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Assessment of developed paper strip based sensor with pesticide residues in different dairy environmental samples

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ABSTRACT

According to the results of this study, the paper strip biosensor can detect pesticide at very low concentration like fungicide, organochlorine, organophosphate, carbamate, and herbicide group ranges from 1 to 10, 1–50, 250–500, 1–50, and 1 μg/L, respectively in animal feed, water, milk and soil. This is a significant improvement from the previous study, which found that the paper strip biosensor could only detect pesticide levels of up to 500 or 1000 μg/L. A total of 436 samples were collected from the dairy farm, including 58 samples of green feed, 54 samples of dry feed, 45 samples of concentrated feed, 41 samples of fermented feed, 49 samples of manure, 54 samples of soil, and 86 samples of milk. PSA (Primary Secondary Amine) and $MgSO_4$ (1:2 ratio) were used to remove pigments from dairy farm samples to prevent the enzyme–pesticide interaction leading to colour development on the strip, which was successfully achieved. Using a strip-based test and an optimized extraction protocol, pesticides were detected in 38.49% in the samples. Limit of Detection of 15 pesticides from the organochlorine, organophosphate, carbamate, neonicotinoid, pyrethroid, ryanoid, strobilurins, and triazole groups recommended for use in dairy farms were evaluated in feed/fodder. Pesticides were being detected in various dairy farm matrices using the newly developed test. The developed technology can be used as a semi-quantitative test for pesticides monitoring in the dairy farm as well as for screening of primary produce under field condition for organic certification of various food/feed commodities.

1. Introduction

Pesticides are being used extensively all over the world and their persistence in the environment has led to widespread contamination of various food commodities such as milk, food grains, vegetables, and animal products (Carvalho, 2006). Pesticides are commonly used in agriculture for a number of reasons, including increasing crop yields to meet the needs of an ever-growing global population and protecting crops from pests and insect-borne diseases. Pesticide residues in food have been linked to a wide range of adverse effects on human health, from short-term irritation to long-term harm (Grewal, 2017). The potential risk of pesticides to public health and their use in agriculture is subjected to constant monitoring. Animal feed and fodder act as the main sources of pesticides in the animal body. When these pesticides enter into an animal's internal system, these toxins get accumulated and affect the animal's body as well as human and human health by the usage of animal origin foods like meat, milk, and milk products. Animal-derived products are frequently found to be contaminated with toxic residues that have a long persistence. Until and unless these pesticides will not be control in feed and fodder, it will continuously enter into animal tissue and therefore, pesticides monitoring programmes need to be focused on animal feed, food crops, fruit and vegetables. Few reports are available regarding the status of pesticide residues in animal feed and fodder (Nag and Raikwar, 2008).

In India, the vast majority of the people are engaged in the agriculture profession and are highly exposed to the pesticides used in

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agriculture. These harmful pesticides pass into the human food chain by the heavy exposure to agricultural crops and degraded compounds in various food chain components like soil, water, and atmosphere and bio-accumulation of persistent pesticides in food products of animal origin like meat, fish, eggs, and milk. In animal feed and fodder, different pesticide residues can be transferred into herbivores through the food chain of the animals.

Monitoring the presence of toxic compounds like pesticides is important because their presence at trace levels requires highly sensitive techniques (Mishra et al., 2012). For the detection of pesticide residues in the samples of dairy farm, both qualitative as well as quantitative methods are available, that are mostly conventional methods, including AOAC approved Gas Chromatography-Mass Spectrometry (GC-MS), Liquid Chromatography-Mass Spectrometry (LC-MS) and other spectroscopic methods (Liu et al., 2013). immunoassays are yet another technique for detecting pesticides in the field condition. Hapten creation and antibody preparation are the two stages that are typically involved in immunoassays. To obtain the full antigen of pesticides, hapten formation is necessary (Jin et al., 2017). Building efficient haptens for immunoassay procedures presents significant hurdles because certain pesticides are hydrophobic compounds. These techniques are relatively restrictive for a wide variety of pesticides since they need specific, unique haptens, which makes them difficult to create due to hapten manufacturing procedures (Zhang et al., 2022). These techniques are sensitive, efficient, and reliable, but they are time-consuming, laborious, require complex sample preparation steps, complicated to employ a single test for a wide range of pesticides and are difficult to use under field conditions. So, there is a requirement and an emerging demand for the development of fast screening methods in order to have high throughput analysis of samples at lower cost, time, and resources.

As a low-cost, portable, disposable analytical device that can be used in a wide range of applications, paper-based sensors have emerged as a new alternative technology. Use of paper as a sensing platform is made possible by the unique properties of the material, which allow passive liquid transport, compatibility with chemical and biochemicals, and rapid response. Assessment of paper strip biosensor begins with an appropriate choice and proportioning of paper, fabrication and patterning after that, a quantitative analysis is performed. Research work using strip-based technology employing bacterial spores were initiated in our laboratory. In the early stages of developing a bio-recognition element for antibiotic residues and aflatoxin M1 in milk, bacterial spores were found to be useful because of their ability to germinate and release DPA/marker enzymes (Kumar et al., 2006, 2010, 2014). Most of the pesticide biosensors developed in prior art make use of enzymes especially acetylcholine esterase (AChE) as bio-recognition molecule and their working is based on the monitoring of enzyme inhibition in the presence of pesticides. Our research group explored the detection of pesticides employing novel marker enzyme from prokaryotic source based on the principle of inhibition of specific marker enzyme derived from specific spore-forming bacteria followed by their reaction with chromogenic substrate functionalized on paper-strip indicating semi-quantitative detection of target analyte through colour change. This principle/concept was developed and transformed on the paper strip for its working in milk and milk products, cereal based food and fruit juices (Dasriya et al., 2021; Tehri, 2017; Gopaul, 2015; Morab, 2016; Harshita, 2017; Ritu, 2018). In present study, the scope of application of spore-based biosensor was extended successfully for detection of pesticides in cattle feed, fodder, fermented feed, manure, soil, water for its application in dairy farm system in India.

