http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

OPEN ACCESS Pakistan Journal of Biological Sciences

ISSN 1028-8880
DOI: 10.3923/pjbs.2020.1601.1606

Research Article
Toxicological Characterization of Pesticide Residue in *Phaseolus vulgaris*

[1]E. Adewole, [1]A. Ojo, [1]T. Oludoro and [2]L.A. Enye

[1]Department of Chemical Sciences, college of sciences, Afe Babalola University Ado Ekiti, Nigeria
[2]Department of Anatomy, college of Health Sciences, Afe Babalola University Ado Ekiti, Nigeria

Abstract

Background and Objective: The health implications associated with consumption of food crops preserved with pesticides such as diarrhea, food poisons have been a major challenge to health practitioners and the concerned authorities, the objective of the research was to analyze the pesticide residues and compare the contamination rate with Acceptable Dietary Intake (ADI) and Maximum Residue Limit (MRL). **Materials and Methods:** *Phaseolus vulgaris* were purchased in two different locations, the samples were powdered using a grinder (Sumeet CM/L 2128945). Fifty grams of powdered flour were soaked in 200 mL of Methanol and the crude extracts were concentrated using a rotary evaporator. The extracts were Characterized using GC-MS and percentage compositions of identified pesticide residues were converted into mg/g as contamination rate and the toxic analysis was done by using the Osiris Online server. **Results:** In chromatogram A, identified pesticides residue include Dieldrin (96.1 mg g^{-1}), Indolizine (67.9 mg g^{-1}), permethrin (99.4 mg g^{-1}) and compounds identified in chromatogram B include dichlorvos (8.2 mg g^{-1}), Diazinon (52.3 mg g^{-1}), fenitrothion (17.8 mg g^{-1}) and permethrin (122.0 mg g^{-1}). These pesticide residues exhibited various toxicological effects, such as; mutagenic, tumorigenic effects. Moreover, the contamination rates of the identified residues were higher than both MRL and ADI. **Conclusion:** The research work had shown that the two samples had contamination rates higher than both the ADI and MRL, this could pose health hazards to the populace if consumed and it is recommended that the applications of pesticides in foods should be regulated and MRL and ADI should be adhered to.

Key words: Pesticides, residue, toxicological, characterization, osiris online server, acceptable dietary intake, maximum residue limit

Citation: Adewole, E., A. Ojo, T. Oludoro and L.A. Enye, 2020. Toxicological characterization of pesticide residue in *Phaseolus vulgaris*. Pak. J. Biol. Sci., 23: 1601-1606.

Corresponding Author: E. Adewole, Department of Chemical Sciences, college of sciences, Afe Babalola University Ado Ekiti, Nigeria

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The need to preserve the food crops in order to have high yield is always the hallmark of all farmers and to achieve the target goals at the beginning of farming seasons, they design their agricultural activities by incorporating how to combat insects attack during farming seasons and how to preserve their farm products from being spoilt by an insect. Consequently, adoption of use of pesticides and herbicides inclusive. However, it is a major problem for farmers on how to determine the appropriate dosage applications, most importantly in the preservation of farm products to avoid food spoilage, as human exposure to pesticides can be controlled and decreased by properly implementing pesticide application guidelines such as by setting maximum pesticide residue limits for food[1]. The benefit of pesticides can't be overemphasized as they help improve the quality and quantity of crops produced. Moreover, pesticides can cause environmental problems[2].

Pesticides have been said to be any substance or mixture in the food of humans or animals including any specified derivatives such as degradation and conversion products, metabolites, reaction products and impurities, of toxicological significance [3]. Pesticide residues cause both short- and long-term toxic effects that are hazardous to health, especially at higher levels that can lead to toxicity. It is well documented that chronic exposure to pesticides increases the risk of damage organs, causes mutagenic and carcinogenic transformation and endocrine [4-6].

Phaseolus vulgaris belongs to the family of leguminous plants and can be found in many African countries, Asia, the Middle East and South America. The previous study[7] had shown that they are highly nutritious food and the nutritional qualities have been extensively discussed. The health implications associated with consumption of food crops preserved with pesticides have been a major challenge to health practitioners and the concerned authorities, as many life has been lost due to food poisons, kidney failure, diarrhea and host of others, the need to analyze the pesticide residues present in *Phaseolus vulgaris* and compare the contamination rates with Acceptable Dietary Intake (ADI) and Maximum Residue Limit (MRL) gives rise to the research work.

