Chapter- 4

RESULTS AND DISCUSSION

Morphological diversity of the three species of *Datura*

4. Result and discussion

4.1 Diagnostic characters

- ***Datura metel***

  Annual or biennial undershrub. Stem puberuious or glabrescent. Leaves ovate-elliptic. Flowers white, large, axillary; bracts tomentose; corolla sometimes purple-tinged, lobes 5-cuspidate, acute tip. Capsules subglobose, covered with straight sharp spines. Seeds numerous, compressed, nearly smooth.

Systematic position:-

Order- Solanades

Family- Solanaceae

Subfamily- Solanoidea

Tribe- Datureae

Genus- *Datura* sp.

Species- *D. metel* Linn.

Habitat: Terrestrial, plants are mostly mesophytes.

Habit: Annual or biennial undershrub.

Stem: It is aerial, erect and mostly herbaceous. Glabrescent or puberulous. Deep purple, terete (Fig. 13).

Leaf: Ovate-elliptic, acute, unequal at the base, entire, reticulate venation, green in colour.

FLORAL CHARACTERS

Inflorescence	It is commonly a cymose type. It may be axillary or terminal. In *Datura*, it is terminal and solitary.
Flower	White, large, axillary. Pedicellate, complete, bisexual, actinomorphic, heterochlamydeous, pentamerous and hypogynous. Bracts tomentose (Fig. 13)
Calyx	Sepals 5, gamosepalous and valvate.
Corolla	Petals are 5 gamopetalous and valvate (or) twisted aestivation. Sometimes purple-tinged, acute-tip.
Androecium	It consists of five, stamens are alternating with petals. Anther lobes are large, dithecous, introrse, basifixed and the dehiscence may be longitudinal in *Datura*.
Gynoecium	It is bi-carpellary and syncarpous. Ovary is superior, bilocular with many ovules on swollen axile placentation. In *Datura*, ovary is tetralocular due to the development of false septum.
Pollination	It is commonly entomophilous (cross pollination through insects). Flowers are usually protandrous
Fruit	Capsules sub-globose, covered with straight sharp spine.
Seed	Numerous, compressed, nearly smooth.

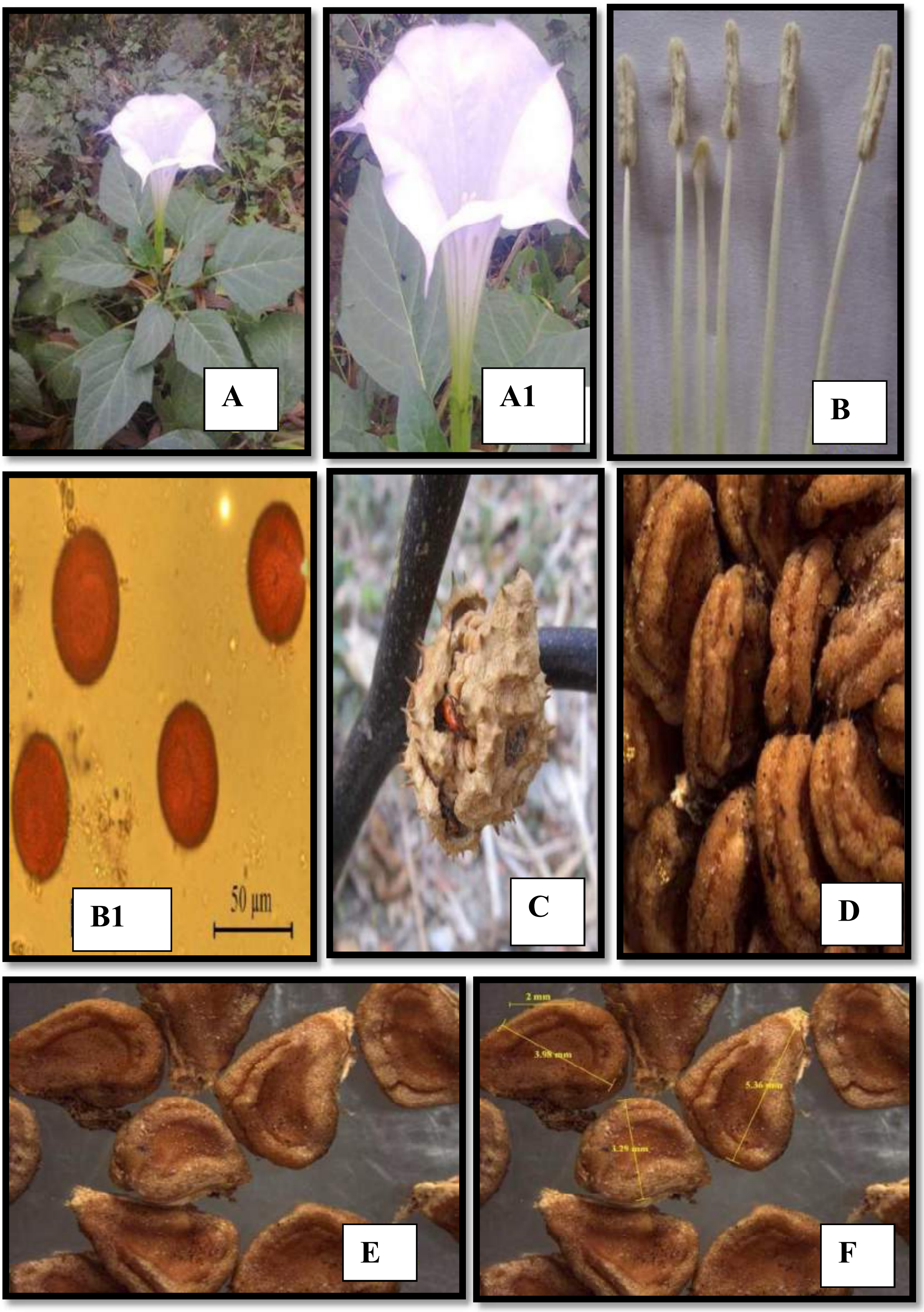


Fig. 13: A. Flowering twig of *Datura metel*, A1. A single flower, B. Androecium and Gynoecium of *Datura metel*, B1. Pollen (L.M model no Leica Dm-1000, magnification 40X), C. Seed dispersal, D& E Seeds, F. Dimensions (size) of seeds. (L.M model no Leica Dm-1000, magnification 40X)

- ***Datura stramonium***

Coarse annual glabrous herb. Leaves stalked, ovate, toothed, pale green. Calyx lobes ovate-lanceolate. Corolla white, lobes 5, cuspidate. Capsule erect, ovoid, covered with straight sharp spines. Seed numerous, compressed, nearly smooth.

Systematic position:

Order- Solanades

Family- Solanaceae

Subfamily- Solanoidea

Tribe- Datureae

Genus- *Datura* sp.

Species- *D. stramonium* Linn.

Habitat: Terrestrial, Plants are mostly mesophytes.

Habit: Annual or biennial undershrub.

Stem: It is aerial, erect and mostly herbaceous. Glabrescent or puberulous. Deep purple, cylindrical, terete. (Fig. 14).

Leaf: Stalked, ovate, toothed, pale green, unequal at the base, entire, reticulate venation, green in color.

FLORAL CHARACTERS

Inflorescence	It is commonly a cymose type. It may be axillary or terminal. In *Datura*, it is terminal and solitary.
Flower	White, large, axillary. Pedicellate, complete, bisexual, actinomorphic, heterochlamydeous, pentamerous and hypogynous. Bracts tomentose. **Fig.14**
Calyx	Sepals 5, gamosepalous and valvate. Calyx lobes ovate-lanceolate.
Corolla	Petals are 5, gamopetalous and valvate (or) twisted aestivation. White acute-tip.
Androecium	It consists of five, stamens are alternating with petals. Anther lobes are large, dithecous, introse, basifixed and the dehiscence may be longitudinal in *Datura*.
Gynoecium	It is bi-carpellary and syncarpous. Ovary is superior, binocular with many ovules on swollen axile placentation. In *Datura*, ovary is tetralocular due to the development of false septum.
Pollination	It is commonly entomophilous (cross pollination through insects). Flowers are usually protandrous
Fruit	Capsules erect, ovoid, covered with straight sharp spine.
Seed	Numerous, compressed, nearly smooth.

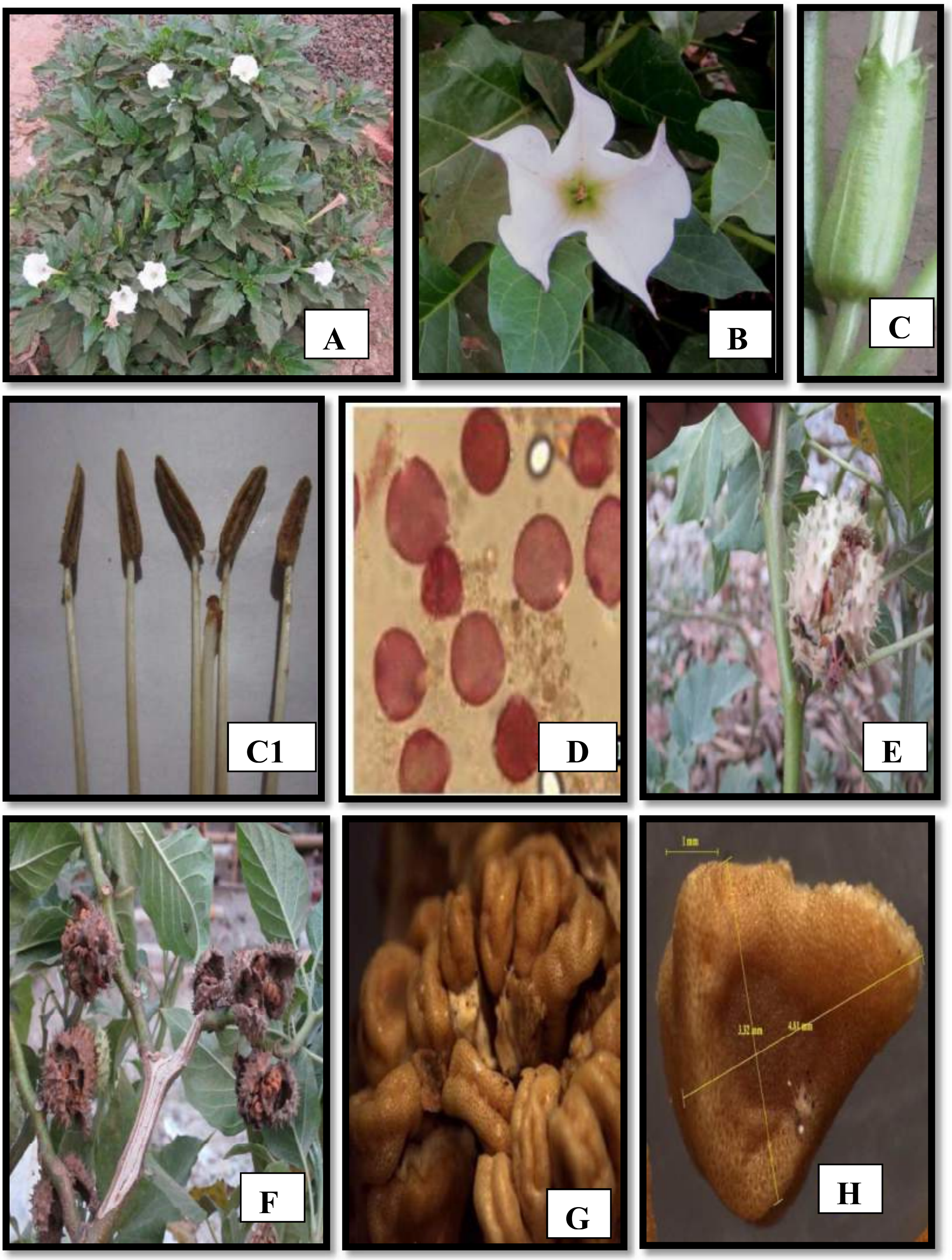


Fig. 14: A. Plant material of *Datura stramonium*, B. A flower of *Datura stramonium*, C. Calyx, C1 Androecium and gynocoecium, D. Pollen (L.M model no Leica Dm-1000, magnification 40X), E&F. Seed dispersal, G. Seed, H. Dimensions (size) of seed (L.M model no Leica Dm-1000, magnification 40X)

- ***Datura inoxia***

  Annual or biennial undershrub. Stem glabrous, deep purple, terete. Leaves ovate - lanceolate or broadly ovate acute, unequal at the base, entire. Flowers purple white, axillary; bracts tomentose. Capsule globose, covered with long spines. Seeds numerous, compressed, nearly smooth.

Systematic position:-

Order- Solanades

Family- Solanaceae

Subfamily- Solanoidea

Tribe- Datureae

Genus- *Datura* sp.

Species- *D. inoxia* Mill.

Habitat: Terrestrial, Plants are mostly mesophytes.

Habit: Annual or biennial undershrub.

Stem: It is aerial, erect and mostly herbaceous. Glabrous, deep purple, terete. (Fig. 15).

Leaf: Leaves are simple, ex-stipulate, ovate-lanceolate or broadly ovate, acute, unequal at the base, entire. Venation is reticulate. (Fig.15)

FLORAL CHARACTERS

Inflorescence	It is commonly a cymose type. It may be axillary or terminal. In *Datura*, it is terminal and solitary.
Flower	The flower is bracteates (or) ebracteate, ebracteolate, pedicellate, complete, bisexual, actinomorphic, heterochlamydeous, pentamerous and hypogynous **(Fig. 15)**
Calyx	Sepals 5, gamosepalous and valvate.
Corolla	Petals are 5, gamopetalous and valvate (or) twisted aestivation. White acute-tip.
Androecium	It consists of five, stamens are alternating with petals. Anther lobes are large, dithecous, introrse, basifixed and the dehiscence may be longitudinal in *Datura*.
Gynoecium	It is bi-carpellary and syncarpous. Ovary is superior, bilocular with many ovules on swollen axile placentation. In *Datura*, ovary is tetralocular due to the development of false septum (Fig. 15).
Pollination	It is commonly entomophilous (cross pollination through insects). Flowers are usually protandrous (Fig. 15)
Fruit	Septifragal capsule, globose, covered with long rather slender spines (Fig.15)
Seed	Numerous, compressed, nearly smooth (Fig. 15)

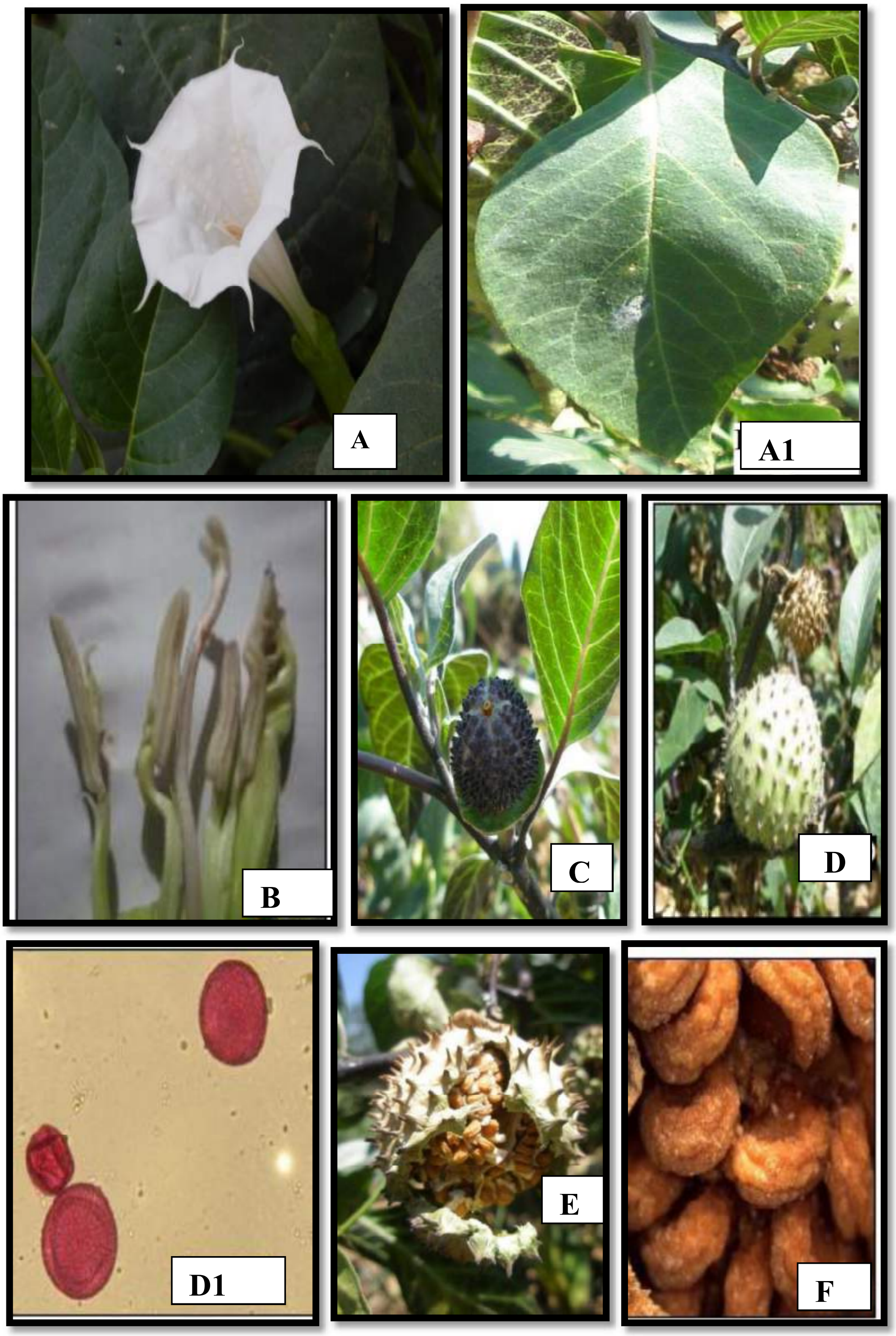


Fig. 15: A. Plant of *Datura inoxia*, A1. A single leaf, B. Androcium and gynoecium, C. Immature fruit, D. Mature fruit, D1. Pollen (L.M model no Leica Dm-1000, magnification 40X), E. Seed dispersal, F. Seed (L.M model no Leica Dm-1000, magnification 40X).

Ultrastructure study of the pollen of *Datura metel*, *Datura stramonium* and *Datura inoxia*

4.2. Study of ultrastructure of pollen

4.2.1 Ultrastructure study by SEM

Belonging to Solanaceae family, *Datura* pollen is tricolporate. It is identified by the presence of tectum. It is striate at mesocolpium. Perforation is present in between the lirae. Ornamentation is coarsely reticulate-regulate towards apocolpium region, lumina with columella, trichomusculate pollen. In Equatorial view the pollen is elliptic while in the polar view it is rounded trilobed. The Colpal membrane is granulated.

All palynological structures and measurements for the 3 species concerning pollen class, Polar (P) measurements, Equatorial (E) measurements, P/E range have been exhibited in Table1, while the other results regarding pollen outline: equatorial view, polar view, P/E ratio, ectoaperture structures concerning the colpi, costae and pollen margins are given in Tables 2-3 (Figs. 16 – 42).

On the basis of the pollen class, it is obvious that palynological structure is same for the 3 species of *Datura* as found in family Solanaceae. All of them were tricolporate and 3 zonoaperturate. These findings are typically similar to those exhibited by other investigators. Polar (P) and equatorial (E) measurements of the pollen grains were found to be generally large for *Datura* sp. different from other species of the family Solanaceae. By depending on the pollen outline it can be noticed that except for the equatorial view of *Datura stramonium* (elliptical), both the polar and equatorial views of *Datura* sp. were spherical in nature. Pollen ectoaperture and endoaperture structures did not show any variations as they were from the same species.

In case of *D. metel* pollen, the exine had been found to be more reticulate in nature at apocolpium with the presence of less amount of columella (not prominent) than the other two species. *D. stramonium* less reticulate pattern was observed. Polar view exhibited fewer perforations. Columella is thicker at the sexine. In *D. inoxia* perforations are less prominent in equatorial view. Columella is coarsely reticulate and less thick than *D. stramonium*. Exine pattern is more striated.

Table 1: Pollen class and dimensions of the three species of *Datura* showing P (polar) and E (equatorial) dimensions in µm with polar and equatorial ratios.

Species	Pollen class	P (µm)	E (µm)	P/E
Datura inoxia	Tricolporate, 3 Zonoaperturate	40 - 45.0	45 - 54	0.88 - 0.83
Datura metel	Tricolporate, 3 Zonoaperturate	48.91 - 48.95	54.6 - 55.09	0.89 - 0.88
Datura stramonium	Tricolporate, 3 Zonoaperturate	40 - 45.5	48.70 - 51.6	0.82 - 0.88

Table 2: Pollen of three species of *Datura* showing outline view (P- Polar; E- Equatorial) ("+"= Present)

Species	E	P	P/E	Trichotomusculate
Datura inoxia	Circular	Circular	Subtransverse	+
Datura metel	Circular	Circular	Subtransverse	+
Datura stramonium	Elliptical	Circular	Subtransverse	+

Table 3: Pollen of three species of *Datura* showing characteristics of exoaperture and endoaperture. ("-"= absent)

Species	Exoaperture			Endoaperture		
	Colpi	Fastigia	Margin	Colpi	Costae	Margins
Datura inoxia	Small	-	Indistinct	Large	-	Indistinct
Datura metel	Small	-	Indistinct	Large	-	Indistinct
Datura stramonium	Small	-	Indistinct	Large	-	Indistinct

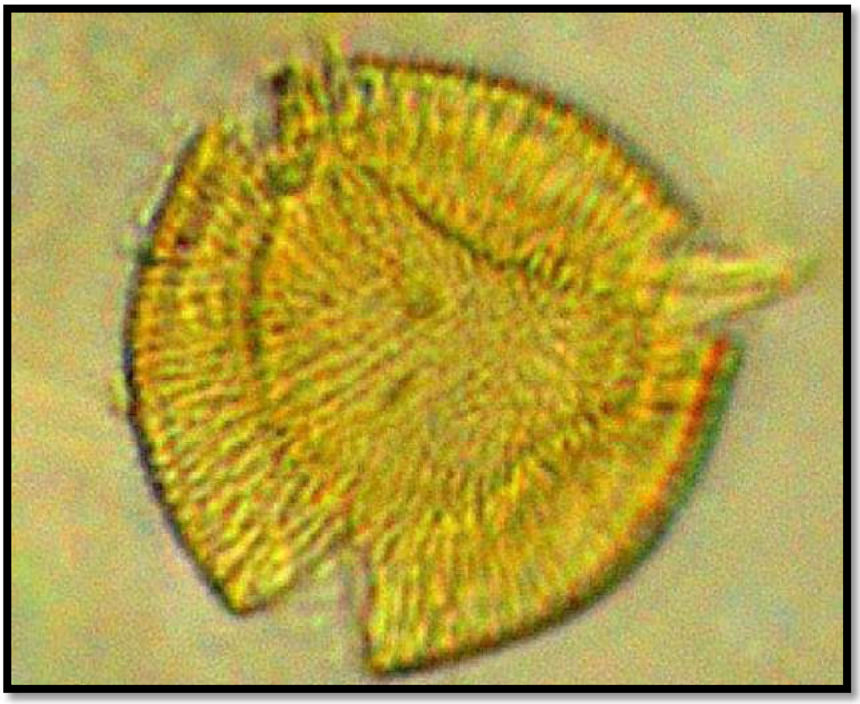


Fig. 16: Light Microscopic image of pollen of *Datura metel* (Mag.40X)

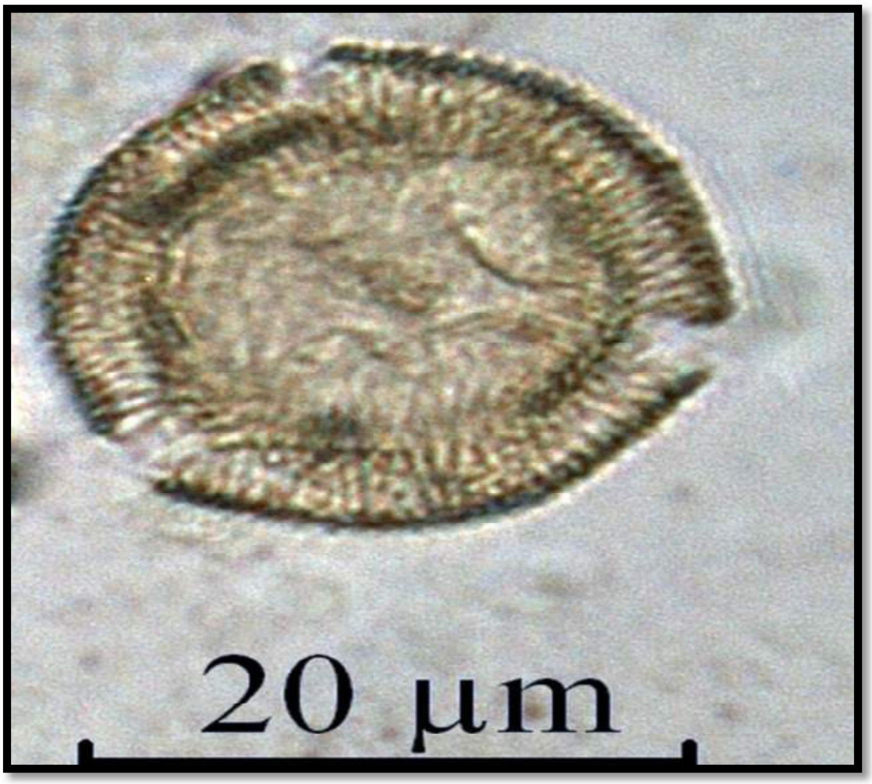


Fig. 17: Light Microscopic image of pollen of *Datura stramonium* (Mag.40X)

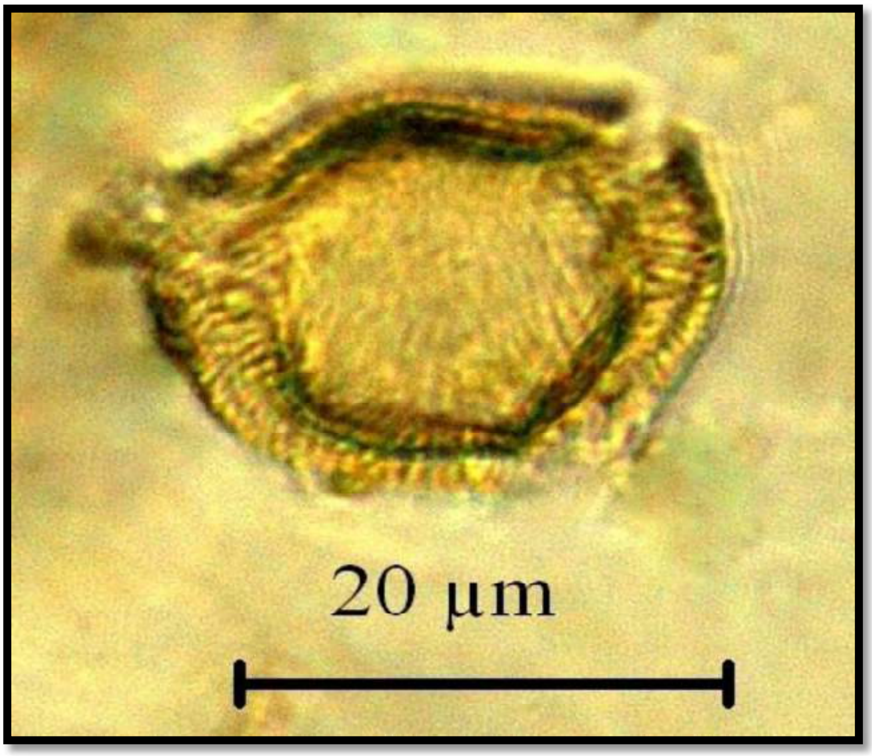


Fig. 18: Light Microscopic image of the pollen of *Datura inoxia* (Mag.40X)

Fig 19: Light Microscopic image of pollen of *Datura metel* showing 100% pollen purity (Mag.10X)

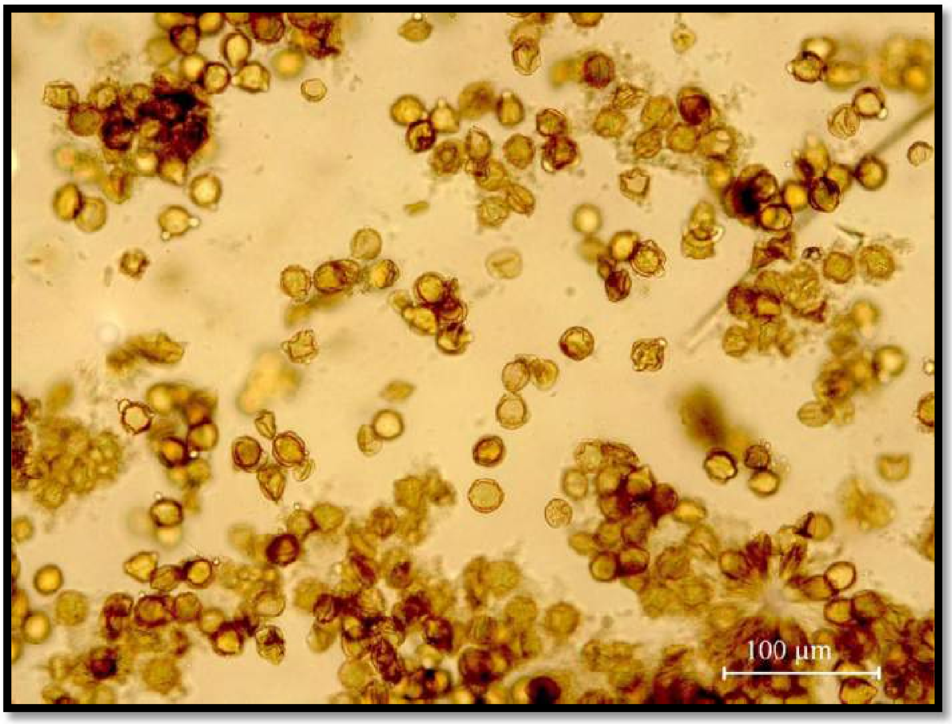


Fig 20: L.M image of pollen of *D. stramonium* showing 100% pollen purity (Mag.10X)

Fig. 21: L.M image of pollen of *D. inoxia* showing 100% pollen purity (Mag.40X)

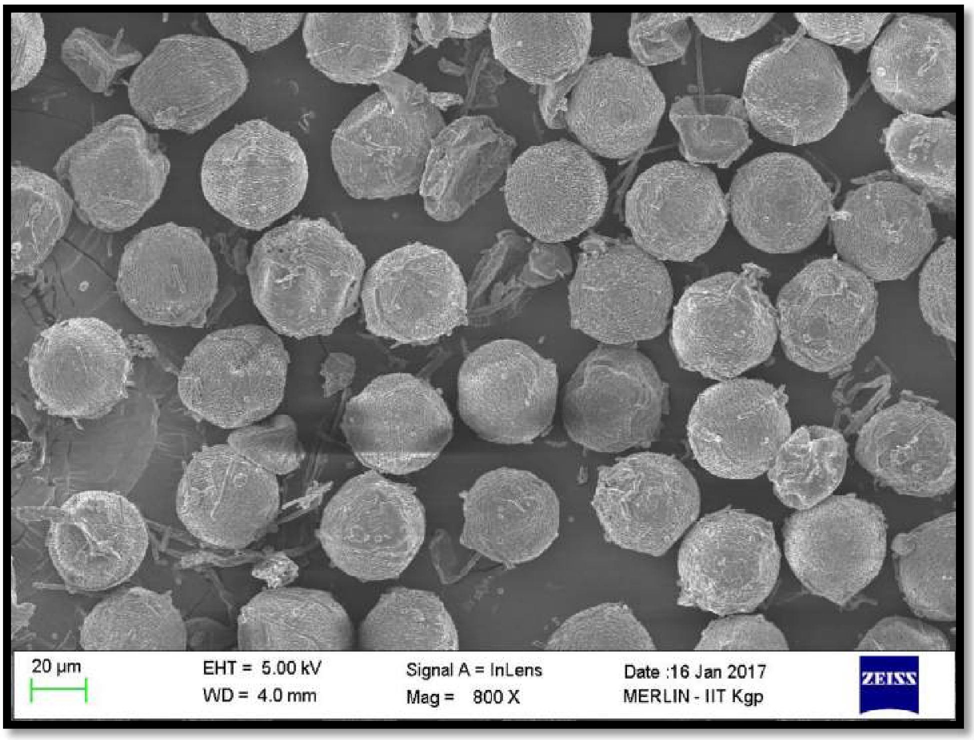


Fig. 22: SEM image of pollen of *D. metel* showing 100% pollen purity (Mag. 800X)

