African Journal of Agriculture and Food Science
Vol.1, No.1, pp.27-35, 2018
www.abjournals.org

ASSESSMENT OF CHLORPYRIFOS RESIDUES IN WATERMELON FRUITS SOLD IN BIRNIN KEBBI MARKETS, KEBBI STATE, NIGERIA

Osesua B.A[1], Aliyu A.K[1], Anyekema M[2] and Tsafe A.I[3]

[1]School of Preliminary and Remedial Studies, Waziri Umaru Federal Polytechnic, Birnin Kebbi.
[2]Department of Science Technology, Waziri Umaru Federal Polytechnic, Birnin Kebbi.
[3]Department of Pure and Applied Chemistry, Usmanu Danfodiyo University, Sokoto.

ABSTRACT: *Watermelon (Citrulus lanatus) samples were randomly collected from seven major markets in Birnin-Kebbi and environs. The samples were divided into three groups: unwashed, detergent-washed and washed with tap water. Samples were extracted with cyclohexane and ethylacetate (1:1) and the chlorpyrifos residues in the sample extracts were determined with GC-MS Perkin-Elmer model fitted with Electron Capture Detector (ECD ^{63}Ni) and Nitrogen Phosphorus Detector (NPD). Results showed that 100% of the samples analysed contained chlorpyrifos pesticide residue. The unwashed watermelon fruits, detergent-washed and tap water washed have mean concentrations of 0.114±0.006µg/g, 0.006±0.002µg/g and 0.009±0.003µg/g respectively. The chlorpyrifos residue levels obtained in this work exceeded the Maximum Residue Limit (MRL) established by FAO/WHO indicating a possible contamination of the fruits. Statistical analysis with one-way ANOVA (SPSS 20.0) showed that there is a decrease in the levels of chlorpyrifos pesticide residues significantly between the unwashed watermelon fruits and the detergent-washed, while there seem to be no difference statistically in residues levels between the detergent-washed fruits and the water-washed watermelon fruits (P ≤0.05).*

KEYWORDS: Watermelon, Chlorpyrifos, Detergent-Washed, Pesticide Residues.

INTRODUCTION

Plant protection products (more commonly known as pesticides) are widely used in agriculture to increase the yield, improve the quality, and extend the storage life of food crops (Fernandez-Alba and Garca-Reyes 2008). Pesticide residues are the deposits of pesticide active ingredient, its metabolites or breakdown products present in some component of the environment after its application, spillage or dumping. Residue analysis provides a measure of the nature and level of any chemical contamination within the environment and of its persistence.

The applied chemicals and/or their degradation products may remain as residues in the agricultural products, which becomes a concern for human exposure. Selected sampling programmes can be used to investigate residual levels of pesticide in the environment, their movement and their relative rates of degradation.

Pesticide residue analysis is tremendously an important process in determining the safety of using certain pesticides. Pesticides polluting the earth and causing problems in human beings and wildlife, the quantity of pesticide being consumed becomes a necessary knowledge.

Analytical quality requirements like trueness, precision, sensitivity and selectivity have been met to suit the need for any particular analysis.

Unfortunately, not all farmers follow legal practices and due to the tremendous number of pesticides and crops in production, a number of analytical methods are designed to determine multiple pesticide residues (Food and Drug Administration, 2009; Luke *et al*., 2010; Lee *et al*., 2011; Andersson and Pålsheden, 2011; Cook *et al*., 2012; General Inspectorate for Health Protection, 2016; Fillion *et al*., 2016; Sheridan and Meola, 2017; Lehotay, 2017).

Watermelon (*Citrulus lanatus*), a member of the Cucurbitaceae family, is related to the cantaloupe, squash, pumpkin and other plants that also grow on vines on the ground. In Nigeria, watermelon is mostly cultivated in the Northern part because it is a warm-loving plant; this makes its production seasonal in the Southern part of the country. The crop is affected by excess water, especially in its last developmental stage (Feher, 2003; Athens, 2008).