2. Materials and methods

2.1. Media and chemicals

All media ingredients for preparation of nutrient agar, tryptone glucose yeast extract, and sporulation media are procured from Himedia. All used chemicals and solvents were of analytical grade and were procured from Sigma Aldrich, U.S.A; Hi-Media, Mumbai, India, Fisher Scientific, HPLC Grade, U.K. and *Supleco, Sigma-Aldrich, U.S.A*. Chromogenic substrates, pesticides, potassium phosphate buffer components sugars (Sucrose) magnesium sulphate, anhydrous, A.R. activated charcoal primary secondary amines are procured from *Supleco, Sigma Aldrich, U.S.A*. Organic solvents, acetone, and acetonitrile were purchased from *Fisher Scientific, HPLC Grade, U.K*.

2.2. Instrumentation

The following instruments were used in our experiments including water purifier (Milli-Q Academic; Milli-Q), vortex instrument (MS 3 basic; IKA), Multimode plate reader (Tecan), Bio-safety Level-II cabinet (Esco Biotech Pvt. Ltd., India), Incubator shaker (Eppendorf, Inc., USA.), centrifuge (Eppendorf, U.S.A.), −20°C Deep Freeze (Bluestar, India) and ultraviolet–visible spectrometer (NanoDrop 2000c; Thermo Fisher Scientific) were available at National Referral centre for milk quality and safety, ICAR-NDRI, Karnal, India.

2.3. Production of B. megaterium MTCC 2949 spore

2.3.1. Microbial cultures

The strains of *Bacillus megaterium* (MTCC 2949), used in this study was procured from Microbial Type Culture Collection (MTCC), IMTECH, Chandigarh, India.

2.4. Revival of cultures

Freeze dried form of strain was transferred in tube containing 5.0 mL of nutrient broth and incubated at 37°C for 24.0 ± 2.0 h for its revival. Following incubation, a loopful of revived culture was streaked on nutrient agar medium and incubated at 37°C for 16.0 ± 2.0 h. Purity of culture was examined microscopically after Gram stain and spore staining.

Single pure colony of *B. megaterium* MTCC 2949 was streaked on nutrient agar plate and incubated at 37°C for 16 ± 2 h. After overnight growth, single pure colony from plate was transferred into 5.0 mL propagation medium (TGY) and incubated at 37°C for 24 h. One hundred mL of growth medium (TGY) was inoculated with overnight grown culture in propagation medium (1%), followed by incubation at 37°C for 48 h. After incubation, culture from growth medium was further inoculated in 100 mL of sporulation medium (7.5%) for spore production. The final incubation was carried out at 37°C for 42 h followed by harvesting of spores by centrifugation at 10,000 rpm for 10 min at 10°C. The pellet containing spores was washed twice using potassium phosphate buffer (pH 6.8, 10 mM) by centrifugation under similar condition. The final suspension was prepared by dissolving the pellet in 10 mM potassium phosphate buffer at pH 6.8 as described in the Fig. 1. The spore suspension was analysed for total viable count and spore count (Feeters et al., 2001) and spores (%) was calculated. The total count that included both the vegetative cells and spores was enumerated using unheated spores suspension, while spores were enumerated after heating the final suspension at 80°C for 10 min in AccuBlock™ Digital Dry Bath (Labnet International, Inc., U.S.A) (Kumar et al., 2015).

The O.D. of spore suspension was set to 0.320 ± 0.02 using 10 mM potassium phosphate buffer (pH 6.8) as a diluent at 595 ± 5 nm using microbiological plate reader. The spores were stored under refrigeration at 4°C, till further use (Kumar et al., 2010)

Evaluate the spores for its enzyme activity before lyophilization of spores by dispensing 20–40 μL of final spore (OD 0.32 ± 0.02) in microcentrifuge containing 30 μL phosphate buffer (10 mM). Add functionalized paper strip followed by incubation at 37°C for 10–20 min. Observed blue colour for recording to test the time taken for the enzyme activity in the spores. Then, lyophilize the 20 μL of spore using lyophilizer at −84 ± 1°C under vacuum of 1 ± 0.5 torr (1 Torr = 133.33 Pa) for

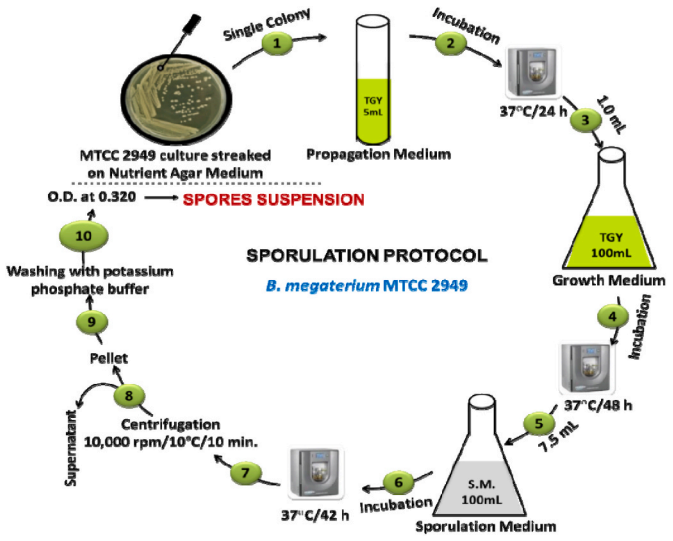

Fig. 1. Spore production in *B. megaterium* MTCC 2949 Lyophilisation of spores.

1hr. After finishing it packed in a plastic bag and stored at −20°C and 4°C.

2.5. Preparation of functional paper strip

The strip making procedure was improved by employing a poly sheet base and onto which a 1.0 cm wide strip of Whatman filter paper was pasted using double adhesive tape. The details procedure for fictionalization of strip was prepared as per the protocol explained in Kumar et al. (2015).

