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RESEARCH ARTICLE

INSECTICIDE RESIDUES LEVEL IN TOMATO FRUITS WASH COLLECTED FROM WAD MEDANI MARKET USING MOSQUITO LARVAE (*Anopheles arabiensis* Patton) AS a BIO-INDICATOR

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ABSTRACT

The aim of the present study was to assess the level of pesticide residues in tomato (*Lycopersicon esculentum L.)* fruits collected from Wad Medani market brought from three different location in Gezira state (Alhoush, Alrahad and Almadina Arab) by using mosquito (*Anopheles arabiensis* Patton) larvae. The 3rd or early 4th instar as a bio-indicator tool instead of high technology and costly analytical equipment was used. A total of 27 samples of tomatoes from market were collected. First the mortality was evaluated by taking 10 larvae/replicate (3 replicates/concentration), and each experiment was repeated three times to obtain the Probit model of insecticides used to control insects and then used later in the study to calculate the residues. The insecticide residues in tape water fruit wash of tomato fruits collected from the market from three localities were investigated by using the mosquito larvae. The mortality results were subjected to the equation of the regression line for each insecticide used to control the insect pest, as the Probit model, to calculate the concentration of the insecticides used in tomato fruits collected from the market. The residues were measured from 500 g and 1 kg of fruits as mg/g tomato fruit. The results provided important information on the current pesticide contamination status of the tomato consumed vegetables and pointed an urgent need to control the use of plant protection products applied. These results also show that the detected pesticides may be considered a public health problem. Malathion and cypermethrin residues in the 0.5kg and 1kg of tomatoes batches brought from the three localities Alrahad, Alhoush and Almadina Arab were found below the Acceptable Daily Intake (ADI) of 0.02mg/kg body weight and 0.05mg/kg body weight, for the two insecticides malathion and cypermethrin respectively. Although, that not means the tomatoes fruit brought to the market free of human health risk. Therefore the organic farming is highly needed to minimize the harmful risk of pesticides to human health.

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INTRODUCTION

Tomatoes (*Lycopersicon esculentum* L.) are the most important vegetable components of the diet and are consumed raw, cooked or processed. Nevertheless, tomato plants are susceptible to several pests and diseases that have been controlled with pesticides to avoid significant yield losses. In fruits and vegetables production, insecticides are used to control insect pests. They are directly applied to the crops and some may still be present as residues in the fruits and vegetables after their harvest. In the Sudan insecticides have been used extensively and intensively for a long period especially in recent years after the introduction of the diesel pumps, which have not only increased the acreage under production but also extended the growing season (Guddoura and Bugstaller 1984).

On the other hand, the tomato crop is susceptible to pest attack throughout the season. The reason with various insects such as fruit worm (*Heliothis armigera*), Epilachna beetles (*Epilachna vigintioctopunctata*), Jassids (*Empoasca devastans*), Tobacco caterpillar (*Spodoptera littoralis*), White fly (*Bemesia tabaci*), and Thrips (*Thrips tabaci* and *Frankliniella intonsa*) (Adalberto, *et al.,* 2006). So, pesticides are extensively used in this culture at various stage of cultivation to control pest and diseases that may cause yield reduction (Adalberto, *et al.,* 2006). Therefore, residues of pesticide could affect the ultimate consumers especially when these commodities are freshly consumed. Also urbanization and migration of rural people to towns stimulated the consumption of vegetables and increased export potential. George and Salama (1984) reported that vegetables are subject to insects attack from the time of planting throughout the growth period and up to the end of harvest. Different vegetables vary in their susceptibility to insect damage but no entirely escape damage. Pesticide residues on vegetables constitute a possible risk to consumers

