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Pesticide Residue Analysis of Raw Milk Samples Using Paper Strip Assay Developed at NDRI andGC-MS/MS: A Case Study of Ajmer City, Rajasthan

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ABSTRACT

Pesticide pollution to the local environment also affects the lives of birds, wildlife, domestic animals, fish and livestock. Therefore, the determination of pesticide residues in the environment and in foods is necessary for ensuring that human exposure to contaminants especially by dietary intake does not exceed acceptable levels for health.Total 37 samples were analyzed using strip based test developed at ICAR-NDRI, Karnal. The result of the study showed that samples 18, 19, 20 and 26 were found positive which depict the presence of pesticides residue while in 33 samples there was absence of pesticides residues. Thefour samples were further analyzed with GC-MS/MS for further confirmation /quantification. The four samples of raw milk were found positive with GC-MS/MS analysis showed the presence of β – endosulfan (2.37-11.09 ppb), DDD (0.14-0.17 ppb), DDT (0.018 ppb). The samples were below regulatory MRL limits.

KEYWORDS: Cattle milk, Pesticide Residues, β-endosulfan, DDD, DDT,

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INTRODUCTION

Pesticides used in agriculture to control pests such as insects, weeds and plant diseases have been subject to considerable legislative, regulatory and consumer scrutiny over the past few decades. Pesticides are toxic in nature; since many pesticides may potentially leave residues on foods available for human consumption, there is much concern regarding the potential health risks of pesticides in the human diet. The problem of contamination of our food commodities especially milk and milk products by pesticide residues constitutes one of the most serious challenges to public health. The information on the levels of pesticide residues occurring in food commodities is essential and can be obtained through regular monitor procedures. The analytical surveys not only provide the current data on pesticide residue Contamination but also serve to indicate whether or not the principles of good agricultural practices are being followed. Moreover, they also provide information regarding the Government policies in context of the restriction or ban on the use of certain persistent chemicals which may pose serious hazards to environment and human. The residues monitoring programs are the key means of ensuring compliance with regulations. These also create a database to help in assessing the levels of pesticide residue and the levels of residue intake. Many countries including India in the world have established analytical laboratories to monitor residue levels in foodarticles (Sharma et al. (2007).

High level of pesticide residues in feedstuff from post-harvest treatment or by drift during commercial aerial application inhaled air or contaminated water. Feedstuff manufactured from plant material that has been treated during growing season with insecticides i.e. contaminated through feed, grass/hay use of insecticides directly on the cattle against disease vectors, use of insectides in stables for the treatment flies in milk processing for hygienic treatment against insects.

GEOGRAPHCAL AREA OF INVESTIGATION

The area under investigation, Ajmer is one of the developing cities known as ‘Heart of Rajasthan’. From ancient time it has been known as a political centre& British also made this as a political headquarter. It is also known as the main education centre. Besides this it has a combination of religious harmony due to famous mosque Dargah Sharif and Pushkar. The district is triangular in shape.

The area under investigation is centrally situated city of Rajasthan lies between 26°25’ to 26°29’ N latitudes and 74°37’ to 74°42’ E longitudes. Area of Ajmer is 8481.40 Sq. km and area of the Ajmer city is 241.56 Sq. km. It is represented by Aravalli hillocks, sand dunes, agricultural fields and fresh water bodies like Anasagar, Foysagar, Pushkar and BudhaPushkar.The entire length of Aravalli is about 692 km running from North-East (near Delhi) to South-West of Gujarat. The

central Aravalli covers the entire Ajmer region. The region has a thickness of argillaceous rocks. The district is endowed with a large number of non-metallic minerals e.g. asbestos, beryl emerald, feldspar, garnet, mica, and vermiculite4.17% of total area is covered under forests.

