Gas Chromatography with Mass Spectrometric (GC-MS) Analysis of Organophosphate Pesticide Residues in Selected Vegetables at Tirunelveli District Tamil Nadu, India

M. Chidambara Priya and P. Vanitha Pappa

Research Journal of Agricultural Sciences
An International Journal

P- ISSN: 0976-1675
E- ISSN: 2249-4538

Volume: 13
Issue: 04

Res. Jr. of Agril. Sci. (2022) 13: 1041–1045

CARAS

Res. Jr. of Agril. Sci. (Jul-Aug 2022)
13(4): 1041–1045

www.rjas.org

ISSN: 0976-1675 (P)
ISSN: 2249-4538 (E)

Full Length Research Article

Gas Chromatography with Mass Spectrometric (GC-MS) Analysis of Organophosphate Pesticide Residues in Selected Vegetables at Tirunelveli District Tamil Nadu, India

M. Chidambara Priya*[1] and P. Vanitha Pappa[2]

Received: 23 May 2022 | Revised accepted: 13 Jul 2022 | Published online: 16 July 2022

ABSTRACT

General agricultural use of pesticides carries with it potential hazards to man and directly by exposure to toxic residues in food and indirectly to the environment. This study was carried out to identify the bioaccumulation of organophosphate pesticide residues in two commonly consumed vegetables (*Abelmoschus esculentus* and *Spinacia oleracea*) collected from random local markets located in the Tirunelveli region, Tamil Nadu (India). The samples were randomly collected from different markets and analyzed by Gas Chromatography-Mass Spectrometry (GC-MS) method for the detection of organophosphate pesticides (OP). Organophosphate pesticide is widely used among farmers for crop protection. The result of the study revealed that collected samples of *Abelmoschus esculentus* and *Spinacia oleracea* were contaminated with some organophosphate substances. There is an urgent need to develop pesticide monitoring and surveillance systems at the farmer's level, educating farmers and promoting the use of greener pesticides to mitigate the health effects of pesticides.

Key words: Bioaccumulation, Environmental pollution, Health hazards, Pesticide residues, Vegetables

Global population growth has increased the demand for food almost everywhere. Food production must be raised to meet increasing consumer demand. There is strong evidence that many countries of the world utilize chemical pesticides extensively to increase agricultural productivity to fulfill a growing demand for food. Globally, about 900 chemical pesticides are used in food, crops, and the treatment of soil for both legal and illegal purposes [1]. The Indian economy is based primarily on agriculture; more than 60-70% of its population depends on agriculture. The population growth in India, which leads to an increase in food grain production, as well as crop loss minimization, is growing [2]. With 109 million metric tons of vegetables produced, India ranks second in production after China, generating 13.4% of global production. Researchers have determined that about 50-70% of vegetables in the country are contaminated with insecticide residue [3]. The most widely used and important group belongs to the organophosphorus group [4]. It is known that many organophosphates are potent nerve agents, and they work by inhibiting acetylcholinesterase (AChE) in nerve cells. Inhalation, ingestion, and dermal absorption are all routes by which organophosphorus may be absorbed. It has long been observed that their chronic effects are more severe than their acute effects. The brain develops through the release of neurotransmitters such as acetylcholine (which can be damaged by organophosphate pesticides), and some organophosphate pesticides are neurotoxic, even at low concentrations [5]. Traditionally, vegetable production has largely been carried out on a commercial basis in the villages. Some farmers have garden plots in their homes. The market serves as the only source of vegetables for urban residents. Most commonly cultivated vegetables include *Abelmoschus esculentus* and *Spinacia oleracea* because they provide farmers with greater returns on their investments. Many farmers apply excessive amounts of pesticides to increase yields and quality as well as to combat insects and pests. Many researchers have reported that this causes the contamination of vegetables with pesticide residues [6-9]. Some developed countries have established a Maximum Residue Limit (MRL) of substances that can be present in food and cosmetics. Among the food products, food units should not exceed the acceptable daily intake (ADI) and potential daily intake (PDI). Pesticides are usually sprayed 1-2 days after vegetables are harvested. Pesticide residue levels should therefore be determined in crops such as okra and spinach. Nowadays, in developed countries, pesticide residue analysis focuses more on subtle issues, such as testing the environment for very low pesticide concentrations.

