu Halima)	0.12	1.53
Khartoum North (Omdom)	0.16	2.6
Khartoum North (Alafon)	0.15	0.47
Omdurman (Markheyat)	0.11	ND
Omdurman (Jamoea)	ND	1.17
Omdurman (Ahamda)	0.13	3.56
MRL	0.1	0.5

ND: not detected

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تقدير متبقيات مبيدات الحشرات في ثمرة الطماطم المزروعة في البيوت المحمية بولاية الخرطوم

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المستخلص: هدفت هذه الدراسه لتقدير متبقيات مبيدات الحشرات الاساسية المستخدمة على محصول الطماطم المزروعة في البيوت المحمية بولاية الخرطوم وايضا لمعرفة مدى وعي المزارعين في التعامل مع المكافحة الكيميائية. تم اعداد وتوزيع استبانة على مزارعى محصول الطماطم فى البيوت المحمية لجمع المعلومات. جمعت تسعة عينات من ثمار طماطم البيوت المحمية من ولاية الخرطوم للإختبار. أُستخدم جهاز كروماتوغرافيا السائل عالي الاداء فى كشف وتقديرمتبقيات المبيدات الحشرية.

اظهرت النتائج بان كل العينات تحتوي علي متبقيات مبيدي لامبدا سيهالوثرين وايميدا كلوبريد. أقل حد للتقدير للاميدا كلوبريد و/او اللامبدا سيهالوثرين تراوحت ما بين 0.3275 و0.02818 واعلي حد للتقدير من 0.1087 و 0.09338 مل/كجم على التوالى. كما بينت النتائج ان مستويات المتبقي للمبيدين في عينات الطماطم في البيوت المحمية أعلي من الحد المسموح بها عدا مبيد الامبدا سيهالوثرين في عينة مشروع سندس الزراعي ومبيد اميدا كلوبريد في عينة العليفون .

بصورة عامة وجد أن نسبة عالية من مزارعي البيوت المحمية لا يضع في الاعتبار طريقة استخدام المبيدات خاصه فيما يتعلق بنوع المبيد , زمن وعدد مرات الرش و زمن الحصاد. لا يلجأ معظم المزارعين لتلقى الخدمات الارشادية.

إقترحت الدراسة بتدريب المزارعين ، استخدام الزراعة العضوية ، إستخدام المكافحه الحيوية ، مراعاة فترة الامان قبل الحصاد واستخدام مبيدات مختارة.