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Foldscope Based Detection of Rice Husk and Saw Dust Adulteration in Feed Ingredients and Thiram Contamination in Cereal Grains

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ABSTRACT

A study was conducted to develop foldscope based methodologies to detect adulteration of rice husk and saw dust in feed ingredients and thiram contamination in cereal grains. Feed ingredients and adulterants were treated with standardized phloroglucinol-hydrochloric acid solution and evaluated based on appearance of pink colour in foldscope images on reaction with lignin. Thiram contaminated or uncontaminated maize and sorghum grains were treated with cuprous iodide based solutions. Pink colour was not observed on treatment with phloroglucinol-hydrochloric acid solution in the foldscope images of soyabean meal, rice bran, de-oiled rice bran and wheat bran while pink colour spots were noticed in groundnut cake, un-decorticated cottonseed cake, decorticated cottonseed cake, sunflower cake, rapeseed meal and red gram chuni. However, both rice husk and saw dust were completely in pink colour without any changes in their cellular structure in foldscope images. Starch granules of maize and sorghum showed white colour while the starch granules of thiram contaminated maize and sorghum were covered with light brown to amber colour on treatment with cuprous iodide in ammonia solution in foldscope images. Rice husk was most frequently found in de-oiled rice bran samples from various dairy farms under field conditions. It is concluded that phloroglucinol-hydrochloric acid solution and cuprous iodide in ammonia solution can be used as reagents to detect adulteration of rice husk and saw dust in feed ingredients and mixed feeds and thiram contamination in cereal grains, respectively on the spot under field conditions using foldscope.

HIGHLIGHTS

- Foldscope can be used to detect adulteration of rice husk and saw dust in feed ingredients with phloroglucinol-hydrochloric acid solution.
- Thiram contamination in cereal grains can be detected with cuprous iodide in ammonia solution using foldscope.

Keywords: Adulteration, Feed, Foldscope, Rice husk, Saw Dust, Thiram

A low cost, portable paper microscope with 140 X magnification known as foldscope (Cybulski *et al*., 2014) can be used to detect adulteration of urea in soyabean meal (Alexander, 2018) and to differentiate calcite powder and dicalcium phosphate of rock and animal origin each other under field conditions (Alexander, 2018). Foldscope can also be used to identify cereal grains such as maize and sorghum in mixed feed (Anitha *et al*., 2022).

Use of quality feed ingredients in the rations of livestock and poultry is essential to ensure desired productivity. Batch to batch variation in nutrient composition,

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presence of inherent anti-nutritional factors, adulterants and contaminants can influence the quality of feed ingredients. Wheat bran, rice bran and de-oiled rice bran are most commonly used feed ingredients in the rations of livestock and poultry. These feed ingredients are often adulterated with ground rice husk and saw dust by the feed manufacturers for financial gain (Uppal *et al*., 2014). Both rice husk and saw dust have no nutritional value for livestock and poultry. Hence, nutritional value of adulterated feed decreases depending on the extent of adulteration with rice husk and saw dust. However, no direct method is available to detect adulteration of rice husk and saw dust in feed ingredients and finished feeds.

Phloroglucinol-hydrochloric acid reagent reacts with cinnamaldehyde end groups of lignin and forms pink colour (Mitra and Loque, 2014). As the lignin content differs among various feed ingredients and adulterants, the pink colour formation on reaction with phloroglucinol-hydrochloric acid may also differ. The differences in area covered with pink colour on feed ingredients with low lignin content and adulterants with high lignin content may be easily recognized under foldscope with 140 X magnification.

Thiram is a fungicide used to treat cereal, cotton and soyabean seeds to protect them from fungal attack and retain germination potential. Thiram treated cereal grains occasionally enter into animal feed and routine testing for thiram is needed to avoid use of thiram contaminated cereal grains (Kavitha *et al*., 2015). Thiram, even at the level of 15 ppm in the diet affected the health and performance of chicken (Subapriya *et al*., 2007). A cuprous iodide based method is used for qualitative detection of thiram in feedstuffs (AOAC, 1995). However, in this method, more volume of chloroform (50 ml) is needed to qualitatively analyse a single sample for thiram.

Thiram dissolved in chloroform reacts with cuprous iodide to form amber colour which is used to recognize the presence of thiram as per the qualitative method of AOAC (1995). The amber colour formation due to reaction of thiram with cuprous iodide may be detected with more sensitivity through observation with foldscope and therefore, the sample quantity and the chloroform required to complete the experiment may be reduced.