As a result of all these factors, the purpose of this study is to evaluate the presence of persistent organic pollutants

* **M. Chidambara Priya**
sidhusri19972013@gmail.com

[1-2] Department of Zoology, Rani Anna Government College for Women (Affiliated under Manonmaniam Sundaranar University), Abisekapatti, Tirunelveli - 627 008, Tamil Nadu, India

(PoPs) as well as determine whether harmful residues of organophosphate pesticides are present in vegetables such as *Abelmoschus esculentus* and *Spinacia oleracea*. The outcome of the investigation will provide the risk associated with exposure to pesticides in *Abelmoschus esculentus* and *Spinacia oleracea* which will be helpful for the scientific communities in the evaluation of pesticide residue propose of safety period and make safe production to the end-user.

MATERIALS AND METHODS

Listed below are the analytical grade chemicals used for each experiment.

1. Acetonitrile, 2. Acetone, 3. Buffer solution (phosphate), 4. Sodium chloride 5. Sodium sulfate, 6. Toluene. The samples for the study were collected from different vendors at the Tirunelveli vegetable market, Tamil Nadu (India)..All the samples were mixed well and made the laboratory samples of 1kg were in clean polythene bags to avoid contamination same procedure for each vegetable. 1.0kg of each of the following two vegetables 1. Okra (*Abelmoschus esculentus*) and 2. Spinach (*Spinacia oleracea)* was obtained from different vendors to prepare composite samples for the detection of pesticides if any were present.

As reported by [10] following acetonitrile extraction methods, for determining organophosphate pesticide levels in vegetables. Using 20g of each vegetable, we added 20mL of distilled water to it. 50mL of acetonitrile was then added to the mixture after standing for 15 minutes, and the mixture was then homogenized with a pestle and mortar. After filtering by suction, the sample was collected. The sample was homogenized and suction filtered once again after 20 mL of acetonitrile was added to the remaining residue on the filter paper. By adding acetonitrile to both filtrates, the volume of the resulting solution was elevated to 100mL. After this was done, 20mL of the resulting solution was added to 10 g of NaCl, followed by 20mL of 0.5 mL/L of phosphate buffer (pH 7.0) and shaken. The aqueous layer was removed by allowing the solution to stand until it became clear. Anhydrous sodium sulfate was used to dry the organic layer at 40˚c and filter it. A ratio of 3:1 between toluene and acetonitrile was then made by adding 2mL from the sample.

To analyze the samples, the following conditions were followed: Gas Chromatography-Mass Spectrometry (GC/MS model GC 2030, Shimadzu).

As a result of the extraction step, acetonitrile/toluene (3:1) was conditioned on a mini-column made of graphite carbon and aminopropylilianized silica gel layer (500 mg/500 mg). As the column eluted (20mL of acetonitrile/3:1), the entire volume of effluent was collected. We concentrated the effluent to 1mL or less at a temperature of 40˚c or lower. The solution can be dried using 5mL of acetone. The residue was made up to 1mL by adding acetone: n-hexane (1:1) mixture.

Finally, GC/MS was used to analyze and determine the level of pesticides present in the extracts. A time-based analysis of the analytical standards was also done to identify the pesticide. GCMS provided information such as identification, quantification, and analysis of the compound. Furthermore, IUPAC name, molecular formula, molecular structure, molecular weight, and fragment pattern were detected.

RESULTS AND DISCUSSION

In this study, (Fig 1) shows the acetonitrile extraction of tested samples and instruments to analyze the status of organophosphate pesticide residues in *Abelmoschus esculentus* and *Spinacia oleracea* was analyzed using Gas Chromatography-Mass Spectrophotometry (GC-MS) and their results were evaluated.

Fig 1 Acetonitrile extraction of *Abelmoschus esculentus* and *Spinacia oleracea* for GC-MS

To conduct this study, GC-MS was used to detect organophosphate pesticide residues on *Abelmoschus esculentus*(okra). Various organic compounds were found in the vegetable samples analyzed by the chromatograph. The following Figure2 illustrates peaks in the *Abelmoschus esculentus* sample due to different organic compounds. The major organic compounds present in *Abelmoschus esculentus* samples according to their peak were Phosphoric acid, dimethyl 1-methyl-3-(methylamino)-3-oxo-1-propenyl ester, (Z)-, Monocrotophos, Phosphoric acid, dimethyl pentyl ester, 3-oxo-18-nor-ent-ros-4-ene-15.beta.,16-acetonide, 2-(Heptyloxycarbonyl) benzoic acid, Diethyl Phthalate.

