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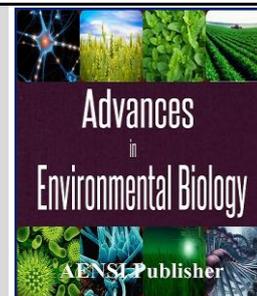
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Health Impact of Khat Chewing and Pesticides: Detection of 8 Pesticides Multi-Residues in Khat Leaves (*Catha edulis*) From Jazan Region, KSA.

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Received 22 June 2016; Accepted 28 August 2016; Available online 31 August 2016

ABSTRACT

BACKGROUND: Khat chewing habit is widespread with a deep-rooted socio-cultural tradition in the southern west region of Saudi Arabia and as such poses a public health problem. Use of pesticides is considered to be indispensable practice for the production of adequate food supply for the increasing world population and the control of insect borne diseases. Thus, pollution of the environment with pesticides as well as the pesticides entry into the food chain is unavoidable especially in most of developing countries. **OBJECTIVE:** Our objective of the current study was to detect the possible pesticides multi-residues contamination of the chewable parts of khat arrested during smuggling into Kingdom Saudi Arabia (unknown origin) and suppose to be consumed in Jazan region. **METHODOLOGY:** A total of 50 khat leaves samples were extracted using QuEChERS method followed by GC/MS analysis. Standard pesticides mixture containing 69 pesticides used in our analysis in addition to Triphenyl Phosphate (TPP) as an internal standard. **RESULTS:** Eight pesticides residues were detected and quantified in 42 / 50 samples (82%) where Lambda-Cyhalothrin and Penconazole were the most abundant pesticides detected (22 / 50 samples; 44%) while Trifloxystrobin was detected in only 1 sample (2%). Five of the pesticides detected were above MRLs (Maximum Residue Limits) while Carbaryl, Keroxime-methyl and Trifloxystrobin were below MRLs. The results of our study necessitate the need for awareness creation among people in our community. **CONCLUSION:** It could be concluded that chewing khat grown with chemical pesticides may cause considerable adverse health effects in khat users.

KEYWORDS: Khat; Pesticides; Health Risk

INTRODUCTION

Khat (*Catha edulis*) leaves are chewed daily by a high proportion of the adult population in Yemen, southern west region of Saudi Arabia and Eastern part of Africa. Cathinone is believed to be the main active ingredient in fresh khat leaves and is structurally related and pharmacologically similar to amphetamine. The habit of khat chewing is widespread with a deep-rooted socio-cultural tradition in these regions and as such poses a public health problem [1].

Fresh leaves of Khat contain two psychoactive constituents which are the stimulant cathinone and cathine. These two compounds act on two main neurochemical pathways- dopamine and noradrenalin. It has been suggested that cathinone, like amphetamine, releases serotonin in the CNS. Both these substances induce the release of dopamine from CNS dopamine terminals thereby increasing the activity of dopaminergic pathways [2].

Pesticides are widely used to protect plant and crop in agriculture from pests and plant diseases. There are different categories of pesticides such as insecticides, fungicides, and herbicides. The European Union reported in 2010 that 208,000 tons of pesticides were used which are biologically active with specific inherent toxicity [3].

With the migration of khat users from Africa and Southwestern part of Arabian peninsula, several health problems have been expanded to different countries around the world [4,5]. Recent study suggested that currently 20 million people regularly used khat worldwide. Khat chewing is known to cause serious health problems [6].

Furthermore, the usage of pesticides been accompanied by human health risk and the environmental contamination because of their toxicity. As well, the chronic effect of pesticides exposure resulted in carcinogenic, genotoxic effects and endocrine disturbances [7].

Recently, use of pesticides has been increased. Therefore, there are many legal limits for pesticides residues in the plants which set by the European Commission (EC) and United States Department of Agriculture to control pesticides usage who has reported that three million pesticide poisonings occur annually and result in 220,000 deaths worldwide. In public health safety and trade, pesticide residue determination in all food products, especially in raw vegetables and fruits, is a very demanding task [8].

One of the most global obstacles that there are no fixed limits on pesticides use. Also, a basic conflict between exporting and importing countries [9]. Furthermore, the low concentration of pesticides and the excessive amount of interfering substances and adversely affect the analysis results [10].