MATERIALS AND METHODS

Sample collection: The two variety of *Phaseolus vulgaris* were purchased in a local market in Akure and Igbara-oke daily market all in Ondo State, Nigeria on the 10th October 2019.

Sample preparation: The dried samples were powdered by using a laboratory scale grinder (Sumeet CM/L 2128945) and sifted through 300 µm sieve to obtain the flour. The flour samples were sealed and packed in airtight containers for further analysis[8].

Crude extract preparation: The powdered *Phaseolus vulgaris* samples of 20 g were soaked in 200 mL of methanol of analytical grade for five days and later filtered using filtered paper and the extract was concentrated using a rotary evaporator at 35°C.

Characterization of the crude extract: The analyses of the compounds in the active fractions were run on a GC-MS system (Agilent Varian GC: 4800/3000). The fused-silica MS capillary column (30 m 0.25 mm ID, the film thickness of 0.25 mm) was directly coupled to an Agilent Varian. The oven temperature was programmed (35°C for 5 min, then 35-300°C at 10°C/min) and subsequently, held isothermal for 20 min. The injector port; was 250°C, the transfer line: 290°C, spitless. The volume injected: 0.2 mL and the column flow rate were 1 mL/min of 1 mg mL^{-1} solution (diluted in chloroform). The peaks of components in gas chromatography were subjected to mass spectral analysis. The MS operate with an EI-source at -70 eV; the solvent delay was 9 min. Scan time 1.5 sec; acquisition rate 10 spectra/second; mass range 50-1000 amu; detector voltage 1800 V and Ion source temperature: 250°C. Data were recorded in TIC mode. The software adapted to handle the mass spectra and chromatograms was Agilent chemstation software. The constituents were identified after comparing with available data in the GC-MS library in the literature. The GC-MS mass spectrum data were analyzed using Mnova 11.0.1 and the database of National Institute Standard and Technology (NIST) was used to interpret analyzed data. A comparison of the mass spectrum of the unidentified components was carried out against the mass spectrum of already known components available in the NIST library. The name, molecular weight and peak area percentage of unknown compounds were evaluated by the software as observed from the chromatogram.

Statistical analysis: Percentage compositions of the pesticide residues from the results of GC-MS was converted to mg g^{-1} by multiplying % by 100 and designated as contamination rate.

RESULTS AND DISCUSSION

The analysis of pesticide residues present in the two different sources of *Phaseolus vulgaris* has shown to be

highly contaminated when compared to the acceptable dietary limit and maximum residue limit. The characterization of methanolic crude *Phaseolus vulgaris* extracts and subsequent identification of pesticides and toxicological properties were done[9]. Figure 1 showed the chromatogram of *Phaseolus vulgaris* crude extract A, having retention times in the range of 8.02 to 46.00 min with varying relative abundance 1000 to 6000, twenty peaks were elucidated and only five pesticide residues were identified with different percentage composition. In Table 1, permethrin insecticide was identified at peak number 16, retention time 40.28 min and had the highest contaminant rate 99.40 mg g^{-1} while cypermethrin had the lowest contaminant rate 29.30 mg g^{-1} and identified at peak number 17, retention time 43.00 min. Furthermore, in a related development, Fig. 2 showed the chromatogram of *Phaseolus vulgaris* crude extract B having varying retention times of 1.56 to 46.10 min and identified peaks were twenty-five. Table 2 showed the pesticide residues identified in the chromatogram of crude extract B and the highest contamination rate was recorded for Iprobenfos (72.80 mg g^{-1}) and the least was Dichlorvos (8.20 mg g^{-1}). Table 3 showed the drug properties of all the identified pesticide residues and they all exhibited high mutagenic, tumorigenic, irritability and reproductive effects, with the exception of indolizine which had 1.3448 drug properties and no toxicology effect. Table 4 showed the comparison of

Table 1: Some identified peaks in the chromatogram of methanolic crude extract *Phaseolus vulgaris* of A

Peak No.	Retention times (RT) (min)	Compound name	Composition (%)	Contamination rate (Mg g^{-1})
10	24.74	Dieldrin	9.61	96.10
16	40.28	Permethrin	9.94	99.40
17	43.00	Cypermethrin	2.93	29.30
20	46.00	Fenvalerate	4.28	42.80
12	26.58	Indolizine	6.79	67.90