Monocrotophos is the only organophosphate compound found in the *Abelmoschus esculentus* sample (Table 1, Fig 2-3) revealed that an organophosphate compound was present in the *Abelmoschus esculentus* extract (monocrotophos). The Monocrotophos insecticide has been found effective for controlling insect pests on different crops [11].

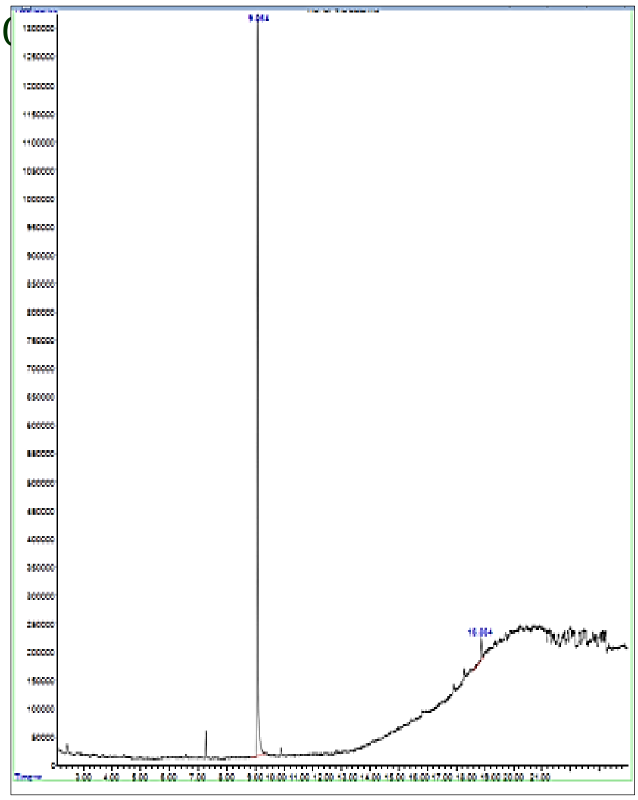
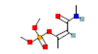
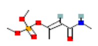
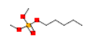
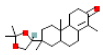
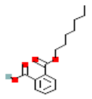
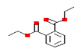


Fig 2 Chromatogram of *Abelmoschus esculentus* in GC-MS

Table 1 List of detected organic compounds with organophosphate pesticides in *Abelmoschus esculentus*

Name of the compound	Molecular weight	Molecular 2D structure	Molecular formula	IUPAC name	Organophosphate compound
Phosphoric acid, dimethyl 1-methyl-3-(methylamino)-3-oxo-1-propenyl ester, (Z)-	223.16 g/mol		C7H14NO5P	dimethyl [(Z)-4-(methylamino)-4-oxobut-2-en-2-yl] phosphate	-
Monocrotophos	223.16 g/mol		C7H14NO5P	dimethyl [(*E*)-4-(methylamino)-4-oxobut-2-en-2-yl] phosphate	√
Phosphoric acid, dimethyl pentyl ester	196.18 g/mol		C7H17O4P	dimethyl pentyl phosphate	-
3-oxo-18-nor-ent-ros-4-ene-15.beta.,16-acetonide	346.5 g/mol		C22H34O3	7-(2,2-dimethyl-1,3-dioxolan-4-yl)-1,4b,7-trimethyl-4,4a,5,6,8,8a,9,10-octahydro-3H-phenanthren-2-one	-
2-(Heptyloxycarbonyl) benzoic acid	264.32 g/mol		C15H20O4	2-heptoxycarbonylbenzoic acid	-
Diethyl Phthalate	222.24 g/mol		C12H14O4	diethyl benzene-1,2-dicarboxylate	-

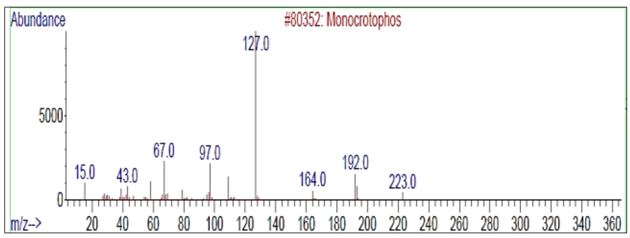


Fig 3 Examination of the gas chromatography of the *Abelmoschus esculentus* extract