With this background, an effort was made to develop appropriate phloroglucinol based reagent to detect adulteration of rice husk and saw dust in feed ingredients and cuprous iodide based reagent to detect thiram contamination in cereal grains using foldscope.

MATERIALS AND METHODS

Collection and processing of samples

This study was conducted at Department of Animal Nutrition, College of Veterinary Science, P.V. Narsimha Rao Telangana Veterinary University, Rajendranagar, Hyderabad. Soyabean meal, groundnut cake, un-decorticated cottonseed cake, decorticated cottonseed cake, sunflower cake, rapeseed meal, de-oiled rice bran, rice bran, wheat bran, red gram chuni, rice husk and saw dust were collected (250 g) from three different sources and stored in polythene zip lock covers. Further, a sub-sample of 25 g was taken from all the collected samples and crushed with mortar and pestle except rice husk which was ground using Wiley mill. The crushed or ground samples were passed through 0.3 mm screen and preserved in plastic containers.

Phloroglucinol solution preparation

Phloroglucinol-hydrochloric acid solution of 0.25% concentration was freshly prepared by dissolving 250 mg of phloroglucinol in 100 ml of 0.5 N hydrochloric acid with modifications (Yeung, 1998). The details pertaining to modification of phloroglucinol-hydrochloric acid solution preparation have been explained under results and discussion section.

Phloroglucinol staining procedure

A minute quantity of powdered sample of feed ingredients/adulterants was taken on a glass slide and 2 drops of 0.25% phloroglucinol-hydrochloric acid solution was added to the sample on the slide with the help of Pasteur pipette. The sample was spread as thin layer and kept undisturbed until the sample was dried completely. The air-dried stained sample was covered with transparent cello tape. The samples were processed in duplicate for each ingredient/adulterant from three different sources.

Foldscope examination and image capturing

The slide was inserted into foldscope and examined using chargeable LED light as light source. The foldscope images were captured using a smart phone camera after adjusting the zoom function (Alexander, 2018; Anitha *et al*., 2022).

Thiram detection

In the qualitative procedure described by AOAC (1995) for detection of thiram, amber colour development was based on the reaction of cuprous iodide with thiram. Preliminary studies revealed that solid cuprous oxide is not suitable for thiram detection using foldscope. Hence, two different solvents i.e. ammonia solution and potassium iodide solution were used to prepare cuprous iodide solution for foldscope based experiments.

Preparation of thiram contaminated samples

Ground maize and sorghum grains were thoroughly mixed with 0.1 g of thiram per 100 g of sample to keep the thiram concentration at 0.1%. Similarly, thiram contaminated maize grain at the concentrations of 0.01 and 0.001% was also prepared by mixing thiram with maize at 0.01 and 0.001 g/100 g, respectively. Then, the thiram contaminated sub-samples were crushed in mortar and pestle and passed through 0.3 mm screen and used for qualitative thiram detection.

Detection of thiram using cuprous iodide in ammonia solution

Cuprous iodide solution was prepared by dissolving 100 mg of cuprous iodide in 100 ml of ammonia solution. The resultant solution was filtered through Whatman No. 1 filter paper and the filtrate was used for the experiment.

A minute quantity of 0.1% thiram adulterated or thiram uncontaminated sample (maize and sorghum) was taken on a glass slide. One drop of chloroform and one drop of cuprous iodide solution were added to the sample and immediately the sample was spread as thin layer. The air-dried sample on the slide was covered with transparent cello tape and examined under foldscope. The foldscope images of samples were photographed using a smart phone camera as indicated earlier.

Detection of thiram using cuprous iodide in potassium iodide solution

Cuprous iodide (10 mg) and potassium iodide (3 g) were dissolved using distilled water in a beaker. The contents were transferred to 100 ml volumetric flask and the volume was made up to 100 ml with distilled water.

A minute quantity of powdered sample (maize/sorghum containing 0.1% thiram or without thiram) was taken on a glass slide. Few drops of hot water were added to the sample and kept undisturbed for five minutes. Then, 2 drops of cuprous iodide solution were added to the sample and the sample was spread immediately as thin layer. The air-dried sample was covered with transparent cello tape and viewed under foldscope. The foldscope images were recorded as mentioned earlier.

Detection of thiram in maize grain contaminated with three different concentrations

Maize contaminated with thiram at 0.1, 0.01 and 0.001% concentration was evaluated for presence of thiram using cuprous iodide in ammonia solution method and foldscope images were observed for presence of amber colour.