Table 2: Identified peaks in the chromatogram of methanolic crude extract *Phaseolus vulgaris* of B

Peak No.	Retention times (RT) (min)	Compound name	Composition (%)	Contamination rate (Mg g^{-1})
4	7.39	Dichlorvos	0.82	8.20
15	24.98	Diazinon	5.23	52.30
18	27.63	Vinclozolin, Carbaryl	7.00	70.00
19	30.02	Fenitrothion	1.78	17.80
21	40.00	Permethrin	12.20	122.00
23	43.51	Cypermethrin	5.33	53.30
25	46.15	Fenvalerate	6.92	69.20
16	25.51	Iprobenfos	7.28	72.80

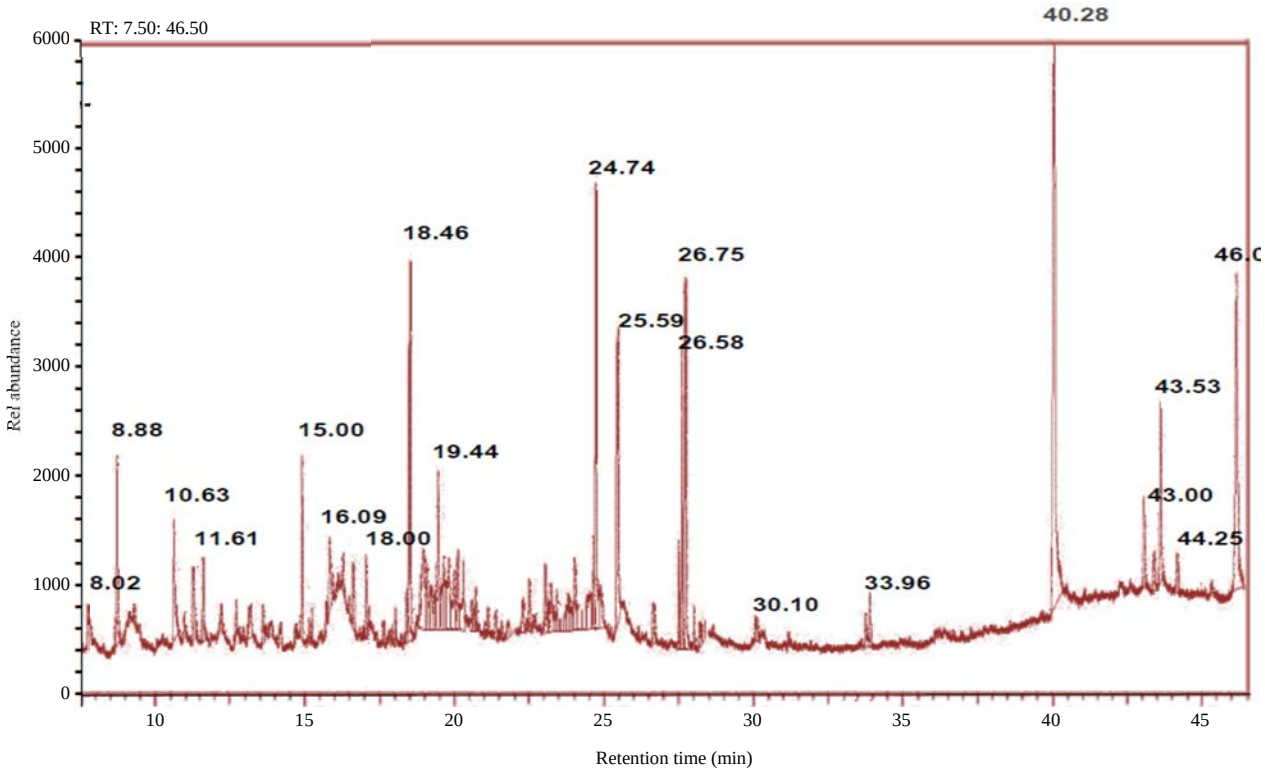

Fig. 1: Chromatogram of methanolic crude extract of *Phaseolus vulgaris* of A

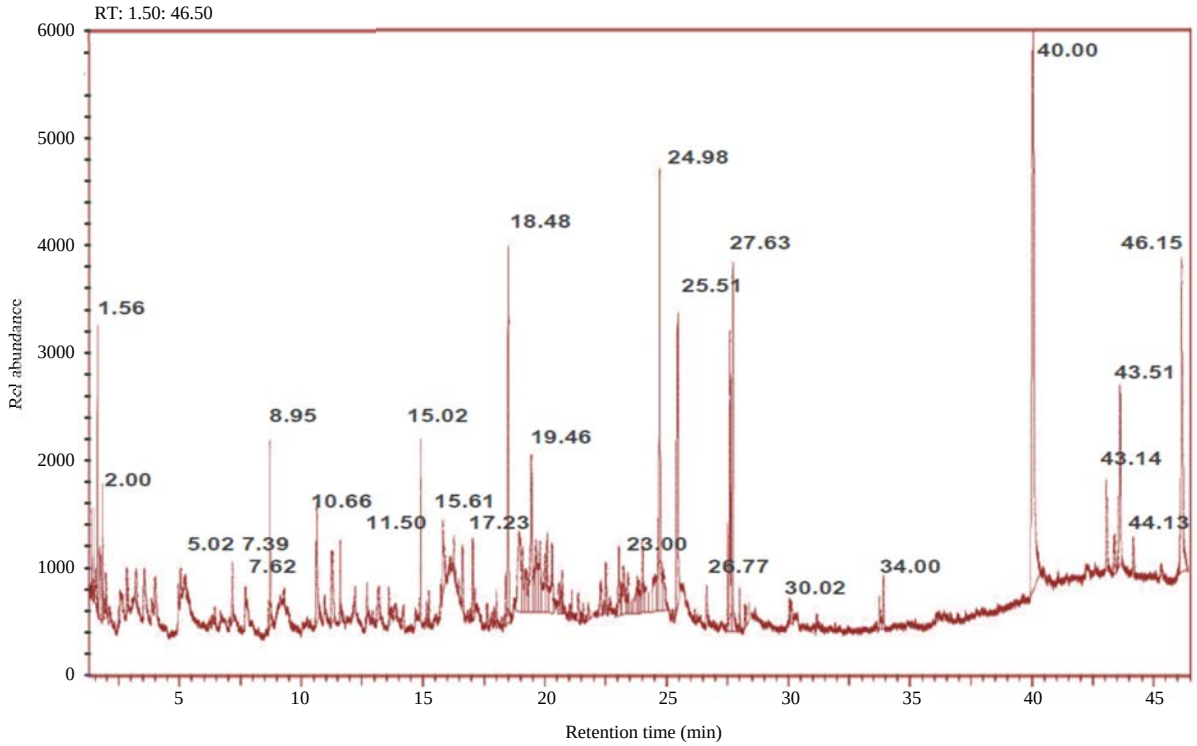

Fig. 2: Chromatogram of methanolic crude extract *Phaseolus vulgaris* of B

Table 3: Drug properties of identified pesticide residues in the methanolic crude extracts of A and B

Compound	Drug likeness	Mutagenic	Tumorigenic	Reproductive effect	Irritability
Fenvalerate	-5.9255	High	Low	High	High
Cypermethrin	-4.5036	High	High	High	High
Permethrin	0.92471	High	High	High	High
Dieldrin	-0.044038	High	Low	High	High
Indolizine	1.3448	None	None	None	None
Diazinon	-9.7111	High	High	High	High
Carbaryl	-3.09	None	None	None	None
Fenitrothion	-9.695	Low	High	High	High
Permethrin	0.92471	High	High	High	High
Cypermethrin	-4.5036	High	High	High	High
Dichlorvos	-18.059	High	High	High	High

Source: Thomas *et al.*[9]