MATERIAL AND METHODS

Paper strip for rapid detection of pesticide residues in milk

A. Extraction of pesticide from milk

Extraction of pesticide from milk: Pesticide are extracted from spiked reconstituted skimmed milk (RSM) / natural milk sample as per following protocol:

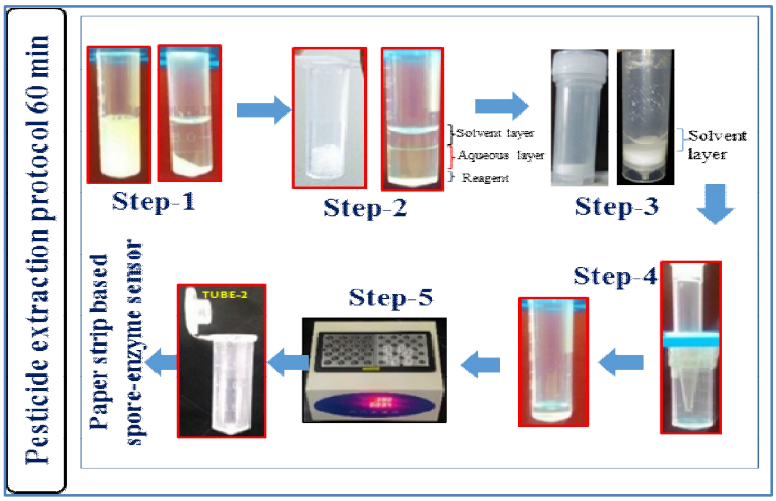
Step-1: Mix equal quantity of milk sample and organic solvent, vortex and centrifuge @ 10000 rpm for 5 min at 37 ^{o}C.

Step-2: Mix supernatant with clean up reagent (I), vortex and centrifuge @ 10000 rpm for 5 min at 37°C.

Step-3: Transfer solvent layer carefully to a tube containing cleanup reagent (II), vortex and centrifuge @ 10000 rpm for 5 min at 37°C.

Step-4: Separate out upper organic solvent layer and filter through specific filter tips.

Step-5: Evaporate filtrate using block heater at 80°C for 40 min.



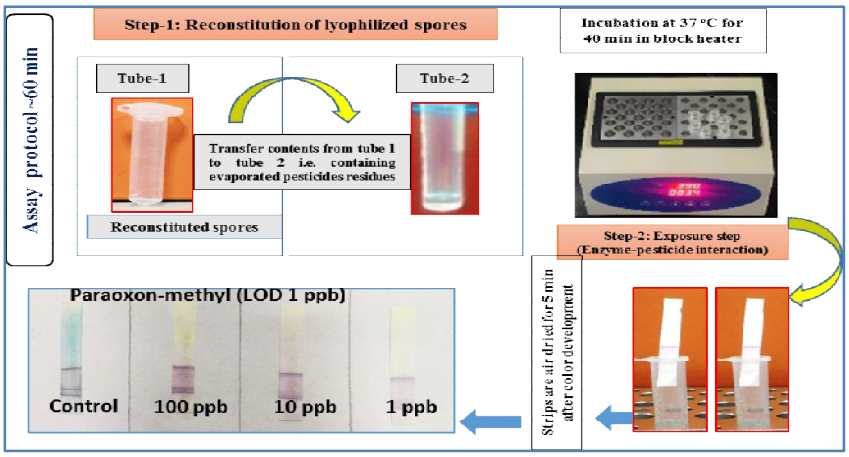
B. Paper-strip protocol

Step-1-Reconstitution of lyophilized spores: Add 30 µL of buffer to reconstitute lyophilized spores (Tube-1)

Step-2-Enzyme pesticide interaction: Transfer reconstituted spores from Tube-1 to Tube-2 containing evaporated pesticide residues from extracts of spiked / natural milk sample and incubate in dry block heater at 37 °C for 40 min and vortex for 25 sec.

Step-3-Addition of paper strip: Add paper-strip functionalized with chromogen to test and control tube and incubate in dry block heater at 37°C for 10-15 min. After incubation, paper strips are air dried for 5 min after color development in control tubes.

