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Comparative light and scanning electron microscopic studies of pollen of two marijuana species causing allergies

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Abstract

Marijuana (*Cannabis*) belongs to family *Cannabaceae*. This plant is a great natural source of cannabinoids, which have several medical applications such as it is used for the treatment of anxiety and psychosis, disorders of motility and epilepsy. However, this plant is also notorious for its pollen allergic reactions. Mainly two species, that is, *C. sativa* L. and *C. indica* Lam. are involved in allergic reactions. In this study, morpho-palynological studies of both the species were conducted. A number of morphological differences were observed in the both species. *C. sativa* is tall with narrowed leaves and fibrous stalk plant while *C. indica* is short, bushier, broad leaves, and having woody stalk plant. Both the species are varied in their pollen characteristics. The polar diameter of *C. sativa* observed was 7.5 μm and equatorial diameter was 8 μm , and its shape was oblate-spheroidal. *C. indica* had 7 μm polar diameter, 7.5 μm equatorial diameter but its shape was similar to *C. sativa*, that is, oblate-spheroidal. About 40%-50% people were affected with both these marijuana species and had symptoms of allergies like dry cough, congestion, itchy eyes, sore throat, nausea, a runny nose, watery eyes, and sneezing. It was concluded that both species' pollen are allergy causing and pollen of both species are different with variations in morphological and allergic symptoms.

Research Highlights

- Marijuana has great therapeutic importance but it is also notorious for its pollen allergic reactions. Two species of Marijuana, that is, *Cannabis sativa* L. and *Cannabis indica* Lam mainly responsible for these pollen allergies. The present research work focused the morpho palynological analysis of these two Marijuana species through light and scanning electron microscopy.

KEYWORDS

Cannabaceae, *Cannabis indica*, *Cannabis sativa*, light microscopy, marijuana, palynology, scanning electron microscopy

INTRODUCTION

Marijuana (*Cannabis*) belongs to a flowering plant family, that is, *Cannabaceae*. There is contradiction over the number of plant species

with reference to genus *Cannabis*. According to some scientists *Cannabis* genus is comprised two species, that is, *Cannabis sativa* L. and *Cannabis indica* Lam. While other researchers report that *Cannabis* has more species other than these two. Majority of researchers report this

genus as one of the highly polymorphic genus containing *C. sativa* as a main species (Raman et al., 2017). The female *Cannabis* plant has significant amount of psychoactive constituents such as tetrahydrocannabinol in its dry fruiting, flowering tops, and leaves. Due to this trait *Cannabis* plant is used as a repellent of pests and killer for insects, mites, fungi, nematodes, and weedy plants. The primary active components of *Cannabis* plant are tetrahydrocannabinol (THC) and cannabidiol (CBN), which play an important role in its psychoactive effects. However, about sixty six (66) other cannabinoids, including: cannabinol (CBN), cannabidiol (CBD), and tetracannabivarin (THCV), also have been reported (Audu et al., 2014).

Geographically, genus *Cannabis* grows at 30°N north latitude and 60°N south latitude. It has great natural distribution in the flora of China, Russia, Iran, Pakistan, and India (Hillig, 2005). All over the world *C. sativa* and *C. indica* are frequently grown as a cultivated species but they grow naturally in mountainous and rural areas of Pakistan. *C. indica* is commonly found in Hindukush, Karakoram, and north mountainous regions. On the other hand *C. sativa* is found abundantly in different parts of province Punjab like Sialkot, D.G khan, Layyah, Bhakar, Muzafargarh, and Mianwali, (Anwar et al., 2006). *C. sativa* plants are erect, annuals, herbs, and mostly dioecious but monoecious sometimes. They are up to 1–6 m in height and its stems are hollow, green, longitudinally ridged, and cylindrical. The leaves are palmate and 3–9 lobed. The lobes are 3–20 cm long, having a width of 1.8 cm and narrowly oblong-lanceolate. At either side of leaf, a primary mid-rib is present. Female and male flowers grow on independent plants. Female plants are normally stronger and healthier while male plants are taller (Raman et al., 2017).

Many researchers and scientists have conflict of interest that *C. sativa* and *C. indica* are truly separate species or not? And it is a matter of debate among them till yet. Jean-Baptiste Lamarck in 1785 described second species of *Cannabis* and published it with the name of *C. indica*. Richard Evans Schultes explained *C. indica* as a tall and densely branched while *C. sativa* was explained as comparatively long and laxly branched. Leaflets of *C. indica* are small but broad whereas *C. indica* has tall and narrow-leaflets.

