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Research Article

Pesticides Residues in Samples of Sweet Peppers (*Capsicum annum*) from Khartoum State, Sudan

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Abstract

This study was carried out to evaluate the level of pesticide residues in samples of fresh sweet pepper collected from vegetables markets (Central vegetables market Khartoum North and Khartoum) and vegetables farms (South Khartoum and North Khartoum) in Khartoum state, Sudan. Three samples were randomly collected, from each location in paper bag and transferred immediately to the Pesticides Laboratory, Faculty of Agriculture, University of Khartoum. Samples were immediately extracted according to official method and analysed by gas chromatography (GC) equipped with flame ionization detector (FID) and GC-MS. Results were expressed in $mgKg^{-1}$ fruit. Detectable levels of heptachlor and dimethoate were found in all samples of both markets while omethoate was found in Khartoum North central market only. Heptachlor was the only pesticide detected in the washings of samples from both markets. The highest frequency of residue detection and the highest load per Kg fruit (3.9 mg Kg^{-1}) was found in Khartoum central market. The highest of residues was found associated with dimethoate with respective average of 0.203 mg Kg^{-1} and 0.187 mg Kg^{-1} in Khartoum and Khartoum North central markets. On the other hand, the lowest level was found associated with heptachlor with an average of 0.051 mg Kg^{-1} and 0.057 mg Kg^{-1} in washing water from Khartoum and Khartoum North central markets respectively, while its level in the washed fruits of both markets is around 0.05 mg Kg^{-1}. On the other hand, detectable levels of heptachlor, dimethoate, diazinon, omethoate and endosulfan were found in at least one or both markets, while chlorpyrifos level was below the detection limit. The highest residue load per Kg fruit (2.912 mg Kg^{-1}) was found in samples from North Khartoum vegetable farms. Heptachlor and omethoate were the only pesticides detected in the washings of samples from both types of farms. The highest residue level was found associated with dimethoate (average of 0.1 mg Kg^{-1}) in washed samples from south Khartoum farms, followed by heptachlor (around 0.04 mg Kg^{-1}) in washed fruits from farms of both locations (South Khartoum and North Khartoum). On the other hand, the lowest level was found associated with diazinon in washed fruits from North Khartoum with an average of 0.013 mg Kg^{-1}. The highest frequency of violation (100 %) in vegetable farms (> MRLs) corresponds to dimethoate, heptachlor, endosulfan beta and omethoate while those in central markets correspond to dimethoate and heptachlor. Washing with distil water seem to completely remove the residues of dimethoate while it reduces heptachlor residues by 44 - 54%. However, it apparently has no effect on other detected pesticides.

Keywords: *Sweet Peppers; Pesticides Residues; Khartoum; Sudan*

Introduction

Sudan is nominated as major producer of vegetable crops for local consumption and plays significant role in alleviating the world food shortage [1,2]. Most of horticultural production in Sudan is under irrigated farming system, along valleys and streams in the areas of high rainfall in the south. Most of the fertile locations in South Kordofan, South Darfur are considered of great potential for horticultural production [3]. Guddoura [4] reported that vegetable and fruit production comprises more than 12% of the total agricultural output compared to 21% contributed by grains, 15% by cotton and 9% by oil seeds in the Sudan. The commercial production of vegetables in Sudan is faced by many problems that worry producers such as the attack by pests and diseases. According to Dabrawski [5], the losses due to pest attack were estimated at 25% of vegetable production. Therefore, farmers tend to over use pesticides, they may apply any pesticides to reduce losses and increase their yield which led to high risks for human health and the environment hazards [5]. Pepper (*Capsicum annuum L.*) is the second most consumed vegetable worldwide and is characterized by its high levels of vitamin C (ascorbic acid), pro-vitamin A (carotene) and calcium. In fact, intakes of 50 - 100g of fresh pepper fruits could provide 100% and 60% of the recommended daily amounts of vitamin C and A, respectively [6,7]. Mature pepper fruits are also rich in carotenoids, compound with antioxidant and anti-carcinogenic capacity. Furthermore, either immature or mature fruits contain a high concentration of antioxidant phenolic compounds [6,8]. Fruit and vegetables are attacked by different pest and diseases during production and storage leading to damages and reduction of the quality and quantity of the crops. In order to reduce the loss and maintain the quality of fruit and vegetables, pesticides are used with other pest management techniques to combat pests and diseases. The use of pesticides for prevention or control of crop pests left behind pesticide residues in and on food crops may result in food contamination and affect human or animal health [9] causing direct or indirect toxic effects such as carcinogenicity, mutagenicity, teratogenicity and interference with endocrine system [10]. To evaluate food quality and to minimize risks to human health, goverments and international organization regulated the use of pesticides by setting the maximum residue limitls (MRLs) in food [11]. The grown of vegetable in Sudan is not under the government control as in case of cotton. The pesticides are used without counting the insect and the farmers are determining the type of pesticides, time of application. The results previous studies indicate the presence of high level of different pesticides residues [12-14]. Furthermore, there is no regular monitoring program of pesticides level in food items in Sudan and levels detected were loosed on sporadic studies done by personal interest or following specific accident [15]. The main objectives of this study are; to estimate residue levels of commonly used pesticides in sweet pepper in main vegetable market and vegetable farms in Khartoum state.

