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IMPROVEMENT OF PURIFICATION METHOD FOR DIAZINON FROM SOIL MATRIX

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ABSTRACT

Diazinon is common pesticides used in the highest amount for paddy production in Bangladesh. In this study, we applied diazinon in different experimental agricultural fields of paddy. The present work was aimed to develop a clean-up method for diazinon from soil matrix. The work also included to the mass of soil extracted (1/5 of the reported) which proportionally will reduce chemicals and solvents used in the reported method. The analysis carried out by GC-ECD, purity of standard diazinon was 97.5%. Solvents and sample blank were injected before analysis of cleaned extracts. For clean-up silica gel and florisil were used; cleaned extract obtained from silica gel column was found to contain less impurity than the cleaned extract of florisil column. Percent recovery of the two columns was found to be 92.70 ± 1.25 and 88.33 ± 1.36, respectively. The best eluting solvent for silica gel column was dichloromethane-methanol mixture (95:5). A few more experiments are necessary to validate the clean-up process. Therefore, for real soil sample extraction will be done taking 10g soil instead of 50g. Silica gel column will be used for clean-up instead of florisil which is cheaper and easily available.

Keywords: Diazinon, Clean-up, florisil, Pesticides, Soil matrix, column, Residual, GC-ECD.

Introduction

Bangladesh is an agricultural country and rice is the staple food. To fulfill the demand of extra foods for increasing population more pesticides including diazinon are used in the agricultural fields for higher food production. To produce high yielding varieties of paddy, more fertilizer, irrigation and pesticides are required. Due to over application of pesticides, water of several rivers of Bangladesh is being polluted day by day and the water quality is more toxic than sewage waste for some major pollutant [18,19]. Different kinds of pesticides are allowed in Bangladesh for rice production and diazinon is at top. It is used all over the country in paddy field starting from seedling to ripe paddy to protect the plant, shoot and paddy from pest. Although there are benefits to the use of pesticides, there are also drawbacks, such as potential toxicity to human health and wildlife. Pesticide use has increased 50-fold since 1950 and 2.3 million tones of industrial pesticides are now used each year. In modern times some sophisticated chemical compounds such as diazinon, malathion, cythion, guthion, acepthate etc. which are carefully researched to ensure their effectiveness against target organisms, are safe to the

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environment and can be used without hazards to the operators or consumers are known as modern pesticides [2]. Inappropriate management and misuse of pesticides have negative impact on surrounding soil, water and environment that reduce biodiversity, nitrogen fixation, pollination, soil productivity, water quality and threatens habitat and endangered species [1].

Diazinon is synthetic pesticide. Diazinon is a contact insecticides which kills by altering normal neurotransmission within the nervous system of target organisms. Non-target organisms can be exposed to diazinon by inhalation, ingestion and dermal exposure [8]. Diazinon had adverse impact such as chronic toxicity, endocrine disruption, carcinogenicity and teratogenicity effects on animals and humans [9]. Diazinon is easily hydrolyzed in both alkaline and acidic media.
Diazinon (in alkaline media) → diethylphosphoric acid + 2-isopropyl-4-methyl-6-oxypyridine.

Diazinon (in acidic media) → diethylphosphoric acid + 2-isopropyl-4-methyl-6-oxypyridine +

small amount of tetraethyl phosphorodithioates + hydrophophorothioates [10].

The fate of diazinon in body is absorption, distribution, metabolism and excretion. Diazinon is highly toxic to bees, birds, ducks and geese, insects and mites, soil microbes, fish and aquatic invertebrates. The chronic Population Adjusted Dose (cPAD) is 0.0002 mg/kg/day [11]. No drinking water standards exist for diazinon. The USEPA has set a lifetime health advisory at 0.001 mg/L for diazinon in drinking water, however this health guidance level is not enforceable [12]. Diazinon is common pesticides used in agriculture. The highest amount pesticides used in paddy production is diazinon. Therefore, a study of residual diazinon in the paddy production system is essential.

In the literature different kinds of extraction and clean-up methods are reported for old (OCs) and modern pesticides residue analysis. In the many cases detection was carried out by liquid chromatography having ultraviolet visible detector & fluorescence detector (LC-UV-VIS & LC-FD), gas chromatograph with electron capture detector (GC-ECD) and gas chromatograph with mass detector (GC-MSD). GC is more sensitive than LC, LOD, and LOQ in much lower than LC. Cost of analysis in LC is much higher than GC. Considering all this factor it was decided to analyzed diazinon by GC-ECD. Moreover, ECD is a specific detector, it detects selectivity compounds which contain loose electron. Diazinon contain two nitrogen and one sulfur having lone pair of electron. Therefore, diazinon is easily detected by GC-ECD. Solid-liquid extraction by ultra-sonication [13], by shaking [14] and by pressurized solid-liquid extraction were found to be good for soil.