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and have been a human health concern. When a chemical is used as recommended on the label of the product, any residues that occur should not exceed the maximum residue levels (MRLs). Residues detected in excess of the MRL rarely constitute a toxicological concern. The levels of pesticide residues are controlled by the maximum residue limits (MRLs), which are established by each country (Zawiyah., *et al.,* 2007; Torres, *et al.,* 1996). A good knowledge of the pesticide concentration is necessary to properly assess human exposure. Health risk assessment of pesticide residues in contaminated vegetables is performed in developed countries (Akoto *et al.* 2013; EFSA 2013); however, these residues are minimally explored in developing countries (Vieira *et al.* 2014). Synthetic pesticides, due to their quick efficacy, are widely used to reduce damage to agricultural crops due to different types of insect pests. However, serious problems can be caused by pesticides and it is estimated that three million cases of pesticides poisoning happen each year. It has been estimated that about 90% of the human exposure to pesticides residues is caused by eating contaminated food (Rathore, 2010). Pesticides in the Gezira scheme are sprayed under the supervision of the Crop Protection Administration entomologists and technicians on field crops, such as cotton and wheat, following the official recommendations. The successful control of pests and diseases by chemicals often depend on the way in which the insecticides are applied, and the substance used. When pesticides are applied, certain points have to be considered e.g. dosage, target, and time of application, physical placement and external hazard to avoid the risk (Hill and Waller, 1982). However, this is not the case with the vegetable farmers within and outside the scheme and other vegetables-producing areas.

Many scientists in the world are working in insecticides residue in field crops for determining the maximum residue levels (Pozzi and Flori, 1996 and Nowacka *et al.,* 2002). Cases of acute poisoning of humans and animals are recorded, but the chronic poisoning is not fully investigated. Studies of insecticide residue analysis in canal water, cotton, vegetables soil, milk, birds, fish and breast milk were conducted and residues were found (Abdalla, 2014). The overall results showed that the intensive use and misuse of insecticides for crop protection notably in vegetables production have led to pesticide contamination of agricultural produce that may carry certain risks for human health (Morzycka, 2002). The result is that vegetables are over-sprayed during the season, using different insecticides. The adverse side effects of pesticides impact the society in general and those who are directly and unjustifiably exposed to their residues, in particular. Pesticide residues in plants may be unavoidable even when pesticides are used in accordance with good agriculture practices (GAP) (Iya and Kwaghe, 2007). The level of contamination of plants and agricultural produce is associated with the intensity of usage of pesticides on farms (Dogenheim *et al*., 1996). Significantly high quantities of pesticide residues have been reported in vegetables and fruits, staple diets such as rice and wheat (Menzer and Thomas,1970; Miyata *et al*., 1994; Chow *et al*., 1971; Hughes *et al*., 1969; Galera *et al*., 1997; Wiersma *et al*., 1972; Gorbach and Wager, 1967; Kumari *et al.,* 2002; Kumari *et al.,* 2003). In an earlier study, 23 out of 108 vegetable samples were found to be contaminated with imidacloprid residues, of them nine samples contained pesticide residues above MRLs. The highest average concentration of the pesticide was found in eggplants (0.81 mg/kg), followed by okra (0.49 mg/kg), and in pumpkins (0.45 mg/kg) (Baig *et al.,* 2012). Surveying the chemical pollution status of a given area is increasingly becoming the task of biological indicators (BIs). The advantage of BIs over chemical or physical detectors is their ability to supply extensive, both spatially and temporally, rather than limited and instantaneous data, thus, making such information more representative. The use of insects and other organisms as test organisms is not new and is progressing very fast. Tomato (*Lycopersicon esculentum* L.) is an important vegetable crop grown extensively in Sudan, and for the control of numerous insect pests, different insecticides have been recommended and used. Many insecticides were found to be used on tomato by the responded farmers. Of these most frequently used insecticides in Sudan were pyrethroids, (namely cypermethrin), fenvalerate, fenpropathrin, λ-cyhalothrin, and deltamethrin (Shinger *et al.,* 2012; Albadri, *et al.,* 2012). The use of insecticides, such as malathion, cypermethrin, and deltamethrin has increased in tomato over the years. Therefore, monitoring their residues is very crucial. Keeping in view the above facts, the present work was designed to monitor and evaluate the level of residues of cypermethrin and malathion in marketed tomato fruits, brought to the market from three localities in Gezira State, using *Anopheles* mosquito larvae as a bio-indicator. The aim of this study was to assess the presence of insecticide residues in vegetables represented here by tomato fruits. This study also aimed to throw light on the influence of tap-water wash on the removal of such residues from field-treated vegetables.

MATERIALS AND METHODS

This study was carried out in the biology lab, Faculty of Agricultural Sciences, University of Gezira, Wad Medani, Gezira State (central Sudan). The insecticides used in the present study comprised of malathion 57% EC, cypermethrin 25% EC. Mosquito larvae (*Anopheles arabiensis*) 3rd or early 4th instar were used for detecting the insecticides residues in tomato fruits collected from the Wad Medani market brought from three localities (Alrahad, Alhowsh and Almadina Arab). Tomato fruits collected form the market previously field treated by insecticides (cypermethrin and malathion) to control the insect pests by farmers were wash using tap water (1000ml). Rearing plates, fish food, cages, mosquito net, Petri dishes, filter paper were used to establish the population of mosquito larvae in the experiment.