Materials and Methods

Sampling and sample preparation

Sampling of fresh sweet pepper fruits was was taken from main vegetable markets and vegetable farms in Khartoum state according to the methods of Pang., *et al*. [16] with minor modifications. Fresh samples were randomly collected from four different locations; two main vegetable markets (Khartoum and Khartoum North Centaral Markets) and two vegetable farms (North Khartoum and South Khartoum). From each location three samples were taken randomly (1 kg each). Each sample was divided into three sub-samples. Collected samples were carefully washed with distilled water and washings were collected in clean conical flasks. The washed fruits were chopped using a pre-cleaned knife and mixed thoroughly to make a homogenise samples prior to extraction.

Chemicals and reagents

Analytical standards of diazinon, malathion, atrazine, chlorpyrifos, alpha and beta endosulfan, pendimethalin and deltamethrin (99% pure) were obtained from the Plant Protection Directorate (Ministry of Agriculture, Khartoum North, Sudan). All solvents (acetone, dichloromethane, toluene and n-hexane) used were of analytical grade or similar quality (99.89% pure). Other chemicals (anhydrous sodium sulfate, Florisil® (60 - 100 mesh) and sodium chloride) were purchased from Lab. Line Co., Sudan.

Extraction and partitioning

Extraction was done according to the methods of Pang., *et al* [16]. Fifty 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 blender jar was rinsed with a few ml of water and acetone and filtered as above. The combined filtrates were collected in a clean Erlenmeyer flask for partitioning. Extracts from each sample were transfered into a 250 ml separation funnel. Fifty ml of dichloromethane and 10 ml of saturated NaCl solution were added. The mixures were carefully shaken for two minutes with frequent opening of the valve to release the pressure. The separatory funnel was left to stand for few minutes to allow separation of layers. The dichloromethane layer was collected a clean conical fask. The aqueous layer was re-extracted with 50, 30 and 20 ml dichloromethane, respectivly. The combined dichloromethane extracts was mixed with 25g of anhydrous Na2SO4, filtered through cotton wool and collected in 500 ml round-bottom flasks. Extracts were again re-filtered through cotton wool with 3 cm layer of anhydrous Na2SO4 in a funnel. The solvent was removed to dryness by a rotary evaporator (Buchi, Postfach, Switzerland) operating under vacuum at 40°C. Dried extracts were reconstituted in 5 ml of n-hexane and kept in closed vials at -10°C for clean-up and pesticide residue analysis.

Clean-up

Sample cleanup followed the methods of Specht and Winkleman [17] and Pang., *et al* [16]. Clean-up was done using a solid phase extraction (SPE) column containing Florisil® and anhydrous Na2SO4. The column was first rinsed with a few ml of n-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 a 20 ml of 19:1 toluene:acetone mixture. The eluates were concentrated to dryness by rotary evaporation. The dry residues were reconstituted in 10 ml of n-hexane, transferred to a 10 ml volumetric flask and stored at -20°C for subsequent residue analysis by GC-FID.