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A very few study was published relating to purification method of diazinon from soil.Villaconta,2003 reveal that, The average recoveries from spiked water and soil samples at various concentration levels exceeded 86% with relative standard deviations of 1.8–5.1%[3]. Fuentes reveals that, an initial approach through experimental design and principal component analysis where recovery of compounds using a water–methanol mixture ranged from 54 to 77% [4]. Another study find out that, High performance liquid chromatography was then used to analyze the treated samples. The ENVI-Carb column recovered 87.99%–95.95% of the diazinon from water and soil with CVs of 5.08%–8.06% [5]. Another article review discusses the advantages and disadvantages, and relative performance, of most of the modern sample-preparation techniques and cites a number of illustrative applications for each of them [6]. In this work solid-liquid extraction by shaking was followed exactly for spiked soil sample reported by Jong-Hyouk Park [14]. IN those papers clean-up were done by solid phase extraction (SPE) cartridge, membrane filtration and florisil column.

The experiment was designed to validate the efficacy of the chosen extraction method (shaking with solvent), the cleanup (silica gel and florisil, various solvents). It is mentioned that the cleanup is necessary to remove impurity from diazinon mixed soil body. It is important to remove these impurities (or matrix components) for sustainable food production and safe from diazinon toxicity into soil and produced food from this soil matrix

This study deals with Validation of extraction and cleanup is needed to accurately analyze diazinon in soil. The work is aimed to develop suitable clean-up method which will be less expensive as well as better than the existing methods i.e. contain less impurity. The work is also aimed to scale down the extraction and clean-up which will be better for food and environment. In the beginning of the recovery experiment, clean-up method reported by Jong-Hyouk Park *et. al* was followed where florisil was used as stationary phase. In the present research 97.5 % pure standard diazinon was used and analysis was carried out by GC-ECD. Standard solutions were made considering the purity of the standard.

2. Materials and Methods

Thirteen experimental paddy fields were made, out of which four fields were controlled. Two applications diazinon were made in the nine fields and samples were collected at 0 (two hours after application), 1, 2, 3,5,7,10,14 days after each application following WHO guidelines.

Sample collection

From our experimental fields, pesticide treated and untreated (control/blank) soil samples were collected from Tista floodplain soil and Chittagong hill tract of Bangladesh because of

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containing high amount of rice and cereal crops cultivated from this regions [15,17]. This floodplain soil is more or less fertile & not having toxicity of any chemical elements except iron [15, 16]. Untreated soil samples were collected to be used for recovery experiments to test the efficacy of the extraction and cleanup methods. In this work untreated soil samples were selected for recovery a part of method validation studies. The samples were collected in poly-ethylene bags and transported to the laboratory. The collected samples were air-dried in shady conditions, then ground into powder, passed through 2 mm sieves, mixed homogenously and stored below 4 degree Celsius until analysis.

A portion of the dried blank soil sample (50 g) was taken in a 300 mL ground joint conical and 1 mL of standard diazinon working solution was added to it to reach spiking level 2 µg/g. Five more replicates spiking were done by following the same procedure. Out of six spiked sample three sample were cleaned up by silica gel column (2.5 cm diameter, 30 cm length). To prepare the column slurry of 10 g of silica gel in n-hexane (50mL) with n-hexane-dichloromethane mixture (1:1; 50 mL) and dichloromethane (50 mL). The eluents were collected in 15 test tubes (10 mL in each). The eluent of each test tube was transferred to 1mL GC vial by evaporation followed by reconstitution in hexane. Other three extract samples were cleaned up by silica gel + florisil column (2.5 cm diameter, 30 cm length). Slurry of 7 g of silica gel in n-hexane was poured into an open preparative chromatographic column; 3 g of florisil was added on the top of the silica gel layer. 5 g of anhydrous sodium sulphate were added to the top of the adsorbent. The sample extract (1mL) was applied to the column and eluted successively with n-hexane (50 mL); n-hexane-dichloromethane mixture (1:1; 50mL). The eluents were collected in 10 test tubes (10 mL in each).