Detection of rice husk and saw dust adulteration and thiram contamination in feed ingredients from different dairy farms

Maize and de-oiled rice bran samples (100 g) were collected from dairy farms located at different districts of Telangana and evaluated using foldscope on the spot. The samples were processed as described earlier in order to reduce the particle size of the sample (0.3 mm). De-oiled rice bran was treated with phloroglucinol-hydrochloric acid solution for detection of adulteration with rice husk and saw dust. Maize samples were evaluated for presence of thiram using cuprous iodide in ammonia solution. In addition, the collected samples were further examined at Animal Nutrition Laboratory for confirmation of results.

RESULTS AND DISCUSSION

Standardization of phloroglucinol-hydrochloric acid solution preparation

At the start of the experiment, phloroglucinol-hydrochloric acid reagent was prepared by dissolving 2 g of phloroglucinol powder in 80 ml of 20% methanol and adding 20 ml of concentrated hydrochloric acid (Yeung, 1998).

The rice husk was completely changed to dark pink colour with crystalline background in 10 minutes. Further studies with higher level of methanol as solvent and separate application of concentrated hydrochloric acid revealed that methanol and concentrated hydrochloric acid were disturbing the sample structure in the foldscope images. Therefore, methanol was omitted and concentration of hydrochloric acid was reduced and concentration of phloroglucinol was increased to prepare phloroglucinol-hydrochloric acid solution. However, crystalline background was observed in the foldscope images even with high concentration of phloroglucinol in the solution.

Therefore, further studies were conducted to assess the effect of different concentrations of phloroglucinol (1, 0.5, 0.25 and 0.125%) in 0.5 N hydrochloric acid on pink colour formation and foldscope image quality. The phloroglucinol concentration of 0.25% in 0.5 N hydrochloric acid showed pink colour with clear background in foldscope images. No pink colour formation was observed when concentration of phloroglucinol was 0.125%. Hence, phloroglucinol concentration of 0.25% in 0.5 N hydrochloric acid was selected for the present investigation.

Foldscope images of powdered oilseed cakes, by-products of cereals and pulses and adulterants stained with phloroglucinol solution

Foldscope images of powdered oilseed cakes, by-products of cereals and pulses treated with standardized phloroglucinol-hydrochloric acid solution are presented in figs. 1A to 1J. Treatment with phloroglucinol-hydrochloric acid solution failed to produce pink colour in soyabean meal (Fig. 1A), de-oiled rice bran (Fig. 1G), rice bran (Fig. 1H) and wheat bran (Fig. 1I). Foldscope images of groundnut cake revealed presence of light or dark pink colour spots (Fig. 1B). Un-decorticated cottonseed cake (Fig. 1C) showed light or dark pink colour spots with orange or yellow background in foldscope images. Dark pink colour spots were noticed in the foldscope images of decorticated cottonseed cake (Fig. 1D). The foldscope images of sunflower cake (Fig. 1E) showed dark pink colour spots. The orange colour of seed coat of rapeseed meal was tinged with pink colour in response to phloroglucinol staining (Fig. 1F). The seed coat of red gram chunni (Fig. 1J) was stained with mixture of dark orange and pink colour which appeared as large or small structures or patches. In contrast, the cellular structures of rice husk (Fig. 2A and 2B) and saw dust (Fig. 2C and 2D) were completely and homogenously stained with pink colour without any structural disturbances.

The pink or red colour formation on treating lignified plant material with phloroglucinol reagent was observed in several previous studies (Tao *et al*., 2009; Mitra and Loque, 2014; Poelking *et al.,* 2015; Roy *et al.,* 2016; Li *et al*., 2018). Reaction of phloroglucinol with cinnamaldehyde end groups of lignin (Mitra and Loque, 2014) might be responsible for appearance of pink colour in some feed ingredients and rice husk and saw dust on treatment with phloroglucinol-hydrochloric acid solution.

The phloroglucinol stained both the rice husk and saw dust completely pink in colour without disturbing their original structure. In contrast, in most of the feed ingredients, phloroglucinol either failed to produce any colour or showed light or dark pink colour spots by disturbing the original structure. The variation in pink colour formation could be due to presence of exceptionally higher level of lignin in both rice husk (Rosado *et al*., 2021) and saw dust (Poletto *et al*., 2010) as compared to feed ingredients (NDDB, 2012). Therefore, standardized phloroglucinol-hydrochloric acid solution used in the present study can be applied confidently to detect adulteration of rice husk and saw dust in most of the routinely used feed ingredients and mixed feeds.