Chromatographic analysis

The pesticides residue analysis was done on a gas chromatograph 2010 Shimadzu, Kyoto system (Japan) with an AOC-5000 auto sampler. The gas chromatograph was equipped with a flame ionization detector (FID) and a DB-5capillary column of 30 ml length and 0.25 mm inner diameter (ID). The stationary phase (0.25 mm thickness) was 5% phenyl, methylpolysiloxane. The detector and injector temperatures were 280 and 300°C, respectively. Helium (purity ≥ 99.999%) was used as a carrier gas at a flow rate of 1.69 ml/min. The split less injector mode was followed. The oven temperature was programmed from initial temperature of 50°C held for one minutes, then increased at 20°C per minute until 75°C, where it was held for 5 minutes, then increased to 230°C at 3°C per minute where it was held for held for 5 minutes and finally increased to 280°C at 10°C per minute, where it was held for 2 minutes. The solvent delay was set to 4 minutes. One µl of each sample was injected into the GC using a syringe of 5 cm long needle. Three concentrations (2, 0.2, 0.02 mg/ml) were prepared for each analytical standard and 1 µL of each pesticide standard was injected into the GC and response (peak height) was used to construct the standard curve. The retention time of standard pesticides and the detection limits were given in table 1. The recovery of the method ranged from 70 - 120%.

Pesticides	Retention time (minutes)	Minimum detected level (mg/kg)	Maximum residue limits (MRLs) (EU 2010)
Diazinon	18.992	0.007	0.05
Omethoate	23.391	0.0311	0.02
Dimethoate	26.526	0.08	0.02
Heptachlor	30.764	0.05	0.01
Chlorpyrifos	32.682	0.03	2
Endosulfan alpha	36.808	0.003	0.05
Endosulfan beta	40.434	0.06	0.05

Table 1: *The retention time of standard pesticides and detection limits*.*

Results and Discussion

The pesticide residues in vegetable and other crops grown in Sudan and the rest of the world had received great concern during the last fifteen years (PPD statistics, 2006 - 2010). The current study focuses in estimating the pesticide residues in sweet peppers, a main component of fresh salads consumed in the Sudan, in Khartoum state, Sudan. Twelve samples were collected from four locations (two farms and two markets). The result indicated the presence of organochlorine and organophosphorus pesticides residues. The detected organochlorine pesticides were; heptachlor, endosulfan alpha and endosulfan beta, while the detected organophosphorus pesticides were; dimethoate, omethoate and diazinon.

The results of market samples (Table 2) showed the presence of detectable levels of heptachlor and dimethoate in all samples from both markets while omethoate was found in Khartoum North central market only. On the other hand, detectable levels of diazinon and endosulfan were found in at least one or both markets, while chlorpyrifos level was below the detection limit. The results of farm samples (Table 3) showed the presence of detectable levels of heptachlor, omethoate, dimethoate, endosulfan (α and β isomers) and diazinon in at least one or both farms while levels of chlorpyrifos were below the detection limit. Interviews and questioners with farmers in the area indicated that the detected pesticides were used by farmers in these locations [12-14]. Further previous reports from Sudan indicated the presence of measurable levels of Heptachlor, dimethoate, diazinon, malathion, chlorpyrifos, ethephon, profenofos, oxyfluorfen and dimethoate in vegetable, fruits and soil samples collected farms Sudan [12,14,18-22]. Furthermore, study conducted in Kuwait [23] reported that the pesticides imidacloprid, deltamethrin, cypermethrin, malathion, acetamiprid, monocrotophos, chlorpyrifos-methyl, and diazinon in commonly consumed fruits and vegetables exceeded their MRLs. While Aldrin, an organochlorine pesticide, was below the MRL. On the other hand, Sah., *et al.* [24] found that the residue of cypermethrin and chlorpyrifos in Bihar, India, were above MRL in okra, Brinjal and Cabbage, while endosulfan and quinalphos were detected in Cauliflower.

B: residue inside Sweet pepper tissues		**Locations**				**MRLs (EU 2010)**
Pesticides	**Parameter**	**Khartoum North central market**		**Khartoum central market**		
		Washed fruits	**Washing water**	**Washed fruits**	**Washing water**	
Heptachlor	Average	0.0495	0.05067	0.048	0.05667	0.01
	Range	0.0302-0.0604	0.031-0.062	0.035-0.06	0.035-0.069	
	Median	0.0579	0.059	0.057	0.066	
	Samples tested +ve (%)	100	100	100	100	
	Violative samples (%)	100%	5.06	100%	100%	
	Residue load/kg fruits	0.147	0.152	0.145	0.17	
Endosulfan alpha	Average	ND	ND	ND	ND	0.05
	Range	ND	ND	ND	ND	
	Median	ND	ND	ND	ND	
	Samples tested +ve (%)	ND	ND	ND	ND	
	Violative samples (%)	0	ND	ND	ND	
	Residue load/kg fruits	ND	ND	ND	ND	
Endosulfan beta	Average	ND	ND	ND	ND	0.05
	Range	ND	ND	ND	ND	
	Median	ND	ND	ND	ND	
	Samples tested +ve (%)	ND	ND	ND	ND	
	Violative samples (%)	0	ND	0	ND	
	Residue load/kg fruits	ND	ND	ND	ND	
Diazinon	Average	ND	ND	ND	ND	0.05
	Range	ND	ND	ND	ND	
	Median	ND	ND	ND	ND	
	Samples tested +ve (%)	ND	ND	ND	ND	
	Violative samples (%)	0	ND	0	ND	
	Residue load/kg fruits	ND	ND	ND	ND	

