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Investigation of Organophosphorus and Carbamate Pesticide Residues in Tobacco Produced and Imported in Iran in 1400

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ABSTRACT

Background: The aim of this study is to investigate some pesticides, including organic phosphorus compounds and carbamates, on tobacco leaves, which are commonly used in agricultural activities to increase crop production around the world. Humans are exposed to pesticides through residues left in fields, which have harmful effects on the body.

Methods: The study collected a total of ten samples of tobacco leaves, five from different cultivation areas in Iran and five from five different countries as imported samples. The collection was done three times to ensure that the results were reliable and consistent. To determine the residual concentration of organophosphorus and carbamate pesticides, we collected 210 samples using the QuEChER method to prepare the samples. The residual levels of 26 pesticides were measured using the GC-ECD and UHPLC-MS/MS methods.

Results: Most samples had residual levels of pesticides below the detection limit. However, in Iranian samples, the highest average concentrations were found for Thiodicarb (µg/kg 525.2), Chlorpyrifos (µg/kg 499), Dichlorvos (µg/kg 260.3), Penconazole (µg/kg 208), Thiophanate-methyl pesticides (µg/kg 168.5). For imported samples, Thiodicarb (525.2 µg/kg) and Dichlorvos pesticides (260.3 µg/kg) had the highest average residual concentration.

Conclusion: Despite severe restrictions on pesticide use in the sampled specimens, residual amounts of these chemicals persist, which can contribute to the adverse health effects of smoking. Therefore, more effective monitoring and surveillance measures are necessary.

1. Introduction

Population growth and food shortage have always been serious issues for humans. Consequently, humans have used various methods, such as chemical pest control to increase the productivity of agricultural products [1]. A pesticide is any substance or mixture of substances used to kill, repel or reduce any pest [2]. Pests include insects, rodents, molluscs, other animals such as birds, weeds, fungi, or microorganisms such as bacteria and viruses [3]. According to the Food and Agriculture Organization (2019), Asia consumed the most pesticides (2.1 million tons) and Oceania consumed the least (0.1 million tons) [4]. The entry of pesticides into the human body can threaten health. Pesticide exposure can have a detrimental effect on non-target organisms, including humans. Depending on the amount of the substance (the dose) and the toxicity of the chemical, the severity of the hazard varies [5]. Pesticide exposure causes genetic damage, cancer, non-Hodgkin's lymphoma, neuropsychiatric, immune system disorders, hormonal disorders, and human infertility [6]. Pesticides can decompose in two ways after they are released into the environment. Sunlight, water,