Watermelon is one type of fruit that is often consumed fresh; this fruit is also traded a lot in supermarkets and traditional markets. Its vitamin and mineral contents are very beneficial in increasing nutritional and health qualities. But watermelon production processes often encounter pests and diseases attack. This condition can cause crop shrinkage or loss. The control method that is most frequently performed by the farmers is the usage of pesticides. The use of pesticides can leave any residue that could cause environmental pollution, human health disturbance and impede trade (Miskiyah and Munarso, 2017).

Watermelon diseases caused by fungi, bacteria, nematodes and viruses are one of the main limiting factors in obtaining higher yields. One of the solutions is to apply pesticides at many steps of the cultivation process. Even when applied in accordance with Good Agricultural Practices (GAP), they can leave residues, which can be detrimental to watermelon safety. The presence of pesticide residues in fruits in general and in watermelon in particular, is one important concern for consumers due to their possible long adverse health effects; especially for children, as they consume a higher proportion of fruits in relation to their body weight and are more susceptible to chemicals since they are in early development stages (Zawiyah *et al.*, 2007).

Chlorpyrifos is a broad-spectrum insecticide, a chemical used to kill a wide variety of insects. It was introduced in 1965 (Wagner, 2017). Chlorpyrifos acts on pests primarily as a contact poison, with some action as a stomach poison.

Chlorpyrifos is one of a class of insecticides referred to as organophosphates. These chemicals act by interfering with the activities of cholinesterase, an enzyme that is essential for the proper working of the nervous systems of both humans and insects. Repeated exposure to chlorpyrifos may result in effects include impaired memory and concentration, disorientation, severe depressions, irritability, confusion, headache, speech difficulties, delayed reaction times, nightmares, sleepwalking and drowsiness or insomnia. An influenza-like condition with headache, nausea, weakness, loss of appetite, and malaise has also been reported (Richardson, 2015).

The amounts of pesticides used in Nigeria have been increasing annually. However, most farmers lack awareness regarding proper use of pesticides (Ngowi *et al.*, 2007). Consequently, cases of indiscriminate use of pesticides and non-adherence to good agricultural practices are very common. For example, some farmers spray the fields in the afternoon and pick the fruits early in the next morning for selling in the local markets. These observations suggest that the fruits sold in the markets may have serious pesticides contamination. The aim of this study was therefore to investigate the levels and status of pesticide residues in watermelons one of the main fruits produced locally and consumed by the entire populace.

MATERIALS AND METHODS

Sampling: All the fruit samples were collected randomly from the selected seven major markets in Birnin Kebbi metropolis and environs in Kebbi state Viz; Birnin Kebbi Central Market (BCM), Kalgo Main Market (KMM), Jega General Market (JGM), Aliero Market (AM), Ambursa General Market (AGM), Bunza Main Market (BMM), and D/gari Main Market (DMM). These markets are known for their massive sales of fruits that come from different parts of the state where pesticides are widely used. The samples were collected in December 2017 to March 2018, separately wrapped in aluminium foil, transported in ice box to the laboratory and kept in a refrigerator until extraction, which was conducted within 24 hours

Sample Extraction, Clean-up and Quantification. One kilogram each of fruits collected from the local markets was chopped, sub-sampled and preserved in a freezer till further processing. The method of Tahir et al., (2001) was followed for extraction and cleanup of samples. One kg of the sample was chopped and mixed thoroughly. A subsample of 25 gm was taken out and blended with 50 ml of acetone, 50 gm of anhydrous Sodium sulphate and 50 ml of a mixture of Cyclohexane and ethylacetate (1:1). The mixture was allowed to stand for some time until a clear supernatant was formed and 30 ml supernatant was taken into a round bottom flask. A few drops of 10% propandiol in ethylacetate and about 4-6 glass beads were added. The solvent was evaporated at 40°C under vacuum in rotary evaporator. The contents were reconstituted in 6 ml of cyclohexane and ethylacetate (1:1) and then passed through high-flow super cells. Two ml of this sample was applied on GPC column for further cleanup. After passing through GPC column, the samples were dried under vacuum and reconstituted in 1ml ethylacetate for analysis on Gas Chromatograph (GC).

