

Rapid Detection of Pesticide in Milk, Cereal and Cereal based Food and Fruit Juices using Paper Strip-based Sensor

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Abstract

The study was aimed to validate paper strip sensors for the detection of pesticide residues in milk, cereal-based food, and fruit juices in comparison with GC-MS/MS under field conditions. The detection limit of pesticide using rapid paper strip sensor for organophosphate, carbamate, organochlorine, fungicide, and herbicide group ranges from 1 to 10, 1–50, 250–500, 1–50, and 1 ppb, respectively in milk and milk product, cereal-based food and fruit juices. Among 125 samples of milk samples collected from the market 33 milk samples comprising 31 raw milk and 2 pasteurized milk found positive for pesticide using the strip-based sensor. In Cereal based food and fruit juice samples, 6 cereal flours and 4 fruit juices were found positive for pesticide residues. The pesticide positive samples were further evaluated quantitatively using GC-MS/MS wherein 7 samples comprised of raw milk, pasteurized milk, rice flour, wheat flour, maize flour, apple juice, and pomegranate juice have shown the presence of chlorpyrifos, chlorpyrifos-methyl, α -endosulfan, β -endosulfan DDD and DDT at trace level as well as at above MRL level. It is envisaged that the developed paper strip sensor can be a potential tool in the rapid and cost-effective screening of a large number of food samples for pesticide residues.

Introduction

Pesticides are extensively used to shield agricultural produces from pests and weeds. The widespread of pesticides has created serious problems regarding the effect on ecosystems and concern for human health. Pesticides are known to be a potential carcinogen and their effect on humans and their occurrence in diverse foods including dairy products and cereal-based food. Pesticide deposits in food items have been related to a wide assortment of human illnesses, extending from short to long-term harmful effects with toxigenic impact ^[1]. Due to high human health risks, there is a need to constantly monitoring the pesticide exposure or content in the food. In the total top, 10 brands of processed food made up of cereals were contaminated with different 14 organochlorine pesticide which having the nutritional property, nourishes our body and it is the basic level of food which consume in daily diet. It contributes 60% of total worldwide crops. But it is contaminated with pesticides which contribute to high health risks for infants and young children. According to the Akoto et al. ^[2] evaluation of these pesticides was found above MRLs which raises worries of conceivable cancer-causing nature of pesticide for infant and youngsters. Pesticide utilization constrained by various national and global administrative bodies. In India, the pesticide guidelines are classified by the Central Insecticides Board and Registration Committee (CIBRC) and the Food Safety and Standard Authority of India (FSSAI). FSSAI has given MRLs of various pesticide groups for different food categories including milk, cereals, and fruit juices. Monitoring the presence of the toxigenic pesticide in food commodities needs a tall affectability and precision since these chemicals are frequently found at minute levels ^[3]. These are normally being observed quantitatively as well qualitatively by traditional strategies like chromatography-mass spectrometry (LC-MS), gas chromatography-mass spectrometry (GC-MS), and by other spectroscopic techniques ^[4]. While, traditionally used techniques are reliable, effective, and delicate, but it has also disadvantage like a sophisticated instrument, difficult to handle require a trained technician and also having complex extraction protocol and can't be utilized under field conditions. Consequently, the research concentrated on discovering quick and sensitive devices. Biosensors are being used to explicit sensitive and specific recognition of interested pesticide by using complex combinations of a cellular constituent of microorganisms, spores-based enzyme, chemicals, antibodies, DNA sequences, aptamers, or other compounds. Since biosensors performed as quantitative detection techniques depend on the enzyme-based inhibition in the presence of pesticides ^[5].

Spore-based biosensors are cost-effective, easy to use, portable, less time-consuming, simple, robust, and reasonable techniques ^[6]. Bacterial spore act as a biosensing element for detecting aflatoxin M1 and antibiotic residues in milk is based on the principle that the release of Dipicolinic acid during germination of spores based as a signal ^[7, 8, 9]. In this way, the methodology was additionally extended out to target pathogens, for example, *Enterococci*, *L. monocytogenes*, and *E. coli* in milk ^[10, 11, 12].

Strip-based innovation for targeting pesticides in milk, cereal, and fruit juice by utilizing novel marker catalysts from prokaryotic sources has been created. The spore enzyme-based sensor is based on the principle of *B. megaterium* spore germination -enzyme inhibition in the presence of pesticide followed by the reaction with chromogenic substrate indoxyl acetate and detection using the paper strip by presence or absence of pesticide through a color change, showing semi-quantitative detection of the target analyte. This study's strip-based sensor is very compatible with another chemical, easily available at low cost, passive transport of liquid-like pesticide and organic solvent, versatile in nature with fast response. It is easy to show the result of the presence and absence of pesticides by a change in color.