A portion of the dried sample (10g) was taken in a 100 mL ground joint conical flask and 1 mL of standard diazinon working solution was added to it reach spiking level 0.25 µg/g. the mixtures were thoroughly homogenized and allowed to stand for 30-45 min. five more replicate spiking were done by following the same procedure. In the spiked soil sample (10g), 6mL of 0.2 M ammonium chloride solution was added, then shaked manually. The whole contents were placed in a mechanical shaker and shaked (at 200 rpm) for 1 hour at 25 C. the mixture was filtered (Whattman No.42) using a Buchner funnel under suction. The residue was then washed with another 10 mL of fresh acetone and the combined filtrate was placed into a reparatory funnel (250 mL) into which 10 mL of saturated NaCl solution and 90 mL of distilled water added. The mixture was then partitioned with dichloromethane (10 mL) and the organic layer was dried over 15 g of anhydrous $NaSO_4$ followed by evaporation to dryness under vacuum at 40˚C. The extract was reconstituted in 0.5 mL of n-hexane and subjected to clean-up.

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A gas chromatograph (SHIMADZU-2010) having Electron Capture Detector (ECD) was used for identification and quantification of Diazinon. The retention time of standard solutions of Diazinon was detected 8.97 min. In the literature different kinds of extraction and clean-up methods are reported for old (OCs) and modern pesticides Residue analysis. In the many cases detection was carried out by liquid chromatography having ultraviolet-visible director and fluorescence detector (LC-UV-sis & LC-FD), gas chromatography with nitrogen Phosphorus detector (GC-NPD), gas chromatograph with electron capture detector GC-ECD and gas chromatograph with mass detector (GC-MSD).

4. Result and discussions

All blanks and extracts from the silica gel and silica gel + florisil columns were below the LOD**.** To find out right solvent for elution of diazinon and also to save stationary phase a small silica gel column was made. 0.25 pg of diazinon in solution of 0.5 mL n-hexane was applied to the top of the column. The column was eluted with n-hexane (2 mL), n-hexane-dichloromethane (1:1; 2 mL), dichloromethane (2 mL), and dichloromethane-methanol (2 mL; 98:2 & 3 mL; 95:5). All the eluted fractions were evaporated and reconstituted in hexane analyzed by GC-ECD. The results are in Table-1. From the Table-1, it was found that diazinon was eluted by DCMMeOH mixture. 20.27% was eluted with DCMMeOH (98:2) and 78% was eluted with DCMMeOH (95:5). Total recovery was 98.27%. Two more recovery experiments were carried out keeping all chromatographic conditions same. Elution was carried out with hexane, hexane-DCM, DCM and DCMMeOH mixture (95:5). Results of the two recoveries are also presented in the Table-1. In the two later columns recoveries were 90.81% and 91.44%.

Table-1: Recovery experiment of Diazinon using silica gel column

Sample no.	Diazinon n added (ppm)	Fraction ID	Peak Area	Recovery (%)	Recovery (%) (mean + SD)
1	2.5	Hexane	0.0	-	93.51+4.14
		Hexane-DCM 1:1	0.0	-	
		DCM	0.0	-	
		DCMMeOH(98:2)	196559	20.27	
		DCMMeOH 95:5	756124	78.00	
2	2.5	Hexane	0.0	-	

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		Hexane-DCM 1:1	0.0	-	
		DCM	0.0	-	
		DCMMeOH(95:5)	880388	90.81	
3	2.5	Hexane	0.0	-	
		Hexane-DCM 1:1	0.0	-	
		DCM	0.0	-	
		DCMMeOH(95:5)	886455	91.44	

Total recovery was 88.21%. Two more recovery experiments were carried out keeping all chromatographic conditions same. Elution was carried out with hexane, hexane-DCM, DCM and DCMMeOH mixture (95:5). Results of the two recoveries are also presented in the Table-2. In the two later columns recoveries were 91.90% and 92.11%.

Table-2: Recovery experiment of Diazinon using florisil gel small column

Sample no.	Diazinon added (ppm)	Fraction ID	Peak Area	Recovery (%)	Recovery (%) (mean + SD)
1	2.5	Hexane	0.0	-	
		Hexane-DCM 1:1	0.0	-	
		DCM	532212	54.90	
		DCMMeOH 95:5	322937	33.31	
2	2.5	Hexane	0.0	-	90.74 +2.19
		Hexane-DCM 1:1	0.0	-	
		DCM	486565	50.19	
		DCMMeOH(95:5)	404360	41.71	
3	2.5	Hexane	0.0	-	
		Hexane-DCM 1:1	0.0	-	

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		DCM	419853	43.31	
		DCMMeOH(95:5)	473139	48.80	