And also carry in the same study, GC-MS was used to identify organophosphate pesticide residues on *Spinacia oleracea* (spinach). (Fig 4) showing the peaks detected in the *Spinacia oleracea* sample due to different organic compounds has been provided. As determined by their peak concentrations in *Spinacia oleracea* samples, the most prevalent organic compounds were Cyclohexanone, 2-cyclohexylidene, 2-(1Cyclohexenyl) cyclohexanone, Phosphoric acid, dimethyl 1-methyl-3-(methylamino)-3-oxo-1-propenyl ester, (Z)-, Monocrotophos, Bis (2-ethylhexyl) phthalate, Diisooctyl phthalate,2-(Heptyloxycarbonyl) benzoic acid. Among the seven compounds, Monocrotophos and Bis (2-ethylhexyl) phthalate are documented as organophosphate compounds in *Spinacia oleracea* samples (Table 2, Fig 5) illustrates the presence of (Bis(2-ethylhexyl) phthalate) which is used as a semivolatile pesticide. (Fig 6) displays another pesticide is known as Monocrotophos.

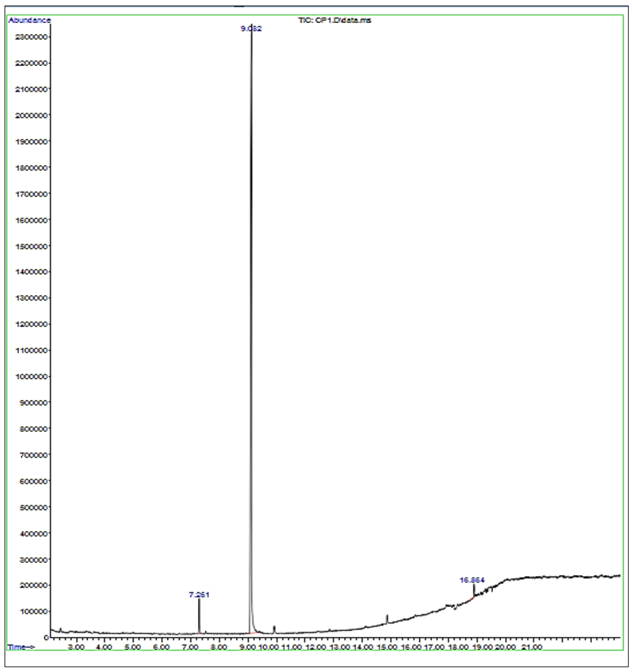
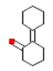
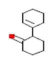
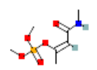
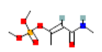
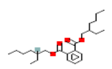
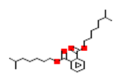
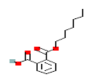


Fig 4 Chromatogram of *Spinacia oleracea* in GCMS

Table 2 List of detected organic compounds with organophosphate pesticides in *Spinacia oleracea*

Name of the compound	Molecular weight	Molecular 2D structure	Molecular formula	IUPAC name	Organophosphate compound
Cyclohexanone, 2-cyclohexylidene-	178.27 g/mol		C12H18O	2-cyclohexylidenecyclohexan-1-one	-
2-(1-Cyclohexenyl) cyclohexanone	178.27 g/mol		C12H18O	2-(cyclohexen-1-yl)cyclohexan-1-one	-
Phosphoric acid, dimethyl 1-methyl-3-(methylamino)-3-oxo-1-propenyl ester, (Z)-	223.16 g/mol		C7H14NO5P	dimethyl [(Z)-4-(methylamino)-4-oxobut-2-en-2-yl] phosphate	-
Monocrotophos	223.16 g/mol		C7H14NO5P	dimethyl [(E)-4-(methylamino)-4-oxobut-2-en-2-yl] phosphate	√
Bis(2-ethylhexyl) phthalate	390.6 g/mol		C24H38O4	bis(2-ethylhexyl) benzene-1,2-dicarboxylate	√
Diisooctyl phthalate	390.6g/ mol		C24H38O4	bis(6-methylheptyl) benzene-1,2-dicarboxylate	-
2-(Heptyloxycarbonyl) benzoic acid	264.32g /mol		C15H20O4	2-heptoxycarbonylbenzoic acid	-