contamination rate with Acceptable Dietary Limit (ADI) and all the compounds had higher contaminants than the ADI[10], for crude extract A, dieldrin has contamination rate 96.100 mg g^{-1} (ADI 0.001 mg kg^{-1}), cypermethrin 29.30 mg g^{-1} (ADI 0.05 mg kg^{-1}), crude extract B has dichlorvos 8.20 mg g^{-1} (ADI 0.004 mg kg^{-1}). Table 5 showed the classification of the pesticides into 7 insecticides and 1 fungicide[11]. Moreover, these identified residue contamination rates were found to be higher than the Maximum Residue Limits (MRL). The MRLs for; Diazinon to be (0.02 mg kg^{-1}), Cypermethrin (0.05 mg kg^{-1}), Dichlorvos (0.20 mg kg^{-1}), Fenvalerate Soya bean (dry) (0.1 mg kg^{-1}), Fenitrothion (0.5 mg kg^{-1})[12]. The use of different types of pesticides such as insecticides and fungicides to control the insect attacks on foods have been found to have very serious health implications on the final consumer's health. Aly [11] had enumerated the different health implications of pesticide residue in foods. The implications of exceeding the maximum residue limit as a result of excessive use have been found harmful to non-target organisms, including birds, fish and humans after being exposed to and harmed by organophosphorus residue[13-17]. Nature of pesticides, dosage and duration of exposure are important