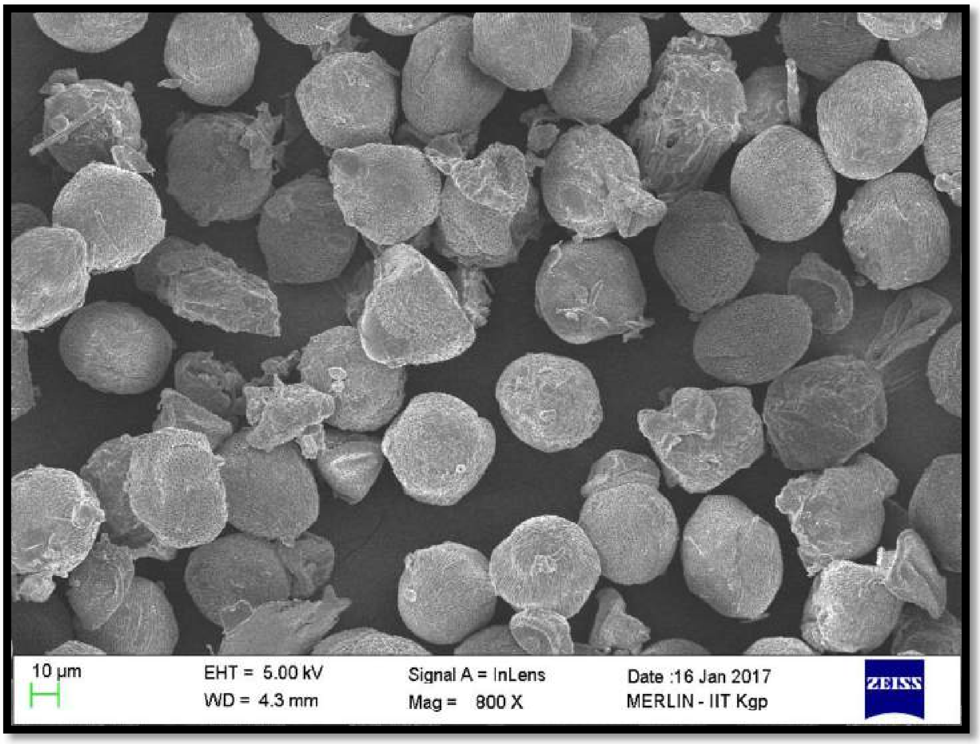


Fig. 23: SEM image of pollen of *D. stramonium* showing 100% pollen purity (Mag. 800X)

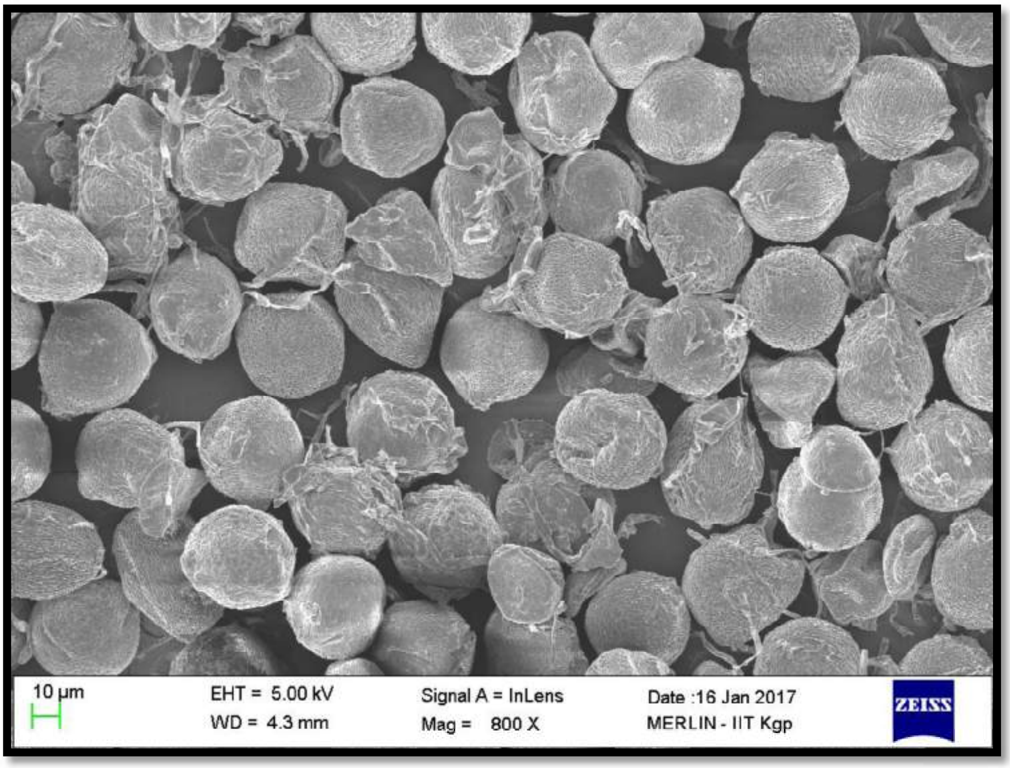


Fig. 24: SEM image of pollen of *D. inoxia* showing 100% pollen purity (Mag.800X)

SEM images of pollen of *D*atura *metel*

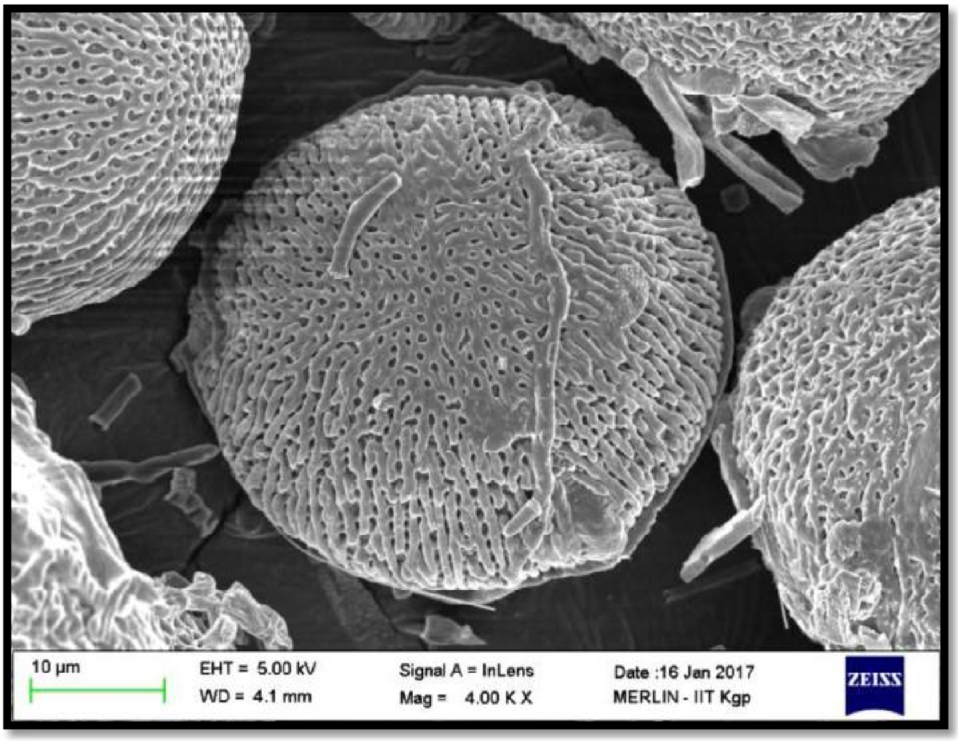


Fig. 25: Polar view of pollen of *D. metel* (Mag. 4.00 K X)

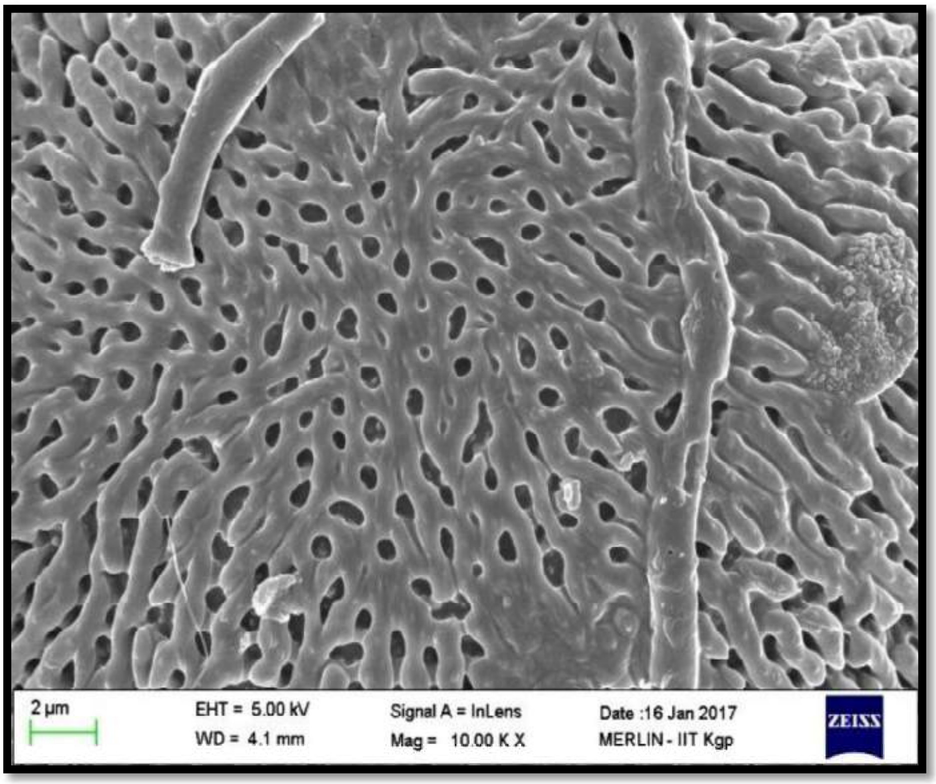


Fig. 26: Exine pattern at polar view of *D. metel* (Mag.10.00 K X)

SEM images of pollen of *Datura metel*

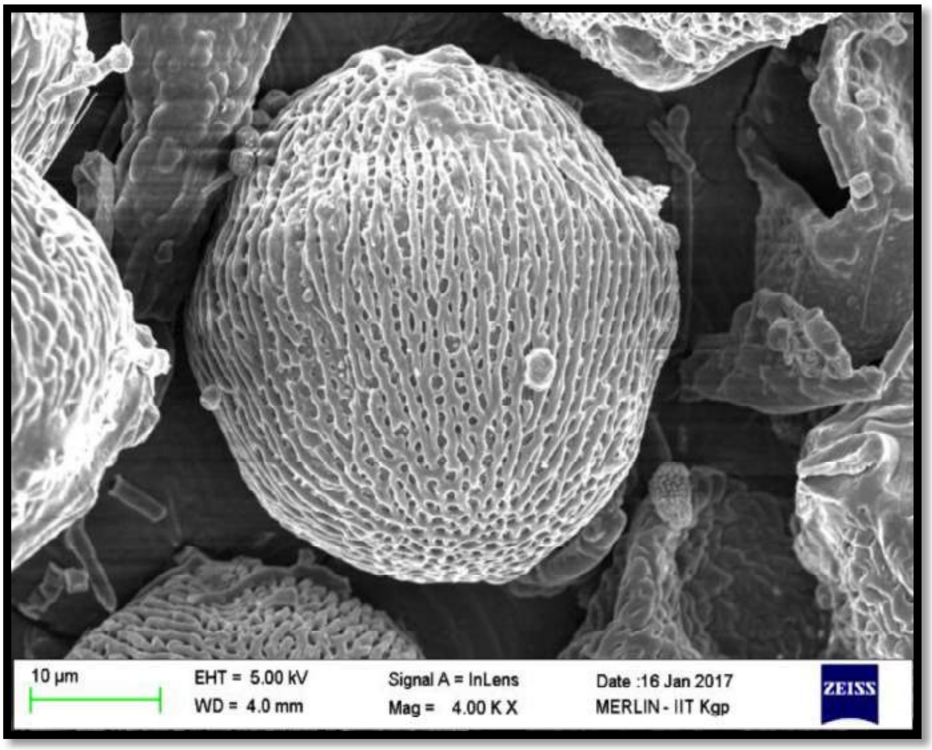


Fig. 27: Equatorial view of pollen of *D. metel* (Mag.4.00 K X)

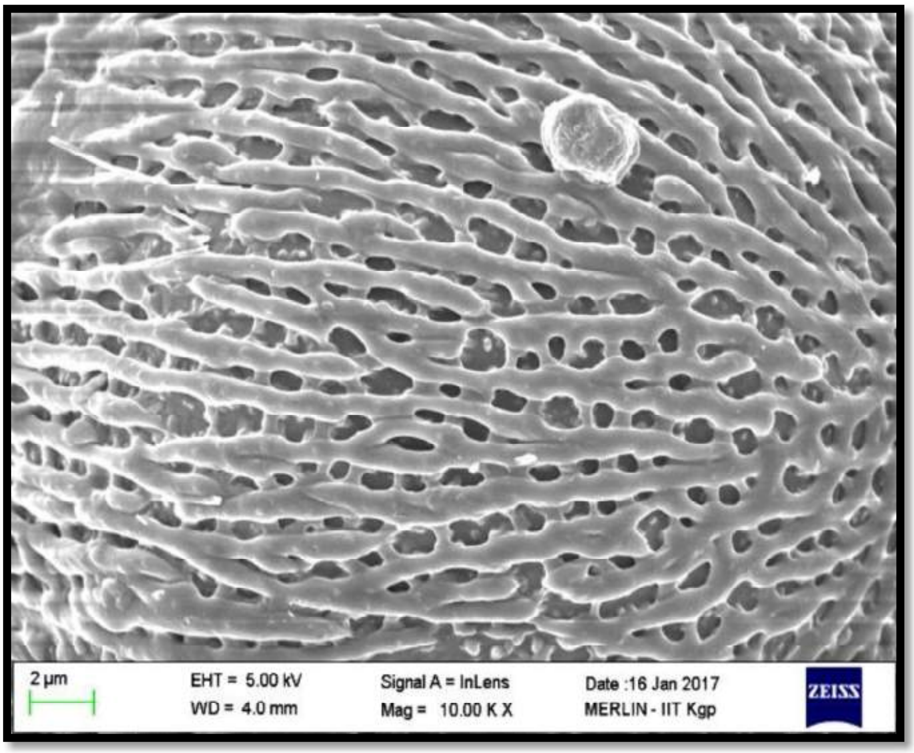


Fig. 28: Exine pattern at equatorial view (Mag.10.00 K X)

SEM images of pollen of *Datura metel*

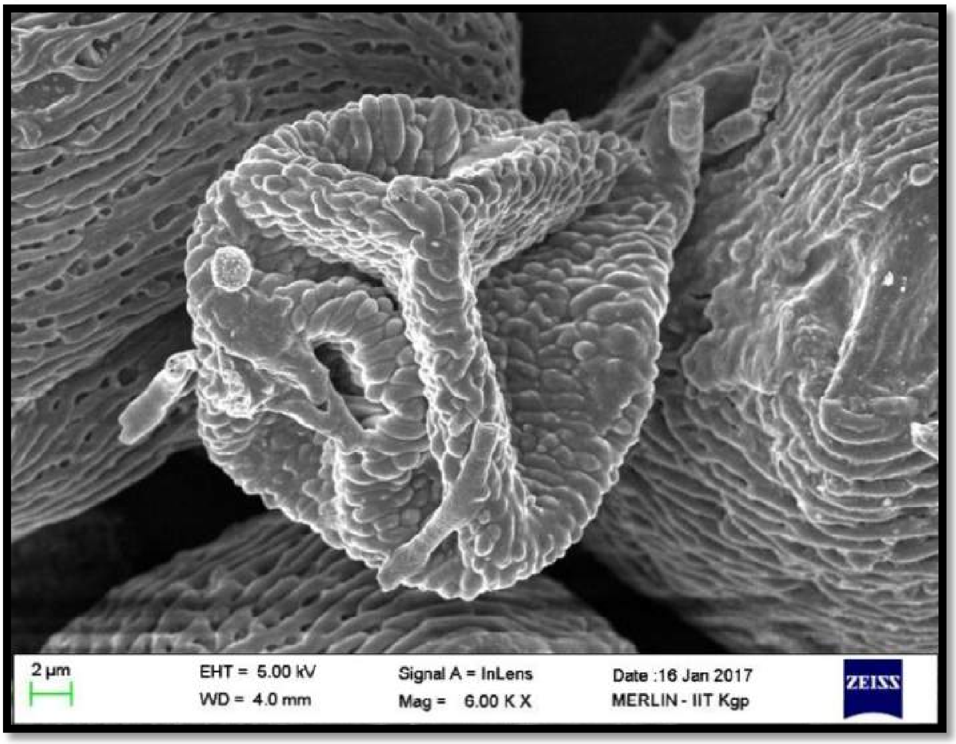


Fig. 29: Pollen of *D. metel* showing trichotomusculature (Mag.6.00K X)

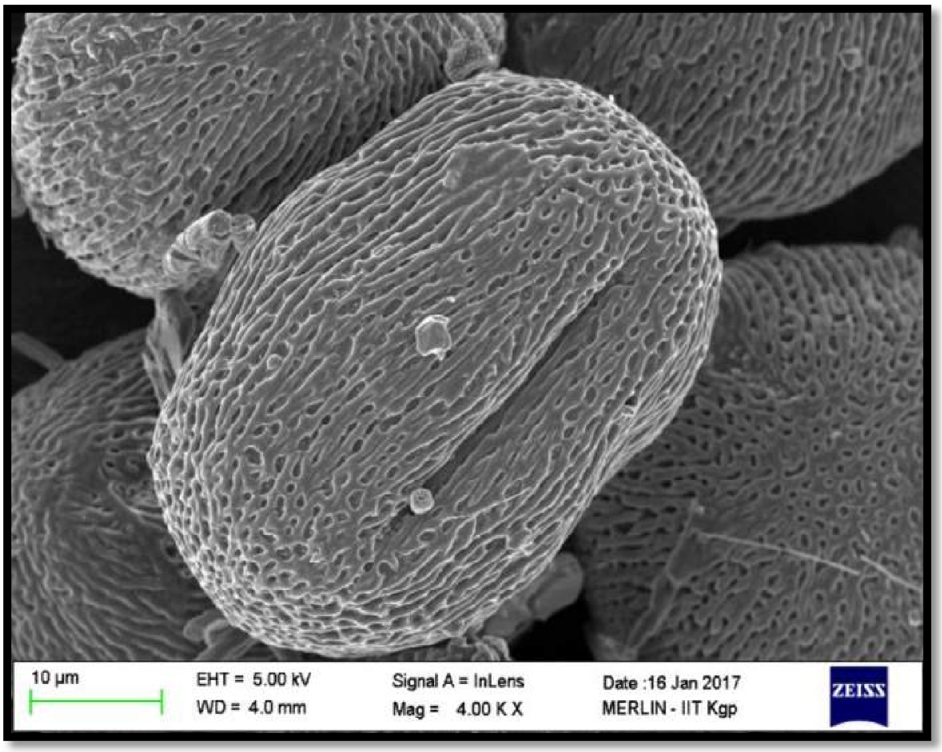


Fig. 30: Pollen showing furrow (Mag. 4.00K X)

SEM images of pollen of *Datura stramonium*

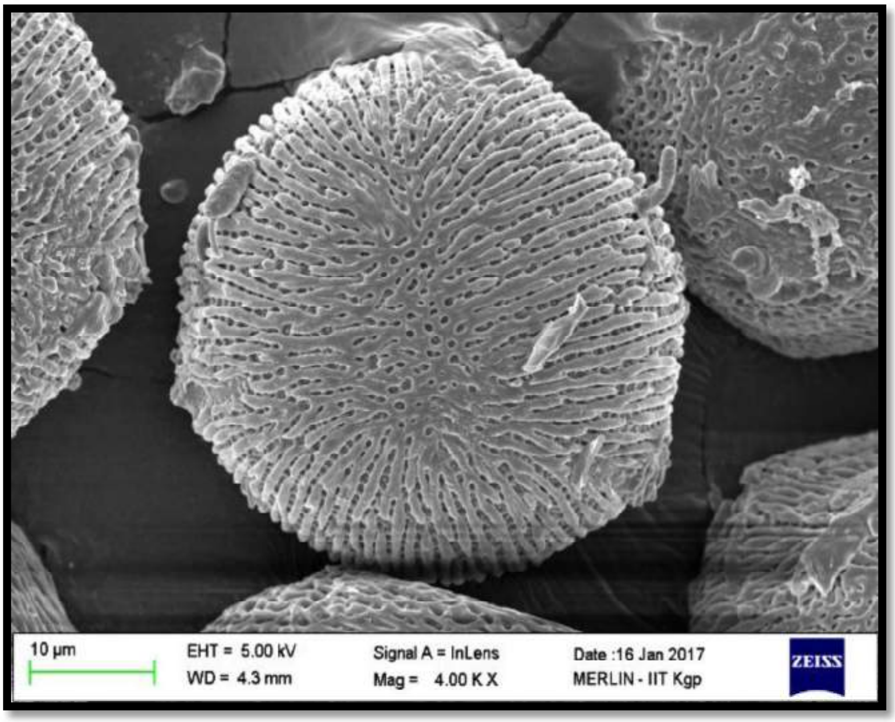


Fig. 31: Polar view of pollen of *D. stramonium* (Mag.4.00K X)

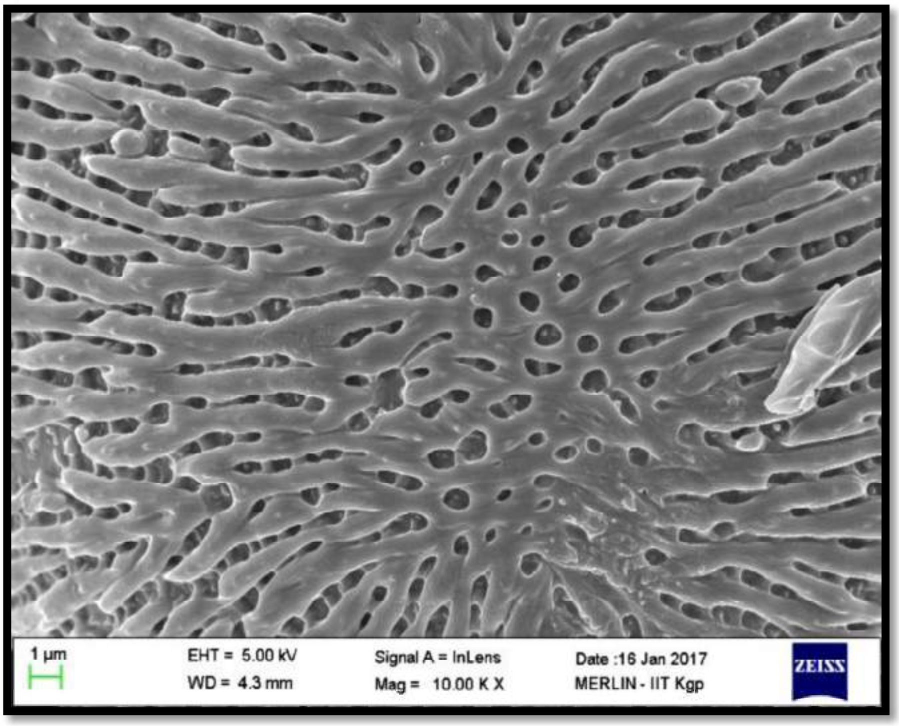


Fig. 32: Exine pattern at polar view of *D. stramonium* (Mag.10.00K X)

SEM images of pollen of *Datura stramonium*

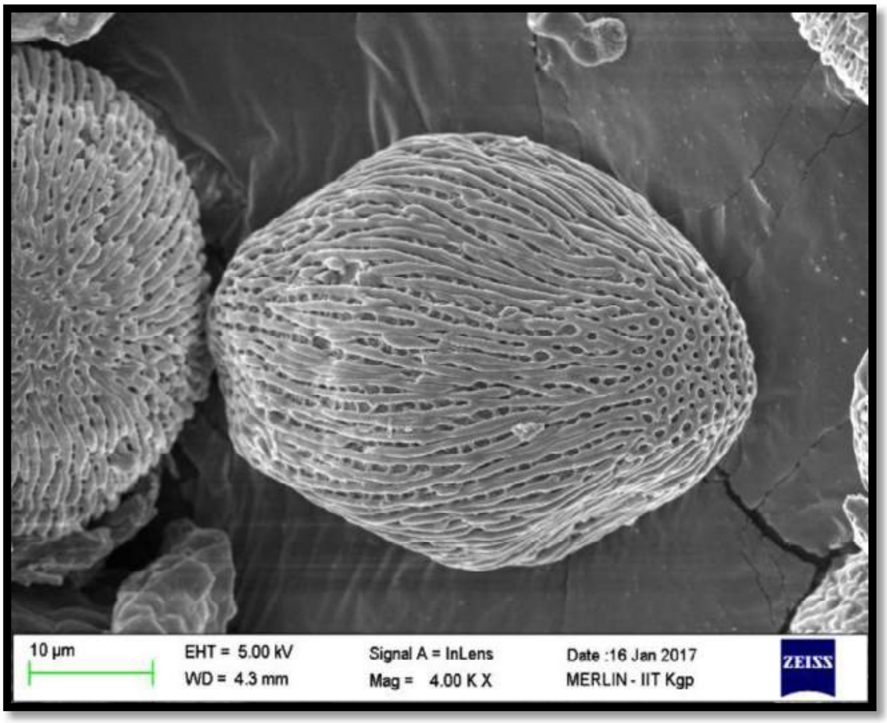


Fig. 33: Equatorial view of pollen of *D. stramonium* (Mag.4.00K X)

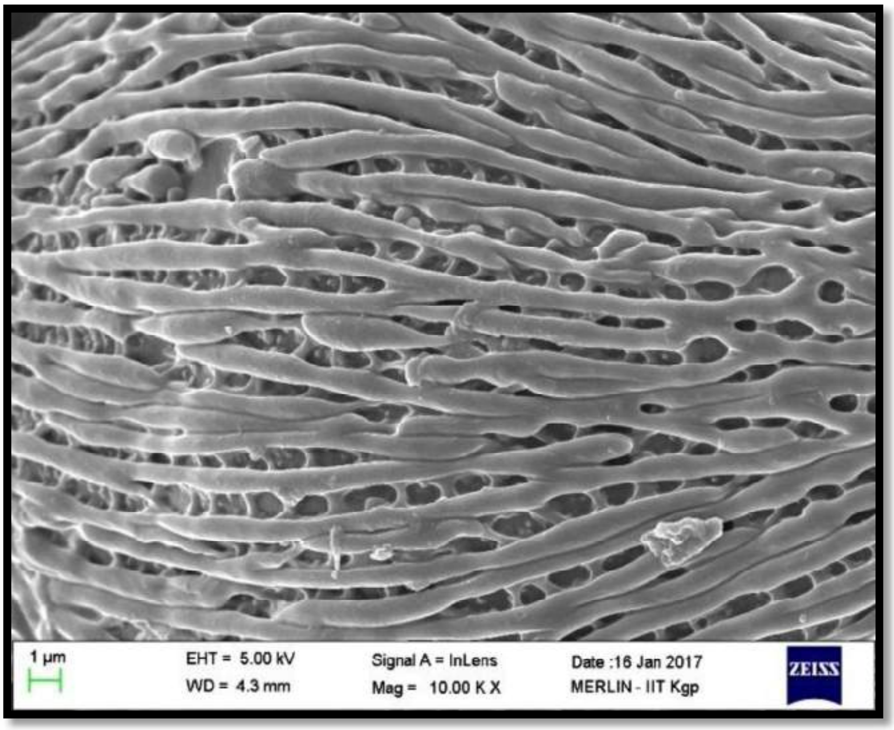


Fig. 34: Exine pattern at equatorial view *D. stramonium* (Mag.10.00K X)

SEM images of pollen of *Datura stramonium*

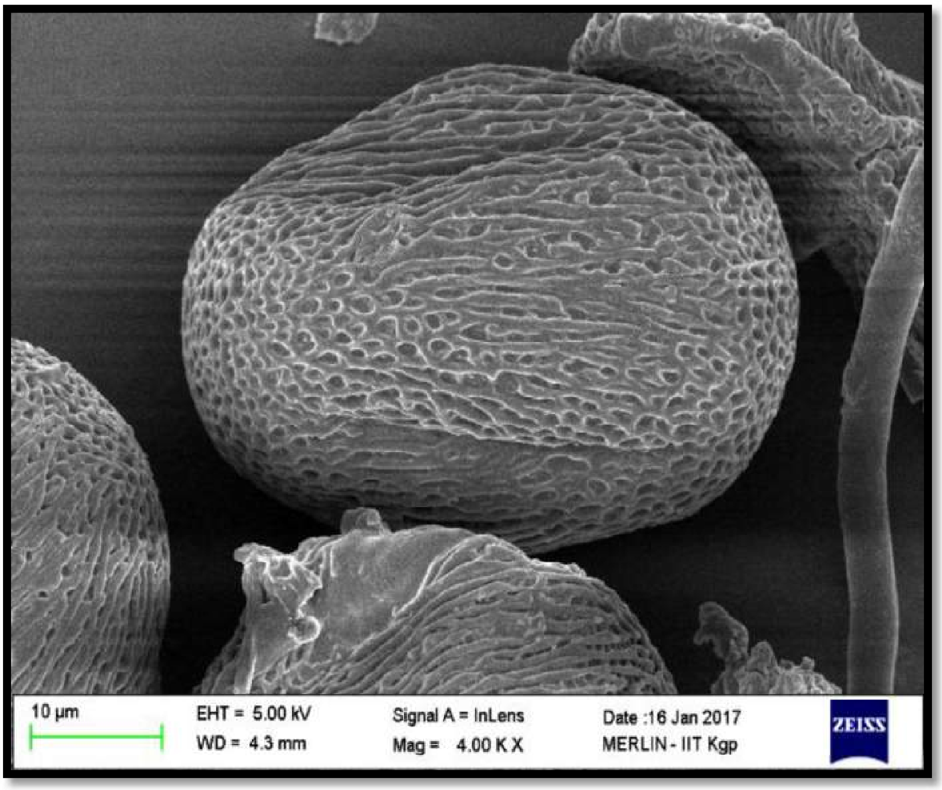


Fig. 35: Pollen of *D. stramonium* showing furrow (Mag.4.00K X)

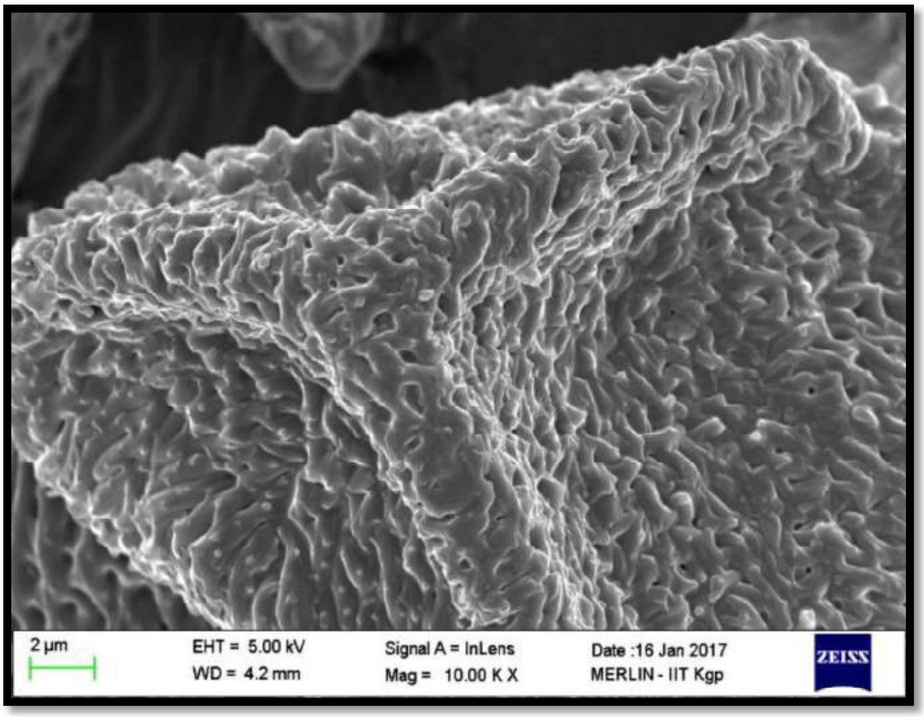


Fig. 36: Pollen of *D. stramonium* showing trichotomusculature (Mag.10.00K X)