Acetylcholine esterases represent a various group of hydrolases catalyzing cleavage and formation of ester bonds and are also known as ester hydrolases [13]. These are broadly distributed in animals, flora, and microorganisms [14]. Membrane sure localization of AChE had been mentioned in many microbes. The presence of mobile esterases in *B. megaterium* 20 – 1 was found by Jung et al [15]. Colorimetric esterase assay normally makes using chromogenic substrates like indoxyl acetate [16, 17]. This principle was applied for the milk and milk products, cereals, and fruit juices by extracting pesticide and detecting it with the paper strip-based sensor. Keeping in perspective on current guidelines and the presence of disease-causing pesticides in our basal diet, there is a need for a fast and sensitive method for detecting pesticides in milk and cereal-based food as well as fruit juices.

Material And Method

Media and their composition

All media ingredients for preparation of nutrient agar, tryptone glucose yeast extract, and sporulation media are from Himedia. Tryptone glucose yeast extract (pH=7.0) prepares by addition of tryptone (5.0 g/L), dextrose (2.5 g/L), and yeast extract (1.0 g/L). Preparation of sporulation media require Beef extract (0.074 g/L), Peptone (0.374 g/L), Sodium chloride (0.374 g/L), and yeast extract (0.150 g/L).

Reagents

Propagation (TGYE) medium was obtained by dissolving tryptone (0.5%), dextrose (0.1%), and Yeast extract type-1 (0.25%) in distilled water. The pH of the medium was adjusted to 7.0 followed by sterilization at 121°C for 40 min. Store it at room temperature. The chromogenic substrate was prepared in 1 mL organic solvent with addition of 3.5 mg indoxyl acetate (chromogenic substrate) (Sigma Aldrich, U.S.A; Hi-Media, Mumbai, India). Pesticide (Supleco, Sigma-Aldrich, U.S.A.) solution prepared by diluting standard stock solution in acetonitrile (Fisher Scientific, HPLC Grade, U.K.) and stored at 4°C. Potassium Phosphate Buffer (Hi-Media, India) (pH 6.8) was prepared by dissolving dipotassium hydrogen phosphate (K_2HPO_4) (0.174g) and potassium dihydrogen phosphate (KH_2PO_4) (0.136g) in 100.0 mL of distilled water and stored at room temperature.

Instrumentation

Inoculation and reconstitution of spore carried out in Bio-safety Level-II cabinet (Esco Biotech Pvt. Ltd., India). Incubator shaker (Eppendorf, Inc., USA.) was used for the incubation of spore. Separation of pellet done in centrifuge (Eppendorf, U.S.A.). Spore stored in -20°C Deep Freeze (Bluestar, India). Lyophilization of spores were carried out in Lyophilizer (Labconco, U.S.A.). Microbiological plate reader (*Perkin Elmer, U.S.A.*) used for adjustment of spore O.D. Immobilization of substrate on paper strip carried out using the Easy printer (Advanced sensor system Pvt. Ltd., India). Usually block heater (Labnet International, Inc., U.S.A) used for evaporation of organic solvent from extracted pesticide solvent of food sample.

Production of *B. megaterium* MTCC 2949 spore

Production of *B. megaterium* MTCC 2949 spore was carried out as per the protocol explained by Kumar et al. [8].

Preparation of functionalized paper strip

Cut the paper strips in dimensions of 0.5 x 3.5 cm. immobilized the chromogenic substrate indoxyl acetate using an easy printer. Approximately 400 μL substrate dispense to needle and loaded substrate using printer needle on the paper. Developed functionalized paper strip vacuum-packed INDVAC vacuum packaging machine.

Lyophilisation of spores

Dispense 20-40 μL of final spore (OD 0.32 ± 0.02) and reconstitute it with 30 μL phosphate buffer (10 mM). Add functionalized paper strip followed by incubation at 37°C for 10-20 min. Observed blue color for a recording test time of enzyme activity at the time interval between the incubation periods before the lyophilization. Lyophilized 20 μL of spore to lyophilizer at $-84 \pm 1^\circ\text{C}$ under vacuum of 1 ± 0.5 torr (1 Torr = 133.33 Pascal) for 1hr. After finishing it packed within a plastic bag and stored at -20°C and 4°C .

Extraction of pesticide from cereal based foods

Take equal volume (500 μL) sample and acetonitrile was taken in a micro-centrifuge tube and vortex it for 1 min. Centrifugation was done at 10000 rpm for 5 min at 37°C . The supernatant (≈ 750 μL) was separated in to tubes containing 0.25g sucrose (reagent 1) (Hi-Media, Mumbai, India) and subsequently, vortex for 1 min. Centrifugation was done at 10000 rpm for 5 min at 37°C . Then separate, a top layer containing solvent ~ 400 μL and collected it into a tube containing 0.25g PSA (reagent 2) (Supleco, Sigma-Aldrich, U.S.A.) and MgSO_4 (Hi-Media, Mumbai, India)) at ratio of 1:2 and vortex it properly followed by centrifugation at 10000 rpm for 5 min at 37°C . Top layer containing solvent ~ 200 μL was separated and filtered through specialized filter tips. The filtrate was collected in a micro-centrifuge tube and allowed to evaporate using a dry block heater at 80°C for 15-20 min. Finally, the tube containing pesticide residue (Tube-2) was used to carry out paper strip assay [13].