Table 4: Comparison of contamination rate of pesticide residues such as Dichlorvos, cypermethrin, Fenitrothion with acceptable dietary intakes

	Pesticide	Contamination rate (mg g^{-1})	Acceptable Dietary Intake (ADI) (mg/kg/day)
Crude extract A	Dieldrin	96.100	0.001
	Permethrin	99.400	0.050
	Cypermethrin	29.300	0.050
	Fenvalerate	42.8	0.060
Crude extract B	Dichlorvos	8.200	0.004
	Diazinon	52.300	0.001
	Fenitrothion	17.800	0.002
	Carbaryl	70.000	0.01
	Permethrin	122.000	0.050
	Cypermethrin	53.300	0.050
	Fenvalerate	69.200	0.060

Source: Dennis[10]

Table 5: Classification of pesticide residues into different classes

Compound name	Class of pesticides
Diazinon	Insecticide
Fenitrothion	Insecticide
Iprobenfos	Fungicide
Dichlorvos	Insecticide
Carbaryl	Insecticide
Permethrin	Insecticide
Cypermethrin	Insecticide
Dieldrin	Insecticide

Source: Aly *et al.*[11]

factors in the determination of adverse health effect as a result of contaminated foods[18], symptoms of exposure to organophosphates such as diazinon, fenitrothion include diarrhea, salivation[19], anxiety, depression, coma and convulsions are acute psychological and behavioral effects of organophosphorus pesticide exposure, while chronic exposure leads to cognitive and emotional deficits. Organophosphates act directly on the nervous system by inhibiting the enzyme acetylcholinesterase (AChE)[20]. Severe organophosphate exposure is clinically manifested by marked miosis and loss of the pupillary light reflex, fasciculations, flaccid paralysis, pulmonary rales, respiratory distress and cyanosis with less than 10% of the normal value of serum cholinesterase[21]. The legally permitted maximum residue limit represents the highest concentration (mg kg^{-1}) expected to be found in food items. Exceeded MRLs are an indication of a violation of good agricultural practices[22-24]. The results of contamination rates of the research work, when compared to MRL and ADI, had shown that the rule of pesticide applications to *Phaseolus vulgaris* bought from the two locations have been strongly violated.

CONCLUSION

However, the pesticide residue analysis of *Phaseolus vulgaris* bought in two different locations in Ondo state had shown to be highly contaminated and the rules of application of pesticides to farm products have been strongly violated, this could part of factors responsibly for health problems associated with consumption of food laden with pesticide residues, there is need for Agricultural agencies to continue monitoring the use of these pesticides in order to mitigate the different diseases caused by these harmful chemicals.

SIGNIFICANCE STATEMENT

This study discovered the violation of abiding with the recommended amount of pesticides applications to farm crops by geometrically exceeding the Maximum Residue Limit and Acceptable Dietary Intake, this study will help the researchers to uncover the critical areas of risk assessment associated with excessive use of pesticide applications and toxicological characterizations that many researchers were not able to explore. Thus, a new theory on monitoring and evaluation of pesticide residues may be arrived at.

ACKNOWLEDGMENT

I want to sincerely appreciate Professor Jamshed Iqbal, Head, Center for Advance Drug Research (CADR), COMSATS Institute of Information Technology, Pakistan for the software and the training on how to use the software during my Postdoctoral fellowship period under TWAS/ CIIT partnership in 2017.

REFERENCES

1. The Japan food chemical research foundation, 2006. Japanese positive list system for agricultural chemical residues in foods. Official release of Japanese Ministry of Health, Labour and Welfare.
2. Spiewak, R., 2001. Pesticides as a cause of occupational skin diseases in farmers. Ann. Agric. Environ. Med., 8: 1-5.
3. McNaught, A.D. and A. Wilkinson, 1997. IUPAC Compendium of Chemical Terminology. 2 Edn., Blackwell Science Ltd., Oxford, UK., ISBN: 0865426848.