Thiram detection

A cuprous iodide based method is used for qualitative detection of thiram in feedstuffs (AOAC, 1995). In this method, more volume of chloroform is needed to conduct the experiment. Therefore, an effort was made to develop a more simplified method to detect thiram in cereal grains using foldscope. Initially, after adding chloroform to the powdered thiram contaminated or un-contaminated maize sample, a minute quantity of cuprous iodide powder was added and the sample was air dried and examined under foldscope. However, amber colour was observed in foldscope images from both thiram contaminated and un-contaminated maize sample. Therefore, it was decided to apply cuprous iodide in liquid form for detection of thiram.

Initially, chloroform was used as solvent to dissolve cuprous iodide. However, cuprous iodide was not readily

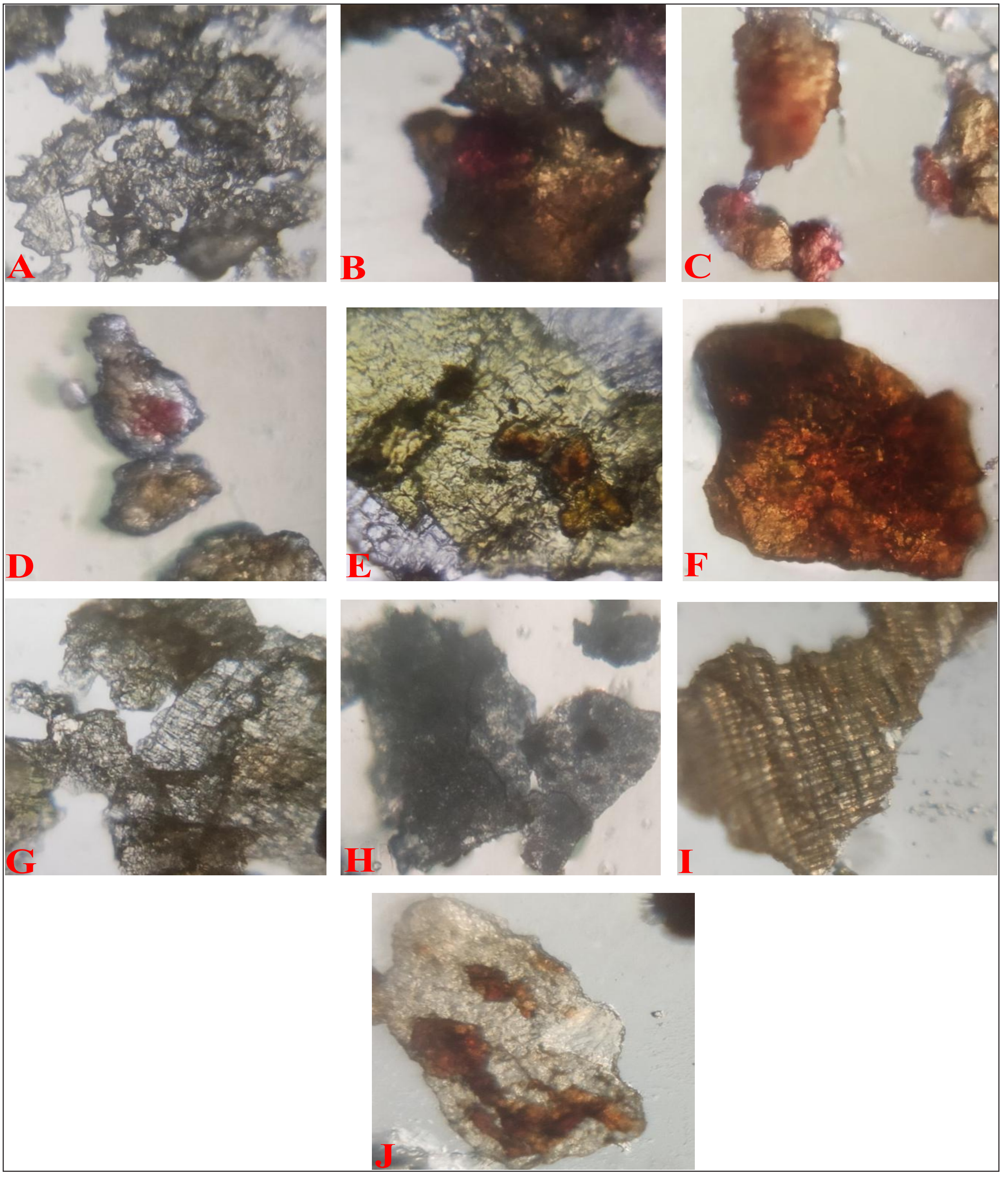

Fig. 1: Foldscope images of powdered feed ingredients stained with phloroglucinol-hydrochloric acid solution: **(A)** Soyabean meal; **(B)** Groundnut cake; **(C)** Un-decorticated cottonseed cake; **(D)** Decorticated cottonseed cake; **(E)** Sunflower cake; **(F)** Rapeseed meal; **(G)** De-oiled rice bran; **(H)** Rice bran; **(I)** Wheat bran; **(J)** Red gram chuni