Assay protocol for pesticide detection using a paper strip

Transfer reconstituted spore with 30 μL of potassium phosphate buffer (pH 6.8) to tube containing residues left after evaporation of ~ 250 μL and mixed by vortexing for 1 min. After mixing it properly, tubes were allowed to incubate at 37°C for a period of 40 min (exposure time). After exposure, the mixture was vortexed for 25 sec. and the paper strip was added and followed by incubation at 37°C for 10-15 min. During incubation, the tubes were observed for the color development on the paper strip. Development of blue color indicates an absence of pesticide residues and the presence of pesticide residues was indicated by less or no blue color development. For the comparison of results, positive control standard pesticide (Paraxon methyl) was used and for the negative control, the sample was screened from the Acetate QuEChERS method.

Protocol for the limit of detection (LOD) of pesticide from food products:

Initially, the different concentrations of pesticide prepared in organic solvent i.e., acetonitrile, and followed the protocol of pesticide detection. Similarly, a homogenous mixture of products spiked with different quantities of pesticide. The spiked

concentration of pesticide from 100ppm to 1ppb. Spiked product proceeds for extraction of pesticide and then follows assay protocol for pesticide detection using a paper strip.

GC-MS/MS analysis

Sample preparation

Equal quantity of homogeneous food sample and acetonitrile transfer in a 50 mL polypropylene tube. Kept this mixture for 5 min at room temperature followed by the addition of 6.0 g of MgSO₄ and 1.5 g of sodium acetate followed by vortex for proper mixing and 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, 1mL of the clear extract was injected into GC for analysis.

Conditions for GC-MS/MS

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 quadrupole 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 70eV with solvent delay time 3min. The quadrupole detector voltage was 0.6 KV. The injection temperature was 250°C and the carrier gas used was helium.

Results And Discussion

LOD of paper strip for pesticide in spiked food sample

In the present study, different groups of pesticide concentrations are prepared in organic solvent and detected using the paper strip assay (Figure 1A). In the presence of pesticide residues like organophosphate, carbamate, organochlorine, and fungicide and herbicide group, the bacillus spores present in the tubes were germinated into vegetative cells followed by the release of marker enzyme, and activity of the marker enzyme get inhibited due to the presence of pesticides residues present in the food system. Therefore, there was no color change due to no hydrolysis of a specific chromogenic substrate, and the strip remains colorless. In the absence of pesticide residues in food, marker enzyme released during spore germination will be intact and hydrolyze the specific chromogenic substrate with the production of blue color (Figure 1B).

The developed paper strip assay was evaluated with a different group of pesticides including organophosphate, carbamate, organochlorine, and fungicide and herbicide group spiked in a pure system as well as a different food system in the range from 100 ppm to 1ppb in an organic solvent for the detection of Limit of Detection (LOD) of the developed assay. The limit of detection in a pure solvent system ranges from 1 to 10 ppb, 1-50ppb, 250-500ppb, 1-50ppb, and 1ppb for organophosphate, carbamate, organochlorine, and fungicide, and herbicide, respectively (Table 1). Further, different concentrations of pesticides spiked inhomogeneous food samples like milk, cereal-based food, and fruit juice sample. After extraction of pesticide, the pesticide residues analyzed by paper strip assay protocol and the LOD of an organophosphate pesticide, carbamate pesticide, organochlorine pesticide, fungicide, and herbicide group in milk ranges from 1-10 ppb, 1-50ppb, 250-500ppb, 1-50ppb, and 1ppb, respectively by strip-based sensor same as obtained in pure system. The LOD for cereal-based food was found to be in the range of 1-100ppb, 10ppb, 1ppb, and 1-100ppb for organophosphate, organochlorine, fungicide, pyrethroid ester, and herbicide, respectively. The LOD for fruit juices are in the range from 1ppb, 1-10ppb, and 1ppb, respectively for organophosphate, fungicide, and herbicide groups. There was no difference in a detection limit of pesticide in the pure system and food sample was observed using paper strips.

Screening of food sample using paper strip assay

Cereal based foods

Cereal-based foods, fruit juices, and milk were screened for pesticide detection. Cereal-based food comprises a total of 19 samples of rice flour, 23 samples of wheat flour, 12 samples of maize flour, 3 samples of cornflakes, and 13 samples of cookies were screened. Among these, 3 each sample of wheat flour, rice flour, and maize flour samples have shown the presence of pesticide residues by not changing the color from colorless to blue color (Figure 2A). There were no positive samples found for corn flakes and cookies. While the using modified QuEChERS and GC-MS were used for the detection of pesticide in whole wheat flour samples collected from the south Brazil Region ^[18]. The detection of fenitrothion in 20% of maize flour samples found less than permissible limits ^[19]. The results indicate that different flour and products need continuous checking to warrant food safety.