2.6. Preparation of paper strip

The chromogenic substrate (20 mM) solution was prepared by dissolving 3.5 mg in 1 mL of acetone and was loaded on paper having a dimension of 30 × 0.5 cm2 using the Easy printer. This system enabled precise dispensing of microliter volumes of substrate at specific locations in the form of a band on paper. First, the substrate was loaded into the reservoir using a micropipette (approx. 400 μL), the needle dispenses the substrate as a stream or minute droplets at a specific location on the paper to form pink band. This paper was further allowed to dry at 37°C for 30 min, followed by cutting of paper to form strips with dimensions of 0.5 × 3.0 cm and stored. Trials were conducted with acetonitrile and model pesticide using these prepared paper-strips functionalized with substrate to check for their working performance, consistency, and reproducibility of the results using developed sensor. Furthermore, the produced strips were vacuum packed using an INDVAC vacuum packaging machine.

2.7. Optimization of pesticide extraction protocol

Pesticide residues from milk samples were extracted according to the method of the Acetate QuEChERS method. For the simplicity and commercial point of view, the extraction procedure was optimized according to the nature of sample. Feed samples are mainly composed of starchy materials, which change their nature after treatment (dry, green, fermented, and concentrated). After optimization of all parameters like quantity of sample, exposure time, volume of homogeneous mixed sample, clean up reagent, amount of reagent 1 (Sucrose), a consolidated protocol was finalized. This protocol was applied for the extraction of pesticide residues from different cattle yard samples, dried in a block heater and tested with a developed paper strip test.

The following are the steps for the extraction of pesticides from different cattle feed. One gram of feed sample was taken in 10 ml of distilled water and vortexed for 1 min after vertexing, the sample was kept for 30 min without any disturbance. An equal volume of reconstituted feed sample and acetonitrile (0.75 ml) was taken in a micro-centrifuge tube, vortexed for 1 min and further centrifuged at 10,000 rpm for 5 min at 37°C. A supernatant of 1200–1300 μL was added to a micro-centrifuge tube followed by addition of 0.25g of sucrose into the centrifuge tube and vortexed until all sucrose particles were completely dissolved. Further, the preparation was centrifuged at 10,000 rpm for 5 min at 37°C (Fig. 2). The top solvent layer was removed and transferred into a centrifuge tube containing 0.25 g of clean-up reagent 2 (PSA and MgSO4 in 1:2 ratio) and vortexed. The preparation was centrifuged at 10,000 rpm for 5 min at 37°C. The upper layer, ~250 μL, was collected, filtered through specialised filter tips into a micro-centrifuge tube, and evaporated using a dry block heater at 80°C for 40 min. The tube containing pesticide residue (Tube-2) was used to carry out paper–strip assay as explained by Dasriya et al. (2021).

2.8. Strip assay

Assay protocol for spore-based biosensor on paper-strip, Morab (2016) optimized spore enzyme-based sensor for detection of pesticides after extraction and drying consisted of three distinct steps: reconstitution of lyophilized spores, enzyme-pesticide exposure and enzyme substrate reaction (Morab, 2016). Initially, reconstitution of lyophilized spores (O.D. 0.320 ± 0.02) with 30 μL of potassium phosphate buffer (pH 6.8). The reconstituted spores were transferred to tubes containing residues left after evaporation of ~250 μL. Contents of each tube were then mixed by vortexing for 1 min. After mixing, tubes were allowed to incubate at 37°C for a period of 40 min. Followed by enzyme substrate reaction after exposure, the tubes were vortexed for 25 s and the functionalized paper-strips were added to each tube and subsequent incubation was carried out at 37°C for 10–15 min. Following incubation, tubes were observed for blue colour development in acetonitrile (control) for qualitative determination.The interpretation of the results is that the development of blue colour on paperstrip indicated the absence of pesticide residues, and less or no blue colour development on paper-strip when observed visually indicated the presence of pesticide residues.

2.9. GC–MS/MS analysis

In a 50 mL polypropylene tube, combine an equal amount of the homogeneous feed sample and the acetonitrile. After keeping this mixture at room temperature for 5 min, 6.0 g of MgSO4 and 1.5 g of sodium acetate were added. Vertex was then used to ensure proper mixing before the mixture underwent centrifugation at 6000 rpm for 10°C. Then, collect 1.5 mL of supernatant and transferred it to dispersive solid-phase extraction tubes (d-SPE). Afterward vortex it properly for 1 min and centrifuge was carried out at 6000 rpm at 10°C for 5.0 min approximately, 1 mL of the clear extract was injected into GC for analysis. Estimation of pesticide performed by GC–MS/MS composed with SLB-5MS, (30 m × 0.25 mm × 0.25 μm, Supelco, Sigma Aldrich) and TQ 8030 triple quadruple detector. The initial temperature of the GC oven was 80°C for 2 min and raised at 20°C/min up to 180°C with no holding period and further, it was raised for 5°C/min up to 300 °C for 3 min. Electron impact mode was used for performing mass spectrometry and ionization energy was 70 eV with solvent delay time 3 min. The quadruple detector voltage was 0.6 kV. The injection temperature was 250°C and the carrier gas used was helium.