Chlorpyrifos	Average	ND	ND	ND	ND	2
	Range	ND	ND	ND	ND	
	Median	ND	ND	ND	ND	
	Samples tested +ve (%)	ND	ND	ND	ND	
	Violative samples %	0	ND	0	ND	
	Residue load/kg fruits	ND	ND	ND	ND	
Dimethoate	Average	0.187	ND	0.203	ND	0.02
	Range	0.115 - 0.229	ND	0.124 - 0.248	ND	
	Median	0.219	ND	0.238	ND	
	Samples tested +ve (%)	100	ND	100	ND	
	Violative samples%	100%	ND	100%	ND	
	Residue load/kg fruits	0.563	ND	0.61	ND	
Omethoate	Average	ND	ND	0.995	ND	0.02
	Range	ND	ND	0.607 - 1.213	ND	
	Median	ND	ND	1.164	ND	
	Samples tested +ve (%)	ND	ND	100	ND	
	Violative samples (%)	ND	ND	20.3	ND	
	Residue load/kg fruits	ND	ND	2.984	ND	
Total load in washing		0.152		0.17		
Total load in tissues		0.71		3.734		
Total load in fruits		0.862		3.904		

Table 2*: Levels of pesticides residues (mg kg $^{-1}$) detected in samples collected from markets (Khartoum North and Khartoum central market) of Khartoum State.*

ND: Not Detected; MRLs: Maximum Residue Limits.

Pesticides	Parameters	Locations				MRLs (EU 2010)
		South Khartoum		North Khartoum		
		Washed fruits	Washing water	Washed fruits	Washing water	
Heptachlor	Average	0.047	0.037	0.050	0.044	0.01
	Range	0.029 - 0.058	0.02 - 0.05	0.031 - 0.061	0.027 - 0.054	
	Median	0.055	0.04	0.058	0.051	
	Samples tested +ve (%)	100	100	100	100	
	Violative samples (%)	100%	100%	100%	100%	
	Residue load/kg fruits	0.142	0.11	0.15	0.132	
Omethoate	Average	ND	ND	ND	1.084	0.02
	Range	ND	ND	ND	0.677 - 1.314	
	Medium	ND	ND	ND	1.26	
	Samples tested +ve (%)	ND	ND	ND	100	
	Violative samples (%)	0	0	0	100%	
	Residue load/kg fruits	ND	ND	ND	3.252	
dimethoate	Average	0.101	ND	ND	ND	0.02
	Range	0.061 - 0.123	ND	ND	ND	
	Median	0.118	ND	ND	ND	
	Samples tested +ve (%)	100	ND	ND	ND	
	Violative samples (%)	100%	0	0%	ND	
	residue load/kg fruits	0.302	ND	ND	ND	

Endosulfan Alpha	Average	ND	ND	0.017	ND	0.05
	Range	ND	ND	0.044 - 0.088	ND	
	Median	ND	ND	0.084	ND	
	Samples tested +ve (%)	ND	ND	66%	ND	
	Violative samples (%)	0	0	1.7	0	
	Residue load/kg fruits	ND	ND	0.216	ND	
Endosulfan beta	Average	0.014	ND	ND	ND	0.05
	Range	0.044 - 0.088	ND	ND	ND	
	Median	0.088	ND	ND	ND	
	Samples tested +ve (%)	100	ND	ND	ND	
	Violative samples (%)	66%	0	0	0	
	Residue load/kg fruits	0.216	ND	ND	ND	
Diazinon	Average	ND	ND	0.013	ND	0.05
	Range	ND	ND	0.033 - 0.066	ND	
	Median	ND	ND	0.063	ND	
	Samples tested +ve (%)	ND	ND	100	ND	
	Violative samples (%)	0%	0	33%	0	
	Residue load/kg fruits	ND	ND	0.162	ND	
Chlorpyrifos	Average	ND	ND	ND	ND	2
	Range	ND	ND	ND	ND	
	Median	ND	ND	ND	ND	
	Samples tested +ve (%)	ND	ND	ND	ND	
	Violative samples (%)	0	0	0	0	
	Residue load/kg fruits	ND	ND	ND	ND	
Total load (washing)		0.11		3.384		
Total load in tissues		0.66		0.528		
Total load in fruits		0.77		2.912		

Table 3: *Levels of pesticides residues (mg kg^{-1}) detected in samples collected from farms (South and North Khartoum), Khartoum State.*

ND: Not Detected; MRLs: Maximum Residue Limits.