For the recovery of diazinon from spiked soil sample silica gel column was prepared as like as standard diazinon sample. The sample extract in n-hexane (5OO µL) was applied to the top of the column. When sample was adsorbed in adsorbent then the adsorbed sample was successively eluted with n-hexane (2 mL), n-hexane dichloromethane (1:1; 2 mL), dichloromethane (2 mL), and dichloromethane methanol (4 mL; 95:5). The results are given in the Table-3. From the Table-3, it was found that diazinon was eluted by DCMMeOH (95:5) mixture. Recovery was 91.72%. Two more recovery experiments were carried out keeping all chromatographic conditions same. Results of the recoveries are also presented in the Table-3. In the two later columns recoveries were 94.11% and 92.26%.

Table-3: Recovery experiment of diazinon using florisil gel small column

Sample no.	Diazinon added (ppm)	Fraction ID	Peak Area	Recovery (%)	Recovery (%)(mean + SD)
1	2.5	Hexane	0.0	-	
		Hexane-DCM 1:1	0.0	-	
		DCM	0.0	-	
		DCMMeOH 95:5	889127	91.72	
2	2.5	Hexane	0.0	-	
		Hexane-DCM 1:1	0.0	-	92.70 +1.25
		DCM	0.0	-	
		DCMMeOH(95:5)	912383	94.11	
3	2.5	Hexane	0.0	-	
		Hexane-DCM 1:1	0.0	-	
		DCM	0.0	-	

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		DCMMeOH(95:5)	894357	92.26	

In case of recovery of diazinon from spiked soil sample florisil gel column was prepared as like as standard diazinon sample. The sample extract in n-hexane (500 µL) was applied to the top of the column. When sample was adsorbed in adsorbent then the adsorbed sample was successively eluted with n-hexane (2 mL), n-hexane dichlommethane (1:1; 2 mL), and dichloromethane (4 mL). The results are given in the Table-4. From the Table-4, it was found that diazinon was eluted by dichloromethane (DCM). Recovery was 87.15%. Two more recovery experiments were carried out keeping all chromatographic conditions same. Results of the two recoveries are also presented in the Table-4. In the two later columns recoveries were 89.82% and 88.03%.

Table-4: Recovery experiment of Diazinon using silica gel column.

Sample no.	Diazinon added (ppm)	Fraction ID	Peak Area	Recovery (%)	Recovery (%) (mean + SD)
1	2.5	Hexane	0.0	-	
		Hexane-DCM 1:1	0.0	-	
		DCM	844899	87.15	
2	2.5	Hexane	0.0	-	
		Hexane-DCM 1:1	0.0	-	88.33 +1.36
		DCM	870727	89.82	
3	2.5	Hexane	0.0	-	
		Hexane-DCM 1:1	0.0	-	
		DCM	894357	92.26	

Conclusion

Solvent and sample blank were injected before analysis of cleaned extracts. Percent recovery of the two columns was found to be 92.70 + 1.25 & 88.33 + 1.36 respectively. The best eluting solvent for silica gel column was dichloromethane-methanol mixture (95:5) and for florisil gel column it was dichloromethane. From the present research it is clear that recovery of diazinon by

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silica gel and florisil gel column are almost same. But extra peak are found in florisil gel. Clean-up are better by silica gel compare to florisil column. Silica gel column can be used for clean-up instead of florisil where the former is cheaper and easily available in Bangladesh than that of the later one. The work also includes to reduce the soil matrix (1/5 th of the reported) which proportionally will reduce chemicals and solvent used in the reported method.

References

1 Kamrin, M.A. 1997. Pesticide Profiles: Toxicity, Environmental Impact and Fate. CRC Press.)

2 Hock, W. et al. 1991. Farm Chemicals Manual: A Guide to Safe Use and Handling. The Agric. And Vet. Chem. Assoc. Aust. Ltd.

3 M. E. Sánchez, R. Méndez, X. Gómez & J. Martín-Villacorta. Determination of Diazinon and Fenitrothion in Environmental Water and Soil Samples by HPLC. Journal of Liquid Chromatography & Related Technologies. 26(3); 2003

4 EdwarFuentes,MaríaE.Báez .Ronnie Labra.,2007. Parameters affecting microwave-assisted extraction of organophosphorus pesticides from agricultural soil. Journal of Chromatography. 1169(1-2):pp 40-60

. .