Fruit juices

In the case of fruit juices, a Total of 57 samples of fruit juice collected from the market comprises 12 grapes juice samples, 15 apple juice samples, 15 mixed fruit samples, and 14 pomegranate juice samples. All sample screens for pesticide detection using the paper strip-based sensors. Among the 57 samples, 2 raw un-washed pomegranates and 2 raw un-washed apple juice found positive for pesticide at their MRL level (Figure 2B). However, these pesticides are harmful and not deteriorate naturally and they will present on plant tissue and appear in the pulp and juice. However, the pesticide cannot completely remove from the pulp and juice. Since wherein natural fruit juice has less concentration of pesticide than the processed juice ^[20]. Children consume more juice than adults and thus the children are more susceptible than adults ^[21].

Milk samples

Milk is considered a complete food with a source of protein and major minerals. A total of 125 samples of milk collected which comprises raw milk, pasteurized milk, and UHT milk were screened for pesticide using paper strip-based sensor. Among 125 samples of milk comprising 89 raws, 12 pasteurized and 24 UHT milk among that 31 samples of raw milk and 2 samples of pasteurized milk found positive for pesticide (Figure 2C). The assessment and detection of organochlorine pesticide (OC) residues in bovine milk of different places in the Bundelkhand region of India were studied by Nag and Raikwar ^[22]. They revealed that the concentration of pesticide levels in milk was decreased over the previous studies of particular in India but still contamination present in low concentration. Overall, 33 milk samples, 6 cereal flour samples, and 4 fruit juice samples were found positive for pesticide residues (Figure 2D) by developed paper strip sensors for detection of pesticide residues.

GC-MS/MS analysis

Among 43 positive samples out of 159 samples including milk, cereal products, and fruit juice samples by paper strip sensor for detection of pesticide residues were evaluated for pesticide residues quantitatively by GC-MS/MS. The condition required for multiple reactions monitoring (MRM) method using GC-MS/MS was optimized for the analysis of pesticide residues in food samples (Table 2). In GC-MS/MS analysis about 11 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 the 43 samples, 7 samples (Raw milk, pasteurized milk, rice flour, wheat flour, maize flour, apple juice, and pomegranate juice) were found positive for different groups of pesticides at trace levels or above the MRL using GC-MS/MS analysis (Figure 3). Three samples of pasteurized milk, rice

flour, and wheat flour were positive for the presence of Chlorpyrifos and chlorpyrifos-methyl pesticide residues, respectively at above MRL level prescribed by the Codex Alimentarius Commission (Table 3). In case of other pesticide residues found at trace level that includes DDT, DDD, α -Endosulfan, β – Endosulfan that are found at the level below MRL using GC-MS/MS and these samples were showing positive by our developed paper strip assay. Based on the above comparative study it can be concluded that the developed paper strip assay can be a potential tool in the screening of pesticide residues in a large number of milk samples, cereal flour samples, and fruit juice samples. Similar results were reported by Kowalska et al. [23] in the products of plant origin and the pesticides identified by HPLC-MS/MS includes azoxystrobin (22.5%), linuron (20.6%), chlorpyrifos, and carbendazim (8.1%), metalaxyl and metalaxyl M (6.9%), and acetamiprid (4.4%). In India, Dwivedi et al. [24] have reported the presence of Chlorpyrifos in orange juice (1.08 mg/kg) & Deltamethrin (1.28 mg/kg) in ginger garlic at or above the permissible limit of FSSAI. Garcí'a-Reyes et al. [25] were also reported the presence of pesticide residues in fruit-based soft drinks extracts based on the application of liquid chromatography-electrospray time of-flight mass spectrometry (LC-TOF MS). The contamination of milk with hexachlorocyclohexane (HCH), dichloro-diphenyl trichloroethane (DDT), endosulfan, cypermethrin, cyhalothrin, permethrin, chlorpyrifos, ethion, and profenophos pesticides was also reported in peri-urban bovine milk at or above respective maximum residue limits (MRLs) for pesticide using GC-MS [26].

Conclusion

The current study shows that developed extraction protocol gives better detection of pesticides from milk, cereal-based food, and fruit juices. These results show a wide range of applications of paper strip sensors and found to be a cost-effective, robust, and rapid method for the detection of pesticides from samples. The comparative result obtained of pesticide-positive samples using GC-MS were ranged between 0.012–499.59 ppb. There are no losses of pesticides during extraction from food samples. The detection limit of pesticide from the food sample ranges from 1-500ppb which is below the MRL of pesticide according to FSSAI and EU regulatory standards.