Chlorpyrifos level was below the detection limit in both farm and market samples. It is worth to mention that chlorpyrifos is not registered in vegetables in Sudan and was not claimed to be used by the interviewed farmers in this area (PPD statistics, 2006-2010) [22,25]. Golge., *et al.* [26] found that propamocarb and chlorpyrifos were the main contributor to hazards index in green pepper and cucumber. In a similar study Tomás., *et al.* [27] found that the insecticides chlorpyrifos has the highest frequency of detection in vegetable and fruits tested. The mostly pesticide contaminated vegetables in India were okra, brinjal, lettuce, cucumber and tomato [28]. Pesticides found in most of the vegetable samples in the preliminary observations were chlorpyrifos, monocrotophos, endosulfan, DDT and lindane [28].

Heptachlor was the only pesticide detected in the washings of samples from both markets and farms samples (Table 2 and 3). The current result agrees with Aldawi., *et al.* [29] that washing of fruits seems to decrease the levels of residue load of some pesticides and that the reduction corresponds mainly to the level of heptachlor and sometime dimethoate. Robertson and Jaskukle [30] reported that washing can cause a significant reduction in pesticides residues especially in smooth surface fruits as in the case of tomato. He added that 90% of the surface residues could be removed by washing with water. Albach and Lime [31] mentioned that washing of orange fruits sprayed with malathion, parathion and dimethoate eliminated from 8 - 35% of the residue percent in the unwashed fruits. The highest frequency of residue detection and the highest residue load in market samples (3.9 mg Kg^{-1} fruit) was found in Khartoum central market (Table 2). On the other hand, the highest residue load in farm samples (2.912 mg Kg^{-1} fruit) was found in samples from North Khartoum vegetable farms (Table 3). Interviews and questioners indicated that farmers in this area tend to use high doses of pesticides [12,13,29,32]. The highest residue level in both market and farm samples was found associated with dimethoate with an average of 0.203 mg Kg^{-1} and 0.187 mg Kg-1 in Khartoum and Khartoum North central markets (Table 2) and 0.1 mg Kg^{-1} in South Khartoum farms (Table 3). Dimethoate is the most heavily used insecticides in the Sudan. It has been registered under more than ten trade names with annual imports exceeding 370 tons (PPD statistics, 2006 - 2010; [22,25]. On the other hand the lowest level in market samples was found associated with heptachlor with an average of 0.051 mg Kg^{-1} and 0.057 mg Kg^{-1} in washing water from Khartoum and Khartoum North central markets, while its level in the washed fruits of both markets is around 0.05 mg Kg-1 (Table 2). Heptachlor level in farm samples is around 0.05 mg Kg^{-1} in washed fruits from farms of both locations (Table 3). Heptachlor was not registered for use in vegetables in Sudan and even its other uses were severely restricted in 1994 to termite control in Sugarcane plantation and cotton and later was completely banned in 2005 [33]. On the other hand, the lowest level (0.013 mg Kg^{-1}) in farm samples was found associated with diazinon in washed fruits from North Khartoum. The highest frequency (100 %) of violation (> FAO Codex MRLs, [34]) in central markets samples corresponds to dimethoate and heptachlor while those in farm samples corresponds to dimethoate, heptachlor, endosulfan and omethoate (Tables 2 and 3).

Conclusions

Pesticides residues were detected in all sweet pepper samples from farms and central markets. Organophosphorus pesticides were found at a relatively higher level compared to organochlorine. The detected pesticides were heptachlor, endosulfan alpha, endosulfan beta, dimethoate, omethoate and diazinon. The level of chlorpyrifos was below the detection limits. Washing of fruits reduced the level of contamination by some pesticides. Violations in farm samples correspond to dimethoate, heptachlor, endosulfan and omethoate while those in market samples correspond to dimethoate and heptachlor.

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