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MONITORING OF ORGANOPHOSPHATE PESTICIDES RESIDUE IN MILK COLLECTED FROM JALGAON DISTRICT, MAHARASHTRA

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ABSTRACT

Total 60 buffalo milk samples were collected from the different regions of district Jalgaon, Maharashtra and it was monitored for the presence of pesticide residues. Analysis of pesticides was performed by using multiresidue method which is validated in laboratory. The identification and quantification of the pesticides e.g. Chlorpyrifos, Cyhalothrin, Cypermethrin, Dichlorvos, Dimethoate, Deltramethrin, Profenofos, Quinolphos, Trizophos, and Phorate have done in the present review. The analysis was done by using the GC- ECD by which degree of contamination could be determined. The analysis showed some of the milk samples were contaminated by pesticides. About 10 % of the milk samples were contaminated with pesticide residues. Dichlorvos was most significantly found the milk sample. Chlorpyrifos was also found in the milk sample which was the measurable amount of pesticide residues; this detection of pesticides was recommended by Codex. Phorate was also found in traces amount. Other pesticides were found below detection limit. The presence of these pesticides residues are harmful for the human health, the monitored data of the presence of pesticides residues in milk may assist for awareness for farmers and local peoples as well as in future scientific assessment.

Keyword: Organophosphate, pesticides , pesticide residue, Milk

1. INTRODUCTION

Pesticides are used all over the world to enhance productivity, yet many pesticide residues remain in the environment, posing a threat to human health. Many countries

use the pesticides and several pesticidescontain noxious substances which remain in the environment for long period of time. Insecticides are required for commercial agricultural productivity**[1, 2].** Pesticides consist of the organophosprous and organochlorine and it can be identified in the milk samples.Organochlorines (OC) and organophosphates (OP) are insecticides that are commonly employed in public health, agriculture, and veterinary medicine.The organophosphate esters have a well-established place in the pesticide market. Furthermore, the organophosphate esters have a wider range of applications in veterinary medicine than the organochlorine compounds. The main sources of these pesticides found in the milk are feed of the animals like buffalos or cow. Presence of these types of pesticides in the milk can be harmful to the human health. Main purpose of using pesticides is to kill the pest on crop or to make them ineffective, likewise these can affect the human body as well unintentionally. The pesticides residues can make the harmful effects on the human body hence these should be monitored and these are having greater concern with the milk and milk products**[3-5].**Animal milk can be contaminated with OC and OP pesticides in a variety of ways. It can occur as a result of ingesting tainted feed or fodder, or as a result of licking a pesticide intended to control parasites on an animal's body. There are many survey found to detect the pesticides content in milk and milk products**[6-8].**Mukherjee *et al* studied and investigated organochlorine pesticide in the dairy milk from Delhi area. It was analysed in the 28 samples of butter brands available. The analysis was carried out by using the technique GC-ECD. All the samples showed detectable residues like DDT, HCH **[9].**Kumar *et al*, Aslam *et al*, Gupta *et al*, Surendra *et al*, John *et al* andKalra *et al* studied and estimated the analysis of the pesticides from milk from different regions. The study involved the determination residual concentration of pesticides in milk and milk products from different regions of India**[10-15].** These residues are far too long-lasting. They build up in body fat and even breast milk. Pesticide residues that enter the human body through milk samples cause a variety of health issues, including heart disease, endocrine disruption, and cancer.Regular pesticide applications first have an acute effect, then a chronic one, and can even result in mortality.Different organochlorine pesticides have been found in crops, human fluids, and meat, according to evidence. These herbicides also result in low sperm counts, increased testicular cancer, various birth abnormalities, and other reproductive

issues.Farmers utilise a wide range of pesticides around the world due to their broad spectrum activity and higher efficiency.Another reason for their widespread use is their low cost. Organochlorine insecticides were banned in several nations for various periods of time due to their detrimental effects **[16, 17].**

Synthetic pesticides and biological pesticides are the two types of pesticides that can be used. Pesticides with a broad range of action can kill any species, whereas pesticides with a narrower target kill just the species for which they were designed.Pesticides that are absorbed by the plant are known as systemic pesticides. These insecticides enter the plant's circulatory system.DDT was a well-known insecticide that was widely used in the past.Pesticides are used by developed countries in 75 percent of cases.Pesticides help farmers save money by eradicating undesired insects and pests in their crops while also enhancing output yield.Endocrine glands are being disrupted by pesticides. Hormones are essential for optimal development, and pesticides disrupt hormones, resulting in aberrant development in the early stages.Pesticides like carbamate and organophosphate reduce acetyl cholinesterase activity, which blocks nerve impulses.The neural system of the insect is targeted by the majority of pesticides. Although the neurological systems of insects and mammals differ significantly, the toxicity mechanism is the same in both. Different tests are employed to determine the maximum residue limits of pesticides **[1].**

In this research study, buffalo milk samples collected from the district Jalgaon area and were analysed for the presence of pesticides residues. The main purpose of this study was to determine whether milk was contaminated with pesticides residues or not and what was the limit of the residues if present in the milk. This study can be helpful for the farmers and general public that they should use pesticides with precautions.