The study of pollen features of a plant is called as palynology and many scientific researches utilized the information obtained from pollen studies in forensic studies, systematics, and genetics (Sufyan et al., 2018). *Cannabis* produces enormous pollen. One flower can produce nearly 350,000 pollen and a plant has hundreds of flowers. The pollen components of *C. sativa* are cannabinoids, THC, and THCA (Small & Antle, 2003). However, *C. indica* has high amount of THC content than *C. sativa* strains. Variation is produced due to human intervention within the species in this genus and only one species is recognized by some authorities that had selective pressure to produce plants with high THC content. Although a large variability exists between the two species and there is a need of elaborated discussion that whether existing paradigm is sufficient to delimit these two species of genus *Cannabis* (Chatkin et al., 2019).

The symptoms of inhalation of *Cannabis* pollen are allergic rhinitis (sneezing and nasal congestion) and conjunctivitis (eye redness and pruritus), and it also causes asthma (wheezing, cough, and dyspnea). Allergic manifestations like wheezing, sinusitis, rhinitis, periorbital angioedema,

conjunctivitis, and throat edema can also be a result of pollen allergy caused by marijuana pollen (Alyaset al., 2021).

Despite of these allergies *Cannabis* has been used as a source of oil and medical applications in different regions of the world. Since ancient times *Cannabis* has been used for medical purposes such as inflammation, pain, anxiety, and food intake (Andre et al., 2016). After D9-THC, the second most abundant phytocannabinoid is CBD, it has pharmacotherapy potential for treating the various neuropsychiatric disorder symptoms like anxiety, psychosis, addiction, epilepsy, and disorders of motility (Boggs et al., 2018).

All the above cited literature shows the immense value of *Cannabis* plant. So the main aim of study was the identification of pollen of the two studied Marijuana (*Cannabis*) species and make comparison on morpho-palynological basis. The morphological and palynological characteristics of both *Cannabis* species pollen were studied by using both Light and Scanning Electron Microscopic techniques.

1 | MATERIALS AND METHODS

1.1 | Sample collection and identification

Various Field trips were conducted to collect the pollen samples of family *Cannabaceae* (*C. sativa* and *C. indica*) in different areas of district Lahore (Bagh-e-Jinnah, PHA Nursery, Cantt Area, R.A Bazar Nursery, and Jallo Mor Area). After collection, all the samples were preserved. By using the different herbaria (LCWU, Lahore, NARC, Islamabad and QAU, Islamabad) and by consulting the flora of Pakistan (<http://www.eflora.org/index.aspx>), collected samples were identified. To get the right botanical name, a website (www.ipni.org) named as International Plant Name Index (IPNI) was also utilized.

1.2 | Method of preparation of pollens

The collected and preserved pollen were prepared for microscopy by the following method.

1.2.1 | Acetolysis

According to the standard procedure of Younis et al. (2021), pollen were acetolyzed. Preserved samples of flowers having pollen were put in to 5 ml glacial acetic acid and then crushed with help of glass rod. After crushing, pollen material was centrifuged for about 20 min at 4000 rpm. After centrifugation glacial acetic acid was descended and pollen material was separated from it. Then one part of concentrated sulfuric acid and nine parts of acetic anhydride were added to the separated pollen residue. This mixture was heated in a water bath up to boiling point for 3–4 min. Then after cooling, the mixture was centrifuged for about 20 min at 40000 rpm and the liquid part was descended. The separated residual material was washed with distilled water. The washed and sieved material was again centrifuged. After centrifugation, material was divided into two parts for scanning and light microscopy testing.

1.3 | Palynological studies

1.3.1 | Preparation of glycerin jelly

For the preparation of glycerin jelly, method of (Alyas et al., 2021) was used. Fifty (50) ml distilled water was taken and boiled on a hot plate, then 35 grams of gelatin was added and the temperature was set up to limit of 70–80°C. A viscous solution was formed. That solution was kept on a hot plate for an hour and then 35 grams of glycerin/glycerol was mixed in it with few phenol crystals. To give pink color to jelly 0.1% safranin was added to it. Then for stabilization the gel was set at room temperature.

1.3.2 | Palyno-morph characteristics

The pollen slides prepared with glycerin jelly were examined by using the light and scanning electron microscope. Both the qualitative and quantitative characteristics were observed. Shape of pollen in polar as well as in equatorial view, spine and colpi presence, presence and absence of pore and pore shape, pores number, sculpturing of pollen, P/E ratio, polar and equatorial diameter, spines number, colpi number, colpi length and width, length of spine and thickness of intine and exine were studied.