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other chemicals, or microorganisms such as bacteria may decompose them. As a result, the decomposition process, less harmful products are usually formed. However, in some cases, toxic byproducts can be formed. Furthermore, some pesticides are resistant to degradation and remain unchanged in the environment for a long period. These pesticides, known as persistent pesticides, can travel longer distances, accumulate in living organisms, and pose a risk to human health and the environment [7]. Three important types of pesticides are organophosphates, organochlorines, and carbamates [8]. One of the most important groups of pesticides is organophosphates which are derived from phosphoric acid. They typically contain a central phosphorus atom and numerous side chains [7]. In addition to being insecticides, these compounds are also herbicides and fungicides [7, 9]. Compared to other pesticides, organophosphates have a milder effect and a shorter shelf life in nature. They are often used as alternatives to organochlorine pesticides, which are persistent and harmful to the environment [3, 7]. One of the most common effects of organophosphorus pesticides is the inactivation of Acetylcholinesterase (AChE), an enzyme required for the proper functioning of the nervous system in humans, vertebrates, and insects [7]. Carbamates are another type of pesticide that is relatively polar, highly soluble in water, and chemically reactive. Carbamates tend to hydrolyze easily, resulting in a low persistence level in soil and water. Similar to organophosphates, carbamates have an acetylcholinesterase inhibitory effect [10]. In contrast to organophosphate poisoning, carbamate poisoning occurs more rapidly because the inhibition of AChE by carbamates is reversible. Additionally, carbamates are metabolized more quickly [11]. Tobacco is a plant primarily cultivated for using its leaves, which contain a diverse array of chemical components. These components include cellulose products, starch, protein, sugar, alkaloids, hydrocarbons, phenols, fatty acids, isoprenoids, sterols, and minerals [12]. Tobacco is a plant belonging to the *Solanaceae* family and *Nicotiana* genus, which comprises 60 species divided into three subgenera: Rustica, Petunioides, and Tabacum. The majority of commercial tobacco belongs to the subgenus *Tabacum* [12, 13]. Originally native to the American continent, it is now grown worldwide due to its significant commercial value [14, 15]. Tobacco cultivation occupies over 5 million hectares around the world, with an annual production exceeding 7 million tons [16]. The largest tobacco producers in the world are China, Brazil, and India, with a combined production of 3.2 million tons [17]. According to a recent report published by Iran′s Ministry of Agricultural Jihad, 9,563 hectares of tobacco is cultivated in Iran and produced 19,413 tons of tobacco in the 2017 crop year [18]. Tobacco is commonly used as a dried and fermented product [19]. However, tobacco plants are vulnerable to a range of pests [12]. Since tobacco products are susceptible to various types of fungi, insects, and viruses from the seed stage to the storage stage in warehouses [20], farmers and tobacco companies often use pesticides to improve the quality of the product. Tobacco plants absorb some of the chemicals, and the residues may remain in the final product [12]. In addition, tobacco leaves have a higher surface-to-weight ratio than other crops, and excessive pesticide use increases the probability of pesticide residue accumulation [21]. Tobacco can be consumed through non-smoking (in snuff) or smoking (in cigarettes, pipes, etc.) methods [22]. Studies show that smoking is the most common form of tobacco use worldwide [23]. However, it should be noted that pesticide residues can remain after harvesting and processing tobacco [20]. These residues can not only enter the body of smokers directly but also expose non-smokers to tobacco pesticides through inhaled cigarette smoke [24]. In most cases, cigarettes contain a blend of different types of tobacco [12]. Despite the well-documented health risks associated with tobacco use, little is known about the potential effects of pesticides on tobacco and tobacco products. There is currently a dearth of information on the levels of pesticide residues in tobacco samples consumed in Iran. The present study aimed to address this knowledge gap by determining the residual amount of organophosphorus and carbamate pesticides in tobacco samples. The findings of this study have the potential to shed light on the state of the tobacco used and any harmful effects on human health associated with pesticide residues [25].

2. Materials and Methods

Chemical materials were utilized in the preparation and measurement of the phosphorus and carbamate pesticides in tobacco samples. Acetonitrile (CH_3CN) and primary secondary amine (PSA) were obtained from CARLO ERBA Co. (CARLO ERBA, Milano, Italy) and Agilent Technologies Co. (Agilent Technologies, Santa Clara, the USA), respectively. Other materials required for the study, including graphitized carbon black, sodium chloride (NaCl), anhydrous magnesium sulfate (Mg SO_4), and sodium acetate ($C_2H_3NaO_2$), were obtained from Merck Co. (Merk, Darmstadt, Germany).

2.1 Data collection

In this study, we selected five varieties of Iranian and imported tobacco leaves, which were sampled three times from seven different parts of the mass of tobacco leaves, including the sides and center of an imaginary cube. The samples were mixed with equal weights, resulting in a total of 30 samples that were transported to the laboratory in closed containers for analysis. The average concentrations of pesticides were calculated and recorded for each type of tobacco at each time point. A limit of detection (LOD) of 0.5 was considered for values below the LOD.