2. MATERIAL AND METHOD

2.1 Sample Collection

Buffalo milk samples were collected from the different villages located in the different Taluka of Jalgaon district.The sampling was carried out during august 2018 to January 2019.Total 60 milk samples were randomly collected at morning sessions from the farmers. The samples were undergone sterilization which was stored in the 500 ml plastic bottles having the tight stopper in cool storage condition to avoid any contamination. The samples were immediately transferred to the school of environmental & earth science department laboratory at Jalgaon University. The

samples were kept in deep freeze until analysed.

2.2 Sample extraction and preparation

Sample preparation method was done by using the QuEChERS analytical method for determination of residues in the milk sample with slight alternation. Acetic acid (1%) buffer solution was used to improve the problematic analyte. QuEChERS is a sample preparation technique that vastly simplifies the analysis of pesticide residues. It's quick, easy, cheap, effective, rugged, and safe. QuEChERS is primarily viewed as a two-step procedure: The first stage is extraction, which involves extracting analyte of interest from your sample.The second step is clean up, which involves removing various potential interferences from the extract.This buffer QuEChERS method as primarily registered as an AOAC official method determination of pesticide residues in foods by acetonitrile extraction and partitioning with magnesium sulphate.

About 10 ml of milk sample was taken from previously collected milk sample. Transferred it into the 50 ml capacity of polypropylene tube. Distilled water (10 ml) was added slowly and shake the tube slightly and kept it in deep freezer for 10-12 min. About 20 ml of 1% acetic acid solution in acetonitrile (v/v) was added into the tube and mixed itvigorously for 1 minute by using a vortex mixer at maximum speed at this time polypropylene tube was tightly closed. Anhydrous MgSO4 (6 g) and anhydrous NaCl (1g) were added to the tube andit was mixed vigorously for 1 min then centrifugation of the content was done at2000 rpm for 1 min. Upper supernatant liquid (6ml) was transferred into the 15 ml centrifuge tube containing 300 mg PSA + 900 mg $MgSO_4$. The tube was robustly mixed for 30 s using a vortex mixer and then centrifuged the sample mixture for 1 min at 2000 rpm. Supernatant liquid (4ml) was taken and evaporate it to dry and make up with hexane: acetone (3:1, v/v), it was filtered through 0.2 micron filter and transferred into an auto sampler bottle for GC–MS Injection.

2.3 Instrument analysis

Organophosporus pesticides Analysis was performed by using GC-2010 equipped with a 63Ni electron capture detector (ECD) having DB-35 MS capillary column (30 mx0.25 mmx0.25 μm film thickness). The different operating conditions were like this, the column oven temperature was initially held at a temperature of 80°C for 0.0 min, and then ramped at 7 °C min-1 to 180 °C for 1.0 min, and then ramped at 5 °C min-1 to 285 °C for 10.0 min. The temperature of injector and detector were 250 °C, Nitrogen were used as carrier gas with a flow rate of 1.5 ml min-1.Sample inoculation was done with auto-sampler

(AOC- 20i) in the split injection inlet in the ratio of 1:5 while injection volume was 1.0 µl.

Validation of analytical method was performed on basis of estimating linearity, accuracy and precision, limits of detection and limit of quantification, and repeatability. Selected pesticides standards were added to matrix blank extract with five various concentrations to correct for background interferences. Linearity was determined by constructing calibration curves with standard solutions of concentration 0.05, 0.1, 0.25, 0.4, and 0.5µg/ml. Limits of detection and quantification were calculated Accuracy data was obtained from recovery studies. Repeatability was evaluated through within-run precision of the method.