Therefore, efficient analytical methods required to determine the pesticides residue limit. The Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) method is considered one of the most efficient multi-residue analytical technique including wide polarity range. The QuEChERS method replaces many complicated analytical steps which conducted in the traditional methods. This method is relying on an extraction with acetonitrile and partitioning with salt [11].

First published data about QuEChERS for pesticide extraction was in 2003 [11]. It is used for all food stuff [12] and others like soil [13]. There are various applications for QuEChERS in the field rather than pesticides including, pharmaceutical uses (drug analysis; hormones and steroids), mycotoxin analysis and environmental studies of toxic compounds such as acrylamide [14,15].

The QuEChERS method is the most successful routine lab test and famous for more than ten years. It requires few steps and minimum solvent in comparing with conventional sample preparation techniques. It is characterized by 95% recovery range and repeatability of <5%, and cost-effective strategy (<30 min and \$1 per sample). It was recommended to be used in international standard methods such as the AOAC official method 2007.01 and the CEN's, European Committee for Standardization, standard method EN 15662 [16].

Several reviews on the adverse health effects of chewing khat documented negative health impact of the active compounds in khat and in some cases in association with smoking (commonly used in khat chewing sessions) but did not account the role that may be contributed by pesticides [17]. For instance, Malathion has been reported to produce chromosomal aberrations and micronuclei in experimental animal studies, and immune system alterations at a subclinical level [18,19]. Daba *et al.* (2011) found that khat samples collected from different places in Ethiopia contained some pesticides residues such as DDT and Diazinon [20].

MATERIAL AND METHODS

Study area:

Jazan City, South Province of Saudi Arabia.

Sampling:

As a total, 50 samples of the chewable parts of khat arrested during smuggled into Kingdom Saudi Arabia were randomly selected during the period January – April, 2016.

Sample processing:

The selected fresh chewable part (20 g) from each sample was homogenized in a blender. Then, 10 grams of the homogenate were weighed in 50 ml falcon tubes. Acetonitrile solution (15 ml) was added to each sample and vortexed vigorously for 2 minutes.

Internal standard and QuEChERS powder kit (UCT, USA) was added to each tube slowly and vortexed for more 2 minutes. Then, all samples were centrifuged at 3000 rpm for 5 minutes. The supernatant (organic layer) was transferred to chlorophyll clearance tubes (UCT, USA), then vortexed for 30 seconds and centrifuged at 3000 rpm for 5 minutes. The clear solution was finally transferred to GC vials for analysis.

Standard Solutions:

A standard mixture solution of 69 pesticides was used in our study which was given as a gift from Dr. Mohammed Al Tufail, Head of Toxicology Lab, KFSHRC. Triphenyl Phosphate (TPP) was used as internal standard in a concentration of 500 ng/ml. For quantitative analysis, a calibration curve was prepared by using standard mixture solutions prepared in four concentrations (100, 500, 1000, 2000 ppb).

GC-MS Analysis:

All samples were analyzed by GC/MS instrument manufactured by Shimadzu (Model GCMS-QP2010 Ultra) using Rxi®-5ms (fused silica) capillary column (30 m - 0.25 mm ID, 0.25 ml film thickness, low polarity phase; Crossbond® diphenyl dimethyl polysiloxane). Helium gas was used as the carrier gas at a flow rate of 1 ml/min. A volume of 2 µl mixture of standard or sample extract was injected in a split mode.

The injection port was set at a temperature of 270°C, and the temperature of the oven was initially held at a temperature of 70°C for 1 min. Then it was ramped to 190°C at a rate of 30°C/min, and finally at 10°C/min to 300°C (held for 5 min). The mass spectrometer was operated with an electron ionization (EI) source in the selected ion monitoring mode. The electron energy was 70 eV and the ion source, and the interface temperature was maintained at 240°C and 210°C, respectively. The electron multiplier voltage was 1 kV, and the solvent delay was set to 15 min.

