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Evaluation of Pollen Extraction Methodologies from Soil Substrate for Forensic Applications

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ABSTRACT

Forensic palynology has been important in criminal investigation since the 1950s and often provides evidence that is vital in identifying suspects and securing convictions. Soil is commonly used in forensic casework to provide discriminatory power to link a suspect to a crime scene. Therefore, in the present study, an attempt was made to find out the best method for pollen extraction. Soil samples from 10 different sites of Mohali were collected. The efficiency of previously used forensic laboratory protocols for retrieving pollen including centrifugation method, acetolysis method, and density separation method was tested. It was found that Bromine density separation method was found to be the best suitable method as compared to other methods for extraction. The present study is thought to be helpful in extracting pollens from soil or cloth substrates and help the investigating officers to link victim suspect or crime scene with each other.

Key words : Forensic science, palynology, biology, soil examination, microscopic techniques, botany

INTRODUCTION

Pollen is considered as a fine powdery substance consisting of micro-gametes that produce and carry male gametes. They contain pollen grains which have a hard coat that protects the gametophyte during the process of their movement from stamens to the pistil of a flowering plant (Kelso and Miller, 2016; Ochando *et al.,* 2018). As soon as the pollen grains fall on a compatible flowering plant, these germinate and give rise to a pollen tube which runs through the style part of female reproductive organ and transfers the male gamete to the ovule where one of the male gamete fuses with the female gamete or egg to give rise to an embryo, now-a-day's palynology is also studied in forensic so as to solve crime and to find a connection between the crime scene and the suspect (Uitdehaag *et al.*, 2016; Procter *et al.,* 2019; Guo *et al.,* 2022). Pollens are transferred by means of air, water, animals and can drop in the soil and various objects during transfer from male sporangia to female reproductive organs. They are minute structure and cannot be seen by naked eye which makes them hard to be found and use as evidence. They can be collected from the soil, mud on the shoes, clothes of victim and suspected and can be used as evidence (Munuera-Giner and Carrion, 2016; Abdulrahaman *et al.,* 2018; Allwood *et al.*, 2020; Phuphumirat *et al.*, 2021). Pollen is small in size and cannot be cleaned or wiped from the crime scene and thus helps investigator to solve crime. It also helps in the identification of the crime scene as certain plants are found at a particular location and identification of their pollen and spore can help in determining the location of crime. Pollens can be found on clothing, carpets, soil and mud on the shoes and can be identified with the help of pollen morphology. In forensic, pollens which are found commonly are of less use than the pollens which are less common and found in a specific small area (Langgut *et al.,* 2015; Shafeek *et al.,* 2015; Selamoglu *et al.,* 2016; Fløjgaard *et al.*, 2019; Guo *et al.*, 2021).

In the present study, different pollen extraction methods were compared and validated in order to determine their practical applicability and efficiency to identify pollens and their genus. Further attempt was made to study the retention of morphological characteristics of pollen in soil and cloth substrate with different extraction methods.

MATERIALS AND METHODS

Soil samples were collected from 10 different regions of Punjab and were marked with

voucher number. During collection, it was taken care that good amount of top layer of soil was collected and along with the soil samples, flowering plants in that region were also collected as a control to identify the family of pollens found in soil sample during analysis. Soil samples were taken and oven-dried at 105°C as soon as possible after collection in order to inhibit any possible microbiological activity. The soil was then digested with 10% NaOH.

Centrifugation method: The material was washed and centrifuged with distilled water at 3000 RPM several times to remove all caustic soda and then taken up in 5 ml of a 1:1 glycerine-water mixture, to which a 0.5% alcoholic solution of safranin was added at the rate of 1 ml/50 ml. In highly humic samples and especially if finely divided charcoal was present, it was often necessary to add extra stain. At this stage, the material was stored for a considerable time without deterioration. For analysis, the solution was thoroughly shaken up and a little of the suspension was taken on slide and observed under microscope.

Acetolysis method: Soil samples were taken and were oven-dried at 105°C as soon as possible after collection in order to inhibit any possible microbiological activity. The soil was then digested with 10% NaOH. The material was washed and centrifuged with distilled water at 3000 RPM several times to remove all caustic soda. Washing with glacial acetic acid was done to replace water with acetic acid. Freshly prepared acetolysis mixture consisting of acetic anhydride and concentrated H_2SO_4 (sulphuric acid) in the ratio of 9 : 1 was taken. Acetolyzed pollen suspension was centrifuged and supernatant liquid was decanted and pellet was observed under microscope. For analysis, the solution was thoroughly shaken up and a little of the suspension was taken on slide and observed under microscope.

Washing method: Soil samples were taken and were oven-dried at 105°C as soon as possible after collection in order to inhibit any possible microbiological activity. The soil sample was then washed with water and kept for some time. The upper layer was washed to remove any soil particle. Smear of this sample was made on a slide and observed under microscope.

Bromine density separation: Soil samples were taken and were oven-dried at 105°C as soon as possible after collection in order to inhibit any possible microbiological activity. The soil was then digested with 10% NaOH. Equal amount of bromine water was added to the soil sample and shaken thoroughly. For analysis, the upper layer was taken on slide and observed under microscope.