The retention time was within ± 2% of that of the standard. The method validation consisted of three sample sets. Each set included three levels of fortification (0.01, 0.05 and 1.0 ppb) and a method blank. All spikes and method blank samples were processed through the entire analytical method. Quantification was based on external standard calculation using the peak area.

Instrumentation and Reagents: Gas Chromatograph, Perkin-Elmer, Microprocessor, Autosystem was fitted with Electron Capture Detector (ECD-^{63}Ni) and Nitrogen Phosphorous Detector (NPD). Nitrogen and Air Generator Peak Scientific. Hydrogen Generator, Peak Scientific, Gel Permeation Chromatograph (GPC), Mikrolab Arhus A/S, USA. Rotary Evaporator, made Buchi R-114/A, Switzerland. Food Blender, Germany. FlASK Shaker SF1, Sartatorius single pan analytical balance and Refrigerator/Freezer.

The analytical grade standard of chlorpyriphos was purchased from Sigma Aldrich Germany through Bristol Scientific Nigeria Ltd Lagos. Stock solutions and required working dilutions were prepared in ethylacetate. All other solvents and reagents were of extra pure GC/HPLC grade. Acetone (Merk) Ethylacetate (Merk), cyclohexane (BDH) and n-Hexane (BDH). Anhydrous sodium sulphate (Merk), dichloromethane (Lock-light Ltd.), propane 1, 2-diol (Pharmacos Ltd., UK), high flow super cells (BDH) bio-beads, SX3 200-400 (Reidel-de Haën), sodium hydroxide (Merck), potassium dichromate (Merck), sodium chloride (Merck) and Millipore distilled water.

Table 1: Concentration of Chlorpyrifos in Watermelon Fruits (Conc. µg/g)

Sample Site	Unwashed-Watermelon	Detergent-Washed Watermelon	Water-Washed Watermelon
B/K Cen. Market (BCM)	0.088±0.003	0.017±0.005	0.020±0.008
Kalgo Main. Market (KMM)	0.102±0.007	0.004±0.001	0.007±0.002
Jega Gen. Market (JGM)	0.097±0.003	0.002±0.001	0.006±0.001
Aliero Market (AM)	0.143±0.008	0.010±0.005	0.011±0.004
Ambursa Gen. Mark. (AGM)	0.110±0.006	0.006±0.002	0.009±0.002
Bunza Main Market (BMM)	0.146±0.009	0.003±0.001	0.009±0.004
D/Gari Main Market (DMM)	0.109±0.007	0.004±0.001	0.005±0.001
Mean	0.114±0.006	0.006±0.002	0.009±0.003
Minimum Residues	0.088±0.003	0.002±0.001	0.005±0.001
Maximum Residues	0.146±0.009	0.017±0.005	0.020±0.008