RESULTS AND DISCUSSION

Our results revealed a detection of 8 different pesticides residues in all khat samples collected. The average concentration ranged from 28 ± 8 µg/Kg for Keroxim-methyl to 1104 ± 488 µg/Kg for Lambda-Cyhalothrin pesticides. Five of the detected pesticides exceeded MRLs which are Penconazole, Triademinol, Tebuconazole, Bifenethrin and Lambda-Cyhalothrin. On the other hand, Carbaryl, Keroxim-methyl and Trifloxystrobin levels were below MRLs (Table 1, Figure 4).

Quantitative estimation of pesticides residues detected in khat samples were done using a calibration curve prepared from standard mixture of pesticides 100, 100, 500 and 2000 µg/kg (ppb) as well as the specific target ions for each pesticide (Figure 1). The R and R² values were not less than 0.998 and 0.997, respectively.

Lambda-Cyhalothrin and Penconazole were detected in 22 / 50 samples tested (44%) while Bifenthrin and Carbaryl were found to contaminate 17 (34%) and 15 (30%) of all samples, respectively. Only one sample was contaminated by Trifloxystrobin representing 2% of all samples (Figure 2, 3).

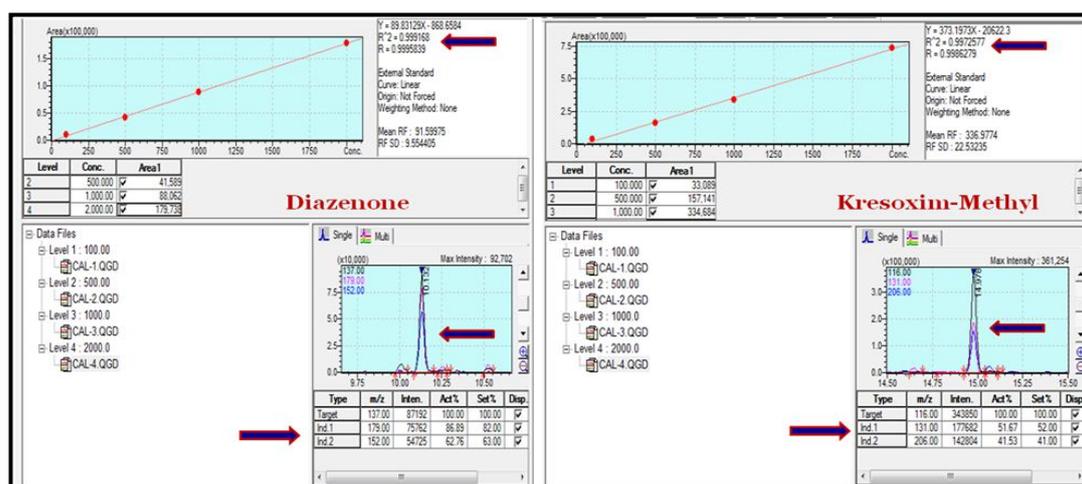


Fig. 1: Quantitative determination of pesticides detected in all samples depending on calibration curve prepared and the target ions specific for each pesticide (Diazenone & Kresoxim – Methyl).

Table 1: Quantification data of pesticides residues detected in all khat samples as $\mu\text{g}/\text{Kg}$ (ppb).