2.2 Sample preparation

After grinding, the samples were homogenized. 5 g of the sample was added to 15 mL of deionized water and stirred using an incubator shaker at room temperature for 1 h. subsequently, 15 mL of acetonitrile containing 1% CH_3COOH was incorporated, and the resultant mixture was firmly shaken by hand for 1 min. Mg SO_4 (4 g), NaCl (1 g), and $C_2H_3NaO_2$ (1.5 g) were added to the solution, and the solution was mixed with a vortex machine for 1 min. The solution was then centrifuged at 3450 rpm for 5 min, yielding four distinct layers of supernatant, solid, water, and additional extractive salt from top to bottom. To remove any residual impurities, 5 mL of the supernatant was mixed with 250 mg of PSA absorbent, 1.5 g of Mg SO_4, and 80 mg of GCB, followed by stirring with a vortex machine for 1 min. the resulting mixture was then centrifuged at 3450 rpm for 3 min, yielding two distinct layers. Finally, 1 mL of the obtained supernatant was filtered through a syringe head filter (PTFE) and injected into an analytical instrument.

2.3 Gas Chromatography Device

In the present study, a Shimadzu-2010 gas chromatography system equipped with an HP-5MS column having a diameter of 0.32 mm and a stationary phase layer thickness of 0.25 mm was used, complemented by an Electron Capture Detection. Also, the carrier gas was selected as nitrogen, with a flow rate of 2.91 mL/min. The gas was subjected to split injection mode at a ratio of 5:1. The injection chamber was maintained at a temperature of 260 °C, while the detector was operated at 280 °C. The temperature programming of the ECD-GC instrument involved a hold time of 1 min at 100°C, followed by an increase in temperature to 200°C at a gradient of 10°C per minute, and the system was held at 200 °C for 10 min. The overall duration of the sample analysis was 21 min. The compounds under investigation using this instrument were Chlorpyrifos, Bromophos.

2.4 Liquid Chromatography Device

The liquid chromatograph (Agilent 6410) was equipped with a triple quadrupole mass spectrometer consisting of an SL 1200 series binary pump with degasser and an Agilen 1200 series autosampler. Liquid chromatography was performed on an Agilent-RR Zorbax Eclipse XDB-C18 column with a particle size of 1.8 µm, a diameter of 4.6 mm, and a length of 50 mm. The column temperature was maintained at 25°C, while the active phase consisted of acetonitrile (A) and an aqueous solution containing 0.1% formic acid (B). The washing program employed was as follows: 10% of phase A and 90% of phase B at min 0, 100% of phase A and 0 % of phase B at min 13, and 10% of phase A and 90% of phase B at min 20. The injection volume was 10 µL and the temperature of the automatic sampling tray was 25 °C. The system had a maximum pressure of 400 bar, a flow rate of 0.6 ml/min, and a running time of 20 min. The mass spectrometer used an Agilent 6410 Triple Quadrupole system (Agilent 6410 Triple Quadrupole, the USA). The mass spectrometry parameters included a gas temperature of 350 °C, a gas flow of 6 L/min, a nebulizer pressure of 10^{-5} psi, a capillary voltage of 4000 V, a dwell time of 70 ms, and an ESI-MS interface in positive ion mode. The pesticides analyzed using this device included Buprofezin, Carbaryl, Diazinon, Dichlorvos, Difenoconazole, Dimethoate, Fenitrothion, Iprodione, Kresoxim methyl, Malathion, Metribuzin, Penconazole, Phosalone, Pirimicarb, Pirimiphos-methyl, Propamocarb, Propargite, Propiconazole, Pymetrozine, Spiromesifen, Spirotetramat, Tebuconazole, Thiodicarb, and Thiophanate-methyl.

2.5 Validation

The precision and accuracy of analysis methods were calculated using the relative standard deviation (RSD), recovery rate, limit of detection (LOD), and limit of quantitation (LOQ).