RESULTS AND DISCUSSION

In the present study, four extraction methods were tested in order to find out best extraction method to extract pollens for the purpose of forensic applications. The pollen extraction procedure was validated with respect to soil as well as cloth substrate (Table 1). Low quality or degraded pollens were found in soil sample using centrifugation method. Only two types

Table 1. The observation of pollens with different pollen extraction methods

S. No.	Samples	Centrifugation method	Acetolysis method	Washing method	Bromine density method
1.	Sample 1	-	-	-	+
2.	Sample 2	-	-	-	+
3.	Sample 3	-	-	+	+
4.	Sample 14	+	-	-	+
5.	Sample 15	-	-	+	+
6.	Sample 16	+	-	+	+
7.	Sample 17	-	-	+	+
8.	Sample 18	-	-	+	-
9.	Sample 19	-	-	-	+
10.	Sample 10	-	-	-	-
11.	Sample 11	-	-	+	+

+ sign denotes species observed under microscope and - sign denotes species absence.

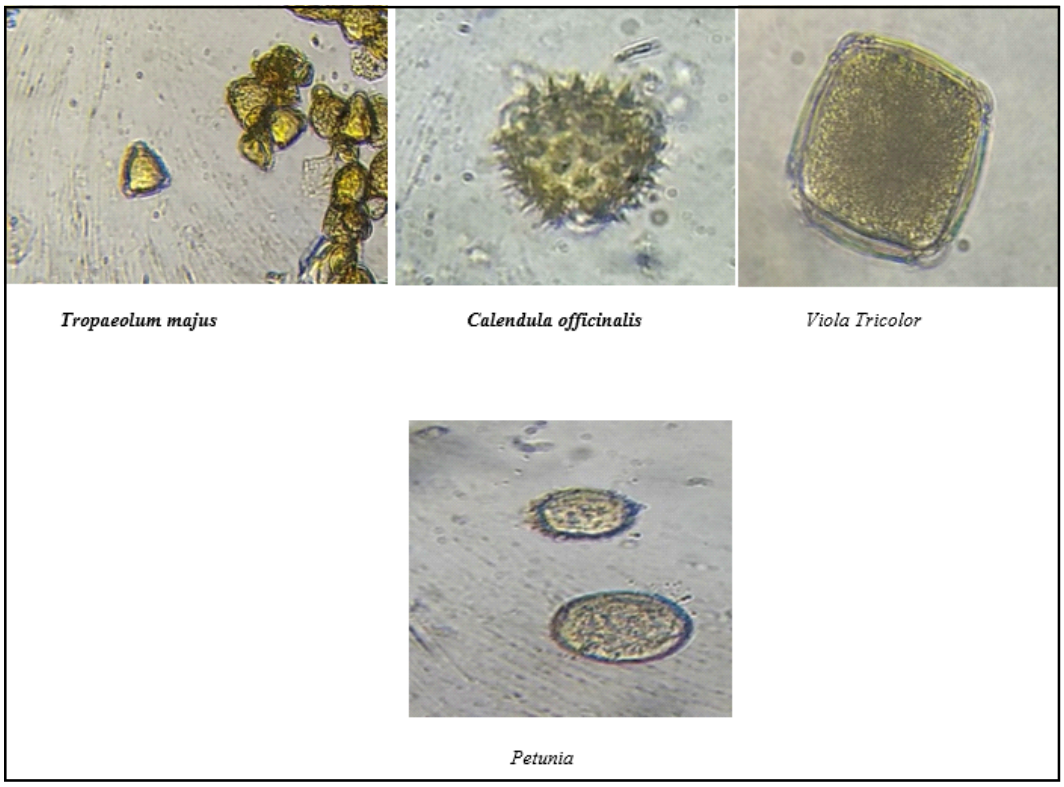

Fig. 1. The pollen diversity extracted from soil samples with the help of Bromine density method.

of pollen were found out of 10 standards. The frequency and number of pollens in the sample were also very less to give good results using centrifugation method. Least number of pollens was found in soil sample using Acetolysis method i. e. out of 10 standards no pollens were found in the sample. The frequency and number of pollens in the sample were close to zero making this method insignificant to be used for criminal investigative purposes.

Medium to high quality pollens were found in soil sample using washing method which were easily decipherable. The frequency and number of pollens in the sample were quite good making this method significant over the above given extraction method and can be used for criminal investigative purposes.

Medium to high quality pollens were found in soil sample using bromine density separation method which were easily decipherable, eight different types of pollen were found in the sample (Fig. 1). The frequency and number of pollens in the sample were quite good making this method significant over the above given extraction method and can be used for criminal investigative purposes.

Medium to high quality pollens were found in cloth sample when sample was observed with centrifugation method. The frequency and number of pollens in the sample were significant and can be used for criminal investigative purposes. The best and efficient method for extracting pollens from soil was found to be Bromine density separation method (80%) >Washing method (60%) >Centrifugation method (20%) >Acetolysis (0%).

CONCLUSION

The study was undertaken to find out the best method for pollen extraction. For the extraction of pollens from soil samples Bromine density separation method was found to be the best suitable method as compared to other methods for extraction. The present study was thought to be helpful in extracting pollens from soil or cloth substrates and help the investigating officers to link victim suspect or crime scene with each other.

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