2.10. Statistical analysis

Pesticide detection data generated through paper strip sensor and GC–MS/MS analysis were assessed for accuracy, repeatability, precision, limit of detection, correlation coefficient, limit of quantification, reproducibility depend on the method given by NATA 22 (2012). All the

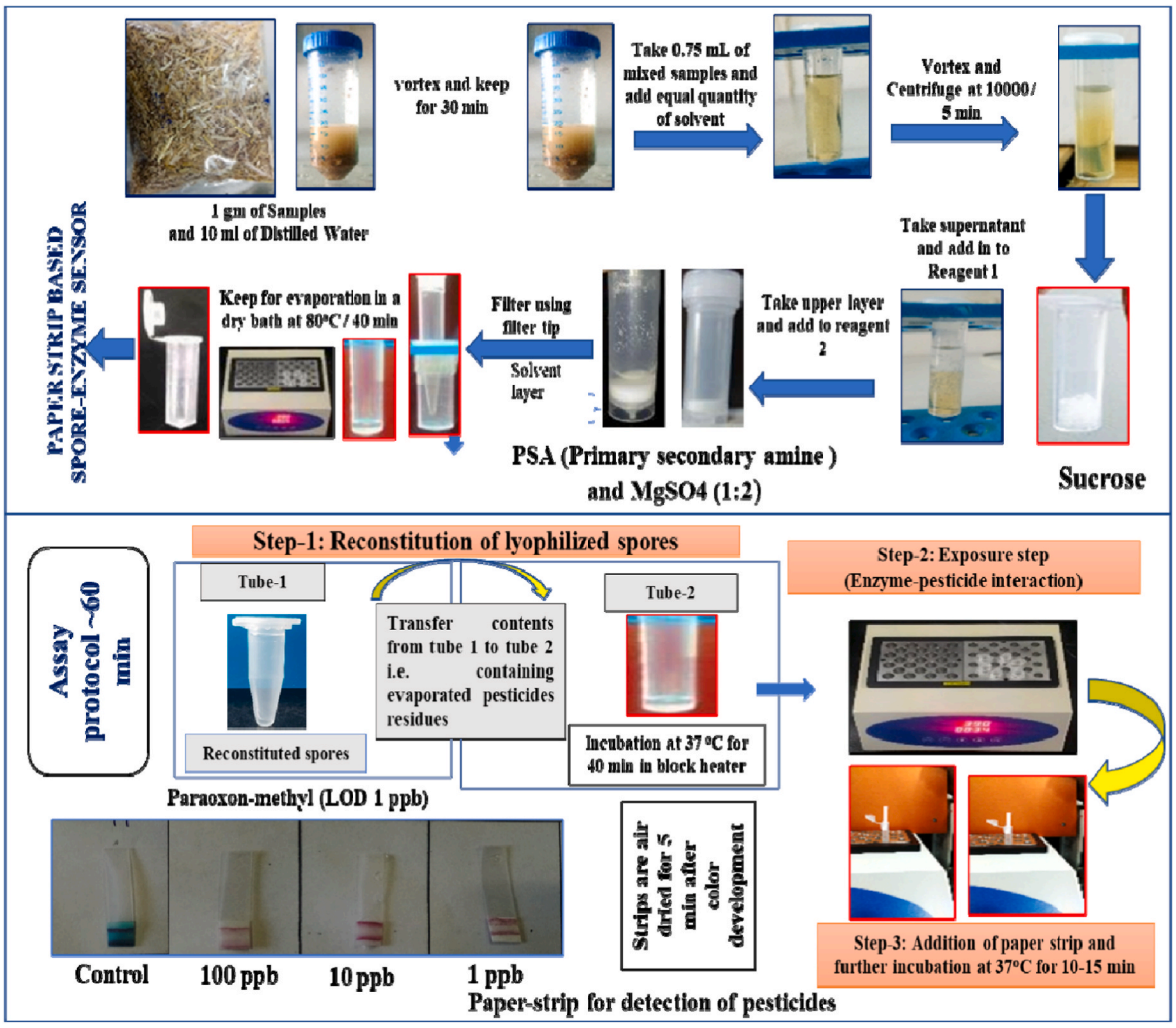

Fig. 2. Overall extraction and Paper strip assay for rapid detection of pesticide residues in animal feed (A) Protocol for extraction and screening of Pesticide residues in food products. (B) Color of Paper strip before (Colorless) and after (Blue) incubation in the detection of pesticide residues. (− ve) sample blue color, (+ ve) sample—Colorless. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 1
LOD of different group of pesticide from animal feed sample. Wheat straw, Rice straw, Soybean straw, Alfalfa straw, Barley straw, Maize fodder, Fodder of cereal grains.

S. No	Pesticide	Target	Matrix	MRL (mg/L)	LODs in pure system (mg/L)	LODs Spiked (mg/L)
1	Bitertanol	Fungicides	Wheat straw/fodder	0.05	0.05	0.05
2	Carbaryl	Insecticide	Wheat straw/fodder	30	0.2	0.2
3	Carbofuron	Insecticide	Soybean fodder	0.5	0.5	0.5
4	Chlorantraniliprole	Insecticide	Alfalfa straw/fodder	0.05	0.01	0.01
5	Dinotefuron	Insecticide	Rice straw/fodder	6	0.05	0.05
6	Dimethioate	Insecticide	Wheat straw/fodder	1	0.05	0.05
7	Fenpropathrin	Insecticide	Soybean straw/fodder	2	0.05	0.05
8	Flubendiamide	Insecticide	Soybean straw/fodder	0.1	0.05	0.05
9	Indoxacarb	Insecticide	Maize fodder	25	0.05	0.05
10	Imidaclopride	Insecticide	Barley straw/fodder	1	0.5	0.5
11	Lindane	Insecticide	Fodder of cereal grains	0.01	0.05	0.05
12	Melathion	Insecticide	Maize fodder	0.05	0.05	0.05
13	Phorate	Insecticide	Maize fodder	50	0.05	0.05
14	Pyraclostrobin	Fungicides	Straw/Fodder of cereal grains	0.03	0.01	0.01
15	Thiamethoxam	Insecticide	Wheat straw/fodder	2	0.01	0.01

experiments were carried out in triplicate (n = 3).

3. Results and discussion

3.1. Limit of detection (LOD) of paper strip for pesticide in spiked food sample

In the current study, organic solvent was used as a diluent to prepare various pesticide concentrations for use in paper strip assays for LOD detection and verification. To check existence of pesticide residues these group of pesticides have been used like organochlorine, carbamate, organophosphate, fungicide and herbicide. The spores of bacillus species germinate into vegetative cells in the tube (2 ml), followed by the release of marker enzyme, and the activity of the marker enzyme is hindered due to presence of pesticide residues. Different concentration for each group of pesticides has been prepared in the organic solvent and detected using the paper strip assay. LOD's of pesticide in pure system and after extraction from different cattle feed are as shown in Table 1.