Collection and rearing of mosquito larvae

Larval populations were reared in the insectaries of BNNICD after being collected from ponds around Wad Medani town. These populations were expected to be homogeneous, since these were established in the BNNICD insectaries for generations according to the standard methods of WHO (1980). The adults were reared in cages measuring 50x50x50 cm. The height of cage is important for mating. The cages and rearing plates were covered with mosquito net. A Petri dish, covered with a piece of cotton and filter paper wetted with water, was placed inside each cage for oviposition of adults. A piece of cotton saturated with sugar solution was also placed inside the cages to provide adults with energy. Blood meals were taken from a rabbit that was held in a holding cage inside the adult's cage after shaving the dorsal parts of its skin. Laboratory hatched larvae (first generation) were reared in 20 enamel plates, half filled with water. Fish food was offered to larvae for feeding on daily basis until used for the test. The insectary conditions were: 280C temp., 70% R.H. and 12:12 L:D period. The insectary was furnished with all the required equipment and glassware, such as, conical flasks, pipettes for capturing the larvae, measuring cylinders, needles, nets and paper cups used for capturing the larvae from the field. In each test the first generation of the larvae was used as a bio-indicator to evaluate insecticides residues in tomato fruits.

Preparation of Insecticides concentrations

Two insecticides used to control insect pests in tomato field in three localities at different concentrations were used to obtain base-line data as follows:

- Malathion 57% EC (OP): 0.08, 0.04, 0.02, 0.01, 0.005, 0.0025 and 0.001 ppm
- Cypermethrin 25% EC (Pyrethroids): 10, 1, 0.8, 0.7, 0.6, 0.5 and 0.4ppm

Dilutions were prepared by using tap water

Mosquito larvae of *A. arabiensis* were used in this bioassay as a bio-indicator (BI) to determine the toxicity of each of the two insecticides in the laboratory (This was later used to measure the residues on tomato fruits collected from the market which brought from three localities. The 3rd or early 4th (L3 or L4) instars larvae of *A. arabiensis* were exposed to concentrations of pesticides mentioned above to determining the toxicity (each concentration replicated 3 times and the experiment repeated 3 times for each insecticides). Mortality among larvae at each concentration for each insecticide was recorded after 24 hr (acute toxicity) to obtain base-line data as the model.

Collection of tomato fruit from the market

Tomato collections were carried out at Wad Medani market brought from the three localities Alrahad Alhosh and Almadina Arab, Gezira State. In this study, 27 samples were collected during study 2016, the tomato collected in three different days and in each day three random groups (500g each) will collect the time brought to the market form three localities (3x3x3=27 samples).

Bioassay

Bioassay was performed to determine the insecticides residue and calculate their amount. Freshly collected mosquito larvae or laboratory-reared L3 or L4 instars were exposed to tap-water fruit wash (1000ml) of insecticides residues of malathion (Alrahad) and cypermethrin (Alhosh and Almadina Arab) the chemicals applied by the farmers to control insects from tomato fruits samples which were collected from the market brought from three localities. Mosquito larvae exposed to tap water served as control. Each replicate consisted of 10 larvae and each experiment was repeated 3 times on each day of collection. The observation was recorded after 24 hr as mortality percentage (% M) as per the standard formula.

Data analysis

Data were collected from the laboratory bioassay and analyzed by using Probit analysis, then subjected to Microsoft office Excel (2003) program. Log-dose probability lines (Ld-p lines) were calculated for each insecticide and then regression line equations were obtained as Probit model.

The data collected from the tomato collect from the market (% mortality) after exposing the larvae to tap-water wash of tomato fruits collected from market, per locality were subjected to the Probit model obtained from the bioassay study to determine/calculate the approximate concentration of insecticide residues washed from the fruits. Data were presented in tables and figures.

RESULTS AND DISCUSSION

The present work was completed in two phases. In the first phase, a laboratory bioassay was conducted for determining the base-line data to develop probit model (Fig. 1 and Fig.2) to be used later in Phase II for monitoring/estimating the residue levels/concentrations of the previously mentioned two insecticides applied by farmer in the field to control tomato insect pests.