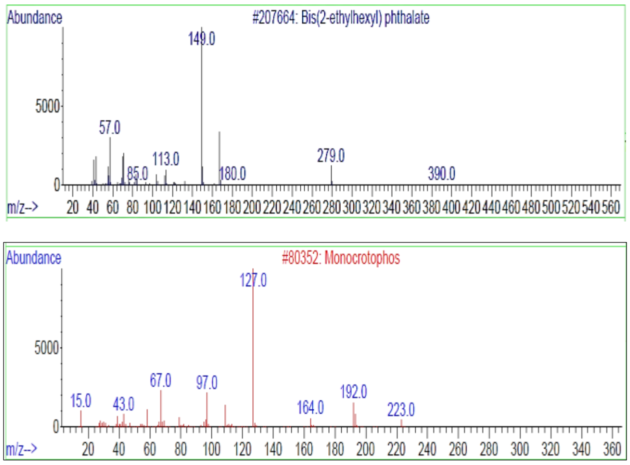


Fig 5-6 Examination of the gas chromatography of the *Spinacia oleracea* extract

Study findings of the chromatogram of *Abelmoschus esculentus* (okra) and *Spinacia oleracea*(spinach) Samples show proof of efficacy *Abelmoschus esculentus* contains six (6) organic compounds of which one (1) compound Monocrotophos was marked as Organophosphate compound. In the *Spinacia oleracea* sample, there was evidence of seven (7) organic compounds of which two (2) compounds Bis(2-ethylhexyl) phthalate and Organophosphates were marked as Monocrotophos. As a result, Monocrotophos is highly toxic through all routes of exposure. Symptoms of exposure, regardless of route, may include pallor, abdominal cramps, vision changes, eye pain, dizziness, stomach cramps, and nausea. A detailed set of guidelines for classifying pesticides by hazard and the recommended WHO edition from 2004 are available [12]. In terms of WHO classification, monocrotophos is classified as class 1b (i.e.) as a highly toxic hazardous pesticide. An irregular heartbeat, involuntary defecation, and affect the central nervous system are also possible in severe cases. It is possible to die from respiratory failure. It has been shown that pesticide exposure can reduce both women's and men's fertility [13-14].

Abelmoschus esculentus(okra) is an extremely fast-growing vegetable and needs to be harvested every three days, the longer residual life after consumption could harm human health. Because Monocrotophos is most effective on okra crops, it should only be applied for this purpose. Pesticide applications are safe if the okra is harvested after the 15th, 17th, and 19th days following application [15]. Further, good agricultural practices help minimize pesticide residues in food.

A similar result was also reported by [16] found In Indian spinach also 14 compounds were detected as organic of which 3 were chlorinated and they were pentane, 3-(2,2-dichloro-3-methylcyclopropyl), 1-chloro heptacosane, and nomanoyl chloride-SS. According to [17], spinach was contaminated with permethrin. Although all concentrations were below the respective action limits set by the EPA, all were still below the levels of concern. Organic-labeled samples had significantly less pesticide content than non-organic samples, but some pesticides were still detected. Permethrin was detected even in samples from the laboratory with only 1ppb. Pumpkin leaves, spinach leaves, and sorrel leaves that were found to contain residues of pesticides [18]. This study confirmed that most growers using agrochemicals were responsible for residue levels in vegetables that exceeded the maximum residue levels and posed a health issue since these popular or common vegetables are regularly consumed by the population. And also [19], observed that Nursery and primary school children were intake Bis (2-ethylhexyl) phthalate (DEHP) via school lunches. The lowest concentration of DEHP was found in spinach. The phthalate esters in soils contaminated with phthalate esters appear to inhibit the growth of spinach and pea seedlings [20]. These concentrations are similar to what may be found in the environment, for example in landfills and other areas where plastic products may leach esters.

According to [21-22] this Bis(2-ethylhexyl) phthalate is rapidly absorbed from the gut, primarily as mono(2-ethylhexyl) phthalate. Added to pesticides, it functions as a semi-volatile mixing agent. It has been reported to have liver and kidney effects [23-24]. High doses have also been reported to cause reduced hemoglobin and packed cell volume [25-26]. The report [27] found that pesticide residues can persist in plant tissues or are visible on their surfaces even after they are harvested. Food chains at the beginning can introduce pesticides to vegetables. Fresh vegetables are one of the most important factors determining the amount of pesticide residue in them [28] and the number of pesticide residues differs from part to part of plants [29]. To ensure maximum protection against pesticide residues being exceeded in produce that reaches the market, it is of utmost importance to conduct continuous monitoring [30]. The vast majority of research suggests washing and cooking do reduce some pesticide residues on plants, however food preparation, and in-home environments can concentrate levels [31]. Pesticide levels in vegetables depend on the biodegradation of pesticides in farmland.