3.2. Detection of pesticide residues

3.2.1. Soil samples

Among 54 soil samples, 6 samples were found to be positive for pesticide residues with an overall incidence of 11.11% ((more than one in ten) shown in Fig. 3. The survey conducted by Ahad and their co-worker (2010) found that soil is mainly contaminated with DDTs (Dichlorodiphenyltrichloroethane) followed by BHC (Benzene Hexachloride).

3.3. Manure samples

In total, 2 of the 49 samples of manure tested positive for pesticides residues. There were 49 samples of manure collected in the northern state of India. As per the protocol, the samples were tested for pesticide residues using a paper strip-based biosensor. It was found that 4.081% of the 49 samples tested positive for pesticide residue. (Fig. 4). Pesticides such as DDT and lindane were found in high concentrations in water samples taken from Punjab, according to one survey (Ahad et al., 2010).

3.4. Water samples

Forty nice water samples were collected from different part of north India, among these collected samples 3 samples got positive (Fig. 5) using evaluated developed paper strip-based biosensor using the protocol described in the Fig. 2. It was reported in one of the survey studies that water samples collected from Punjab were heavily contaminated with pesticides like DDT, Lindane etc. (Ahad et al., 2010)

3.5. Raw milk

Paper strip-based biosensors based on colour change from pink or colourless to blue colour on paper strip were used to test 86 milk samples for pesticide residues sourced from the local dairy farm and market. Overall, 15.116% of the 86 samples tested for pesticides; this included 13 samples (7 raw and 6 pasteurized milk) (Fig. 6).

3.6. Cattle feed and fodder sample

Among 54 samples of green feed samples that were evaluated for pesticide residues using a developed paper strip based biosensor, 5 samples were found positive for pesticide residues with an overall incidence of 9.259%. In the case of dry feed samples, 3 out of 58 samples were found to be contaminated with pesticide residues (5.1724%). Among 45 concentrated mix samples, 4 samples were found to be positive for pesticide residues (8.88%), and of 41 fermented feed samples, 2 samples were found to be contaminated with pesticide residues. (4.87%). Overall, in cattle feed and fodder samples were analysed for pesticide residues using paper strip based biosensors (n = 198), about 12 samples did not show any blue colour (remain white) on the paper strip biosensor after incubation at 37°C for an exposure time of 30 min, with an overall incidence in cattle feed and fodder samples were found to be 7.070% (Fig. 7).

3.7. GC–MS/MS analysis

The 38 samples were found positive among 436 samples for pesticide residues including green feed, dry feed, concentrated feed, fermented feed, manure, water, soil and milk samples using paper-based strip sensor. Pesticide contaminated positive samples were evaluated for pesticide residues quantitatively by GC–MS/MS. The required condition for "multiple reactions monitoring (MRM) method "with the GC–MS/MS was optimized for the study of pesticide residues in food samples (Table 2). In GC–MS/MS analysis about 12 pesticides were targeted from two groups that include organochlorine (Aldrin, dieldrin, endosulfan, and DDT) and organophosphate (Fenithrothion, Chlorpyrifos-methyl, Monochrotofos, Diazinon, Malathion, Phorate, and Chloropyrifos). Among 38 positive samples out of 436 samples (including green feed, dry fees, concentrated feed, fermented feed, manure, water, soil and milk) shows contamination of 4 different group of pesticide at below as well as above the MRL by the usage of GC–MS/MS analysis. The samples of green feed, dry feed, and concentrated feed were found positive for the presence of Dichlorvos, Chlorpyrifos, Malathion and Dichlorvos pesticide residues at above MRL level prescribed by the Codex Alimentarius Commission (Table 1). α- Endosulfan, β-Endosulfan, Fenitrothion, DDT and DDD these are some other pesticides which were found at very low concentration that are detect below the MRL level using GC–MS/MS and same result (positive) were shown by our developed paper strip biosensor. The developed paper strip biosensor can be a

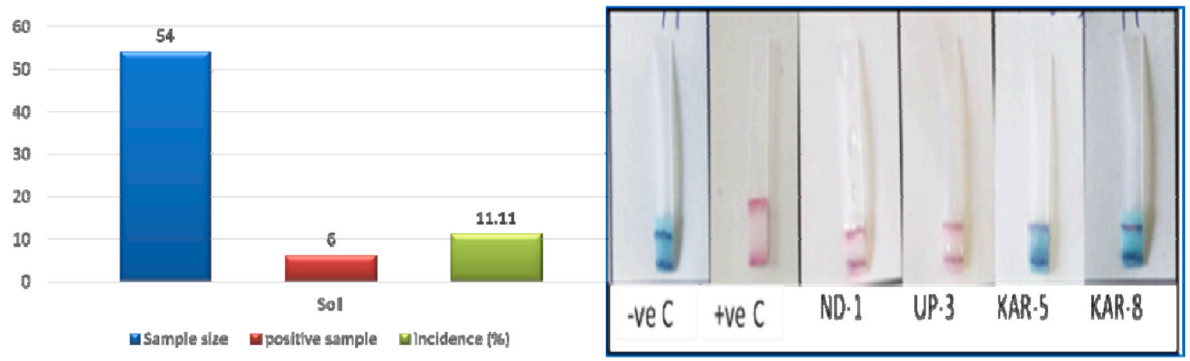

Fig. 3. Incidences of pesticides in soil samples.