SEM images of pollen of *Datura inoxia*

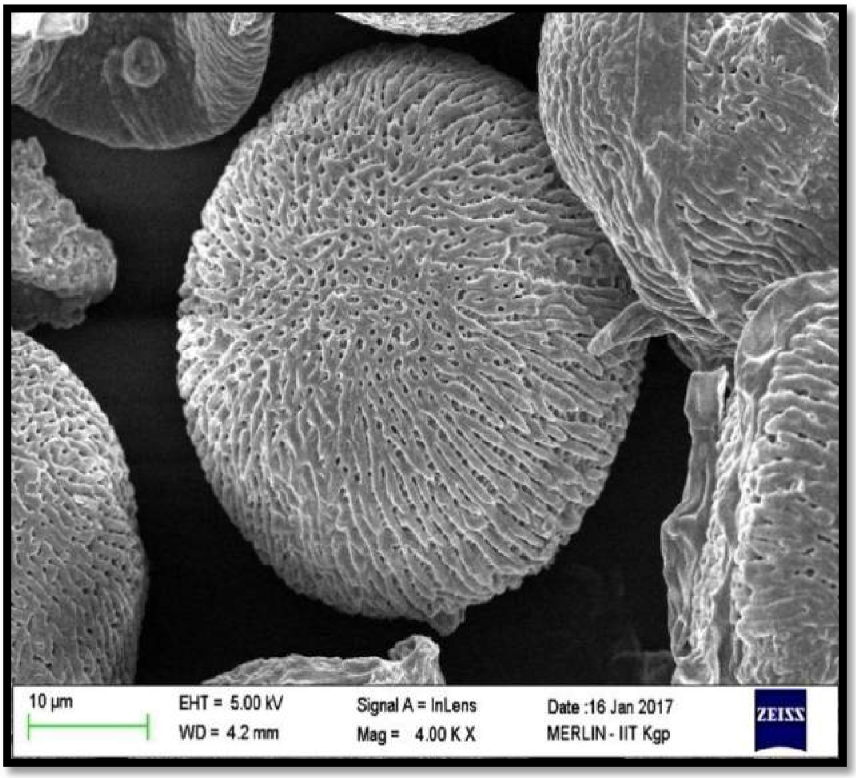


Fig. 37: Polar view of pollen of *D. inoxia* (Mag.4.00K X)

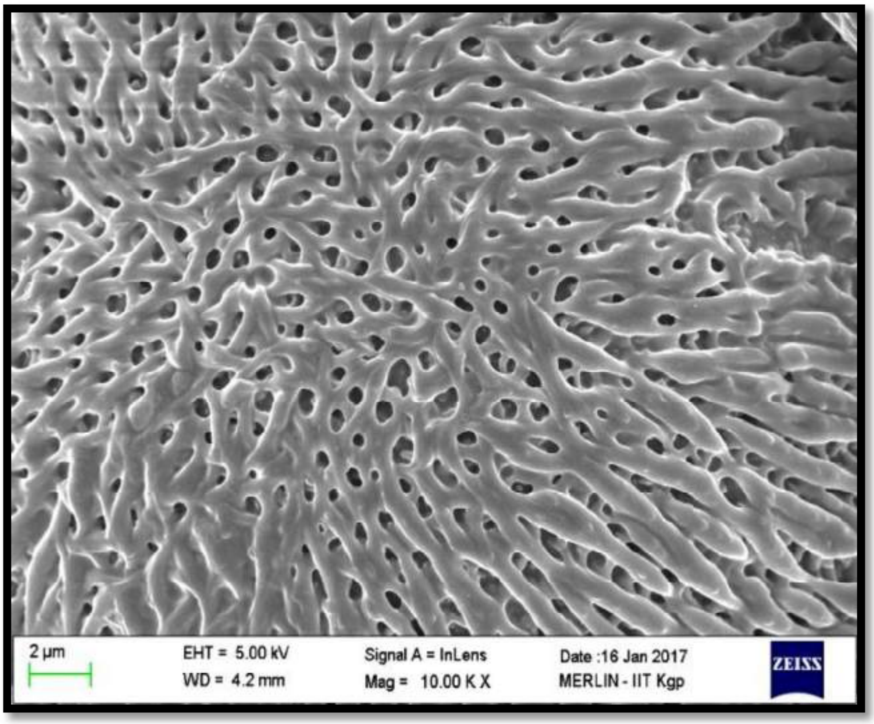


Fig. 38: Exine pattern at polar view of *D. inoxia* (Mag.10.00K X)

SEM images of pollen of *Datura inoxia*

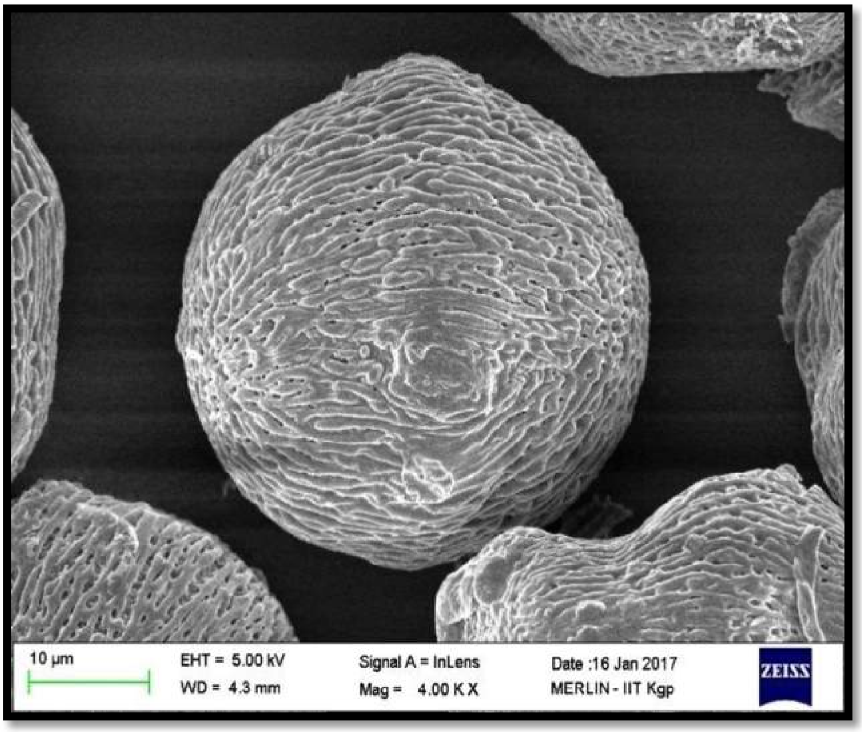


Fig. 39: Equatorial view of pollen of *D. inoxia* (Mag.4.00K X)

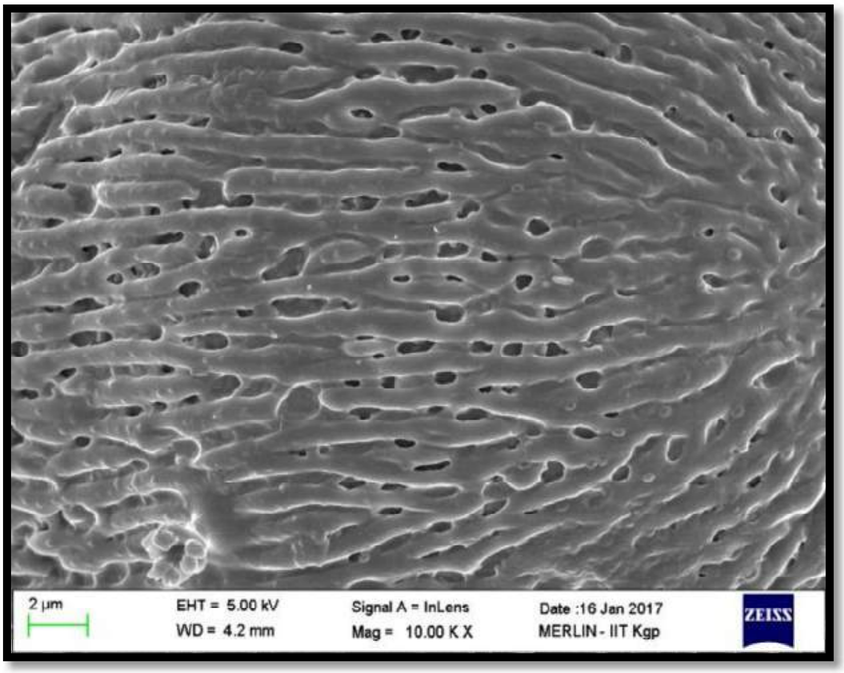


Fig. 40: Exine pattern at equatorial view (Mag.10.00K X)

SEM images of pollen of *Datura inoxia*

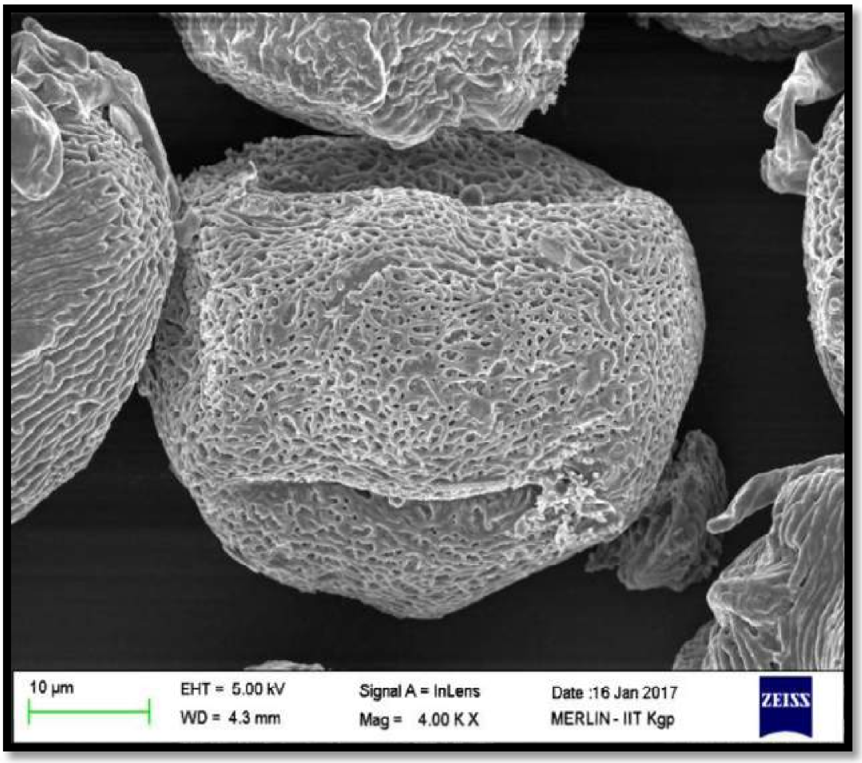


Fig. 41: Pollen of *D. inoxia* showing furrow (Mag.4.00 K X)

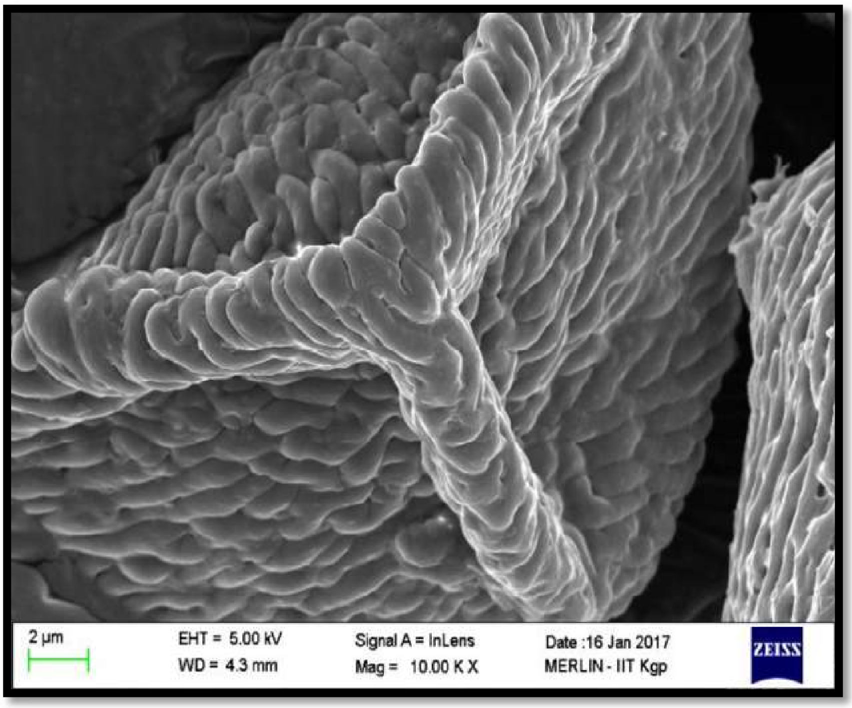


Fig. 42: Pollen of *D. inoxia* showing trichotomusculature (Mag.10.00 K X)

4.2.2 Ultrastructure study by TEM

Pollen allergens are generally glycoproteins or proteins and water soluble in nature with molecular weight ranging from 5 and 80 kDa. (Grote et al., 2000, 2005; Knox and Suphioglu, 1996). They are easily accessible which enables them to cross the mucosal barriers of the nasal passage (Aalberse, 2000; Castells et al., 2002; Vrtala et al., 1993). Because of these properties it is very difficult to locate pollen allergens within the pollen grains using conventional aqueous fixation methods (Grote, 1999). Hence it requires methods involving anhydrous fixation or cryofixation. These techniques have shown that majority of the pollen allergens reside in the cytoplasm of dry pollens, amyloplast or sometimes associated with organelles only and are hardly spotted in the pollen wall (Alche et al., 2004; Castells et al., 2002; Grote, 1999; Grote et al., 1994, 2000).These proteins upon hydration are released within minutes when pollen grains are introduced with moist and warm surface of mucosa leading to the development of allergenic rhinitis (Casas et al., 1996; Castells et al., 2002; El-Ghazaly et al., 1999; Grote, 1999; Grote et al., 2005). Allergenic substances may also be released by discharge of cytoplasmic content through aperture or via rupturing of pollen grains upon hydration. (Bacsi et al., 2006; El-Ghazaly et al., 1999; Grote et al., 2000, 2001, 2003; Taylor et al., 2004).

Diethart et al. (2007) have reported that the pollen wall of many minor allergenic plants belonging to families like Fagaceae, Salicaceae or Ulmaceae, show the typical organization of pollen wall consisting of intine, a compact endexine and ektexine. Same pollen wall organization was exhibited by some well known allergy causing plant families like Platanaceae and Oleaceae. However some major allergenic pollen including Betulaceae, Chenopodiaceae, Poaceae, etc. is characterized by the lack of a compact endexine and have a discontinuous, slightly or not detectable endexine. Microchannels have also been observed in the exine of the Betulaceae and Poaceae pollen investigated by them.

Transmission electron microscopy (TEM) of the pollen of *Datura* was done to study the details of the architecture of the pollen wall in order to get an insight into some of the important ultrastructural as well as morphological features of the pollen and to correlate these features with the possible mechanism of the release of the allergen as well as the site of storage of these antigenic proteins.

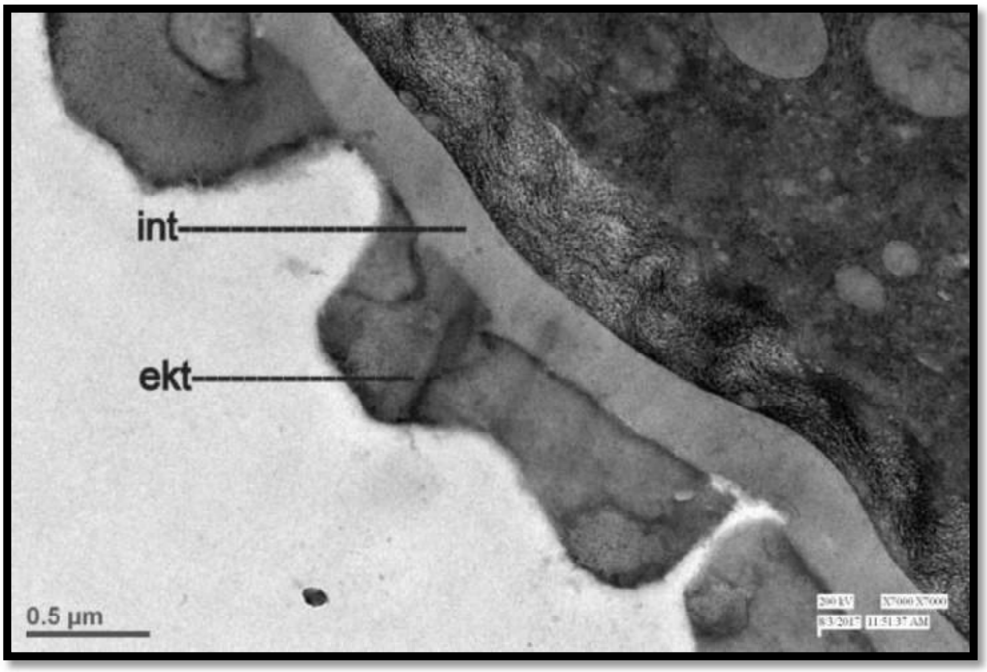


Fig. 43: TEM images of pollen wall of *Datura metel* (int- intine, ekt- ektexine, Mag X 7000 X 7000)

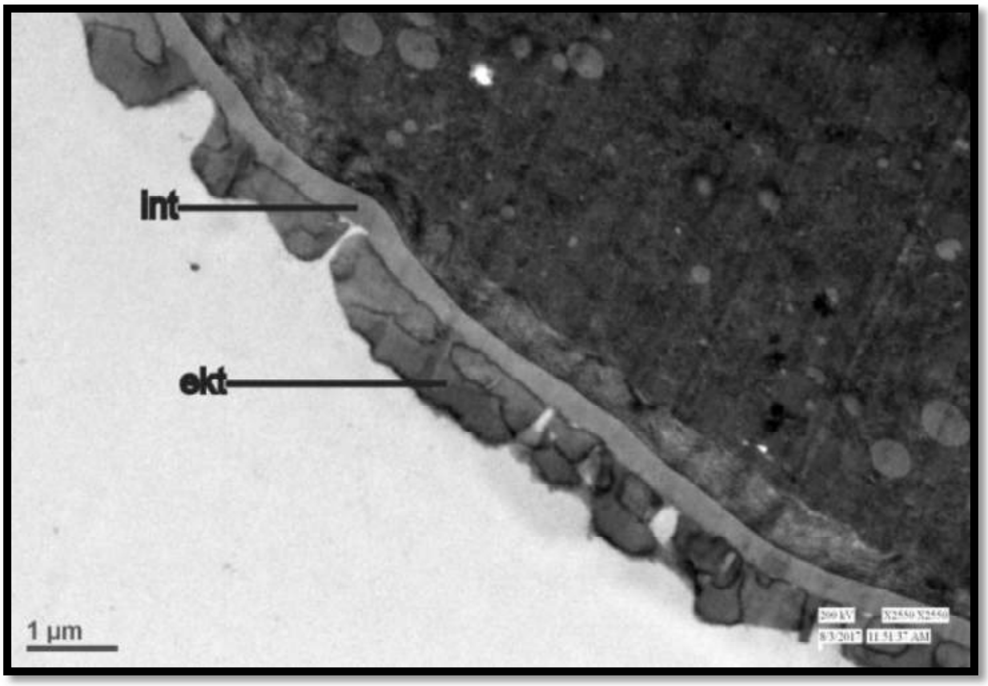


Fig. 44: TEM images of pollen wall of *Datura metel* (int- intine, ekt- ektexine, Mag. X 2550 X 2550)

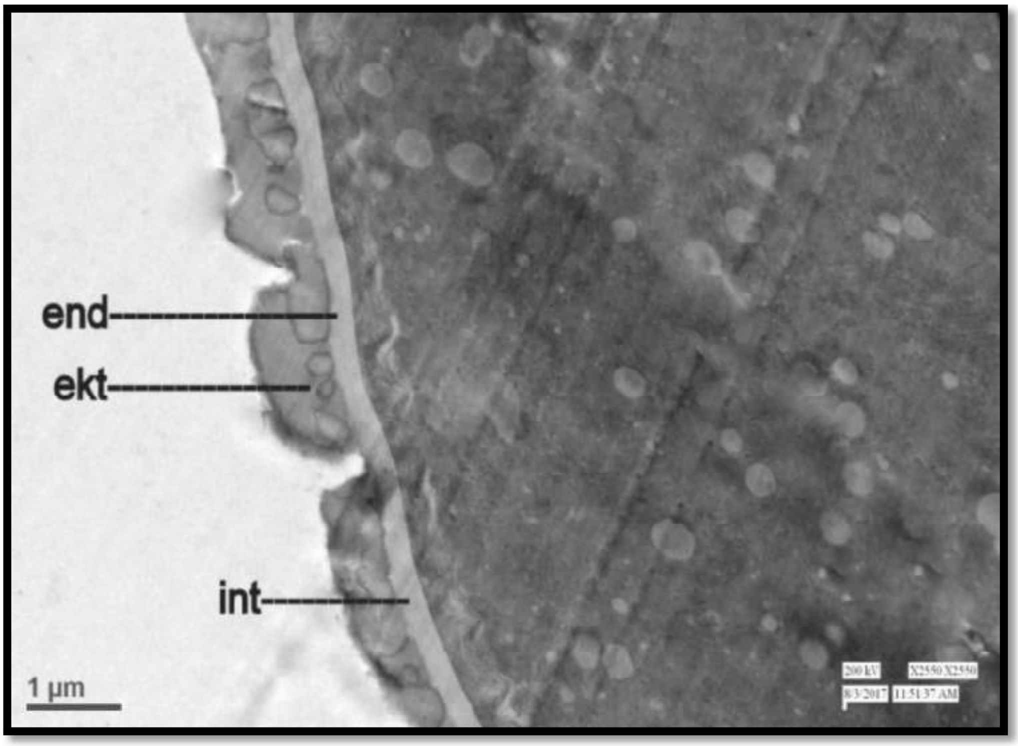


Fig. 45: TEM images of pollen wall of *Datura metel* (end-endexine, ekt-ektexine, Mag. X 2550 X 2550)

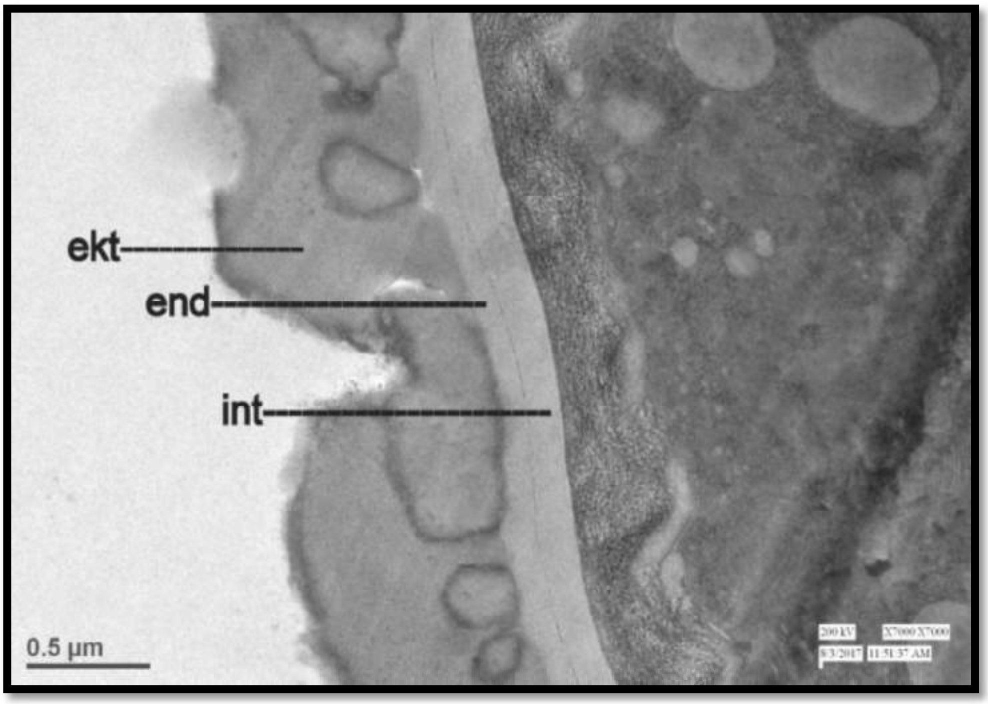


Fig. 46: TEM images of pollen wall of *Datura metel* (end-endexine, ekt-ektexine, int- intine, Mag. X 7000 X 7000)

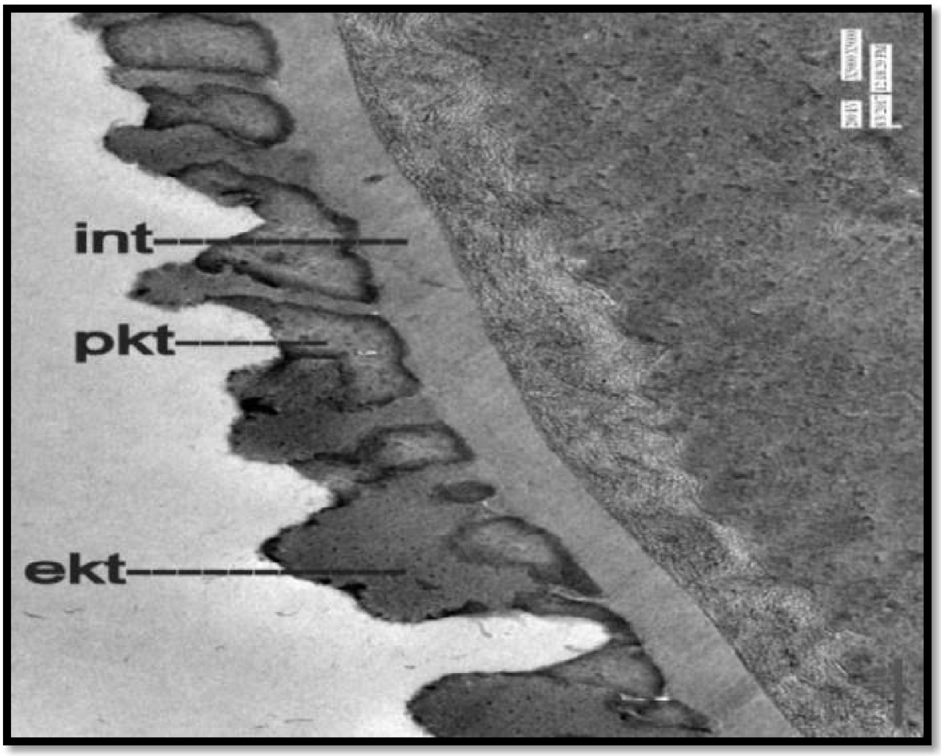


Fig. 47: TEM images of pollen wall of *Datura stramonium* (end-endexine, ekt-ektexine, int- intine, Mag. X 5000 X 5000)

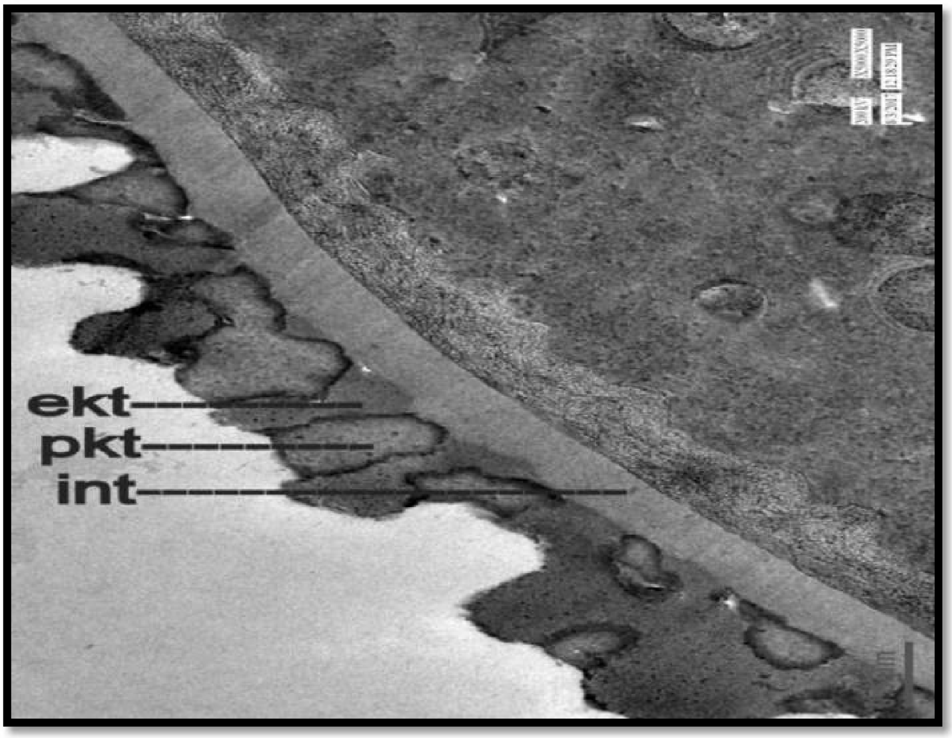


Fig. 48: TEM images of pollen wall of *Datura stramonium* (end-endexine, ekt-ektexine, int- intine, Mag. X 5000 X 5000)

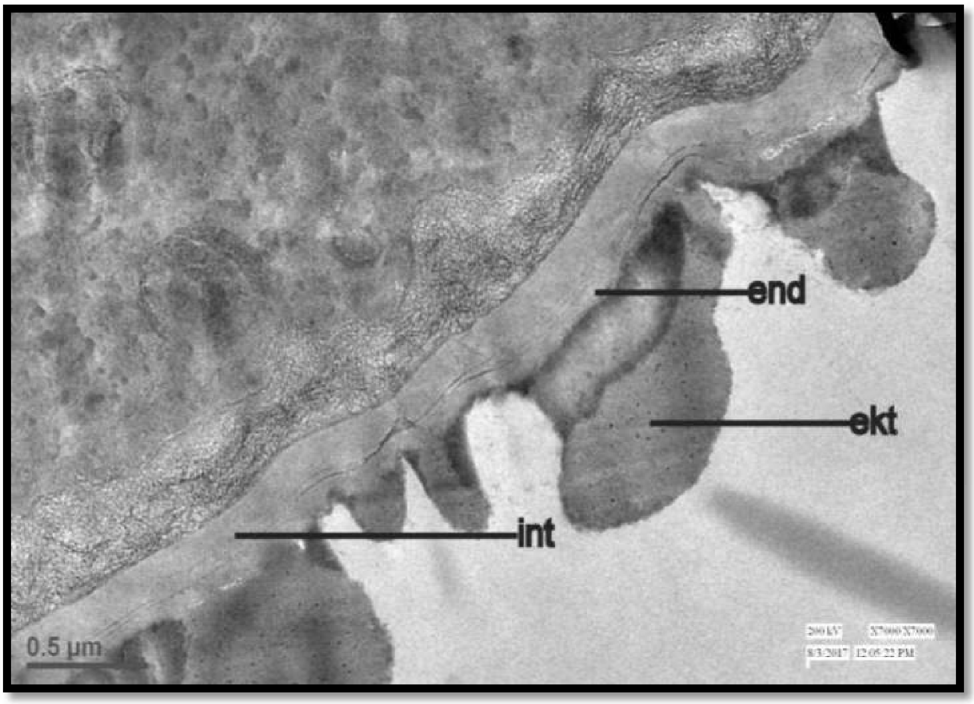


Fig. 49: TEM images of pollen wall of *Datura inoxia* (end-endexine, ekt-ektexine, int- intine, Mag. X 7000 X 7000)

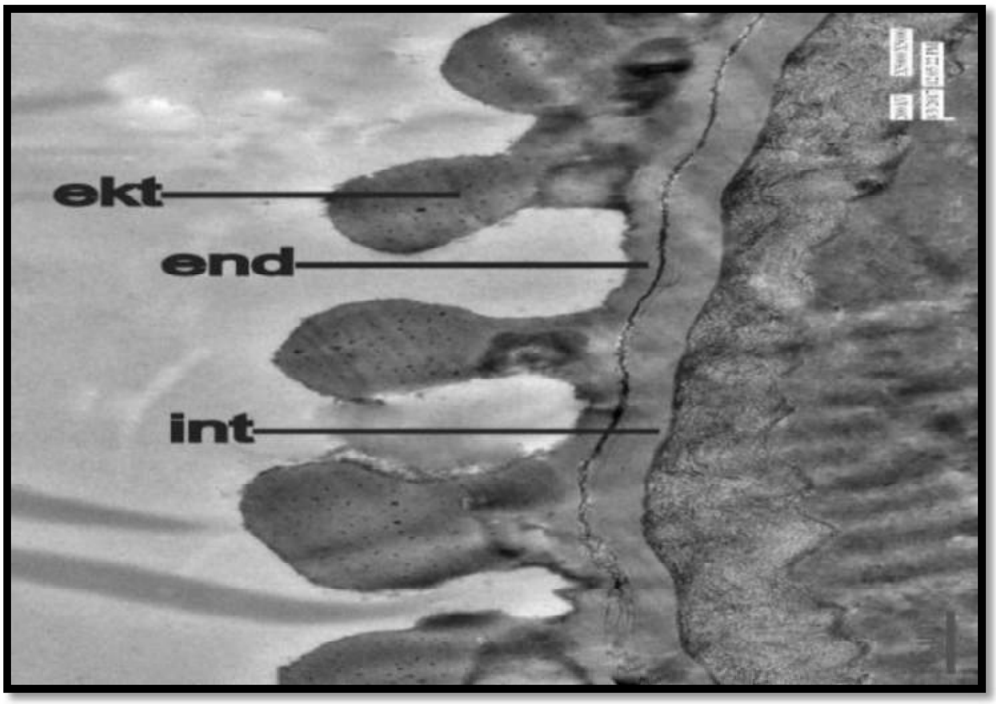


Fig. 50: TEM images of pollen wall of *Datura inoxia* (end-endexine, ekt-ektexine, int- intine, Mag. X 5000 X 5000)

4.2.3 Analysis of TEM study

Based on the TEM studies, the following results were obtained

Datura metel

The pollen of *Datura metel* showed a typical pollen wall organization with inner intine and outer exine. The ektexine is semitectatecolumellate, while endexine is discontinuous to absent. The intine is thinner than exine and forms a continuous layer (Figs. 43-46).