3. RESULT AND DISCUSSION:

Milk sample analysis for presence of pesticide residues showed, presence of Chlorpyrifos in three samples out of 60 samples. The amount of residues found 0.082, 0.079, 0.091 µg/mL.Dichlorvos was found in significant amount 0.166 µg/mL in one milk sample and Phorate was found in traces of amount i. e. 0.025, 0.028 in two samples out of 60 milk samples. The pesticides residues like Cyhalothrin, Cypermethrin, Dimethoate, Deltramethrin, Profenofos, Quinolphos, Trizophos were found in below detection limit.(Table 1).

There are many researchers who have studied regarding the presence of pesticides in milk. Nigam *et al* studied on 2205 samples of dairy milk which were gathered from rural and urban areas of 12 states representing different geographical regions of India as part of a multicentre study undertaken by the Indian Council of Medical Research **[18].**In the investigation of Battu*et al*, HCH residues were found in 53.3 percent of the samples collected in the Ludhiana district **[19].** Only 5% of bovine milk samples from Haryana exceeded the lindane MRL which studied by Sharma *et al*. **[20].** In milk samples obtained from the Bundelkhand region of India, Nag and Raikwar (2008) reported a 28.98 percent detection rate for lindane**[21].** Pesticide contamination of feed and fodder has been noted by many researchers.Some substances, such as chlorpyrifos, are easily metabolised and eliminated from an animal's body, whereas others, such as lindane, leave measurable residues in several matrices, such as egg, milk, and body fat **[22].** Residue of Chlorpyrifos was found in three samples which are 0.082µg/mL, 0.079 µg/mL, 0.091µg/mL. The detected values are higher than the MRL values of Chlorpyrifos i.e. 0.002 µg/mL**[23].**It means only 5% of the sample was contaminated withChlorpyrifos. RegardingDichlorvos, only 0.6% of the

milk sample was contaminated with Dichlorvos and about 1.2% of sample was contaminated with Phorate residues.Figure 1 shows graphical representation of the pesticides residues in µ/ml**.** The percentage contamination of the milk sample is very low as compared to higher levels (59%) which are found in the vegetables, cereals, grains, oils, eggs meat, fish and other poultry products and it was reported by Bhushan**[24].** It was happened because; only milk was tested for pesticide residue in this study, as opposed to other foods such as egg, meat, fish, and poultry. The decrease in residual levels in milk could possibly be attributed to the introduction of newer, target-specific compounds or improvements in insect pest management strategies.

Table 2 represents the LOD, LOQ, of the ten pesticides with different spiking concentrationwhich ranges from 0.05 to 0.5µg/ml**.**The linearity of the pesticides ranged between 0.05 to 0.5µg/mlwith a coefficient of correlation higher than 0.98. The repeatability of all the pesticides was found to be satisfactory and the result of method validation indicates that multiresidue sample preparation coupled with GC–ECD analysis is suitable for analysis of these pesticides. The result of the present study shows the data on the pesticides residues in buffalo milk from the different regions of Jalgaon district. The data will assist in valuable assessment of the future scientific research on pesticide contamination also, data can suggest monitored area should be extended further to different regions to make strongest scientific basis of pesticide management.

Table 1: Pesticide residue in buffalo milk samples

Pesticides	Number of Sample	Contaminated sample	Residue in µg/mL	LOD µg/mL	LOQ µg/mL
Chlorpyrifos	60	3	0.082, 0.079, 0.091	0.076	0.231
Cyhalothrin	60		BDL	0.099	0.301
Cypermethrin	60		BDL	0.121	0.368
Dichlorvos	60	1	0.166	0.156	0.473
Dimethoate	60		BDL	0.08	0.244
Deltramethrin	60		BDL	0.114	0.347
Profenofos	60		BDL	0.058	0.177
Quinolphos	60		BDL	0.071	0.216
Trizophos	60		BDL	0.079	0.242
Phorate	60	2	0.025, 0.028	0.02	0.06