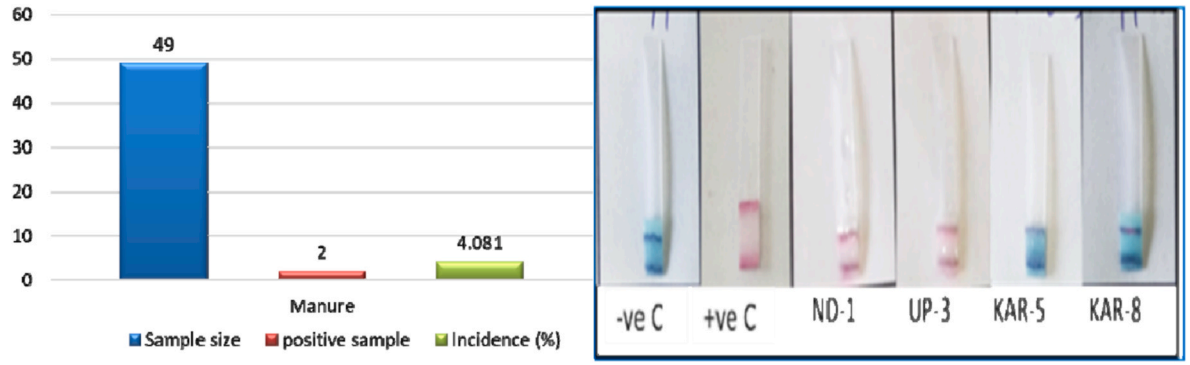

Fig. 4. Incidences of pesticides in manure.

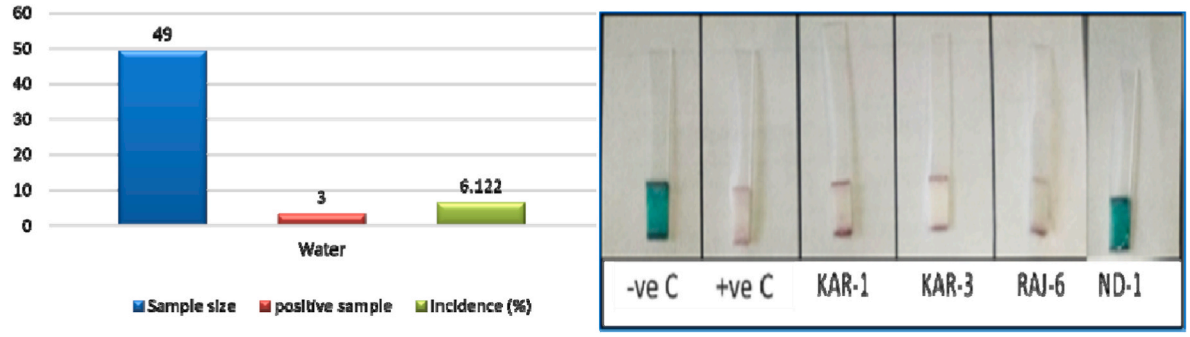

Fig. 5. Incidences of pesticides in water sample.

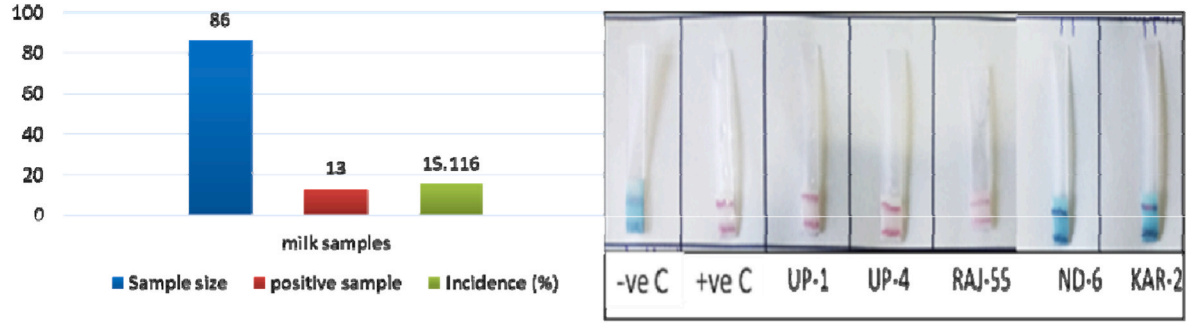

Fig. 6. Incidences of pesticides in milk sample.

promising tool in the screening of pesticide residues in a large number of milk samples, green feed, dry feed, concentrated feed, fermented feed, manure, water, soil samples, based on the above comparison analysis (see Fig. 8).

The developed paper strip biosensor can be a promising tool in the screening of pesticide residues in a large number of milk samples, green feed, dry feed, concentrated feed, fermented feed, manure, and water, soil samples, based on the above comparison analysis. In one of the studies, 301 out of 533 feed samples were found positive for pesticides like endosulfan, DDTs, HCH Isomers and dicofol (Nag and Raikwar, 2008). In a similar study conducted using GC-MS, dry and green fodder from rural areas of Haryana were found to be positive for organochlorine pesticides (OCPs) and endosulfan residues (Koli and Bhardwaj, 2018). Studies using GC–Mass Spectrometer carried out as a part of various surveys indicated the presence of Lindane in feed sample (Bedi et al., 2013). Lindane contamination was also reported in water samples (78–89%) using conventional GC method (Ahad et al., 2010). In a similar study carried out by Klarich Wong et al. (2019) reported the presence of Thiamethoxam in all the tap water samples analysed by LC-MS at a concentration ranging between 0.24 and 4.15 ng/L. Karabasanavar et al. (2015) reported the presence of endosulphan residues in 40% of feed and 44% of fodder analysed in which 22.5% of animal feed samples contained endosulphan residues in excess of the prescribed MRL. The Optimized conditions of multiple reactions monitoring (MRM) method for pesticide analysis were given in Table 2. The confirmation of pesticides residues in environmental, milk and cattle feed samples quantitatively by GC–MS/MS were shown in Fig. 8.