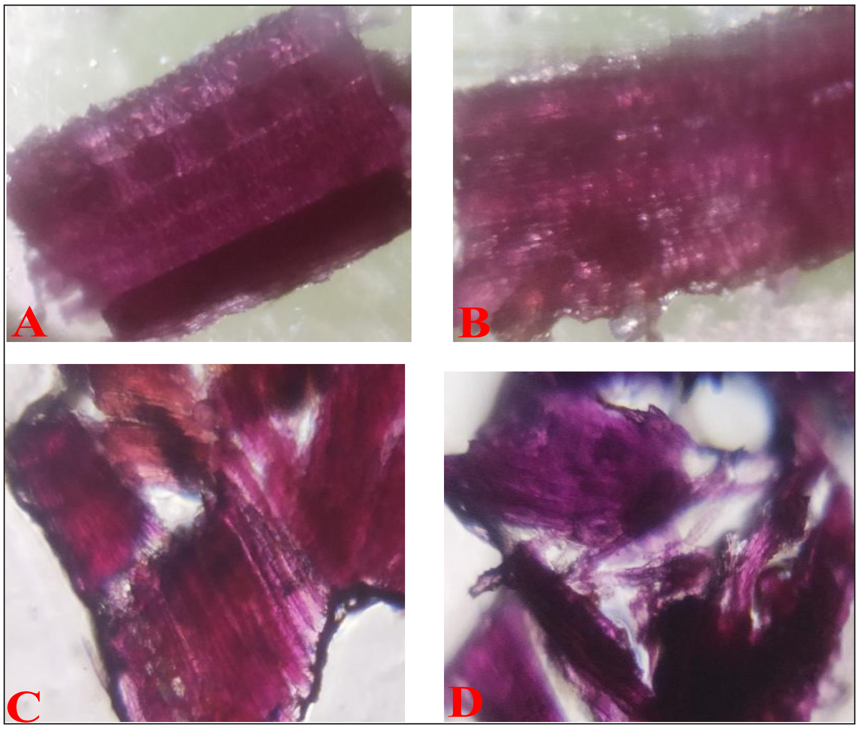

Fig. 2: Foldscope Images of adulterants stained with phloroglucinol-hydrochloric acid solution: **(A-B)** Rice husk; **(C-D)** Saw dust

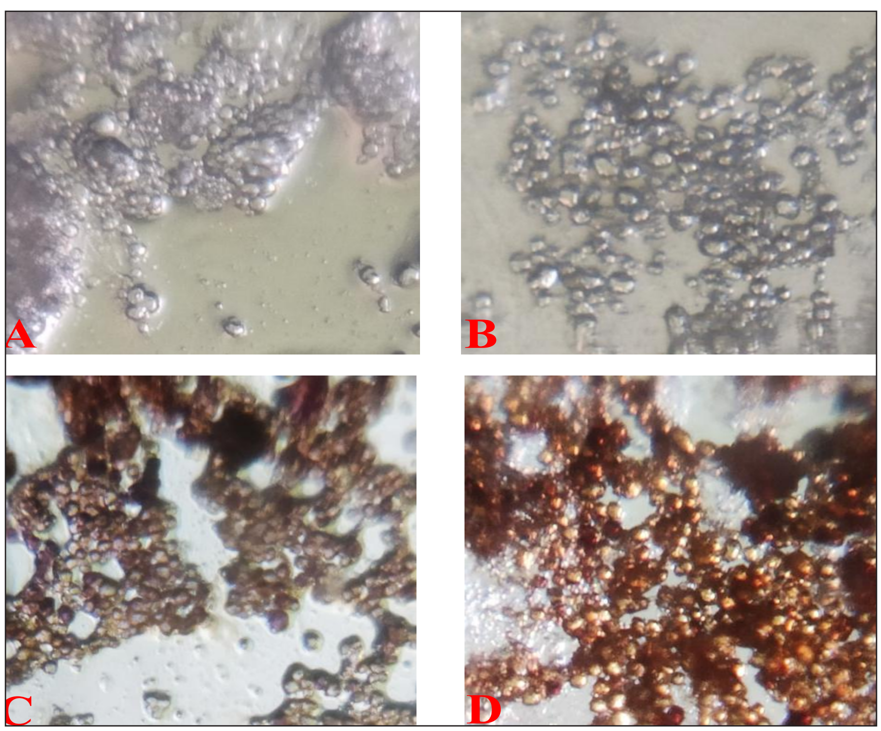

Fig. 3: Foldscope images of powdered cereal grains treated with cuprous iodide in ammonia solution: **(A)** Maize; **(B)** Sorghum; **(C)** Thiram (0.1%) contaminated maize; **(D)** Thiram (0.1%) contaminated sorghum