CONCLUSION

This study investigated the contamination of pesticide residues in selected vegetables from the Tirunelveli market. The results indicated that samples were contaminated with pesticide residues. From a public health perspective, pesticide residues pose a potential health risk to consumers. Therefore, to reduce the risk, sensitization of farmers to better pesticide safety practices and the need for continuous pesticide residue monitoring is highly recommended.

Acknowledgment

I wish to acknowledge my supervisor, Dr. P. Vanitha Pappa, who guided me throughout this research work, my friends, and the help provided by the technical and support staff in the zoology department of Rani Anna Government College for Women, Tirunelveli, Tamil Nadu.

LITERATURE CITED

1. Mills MS, Thurman EM. 1994. Preferential Dealkylation Reactions of s-Triazine Herbicides in the Unsaturated Zone. *Environmental Science and Technology* 28(4): 600–605. https://doi.org/10.1021/es00053a011
2. AK P. 2017. A review on microbial degradation of organophosphorous pesticide: Methyl parathion. *Austin Journal of Biotechnology and Bioengineering* 4(1): 1–6. https://doi.org/10.26420/austinjbiotechnolbioeng.2017.1074.
3. Hanak E, Boutrif E, Fabre P, Pineiro M, Karanth NGK. 2002. *Food Safety Management in Developing Countries*. *December* 2000: 11–13.
4. Yamarthi A, Pandey R, Aparna Y. 2019. Biodegradation of Organo phosphorous pesticide dichlorvos by bacteria isolated from field sample "Screening of Microorganisms for quorum sensing Inactivation" View project Biodegradation of Organo