1.4 | Light microscopy (LM)

Then separated pollen material was transferred to glass slide with drop of glycerin jelly. The slide got dried and then examined under light microscope.

1.5 | Scanning electron microscopy

For scanning, the acetolyzed pollen were suspended in 90% ethanol and then transferred on a double adhesive tape of metallic stub. For an hour, the stub was placed at room temperature to dry. Then the pollen material was examined by Scanning Electron Microscope, JEOL (JSM-6480LV).

2 | RESULTS AND DISCUSSION

In different areas of Pakistan, marijuana plant of family Cannabaceae is widely distributed. This plant has immense medicinal and industrial value (McPartland, 2017). Although both species resemble much with each other but still have many morphological differences. Previous literature showed that the palynomorph studies play an important role to evaluate pollen diversity and taxonomic significance (Liao et al., 2021). Morphological differences between the *C. Sativa* and *C. indica* were observed. *C. sativa* plant was tall with narrowed leaves and fibrous stalk with early maturation while *C. indica* plant was short, bushier with broad leaves and woody stalk with late maturation (Table 1).

TABLE 1 Qualitative morphological comparative description of *Cannabis sativa* and *Cannabis indica*.

Botanical name	Family	Origin	Habitat	Distribution in world	Distribution in Pakistan	Flowering period	Morphological description
<i>Cannabis sativa</i>	Cannabaceae	Originated from Temperate Central Asia and Indigenous to Eastern Asia.	Roadside, inhabited and open areas.	America, Russia, China, India, Pakistan, and Iran.	Lahore, Sialkot, Layyah, Bhakar, Mianwali, Muzafargarh, D. G khan, hilly area of Islamabad, and Quetta.	April–June	Tall, narrowed leaves and fibrous stalk plant with early maturation.
<i>Cannabis indica</i>	Cannabaceae	Originated from Hindu Kush Mountain Range.	Harsh, dry, and turbulent climate.	Drier Areas of Southern and Central Asia climates like India, Afghanistan, Morocco, Turkey, South China, and Pakistan.	Peshawar, Karachi, Punjab, Himalaya, Bhakar, Mianwali, Hilly Areas of Islamabad and Quetta.	May–July	Short, bushier, broad leaves and woody Stalk plant with late maturation.

Mostly, *Cannabaceae* pollen grains are recovered in sediments from the bottom of wells, ponds, and lakes. Typically, *Cannabaceae* pollen consists of trizonoporate grains and less than 50 microns in diameter (Fleming & Clarke, 2008). Morphology of pollen is an important taxonomic tool in the classification and identification of plants (Ullah et al., 2019). The palynology of *C. sativa* under LM and SEM showed that the type of pollen of *C. sativa* specie is triplicate. From polar and equatorial view, the shape of pollen seen was oblate or spheroidal. The polar diameter of *C. sativa* pollen was 24.5 (24–25) μm and the equatorial diameter was 22.5 (20–25) μm . According to the polar and equatorial diameter, the P/E ratio of pollen of *C. sativa* was 1.08 and the pollen fertility observed was 93.75%. The outer covering of pollen grain is called exine and the exine thickness of pollen of *C. sativa* observed was 1.0 μm . Shinwari et al., (Shinwari et al., 2015) carried out the palynological study of *C. sativa* but his results were a bit different from the present studies. According to Shinwari results the polar diameter of *C. sativa* was 21.04 μm (18.41–23.67 μm) and the equatorial diameter was 21.04 μm (18.41–23.67 μm) (Plate 1). The palynology of *C. indica* under LM and SEM showed that the type of pollens of *C. indica* specie is triplicate (Plate 2). The shape of pollen was same as *C. sativa*, that is, oblate or spheroidal. (Plate 3). The polar diameter of *C. indica* pollen was 30 (25–35) μm and the equatorial diameter was 30 (25–35) μm . According to the polar and equatorial diameter, the P/E ratio of

pollens of *C. indica* was 1.0 and the pollen fertility observed was 97.33%. The exine thickness of pollen of *C. indica* was 1.2 μm (Plate 2). The results of the present studies are in agreement with the studies of Liao et al., which concluded that the pollen variations are useful for taxonomic revisions at the species level.

In this study, the relationship of marijuana pollen with allergies was also observed. Pollen are the main cause of pollen allergy that are wind pollinated from grasses, weed, and trees. In 1995, a survey was published by Pakistan Medical and Research Council and according to it 45% residents of Islamabad and Rawalpindi had allergies to the pollen of *Cannabis* and paper mulberry (Sufyan et al., 2018). The studies on pollen allergy in Pakistan are not comprehensively addressed but there are few studies found in which the problem of pollen allergy was found very crucial (Shinwari et al., 2015).