4. Conclusions

It is concluded that the developed extraction protocol with novel intervention is robust as well as unique in terms of pesticides recovery from complex matrices like feed and fodder. The developed technology has repeatability, reproducibility and portable, ready to use under field conditions. LOD's of the pesticides under consideration were achieved at or below their MRLs in different cattle feed and fodder samples as defined by the regulatory bodies. It was observed that developed extraction and assay protocol is sensitive to broad spectrum group of

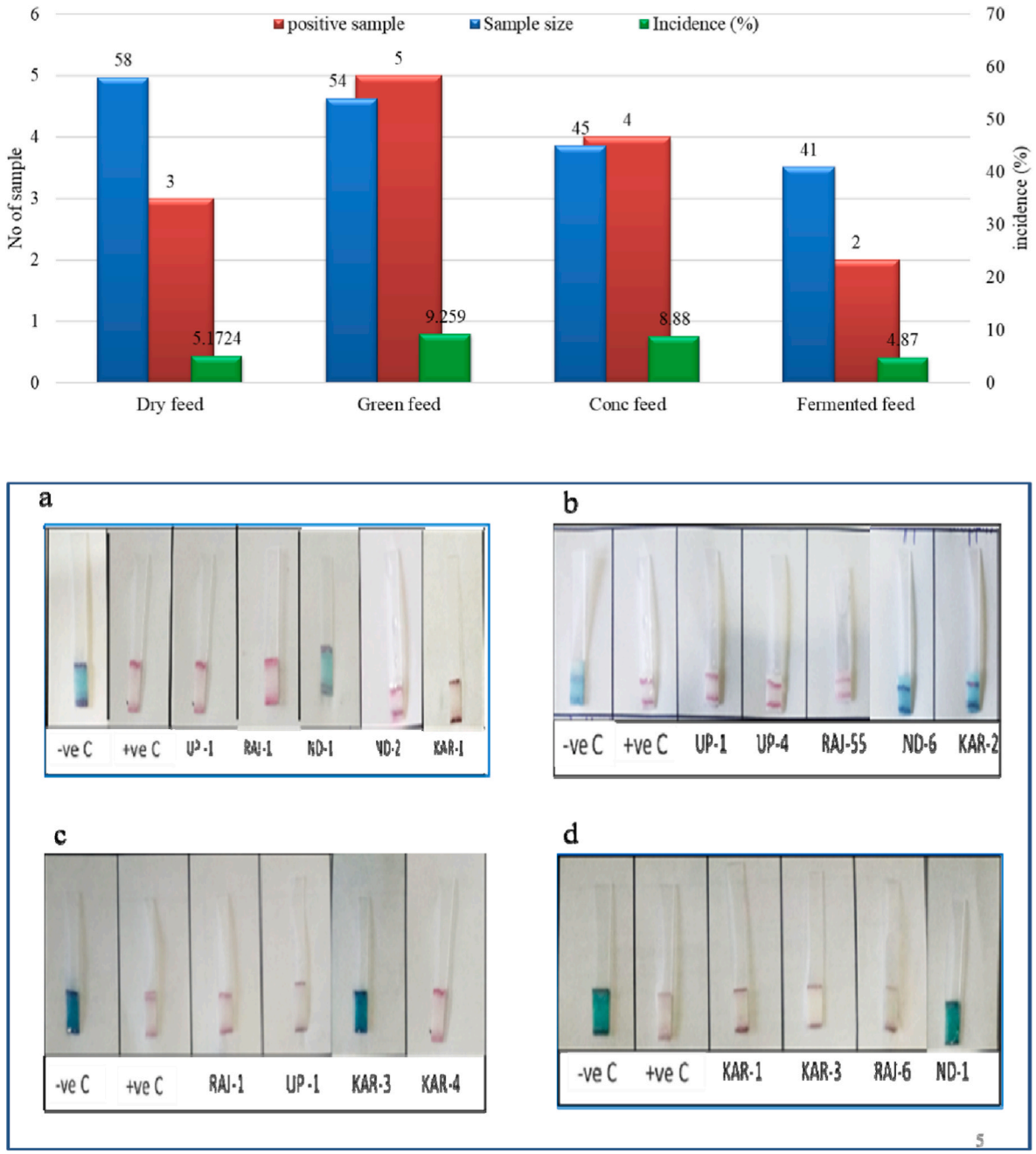

Fig. 7. Evaluation of developed assay under field conditions and incidences of pesticide residues (a-Dry feed, b-Green feed, c-Concentrated feed and d-Fermented feed). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

pesticides even at 10–100 μg/L concentration. There was no interference of matrix components like pigments. Developed assay can be explored as rapid and cost-effective technology and a substitute for screening of pesticide residues in different food and feed industries.

Funding

(information that explains whether and by whom the research was supported)

Ethics approval

(include appropriate approvals or waivers)

Consent to participate

(include appropriate statements)

Consent for publication

(include appropriate statements)

Availability of data and material

(data transparency)

Code availability

(software application or custom code)

Table 2
Optimized conditions of multiple reactions monitoring (MRM) method for pesticide analysis.

Compound	Start time	End time	Event time	CH-1 (m/z)	CE	CH-2 (m/z)	CE	Q1 Resolution	Q3 Resolution
Dichlorvos	2.5	3.12	0.15	121.10 > 103.00	12	121.10 > 103.00	14	Low	Low
Malathion	8.2	8.55	0.05	170.10 > 97.00	14	170.10 > 127.00	6	Low	Low
Chlorpyrifos	8.5	9.15	0.06	318.60 > 240.30	14	319.90 > 290.10	8	Low	Low
Monocrotophos	9.30	10.35	0.15	125.00 > 110.10	12	125.00 > 95.00	16	Low	Low
Phorate	9.25	10.65	0.15	259.00 > 73.00	8	259.00 > 232.10	6	Low	Low
Diazinon	11.44	12.55	0.3	307.00 > 180.10	8	307.00 > 165.00	6	Low	Low
Chlorpyrifos-methyl	12.25	14.11	0.06	288.00 > 92.80	22	288.00 > 271.00	14	Low	Low
Fenitrothion	12.11	14.66	0.05	276.90 > 261.00	6	276.90 > 108.10	14	Low	Low
Aldrin	12.35	14.47	0.05	263.00 > 192.50	28	263.00 > 202.90	26	Low	Low
alpha-Endosulfan	15.75	18.32	0.10	339.00 > 161.00	18	339.00 > 267.00	8	Low	Low
Dieldrin	15.75	18.32	0.12	277.10 > 240.00	10	277.10 > 171.00	38	Low	Low
beta-Endosulfan	17.25	18.66	0.16	339.00 > 159.90	18	339.00 > 267.00	8	Low	Low
p,p'-DDD	18.11	19.95	0.18	236.00 > 164.00	24	236.00 > 200.00	14	Low	Low
p,p'-DDT	19.61	20.75	0.3	234.80 > 166.00	22	234.80 > 198.00	18	Low	Low