Note: Results of triplicate

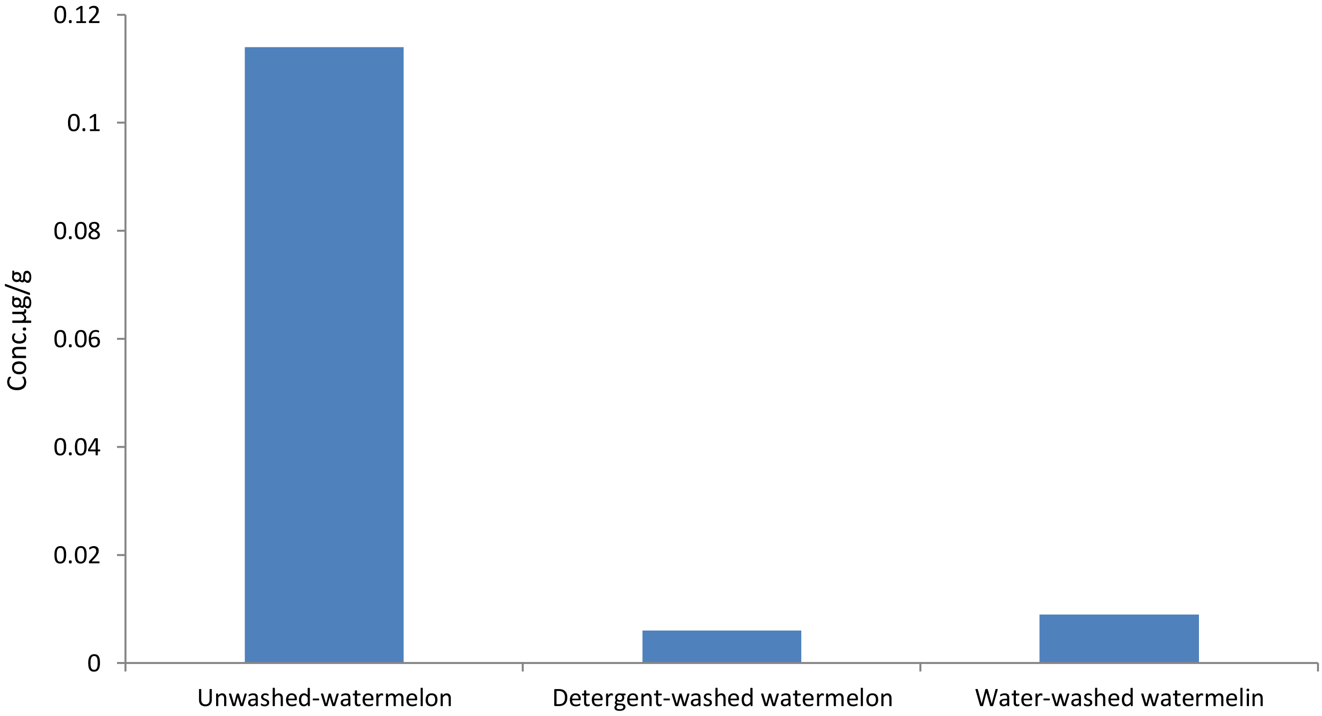

Figure 1: Mean Concentration of Chlorpyrifos Pesticide Residues in the Samples

DISCUSSION

The results from this study revealed the frequent presence of chlorpyrifos residues in all the samples analysed (Table 1). The highest chlorpyrifos residues concentration (0.146±0.009µg/g) was obtained from samples collected from (BMM) closely followed by (0.143±0.008µg/g) obtained in samples from (AM). These mean concentration levels are however higher than the Maximum Residues Limit (MRL) of chlorpyrifos concentration (0.003µg/g) in fruits set by FAO/WHO, (2013). Munarso *et al.*, (2009) and Allen *et al.*, (2013) reported similar levels of residues in watermelon and tomatoes fruits. The high level of residues observed in this work could be directly attributed to highly intensive usage of pesticides formulations containing chlorpyrifos by farmers in these areas, the last-spraying time before samples were taken as well as the pesticide application techniques used.

Detergent-washed samples showed chlorpyrifos residues reduction levels of about 80%. There was no significant difference between the percentage reduction of residues of chlorpyrifos in detergent-washed and water-washed samples. The high residues reduction levels observed in detergent and water washed samples (Figure 1) could be attributed to the high solubility potentials of chlorpyrifos in water 1:20 (Irie, 2017) as well as chemical event eg detergent washing. But detergent usage could be dangerous if detergent residues remained because of incomplete rinsing. Beside soap usage, there are natural chemicals that are recommended for pesticide residues reduction purpose e.g salt (NaCl), sodium bicarbonate ($NaHCO_3$) and acetic acid (CH_3COOH) (FAO/WHO, 2013)

Chlorpyrifos residues level in unwashed, detergent washed and running water washed were found to exceed the MRL established by Food and Agricultural Organisation and World Health Organisation (FAO/WHO. 2013). Fruits that positively contain pesticide residues could be dangerous if consumed on and on. The residues will accumulate in the body and influenced nerve dysfunctions and are stored in fatty tissues. Residues that are stored in the fatty tissues cannot be excreted and may be accumulated endlessly and could cause tissues damage and may predispose to cancer (Chen *et al*., 2015). Exposure towards pesticides from the organophosphate groups for a long period and high relative amount will inhibit functions of acetyl cholinesterase enzyme and this can lead to several health complications including salivation, dizziness, difficulty in breathing and even comma (Feldsine *et al.*, 2015).