soluble in chloroform. As per the chemical property, cuprous iodide is known to be soluble in ammonia and potassium iodide solutions. Accordingly, cuprous iodide readily dissolved when added in ammonia solution. Therefore, ammonia was selected as solvent to dissolve cuprous iodide and the experiments were conducted using cuprous iodide in ammonia solution for the detection of thiram in maize and sorghum grain.

Both chloroform and ammonia which are necessary for thiram detection in cereal grains may not be freely available for use under field conditions. Therefore, in an alternate method, hot water was used to extract thiram from samples and potassium iodide solution was used to dissolve cuprous iodide.

Detection of thiram using cuprous iodide in ammonia solution

In the foldscope images of thiram un-contaminated samples of maize grain (Fig. 3A) and sorghum grain (Fig. 3B), starch granules appeared white in colour while 0.1% thiram contaminated samples of maize grain (Fig. 3C) and sorghum grain, starch granules (Fig. 3D) were covered with brown to amber colour. However, in thiram contaminated samples, the edges of the slide had more area under brown to amber colour due to movement of chloroform to edges along with thiram.

Detection of thiram using cuprous iodide in potassium iodide solution

Foldscope images of starch granules of maize (Fig. 4A) and sorghum (Fig. 4B) showed white colour while the starch granules of thiram contaminated maize (Fig. 4C) and sorghum (Fig. 4D) were covered with light brown to amber colour. The brown colour area and the colour intensity were relatively less in foldscope images at the same concentration of thiram in cereal grains when samples were extracted with hot water and treated with cuprous iodide in potassium iodide solution than those

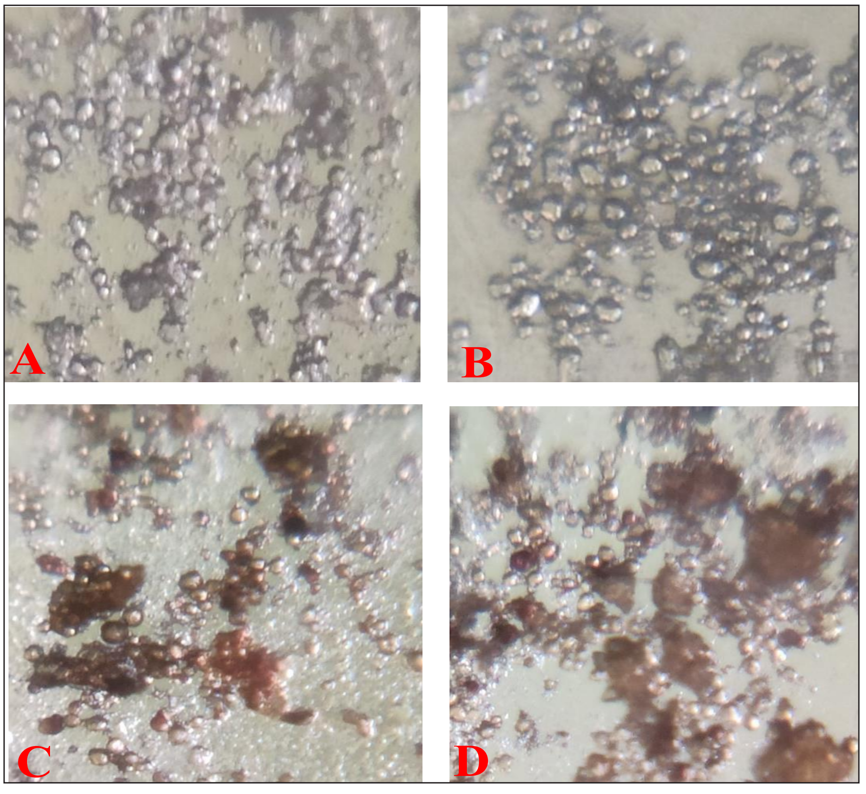

Fig. 4: Foldscope images of powdered cereal grains treated with cuprous iodide in potassium iodide solution: **(A)** Maize; **(B)** Sorghum; **(C)** Thiram (0.1%) contaminated maize; **(D)** Thiram (0.1%) contaminated sorghum