Base-line (Regression line equations)

First generation larvae of *A. arabiensis* from field collection were used in two phases as a BI to determine the toxicity of each of the two insecticides in the laboratory bioassay. The results were based on the % mortality of the larvae. Table 1 shows the equations obtained from the baseline data obtained from laboratory bioassay as Probit model for calculation of insecticides residues in tomato. The equation of the regression line for each chemical was later used as the model to calculate the residues of insecticides in tomato fruits collected from the local markets. Figs. 1 and 2 show the equation, and slope of malathion and cypermethrin, respectively. The LC_{50} for the malathion was found to be 0.004 ppm and for the pyrethroid cypermethrin, it was calculated to be 0.54 ppm, The higher slope (6.04) was that for cypermethrin while malathion showed low slope (0.96), which reflects the homogeneity response of mosquito larvae to cypermethrin and heterogeneity to malathion.

Determination of residues

The concentrations of pesticide residues found in vegetables sampled from the local markets was determined mortality percentages of the larvae exposed to tap water wash of tomato fruits collected from market previously sprayed with the two insecticides (malathion and cypermethrin) by farmers are shown in Tables 2, 3 and 4, which reflect their residues in the market collected tomato fruits brought from three localities. The % mortality of the larvae as the result of exposure to the tap-water wash of the tomato fruits obtained from the bioassay was subjected to Probit transformation, and then the resulting Probit was applied to the regression line equation of each insecticide that applied to control insect in each locality presented in (Table 1) to calculate residues of the two insecticides, malathion and cypermethrin.

Table 1. Regression line equation as a Probit model obtained from bioassay of tested insecticides using 3rd and 4thinstars of *Anopheles arabiensis* as bio-indicators

Insecticides	Equation(model)	LC_{50} in ppm	Slope
Malathion	Y=0.962X+4.405	0.004	0.96
Cypermethrin	Y= 6.04X-11.49	0.54	6.04

The amount pesticides residues obtained presented in tables (2, 3 and4). For tomato collected from the market that brought from Alhoush previously treated with cypermethrin by farmers the amount of cypermethrin residue in ppm shown in Table 2 indicated the calculated concentrations of cypermethrin residues in the three batches (I, II and III). The calculated concentrations in 500 g tomatoes were Ω 0.44, 0.49 and 0.44 ppm, respectively. The calculated concentrations, when the sample weight was I kg fruit for the same periods of time were Ω 0.88, 0.98 and 0.88ppm/kg fruits, respectively. The high concentration found in batch II which is 0.02mg/kg bw less than the ADI limit for cypermethrin (0.05mg/kg bw). For tomato collected from the market that brought from Alrahad previously treated with cypermethrin by farmers the amount of cypermethrin residue in ppm shown in Table 3 indicated the calculated concentrations of cypermethrin residues in the three batches (I, II, and III). The calculated concentrations in 500 g tomatoes were Ω 0.74, 0.55 and 0.49 ppm respectively. The calculated concentrations, when the sample weight was I kg fruit were Ω 1.48, 1.1 and 0.98ppm/kg fruits, respectively. because Log concentration less than one is negative according to Busvine (1971).

Effect of insecticides residues on *A. arabiensis* larvae

In laboratory bioassay tests, the mortalities resulting from the residues of cypermethrin in tomatoes brought from Alhowsh in three batches (I, II and III) were 30, 40 and 30% respectively. While the larval mortalities' caused by cypermethrin residues in tomatoes brought from Alrahad in three batches (I, II and III) were 80, 53, and 40 % following the same order. However, mortalities by the residues of malathion in tomatoes brought from Almadina Arab in three batches (I, II and III) were 10, 67 and 37% respectively (Table 4). In the probit model of insecticides, the concentrations causing death of 50% of larval population by malathion was 0.004 ppm (Table 1),

Table 2. Insecticides residue in tomato from Al housh locality

Insecticide use to control insect pests	Bach I			Batch II			Batch III		
	% mortality	Approx. Residue (ppm)		% mortality	Approx. Residue (ppm)		% mortality	Approx. Residue (ppm)	
		Sample wt. in Kg			Sample wt. in Kg			Sample wt. in Kg	
		0.5 kg	1 kg		0.5 kg	1 kg		0.5 kg	1 kg
cypermethrin	30	0.44 ppm	0.88 ppm	40	0.49 ppm	0.98 ppm	30	0.44 ppm	0.88 ppm
Residue mg/kg bw			0.015			0.016			0.015