Datura stramonium

The pollen of *Datura stramonium* also showed the same typical pollen wall organization as *Datura metel* with compact continuous thin intine but tectat ecolumellate ektexine (Figs. 47-48). The endexine is almost absent. However a major difference between the two pollen is the presence of a moderate amount of pollenkitt which is an adhesive electron-dense, homogeneous material as is a characteristic of almost all angiosperms pollinated by animals. This pollen kit is present not only on the tectum surface but also in the exine cavities between the columellae.

Datura inoxia

Datura inoxia unlike the other two species shows the presence of a thin compact continuous endexine and columellateektexine (Figs.49-50). Even the intine is also thin and continuous. There is the presence of very little amount of pollenkitt material.

Datura metel

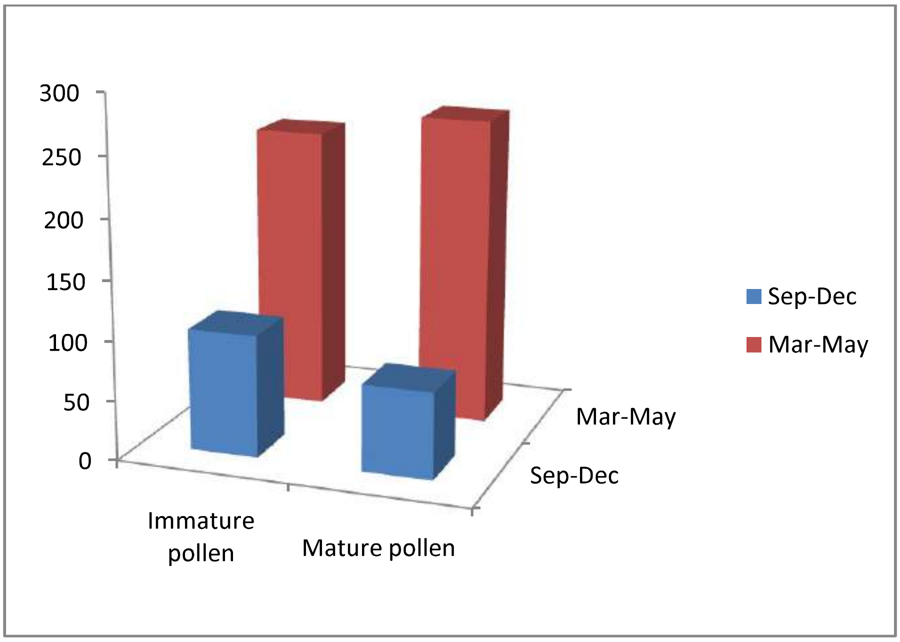
4.3 Protein extraction, isolation and characterization of *Datura metel*

4.3.1 Protein concentration of the pollen of *Datura metel*

The protein concentration study revealed that both mature and immature pollen of *Datura metel* exhibited a seasonal variation in protein concentrations with an increase during summer months. The protein concentration amounted to an average of 102.71 µg/ml during winter and 240 µg/ml during the summer months in immature pollen. Contrary to this, the mature pollen protein showed a concentration varied from a mean of 72.71 µg/ml to 260 µg/ml from winter to summer months. Over all protein content of immature pollen was noticed to be greater than mature one. The pollen protein concentration also showed a variation with maturity. Compared to the immature pollen, the protein concentration increased with maturity (Tables 4, Graph 1).

Table 4: Seasonal variation of protein concentration in the pollen of *Datura metel*

AVERAGE PROTEIN CONCENTRATION IN IMMATURE POLLEN		AVERAGE PROTEIN CONCENTRATION IN MATURE POLLEN	
September-December (Winter)	March-May (Summer)	September-December (Winter)	March-May (Summer)
102.71 µg/ml	240 µg/ml	72.71 µg/ml	260 µg/ml



Graph 1: Graphical representation of the seasonal variation in protein concentration in the pollen of *Datura metel* (X axis- types of pollen and Y axis- concentration of pollen proteins in µg/ml)

4.3.2 Analysis of SDS-PAGE Protein Profile Study of *Datura metel*

The SDS-PAGE protein profile of the pollen of *Datura metel* showed a total of 20 protein bands which were designated as d1 to d20, the molecular weights ranging between 29 kDa to 205 kDa (Figs. 51-53, Graph 2). A deviation in the protein profile between mature and immature pollen was noticed. The number of protein bands in the immature pollen were greater than those found in mature pollen (17) [Table 5]. The immature pollen profile exhibited all the 20 bands. Two of these bands (205 kDa, 43 kDa) were also present in the other two species. Four bands (97.4 kDa, 66.0 kDa, 56.2 kDa and 33.2 kDa) were common with *Datura inoxia* while two protein bands (43 kDa and 29 kDa) were common between *Datura stramonium* and *Datura metel.*

A comparison with the protein profile reported earlier in the mature pollen of *Datura metel* collected from Shantiniketan (Parui and Mandal, 1998) considerable variation was observed with several bands absent (108.2, 56.2, 43, 35.3, 33.2 and 29 kDa) and no bands above the range of 127.2 kDa was reported in the previous study. The present study recorded 7 bands above this range which were not observed earlier. Contrary, 4 bands of molecular weights 127.2, 87.0, 79.1 and 54.5 kDa which were recorded earlier were completely absent in the pollen which was collected from Kolkata. This shows that there can be a wide variation in the protein banding pattern of pollen collected from different regions. This is a major hurdle faced by pharmaceutical industries in the preparation of standardized immunotherapeutic vaccines.

Table 5: SDS-PAGE protein profile of the pollen of *Datura metel*

M.W of Marker Proteins (kDa)	Proteins bands from immature pollen		Proteins bands from mature Pollen		Protein bands as observed in previous study
	Protein band	M.W in (kDa)	Protein band	M.W in (kDa)	M.W in (kDa)
	d 1	>205	-	-	-
	d 2	>205	-	-	-
205	d 3	205.0	d 3	205.0	-
	d 4	183.5	d 4	183.5	-
	d 5	172.7	d 5	172.7	-
	d 6	152.0	d 6	152.0	-
	d 7	129.7	d 7	129.7	-
	-	-	-	-	127.2
	d 8	108.2	d 8	108.2	-
97.4	d 9	97.4	d 9	97.4	97.4
	-	-	-	-	87.0
	-	-	-	-	79.1
66	d 10	66.0	d 10	66.0	66.0
	d 11	59.4	d 11	59.4	59.4
	d 12	56.2	d 12	56.2	-
	-	-	-	-	54.5
	d 13	49.8	d 13	49.8	49.8
43	d 14	43.0	d 14	43.0	-
	d 15	40.5	d 15	40.5	40.5
	d 16	37.5	d 16	37.5	37.5
	d 17	35.3	d 17	35.3	-
	d 18	33.2	d 18	33.2	-
	d 19	31.6	-	-	31.6
29	d 20	29.0	d 20	29.0	-
	-	-	-	-	18.0

- The green color highlighting protein bands common to *Datura metel, Datura inoxia and Datura stramonium.*
- The blue color highlighting protein bands common to *Datura metel* and *Datura stramonium* including 43 kDa band.

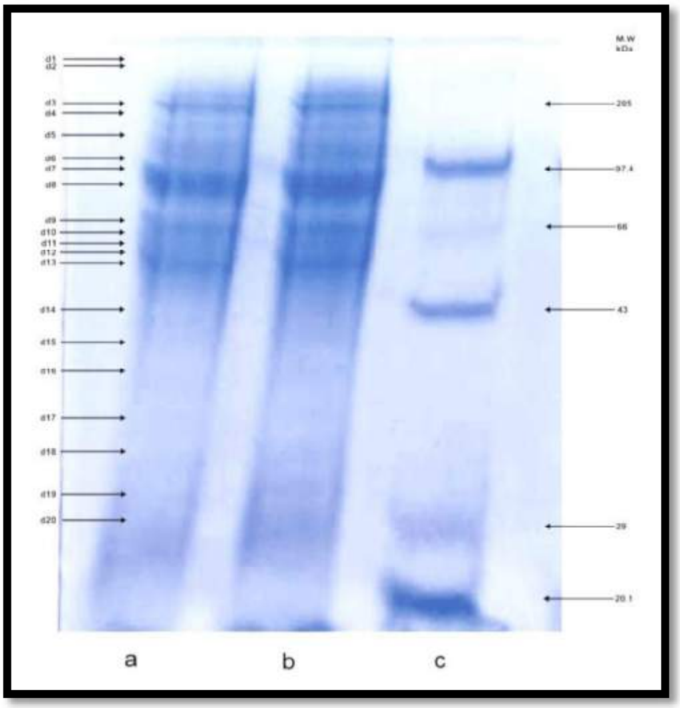


Fig. 51: SDS-PAGE protein profile of the immature pollen of *Datura metel* (a) & (b) 15µl, 10 µl of protein sample respectively, (C) molecular marker.

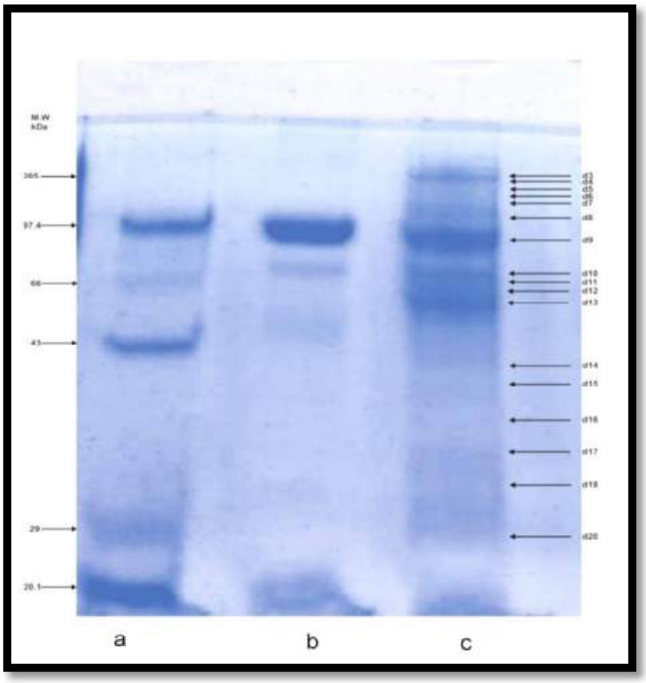


Fig. 52: SDS-PAGE protein profile of the mature pollen of *Datura metel*.
(a) Molecular weight marker (b) BSA (66 kDa) (c) 40µl of protein sample.

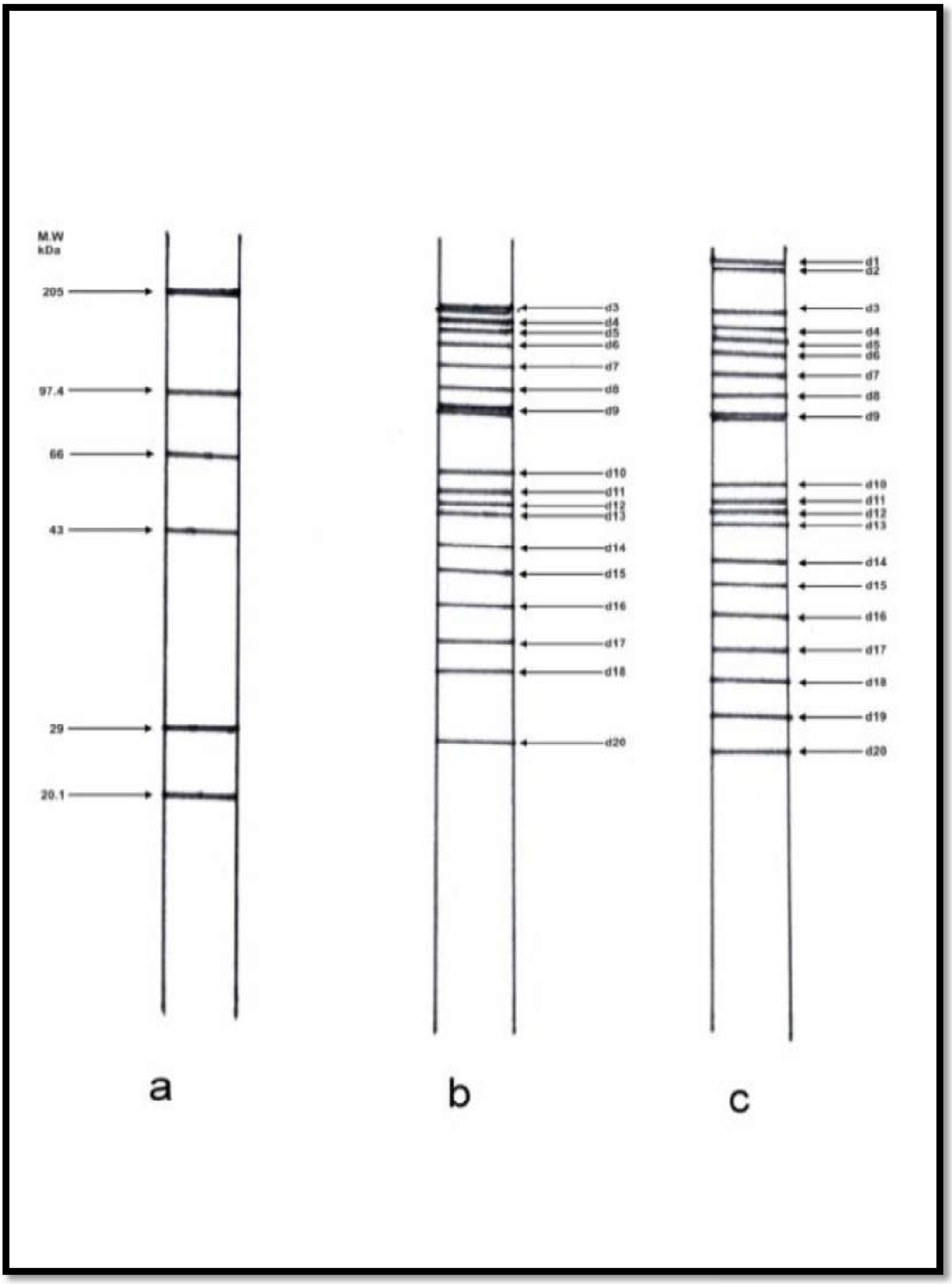
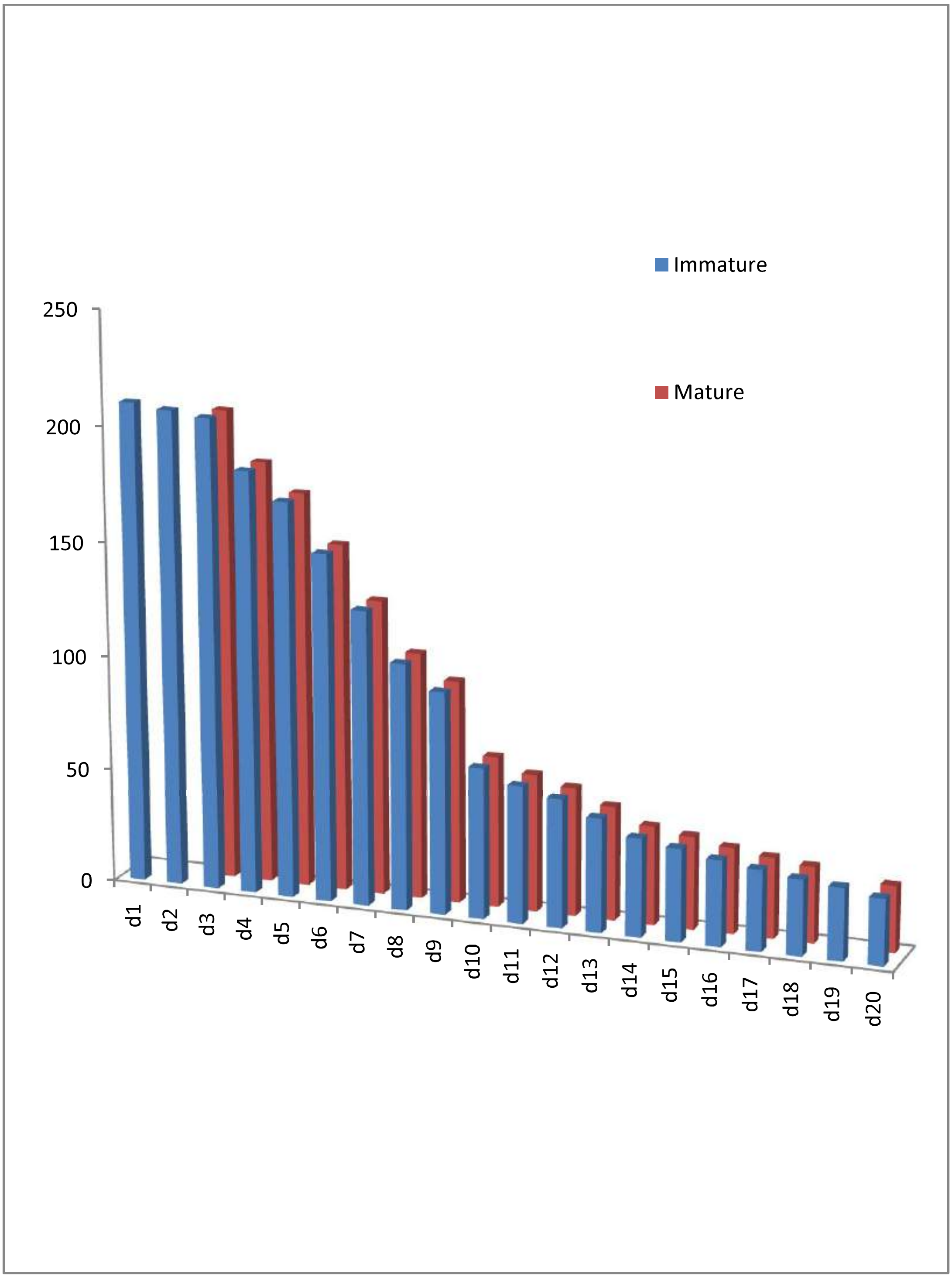


Fig. 53: Comparative protein profile of the pollen of *Datura metel* (a. Molecular weight markers, b. mature pollen, c. immature pollen)



Graph 2: Graphical representation of the molecular weights of the proteins of immature and mature pollen of *Datura metel* (X axis-protein bands and Y axis - molecular weight of proteins in kDa)

4.3.3 Isolation of individual protein fractions by gel filtration

The individual protein fractions were isolated and purified by using gel filtration chromatography and the allergenic fractions were identified by immunodiffusion as well as ELISA using the pooled blood serum of sensitive patients (patients who showed a positive response to the total antigenic extract of the pollen of *Datura metel*).

4.3.4 Analysis of protein fractions isolated by gel filtration

Gel filtration of the total crude protein extract gave 16 fractions (F1-F16) [Table 6]. F8 had a 127.8 kDa protein which gave 2 bands in SDS-PAGE (40.5kDa and 37.5 kDa). Similarly F9 had a 125.4 kDa protein which gave 2 bands in SDS-PAGE (66 kDa and 59.4 kDa). These fractions gave single bands on native gel.

Table 6: Molecular weight of the proteins of *Datura metel* in different fractions obtained by gel filtration

Fraction	Protein	Molecular weight in kDa	
F1	d1		>205
F2	d2		>205
F3	d3		205.80
F4	d4		184.63
F5	d5		172.7
F6	d6		152.0
F7	d7		129.02
F8	d15	40.5	127.8
	d16	37.5	
F9	d10	66.05	125.4
	d11	60.06	
F10	d8		107.79
F11	d9		97.2
F12	d12		56.2
F13	d13		49.8
F14	d14		43.0
F15	d17		35.3
F16	d18		33.2
F17	d19		31.6
F18	d20		29.02

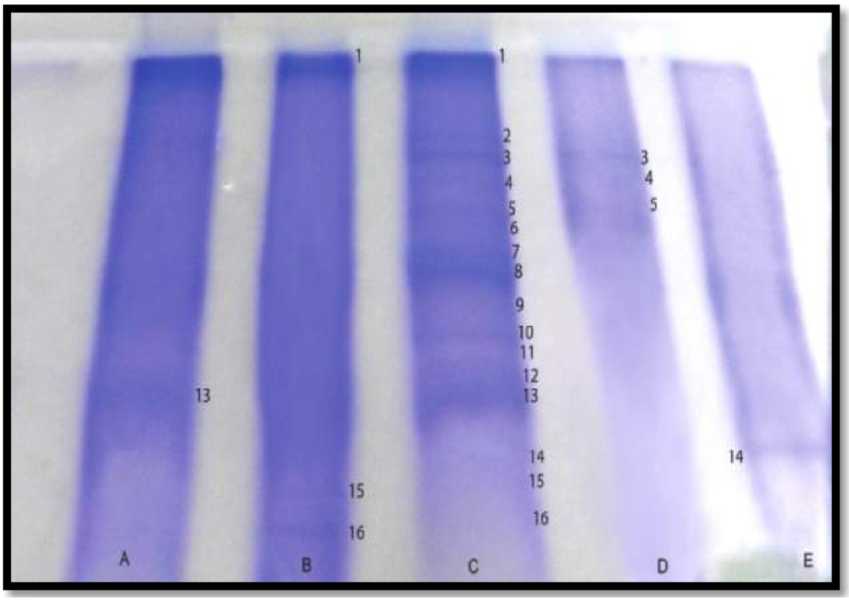


Fig. 54 : SDS-PAGE profile of isolated protein fractions of *Datura metel*

A) F13 - d13
B) F8 - d15, d16
C) F - Total protein extract
D) F3, F4 & F5 - d3, d4, d5
E) F14 - d14

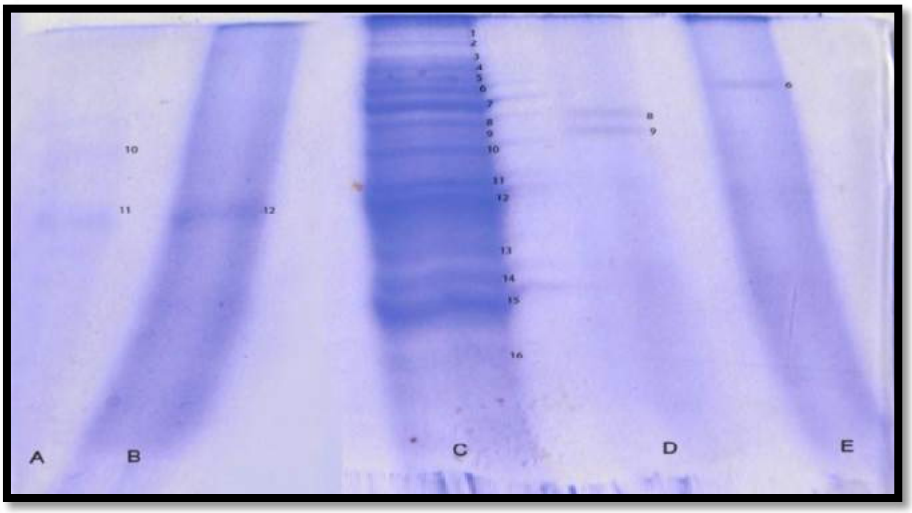


Fig. 55: SDS-PAGE profile of isolated protein fractions of *Datura metel*

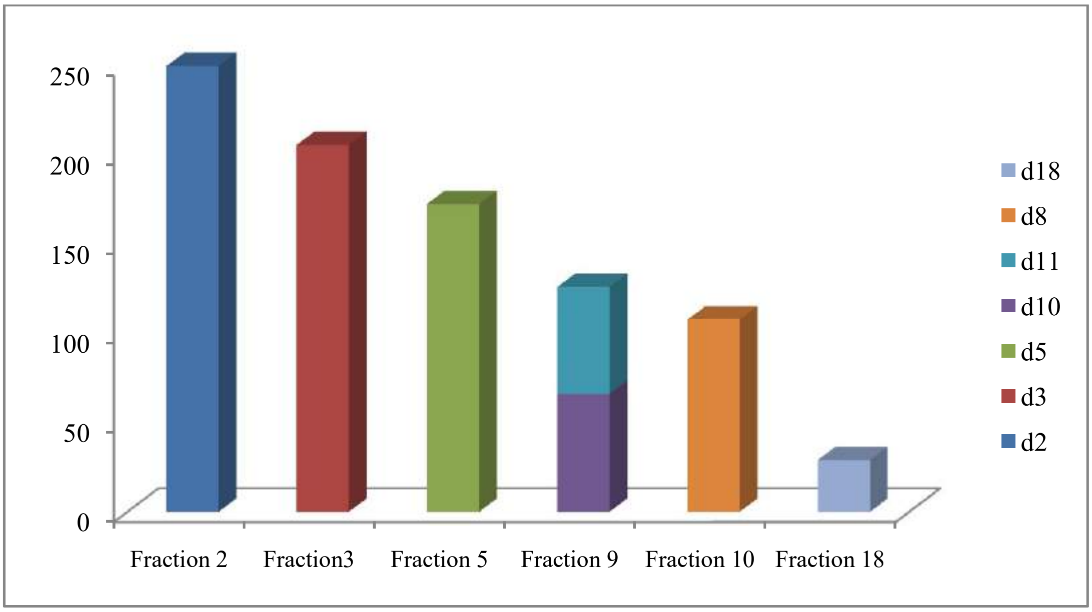
A) F9 - d10, d11
B) F12 - d12
C) F - Total protein extract,
D) F10 & 11 - d8, d9
E) F6 - d6

4.3.5 Identification of antigenic fractions by immunodiffusion

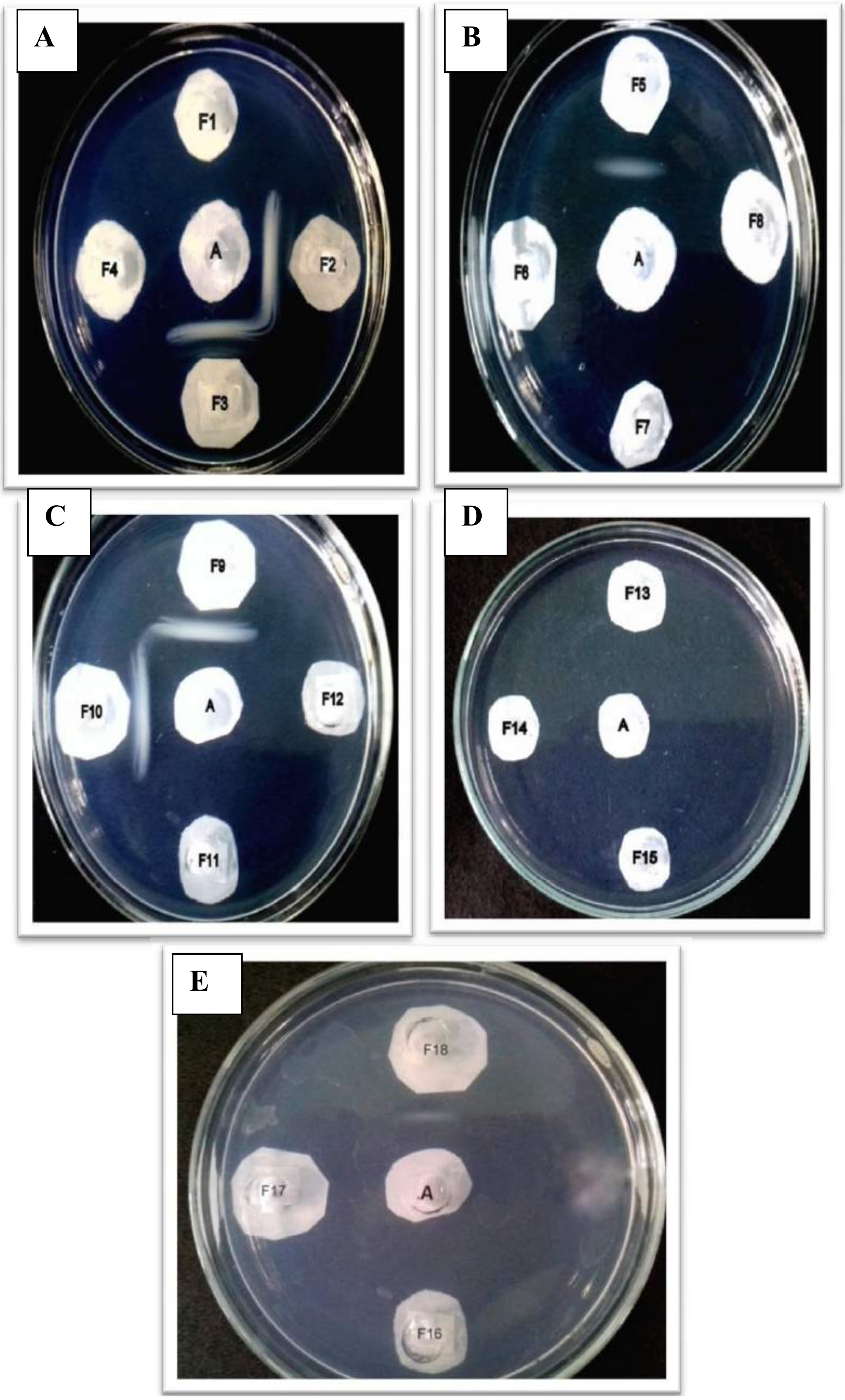
Immunodiffusion of the pooled antiserum of the patients helped in the identification of the allergic protein fractions of *Datura metel* (Fig.56). The precipitation arcs were obtained with fractions F2, F3, F5, F9, F10 and F18. No precipitation reaction was observed with F1, F4, F6, F7, F8, F11-17. Thus 7 proteins (Table 7, Graph 3) with molecular weights >205 kDa, 205 kDa, 172.7 kDa, 108.2 kDa, 66.0 kDa, 59.4 kDa and 29.0 kDa proved to be allergenic in case of *Datura metel* pollen.

Table 7: Allergenic proteins of *Datura metel* and their molecular weights

Fraction 2		Fraction 3		Fraction 5		Fraction 9		Fraction 10		Fraction 18	
Name of the proteins	M.W (kDa)	Name of the proteins	M.W (kDa)	Name of the proteins	M.W (kDa)	Name of the proteins	M.W (kDa)	Name of the proteins	M.W (kDa)	Name of the proteins	M.W (kDa)
d2	>205	d3	205	d5	172.7	d10	66	d8	108.2	d18	29.0
						d11	59.4				



Graph 3: Graphical representation of the molecular weights of allergenic protein fractions of *Datura metel* (X axis- no of protein fractions and Y axis- molecular weight of proteins in kDa)


A
F1
A
F4
F2
F3
B
F5
F8
A
F6
F7
C
F9
F10
A
F12
F11
D
F13
F14
A
F15
E
F18
F17
A
F16

Continued

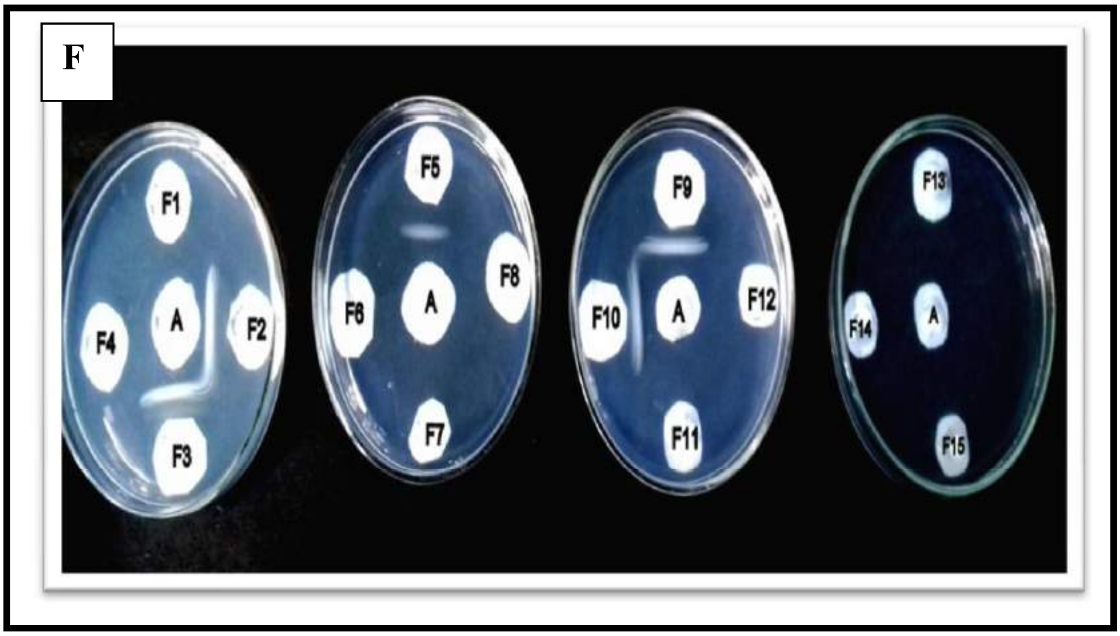


Fig 56: Serological precipitation reaction between pooled antiserum of *Datura metel* pollen sensitive patients and individual protein fractions of *Datura metel* "A" represents pooled human antiserum (25 µl containing 150 µgm total protein) and F1 to F18 contain 50 µgm of the different protein fractions.