Result interpretation:Development of sky blue color on paper strip, indicates absence of pesticide and no blue color indicates presence of pesticide in milk as depicted in below mentioned figure



GC procedure for confirmation / quantification of pesticide residues in milk

Sample preparation protocol for GC

Step.1: 5mL of milk sample was taken in dry mortar, add 20g of silica gel and 15g of anhydrous sodium sulphate and mix using pestle to make free flowing powder

Step.2: Prepare a glass column by plugging glass wool and add 40mL of Dichloromethane (DCM)

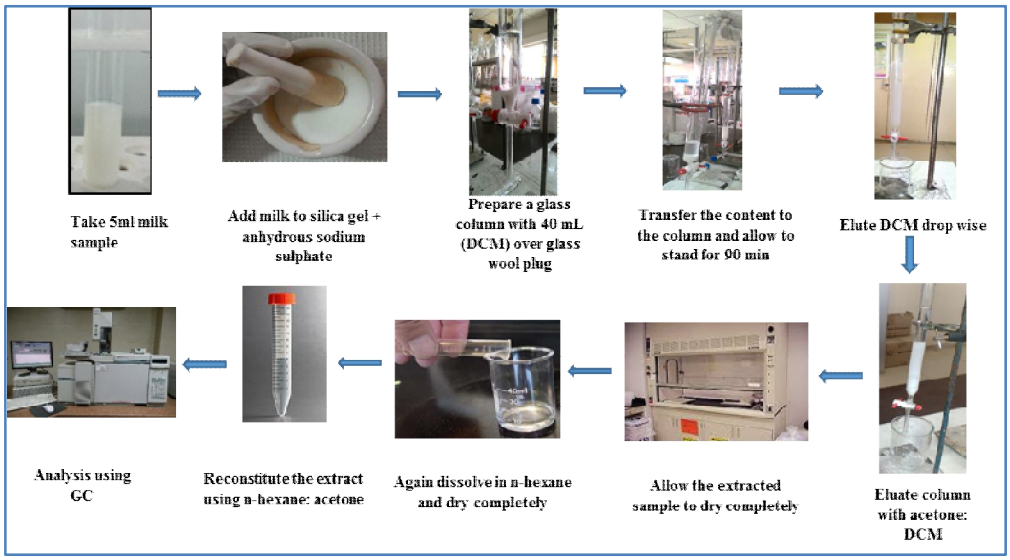
Step.3: Transfer the prepared content from step-1 into glass column. Allow to stand for 90 min.

Step.4: Elute DCM drop wise @ 20 drops/ min. Again eluate the column using 150mL of acetone and DCM in the ratio of 2:1 (v/v).

Step.5: Elute the solvent again and evaporate by keeping in fume board till the volume get concentrated.

Step.6: Dissolve the concentrate obtained using n-hexane by thorough shaking and again evaporate by keeping in fume board to ensure complete removal of DCM.

Step.7: The obtained extract or concentrate is reconstituted using 5mL of n-hexane and acetone in the ratio of 1:1 and used for further GC analysis.



RESULTS AND DISCUSSION

Table 1: Pesticide residue analysis of raw milk samples using paper strip assay developed at NDRI

Sample No.	Result interpretation on paper strip	Sample No.	Result interpretation on paper strip
1	Negative	21	Negative
2	Negative	22	Negative
3	Negative	23	Negative
4	Negative	24	Negative
5	Negative	25	Negative
6	Negative	26	**Partial positive**
7	Negative	27	Negative
8	Negative	28	Negative
9	Negative	29	Negative
10	Negative	30	Negative
11	Negative	31	Negative
12	Negative	32	Negative
13	Negative	33	Negative
14	Negative	34	Negative
15	Negative	35	Negative
16	Negative	36	Negative
17	Negative	37	Negative
18	**Partial positive**		
19	**Partial positive**		
20	**Positive**		

Result interpretation: 37 samples of raw milk brought which were analyzed using strip based test developed at ICAR-NDRI, Karnal. Out of total 33 samples showed typical blue color on paper strip were found negative while four samples (18, 19, 20 and 26) were found suspected for the presence of pesticide residues as shown in table no 1. These samples were further analyzed with GC-MS/MS for further confirmation /quantification (Table no 2).