Statistical analysis of results with One-way ANOVA (SPSS 20.0) revealed that chlorpyrifos residue levels in unwashed samples was significantly different compared to running-water-washed and detergent-washed samples ($p \leq 0.05$). Whereas, running-water-washed watermelon fruit samples and detergent washed samples showed no difference statistically in residues levels ($p \leq 0.05$) indicating similar mode of residues contamination of the fruits.

The findings of this study confirm previous studies that pointed to high levels of pesticide residues in fruits and vegetables (Saeed *et al*., 2011; 2013; 2015). However, in comparison, the levels of the residues in the tested fruits were higher than those previously reported in other studies (Lozowicka, 2015; Osman *et al*., 2015; Bempah *et al*., 2011). Chlorpyrifos, a systemic neonicotinoid insecticide used to control various pests of fruits and vegetables is the most commonly detected pesticide (Jallow *et al*., 2017). Although chlorpyrifos is classified as moderately hazardous (WHO, 2016) this pesticide has been associated with human neurotoxicity (Tomizawa and Casida, 2013).

The occurrence of high levels of residues in all the samples analyzed is likely to be a consequence of indiscriminate and persistence applications of pesticides (as chlorpyrifos forms a major component of several pesticide formulations) to protect crops against different insect pests and diseases, especially vegetable crops in temperate environments where the incidence of pests can be extremely high (Montory *et al.*, 2017). From the results, it is plausible to state that farmers were not following proper precautions with regard to the use of pesticides in appropriate dosages and at standard pre-harvest intervals. Consequently, a large number of the fruit's samples were contaminated with pesticides. The high pesticide levels in the samples would suggest that the pesticide has been used indiscriminately, which could lead to health problems not only to the farmers but also to the general consumers. The widespread and overuse of pesticides in agriculture, especially in vegetable production, is a serious problem. Faced with several pest complexes, farmers simply rely on pesticides to address pest problems because of lack of viable alternative methods of pest control. Jallow *et al*., (2017) reported that 58% of smallholder vegetable farmers in developing countries overused pesticides. Pesticide application frequency in vegetable crops ranged from two times a month to once a week, depending on the crop (Jallow *et al.,* 2017). This problem is further exacerbated by farmers' limited knowledge of pesticide safety.

Greater priorities must be given to develop strategies for pesticide reduction in agriculture through farmer training in judicious and safe pesticide use, and promote alternatives to chemical pest control such as biological control. Intervention strategies by regulatory agencies to strengthen the enforcement mechanisms of current pesticide laws at the farm and retail level are a necessity in promoting safe pesticide use. Adherence to pesticide label instructions, especially pre-harvest intervals, needs to be ensured. It is also critical to raise awareness among the general public, who may be directly or indirectly exposed to pesticides, about the risk of these chemicals and how to reduce this risk. Consumers should be aware of practical measures to reduce the contamination of pesticides in fresh agricultural produce, especially fruits and vegetables that may be consumed raw. For example, washing and peeling, have been demonstrated to reduce pesticide residues on fruits and vegetables (Osman *et al*., 2017; Qin *et al*., 2016). Consequently, a follow-up investigation is needed to determine whether peeling, in particular, could reduce the dietary intake of pesticide residues. Finally, due to increasing trend in pesticide use in agriculture, routine monitoring of pesticide residues in agricultural produce is a necessity to ensure the safety of consumers.

CONCLUSION

This study investigated the levels of organophosphate pesticide residue (chlorpyrifos) in commonly used fruit (watermelon) in Birnin kebbi. The results indicated that all of the fruit samples were contaminated with pesticide residue, with concentrations above the MRL. From a public health perspective, the observed levels of pesticide residue pose a potential health risk to consumers. Therefore, to reduce this risk, sensitization of farmers to better pesticide safety practices, the need to properly wash raw fruits from the farm with running clean water always before consumption and continuous pesticide residue monitoring is highly recommended.