Declarations

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References

1. Grewal, A. S. Pesticide Residues in Food Grains, Vegetables and Fruits: A Hazard to Human Health. *J. Med. Chem. Toxicol.* **2** (1), 1–7 (2017).
2. Akoto, O., Gavor, S., Appah, M. K. & Apau, J. Estimation of human health risk associated with the consumption of pesticide-contaminated vegetables from Kumasi. *Ghana. Environ. Monit. Assess.* **187** (5), 244 (2015).
3. Mishra, K. & Sharma, R. C. Assessment of organochlorine pesticides in human milk and risk exposure to infants from North-East India. *Sci. Total Environ.* **409** (23), 4939–4949 (2011).
4. Liu, B. *et al.* Detection of pesticides in fruits by surface-enhanced Raman spectroscopy coupled with gold nanostructures. *Food Bioproc. Tech.* **2013**, *6*(3), 710–718.
5. Bucur, B., Munteanu, F. D., Marty, J. L. & Vasilescu, A. (2018). Advances in enzyme-based biosensors for pesticide detection. *Biosens.* **2018**, *8*(2), 27.
6. Naresh Kumar, N. *et al.* (2015). Rapid spore enzyme based miniaturized assay (s) for detection of pesticide residues. Indian Patent Reg. No. 3819/ DEL/ **2015**. Patent Journal 21/2017 dated 26/05/2017.
7. Kumar, N., Sawant, S., Patil, G. R. & Malik, R. K. Development of Analytical Process for Detection of Antibiotic Residues in Milk Using Bacterial Spores as Biosensor. Indian Patent Reg No. 2006;1439.

8. Kumar, N., Singh, N. A., Singh, V. K., Malik, R. K. & Raghu, H. V. Process of preparing a spore inhibition based enzyme substrate assay for monitoring Aflatoxin M1 in milk. Indian patent no.292836. 2010
9. Kumar, N. *et al.* (2012). Spore germination based assay for monitoring antibiotic residues in milk at dairy farm. *World J. Microbiol. Biotechnol.* **2012**, 28(7), 2559–2566.
10. Kumar, N. *et al.* K. Real time detection of Enterococci in dairy foods using spore germination based bioassay. Patent Reg # IPR 119/DEL/2012 – Published in Indian patent Journal No. – 19/2015 dated 08/05/2015.
11. Balhara, M. *et al.* ; Shabnam. Two Stage Enzyme Assay for Detection of *Listeria monocytogenes* In Milk Products. Patent published and Examination awaited (2/02/2015) Patent # 1357/DEL/2013. Journal No. 50/2014 dated 2014/12/12.
12. Kumar, N. *et al.* Spore-sensor for rapid detection of *coli* in milk employing marker enzymes and marker sugar as germinants”. Patent: Reg # IPR 2214/DEL/2014. Patent Journal 7/2016 dated 2016/02/12.
13. Tehri, N. Spore based sensor for pesticide residues in milk, 2017. PhD thesis submitted NDRI Deemed University for the partial fulfilment of doctoral degree.
14. Bornscheuer, U. T. Microbial carboxyl esterases: classification, properties and application in biocatalysis. *FEMS Microbiol. Rev.* **26** (1), 73–81 (2002).
15. Jung, Y. J., Lee, J. K., Sung, C. G., Oh, T. K. & Kim, H. K. Nonionic detergent-induced activation of an esterase from *B. megaterium* 20 – 1. *J. Mol. Catal. A-Enz.* **6** (3–6), 223–229 (2003).
16. Huggins, C., Lapides, J. & Chromogenic substrates, I. V. Acyl esters of p-nitrophenol as substrates for the colorimetric determination of esterase. *J. Biol. Chem.* **170** (2), 467–482 (1947).
17. Kaiser, P. *et al.* N. A novel esterase from *Bacillus subtilis* (RRL 1789): Purification and characterization of the enzyme. *Protein Expr. Purif.* **45** (2), 262–268 (2006).
18. Bordin, A. B., Minetto, L., Filho, I., Beal, L. L. & Moura, S. Determination of Pesticide Residues in Whole Wheat Flour Using Modified QuEChERS and LC–MS/MS. *Food Anal. Methods.* <https://doi.org/10.1007/s12161-016-0542-2> (2016).
19. **2019**, 54(11), 892–897.
20. Cabras, P. & Angioni, A. Pesticide residues in grapes, wine, and their processing products. *J. Agric. Food Chem.* **48** (4), 967–973 (2000).
21. Topuz, S., Özhan, G. & Alpertunga, B. Simultaneous determination of various pesticides in fruit juices by HPLC-DAD. *Food Control.* **16** (1), 87–92 (2005).
22. Nag, S. K. & Raikwar, M. K. Organochlorine Pesticide Residues in Bovine Milk. *Bull Environ. Contam. Toxicol* 2007 (2008) 80:5–9 DOI 10.1007/s00128-007-9276-6.
23. Kowalska, G., Pankiewicz, U. & Kowalski, R. Estimation of Pesticide Residues in Selected Products of Plant Origin from Poland with the Use of the HPLC-MS/MS Technique. *Agri.* **10** (6), 192 (2020).
24. Dwivedi, B. C., Tiwari, H. & Gaur, V. K. Assessment of 27 pesticide residues in fruit juices & vegetables paste by gas chromatography with mass spectrometry (GC-MS). *Int. J. Chem. Stud.* **5** (1), 259–285 (2017).
25. Garcia-Reyes, J. F., Gilbert-Lopez, B., Molina-Diaz, A. & Fernandez-Alba, A. R. (2008). Determination of pesticide residues in fruit-based soft drinks. *Anal. Chem.* **2008**. 80(23), 8966-74.
26. Gill, J. P. S. *et al.* Pesticide residues in peri-urban bovine milk from India and risk assessment: A multicentre study. *Sci. Rep.* **10** (1), 1–11 (2020).