5 Jianshe Tang Min Zhang Guohua Cheng Yitong,. 2009. Diazinon Determination Using High Performance Liquid Chromatography: A Comparison of the ENVI-Carb Column with the Immunoaffinity Column for the Pretreatment of Water and Soil Samples. Bulletin of Environmental Contamination and Toxicology. 83:626

6 Sjaak de Koning, Hans-Gerd, Janssen Udo A. Th. Brinkman. 2009. Modern Methods of Sample Preparation for GC Analysis. Chromatography. 69(33)

7 I.R.E.D Facts Diazinon; U.S. Environmental Protection Agency, Office of Prevention, Pesticides and Toxic Substances, Office of Pesticide Programs. May 2004.

8 Environmental Risk Assessment for Diazinon; U.S. Environmental Protection Agency, Office of Prevention, Pesticides and Toxic Substances, Office of Pesticide Programs, U.S. Government Printing Office: Washington, DC, 2000.

9 Diazinon Revised HED Human Health Risk Assessment for the Reregistration Eligibility Decision (RED); U.S. Environmental Protection Agency, Office of Prevention, Pesticides and

Vol. 2, No. 04; 2017

ISSN: 2456-8643

Toxic Substances, Office of Pesticide Programs, U.S. Government Printing Office: Washington DC, 2000.

10 Gruzdyev, G.S., V.A. Zinchenko, V.A. Kalinin, R.I. Slovtsov; The chemical protection of plants; Mir Publishers Moscow; pp. 201-202.

11 Reregistration Eligibility Decision (RED) Diazinon; EPA 738-R-04-006; U.S. Environmental Protection Agency, Office of Prevention, Pesticides and Toxic Substances, Office of Pesticide Programs, U.S. Government Printing Office: Washington, DC, 2006.

12 Drinking Water Standards and Health Advisories; U.S. Environmental Protection Agency. (Accessed on December 2007), updated 2006.

13. M.C Akogbeto, R. F Djouaka and D. A. Kinde-Gazard, screening of pesticide residue in soil and water samples from agricultural settings; Malaria journal 2006, 5:22

14. Jong-Hyouk Park, M. I. R. Mamun, Jeong-Heui Choi, A.M. Abd El-Aty, M.E. Assayed, W. J. Choi, K.S. Yoon, Seong-Soo Han, H. K. Kim, B.J. Park, K.S. Kim, S. D. Kim, H.G.Choi and Jae-Han Shim; Development of a multiresidue method for the determination of multiclass pesticides in soil using GC; Biome. Chromatogr. 2010; 24, 839-901

15. Hassan M, Ahmed AA, Hassan MA, Nasrin R, Rayhan AB, Salehin SM, Rahman MK. Changes of Soil Fertility Status in Some Soil Series of Tista Floodplain Soils of Bangladesh, during 1996-2016. Asian Research Journal of Agriculture. 2017 Jul 13; 5(3):1-9.

16. Ahmed AA, Hassan M, Ahmed SU, Rahman MK. Fertility Status on Some Tista Floodplain Soils of Bangladesh. International Journal of Scientific & Engineering Research. 2017 Jun; 8(6):579-82.

17. Hassan M, Hassan R, Pia HI, Hassan MA, Ratna SJ, Aktar M. Variation of Soil Fertility with Diverse Hill Soils of Chittagong Hill Tracts, Bangladesh. International Journal of Plant & Soil Science. 2017 Aug 5; 18(1):1-9.

18. Hassan M, Hassan R, Mahmud MA, Pia HI, Hassan MA, Uddin MJ. Sewage Waste Water Characteristics and Its Management in Urban Areas- A Case Study at Pagla Sewage Treatment Plant, Dhaka. Urban and Regional Planning. 2017 Jul 14;2(3):13-6.

Vol. 2, No. 04; 2017

ISSN: 2456-8643

19. Mahmud MA, Hussain KA, Hassan M, Jewel AR, Shamsad SZ. Water quality assessment using physiochemical parameters and heavy metal concentrations of circular rivers in and around Dhaka city, Bangladesh. International Journal of Water Research. 2017 Jun 7;7(1):23-9.

18. Hassan M, Hassan R, Mahmud MA, Pia HI, Hassan MA, Uddin MJ. Sewage Waste Water Characteristics and Its Management in Urban Areas- A Case Study at Pagla Sewage Treatment Plant, Dhaka. Urban and Regional Planning. 2017 Jul 14;2(3):13-6.

19. Mahmud MA, Hussain KA, Hassan M, Jewel AR, Shamsad SZKM. Water quality assessment using physiochemical parameters and heavy metal concentrations of circular rivers in and around Dhaka city, Bangladesh. International Journal of Water Research. 2017 Jun 7;7(1):23-9.