3. Results and Discussion

Experiments conducted by GC-ECD device showed that Chlorpyrifos pesticide had the RSD of 7% and 12% for two concentrations of 10 µg/kg and 50 µg/kg, while Bromophos pesticide had RSD of 5% and 4%. The RSD of the analyzed pesticides by the LC-MS/MS device for Diazinon, Dichloros, Dimethoate, Fenitrothion, Malathion, Fuzalon, Primiphos methyl, Carbaryl, Primicarb, Propamocarb, Thiodicarb, Thiophanate methyl, Iprodione, Cresoxim methyl, P. metrozine, Spiromsifen, Spirotetramat, Penconazole, Metribozin, Propargit, Propiconazole, Tebuconazole, Difenoconazole, and Buprofezin were less than 10%. GC-ECD measurements revealed that the LOD for Chlorpyrifos and Bromophos pesticides was 0.3 µg/kg, while the LOQ was 1 µg/kg. The LOD for Diazinon, Dichloros, Dimethoate, Fenitrothion, Malathion, Fuzalon, Primiphos methyl, Carbaryl, Primicarb, Propamocarb, Thiodicarb, Thiophanate methyl, Iprodione, Cresoxim methyl, Pmetrozine, Spiromsifen, Spirotetramat, Penconazole, Metribuzin, Propargit, Propiconazole, Tebuconazole, Difenoconazole, and Buprofezin pesticides measured by LC-MS/MS device was 2 µg/kg, and the LOQ was 6 µg/kg. This study involved the analysis of 26 pesticides, including organophosphorus compounds, carbamates, triazoles, conazoles, and triazinones. The recovery rate for all pesticides was observed to be 80% and 120%, except for Bromophos, which had a recovery rate of 40%. Tables 1,2, and 3 display the average concentrations of pesticides in tobacco leaf samples. These tables represent pesticides with concentrations higher than the LOD at least in one sample, while samples falling below the LOD are shown with 0.5 LOD. This study found that the most toxic organophosphorus and carbamate pesticides were Chlorpyrifos (499 µg/kg) and Thiodicarb (525.2 µg/kg), respectively. Additionally, Panconazole has the highest concentration (208 µg/kg) among other pesticides. Table 3 compares the levels of pesticides in Iranian and imported samples based on the Cooperation Centre for Scientific Research Relative to Tobacco (CORESTA) guidelines. Compared to imported samples, Iranian samples had a higher level of pesticides. Thiodicarb pesticide had the highest concentration among Iranian samples (525.2 µg/kg), while Dichloros pesticide had the highest concentration in imported samples (260.3 µg/kg). The results of the present study were consistent with Gedge et al.ʼs findings (2020) in the United States. They found that Chlorpyrifos had the highest concentration in one of the samples [26].

Table 1: The average concentration of pesticides in Iranian samples in µg/kg

Pesticides	samples of Iranian tobacco leaves					Average
	Province A	Province B	Province C	Province D	Province E	
Carbaryl	11.3	1	1	7.3	1	4.32
Diazinon	1	2.7	1	1	19.2	4.98
Dichlorvos	94	1	1	1	1	19.6
Penconazole	1	53.7	77	208	1	68.14
Propamocarb	2.3	8	7	1	14	6.46
Propargite	1	1	11	1	1	3
Propiconazole	1	11.3	1	1	1	3.06
Pymetrozine	1	2	4.7	1	2.2	2.18
Tebuconazole	3.7	1.3	1.3	2	1.8	2.02
Thiodicarb	196.3	55.7	29.3	59	525.2	173.1
Thiophanate-methyl	7	4	10	6.7	168.5	39.24
Chlorpyrifos	52.8	0.5	0.5	0.3	499	110.62

Table 2: Average concentration of pesticides in imported samples in µg/kg

Pesticides	samples of imported tobacco leaves					Average
	Country A	Country B	Country C	Country D	Country E	
Diazinon	1	1	3	1	1	1.4
Dichlorvos	1	260.3	1	102	88	90.46
Difenoconazole	3.3	36.7	3	25.3	25.7	18.8
Propamocarb	1	1	9.7	1	1	2.74
Pymetrozine	1	1	2.3	3.3	1.7	1.86
Tebuconazole	6	11.7	6	12.3	3.3	7.86
Thiophanate-methyl	1	1	1	2	3	1.6
Chlorpyrifos	0.2	0.2	0.3	0.2	0.3	0.24