In summer season when pollen allergies grows, due to *C. sativa* pollen allergy people suffer from the dry cough, sneezing congestion, itchy eyes, sore or itchy throat, nausea, asthma, rhinitis, rashes under the skin and cramping. It also causes severe allergic reactions called anaphylaxis (dizziness, fainting, vomiting and difficulty in breathing) (Figure 1). On the other hand the *C. sativa* produce Δ^9 -tetrahydrocannabinol (THC) more than cannabidiol (CBD) and produce a terpenoid that has herbal and sweet smell. It imparts uplifting and is suggested for the treatment of headaches, depression, and loss of appetite (Sufyan et al., 2018).



PLATE 1 Inflorescence of (a) *Cannabis sativa* (b) *Cannabis indica*.

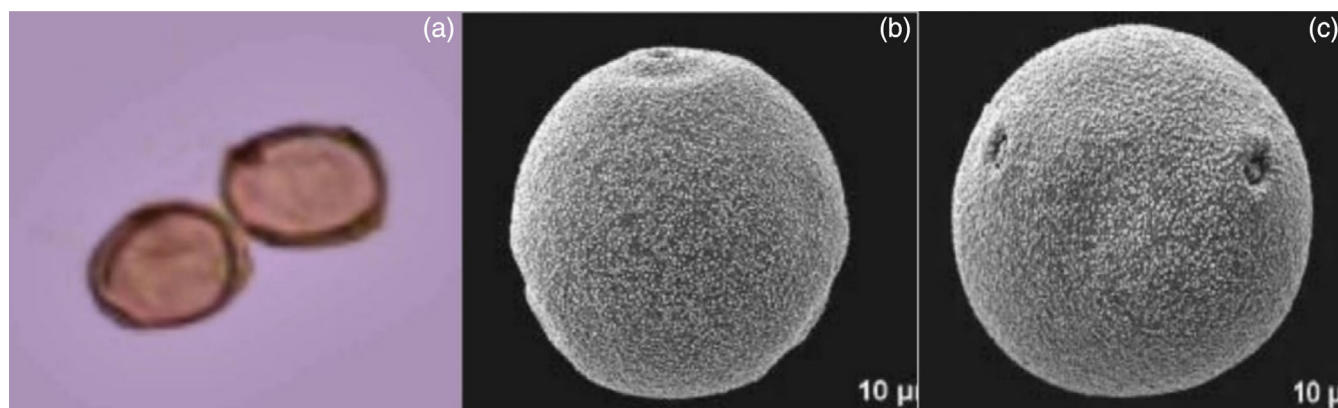


PLATE 2 (*Cannabis sativa*): (a) the light micrographs of pollen (b) scanning electron microscopy showing polar view of pollens of *Cannabis sativa* and (c) scanning electron microscopy showing equatorial view of pollen of *Cannabis sativa*.

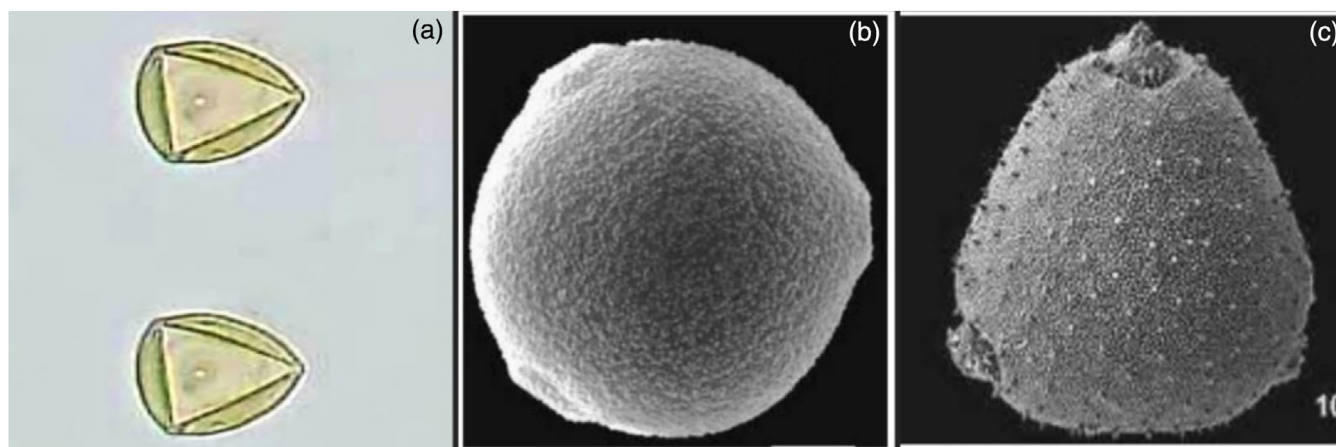


PLATE 3 (*Cannabis indica*): (a) the light micrographs of pollen (b) scanning electron microscopic polar view of pollen of *cannabis indica* and (c) scanning electron microscopic equatorial view of pollen of *Cannabis indica*.