Datura stramonium

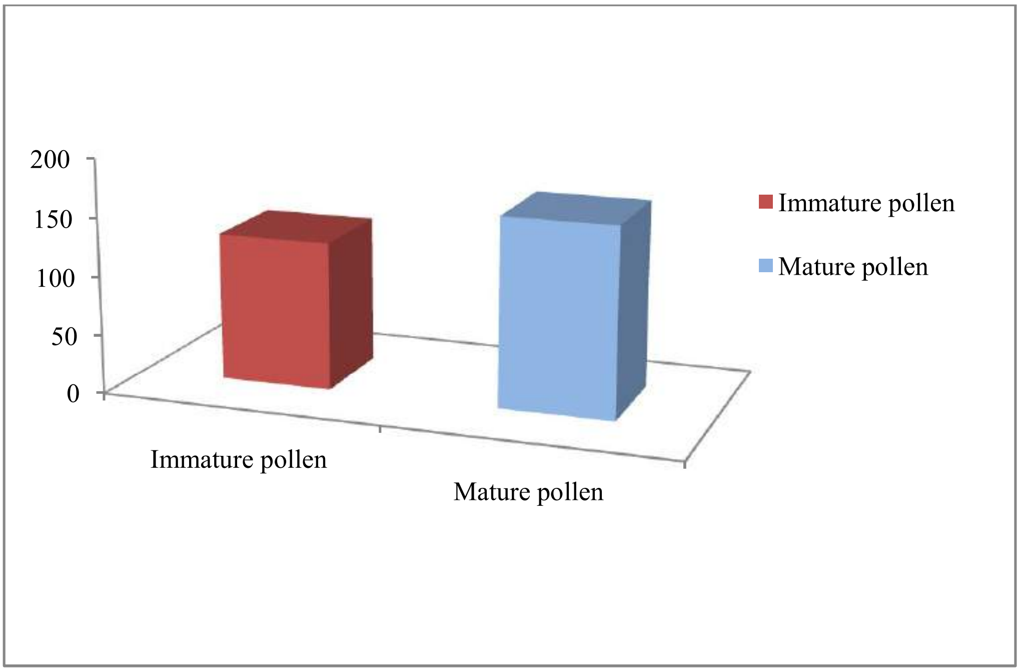
4.4 Protein extraction, isolation and characterization of *Datura stramonium*

4.4.1 Protein concentration of the pollen of *Datura stramonium*

The total concentration of protein in the pollen of *Datura stramonium* also showed a variation with the developmental stages of pollen. The protein concentration of mature pollen was found to be higher than in mature pollen (Table 8, Graph 4). However the protein concentration in the *Datura stramonium* pollen was much lower than that of the other two species. This may be due to the considerable amount of hydrophobic pollenkit present on the ektexine of this pollen which acted as a barrier for the extraction of the water soluble proteins.

Table 8: Protein concentration in the pollen of *Datura stramonium*

AVERAGE PROTEIN CONCENTRATION IN IMMATURE POLLEN	AVERAGE PROTEIN CONCENTRATION IN MATURE POLLEN
127 µgm/ml	162 µgm/ml



Graph 4: Graphical representation of the concentration of pollen proteins of *Datura stramonium* at stages of maturity (X axis- types of pollen and Y axis- concentration of pollen proteins in µg/ml)

4.4.2 Analysis of SDS-PAGE protein profile of *Datura stramonium*

The protein profile by SDS-PAGE exhibited 16 protein bands from 15 kDa to 205 kDa molecular weight range (Fig. 57-59). Mature pollen showed 15 bands with one protein band of 48.8 kDa and 7 low molecular weight protein bands between 15 kDa and 21.61 kDa (D’10-D’16) absent in case of immature pollen. Immature pollen showed nine bands (Table 9, Graph 5). Five of these bands (205.80 kDa, 97.2 kDa, 87 kDa, 60.06 kDa and 43 kDa) were common to the other two species while a 29.02 kDa protein was also found in *Datura metel*. Two proteins (87.0 kDa and 21.61 kDa) were common to *Datura inoxia* and *Datura stramonium.*

Table 9: SDS-PAGE protein profile of the pollen of *Datura stramonium*

M.W of Marker Proteins (kDa)	Proteins bands of Immature pollen		Proteins bands of Mature Pollen	
	Protein band	M.W in (kDa)	Protein band	M.W in (kDa)
205	D’1	205.80	D’1	205.80
	D’2	133.3	D’2	133.3
97.4	D’3	97.2	D’3	97.2
	D’4	87.0	D’4	87.0
	D’5	66.05	D’5	66.05
	D’6	60.06	D’6	60.06
66.05	-	-	-	-
	-	-	D’7	48.8
43	D’8	43.0	D’8	43.0
29	D’9	29.02	D’9	29.02
	-	-	D’10	21.61
20.1	-	-	D’11	20.1
			D’12	19.34
			D’13	18.3
			D’14	17.6
			D’15	16.6
			D’16	15.0
14.3	-	-	-	-

The blue color highlighting protein bands common in *Datura inoxia, Datura metel* and *Datura stramonium*

Thered color highlighting protein bands common to *Datura metel* and *Datura stramonium*

The green color highlighting protein bands common in *Datura inoxia* and *Datura stramonium*

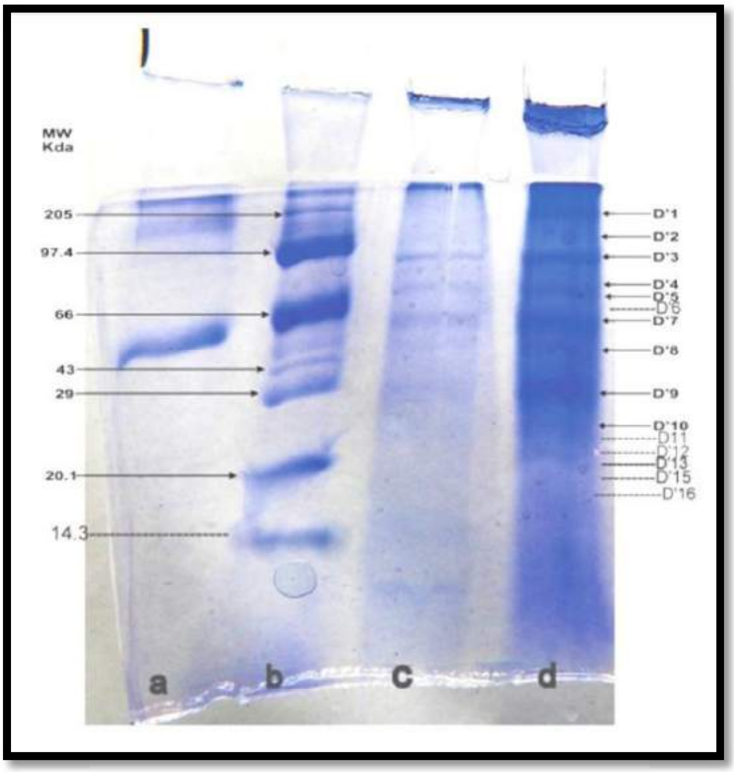


Fig. 57: SDS-PAGE protein profile of mature pollen of *Datura stramonium.*
(a) BSA [66 kDa], (b) molecular weight marker & (c) & (d) 40µl and 20µl of protein sample

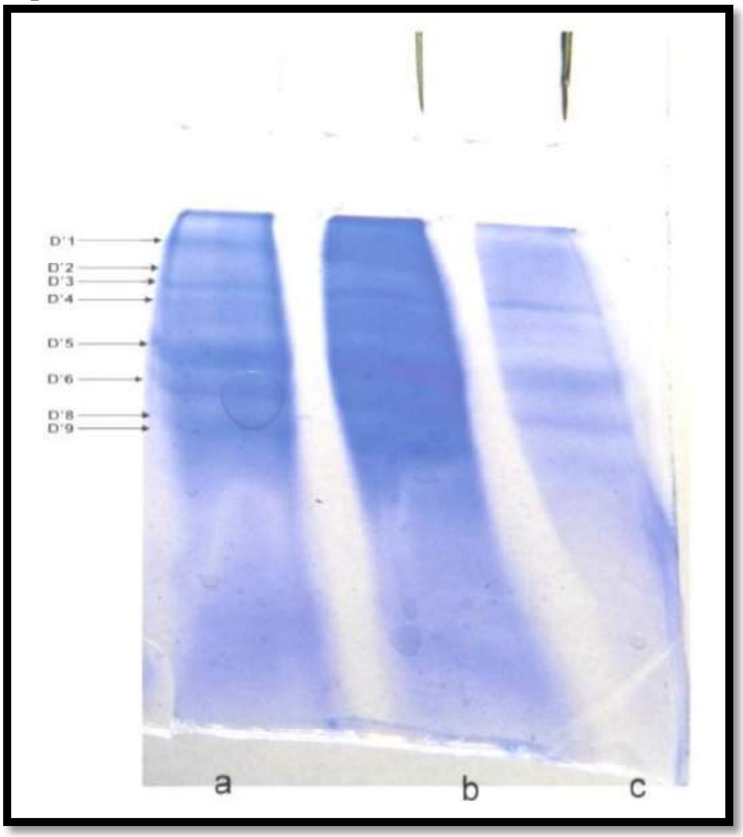


Fig. 58: SDS-PAGE protein profile of the immature pollen of *Datura stramonium*
(a), (b) & (C) 15µl,10 µl and 5ul of protein sample respectively

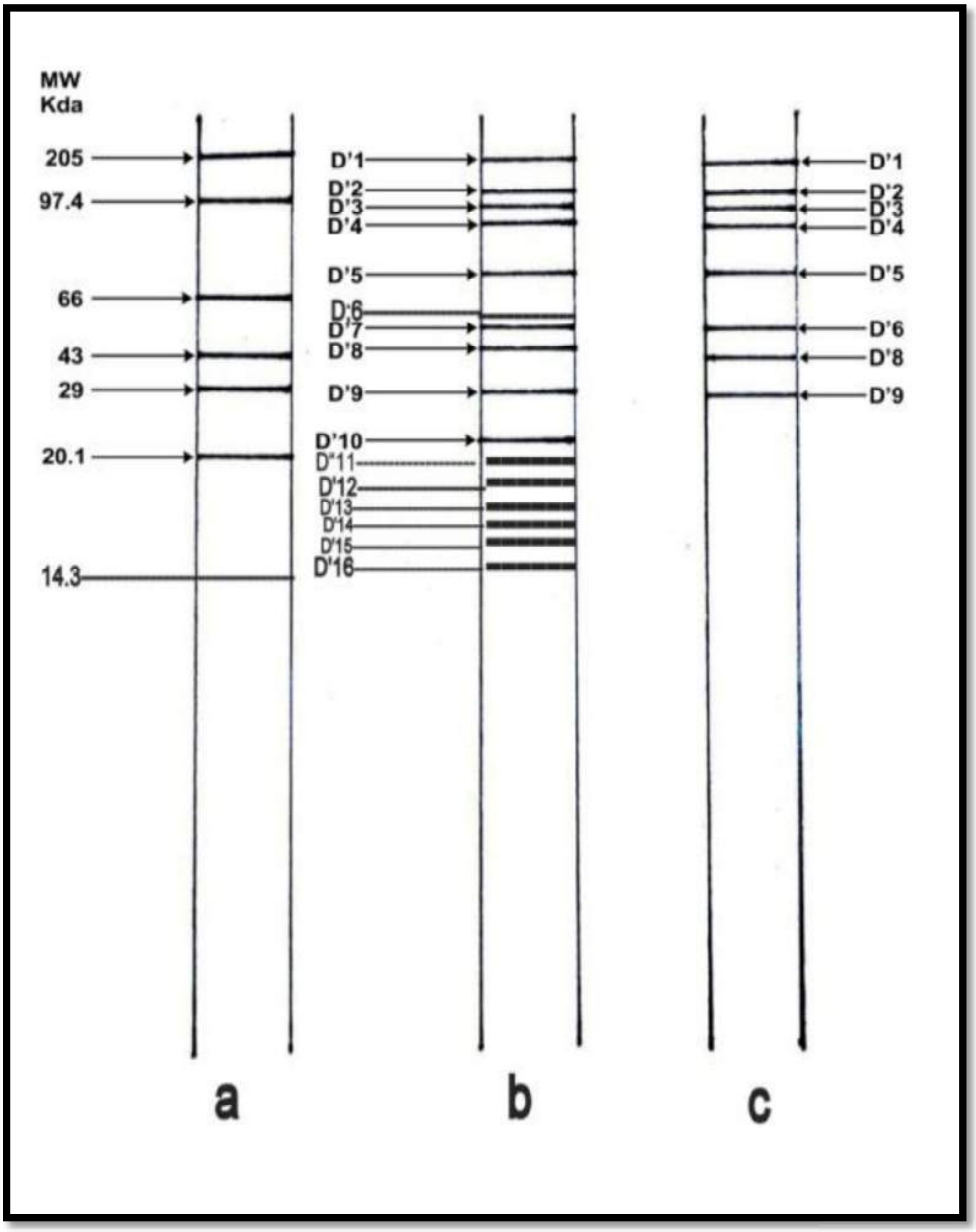
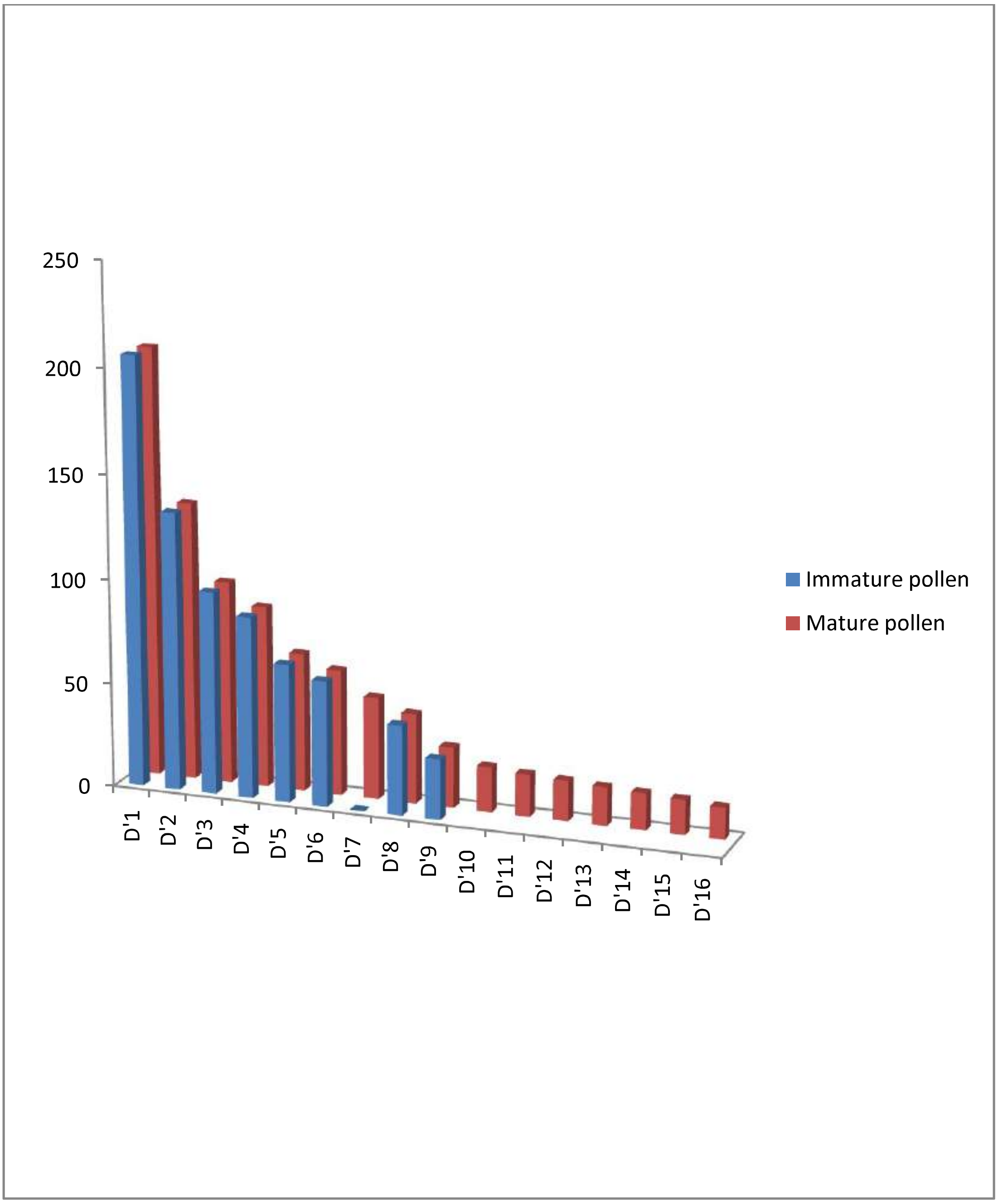


Fig. 59: Comparative protein profile of the pollen of *Datura stramonium*
a. Molecular weight markers, b. mature pollen, c. immature pollen



Graph 5: Graphical representation of the molecular weight of immature & mature pollen proteins of *Datura stramonium* (X axis- protein bands and Y axis- molecular weight of proteins in kDa)

4.4.3 Analysis of protein fractions isolated by gel filtration

The different protein fractions from the pollen were isolated and purified by using gel filtration and the allergenic fractions were identified by immunodiffusion using the pooled blood serum of sensitive patients. Gel filtration yielded 14 fractions (F1-F14) as shown in Table 10 (Figs. 50 and 51). F8 had a 34.0 kDa protein which gave 2 bands in SDS-PAGE (17.6 kDa and 16.6kDa). Similarly F3 had a 125.4 kDa protein which gave 2 bands in SDS-PAGE (66.05kDa and 60.06kDa). These fractions gave single bands on native gel.

Table 10: Molecular weight of the proteins of *Datura stramonium* in different fractions obtained by gel filtration

Fraction	Protein	Molecular weight in kDa	
F1	D'1		205.80
F2	D'2		133.3
F3	D'5	66.05	125.4
	D'6	60.06	
F4	D'3		97.2
F5	D'4		87.0
F6	D'7		48.8
F7	D'8		43.0
F8	D'14	17.6	34.0
	D'15	16.6	
F9	D'9		29.02
F10	D'10		21.61
F11	D'11		20.1
F12	D'12		19.34
F13	D'13		18.3
F14	D'16		15.0

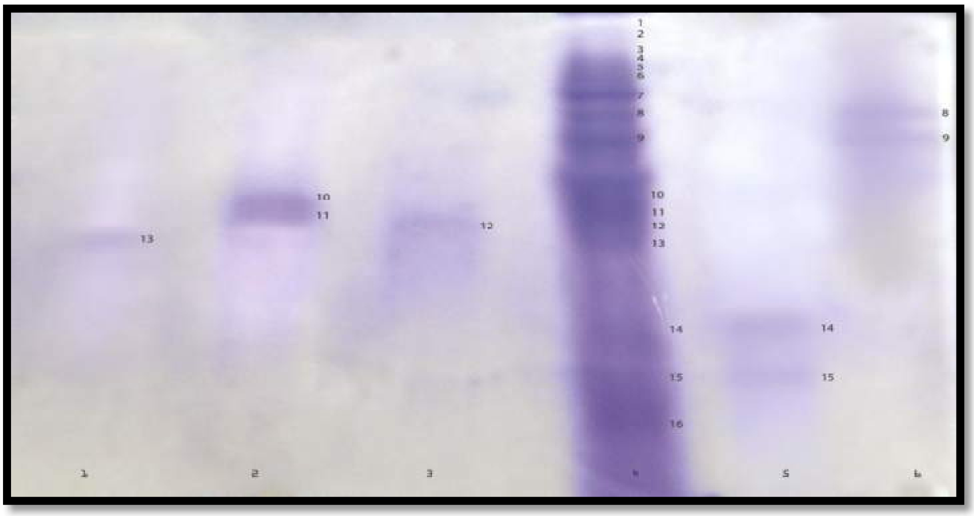


Fig 60: SDS-PAGE profile of isolated protein fractions of *Datura stramonium*.

Lane 1 - F13 (D'13)
Lane 2 - F10 & F11 (D'10 &D'11)
Lane 3 - F12 (D'12)
Lane 4 - Total protein extract
Lane 5 – F8 (D'14 &D'15)
Lane 6 – F7 & F9 (D'8&D'9)

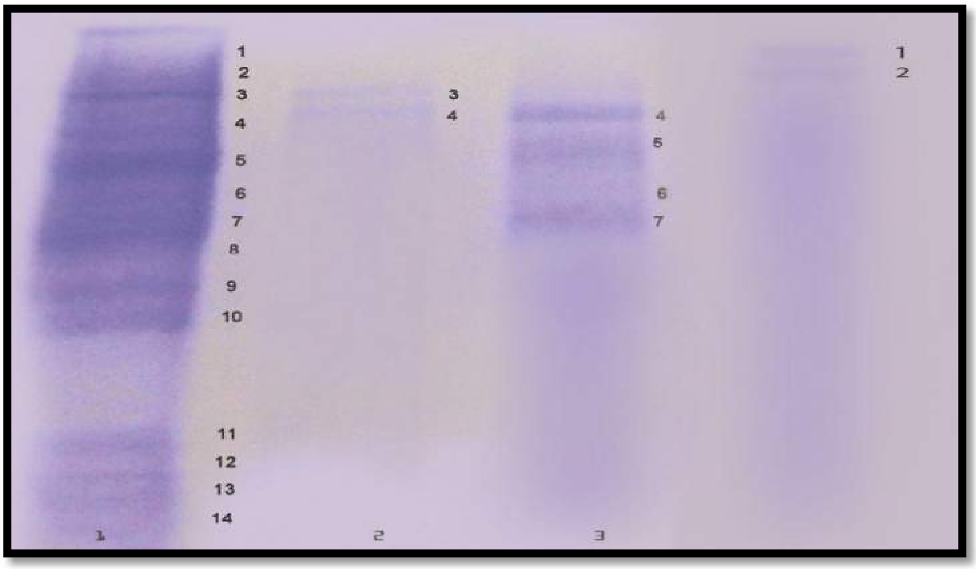


Fig. 61: SDS-PAGE profile of isolated protein fractions of *Datura stramonium*.

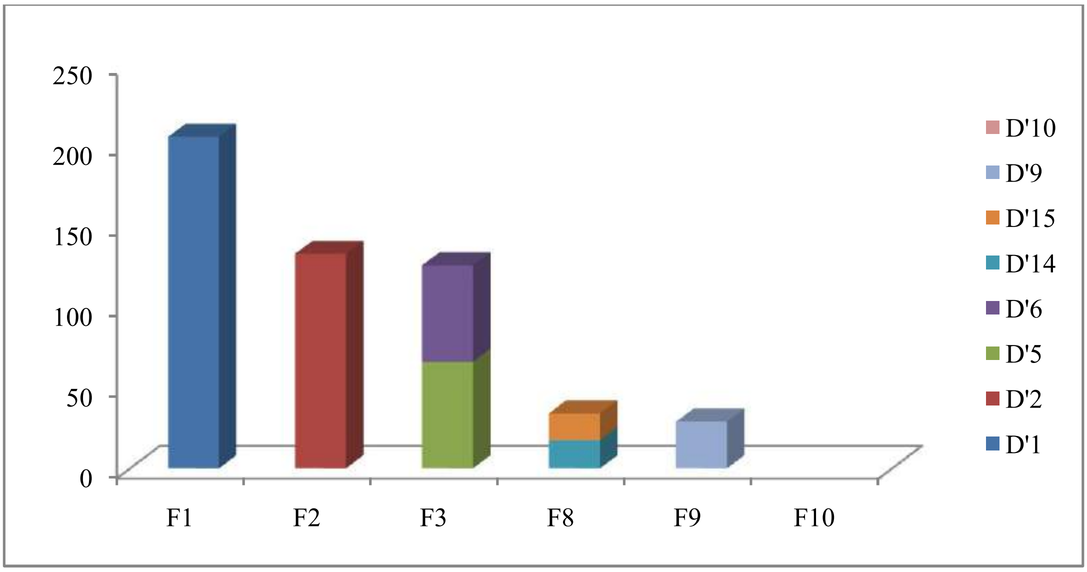
Lane 1 - Total protein extract
Lane 2 – F4 & F5 (D'3&D'5)
Lane 3 - F5, F3 & F6 (D'4; D'5 &D'6; D'7)
Lane 4 – F1 & F2 (D'1 &D'2)

4.4.4 Identification of antigenic fractions by immunodiffusion

Immunodiffusion of the pooled antiserum of the patients helped to detect the allergenic protein fractions of *Datura stramonium* (Fig. 62). The precipitation arcs were obtained with fractions F1, F2, F3, F8, F9 and F10. A very faint precipitation arc was obtained in case of F1. No precipitation reaction was observed with F4, F5, F6, F7 and F11 - F14.Thus 8 proteins (Table 11, Graph 6) with molecular weights 205.80 kDa, 133.3 kDa, 66.05 kDa, 60.06 kDa, 29.02 kDa, 21.61 kDa, 17.6 kDa and 16.6 kDa were detected as the allergenic proteins in case of *Datura stramonium* pollen.

Table 11. Allergenic proteins of *Datura stramonium* and their molecular weights

F1		F2		F3		F8		F9		F10	
Name of the proteins	MW (kDa)	Name of the proteins	MW (kDa)	Name of the proteins	MW (kDa)	Name of the proteins	MW (kDa)	Name of the proteins	MW (kDa)	Name of the proteins	MW (kDa)
D'1	205.0	D'2	133.3	D'5	66.0	D'14	17.6	D'9	29.0	D'10	21.0
				D'6	59.4	D'15	16.4				



Graph 6: Graphical representation of the molecular weights of the allergenic protein fractions of *Datura stramonium* (X axis- no of protein fractions and Y axis- molecular weight of proteins in kDa)

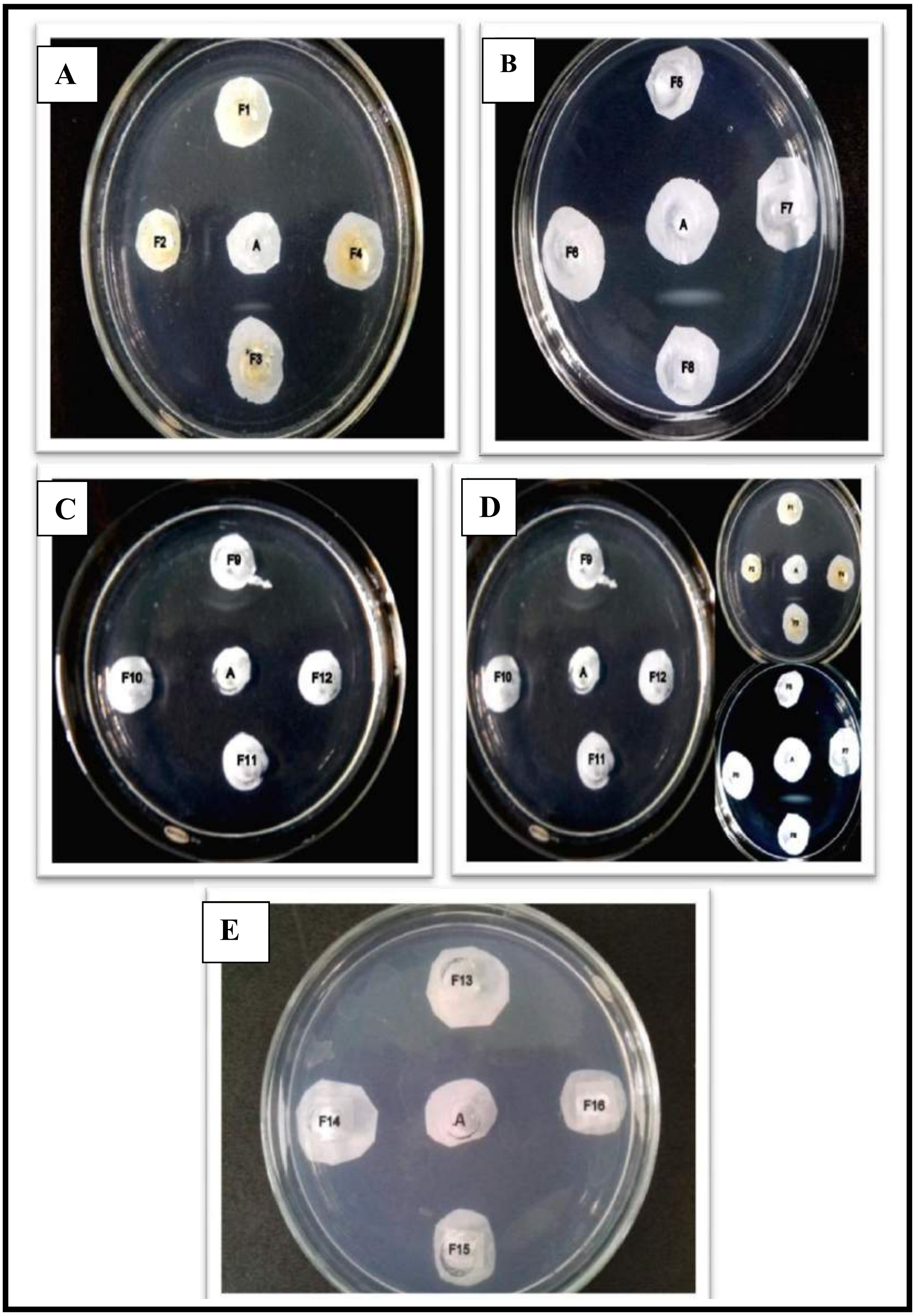


Fig. 62: Serological precipitation reaction between pooled antiserum of *Datura stramonium* pollen sensitive patients and individual protein fractions of *Datura stramonium*" A" represents pooled human antiserum (25 µl containing 150 µgm total protein) and (A) F1 to F4; (B) F5 to F8; (C) F9 to F12; (D) F1-F12 contain 50 µgm of protein fractions.

Datura inoxia

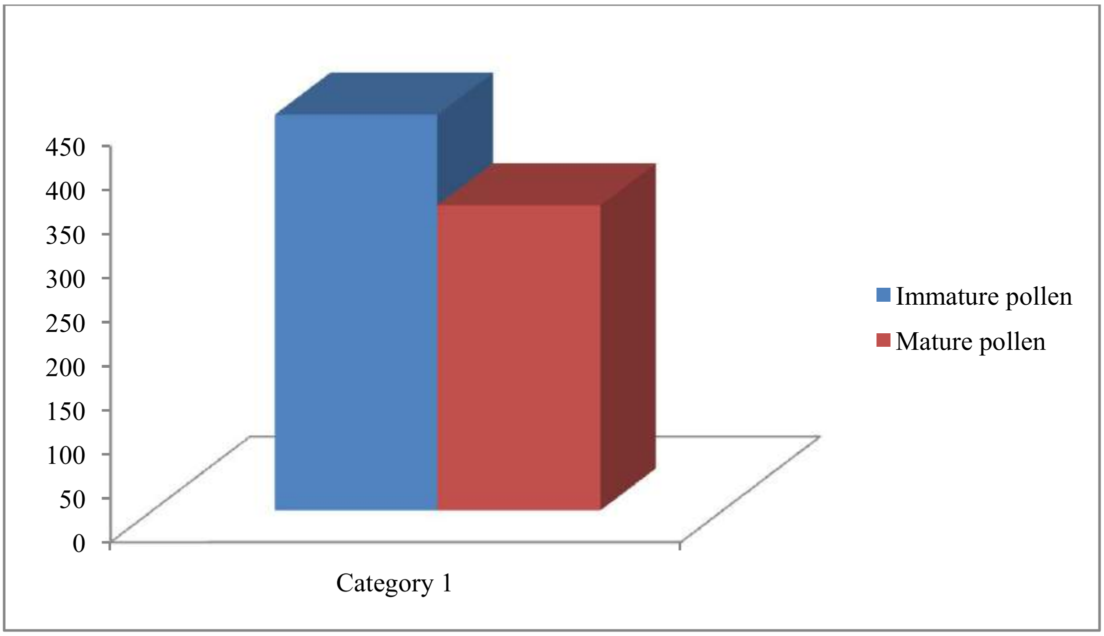
4.5 Protein Extraction, Isolation and Characterization of *Datura inoxia*

4.5.1 Protein concentration of the pollen of *Datura inoxia*

Datura inoxia pollen showed the highest protein concentration among the 3 species. Unlike *Datura stramonium*, the protein concentration of mature pollen was lower than that of immature pollen (Table 12, Graph 7) of *Datura inoxia.*

Table 12: Protein Concentration of the pollen of *Datura inoxia*

AVERAGE PROTEIN CONCENTRATION IN IMMATURE POLLEN	AVERAGE PROTEIN CONCENTRATION IN MATURE POLLEN
450 µgm/ml	347 µgm/ml



Graph 7: Graphical representation of the concentration of pollen proteins of *Datura inoxia* at different stages of maturity maturity (X axis – pollen types and on Y axis- protein concentration in µgm/ml)

4.5.2 Analysis of SDS-PAGE protein profile of *Datura inoxia*

The SDS-PAGE profile of the protein of *Datura inoxia* revealed 16 protein bands ranging from 20.6 kDa and 205.80 kDa. Of these 5 bands were found common to immature and mature pollen (Table 13). Eight protein bands were found in immature pollen only. These include 205.80 kDa, 184.63 kDa, 92.1 kDa, 87 kDa, 70.78 kDa, 56.8 kDa, 24.9 kDa and 22.4 kDa proteins. Three proteins (66.05 kDa, 21.61 kDa and 20.6 kDa) were unique to the mature pollen (Figs. 63-65, Graph 8). Three of these bands (205.80 kDa, 97.2 kDa and 43 kDa) were also common to the other two species while four more bands (184.63 kDa, 108.9 kDa, 66.05 kDa and 56.2 kDa) were common between *Datura metel* and *Datura inoxia*. D6 (87 kDa) was the protein found in *Datura stramonium* and *Datura inoxia* only.