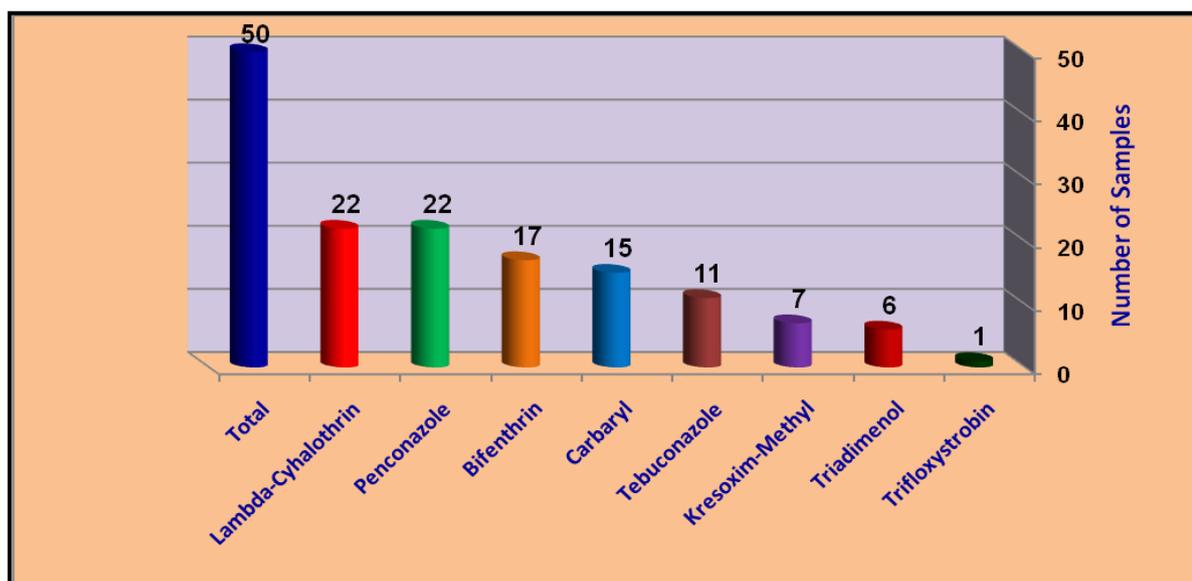
Pesticide Name	R T (min.)	m/z			Conc. (ppb) Mean \pm SD (Positive Samples; %)	MRLs# (ppb)
		Main Ion	2 nd Ion	3 rd Ion		
Carbaryl	7.378	144	115	116	125 \pm 28 (15; 30%)	500
Penconazole	13.606	248	159	161	449* \pm 209 (22; 44%)	300
Triadimenol	13.845	112	57	168	260* \pm 308 (6; 12%)	200
Kresoxim-methyl	15.006	116	131	206	28 \pm 8 (7; 14%)	100
Trifloxystrobin	16.165	116	131	59	41 \pm 0 (1; 2%)	300
Tebuconazole	16.992	125	70	83	392* \pm 215 (11; 22%)	100
Bifenthrin	17.413	181	166	165	194* \pm 151 (34; 68%)	100
Lambda - Cyhalothrin	18.464	181	197	208	1104* \pm 488 (22; 44%)	300

Maximum Residue Limits

* Above MRLs

The increased prevalence of smoking, hypertension and diabetes may play a vital role in the development of cardiac diseases like heart failure which make it difficult to determine the effect of khat [21,22]. A recent study revealed the effects of concurrent tobacco and khat use and suggested that the adverse effects of khat use may lie in its association with tobacco use [6].

The application of pesticides by farmers for better khat production results in exposure of khat chewers to different residues of pesticides which was found to be another risk factor for cardiac complications among khat users [23]. The presence of pesticides in khat was implicated in the inhibition of serum acetyl cholinesterase activity and may contribute to the presence of high levels of free radicals [24]. Another study showed that the production of oxidants may be due to khat use. These oxidants are responsible for reduction in antioxidant defense system [25].

**Fig. 2:** Frequency of individual pesticides residues detected in all samples tested.

Chewing khat leaves without any cleansing (such as washing, soaking in hot water, or thermal treatments), which is the traditional way of using the product, may lead to higher exposure to pesticides. Furthermore, it has been reported that khat farmers do not follow the safety instructions. For example, a substantial proportion of khat farmers in Yemen ignored the required period to harvest after the last spraying. Al-Haj *et al.* (2005) reported that 50% of farmers interviewed stated that a period of 7–10 days was required between harvesting khat and the last spraying, and that half of the farmers said that the period should be 10–20 days [26].

A recent study from Ethiopia indicated that some khat farmers harvested a recently sprayed khat for sale and self-consumption, potentially putting themselves and their consumers at higher risk of oral exposure to and ingestion of pesticides. Some khat farmers prefer to use higher doses of pesticides than that recommended on the label²⁷. For example, previous study reported that 40% of the farmers restricted their use to concentrations of pesticides on the label, but 60% did not follow the instructions. Khat harvesting during the waiting period after pesticide application and the use of high pesticide concentrations for spraying will accordingly increase the amount of pesticide residues on khat [26].