Acceptable daily intake of cypermethrin is 0.05mg/kg bw; bw= Body weight wt=weight

Table 3. Insecticides residue in tomato from Al rahad locality

Insecticide use to control insect pests	Bach I			Batch II			Batch III		
	% mortality	Approx. Residue (ppm)		% mortality	Approx. Residue (ppm)		% mortality	Approx. Residue (ppm)	
		Sample wt. in Kg			Sample wt. in Kg			Sample wt. in Kg	
		0.5 kg	1 kg		0.5 kg	1 kg		0.5 kg	1 kg
cypermethrin	80	0.74 ppm	1.48 ppm	53	0.55 ppm	1.10 ppm	40	0.49 ppm	0.98 ppm
Residue mg/kg bw			0.02			0.02			0.02

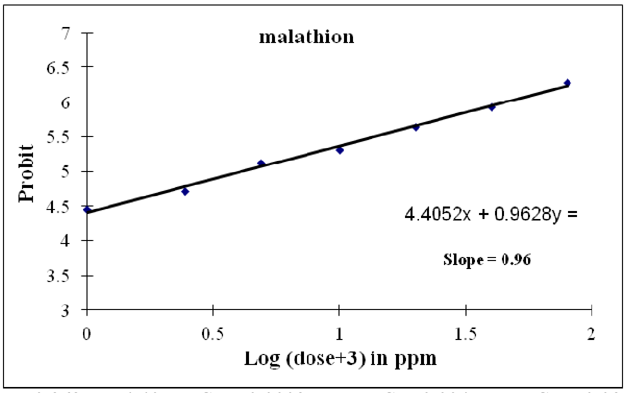
Acceptable daily intake of cypermethrin is 0.05mg/kg bw; bw= Body weight wt=weight

Table 4. Insecticides residue in tomato from Al madina Arab locality

Insecticide use to control insect pests	Bach I			Batch II			Batch III		
	% mortality	Approx. Residue (ppm)		% mortality	Approx. Residue (ppm)		% mortality	Approx. Residue (ppm)	
		Sample wt. in Kg			Sample wt. in Kg			Sample wt. in Kg	
		0.5 kg	1 kg		0.5 kg	1 kg		0.5 kg	1 kg
malathion	10	0.0002 ppm	0.0004 ppm	67	0.012 ppm	0.024ppm	37	0.002 ppm	0.004 ppm
Residue mg/kg bw			0			0.0004			0

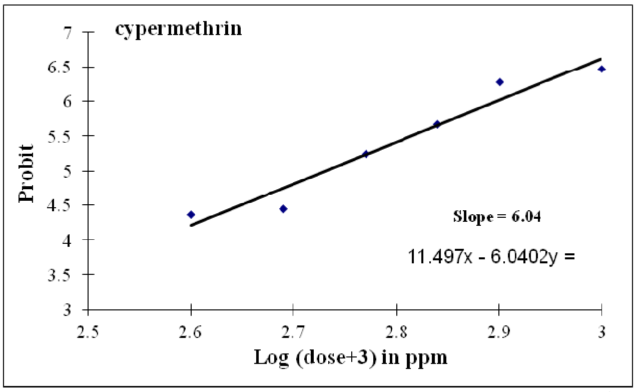
Acceptable daily intake of malathions 0.02 mg/kg bw; bw= Body weight wt=weight

The concentration of residue found in batch I which is 0.02 mglkg bw is less than the ADI limit for cypermethrin (0.05mg/kg bw). For tomato collected from the market that brought from Almadina Arab previously treated with malathion by farmers the amount of malathion residue in ppm shown in Table 4 indicated the calculated concentrations of malathion residues in the three batches (I, II and III). The calculated concentrations in 500 g tomatoes were Ω 0.0002, 0.012 and 0.002 ppm, respectively. The calculated concentrations, when the sample weight was I kg fruit were Ω 0.0004, 0.024 and 0.004 ppm/kg fruits, respectively. The concentration of residue found in batch II which is 0.0004 mglkg bw is less than the ADI limit for malathion (0.02mg/kg bw). In case of malathion and cypermethrin (dose+3) 3 unit was added to log of the concentration used for easy calculation when compared with results of malathion residues more than 50% of larval population died on the tap water wash (concentration= 0.012 ppm) of the 2nd batch and the concentration of malathion residue on 1st and 3rd batches was 0.0002 ppm, 0.002ppm in 500g sample of tomatoes fruits, respectively and 0.0004 ppm, 0.004 in the one kg sample of tomatoes fruits, respectively (Table 4). The amount of malathion residue found in tomatoes tap water wash in batch II was very high but not higher than the acceptable daily intake of 0.02mg/kg bw recommended by WHO/FAO. Thus the residue amounts of 0.0004 mg/kg on batch II not exceeded this acceptable daily intake, although the tap water wash give 67% mortality of the bio-indicator; while the amounts found in the tap water wash for batch I and III is far less than the acceptable daily intake (Table 4).