Table 13: SDS-PAGE protein profile of the pollen of *Datura inoxia*

M.W of Marker Proteins (kDa)	Proteins bands from Immature pollen		Proteins bands from Mature pollen	
	Protein band	M.W in (kDa)	Protein band	M.W in (kDa)
205	D1	205.80	-	-
	D2	184.63	-	-
	D3	107.79	D3	107.79
97.2	D4	97.2	D4	97.2
	D5	92.1	-	-
	D6	87.0	-	-
	D7	82.91	D7	82.91
	D8	70.78	-	-
	-	-	-	-
66.05	-	-	D9	66.05
	D10	60.06	-	-
	D11	43.0	D11	43.0
43	-	-	-	-
	D12	32.5	D12	32.5
29.02	-	-	-	-
	D13	24.9	-	-
	D14	22.4	-	-
	-	-	D15	21.61
	-	-	D16	20.6
20.1	-	-	-	-
14.3	-	-	-	-

The green color highlighting protein bands common to *Datura metel, Datura stramonium* and *Datura inoxia*

The red color highlighting protein bands common to *Datura stramonium* and *Datura inoxia*

The blue color highlighting protein bands common to *Datura metel* and *Datura inoxia*

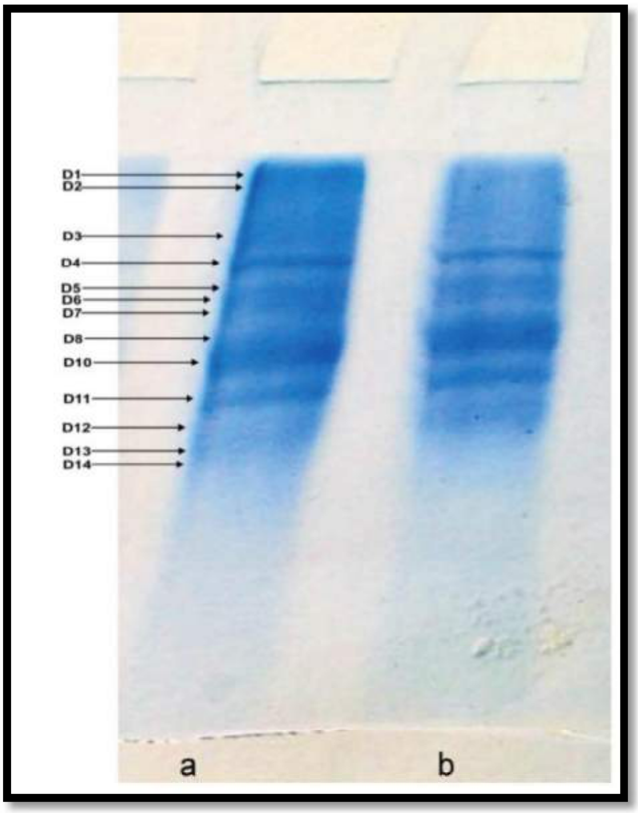


Fig 63: SDS-PAGE protein profile of the pollen of immature pollen of *Datura inoxia* (a) & (b) 5µl and 10 µl of protein sample

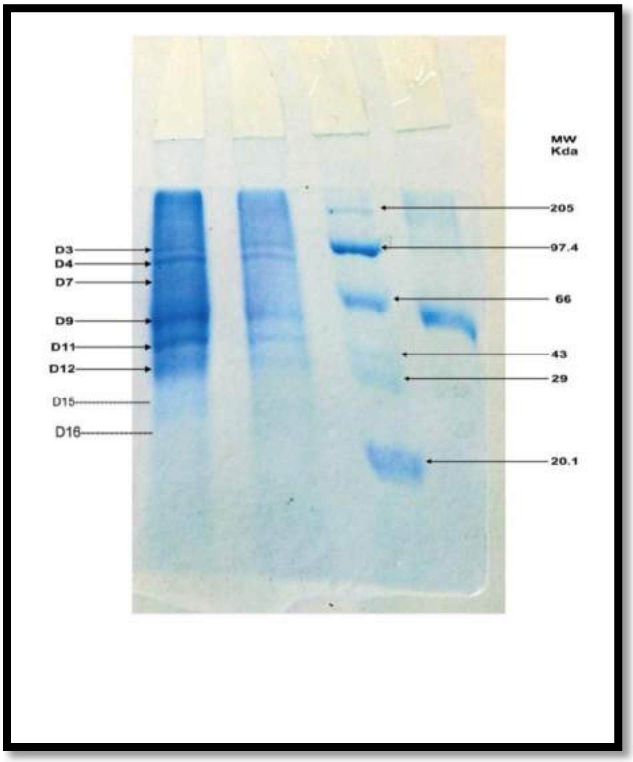


Fig 64:SDS-PAGE protein profile of the pollen of mature pollen of *Datura inoxia* (a) & (b) 40µl and 20µl of protein sample (c) molecular weight marker (d) BSA (66 kDa)

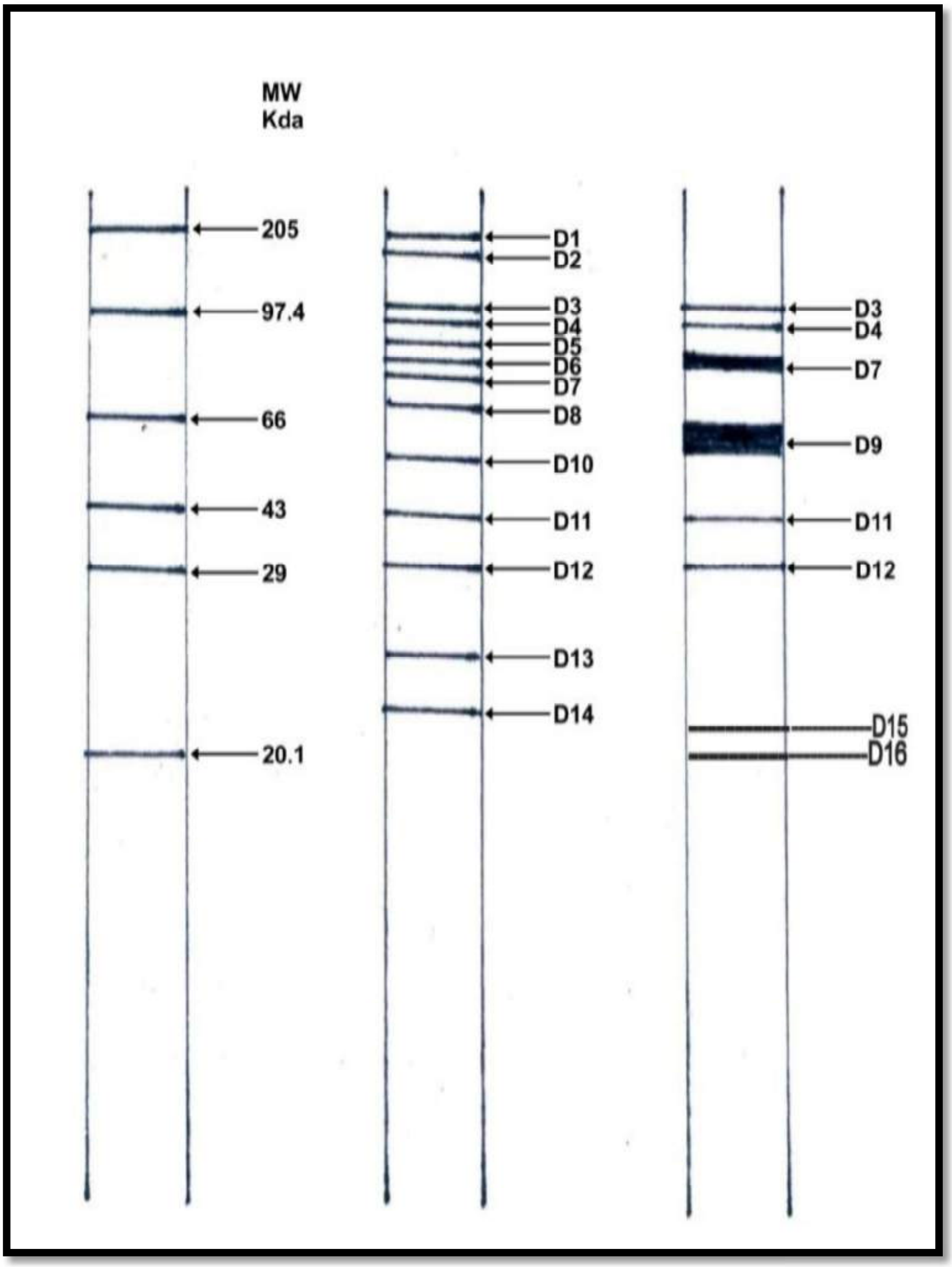
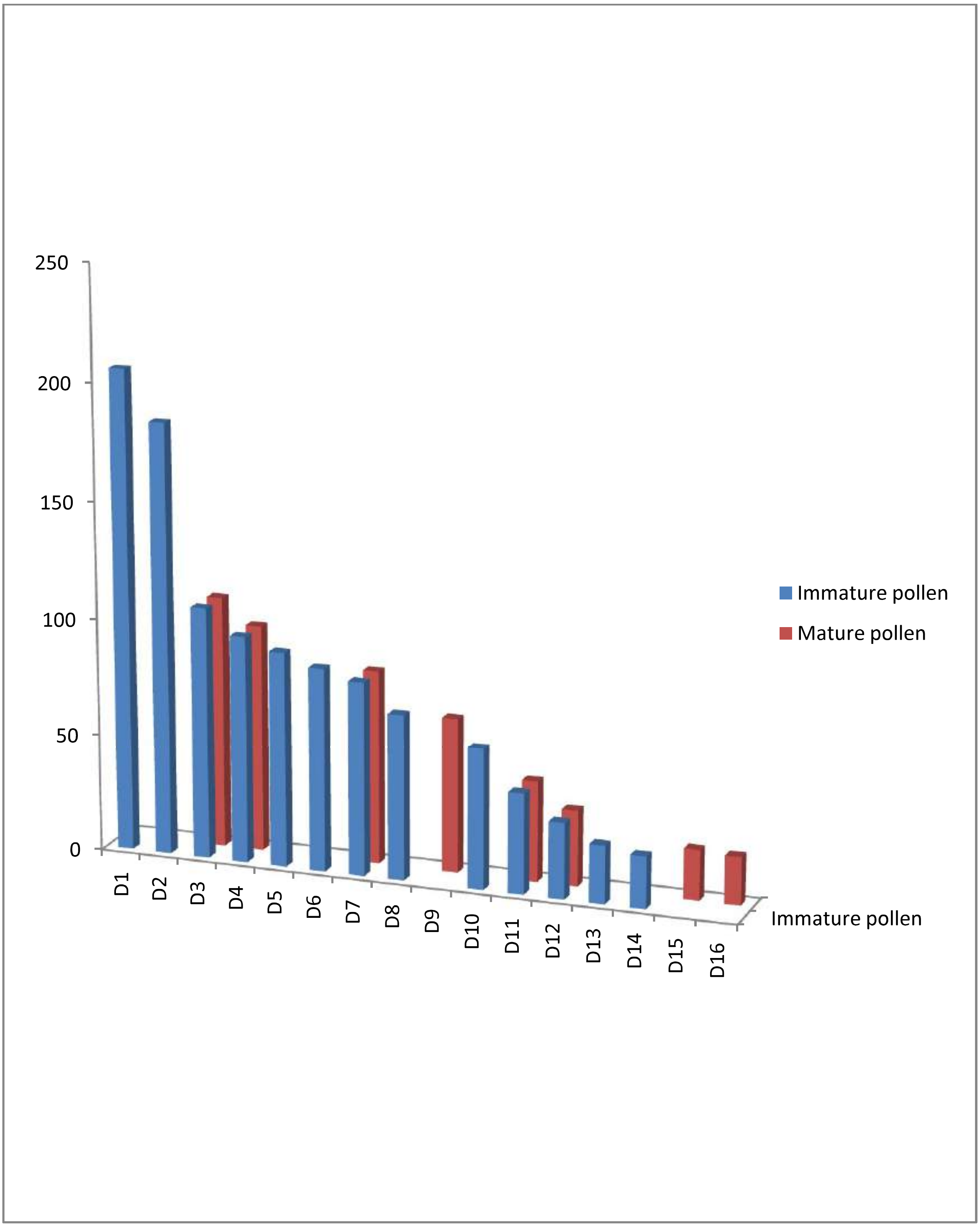


Fig 65: Comparative protein profile of the pollen of *Datura inoxia* a. Molecular weight markers, b. immature pollen, c. mature pollen



Graph 8: Graphical representation of the molecular weight of immature & mature pollen proteins of *Datura inoxia*(X axis- protein bands and Y axis- molecular weight of proteins in kDa)

4.5.3 Analysis of protein fractions isolated by gel filtration

Fourteen protein fractions (F1 - F14) were isolated and purified by using gel filtration (Table 14) and the allergenic protein fractions were identified by immunodiffusion using the pooled blood serum of sensitive patients. F10 had a 63.6 kDa protein which gave 2 bands in SDS-PAGE (43.0 kDa and 20.6 kDa). Similarly F9 had a 125.4 kDa protein which gave 2 bands in SDS-PAGE (66.05 kDa and 60.06 kDa) [Figs.66-67]. These fractions gave single bands on native gel.

Table 14: Molecular weights of the proteins of *Datura inoxia* in different fractions obtained by gel filtration

Fraction	Protein	Molecular weight in kDa	
F1	D1		205.80
F2	D2		184.63
F3	D3		107.79
F4	D4		97.2
F5	D5		92.1
F6	D6		87.0
F7	D7		82.91
F8	D8		70.78
F9	D9	66.05	125.4
	D10	60.06	
F10	D11	43.0	63.6
	D16	20.6	
F11	D12		32.5
F12	D13		24.9
F13	D14		22.4
F14	D15		21.61

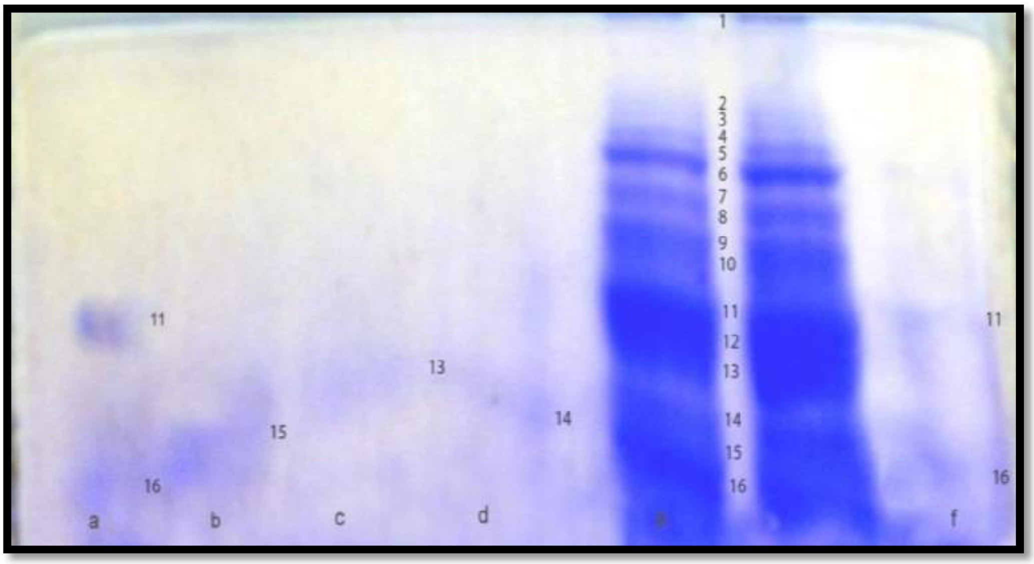


Fig. 66: SDS-PAGE profile of isolated protein fractions of *Datura inoxia*

a) F 10 - D11 & D16
b) F14 - D15
c) F12 - D13
d) F13 - D14
e) Total protein extract
f) F10 - D11& D16

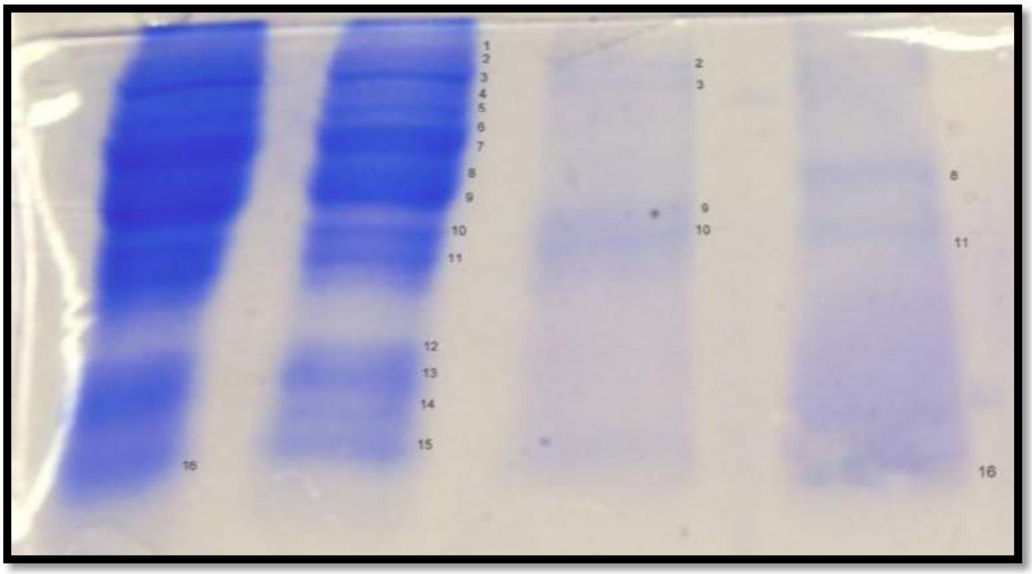


Fig. 67: SDS-PAGE profile of isolated protein fractions of *Datura inoxia*

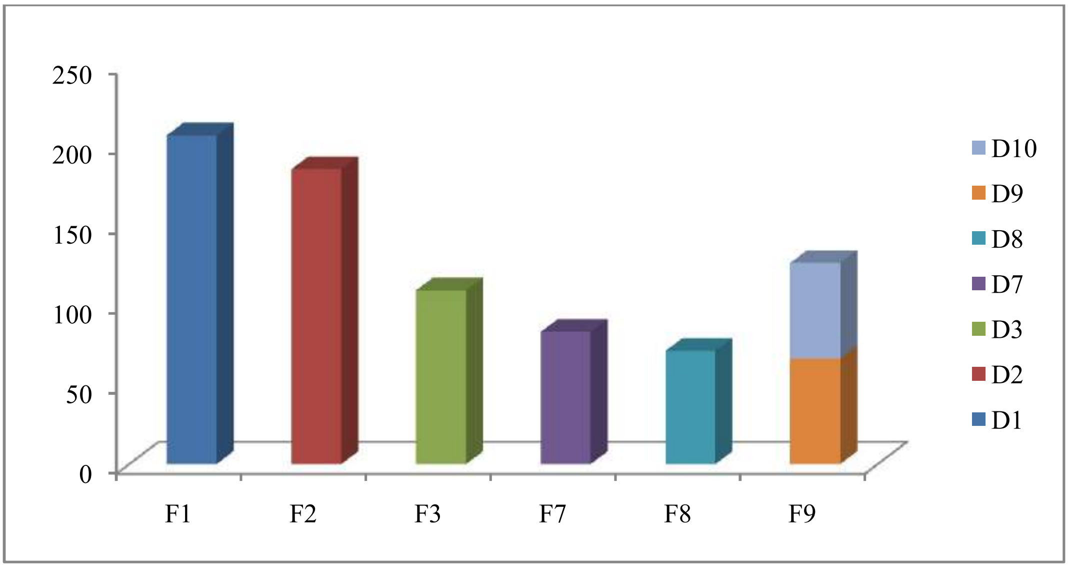
a & b) total protein extract
c) F9, 2 & 3 - D9, D10, D2 & D3
d) F8 & 10 - D8, D11, D16

4.5.4 Identification of antigenic fractions by immunodiffusion

Immunodiffusion of the pooled antiserum of the patients helped in the identification of allergenic protein fractions of *Datura inoxia* (Fig 68). The precipitation arcs were obtained with fractions F1, F2, F3, F7, F8 and F9. A very faint precipitation arc was obtained in F1. No precipitation reaction was observed with F4, F5, F6, F10 - F14. Thus 7 proteins (205.80 kDa, 184.63 kDa, 108.9 kDa, 82.91 kDa, 70.78 kDa, 66.05 kDa and 60.06 kDa) were confirmed to be allergenic in the pollen of *D.inoxia* (Table 15, Graph 9).

Table 15. Allergenic proteins of *Datura inoxia* and their molecular weights

F1		F2		F3		F7		F8		F9	
Name of the proteins	MW (kDa)	Name of the proteins	MW (kDa)	Name of the proteins	MW (kDa)	Name of the proteins	MW (kDa)	Name of the proteins	MW (kDa)	Name of the proteins	MW (kDa)
D1	205.0	D2	183.5	D3	108.9	D7	82.0	D8	70.0	D9	66.0
										D10	59.4



Graph 9: Graphical representation of the molecular weights of the allergenic protein fractions of *Datura inoxia* (X axis- protein fractions and Y axis-molecular weight of proteins in kDa)

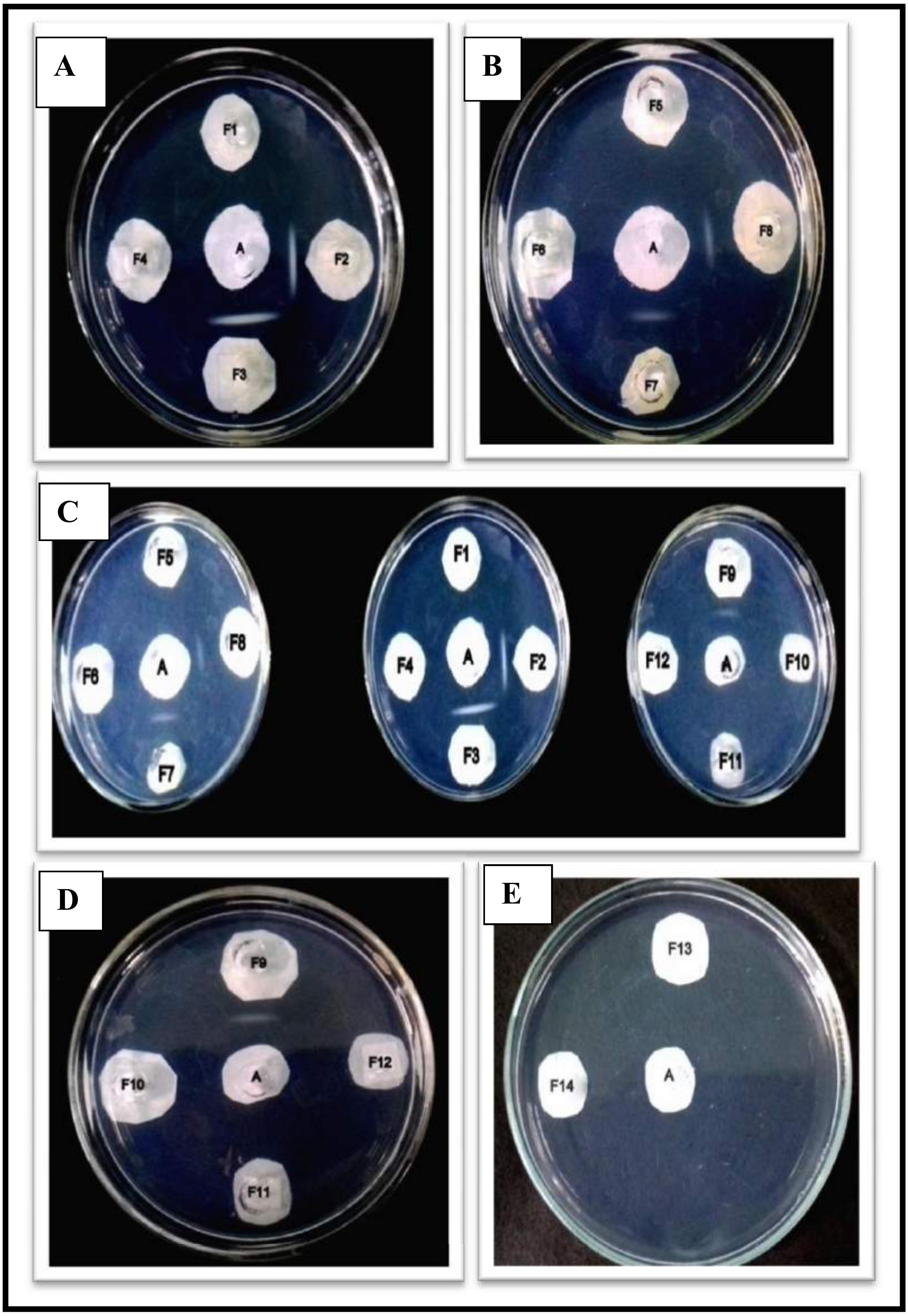


Fig. 68: Serological precipitation reaction between pooled antiserum of *Datura inoxia* pollen sensitive patients and individual protein fractions of *Datura inoxia* ”A” represents pooled human antiserum (25 µl containing 150 µgm total protein) and (A) F5 to F8; (B) F1 to F4; (C) F9 to F12; (D) F1-F12 and (E) F13-F14 contain 50 µgm of protein fractions.

Study of cross reactivity

4.6 Study of Cross-Reactivity

Cross-reactivity in allergic reactions occurs when proteins of two substances (here two pollens belonging to different species) are similar to each other. As a result, the immune system sees them as the same. According to Knox and Suphioglu (1996) and Grote et al. (2000, 2005), pollen allergens are low molecular weight proteins or glycoproteins which are water soluble ranging between 5 kDa to 80 kDa. A comparison of the sequence analysis has shown that the allergenic proteins of pollen belong to only 29 of the 2615 protein families found in seed plants including profilins, expansions, pathogenesis-related proteins, and calcium-binding proteins (Chapman et al., 2007; Radauer and Breiteneder, 2006). As a consequence of cross reactivity a remarkable sequence similarities is shown in many allergens which cause the phenomenon of cross-reactivity. Thus, although a person may not have been exposed to a particular allergen earlier, he or she might show the allergic reaction due to cross-reactivity to a similar IgE epitope of a similar protein to which the patient had been exposed to earlier.

Ouchterlony immunodiffusion and ELISA were performed to check the cross reactivity reactions of whole protein extract and the isolated protein fractions among the three species of *Datura* viz. *Datura metel, Datura stramonium and Datura inoxia.*

4.6.1 Cross-reactivity between total extract of pollen

Serological precipitation reaction was obtained between antiserum of white rat injected with the total pollen protein extract of *Datura metel, D. stramonium and D.inoxia* and the total protein extracts of the pollen of *Datura inoxia* (Di)*, Datura metel* (Dm) and *Datura stramonium* (Ds) [Fig. 69].

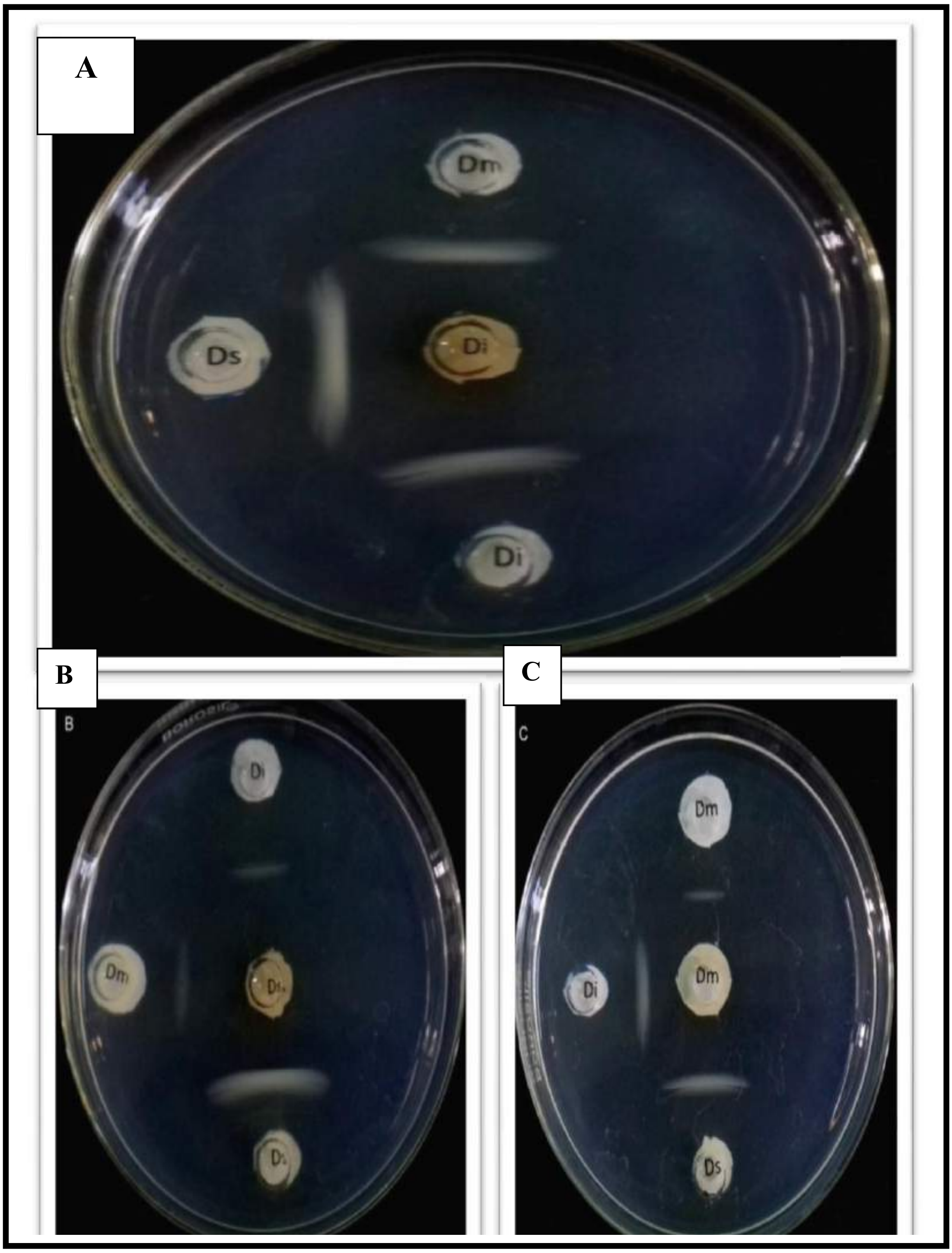


Fig 69: Serological precipitation reaction between antiserum against A) *D. inoxia*, B) *D. stramonium* and C) *Datura metel* pollen developed in white rat (each centre well containing 25ul of blood serum) and the total protein extracts of the pollen of *Datura inoxia* (Di), *Datura metel* (Dm) and *Datura stramonium* (Ds) in the outer wells containing 50 µg of protein.

4.6.2 Cross-reactivity between different protein fractions of the three species of pollen

Serological precipitation reaction was also obtained between antiserum of white rat injected with the total pollen protein extract of *Datura metel, D. stramonium and D. inoxia* and the isolated protein fractions of *Datura inoxia* (Di), *Datura metel* (Dm) and *Datura stramonium* (Ds) obtained by gel filtration.

4.6.2.1 Antiserum of *Datura metel* and protein fractions of *Datura inoxia*

Precipitation arcs were obtained in the Fractions 1,2,3,4,9 and 10 both in immunodiffusion as well as in ELISA (Fig 70: A & B).