TABLE 2 Comparative palyno-morph analysis of *Cannabis sativa* and *Cannabis indica*.

Sr. no	Plant specie name	Common name	Polar diameter (μm)	Equatorial diameter (μm)	P/E ratio (%)	Pollen shape	Pollen type	Exine thickness (μm)	Pollen fertility
1.	<i>Cannabis sativa</i>	Hemp, Marijuana	24.5 (24–25)	22.5 (20–25)	1.08	Oblate spheroidal	Triporate	1.0 μm	93.75%
2.	<i>Cannabis indica</i>	Purple Kush, Charas, Hashish	30 (25–35)	30 (25–35)	1.0	Oblate spheroidal	Triporate	1.2 μm	97.33%

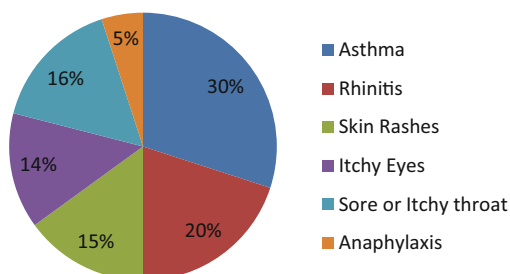


FIGURE 1 *Cannabis sativa* allergy symptoms.

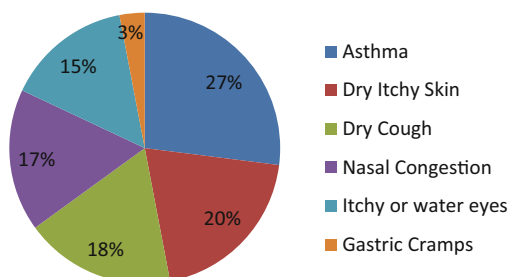


FIGURE 2 *Cannabis indica* allergy symptoms.

Due to the presence of THC and CBN, *C. indica* has some common pollen allergic symptoms which differ very slightly from *C. sativa*. Along with red, itchy and watery eyes, dry cough, nasal congestion, sneezing,

dry itchy skin people also suffer from gastric cramping and rashes under the skin surface. Asthma and rhinitis are also the symptoms observed by *C. indica* pollen allergies (Figure 2). *C. indica* produce about equal ratio of THC and CBD and its terpenoid has a skunky or acrid aroma. *C. indica* imparts sedating, relaxing, and pain-reducing effects and it is recommended for treating the treating pain, inflammation, insomnia, muscle spasms, glaucoma, and epilepsy (McPartland, 2017).

Typically, before dispersion pollen grains are dehydrate and when they land on stigma they rehydrate by getting water from it. After 72 h, the viability of *C. sativa* pollen fell off from about 70% to less than 5% at anthesis (opening of anthers) (Zafar et al., 2011). According to a study done by Small and Antle in 2003 under the favorable weather conditions, *Cannabis* pollen viability may reach 90% but usually ranges from 60% to 80%; and storage pollen viability is about 50% after 3 days and after 7 days it's about 16%.

3 | CONCLUSIONS

Weed pollen are common allergies sources worldwide. In Lahore marijuana (*Cannabis*) exists in many areas and its pollen are considered to be a cause of pollen allergy in residents of Lahore during fall season. The prevalence of allergies due to marijuana is increasing like asthma and rhinitis. According to a survey above 50% people have symptoms of allergies due to marijuana pollen. Morphology and palynology of pollen of marijuana showed the qualitative and quantitative features.

The observed pollen features through LM and SEM are a good source of taxonomic information that can help the species and genera delimitation and identification. *Cannabis* (Marijuana) is illegal plant in Pakistan so we ever gave it less importance but we can cultivate it in the areas far from cities and residency places so that people can be saved from the allergies caused by it but on the other hand due to its medicinal importance we can use it for many clinical purposes because it is used for the treatment of epilepsy, anxiety and psychosis, disorders of motility, epilepsy and as painkiller.

DATA AVAILABILITY STATEMENT

All the data mentioned in the article is available and all the necessary citations are given in the manuscript.

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