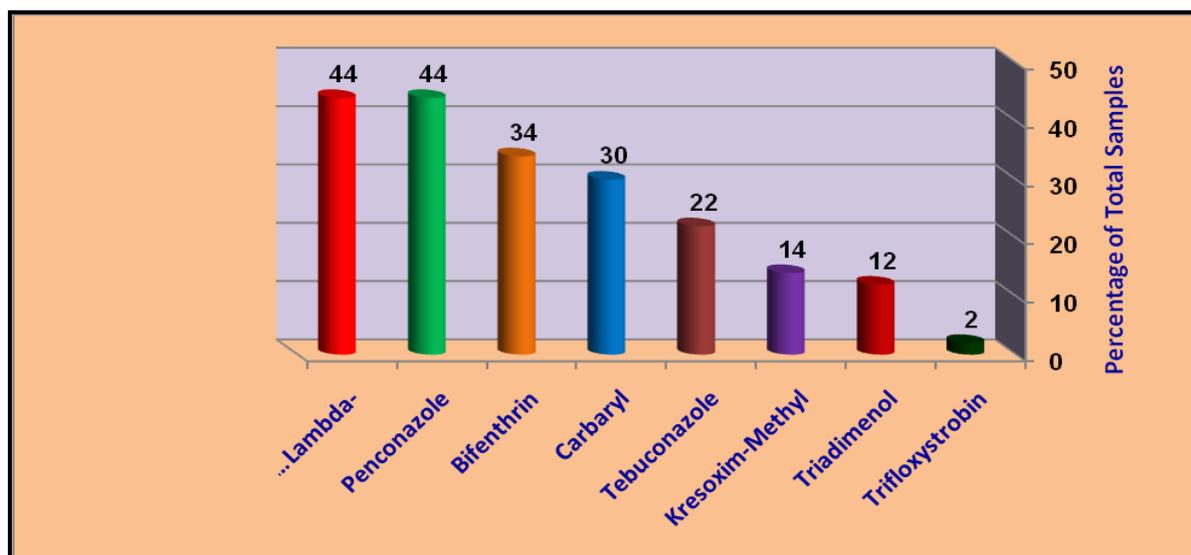


Fig. 3: Percentage of detected pesticides residues in all samples tested.

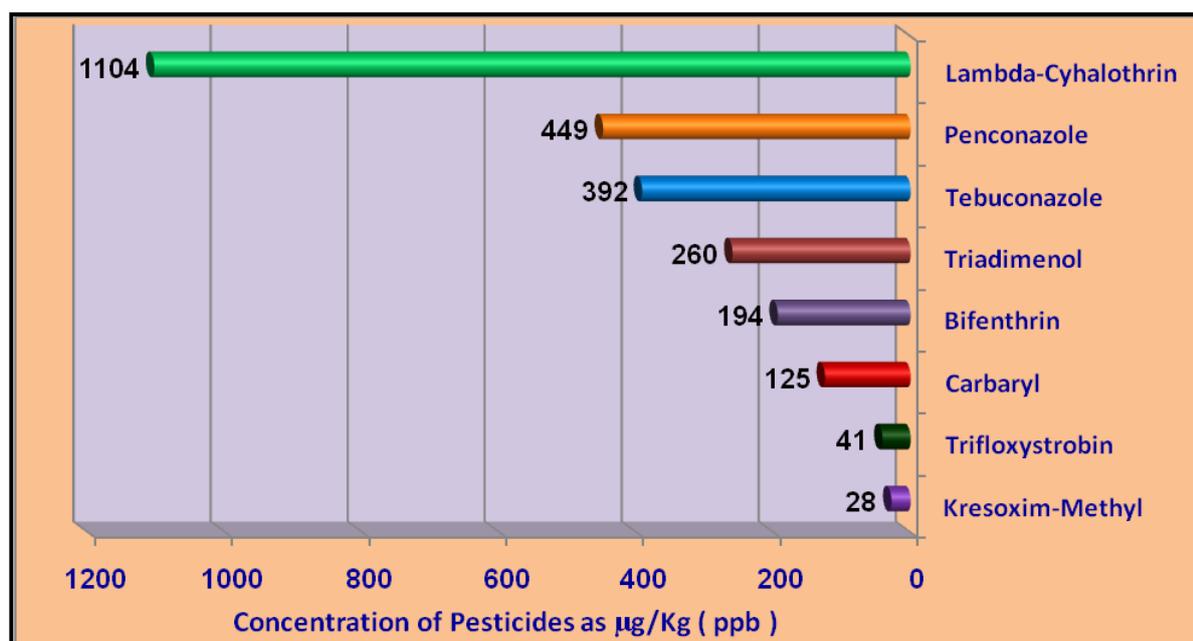


Fig. 4: Average concentration of quantified pesticides residues expressed as $\mu\text{g}/\text{kg}$ (ppb).