Table 3: Comparison of pesticides in Iranian and imported samples

Pesticides	Average concentration range in Iranian samples (µg/kg)	Average concentration range In imported samples (µg/kg)	GRL CORESTA[a] (µg/kg)	Examples close to GRL
Chlorpyrifos	0.3-499	0.2-0.3	500	1 Iranian sample
Carbaryl	1-11.3	1	500	
Diazinon	1-19.2	1-3	100	
Dichlorvos	1-94	1-260.3	100	2 imported samples and 1 Iranian sample
Difenoconazole	1	3-36.7	12000	
Penconazole	1-208	1	1000	
Pirimicarb	1-1.3	1-1.3	500	
Pirimiphos-methyl	1	1-1.3	100	
Propamocarb	1-14	1-9.7	13000	
Propargite	1-11	1	ND[b]	
Propiconazole	1-11.3	1	ND	
Pymetrozine	1-4.7	1-3.3	100	
Tebuconazole	1.3-3.7	3.3-12.3	18000	
Thiodicarb	29.3-525.2	1	1000	
Thiophanate-methyl	4-168.5	1-3	2000	

[a]: Guidance Residue Level, Cooperation Centre for Scientific Research Relative to Tobacco 2021.
[b]: ND stands for Not Defined.

In addition, Daniel et al. (2019) investigated pesticide levels in six marijuana samples in Brazil. In their study, it was found that pesticide contamination is common in marijuana. In this study, 67% of the analyzed samples were contaminated with Metalaxyl, Buprofezin, metazachlor and imidacloprid. Buprofezin had the lowest level in the present study [27]. In a study, Hao et al. (2017) determined the residues of organophosphorus and organochlorine pesticides in raw tobacco and commercial cigarettes. They reported 13 pesticides, including Phosphorus dimethoate, Chlorpyrifos, Diazinon, Methamidophos, and Epirubbenphos [28]. Bernardi et al. (2016) conducted research to determine the level of pesticide residues of 55 in tobacco in Brazil. The outcome showed that the pesticide residues in the samples were lower than the residue levels of the CORESTA guidelines, and residue amounts in the compounds of Primiphos methyl and Isofenphos were in the range of µg/ml. 35-51. In the current study, the average residual concentration of most pesticides in the samples was lower than the residual levels of CORESTA guidance. However, the average residual concentration of Dichloros pesticide in two imported samples was higher than the residual levels of CORESTA guidance. Also, the average residual concentration of Chlorpyrifos and Dichloros in two Iranian samples was close to the residual levels of CORESTA guidelines [29, 30]. Schneider et al. (2014) identified seven pesticides in Luxembourg hemp plants, where fungicides were found to be the most toxic in the samples. In contrast, the present study found that insecticides were the most common pesticides [31]. In the same vein, Sullivan et al. (2013) investigated pesticide residues in cannabis in the United States. Their findings revealed that pesticide residues in cannabis were directly transferred to mainstream smoke and ended up in the consumer, which may pose a significant toxicological threat without adequate regulatory frameworks. This study highlights the high level of transfer of pesticides from cigarette smoke to humans, which may lead to more health complications in smokers [32].

4. Conclusion

The results of this study, along with similar studies, demonstrate that despite the pesticide ban on using pesticides in agriculture, residues of these compounds can still be found in tobacco and tobacco products. Thiodicarb insecticide from the carbamate category had the highest average residual concentration (525.2 µg/kg) in Iranian samples, while Dichloros from the organophosphorus category had the highest average residual concentration (260.3 µg/kg) in imported samples. These findings underscore the need for continued monitoring of pesticide residues in tobacco products and for strict enforcement of regulations to prevent exposure to these harmful chemicals.