Figure no 1: Showing positive and negative results in milk

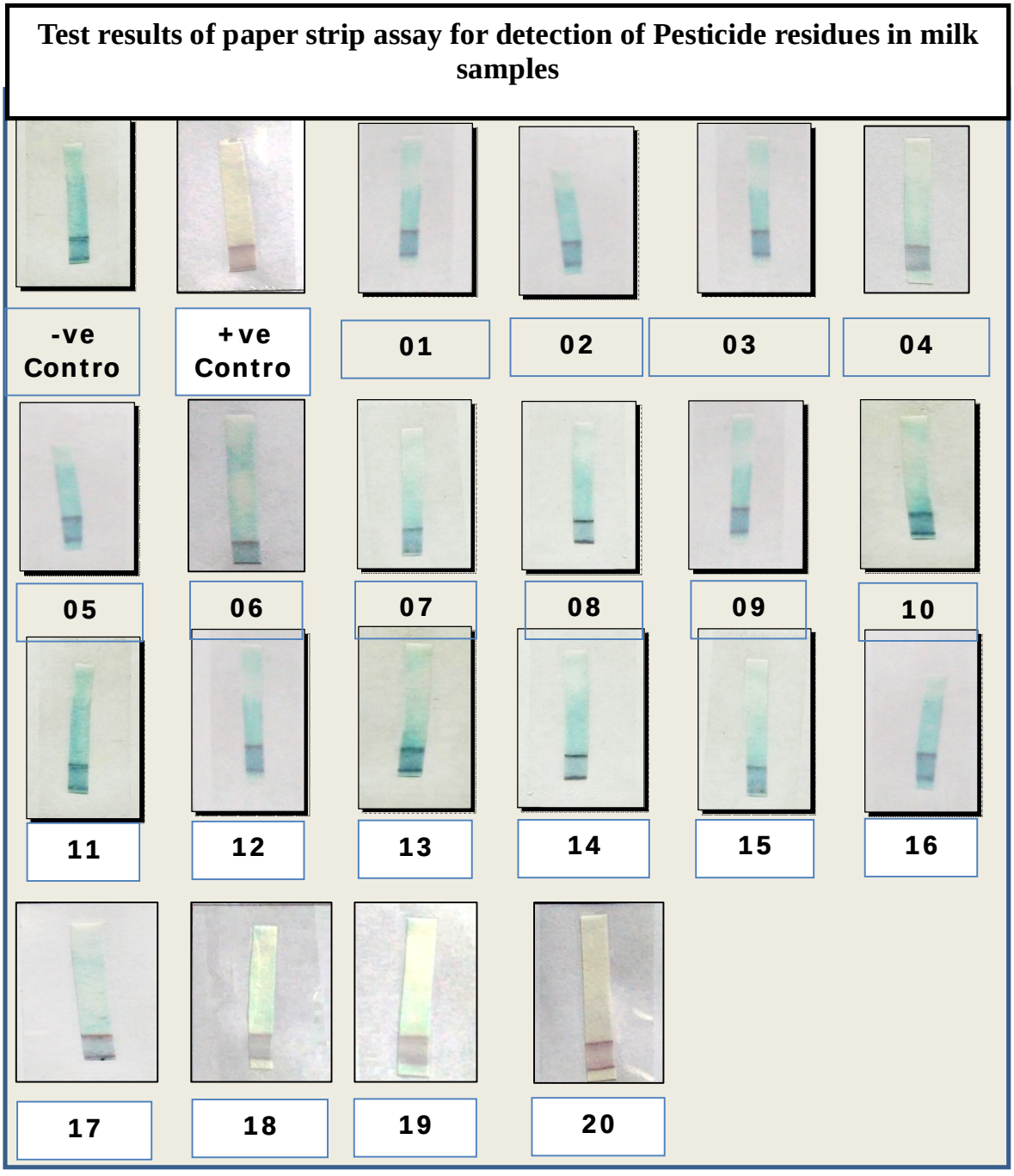


Table no 2 GC-MS/MS analysis of suspected samples of Raw milk

Sample Code	Results obtained by GC-MS/MS analysis		
	Name of Pesticide	Concentration (ppb)	Regulatory MRL (ppb)
Control	Pesticide negative raw milk	0.00	Absent
18	β - endosulfan	5.40	50
	DDD	0.17	150
19	β - endosulfan	2.37	50
	DDT	0.018	150
20	β - endosulfan	11.09	50
26	β - endosulfan	6.19	50
	DDD	0.14	150

Result interpretation: 4 suspected samples of raw milk were found positive with GC-MS/MS analysis. These samples showed the presence of β – endosulfan (2.37-11.09 ppb), DDD (0.14-0.17 ppb), DDT (0.018 ppb) at below regulatory MRL limits. The detailed results are shown in figures enclosed annexure –II.

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