4. Hayes, W.J. and E.R. Laws, 1991. Handbook of Pesticide Toxicology. Academic Press, New York, pp: 55-56.
5. Bunggush, R.A. and T. Anwar, 2000. Preliminary survey for pesticide poisoning in Pakistan. Pak. J. Biol. Sci., 3: 1976-1978.
6. Berrada, H., M. Fernandez, M.J. Ruiz, J.C. Molto, J. Manes and G. Font, 2010. Surveillance of pesticide residues in fruits from Valencia during twenty months (2004/05). Food Control, 21: 36-44.
7. Geil, P.B. and J.W. Anderson, 1994. Nutrition and health implications of dry beans: A review. J. Am. College Nutr., 13: 549-558.
8. Alves, R.M., M.V. Grossmann, C. Ferrero, N.E. Zaritzky, M.N. Martino and M.R. Sierakoski, 2002. Chemical and functional characterization of products obtained from yam tubers. Starch/Stärke, 54: 476-481.
9. Sander, T., J. Freyss, M. Von Korff and C. Rufener, 2015. DataWarrior: an open-source program for chemistry aware data visualization and analysis. J. Chem. Inf. Model., 55: 460-473.
10. Hill, D.S., 2002. Pests of Stored Foodstuffs and Their Control. Kluwer Academic Publishers, New York, Pages: 476.
11. Derbalah, A., R. Chidya, W. Jadoon and H. Sakugawa, 2019. Temporal trends in organophosphorus pesticides use and concentrations in river water in Japan and risk assessment. J. Environ. Sci., 79: 135-152.
12. Pesticide Residues, 2013. Maximum residue limits national bureau of agricultural commodity and food standards ministry of agriculture and cooperatives. Thai Agricultural Standard Tas 9002-2008 Pesticide.
13. Salvi, R.M., D.R. Lara, E.S. Ghisolfi, L.V. Portela, R.D. Dias and D.O. Souza, 2003. Neuropsychiatric evaluation in subjects chronically exposed to organophosphate pesticides. Toxicol. Sci., 72: 267-271.
14. Pazou, E., M. Boko, C. Vangestel, H. Ahissou and P. Laleye *et al*., 2006. Organochlorine and organophosphorous pesticide residues in the Ouémé River catchment in the Republic of Bénin. Environ. Int., 32: 616-623.
15. Reinecke, S.A. and A.J. Reinecke, 2007. The impact of organophosphate pesticides in orchards on earthworms in the Western Cape, South Africa. Ecotoxicol. Environ. Safety, 66: 244-251.
16. Revankar, P.R. and S.K. Shyama, 2009. Genotoxic effects of monocrotophos, an organophosphorous pesticide, on an estuarine bivalve, Meretrix ovum. Food Chem. Toxicol., 47: 1618-1623.
17. Jokanović, M., M. Kosanović, D. Brkić and P. Vukomanović, 2011. Organophosphate induced delayed polyneuropathy in man: An overview. Clin. Neurol. Neurosurg., 113: 7-10.
18. Gupta, R.C., 1994. Carbofuran toxicity. J. Toxicol. Environ. Health, 43: 383-418.
19. Moore, P.C.F., 2009. Children and Pollution: Why Scientists Disagree. 1st Edn., Oxford University Press, Oxford, UK, Pages: 376.
20. Mearns, J., J. Dunn and P.R. Lees-Haley, 1994. Psychological effects of organophosphate pesticides: A review and call for research by psychologists. J. Clin. Psychol., 50: 286-294.
21. Kumar, S.V., M. Fareedullah, Y. Sudhakar, B. Venkateswarlu and E.A. Kumar, 2010. Current review on organophosphorus poisoning. Arch. Applied Sci. Res., 2: 199-215.
22. Nasreddine, L. and D. Parent-Massin, 2002. Food contamination by metals and pesticides in the European union. Should we worry? Toxicol. Lett., 127: 29-41.
23. El-Saeid, M.H., 2003. Pesticide residues in canned foods, fruits and vegetables: The application of supercritical fluid extraction and chromatographic techniques in the analysis. Scient. World J., 3: 1314-1326.
24. EL-Saeid, M.H. and K. Haseeb, 2010. Analysis of Pesticides in Food Samples by Supercritical Fluid Chromatography. Chap. 5. Handbook of Pesticides: Taylor and Frances Group, CRC Press, USA., pp: 93-113.