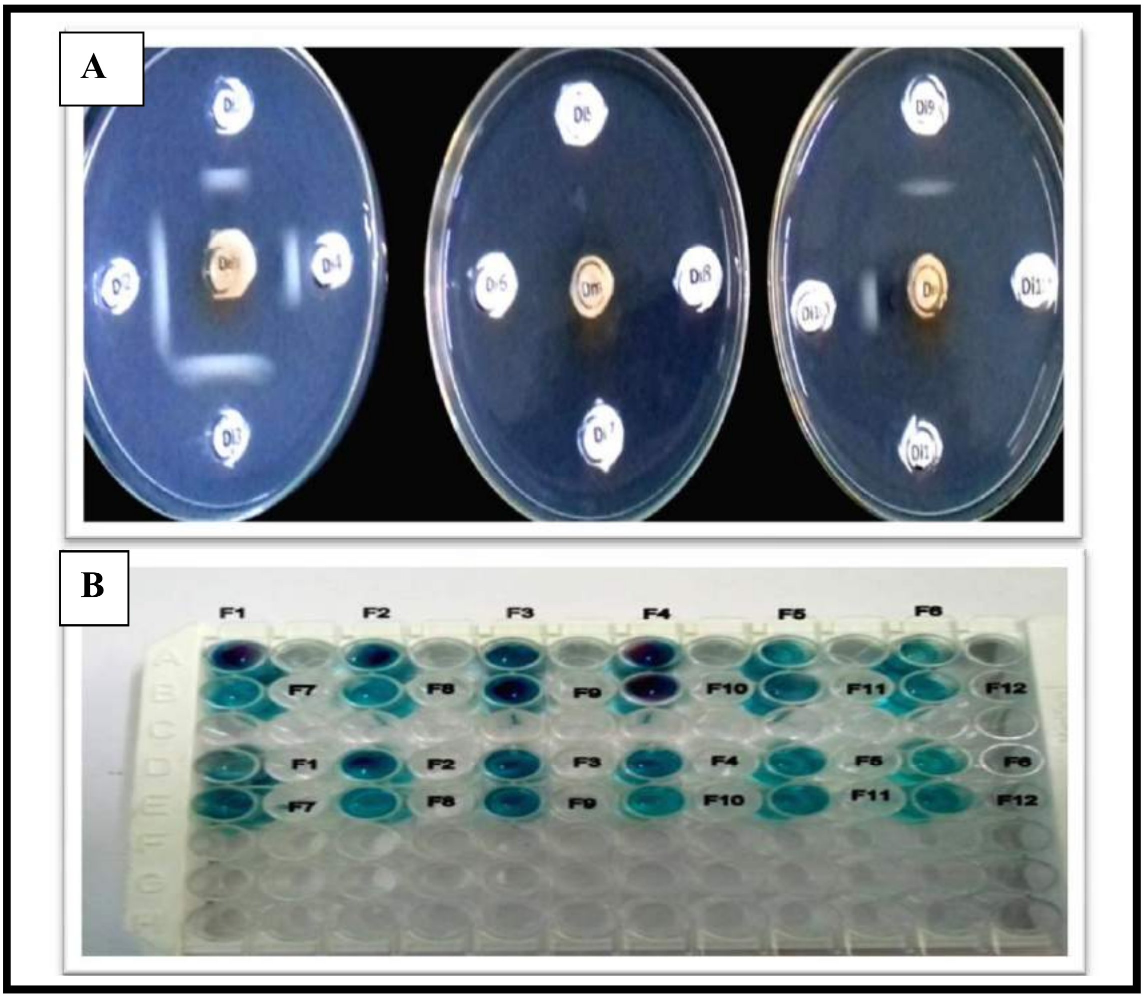


Fig 70: Serological precipitation reaction (A – Immunodiffusion, B – ELISA, Lanes A & B) between antiserum against *Datura metel* (Dm) pollen developed in white rat (each centre well containing 25µl of blood serum) and the individual protein fractions of the pollen of *Datura inoxia* (Di) obtained by gel filtration in the outer wells.

4.6.2.2 Antiserum of *Datura inoxia* and protein fractions of *Datura metel*

Precipitation arcs were obtained in the Fractions 3, 4, 9, 10, 11 and 14 both in immunodiffusion and in ELISA (Fig. 71 A, A1 & B).

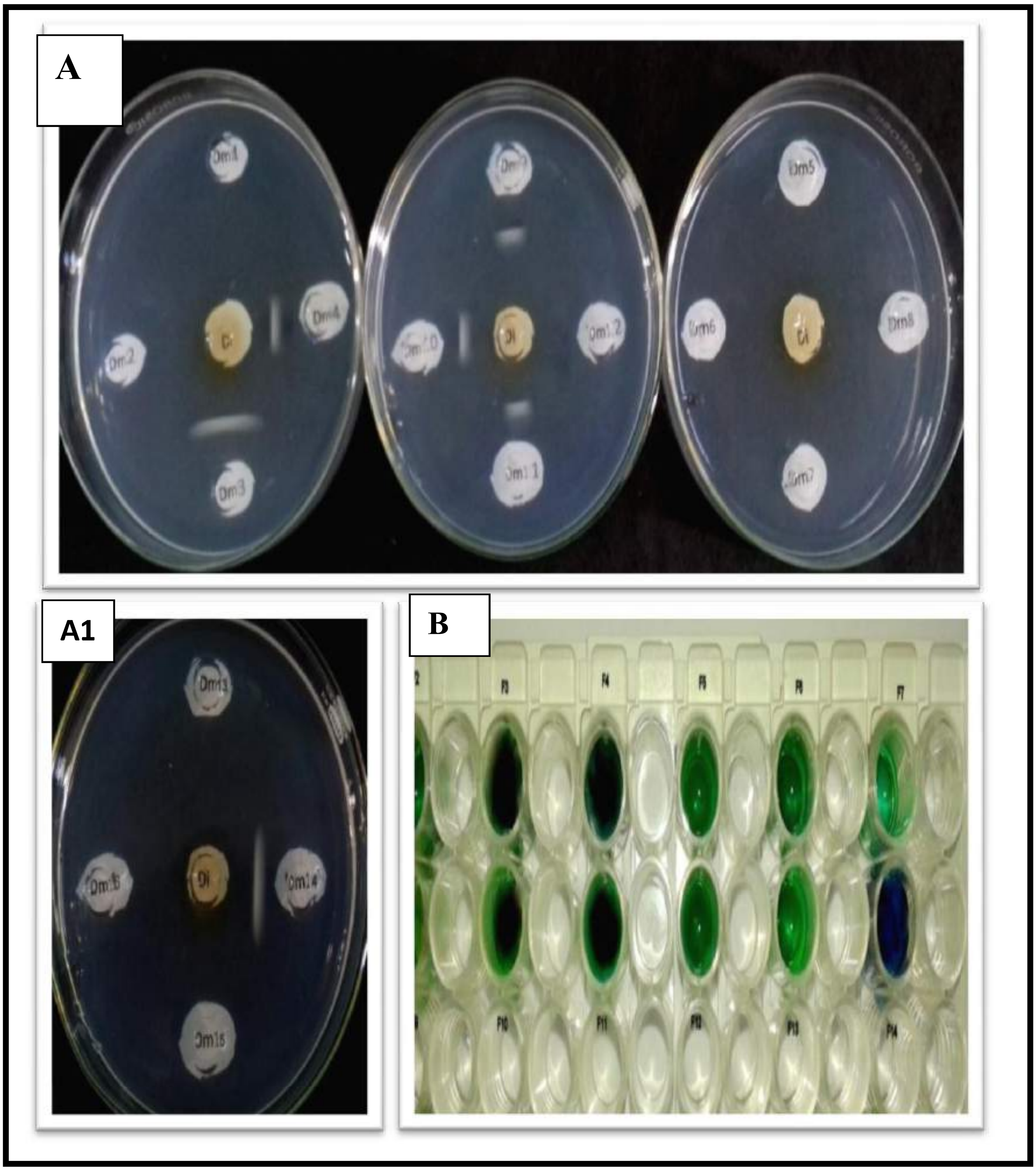


Fig 71: Serological precipitation reaction (A & A1– Immunodiffusion, B – ELISA) between antiserum against *Datura inoxia* (Di) pollen developed in white rat (each centre well containing 25µl of blood serum) and the individual protein fractions of the pollen of *Datura metel* (Dm) obtained by gel filtration in the outer wells.

4.6.2.3 Analysis of cross reactivity reaction between *Datura metel* and *Datura inoxia*

Thus the protein fractions and the corresponding proteins common to *Datura metel* and *Datura inoxia* which showed cross-reactivity reactions include 7 proteins of molecular weights 205.80 kDa, 184.63 kDa, 107.79 kDa, 97.2 kDa, 66.05 kDa, 60.06 kDa and 43 kDa of which 5 proteins have been found to be allergenic as mentioned earlier (Table 16).

Table 16: Proteins showing cross-reactivity between *Datura metel* and *Datura inoxia*

Datura metel			***Datura inoxia***		
Protein fraction from gel filtration	**Name of the proteins**	**M.W. of the proteins**	**Protein fraction from gel filtration**	**Name of the proteins**	**M.W. of the proteins**
3	**d3**	**205.80***	**1**	**D1**	**205.80***
4	**d4**	**184.63***	**2**	**D2**	**184.63***
10	**d8**	**107.79***	**3**	**D3**	**107.79***
11	**d9**	**97.2**	**4**	**D4**	**97.2**
9	**d10**	**66.05***	**9**	**D9**	**66.05***
	d11	**60.06***		**D10**	**60.06***
14	**d14**	**43.0**	**10**	**D11**	**43.0**

*** Allergenic proteins**

4.6.2.4 Antiserum of *Datura metel* and protein fractions of *Datura stramonium*

Precipitation arcs were obtained in the Fractions 1,3,4,7 and 9 in immunodiffusion and in case of ELISA (Fig 72 A, A1, B & B1).

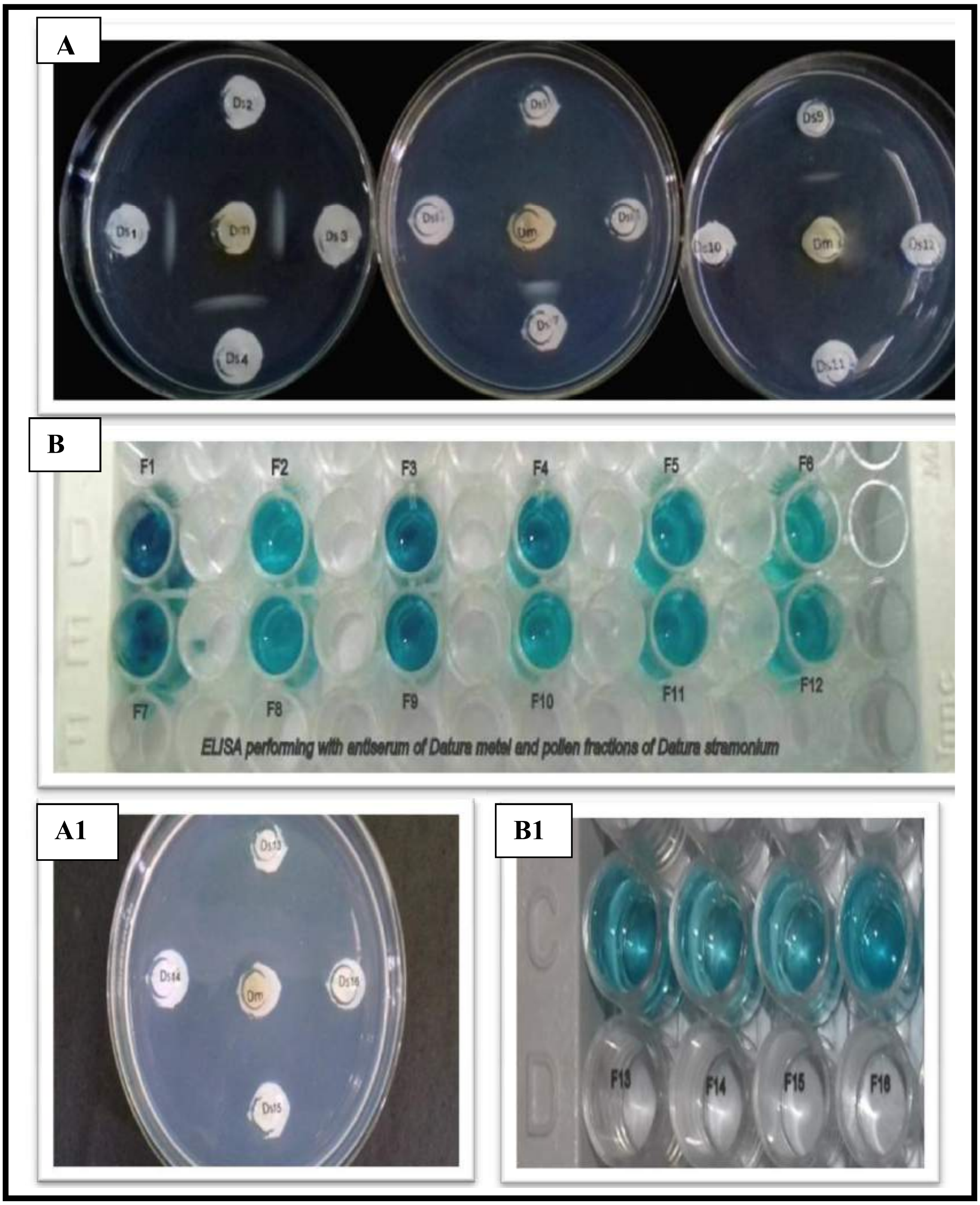


Fig 72: Serological precipitation reaction (A & A1 – Immunodiffusion, B & B1 – ELISA) between antiserum against *Datura metel* (Dm) pollen developed in white rat (each centre well containing 25µl of blood serum) and the individual protein fractions of the pollen of *Datura stramonium*(Ds) obtained by gel filtration in the outer wells.

4.6.2.5 Antiserum of *Datura stramonium* and protein fractions of *Datura metel*

Precipitation arcs were obtained in the Fractions 3, 9, 11, 14 and 18 both in immunodiffusion and in ELISA (Fig 73 A, A1, B & B1).

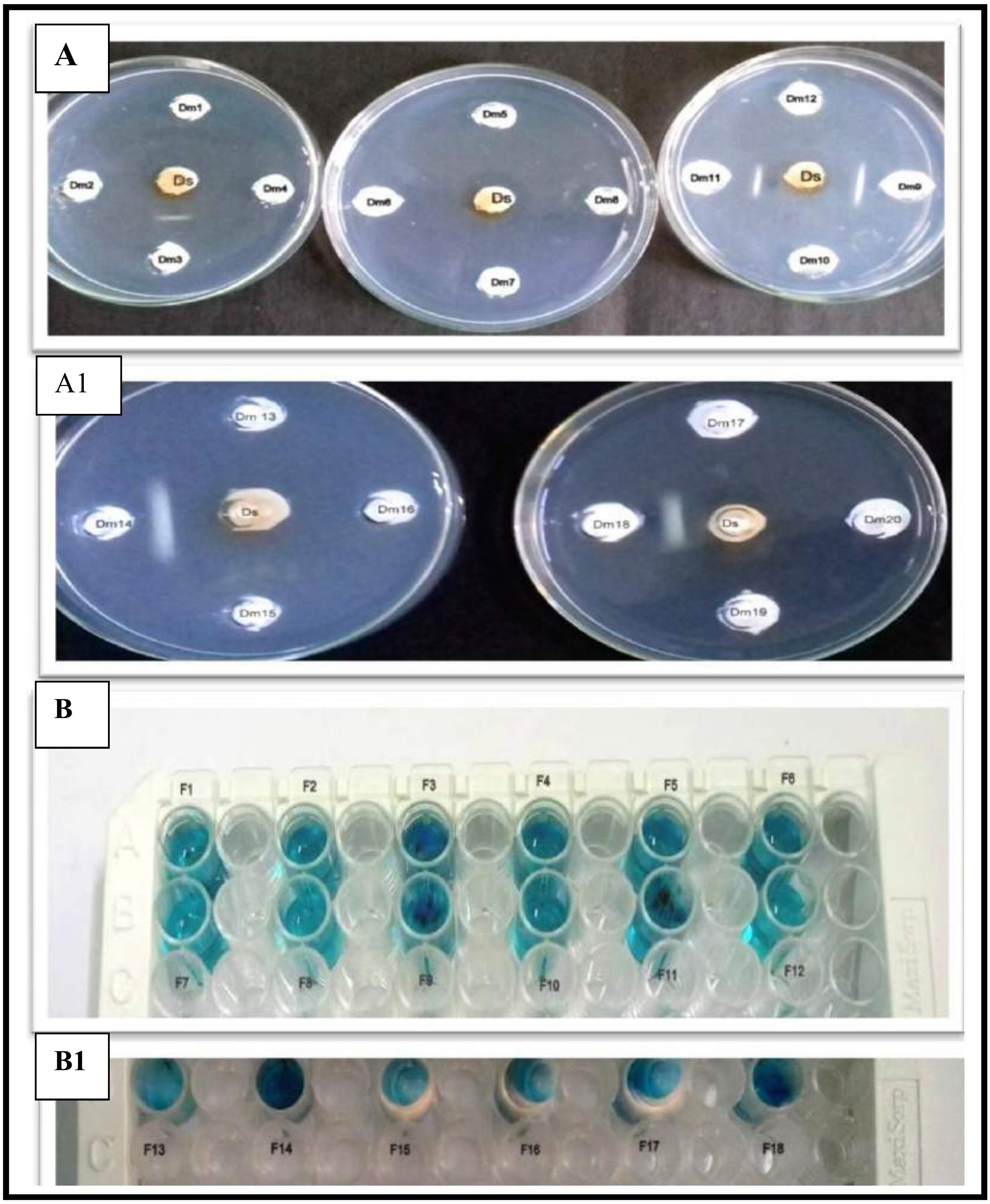


Fig 73: Serological precipitation reaction (A & A1 – Immunodiffusion, B & B1 – ELISA) between antiserum against *Datura stramonium* (Ds) pollen developed in white rat (each centre well containing 25µl of blood serum) and the individual protein fractions of the pollen of *Datura metel* (Dm) obtained by gel filtration in the outer wells.

4.6.2.6 Analysis of cross reactivity reaction between *Datura metel* and *Datura stramonium*

The protein fractions and the corresponding proteins common to *Datura metel* and *Datura stramonium* which showed cross-reactivity reactions include 6 proteins of molecular weights 205.80 kDa, 97.2 kDa, 66.05 kDa, 60.06 kDa, 43 kDa and 29.02 kDa of which 3 proteins have been earlier mentioned to be allergenic from the previous study (Table 17).

Table 17: Proteins showing cross-reactivity between *Datura metel* and *Datura stramonium*

Datura metel			***Datura stramonium***		
Protein fraction from gel filtration	**Name of the proteins**	**M.W. of the proteins**	**Protein fraction from gel filtration**	**Name of the proteins**	**M.W. of the proteins**
3	**d3**	**205.80***	**1**	**D'1**	**205.80***
11	**d9**	**97.2**	**4**	**D'3**	**97.2**
9	**d10**	**66.05***	**3**	**D'5**	**66.05***
	d11	**60.06***		**D'6**	**60.06***
14	**d14**	**43.0**	**7**	**D'8**	**43.0**
18	**d20**	**29.02**	**9**	**D'9**	**29.02**

*** Allergenic proteins**

4.6.2.7 Antiserum of *Datura stramonium* and protein fractions of *Datura inoxia*

Precipitation arcs were obtained in the Fractions 1,4,6,9,10 and 14 both in immunodiffusion and in ELISA (Fig 74 A, A1 & B).

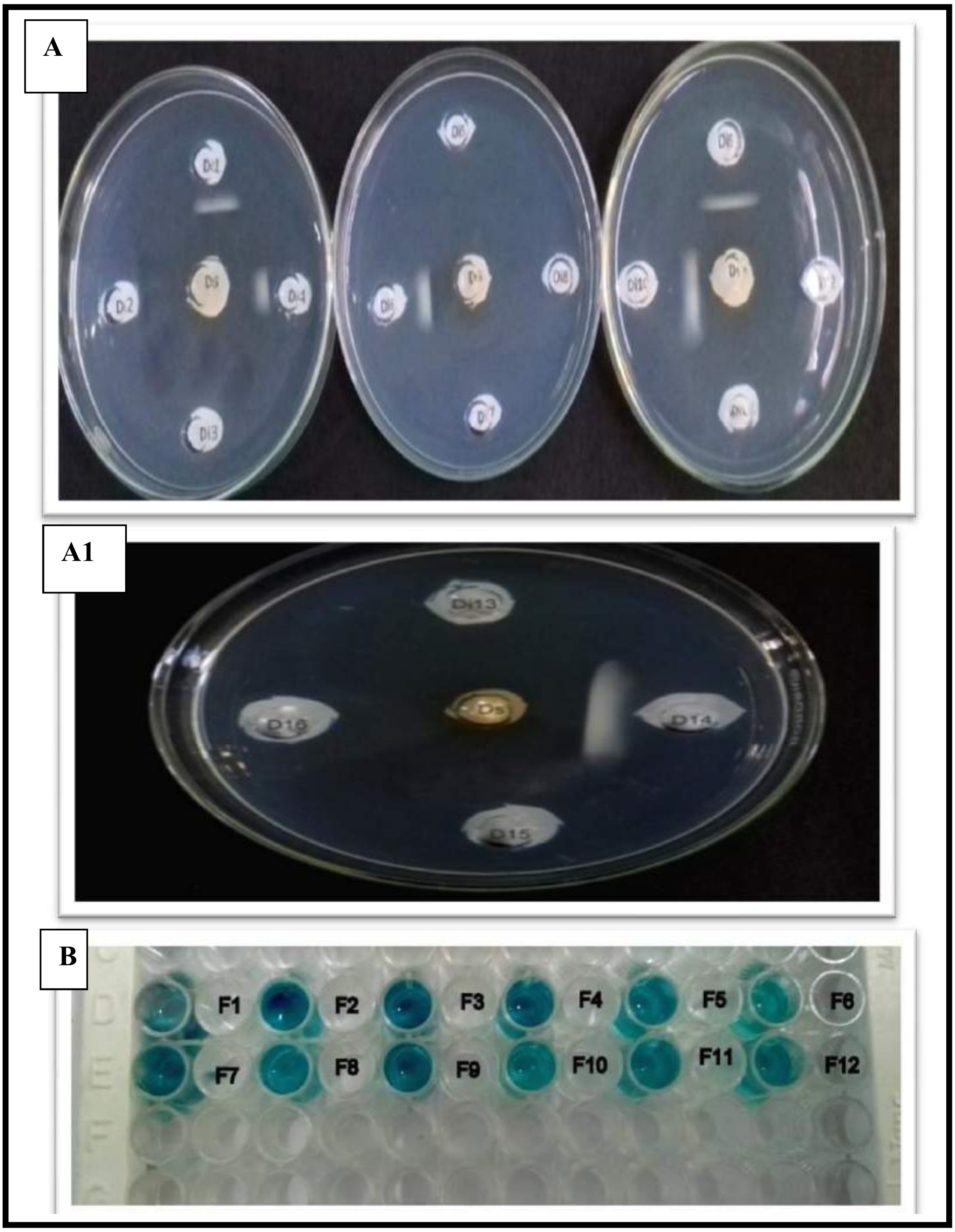


Fig 74: Serological precipitation reaction (A&A1– Immunodiffusion, B – ELISA) between antiserum against *Datura stramonium* (Ds) pollen developed in white rat (each centre well containing 25µl of blood serum) and the individual protein fractions of the pollen of *Datura inoxia* (Di) obtained by gel filtration in the outer wells.

4.6.2.8 Antiserum of *Datura inoxia* and protein fractions of *Datura stramonium*

Precipitation arcs were obtained in the Fractions 1,3,5,7 and 10 in immunodiffusion and Fractions 1,3,4,5,7 and 10 in ELISA (Fig. 75: A, A1 & B).

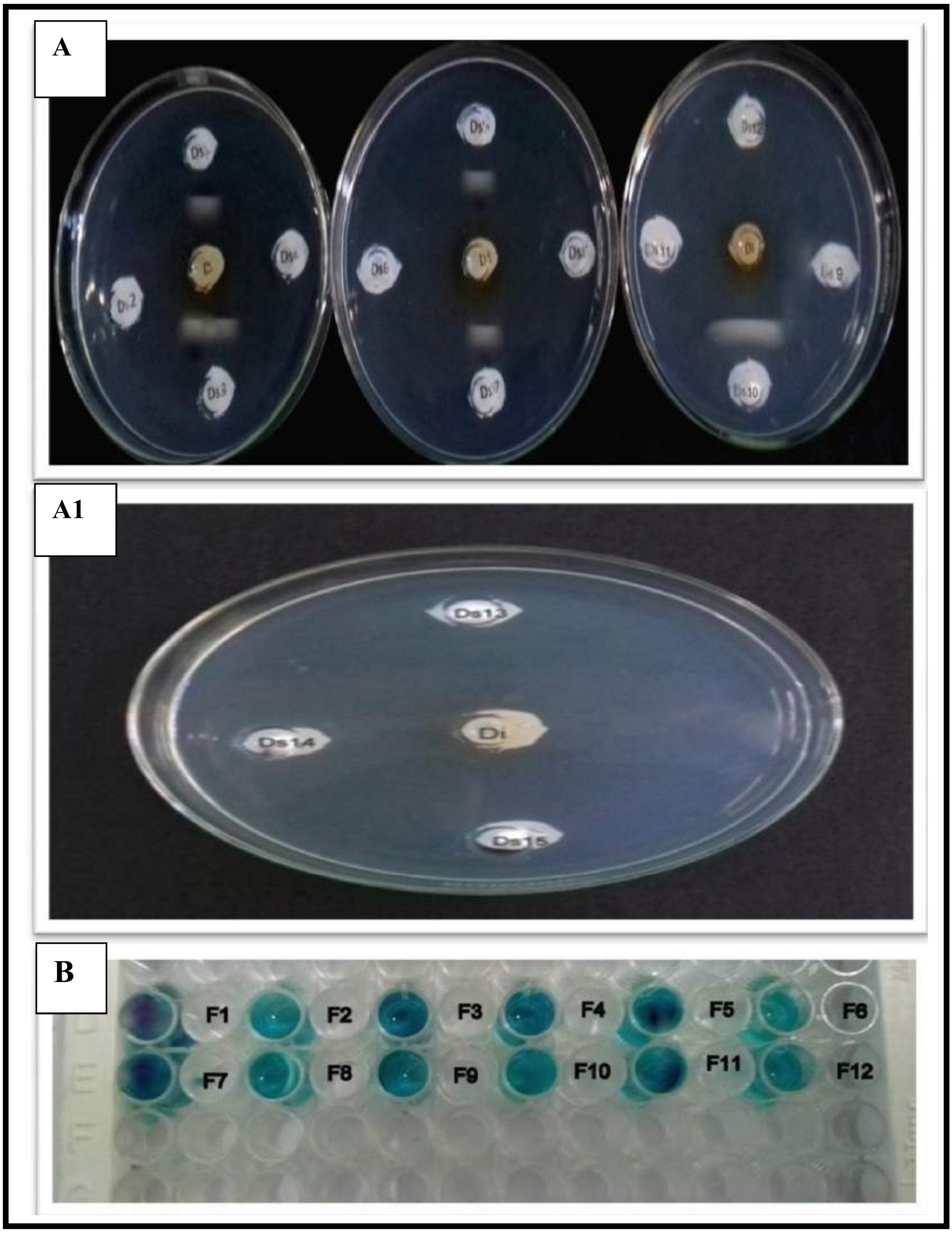


Fig 75: Serological precipitation reaction (A&A1 – Immunodiffusion, B – ELISA) between antiserum against *Datura inoxia* (Di) pollen developed in white rat (each centre well containing 25µl of blood serum) and the individual protein fractions of the pollen of *Datura stramonium* (Ds) obtained by gel filtration in the outer wells.

4.6.2.9 Analysis of Cross reactivity reaction between *Datura stramonium* and *Datura inoxia*

Thus the protein fractions and the corresponding proteins common to *Datura inoxia* and *Datura stramonium* which showed cross-reactivity reactions include 7 proteins of molecular weights 205.80 kDa, 97.2 kDa, 87 kDa, 66.05 kDa, 60.06 kDa, 43 kDa and 21.61 kDa of which 4 proteins have been found to be allergenic as mentioned earlier (Table 18).

Table 18: Proteins showing cross-reactivity between *Datura stramonium* and *Datura inoxia*

Datura stramonium			***Datura inoxia***		
Protein fraction from gel filtration	**Name of the proteins**	**M.W. of the proteins**	**Protein fraction from gel filtration**	**Name of the proteins**	**M.W. of the proteins**
1	**D'1**	**205.80***	**1**	**D1**	**205.80***
4	**D'3**	**97.2**	**4**	**D4**	**97.2**
5	**D'4**	**87.0**	**6**	**D6**	**87.0**
3	**D'5**	**66.05***	**9**	**D9**	**66.05***
	D'6	**60.06***		**D10**	**60.06***
7	**D'8**	**43.0**	**10**	**D11**	**43.0**
10	**D'10**	**21.61***	**14**	**D15**	**21.61***

*** Allergenic proteins**

A comparative study of the protein profile of the pollen of *D. metel, D. inoxia* and *D. stramonium* and degree of allergenicity

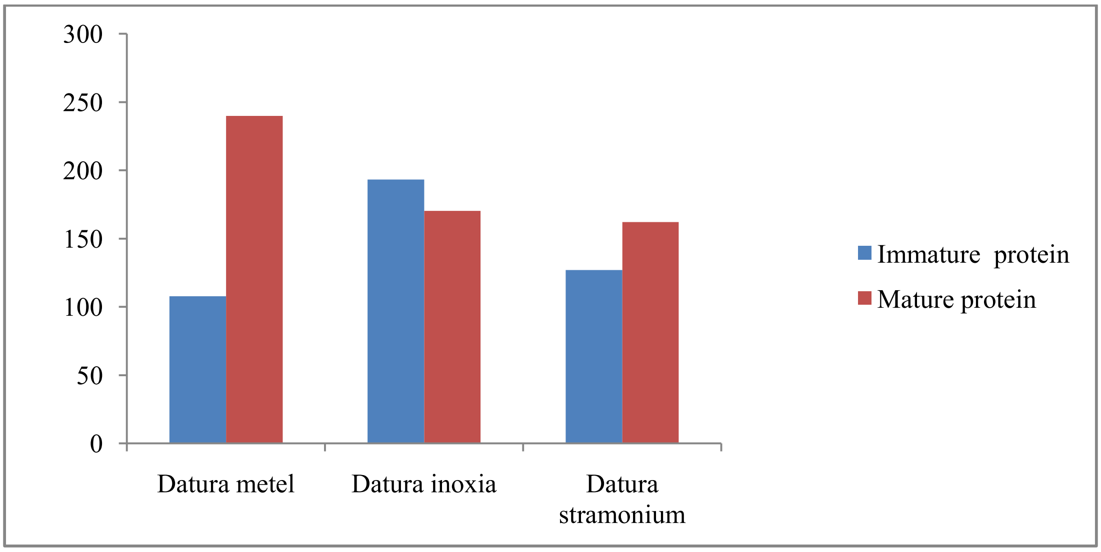
4.7 A comparative study of the protein profile of the pollen of *D. metel, D. inoxia* and *D. stramonium* along with the degree of allergenicity of the pollen proteins.

4.7.1 Study of pollen protein concentration of the three species of *Datura*

A variation in protein concentrations was found in case of mature and immature pollen among all three species. *Datura metel* showed the least variation between the concentration of the pollen proteins of the mature and immature ones. Mature pollen exhibited the concentration of proteins of 260 ug/ml whereas the immature one showed 240 ug/ml of concentration. *Datura stramonium* followed the trend of *Datura metel* and showed the least concentration of protein i.e. 127 ug/ml in case of immature pollen than the mature one which was found to be 162 ug/ml. On the other hand *Datura inoxia* pollen showed a higher concentration of protein i.e. 193.14 ug/ml in immature pollen where as mature pollen showed the concentration of 170.28 ug/ml **(Table 19, Graph 10).**

Table 19: Comparative Protein concentration of the pollen of *Datura metel*, *Datura inoxia* and *Datura stramonium*

Pollen Proteins	Average protein concentration in mature pollen (µg/ml)	Average protein concentration immature pollen (µg/ml)
Datura metel	260	240
Datura inoxia	170.28	193.14
Datura stramonium	162	127



Graph 10: Graphical representation of the protein concentration by mature and immature pollen of *Datura metel, Datura inoxia* and *Datura stramonium* (X axis – 3 species of *Datura* and Y axis - the concentrations of proteins in µg/ml).

