

Biochemical Analysis of Pollen Grains of *Datura innoxia* L. for Screening its Allergenicity

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Abstract

Pollen grains contain number of metabolites which are responsible for physiological and metabolic activities. Pollen grains release the proteins, free amino acids and other chemical metabolites at different rates and quantities. The differences in these metabolites may be responsible for the control of rate and type of allergic reactions caused by pollen grains. *Datura innoxia* is a well-known pollen allergenic plant of India. Quantitative biochemical estimation in the form of carbohydrates, proteins, lipids, free amino acids, ash and moisture was done from the crude extract of the pollen of *Datura innoxia* L. During the present study, the pollen grains were found to be containing 1.28% of total reducing sugar, 3.28% of carbohydrates, 2.48% of nitrogen, 14.9% of crude protein, 0.69% of soluble protein, 0.86% of free amino acid, 1.23% of lipid, 8.77% of moisture and 2.68% of ash. The pollen grains were examined histochemically to assess qualitatively starch and lipid contents. The free amino acids and sugars were identified by thin layer chromatography.

Keywords: *Datura innoxia*, biochemical analysis, allergenic pollen.

1. Introduction

Pollen grains are the major constituent of the organic microbodies in the atmosphere, which cause allergic diseases like hay fever, asthma and dermatitis [1]. Pollen grains contain the number of metabolites like proteins, nucleic acids, carbohydrates, lipids and vitamins which are responsible for physiological and metabolic activities [2]. They release the proteins and other chemical metabolites to the environment at different rates and in different quantities which depend primarily on specific differences. Such differences are responsible for the control rate and type of allergic

reactions caused by different types of pollen [1]. The effect of airborne pollen on human health resulting in respiratory and skin disorders in sensitive patients has also been recognized. Hence, in recent years, a considerable amount of work has been carried out on various aspects of pollen biochemistry. Similar work has been carried out by Chakraborty *et al.*[3]; Mondal *et al.*[4-5]; Boral *et al.*[6] Parui and Mandal [7]; Parui *et al.*[8-9] on chemistry and biochemistry of allergenic pollen grains of various plant species. Identification, characterization and protein profile of the allergenic pollen proteins has been carried out by Mondal *et al.* [6].

Datura innoxia is an annual shrub with showy- white flowers. It contains alkaloids that are quite poisonous and psychoactive. *Datura innoxia* is widely naturalized as a weed and are difficult to control. It is toxic to animals and humans. The pollen of *Datura* Sp. has been found to have a role in causing allergy in sensitive patients [7]. Hence, to know the chemical nature of the allergenic pollen grains of *Datura innoxia*, the plant has been selected for the investigation.

2. Materials and Method

In the present investigation pollen grains of *Datura innoxia* were collected from the Forest area of Hingoli District during the Months of August 2018 to October 2019. Pollen grains were collected in bulk in the morning before anthesis and sun-dried. Microscopic analysis of the samples was done to check the pollen purity up to 95 - 98%.

Total reducing sugar has been quantified according to method suggested by Miller [10]. Recorded the intensity of dark red colour at 510 nm using UV-Vis. Spectrophotometer. The total carbohydrates have been quantified using Anthrone reagent [11]. For total nitrogen content, 50 mg of pollen samples were analysed by Micro-Kjeldahl method [12]. The total amount of crude protein was estimated by using 1:6.25 factor. The lipid content was determined as crude fat from a solvent extract [13] and amount was estimated from the difference in weights [14-15].

Moisture content in pollen was also determined by differences in weights after oven drying at 105°C. The pollen ash was prepared by using Blast Furnace at 500 to 600°C and weighted after cooling. The starch was investigated by using IKI solution and lipids were tested by using Sudan IV dye.

The total content of free amino acids was determined using ninhydrin solution [16]. Pollen sample (50 mg) was homogenized with 5-10 ml of 80% ethanol and centrifuged. The free amino acids were extracted and quantified by quantitative and qualitative estimation [17]. The intensity of the violet colour developed was read using UV spectrophotometer at 570 nm, compared to pure reagent. A calibrated solution of glycine was used as standard. Qualitative analysis of the free amino acids was carried out by thin layer chromatography (TLC) on Aluminium sheets (Merck) using n-Butanol: Acetic acid: Water (80:20:20 v/v) as eluant [18]. Then, 0.1% Ninhydrin in Acetone was used for the detection of amino acids by heating the sheets at 110°C for 5 minutes and the R_f values were calculated following Mondal *et al.* [5].