Another study conducted in 2009 investigated the presence and level of pesticide residues in khat samples collected from various parts of Ethiopia [27]. The khat samples had pp'-DDT concentrations ranging from 141.2 to 973.0 µg/kg. Maximum concentrations were found between 240 and 1200 times the European Union maximum recommended levels for DDT in food (vegetables 10 µg/kg, and cereals 50 µg/kg).

In conclusion. It is clear that most of khat samples tested in this study are contaminated by at least one pesticide which may contribute to possible health problems among chronic khat chewers. Pesticides may lead to pathologic changes in oral cavity and as well may induce production of reactive oxygen species and free radicals. Further study is needed to understand the possible effect of pesticides in chronic khat users.

Recommendation:

Creation a governmental program to design regulations for permanent monitoring and control of using pesticide in agriculture. We recommend routine pesticides screening regimens to be performed on regular periods to monitor control and minimize uncontrolled pesticides usage in agriculture.

ACKNOWLEDGMENT

This work was financially supported by a grant (no. 1008 / 2012) from Substance Abuse Research Center (SARC), Jazan University, KSA.

REFERENCES

- [1] Hassan, N.A., A.A. Gunaid, I.M. Murray-Lyon, 2007. Khat (*Catha edulis*): health aspects of khat chewing. *East Med Health J*, 13(3): 706-718.
- [2] Bempah, C.K. and A.K. Donkor, 2011. Pesticide residues in fruits at the market level in Accra Metropolis, Ghana, a preliminary study. *Environmental Monitoring Assessment*, 175: 551-561.
- [3] Corkery, J.M., F. Schifano, S. Oyefeso, A.H. Ghodse, T. Tonia, V. Naidoo, et al., 2011. "Bundle of fun" or "bunch of problems"? Case series of khat-related deaths in UK. *Drugs Educ Prev Policy*, 18: 408-425.
- [4] Osman, F.A. and M. Söderbäck, 2011. Perceptions of the use of khat among Somali immigrants living in Swedish society. *Scand J Public Health*, 39(2): 212-219.
- [5] Kalix, P. and O. Braenden, 1985. Pharmacological aspects of the chewing of khat leaves. *Pharmacol Rev*, 37(2): 149-64.
- [6] Al'Absi, M., M. Nakajima, A. Dokam, A. Sameai, M. Alsoofi, N. Saem Khalil, et al., 2014. Concurrent tobacco and khat use is associated with blunted cardiovascular stress response and enhanced negative mood: a cross-sectional investigation. *Hum Psychopharmacol*, 29(4): 307-315.
- [7] Bempah, C.K., A. Buah-Kwofie, E. Enimil, B. Blewu, and G. Agyei-Martey, 2012. Residues of organochlorine pesticides in vegetables marketed in Greater Accra, Region of Ghana. *Food Control*, 25: 537-542.
- [8] WHO., 1992. "Our planet, our health. Report of the WHO Commission on Health and Environment. " Geneva, Switzerland: World Health Organization.
- [9] Jaggi, S., C. Sood, V. Kumar, S.D. Ravindranath, A. Shanker A., 2001. Leaching of Pesticides in tea brew. *Journal Agri Food Chem*, 49(11): 5479-83.
- [10] Wilkowska, A. and M. Biziuk, 2011. Determination of pesticide residues in food matrices using the QuEChERS methodology. *Food Chemistry*, 125(3): 803-812.
- [11] Anastassiades, M., S.J. Lehotay, D. Stajnbaher, F.J. Schenck, 2003. Fast and easy multiresidue method employing acetonitrile extraction/partitioning and "dispersive solid-phase extraction" for the determination of pesticides residues in produce. *Journal AOAC International*, 86(2): 412-431.
- [12] Golge, O.B. and B. Kabak, 2015. Determination of 115 pesticide residues in oranges by high-performance liquid chromatography-triple-quadrupole mass spectrometry in combination with QuEChERS method. *Journal Food Composition Analysis*, 41: 86-97.
- [13] Masia, A., K. Vasquez, J. Campo, Y. Pico, 2015. Assessment of two extraction methods to determine pesticides in soils, sediments and sludges. Application to the Turia River Basin. *Journal Chromatography A*, 1378: 19-31.
- [14] Peysson, W. and E. Vulliet, 2013. Determination of 136 pharmaceuticals and hormones in sewage sludge using quick, easy, cheap, effective, rugged and safe extraction followed by analysis with liquid chromatography time-of-flight-mass spectrometry. *Journal Chromatography A*, 1290: 46-61.
- [15] Klinsunthorn, N., A. Petsom, T. Nhujak T, 2011. Determination of steroids adulterated in liquid herbal medicines using QuEChERS sample preparation and high-performance liquid chromatography. *Journal Pharm Biomed Anal*, 55(5): 1175-1178.