Tables

Table 1

LOD of different group of pesticide from cereal based foods sample

Pesticides	Regulatory MRL limits (ppb)	Limit of detections (ppb) in spiked food samples	Limit of detection (ppb) in pure system
Insecticides – organophosphate group			
Primiphos methyl b	500	10	10
Triazophos a	50	1	1
Anilophos a	100	100	100
Insecticides – organochlorine group			
Lindane c	10	10	10
Fungicide			
Iprodione a	10000	1	1
Propineb a	50	1	1
Pyrethroid ester			
Deltametrin b	10	1	1
Herbicide			
Diuron a	500	10	10
Mesosulfuron methyl b	10	1	1
Metribuzin b	30	10	10
Pendimethalin a	50	10	10
Penoxsulum a	100	100	100
Pyrazosulfuron ethyl a	10	10	10
Pretilachlor c	50	10	10
Propiconazole c	10	10	10
Note: ^aRice, ^bWheat, ^cMaize			

Table 2

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

Compound	Start time	End time	Event time	CH-1 (m/z)	CE	CH-2 (m/z)	CE	Q1 Resolution	Q3 Resolution
Monocrotophos	9.86	11.14	0.15	127.10>109.00	12	127.10>95.00	16	Low	Low
Phorate	9.86	11.14	0.15	260.00>75.00	8	260.00>231.00	4	Low	Low
Diazinon	11.14	12.35	0.3	304.10>179.10	10	304.10>162.10	8	Low	Low
Chlorpyrifos-methyl	12.35	14.47	0.06	285.90>93.00	22	285.90>270.90	14	Low	Low
Fenitrothion	12.35	14.47	0.06	277.00>260.00	6	277.00>109.10	14	Low	Low
Malathion	12.35	14.47	0.06	173.10>99.00	14	173.10>127.00	6	Low	Low
Chlorpyrifos	12.35	14.47	0.06	313.90>257.90	14	313.90>285.90	8	Low	Low
Aldrin	12.35	14.47	0.06	262.90>193.00	28	262.90>203.00	26	Low	Low
alpha-Endosulfan	15.75	18.32	0.15	338.90>160.00	18	338.90>266.90	8	Low	Low
Dieldrin	15.75	18.32	0.15	276.90>241.00	8	276.90>170.00	38	Low	Low
beta-Endosulfan	18.32	19.61	0.15	338.90>160.00	18	338.90>266.90	8	Low	Low
p,p'-DDD	18.32	19.61	0.15	235.00>165.00	24	235.00>199.00	14	Low	Low
p,p'-DDT	19.61	20.75	0.3	235.00>165.00	24	235.00>199.00	16	Low	Low

Table 3

Comparative analysis of food samples in Paper strip sensor and GC-MS

S.No.	Sample Code	Results obtained by GC-MS/MS analysis			Results on paper strip
		Pesticide found	Concentration (ppb)	MRL	
	RM (Raw milk)	Chloropyrifos	3.47	Below	Positive
		DDT	0.012	Below	Positive
	PM (Pasteurized milk)	Chloropyrifos- methyl	489.074	Above	Positive
		DDD	0.06702	Below	Positive
	RF (Rice flour)	Chloropyrifos	188.471	Above	Positive
		DDT	0.01700	Below	Positive
	WF (Wheat flour)	Chloropyrifos- methyl	499.59	Above	Positive
		DDD	0.0755	Below	Positive
	MF (Maize flour)	α - Endosulfan	31.57	Below	Positive
1.	AJ (Apple juice)	Chloropyrifos	3.23	Below	Positive
	PJ (Pomegranate juice)	α - Endosulfan	51.8389	Below	Positive
		β - Endosulfan	9.8523	Below	Positive
		DDT	0.0332	Below	Positive