phosphorous Pesticide Dichlorvos by bacteria isolated from field sample. *International Journal of Scientific Research in Biological Sciences* 6(1): 41-45.
5. Cunha SC, Lehotay SJ, Mastovska K, Fernandes JO, Oliveira MBPP. 2010. Sample preparation approaches for the analysis of pesticide residues in olives and olive oils. *Olives and Olive Oil in Health and Disease Prevention* 70: 653–666. https://doi.org/10.1016/B978-0-12-374420-3.00070-X
6. Madan VK, Kumari B, Singh RV, Kumar R, Kathpal TS. 1996. Monitoring of pesticides from farmgate samples of vegetables in Haryana. *Pestic. Res. Journal* 8: 56–60.
7. Kumari B, Kumar R, Madan VK, Singh R, Singh J, Kathpal TS. 2003. Magnitude of pesticidal contamination in winter vegetables from Hisar, Haryana. *Environmental Monitoring and Assessment* 87(3): 311-318. https://doi.org/10.1023/a:1024869505573
8. Kumari B, Madan VK, Kumar R, Kathpal TS. 2002. Monitoring of seasonal vegetables for pesticide residues. *Environmental Monitoring and Assessment* 74: 263-270.
9. Chandra S, Kumar M, Mahindrakar AN, Shinde LP. 2014. Persistence Pattern of Chlorpyrifos, Cypermethirn and Monocrotophos in Okra. *International Journal of Advanced Research* 2(12): 738-743.
10. Hussain Z, Siddique S. 2010. Determination of pesticides in fruits and vegetables using acetonitrile extraction and GC/MS technique. *Journal of Scientific Research* 40(2): 19-29.
11. Nath R, Nath A, Kaur S, Deep G. 2019. Residual study of monocrotophos on okra (*Abelmoschus esculentus* L. Moench). *Journal of Entomology and Zoology Studies* 7(2): 492–494.
12. Forget G. 1994. Balancing the need for pesticides with the risk to human health. *Impact of Pesticide Use on Health in Developing Countries* 2-16. http://scholar.google.com/scholar.
13. Abell A, Juul S, Bonde JP. 2000. Time to pregnancy among female greenhouse workers. *Scandinavian Journal of Work, Environment and Health* 26(2): 131–136. https://doi.org/10.5271/sjweh.522
14. Oliva A, Spira A, Multigner L. 2001. Contribution of environmental factors to the risk of male infertility. *Human Reproduction* 16(8): 1768-1776. https://doi.org/10.1093/humrep/16.8.1768
15. Chandra S, Kumar M, Mahindrakar AN, Shinde LP. 2015. Effects of household processing on reduction of pesticide residues in brinjal and okra. *International Journal of Advances in Pharmacy Biology and Chemistry* 4(1): 98-102.
16. Khan M. 2011. Chlorinated pesticide residue status in tomato, potato and carrot. *Journal of Experimental Sciences* 2: 1-5.
17. AOAC. 2007. Official Method 2007.01: pesticide residues in foods by acetonitrile extraction and partitioning with magnesium sulfate. *Journal of AOAC International* 90(2): 17-26. http://lib3.dss.go.th/fulltext/E_content/1060-3271/2007v90n2.pdf
18. Bempah CK, Buah-Kwofie A, Denutsui D, Asomaning J, Tutu AO. 2011. Monitoring of Pesticide Residues in Fruits and Vegetables and Related Health Risk Assessment in Kumasi Metropolis, Ghana.
19. Cirillo T, Fasano E, Castaldi E, Montuori P, Amodio R. 2011. Children's Exposure to Di(2-ethylhexyl)phthalate and Dibutylphthalate Plasticizers from School Meals. *Journal of Agricultural and Food Chemistry* 59: 10532-10538. https://doi.org/10.1021/jf2020446
20. Herring R, Bering CL. 1988. Effects of phthalate esters on plant seedlings and reversal by a soil microorganism. *In*: *Bulletin of Environmental Contamination and Toxicology* 40(4): 626-632. https://doi.org/10.1007/BF01688390
21. Pollack GM, Li RC, Ermer JC, Shen DD. 1985. Effects of route of administration and repetitive dosing on the disposition kinetics of di(2-ethylhexyl) phthalate and its mono-de-esterified metabolite in rats. *Toxicology and Applied Pharmacology 79*(2): 246–256. https://doi.org/10.1016/0041-008x(85)90346-1
22. Teirlynck OA, Belpaire F. 1985. Disposition of orally administered di-(2-ethylhexyl) phthalate and mono-(2-ethylhexyl) phthalate in the rat. *Archives of Toxicology* 57(4): 226-230. https://doi.org/10.1007/BF00324782
23. Carpenter CP, Weil CS, Smyth HFJ. 1953. Chronic oral toxicity of di-(2-ethylhexyl) phthalate of rats, guinea pigs, and dogs. *A.M.A. Archives of Industrial Hygiene and Occupational Medicine* 8(3): 219-226.
24. 2000T8QN.pdf. (n.d.).
25. Kluwe WM, Haseman JK, Douglas JF, Huff JE. 1982. The carcinogenicity of dietary di(2-ethylhexyl) phthalate (DEHP) in Fischer 344 rats and B6C3F1 mice. *Journal of Toxicology and Environmental Health* 10(4/5): 797-815. https://doi.org/10.1080/15287398209530296
26. Gray TJ, Butterworth KR, Gaunt IF, Grasso GP, Gangolli SD. 1977. Short-term toxicity study of di-(2-ethylhexyl) phthalate in rats. *Food and Cosmetics Toxicology* 15(5); 389-399. https://doi.org/10.1016/s0015-6264(77)80003-5
27. Wilkowska AM, Biziuk MK. 2011. Determination of pesticide residues in food matrices using the QuEChERS methodology. *Food Chemistry* 125: 803-812.
28. Risebrough RW, Menzel DB, Martin DJJ, Olcott HS. 1967. DDT residues in Pacific sea birds: a persistent insecticide in marine food chains. *Nature* 216(5115): 589-590. https://doi.org/10.1038/216589a0
29. Elzorgani GA, Abdulla AM, Ali ME. 1979. Residues of organochlorine insecticides in fishes in Lake Nubia. *Bulletin of Environmental Contamination and Toxicology* 22(1/2): 44-48. https://doi.org/10.1007/BF02026905
30. Li W, Tai L, Liu J, Gai Z, Ding G. 2014. Monitoring of pesticide residues levels in fresh vegetable form Heibei Province, North China. *Environmental Monitoring and Assessment* 186(10): 6341-6349. https://doi.org/10.1007/s10661-014-3858-7
31. Keikotlhaile BM, Spanoghe P, Steurbaut W. 2010. Effects of food processing on pesticide residues in fruits and vegetables: a meta-analysis approach. *Food and Chemical Toxicology: An International Journal Published for the British Industrial Biological Research Association* 48(1): 1-6. https://doi.org/10.1016/j.fct.2009.10.031