Y= 0.962x + 4.405, LC_{10}= 0.0002 ppm; LC_{50}=0.004ppm; LC_{90}= 0.09 ppm, Slope= 0.962.

Fig. 1. Dose-mortality response of *A. arabiensis* larvae exposed to Malathion



Y= 6.04x – 11.49, LC_{10}= 0.33ppm; LC_{50}= 0.54ppm; LC_{90}= 0.88ppm; Slope= 6.04.

Fig. 2. Dose-mortality response of *A. arabiensis* larvae exposed to cypermethrin

Regarding mortalities by the residues of cypermethrin in tomatoes brought from Alhoush in three batches (I, II and III) were 30, 40 and 30% respectively (Table 2). In cypermethrin, the 50% of larval population was killed by 0.54 ppm in the model formulation (Table 1) and in cypermethrin residue (tap water wash), none of the three batches give 50% mortality of the larval population. Thus, the concentration residue of cypermethrin in batches (I, II, and II) of tomatoes fruit tap water wash collected from the market was determined to be 0.44, 0.49 and 0.44 ppm in the 500g sample of tomatoes fruit, and 0.88, 0.98 and 0.88 ppm in the one kg sample of tomatoes fruit. The amount of cypermethrin residue found in tomatoes tap water wash in all batches was very low and less than acceptable daily intake of cypermethrin 0.02mg/kg bw recommended by WHO/FAO.

Thus the residue amounts of 0.02 mg/kg on all batches not exceeded the acceptable daily intake, In case of mortalities by the residues of cypermethrin in tomatoes brought from Alrahad in three batches (I, II and III) were 80, 53 and 40% respectively (Table 4). As per cypermethrin model, 50% kill of the larval population was achieved by 0.54 ppm, as shown in Table 1, while in tap water wash of the tomatoes fruits collected from the market batch I give 80% mortality with concentration of 0.74ppm, while the 50% mortality of the larval population occurred on batch II (Table 4). The residue concentration in batches I, II and III were 0.74, 0.55 and 0.49 ppm in the 500 g tomatoes fruit sample respectively, and 1.48, 1.1 and 0.98 ppm in the one kg of tomatoes fruit sample. Therefore, it was found that the tomatoes fruit brought to the market from Alrahad previously been applied by cypermethrin to control insect is highly contaminated with insecticides, Although, the amount of the residues found in the tap water wash was between 0.02-0.03 which is less than acceptable daily intake of 0.05mg/kg bw recommended by WHO/FAO. The result of this study revealed that all the tomatoes samples collected from the Wad Medani market which brought from the three localities were contaminated with the two insecticides residues. Of course, the two insecticides contaminated samples not exceeded the maximum residue limit (MRL) values as per the FAO/WHO. Therefore, periodic monitoring of market basket vegetables must be carried out to know the prevailing scenario of pesticide contamination of vegetables grown in Sudan, particularly in vegetables growing areas. The results revealed that the most of the tomatoes brought to the Wad Medani market were contaminated. However, the degree of contamination is high on tomatoes brought from Alrahad. From the safety point of view as per the results obtained from the present study, tomatoes fruits will be acceptable if harvested after the safe period at least 7days after last spray. However, this does not mean that the tomatoes fruits were free of insecticides and hence should follow the safety periods recommended by FAO/WHO. In additional to that the residues of the investigated pesticides after pesticide treatments were generally higher but below the MRL the washing once time or several times showed significant efficiency in removing the residues in most cases. In the present study, mosquito larvae as BIs proved to be very sensitive to detect insecticide residues in tomato come in line with study done by Assad, *et al.,* (2014) using mosquito larvae as BIs to determine insecticide residues in okra fruit.

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