Figures

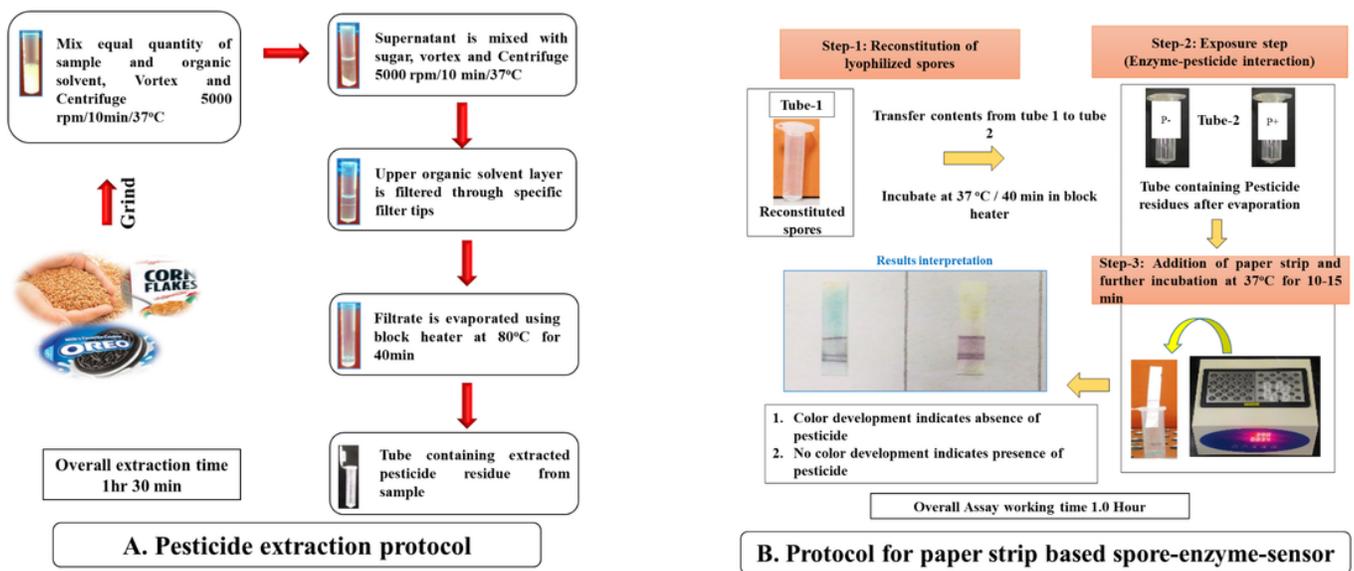


Figure 1

Paper strip assay for rapid detection of pesticide residues in Food. 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 – Colorless; (-ve) sample blue color