REFERENCES

Alen, Y., Habazar, T., Syarif, Z. and Talib, G. (2013): Laporan Penelitian Unggulan Strategis National, Universitas Andalas, *Padang*, 2: 89-90

Andersson, A. and Pålsheden, H. (2011): Comparison of the Efficiency of Different GLC Multi-Residue Methods on Crops Containing Pesticide Residues. Fresenius *J. Anal. Chem.,* 339: 365-367

Athens, F. (2008) Commercial Watermelon Production. Extension Service, University of Georgia, College of Agriculture.

Bempah, C., Donkor, A., Yeboah, P., Dubey, B. and Osei-Fosu, P. (2011): Preliminary Assessment of Consumer's Exposure to Organochlorine Pesticides in Fruits and Vegetables and the Potential Health Risk in Accra Metropolis, Ghana. *Food Chem.*128:1058–1065.

Chen, C., Qian, Y., Chen, Q., Tao, C., Li, C. and Li, Y. (2015): Pesticide Residues in Fruits. *Journal of Food Control*. 22: 1114.

Cook, J., Beckett, M., Reliford, B., Hammock, W. and Engel, M. (2012): Mutiresidue Analysis of Pesticides in Fresh Fruits and Vegetables Using Procedures Developed by the Florida Department of Agriculture and Consumer Services. *J. AOAC Int.,* 82: 1419-1435.

FAO/WHO. (2013): Food Agriculture Organization and World Health Organization Bulletin. *Pesticide*, 7: 56-58

Feher, T. (2003): Watermelon: Citrulluslanatus (thumb) Matsun and Nakai B. O. Pergamum Press, Oxford, U.K.

Feldsine, P., Abeyta, C. and Andrews, W. (2015): Pesticides *JAOAC Int*. 85 (5):1187-1200.

Fernndez-Alba, A. and Garca-Reyes, J. (2008): Large-scale Multi-residue Methods for Pesticides and their Degradation Products in Food by Advanced LC-MS. Trac-Trend. *Anal. Chem.,* 27 (11): 973-990.

Fillion, J. Sauvé, F. and Selwyn, J. (2016): Multiresidue Method for the Determination of Residues of 251 Pesticides in Fruits and Vegetables by Gas Chromatography/mass Spectrometry and Liquid Chromatography with Fluorescence Detection. *J. AOAC Int.,* 83: 698-713.

Food and Drug Administration (2009): Pesticide Analytical Manual Volume I: Multiresidue Methods, 3rd Edition, U.S. Department of Health and Human Services, Washington, DC.

General Inspectorate for Health Protection (2016). Analytical Methods for Pesticide Residues in Foodstuffs, 6th edition, Ministry of Health Welfare and Sport, The Netherlands. http://www.cfsan.fda.gov/~frf/pami3.html

Irie, M. (2017): Pesticide Residues in Food. FAO Plant Production and Protection Paper, Pp191-210.

Jallow, M., Awadh, D., Albaho, M., Devi, V. and Thomas, B. (2017): Pesticide Risk Behaviors and Factors Influencing Pesticide Use among Farmers in Kuwait. *Sci. Total Environ.* 574:490–498.

Lee, S. Papathakis, M. Hsiao-Ming, C. and Carr, J. (2011): Multi-pesticide Residue Method for Fruits and Vegetables: California Department of Food and Agriculture. Fresenius *J. Anal. Chem.* 339: 376-383.

Lehotay, S. (2017): Determination of Pesticide Residues in Nonfatty Foods by Supercritical Fluid Extraction and Gas Chromatography/mass Spectrometry: Collaborative Study. *J. AOAC Int.,* 83: 680-697.

Lozowicka, B. (2015): Health Risk for Children and Adults Consuming Apples with Pesticide Residue. Sci. Total Environ. 502:184–198.

Luke, M. Froberg, J. and Masumoto, H. (2010): Extraction and Cleanup of Organochlorine, Organophosphate, Organonitrogen, and Hydrocarbon Pesticides in Produce for Determination by Gas-liquid Chromatography. *J. Assoc. Off. Anal. Chem.,* 58, 1020-1026.

Miskiyah, K. and Munarso, S. (2017): Pesticide Residues in Fruits. *Journal of Hortikultura*, 19 (1), 101-111.