Authors' Contributions

Zohre Farahmandkia: Conceptualization; Writing-review & editing. Azam Abed: Investigation. Gholamreza Sadeghi, Ali Assadi: Resources; Validation.

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Conflicts of Interest

The authors declare no conflicts of interest.

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References

1. WHO. Safe Use of Pesticides: Twentieth Report of the WHO Expert Committee on Insecticides [Meeting Held in Geneva from 10 to 16 October 1972]. World Health Organization. 1973.

2. EPA. Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) and Federal Facilities Washington DC: United State Environmental Protection Agency. Available from: https://www.epa.gov/enforcement/federal-insecticide-fungicide-and-rodenticide-act-fifra-and-federal-facilities.

3. Koureas M, Tsakalof A, Tsatsakis A, Hadjichristodoulou C. Systematic Review of Biomonitoring Studies to Determine the Association between Exposure to Organophosphorus and Pyrethroid Insecticides and Human Health Outcomes. *Toxicol Lett.* 2012; 210(2): 155-68.

4. Use FP. Pesticides Trade and Pesticides Indicators. Global, Regional and Country Trends. 1990; 2019: 2021.

5. GAO U. Pesticides on Tobacco: Federal Activities to Assess Risks and Monitor Residues. Washington, DC: US General Accounting Office. 2003.

6. Sailaja N, Chandrasekhar M, Rekhadevi P, Mahboob M, Rahman M, Vuyyuri SB, et al. Genotoxic Evaluation of Workers Employed in Pesticide Production. *Mutat Res Genet Toxicol Environ Mutagen.* 2006; 609(1): 74-80.

7. Kamrin MA. Pesticide Profiles: Toxicity, Environmental Impact, and Fate. CRC Press. 1997.

8. Mulla SI, Ameen F, Talwar MP, Eqani SA, Bharagava RN, Saxena G, et al. Organophosphate Pesticides: Impact on Environment, Toxicity, and Their Degradation. *Bioremediat Ind Waste Environ Saf.* 2020: 265-90.

9. Abubakar Y, Tijjani H, Egbuna C, Adetunji CO, Kala S, Kryeziu TL, et al. Pesticides, History, and Classification. Natural Remedies for Pest, Disease and Weed Control. 2020; 29-42.

10. Hamilton MC. Randy D. Horsak, Philip B. Bedient, M. Coreen Hamilton, and F. Ben Thomas. Environmental Forensics: Contaminant Specific Guide. 2010; 143.

11. Karami-Mohajeri S, Abdollahi M. Toxic Influence of Organophosphate, Carbamate, and Organochlorine Pesticides on Cellular Metabolism of Lipids, Proteins, and Carbohydrates: A Systematic Review. *Hum Exp Toxicol.* 2011; 30(9): 1119-40.

12. Geiss O, Kotzias D. Tobacco, Cigarettes and Cigarette Smoke-An Overview. Luxembourg: Office for Official Publications of the European Communities. 2007; 82.

13. Shakhes J, Sarayian A, Zeinaly F. Investigating Morphological and Chemical Characteristics of Tobacco Stalk. *Iran J Wood Pap Sci Res.* 2011; 26(2): 339-51.

14. Paul A, Khan Z, Bhattacharyya A, Majumder S, Banerjee K. Multiclass Pesticide Residue Analysis in Tobacco (Nicotiana Tabacum) Using High Performance Liquid Chromatography-High Resolution (Orbitrap) Mass Spectrometry: A Simultaneous Screening and Quantitative Method. *J Chromatogr A*. 2021; 1648: 462208.

15. Cahn Z, Drope J, Hamill S, Islami F, Liber A, Nargis N, et al. The Tobacco Atlas. Atlanta, American Cancer Society and Vital Strategies. 2018; 58.