BDL: Below detection limit, LOD: Limit of detection, LOQ: Limit of quantitation

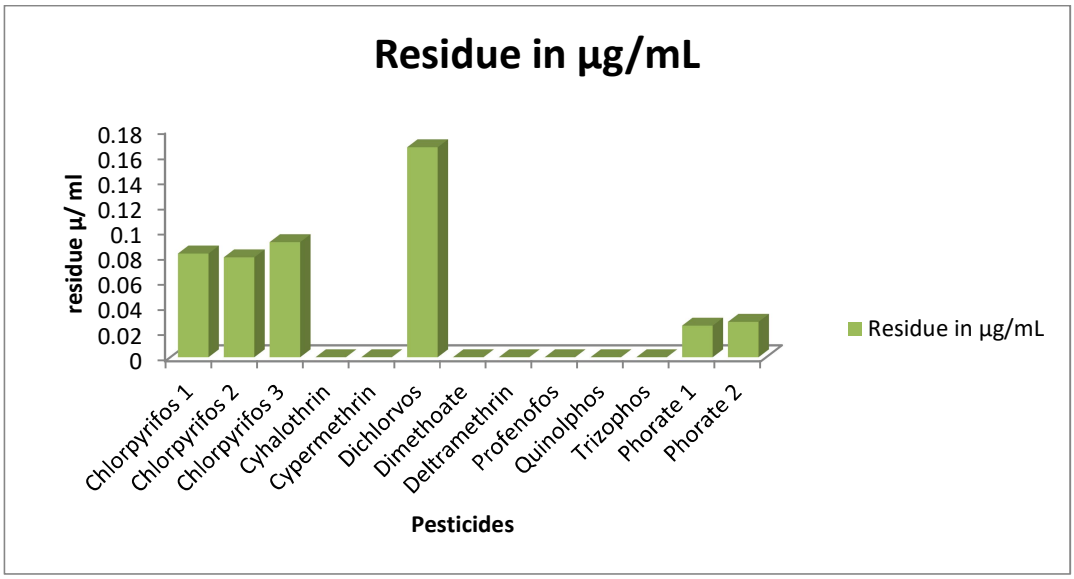

Figure 1: Graphical presentation of pesticides residues in milk sample

Table 2: Limit of detection (LOD), limit of quantification (LOQ), spiked level, recovery and repeatability of 10 pesticides

Sr No	Pesticides	LOD	LOQ	Spiked Level	Recovery	Repeatability %
1	Chlorpyrifos	0.076	0.231	0.05	85.94	5.79
				0.1	77.32	3.44
				0.25	75.63	4.25
				0.4	81.36	3.28
				0.5	88.91	1.49
2	Cyhalothrin	0.099	0.301	0.05	71.61	3.71
				0.1	83.48	2.7
				0.25	81.88	3.31
				0.4	77.61	2.05
				0.5	79.26	2.35
3	Cypermethrin	0.121	0.368	0.05	75.43	7.39
				0.1	82.63	6.82
				0.25	85.63	2.19
				0.4	83.04	1.98
				0.5	86.59	2.21
4	Dichlorvos	0.156	0.473	0.05	88.43	3.82
				0.1	88.39	4.46
				0.25	80.01	1.51
				0.4	79.52	1.93
				0.5	80.24	2.78
5	Dimethoate	0.08	0.244	0.05	81.81	3.01
				0.1	89.47	3.36
				0.25	83.52	2.78
				0.4	89.4	2.22
				0.5	85.72	0.59
6	Deltramethrin	0.114	0.347	0.05	0	0
				0.1	81.03	5.03

				0.25	79.05	6.03
				0.4	87.59	3.67
				0.5	80.52	4.52
7	Profenofos	0.058	0.177	0.05	80.23	0
				0.1	80.23	5.93
				0.25	80.46	6.52
				0.4	81.45	3.06
				0.5	82.35	3.13
8	Quinolphos	0.071	0.216	0.05	72.42	12.32
				0.1	84.09	5.36
				0.25	83.01	2.82
				0.4	86.13	2.15
				0.5	81.34	3.83
9	Trizophos	0.079	0.242	0.05	76.50	0
				0.1	76.5	17.2
				0.25	85.95	6.46
				0.4	81.14	3.45
				0.5	81.85	2.58
10	Phorate	0.02	0.06	0.05	81.26	2.48
				0.1	83.91	1.92
				0.25	83.9	1.29
				0.4	90.17	0.98
				0.5	88.24	1.31

4. CONCLUSION

In this study, 60 milk samples of buffalo were collected from the different regions of Jalgaon district. These samples were analysed by gas chromatography for the presence of pesticides in milk. The pesticides residues are Chlorpyrifos, Cyhalothrin, Cypermethrin, Dichlorvos, Dimethoate, Deltramethrin, Profenofos, Quinolphos, Trizophos, and Phorate. In research study it was found that, about 10 % of milk samples were contaminated with the pesticide residues. The GC method can be used for the identification and separation of pesticides residues in milk samples.The residues which are found in the milk samples are may be due to the unawerness of the farmers and due to lack of knwoldge regarding the pesticides residues and its harardous effects on human health.The research study can be useful for the awareness for the farmers and local peoples about the use of pesticides and their hazardous effects on human being.

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