Montory, M., Ferrer, J., Rivera, D., Villouta, M. and Grimalt, J. (2017): First Report on Organochlorine Pesticides in Water in a Highly Productive Agro-industrial Basin of the Central Valley, Chile. *Chemosphere.* 173:125–136.

Munarso, S. and Miskiyah, B. (2009): *Buletin Teknologi Pasca Panen Pertanian,* 5: 1-7.

Ngowi, A., Mbise, T., Ijan, A., London, L. and Ajayi, O. (2007): Pesticides use by Smallholder Farmers in Vegetable Production in Northern Tanzania. *Crop Protection* 26: 1617–1624.

Osman, K., Al-Humaid, A. and Al-Redhaiman, K. (2015): Monitoring of Pesticide Residues in Vegetables Marketed in Al-Qassim Region, Saudi Arabia. *Ecotoxicol. Environ. Saf.* 73:1433–1439.

Osman, K., Al-Humaid, A., Al-Rehiayani, S. and Al-Redhaiman, K. (2017): Estimated Daily Intake of Pesticide Residues Exposure by Vegetables Grown in Greenhouses in Al-Qassim Region, Saudi Arabia. *Food Control.* 22:947–953.

Qin, G., Zou, K., Li, Y., Chen, Y., He, F. and Ding, G. (2016): Pesticide Residue Determination in Vegetables from Western China Applying Gas Chromatography with Mass spectrometry. *Biomed. Chromatogr.* 30:1430–1440.

Richardson, R. (2015): Assessment of the Neurotoxic Potential of Chlorpyrifos Relative to other Organophosphorus Compounds: a Critical Review of the Literature. *J. Toxicol. Environ. Health* 44 (2), 135-65.

Saeed, T., Sawaya, W., Ahmad, N., Rajagopal, S. and Al-Omair A.(2015): Organophosphorus Pesticide Residue in the Total Diet of Kuwait. Arab *J. Sci. Eng.* 30:17–27.

Saeed, T., Sawaya, W., Ahmad, N., Rajagopal, S., Al-Omair, A. and Al-Awadhi, S. (2011): Chlorinated Pesticides Residues in the Total Diet of Kuwait. *Food Control*.12:91–98.

Saeed, T., Sawaya, W., Ahmad, N., Rajagopal, S., Dashti, B. and Al-Awadhi, S. (2013): Assessment of the Levels of Chlorinated Pesticides in Breast Milk in Kuwait. *J. Food Addit. Contam.* 12:1013–1018.

Sheridan, R. and Meola, J. (2017): Analysis of Pesticide Residues in Fruits, Vegetables, and Milk by Gas Chromatography/tandem Mass Spectrometry. *J. AOAC Int.*, 82, 982-990.

Tahir, S., T. Anwar, I. Ahmed, S. Aziz, M. Ashiq and K. Ahad. (2001): Determination of Pesticide Residues in Fruits and Vegetables in Islamabad Market. *J. Environment. Biol.*, 22(1): 71-74.

Tomizawa, M. and, Casida, J. (2013): Selective Toxicity of Neonicotinoids Attributable to Specificity of Insect and Mammalian Nicotinic Receptors. *Annu. Rev. Entomol.* 48:339–364.

Wagner, S. (2017): Diagnosis and Treatment of Organophosphate and Carbamate Intoxication. *Human Health Effects of Pesticides*; Keifer, M. C., Ed.; Hanley and Belfus: Philadelphia, 12: 239-249.

WHO. (2016): The WHO Recommended Classification of Pesticides by Hazard and Guidelines to Classification. [(accessed on 16 December 2017)]; Available online: http://www.who.int/foodsafety/publications/classification-pesticides/en/

Zawiyah, S., Cheman, Y.B., Nazimah, S.A.H., Chin, C.K., Tsukamoto, I., Hamanyza A.H and Norhaizan, I. (2007). Determination of Organochlorine and Pyrethroid Pesticides in Fruit and Vegetables Using SAX/PSA Clean-up Column. Food Chem,102: 98–103.