- [16] Lehotay, S.J., K.A. Son, H. Kwon, U. Koesukwiwat, W. Fu, K. Mastovska, E. Hoh, N. Leepipatpiboon, 2010. Comparison of QuEChERS sample preparation for the analysis of pesticides residues in fruits and vegetables. *Journal Chromatography A*, 1217(16): 2548-60.
- [17] Al-Habori, M, 2005. The potential adverse effects of habitual use of *Catha edulis* (khat). *Expert Opin Drug Saf*, 4: 1145-1154.
- [18] Amer , S.M., M.A. Fahmy, F.A. Aly, A.A. Farghaly, 2002. Cytogenetic studies on the effect of feeding mice with stored wheat grains treated with malathion. *Mutat Res*, 513: 1-10.
- [19] Johnson, V.J., A.M. Rosenberg, K. Lee, B.R. Blakley, 2002. Increased T-lymphocyte dependent antibody production in female SJL/J mice following exposure to commercial grade malathion. *Toxicology*, 170: 119-129.
- [20] Daba, D.A., A. Hymete, A.A. Bekhit, A.I. Mohamed, A.E. Bekhit, 2011. Multi residue analysis of pesticides in wheat and khat collected from different regions of Ethiopia. *Bull Environ Contam Toxicol*, 86: 336-41.
- [21] Advisory Council on the Misuse of Drugs. Khat: A review of its potential harms to the individual and communities in the UK. ACMD; 2013.
- [22] Ali, W.M., K.F. Al Habib, A. Al-Motarreb, R. Singh, A. Hersi, H. Al Faleh, et al., 2011. Acute coronary syndrome and khat herbal amphetamine use: an observational report. *Circulation*, 124(24): 2681-89.
- [23] Mills, K.T., A. Blair, L.E. Freeman, D.P. Sandler, J.A. Hoppin, 2009. Pesticides and myocardial infarction incidence and mortality among male pesticide applicators in the Agricultural Health Study. *American Journal Epidemiol*, 170(7): 892-900.
- [24] Al-Akwa, A.A., M. Shaher, S. Al-Akwa, S.L. Aleryani, 2009. Free radicals are present in human serum of *Catha edulis* Forsk (Khat) abusers. *Journal Ethnopharmacol*, 125(3): 471- 473.
- [25] Masoud, A.M., B.A. Al-Shehari, L.N. Al-Hattar, M.A. Altaezzi, W.A. Al-khadher, Y.N. Zindal, 2012. Alterations in Antioxidant Defense System in the Plasma of Female Khat Chewers of Tamar City, Yemen. *Jord J Biol Sci*, 5(2): 129-133.
- [26] Al-Haj, M.A., N.A. Awadh, A.A. Ali, 2005. Survey of pesticides used in Qat cultivation in Dhale' and Yafe' and their adverse effects. *Journal Nat Appl Sci.*, 9: 103-10.
- [27] Abdulaziz, M, 2010. An assessment of possible health risks of using DDT and Farmers' Perception towards toxicity of pesticides used on Khat (*Catha edulis*): In Haromaya Woreda, Ethiopia: Addis Ababa University.