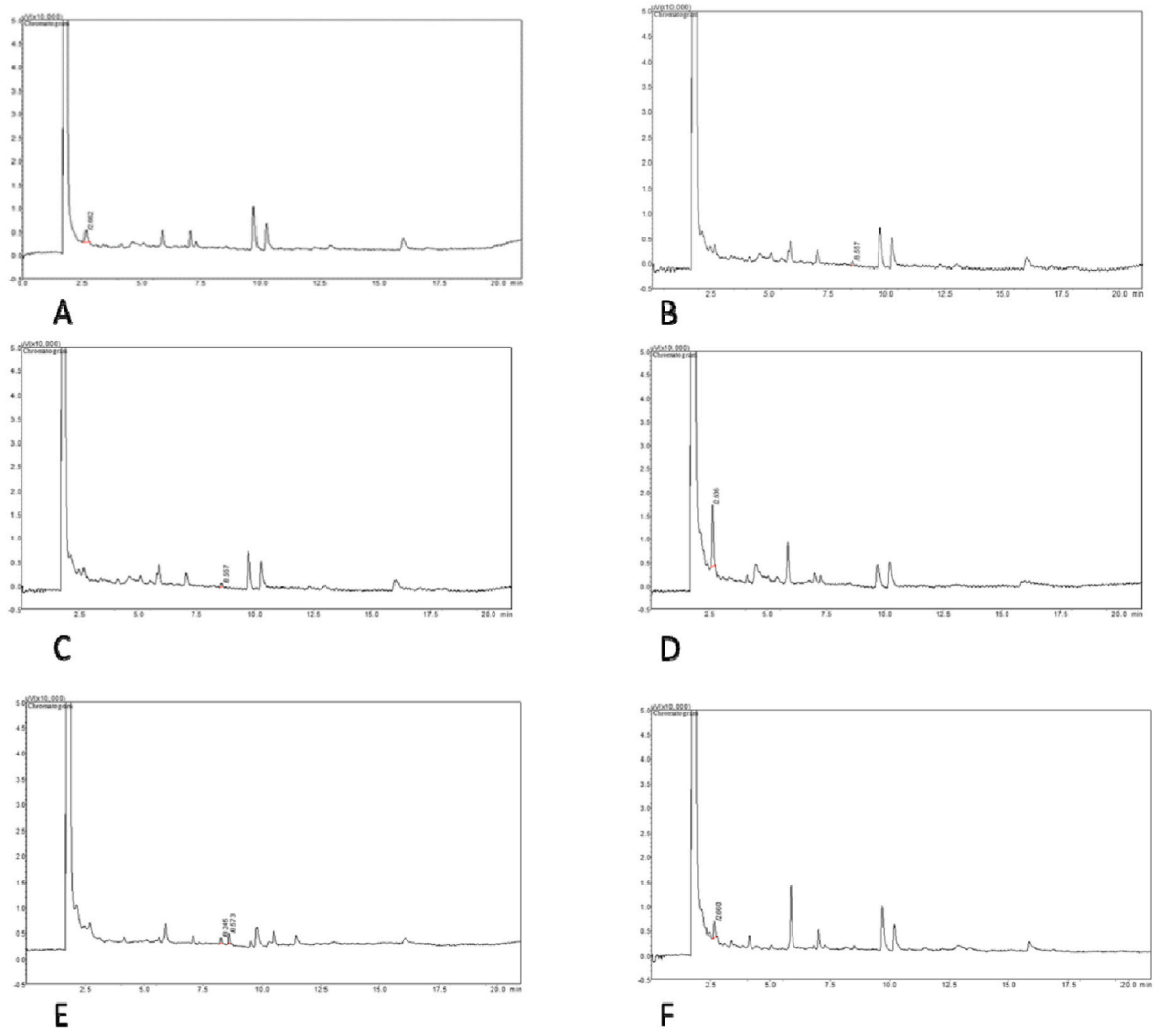

Fig. 8. Confirmation of pesticides residues in soil, water, raw milk, green feed, concentrated feed and dry feed sample quantitatively by GC–MS/MS. [A-Dichlorvos positive 2.6 min (Soil), B-Dichlorvos positive 8.5 min (water), C-Dichlorvos positive 8.5 min (Raw Milk), D-Dichlorvos positive 2.5 min (Green feed) E–Malathion positive 8.2 min, Chlorpyrifos positive 8.5- min (Concentrated feed) F-Dichlorvos positive 2.6 min (Dry feed)].

Authors' contributions

(contribution statements that specify the contribution of every author in order to promote transparency)

Ethics approval

Neither experimental animals nor human subjects were directly involved in the study.

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CRediT authorship contribution statement

Soniya A. Ranveer: Conceptualization, of experiments and conducting of experiments, Formal analysis, writing the paper, edited the paper. **C.G. Harshitha:** Formal analysis, writing the paper. **Vaishali Dasriya:** Formal analysis, writing the paper. **Nimisha Tehri:** Formal analysis, writing the paper. **Naresh Kumar:** Conceptualization, of experiments and conducting of experiments. **H.V. Raghu:** edited the paper.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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