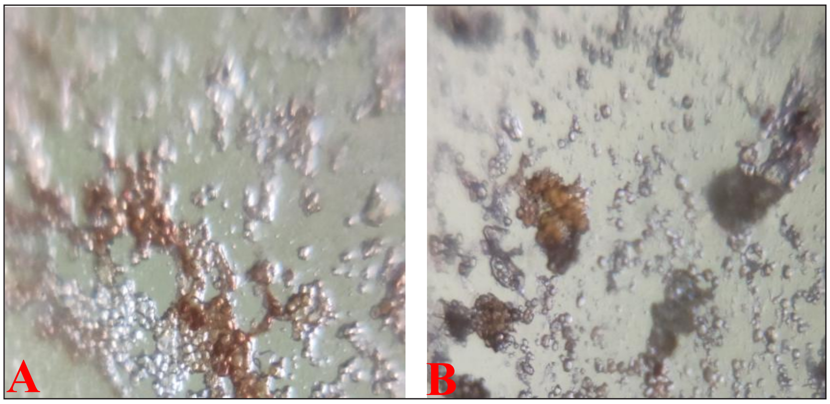

Fig. 5: Foldscope images of powdered maize grain treated with cuprous iodide in ammonia solution: **(A)** Thiram (0.01%) contaminated maize **(B)** Thiram (0.001%) contaminated maize

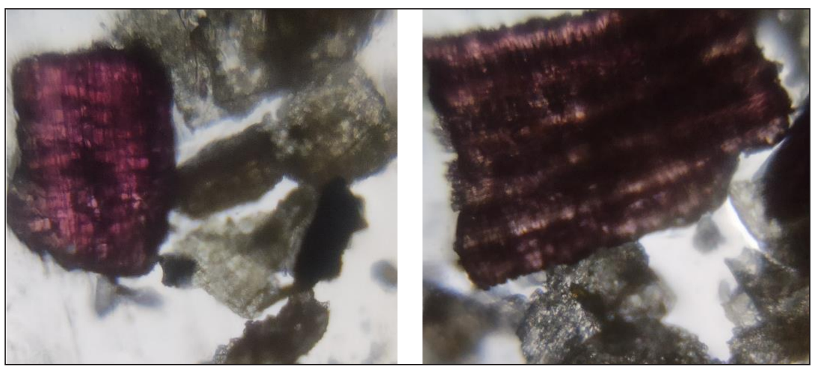

Fig. 6: Foldscope images of rice husk in de-oiled rice bran samples collected from a dairy farm on treatment with phloroglucinol-hydrochloric acid solution

samples extracted with chloroform and treated with cuprous iodide in ammonia solution. This could be due to poor extraction of thiram from samples by hot water as compared to chloroform. Therefore, this modified method may be useful to detect contamination of thiram when its concentration is high in the samples.

Foldscope images of maize grain contaminated with thiram at three different concentrations

As the concentration of thiram decreased in maize grain, area of starch granules covered with brown to amber colour and intensity of brown to amber colour also decreased in foldscope images (Fig. 5A and Fig. 5B). However, the increase in brown to amber colour area and the colour intensity were not in proportion to increase in the thiram concentration in maize grain due to formation of brown to amber colour mainly on the edges of the sample on the slide. This phenomenon might be due to movement of chloroform along with thiram when chloroform was applied on the slide as discussed earlier. Reddy and Reddy (2015) found thiram residues of 1.25 mg/kg in poultry concentrate mixture and 2.25 mg/kg in maize grain. In the present study, starch granules of maize covered with light brown to amber colour in foldscope images (Fig. 5B) was observed even at the thiram concentration of 1 mg/kg. Therefore, sensitivity of this method for detection of thiram in cereal grains may be less than 1 mg/kg.

Detection of rice husk and saw dust adulteration and thiram contamination in feed ingredients from different dairy farms

De-oiled rice bran is one of the commonly used feed ingredients for feeding of different kinds of livestock and poultry. Out of eight samples from different dairy farms, four samples of de-oiled rice bran were adulterated with rice husk (Fig. 6).

Among 10 samples, one sample of maize grain was found contaminated with thiram. Kavitha *et al.* (2015) reported that only one maize sample was found contaminated with thiram out of 213 samples. Therefore, the rate of occurrence of thiram contamination in maize grain in field conditions appears to be low as compared to rice husk adulteration in de-oiled rice bran.