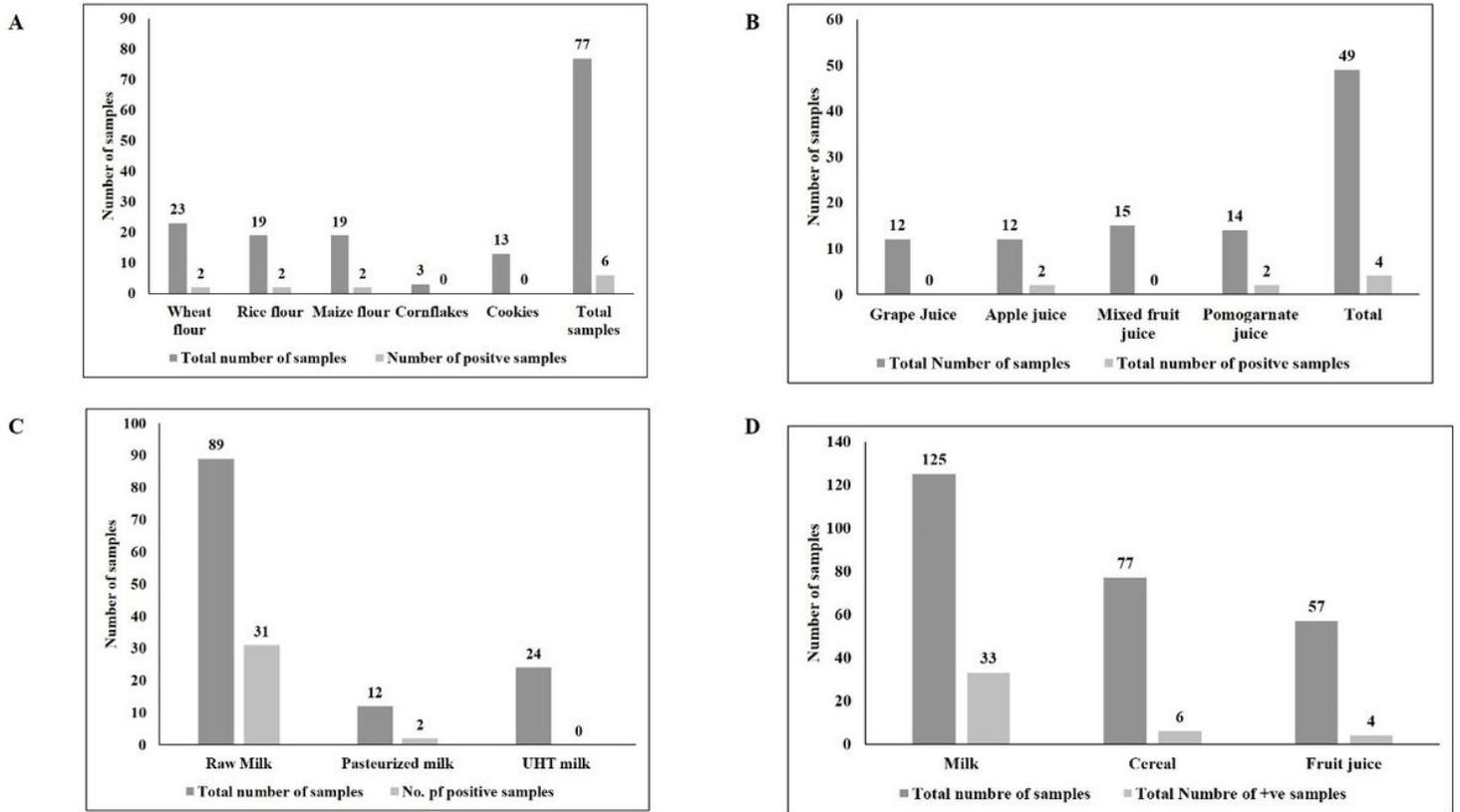


Figure 2

Screening of food sample using paper strip assay. A. Incidence of pesticide residues in cereal based products. B. Incidence of pesticide residues in Fruit juices. C. Incidence of pesticide residues in milk samples. D. Overall incidence of pesticide residues

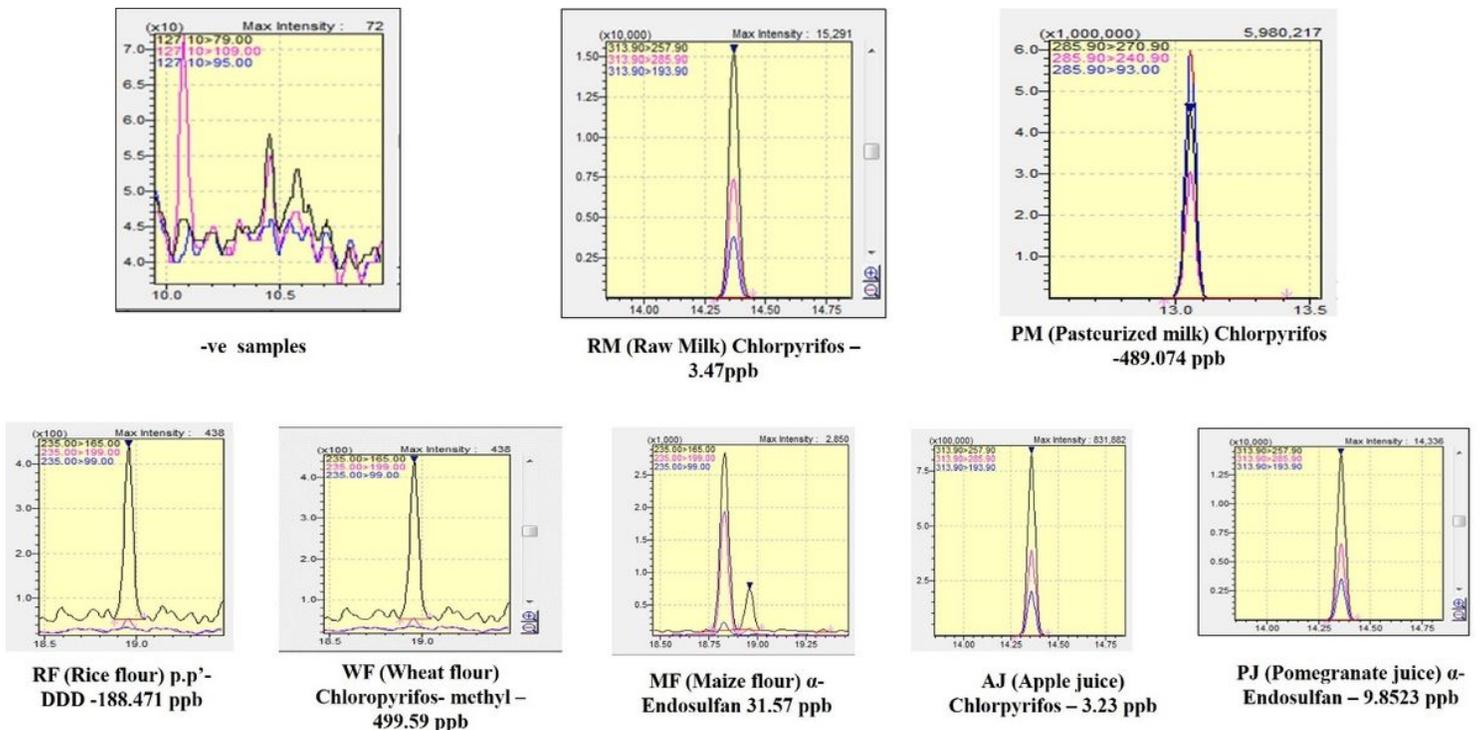


Figure 3

Confirmation of pesticides residues in milk, cereal based foods, and fruit juices quantitatively by GC-MS/MS