Thin layer chromatography (TLC) of major sugars has been carried out according to method suggested by Baron and Economidis (20) using n-Butanol: Acetic acid: Water (40:10:50 v/v) as eluant. Then 5:5:1 of 1% aniline, 1% diphenylamine and orthophosphoric acid was used for detection of sugars.

Total soluble protein was estimated following the method of Lowry *et al.* [19], using bovine serum albumin through standard curve.

3. Results

The carbohydrate level in pollen grains was found to be variable depending upon the species [1]. In the present study the total amount of carbohydrates was found to be 3.28%. The amount of total reducing sugar was found 1.28% (Table 1).

Table 1 The Biochemical Composition of Pollen Grains of *Datura innoxia*

S.N.	Content	Amount in Dry Weight of Pollen	Percentage in Dry Weight of Pollen
1.	Total Reducing Sugar	0.128 mg/10mg	1.28%
2.	Carbohydrates	0.328 mg/10mg	3.28%
3.	Nitrogen	1.24 mg/50mg	2.48%
4.	Crude Protein	0.149 mg/1mg	14.90%
5.	Soluble Protein	0.069 mg/10mg	0.69%
6.	Free Amino Acid	0.43 mg/50mg	0.86%
7.	Lipid	0.123 mg/10mg	1.23%
8.	Moisture	0.877 mg/10mg	8.77%
9.	Ash	0.268 mg/10mg	2.68%
Histochemical Contents			
10.	Starch	Present	+
11.	Lipid	Absent	-
TLC Analysis of Free Amino Acids			
12.	Lysine	Present	+
13.	Glycine	Present	+
14.	Proline	Present	+
TLC Analysis of Sugars			
15.	Glucose	Present	+
16.	Fructose	Absent	-
17.	Sucrose	Present	+

The nitrogen is one of the elements, which is present in proteins, nucleic acid and all type of genetic material. In *Datura innoxia* amount of nitrogen was found to be 2.48 %. Protein is one of the chief constituents of all living matter. Stanley and Linskens [2] reported that protein is one of the major constituents present in the pollen that play vital role during pollen tube growth. The amount of crude protein was found to be 14.90% and soluble protein was found to be 0.69%. Total free amino acids are usually higher in pollen than in leaves or other plant tissues. Free amino acids may comprise up to 6% of the pollen dry weight as found in *Petunia* [2]. In *Datura innoxia* amount of total free amino acids was found to be 0.86%. Todd and Bretherick [20] estimated the total lipid content of the pollen of different plant species which varied from 1 to 20 % of the dry weight. *Datura innoxia* showed 1.23% of lipids present. Moisture content as high as 50% has been reported in *Zea mays* [20]. It was seen that 8.77% of moisture present in *Datura innoxia* pollen. The amount of ash was observed to be 2.68% (Table 1).

The chemical constituents present on the pollen surface were analyzed using histochemical tests. In *Datura innoxia*, presence of lipid is not supported by test, however the starch showed its presence on pollen wall. Regarding the free amino acid composition of different pollen, all the essential amino acids have been reported to be present in pollen and total levels of free amino acids are usually higher in pollen than in leaves and other tissues [2]. The qualitative analysis of free amino acids by TLC showed the presence of Lysine, Glycine and Proline in the pollen grains. However, it is very difficult to draw any conclusion on evolution based upon the data on free amino acid content only, as amino acid composition greatly varies with climatic and nutritional conditions as well as with storage and handling patterns. Proline in the pollen is closely associated with their fertility and is involved in pollen tube formation and in other fundamental metabolic reactions associated with the sexual process. Proline is one of the most abundant free amino acids observed in pollen as earlier reported by Stanley and Linskens [2].

which is also found to be present in *Datura innoxia* pollen grains. Although free amino acids in pollen are not directly involved in the allergic reaction in human beings, they serve as precursors for proteins, which are main allergenic factors. The allergenicity of pollen depends upon their amino acid sequences that may markedly differ between genera and families.

The TLC of sugar was carried for the presence of Glucose, Fructose and Sucrose. In *Datura innoxia*, Fructose was found to be absent (Table 1).

4. Conclusion

During the present investigations pollen of the *Datura innoxia* was studied for biochemical estimations. Major differences have been found in biochemical content between estimated values and reviewed literature. These differences in amount of biochemical content may cause allergy, differing from region to region. Variations in amount of carbohydrates, amino acids and proteins, surely the reason for the allergenicity of *Datura innoxia* pollen.

Conflicts of interest: The author stated that no conflicts of interest.

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