CONCLUSION

Foldscope can be used to detect adulteration of rice husk and saw dust in feed ingredients and mixed feeds with phloroglucinol-hydrochloric acid solution and thiram contamination in maize and sorghum grain using cuprous iodide in ammonia solution on the spot under field conditions.

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REFERENCES

Alexander, G. 2018. Potential of foldscope for quality control of animal feeds. In: Proceedings of 11th Biennial Animal Nutrition Association Conference, Nov. 2018, Patna, India, pp. 31.

Anitha, T., Madhuri, S.B., Alexander, G., Chandra, A.S. and Venkateswarlu, M. 2022. Evaluation of staining procedures for foldscope based identification of cereal grains and oilseed cakes. *Pharm. Innov. J*., **11**(5): 1711-1716.

AOAC. 1995. Official Methods of Analysis. 16th Ed. Association of Official Analytical Chemists, Washington D.C., USA.

Cybulski, J. S., Clements, J. and Prakash, M. 2014. Foldscope: origami-based paper microscope. *PLoS One,* **9**(6): e98781.

Kavitha, R., Senthil Kumar, R.P., Natarajan, A. and Sundaram, T.K. 2015. Occurrence of thiram ectoparasiticide in animal feedstuff(s). *Indian J. Vet. Anim. Sci. Res.,* **44**(2): 132-134.

Li, W., Amos, K., Li, M., Pu, Y., Debolt, S., Ragauskas, A.J. and Shi, J. 2018. Fractionation and characterization of lignin streams from unique high-lignin content endocarp feedstocks. *Biotechnol. Biofuels,* **11**(1): 304.

Mitra, P. P. and Loque, D. 2014. Histochemical staining of *Arabidopsis thaliana* secondary cell wall elements*. J. Vis. Exp.,* **87**: e51381.

NDDB. 2012. Nutritive value of commonly available feeds and fodders in India. Animal Nutrition Group, National Dairy Development Board, Anand, India.

Poelking, V.G.C., Giordano, A., Ricci-Silva, M.E., Rhys Williams, T. C., Alves Pecanha, D., Contin Ventrella, M., Rencoret, J., Ralph, J., Barbosa, M.H.P. and Loureiro, M. 2015. Analysis of a modern hybrid and an ancient sugarcane implicates a complex interplay of factors in affecting recalcitrance to cellulosic ethanol production. *PLoS One,* **10**(8): e0134964.

Poletto, M., Dettenborn, J., Pistor, V., Zeni, M. and Zattera, A.J. 2010. Materials produced from plant biomass: Part I: evaluation of thermal stability and pyrolysis of wood. *Mater. Res.,* **13**(3): 375-379.

Reddy, M.V.B. and Reddy, Y.R. 2015. Pesticide residues in animal feed and effects on animals and its products with special reference to endosulfan. *Int. J. Res. Ayurveda Pharm.,* **6**: 371-374.

Rosado, M.J., Rencoret, J., Marques, G., Gutierrez, A. and del Rio, J.C. 2021. Structural characteristics of the guaiacyl-rich lignins from rice (*Oryza sativa* L.) husks and straw. *Front. Plant Sci*., **12**: 640475.

Roy, S., Acharya, R.N., Harisha, C.R. and Shukla, V.J. 2016. Macro, microscopic and preliminary analytical evaluation of root and leaf of *Globba marantina* Linn.- an extrapharmacopoeial drug of Ayurveda. *Ind. J. Pharm. Sci.,* **78**(4): 469-478.

Subapriya, S., Vairamuthu, S., Murali Manohar, B. and Balachandran, C. 2007. Pathomorphological changes in thiram toxicosis in broiler chicken. *Int. J. Poult. Sci*., **6** (4): 251-254.

Tao, S., Khanizadeh, S., Zhang, H. and Zhang, S. 2009. Anatomy, ultrastructure and lignin distribution of stone cells in two Pyrus species. *Plant Sci*., **176**(3): 413-419.

Uppal, D.S., Ilyas, S.M. and Sikka, S.S. 2004. Quality and safety of animal feeds in India. Central Institute of Post-Harvest Engineering and Technology (ICAR), Ludhiana, India.

Yeung, E.C. 1998. A beginner's guide to the study of plant structure. *In:* Tested studies for laboratory teaching**.** Karcher, S.J., Proceedings of the 19th workshop/conference of the Association for Biology Laboratory Education, June 1997, Calcary, Alberta, Canada, **19**: 125-142.