16. Sajjadi SA, Assemi H. Study of Antifungal Activity of Plant Extracts of Catmint, Tobacco and Thyme on Tobacco Pathogens Fungal. *Biol Control Pest Plant Dis.* 2014; 3(1): 41-52.

17. Organization WH. Tobacco and Its Environmental Impact: An Overview. World Health Organization. 2017.

18. Zamani H, Mobasser H, Hamidi A, Daneshmand A. Evaluation of Germination, Seedling Emergence, and Management of Pest and Diseases in Nicotiana Tabacum cv. K326 in Response to Seed Disinfection. *Iran J Seed Res.* 2021; 7(2): 209-19.

19. Cigarettes and Other Tobacco Products DrugFacts. National Institute on Drug Abuse Website. 2021. Available from: https://www.drugabuse.gov/publications/drugfacts/cigarettes-other-tobacco-products.

20. Chapman S. "Keep a Low Profile": Pesticide Residue, Additives, and Freon Use in Australian Tobacco Manufacturing. *Tob Control.* 2003; 12(3): iii45-53.

21. Mavrikou S, Flampouri K, Moschopoulou G, Mangana O, Michaelides A, Kintzios S. Assessment of Organophosphate and Carbamate Pesticide Residues in Cigarette Tobacco with a Novel Cell Biosensor. *Sens.* 2008; 8(4): 2818-32.

22. Larsson SC, Burgess S, Michaëlsson K. Smoking and Stroke: A Mendelian Randomization Study. *Ann Neurol.* 2019; 86(3): 468-71.

23. WHO. Tobacco. 2022. Available from: https://www.who.int/news-room/fact-sheets/detail/tobacco.

24. López Dávila E, Houbraken M, De Rop J, Wumbei A, Du Laing G, Romero Romero O, et al. Pesticides Residues in Tobacco Smoke: Risk Assessment Study. *Environ Monit Assess.* 2020; 192(9): 1-15.

25. Rahman MA, Chowdhury AZ, Moniruzzaman M, Gan SH, Islam MN, Fardous Z, et al. Pesticide Residues in Tobacco Leaves from the Kushtia District in Bangladesh. *Bull Environ Contam Toxicol.* 2012; 89(3): 658-63.

26. Gedge LM, Hawryluk I, Skelly MG, Vilone G. Probabilistic Modelling of Exposure to Pesticide Residues in Foods and Tobacco. *Int J Environ Agric Biotechnol.* 2020; 5(2).

27. Daniel D, Lopes FS, do Lago CL. A Sensitive Multiresidue Method for the Determination of Pesticides in Marijuana by Liquid Chromatography-Tandem Mass Spectrometry. *J Chromatogr A.* 2019; 1603: 231-9.

28. Hao L, Li H, Lin JM. Fractional-Factorial Design Based Microwave-Assisted Extraction for the Determination of Organophosphorus and Organochlorine Residues in Tobacco by Using Gas Chromatography Mass Spectrometry. *J Sep Sci.* 2017; 40(2): 542-9.

29. Bernardi G, Kemmerich M, Ribeiro LC, Adaime MB, Zanella R, Prestes OD. An Effective Method for Pesticide Residues Determination in Tobacco by GC-MS/MS and UHPLC-MS/MS Employing Acetonitrile Extraction with Low-Temperature Precipitation and D-SPE Clean-Up. *Talanta.* 2016; 161: 40-7.

30. CORESTA. The Concept and Implementation of CPA Guidance Residue Levels. Agro-Chemical Advisory Committee. Seventh Edition. 2021; 1.

31. Schneider S, Bebing R, Dauberschmidt C. Detection of Pesticides in Seized Illegal Cannabis Plants. *Anal Methods.* 2014; 6(2): 515-20.

32. Sullivan N, Elzinga S, Raber JC. Determination of Pesticide Residues in Cannabis Smoke. *J Toxicol.* 2013; 2013.