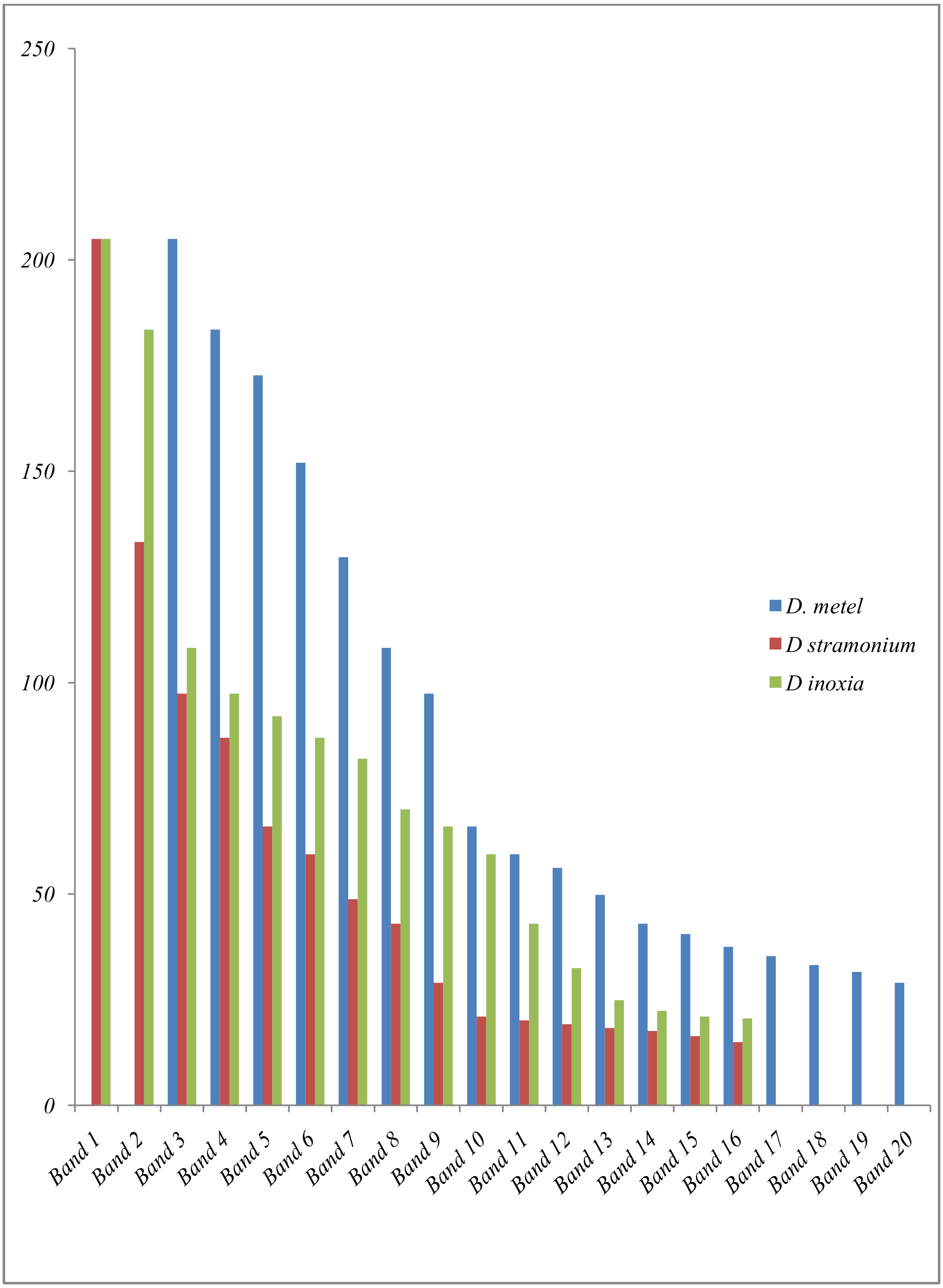
4.7.2 A comparative account of the SDS-PAGE protein profile of the pollen of *D. metel, D. stramonium* and *D. inoxia*

A comparative account of the SDS-PAGE protein profile of the pollen of the three species shows that *Datura metel* exhibited the maximum number of bands (20) and the remaining two species has 16 bands each (Table 20). The molecular weights of the proteins ranged between 205 kDa and 15 kDa. *Datura metel* however exhibited two protein bands above the molecular weight of 205.80 kDa. *Datura stramonium* showed several low molecular weight proteins below 21.61 kDa which were not observed in the other two species. There was also some degree of homology in the protein banding pattern between the three species. Five protein bands of 205.80 kDa, 97.2 kDa, 66.05 kDa, 60.06 kDa and 43 kDa were observed in all the three species. Two protein bands of 184.63 and 107.79 were common in *Datura metel* and *Datura inoxia*. Two protein bands of 87 kDa and 21.61 kDa were common in *Datura stramonium* and *Datura inoxia* while a 29.02 kDa band was common between *Datura metel* and *Datura stramonium* **(Table 10, Graph 11)**.

Table 20: Comparative SDS-PAGE protein profile of the pollen of the 3 species of *Datura*

Proteins bands from the pollen of *Datura metel*		Proteins bands from the pollen of *Datura stramonium*		Proteins bands from the pollen of *Datura inoxia*	
Protein band	**M.W in (kDa)**	**Protein band**	**M.W in (kDa)**	**Protein band**	**M.W in (kDa)**
d 1	>205	-	-	-	-
d 2	>205 *	-	-	-	-
d 3	**205.0 ***	**D'1**	**205.0 ***	**D1**	**205.0 ***
d 4	183.5	-	-	D2	183.5 *
d 5	172.7 *	-	-	-	-
d 6	152.0	-	-	-	-
-	-	D'2	133.3 *	-	-
d 7	129.7	-	-	-	-
-	-	-	-	-	-
d 8	108.2 *	-	-	D3	108.2 *
d 9	97.4	D'3	97.4	D4	97.4
-	-	-	-	D5	92.1
-	-	D'4	87.0	D6	87.0
-	-	-	-	D7	82.0 *
-	-	-	-	D8	70.0 *
d 10	66.0 *	D'5	66.0 *	D9	66.0 *
d 11	59.4*	D'6	59.4 *	D10	59.4 *
d 12	56.2	-	-	-	-
d 13	49.8	-	-	-	-
-	-	D'7	48.8	-	-
d 14	43.0	D'8	43.0	D11	43.0
d 15	40.5	-	-	-	-
d 16	37.5	-	-	-	-
d 17	35.3	-	-	-	-
d 18	33.2	-	-	-	-
-	-	-	-	D12	32.5
d 19	31.6	-	-	--	-
d 20	29.0 *	D'9	29.0 *	-	-
-	-	-	-	D13	24.9
-	--	-	-	D14	22.4
-	-	D'10	21.0 *	D15	21.0
-	-	-	-	D16	20.6
-	-	D'11	20.1	-	-
-	-	D'12	19.2	-	-
-	-	D'13	18.3	-	-
-	-	D'14	17.6 *	-	-
-	-	D'15	16.4 *	-	-
-	-	D'16	15.0	-	-

(red)	Bands common to all three species of *Datura*
(green)	Bands common to *Datura metel* and *Datura inoxia*
(light blue)	Bands common to *Datura metel* and *Datura stramonium*
(dark blue)	Bands common to *Datura stramonium* and *Datura inoxia*
*	Allergenic protein bands



Graph 11: Graphical representation of the SDS-PAGE protein profile of the pollen of *Daura metel, Datura stramonium* and *Datura inoxia* (X axis - protein bands and Y axis - molecular weight of proteins in kDa)

4.7.3 Comparative study of cross reactivity showing common allergenic proteins among the pollen of three species of *Datura*

From the study of cross reactivity reactions between *Datura metel* and *Datura inoxia,* precipitation arcs were obtained in case of 7 proteins. ELISA showed the same result. The proteins were 205.80 kDa, 184.63 kDa, 107.79 kDa, 97.2 kDa, 66.05 kDa, 60.06 kDa and 43.0 kDa. Among them 5 proteins, the molecular weights of those were 205.80 kDa, 184.63 kDa, 107.79 kDa, 66.05 kDa and 60.06 kDa have been proved to be allergenic.

ELISA and Immunodiffusion study showed the presence of 7 common proteins among *Datura metel* and *Datura stramonium*. The proteins were 205.80 kDa, 97.2 kDa, 66.05 kDa, 60.06 kDa, 43.0 kDa and 29.02 kDa respectively. Among them 205.80 kDa, 66.05 kDa and 60.06 kDa proved to be common allergen.

In case of *Datura stramonium* and *Datura inoxia,* a total of 7 common proteins were obtained by ELISA and Immunodiffusion. 205.80 kDa, 97.2 kDa, 87.0 kDa, 66.05 kDa, 60.06 kDa, 43.0 kDa and 21.61 kDa were the proteins which showed cross reactivity reactions among which 4 proteins 205.80kDa, 66.05 kDa, 60.06 kDa and 21.61 kDa were proved to be allergenic.

From the comparative study of cross reactivity, three common allergens were revealed among the three species of *Datura* with molecular weights 205.80 kDa, 66.05 kDa and 60.06 kDa **(Table 21).**

Table 21: Comparative study of cross reactivity showing common allergenic proteins among the pollen of three species of *Datura*

Serial no.	*D.metel & D. inoxia*	*D. metel & D. stramonium*	*D. stramonium* and *D. inoxia*
1.	205.80 kDa*	205.80 kDa*	205.80 kDa*
2.	184.63 kDa*	97.2 kDa	97.2 kDa
3.	107.79 kDa*	66.05 kDa*	87.0 kDa
4.	97.2 kDa	60.06 kDa*	66.05 kDa*
5.	66.05 kDa*	43.0 kDa	60.06 kDa*
6.	60.06 kDa*	29.02 kDa	43.0 kDa
7.	43.0 kDa		21.61 kDa*

(* allergenic proteins)

Epitope mapping

4.8 Epitope mapping

The allergic proteins were evaluated by MALDI. The protein sequencing of twelve allergic proteins were carried out out of thirteen proteins. The thirteenth allergenic protein faractions having a molecular weight greater than 205.80 kDa could not be sequenced.

4.8.1 Allergenic Protein ID

S.No.	[Protein] Protein ID# 0	[Protein] Protein ID# 1	[Protein] Protein ID# 2	[Protein] Source Database
1	A0A1U8HMX4	A0A1U8HMX4	A0A1U8HMX4_CAPAN	UniProt_Solanoideae
2	M1E148	M1E148	M1E148_SOLTU	UniProt_Solanoideae
3	K4B8Q6	K4B8Q6	K4B8Q6_SOLLC	UniProt_Solanoideae
4	A0A1U8GPA8	A0A1U8GPA8	A0A1U8GPA8_CAPAN	UniProt_Solanoideae
5	M1CSI7	M1CSI7	M1CSI7_SOLTU	UniProt_Solanoideae
6	G9IHH6	G9IHH6	G9IHH6_SOLTU	UniProt_Solanoideae
7	K4D473	K4D473	K4D473_SOLLC	UniProt_Solanoideae
8	A0A1U8GY45	A0A1U8GY45	A0A1U8GY45_CAPAN	UniProt_Solanoideae
9	A0A1U8F5H5	A0A1U8F5H5	A0A1U8F5H5_CAPAN	UniProt_Solanoideae
10	K4AVT9	K4AVT9	K4AVT9_SOLLC	UniProt_Solanoideae
11	K4CLY8	K4CLY8	K4CLY8_SOLLC	UniProt_Solanoideae
12	K4D3R0	K4D3R0	K4D3R0_SOLLC	UniProt_Solanoideae

4.8.2 The length, PI value, Molecular Weight. No of peptides and Coverage of the allergenic pollen proteins

S. No.	[Protein] Protein Length	[Protein] Protein pI	[Protein] Protein MW	[Protein] Total Peptides	[Protein] Protein Coverage (%)
1	**149**	**4.6093**	**166.0520.8172**	**2**	**6.0403**
2	**173**	**4.4985**	**19344.5403**	**2**	**18.4972**
3	**191**	**9.6204**	**21.61609.7958**	**1**	**4.1885**
4	**264**	**4.6726**	**29.02021.61.395**	**5**	**5.3031**
5	**542**	**5.21.6126**	**60058.9619**	**3**	**7.5646**
6	**581**	**7.1815**	**66.05048.8059**	**1**	**2.4097**
7	**644**	**5.0712**	**70.78779.21.6105**	**2**	**4.5032**
8	**715**	**6.1779**	**829.0207.305**	**1**	**1.3987**
9	**920**	**6.5157**	**97185.8868**	**1**	**1.7392**
10	**957**	**8.9153**	**107785.1373**	**1**	**1.6719**
11	**1453**	**7.2601**	**184625.7826**	**1**	**0.5506**
12	**2043**	**6.0564**	**205.80802.0445**	**3**	**2.1048**

4.8.3 Description of the allergenic proteins

6	[Protein] Protein Accession Number	[Protein] Protein Description
1	tr|A0A1U8HMX4|A0A1U8HMX4_CAPAN	Translationally-controlled tumor protein homolog OS=Capsicum annuum GN=LOC107879347 PE=3 SV=1
2	tr|M1E148|M1E148_SOLTU	Uncharacterized protein OS=Solanumtuberosum PE=4 SV=1
3	tr|K4B8Q6|K4B8Q6_SOLLC	Uncharacterized protein OS=Solanumlycopersicum GN=101254656 PE=4 SV=1
4	tr|A0A1U8GPA8|A0A1U8GPA8_CAPAN	Uncharacterized protein LOC107868408 OS=Capsicum annuum GN=LOC107868408 PE=4 SV=1
5	tr|M1CSI7|M1CSI7_SOLTU	Uncharacterized protein OS=Solanumtuberosum GN=102584601 PE=3 SV=1
6	tr|G9IHH6|G9IHH6_SOLTU	Apoplasticinvertase OS=Solanumtuberosum GN=InvGF PE=2 SV=1
7	tr|K4D473|K4D473_SOLLC	Uncharacterized protein OS=Solanumlycopersicum GN=101247772 PE=3 SV=1
8	tr|A0A1U8GY45|A0A1U8GY45_CAPAN	Cullin-1-like OS=Capsicum annuum GN=LOC107873724 PE=3 SV=1
9	tr|A0A1U8F5H5|A0A1U8F5H5_CAPAN	Heat shock cognate 70.78 kDa protein 2-like OS=Capsicum annuum GN=LOC107854185 PE=3 SV=1
10	tr|K4AVT9|K4AVT9_SOLLC	Uncharacterized protein OS=Solanumlycopersicum PE=4 SV=1
11	tr|K4CLY8|K4CLY8_SOLLC	Uncharacterized protein OS=Solanumlycopersicum GN=101247843 PE=3 SV=1
12	tr|K4D3R0|K4D3R0_SOLLC	Uncharacterized protein OS=Solanumlycopersicum GN=101261865 PE=4 SV=1

4.8.4 Protein sequence of the allergenic proteins

16.6 kDa

YKELENGVLWEVQGKWVVQGAVDVDIGANPSAEGGDDDEGVDDQA
VKVVDIVDTFRLQEQPSFDKKGFVGYIKKYIKNLTPKLEGEAQDLFKK
NIESATKFLMSKLKDLQFFLGESMHDDGALVFAYYKDGATDPTFLYIA
PGLKEVKC

19.3kDa

MRRYLKSSCILNVSAELRNPGQRFAWGQQWSPSAGPAQGVGSGRDSF
THSCITTKELGTVMRSLGQNPTEAELQDMINEVDADGNGTIDFPEFLNL
MAQKMKDTDSEEELKEAFRVFDKDQNGFISAAELRHVITNLGEKLTDE
EVDEMIREADVDGDGQINYDEFVKVMMAK

21.61kDa

MRTIAARFCPYLWRRTPINSQPRRFSTSSFGRDEQTIEQEAERKVGWLLK
LIFAGTATVIGYQIFPYMGDNLMQQSVSLLQVKDPLFKRMGASRLARFAI
DDERRMKIVDIGGAQHLLNMLESARDDRTRKEALKALFAISKSDAAAVV
LHQAGAMSIIKSTQESGEDAEVGNYKSNLLSRFQDLSFDIRS

29.02 kDa

MDVEFGEWEMLQSNQASESESLTPAGSAESTAGLDEIGGVIQPNYFGID
SQIRHMNGSEVEEDSEESDNPSWIDPTSENIKTPGAGEFWLDSGSEPEA
KSEFDGSFEEEITTETVEESESNPITSAGQNEGILQGNEEKIQVVEQRSND
AGGGGGGYQKRRSIVWWKVPIQLVKYCAFRVSTPVWTISVAAAVMAF
LIVGRRLYKMKKKAKAALQLKVTVDDKNISQFTSRAARLNEAFSIVKR
VPVIRPQLPTAGVTLWPVMR

60.1 kDa

MAKSEGKAIGIDLGTTYSCVGVWQNDRVEIIPNDQGNRTTPSYVA
FTDTERLIAYFNDSQRQATKDAGAIAGLNVMRIINEPTAAAIAYGL
DKKASKNGEKNVLIFDLGGGTFDVSLLTIEEGIFEVKATAGDTHLG
GEDFDNRLVSHFVQEFKRKHKKDITSNARALRRLRTACERAKRTL
SSTSQTTIEVDSLYEGIDFYATITRARFEELNMDLFRKCMEPVEKCL
RDAKMDKSQVHDVVLVGGSTRIPKVQQLLQDFFNGKELCKSINPD
EAVAYGAAVQAAILSGEGDQKVQDLLLLDVTPLSLGIETAGGVMT
VLIPRNTTIPTKKEQIFSTYSDNQPAVLIQVYEGERSLTKDNNLLGK
FELKGIPPAPRGVPQINVCFDIDANGILNVSAEDKTARVKNKITITN
DKGRLSKEEIERMVEEAERYKSEDEAMKRKVEAKNALENYAYNM
RNTIKDEKISGKLDPSEKQKIEKAVDETIEWLDRNQLAEVDEFEDK
LKELEKLCNPIIGKMYQGGAGSDYGTGNSGAGPKIEEVD

66.05 kDa

MDYSSNSRWALPVILVCFFIVLLSNNVVFASHKVFIHLQSQNAVNV
QTVHRTGYHFQPEKHWINDPNAPMYFNGIYHLFYQYNPNGSVWG
NIVWAHSVSKDLINWINLEPAIYPSKPFDQFGTWSGSATILPGNKPV
ILYTGIVDANQTQVQNYAIPANLSDPYLREWIKPDNNPLIVADDSIN
KTKFRDPTTAWMGKDGHWRIVMGSLRKHSRGLAIMYRSKDFMK
WVKAKHPLHSTNGTGNWECPDFFPVALKGTNGIDQYDEEYKYVL
KNSMDLTRFEYYTLGKYDTKKDRYVPDVGSIDSWKGLRFDYGNF
YASKTFYDTSKNRRVIWGWSNESDIFPEDDNAKGWAGIQLIPRKV
WLDASGKQLVQWPVEELETLRTQKVQLSNKKLNNGEKVEVTGIT
PAQADVEVTFSFASLDKAESFDSSWTDMYAQDVCGLKGADVQGG
LGPFGLATLATENLEENTPVFFRVFKAQQNYKVLLCSDAKRSTLKF
NETMYKVSFAGFVDVDLTDKKLSLRSLIDNSVIESFGAGGKTCITS
RVYPTLAINEKAHLFAFNNGTEPITIETLDAWSMGKAKIQY

70.78kDa

MAGKGEGPAIGIDLGTTYSCVGVWQHDRVEIIANDQGNRTTPSYVG
FTDSERLIGDAAKNQVAMNPINTVFDAKRLIGRRFSDASVQSDMKL
WPFKVIPGPGDKPMIVVNYKGEEKQFSAEEISSMVLIKMKEIAEAFLG
TTVKNAVVTVPAYFNDSQRQATKDAGVISGLNVMRIINEPTAAAIAY
GLDKKATSVGEKNVLIFDLGGGTFDVSLLTIEEGIFEVKATAGDTHL
GGEDFDNRMVNHFVQEFKRKNKKDITGNPRALRRLRTACERAKRTL
SSTAQTTIEIDSLYEGIDFYSTITRARFEELNMDLFRKCMEPVEKCLRD
AKMDKSTVHDVVLVGGSTRIPKVQQLLQDFFNGKELCKSINPDEAV
AYGAAVQAAILSGEGNEKVQDLLLLDVTPLSLGLETAGGVMTVLIPR
NTTIPTKKEQVFSTYSDNQPGVLIQVYEGERTRTRDNNLLGKFELSGI
PPAPRGVPQITVCFDIDANGILNVSAEDKTTGQKNKITITNDKGRLSK
EEIEKMVQEAEKYKSEDEEHKKKVEAKNALENYAYNMRNTIKDEKI
ASKLSADDRTKIEDAIEQAIQWLDGNQLAEAEEFEDKMKELESLCNP
IIAKMYQGAGGDMDDEGPAPSGGGAGPKIEEVD

82.9kDa

MEETEEKTIPLEEGMECVQKGINKLKIIIEGEPVTFTSDEYVMLYTTIY
NMCTQKAPHDYSQELYDKYTEAVEDYILTIVLPSLKKKHDEFLLKEL
EKRWQSHKLMVKWLLKFFHYLDKFFIKRAEVPALNEVSLSCFRDLV
YHEVKNRVTDAVIALIDQEREGEKIDRALLKTVINLYIEMGKGKMDY
YVNDFEEAMLRDSACHYSRKASTWIVEDTCPEYMLKADECLKKEKE
RVSHYLHANSETKLLEKVQNQVEDLTRMYSLFHKIPKGIELVAEIFK
QHIAAEGMVVVQQAADAAQNKTESSGSSPEQDFVKKAFEIHDKYM
VYVKGCFADNTIFHKALKEAFEVFCNKSVAGSSTTELLASYCDNTLK
KGGNEQLSDDAIEDTLDKVVKLVTYISDKDVFAEFYRKKLSRRLLFD
RSGNEEHERLILSKLKQQCGGHFTSKMEGMVTDLSLVKENQTHFQE
YISNNPAANPGIDMTVTVLTTGYWPSYKSCDLNLPVEMAKGVESFK
EFYQKKTKHWKLTWIFSLGQCNLNGKFEQKTIELILGIYQAAALLLF
NASDKWSYSDIKSELDLADEDLTRVLASVSCAKYKILNKERSGRTISS
TDTFEFNTQFTDKMRRIRVPLPPVDDRKKMVEEVGKDRRYAIDACL
VRIMKAKKVLTHQQLILECVEQLSKMFKPDVKAIKKRIEDLITRDYL
ERDLENTNTYKYIA

97.2 kDa

MAGKVEGPAIGIDLGTTYSCVAVWKYDRVEIIANDQGNRTTPSYV
GFTDCERLIGDAAKNQVSINPINTVFDAKRLIGRRFSDALVQSDIKH
WPFKVISGHGDKPMIVVNHKGEEKQFAAEEISSMVLVKMREIAEAF
LGSTVKNTVVTVPAYFNDSQRQATKDAGVIAGLNVLRIINEPTAAA
IAYGLDEKETSVGEKNVLIFDLGGGTFDVSLLTIDEGIFEVKATAGD
THLGGEDFDNRMVNHFVQEFKRKNNKDISGNPRSLRRLRTACERA
KRTLSSTAQTTIVIDYLYEDIDFNSTITHARFEELNMDLFRKCMEPV
EQCLRDAKMDKSAIHDVVLVGGSTRIPKVQQLLQDFFNGKELCKSI
NPDEAVAYGAAVQAAILSGEGNKKVKDLLLLDVTPLSLGLETYGG
VMTVLIPRNTTIPAKKEGVFSTCSDNQPDVLIKVYEGERTRTRDSNL
LGKFELSGIRPAPRGVPQITVCFDIDADGILNVAAEDKTTKQKNKITI
TNDNDRLSKEKIDKMVQEAEKYKSEDEAHKKKVDAKNALENCAY
NMRNNIKDERIASKLPEADKKKIEDAIEQVIQWLDANQLAESNELE
DKTKELENICNPIIAKMYEGAGGDIGADMDNDGPAPSSSSGAGPNIE
KEEKKGRRRRLRKQVFNKIKEGELHIGDHIYTWRYGYIYAHHGIYV
SRDIVIHFNPAARQEVETGTALDGIIFSSSTSRRSESPCPICGDRSRNS
GVISTCLECFLSGGKLYRFKYGVSKSVFYAQVRGTCTLATTDSSED
VIHRAKTLLGNNSFGNYKLFKNNCEDFAIYCKTGYNPHFGAGGRG
ASGQVAALSAAAKAPVVSTLHSSWTGYILLSAASAFGGPGPLPIGIT
FVGFPVLFAGMSYCFYRYLSDVGVRKDLKKIPVEELVDASV

107.8kDa

MYLLMESKVDLEQILKELHHRWLLPHEVCQILRNHQSFCLTQQLQ
LKPPAGSIFLYDRKLLPNFCKDGHHWRKNKDGQTIKEAHEKFKAG
SVDVLHCYYVHGEGNKNFQRRSYWMLEEQLEHIVLVHYRDVKEV
FPQQFNDMVVAGYRLGASRLQPVHPGLLLENPDSSSKPCFVFGPAF
QKSHTSNPSLVDLKEQALSSELHSGDSKGLVAFSRSKERFQLNPQV
RAFMSSGFRKFERNLNVMLQRKFYSGHYNLADLRSSKLTYAKLY
AGKAVANNRSRLAITSGKVFEENIHVAPPQIQNISSSQTVVTPDAAV
KTSSLDGGLNSDEVGSLKKLDILGKWMDREFAGGNKSLMSSDSGN
YWNTLDTDNGDKEVSTLSRHLLLEANSVGTSPSQKQLFRIFDFSPQ
WAFSGVETKVLIVGTFLVHRKYLTCLKWSCMFGEVEVSAEVQTQS
IRCQVPFHAPGHVPFYVTCGNRLACSEVREFEYREKSSELALALRP
SDEVHLQVQLVKLLYSGLNKKFLDCSSRECENCKLKTQLCSLKCQ
TGNATERLEDLLAVIECDHINFKDVQIQNFMKDKLYEWLVSRAHE
EDKGPNILNDQGKGVIHLVAALGYEWGLLPLIAAGISPNFRDACGR
TALHWAAHYGREDMVIALIKLGVAAGAVDDPTTASPGGRTAADL
ASSRGYKGIAGYLAESDLTSHHQLLATSKNALDTIGAGLEAEKVYE
SAVQEIVPLNGTIDDDVSLKASLASLRKSAHAAALIQAAFRARSFR
QRQLRESRNDVSEASLDLVALGSLNKVQKVNCFEDYLHSAAINIQ
QKYCGWKGRREFLKVHNQIVKMQALVRGHEVRKQYKKFVWAVS
ILEKGILRWRRKKTGLRGFWPEKTSETGIVEREKEEEYDYLSIGLKQ
KCAGVEKALGRVESMVRHPEARDQYMRMVAKFKSCKLDDGGRE
VNRSSPPV

184.6kDa

MEGGGDILKVSSARLGSSTVWRNSGVDVFSRSSREDYDDEEALKWA
ALEKLPTYLRIRRGILSEEEGQYREVDITKLDLVERRNLLERLVKIADE
DNEKFLLKLKKRIDRVGLDLPTIEVRFEHLNVDAEARVGSRALPTIFN
FTVNIIEDFLNYLHILPSRKKPLPILHEISGIIKPGRMTLLLGPPSSGKTTL
LLGLAGKLDKDLKVSGRVTYNGHGMDEFVPQRTSAYISQNDLHIGE
MTVRETLAFSARCQGVGAKYEILAELSRREKEANIKPDPDVDIFMKS
AWNDGQEANVVTDYTLKILGLEICADTIVGDEMIRGISGGQRKRLTT
GEMMVGPARALFMDEISTGLDSSTTYQIVNSIRQSIHILQGTAVISLLQ
PAPETYDLFDDIILLSDGQIVYQGPRENVLEFFEYIGFKCPQRKGVADF
LQEVTSRKDQEQYWARRDEPYKFITVREFSEAFQSFHVGRKLGDELA
VPFDKSKSHPAALTTERYGVSKKELLKACTAREYLLMKRNSFVYIFK
MIQLTLMATITMTLFLRTEMHRDTMIDGAVFLGALYYAVIMIMFNGF
SELALSIMKLPSFYKHRDLLFFPAWTYALPTWILKIPITLVEVAIWVCM
TYYVIGFEADVGRFFKQLFLLICLNQMASGLFRFLAALGRNVIVANTF
GSCALLIVLVMGGFILSRDNVKQWLIWGYWISPMMYAQNAIAVNEF
LGKSWAHVPPNSTGTDTLGVSFLKSRGIFPEARWYWIGVGALLGYVL
LFNFLFTVALAYLNPFGKPQAVLSEETVAERNASKRGEVIELSPIGKSS
SERGNDVRRSASSRSMSSRVGNIAEGDLNKRKGMILPFEPLSITFDDIR
YAVDMPQEMKAQGFTEDRLELLKGVSGAFRPGVLTALMGVSGAGK
TTLMDVLAGRKTGGYIEGTISISGYPKQQATFARIAGYCEQTDIHSPH
VTVYESLQYSAWLRLPREVDTETRKRFIEEVMELVELKPLREALVGL
PGVNGLSTEQRKRLTVAVELVANPSIIFMDEPTSGLDARAAAIVMRTV
RNTVDTGRTVVCTIHQPSIDIFDAFDELLLLKRGGEEIFVGPLGRHSSH
LIKYFEGIDGVLKIKDGYNPATWMLDITSVAQEAALGIDFTELYRNSE
LYRRNKALIQELSVPAPGSKDLYFETKYSQSFFTQSMACFWKQHWSY
WRNPPYTAVRLMFTFFIALMFGTIFWDLGSKRRRQQDILNAIGSMYA
AVLFLGVQNATSVQPVVAIERTVFYRERAAGMYSALPYAFGQIMIEL
PYIFIQTIIYGVIVYAMIGFEWTVAKFIWYLFFMYFTLLYFTLYGMMT
VAVTPNHSIAAIISSAFYAVWNLFSGFIVPKTRMPVWWRWYFYICPIS
WTLYGLVASQFGDLQDKLETKETVEEFIESFFDFKYDFVGYVALILVG
ISVGFLFIFAYSIKAFNFQKR

205.80 kDa

MDTGSSPESDTLTPMERILKRLDILGVPAEYLELLQPGLVAYVKNNKSQIAE
LVPALFPTNEEAVEIIAEQQIQSPRSMVSSSVNVKDLFQESMEWIQWLMFD
GEPSRALEQLEDTGQRGVCGAVWGNNDIAYRCRTCEHDPTCAICVPCFQN
GNHKDHDYSIIYTGGGCCDCGDVTAWKREGFCSKHKGAEQIQPLPEEFAN
SMGPVLDLLLSCWRKRFLFPDSISGRNPRKNDHSTELKMVTDELTSAVVK
MLLKFCKHSESLLSFISRRVSSSAGLLDILVRAERFMIIEENVKKIHELLLKLL
GEPQFKYEFAKVFLSYYPTVVNEATSECNDSVYNKYPLLSTFSVQIFTVPTL
TPRLVKEMNLLPMLLGCLGDIFASCAGEDGKLQVMKWSNLYETTLRVVED
IRFVMSHSVVPRYVTHERRDILRTWMKLLAFVQGANPQKRETGIHVEEENE
NMHLPFVLGHSIANIHSLLVSGAFSTSSTEDGADAFFNTHREDFEDQDSQRH
AKVGRLSQESSVCSMAGRSPLEHASRVLEVHYDSSPISSSVLCLTFECLRAI
ENWLIVDNTSGPLLHILCPKTSSTPGNNFSVLKKTLSKFRRGREMFKSQSPP
SNDVRLVTSAEGYNKQYSNPSLNGRTILDSGLGSGQEPACLGGHDDSMLE
GDNASELGELRLLSLSDWPDIVYKVSLQDISVHNPLQRLLSMVLQKALGKC
YGENAQPVASSAKLSSSVHYDFFGHILGVYHPQGFSAFIMEHALRIRVFCA
QVYAGMWRRNGDSAILSCEWYRSVRWSEQGLELDLFLLQCCAALAPADL
YISRILERFELSNYLSFNLERPSEYEPALVQEMLTLIIQILKERRFCGLTSSECL
QRELVYRLSIGDATHSQLVKSLPRDLSKIDKFQEVLDKIAIYSNPSGMNQG
MYKLRLPYWKELDLYHPRWNSRDLQVAEERYMRFCNASALTTQLPGWSK
IYPPLGRIAEVATCRTVLQIVRAVVSYAVFSDASNASCAPDGVLLRALHLLS
LALDICHAHRESGEHSCSNGDVIPILALACEEISVGKFGDQSLLSLLVLLMR
KHKKENYFVEAGMLNLLSLVESVLKKFAELQPECMKKLQDLAPDVVNQL
SRSFPAGDMNSFKSVSDSDKHKAKARERQAAMLEKMRVQQSKFLASIDSK
TDVAADDSKHGKDLCDSDGRPRSEEATPVICSLCRDPNSRSPVSYLILLQKS
RLLSCTNRGPPSWEQTRRPGKEPTSCAKHVPNISSERSNLSRSSEITSSSCLM
QLIQNKVNEFALEGQPKEVEAFLEYIKEKFPSMKNIQPSCASSTVKKKTSSS
FEMLEEHMYSLIWEEMDANSWNWDLLKNDRKLSALGDNGSAESLLLGRY
ISALSRECSPSASTNSRKAQLESSMLLPTYNGFGPSDCDGIYLSSCGHAVHQ
GCLDRYLSSLKERYTRQIVFEGGHIVDPDQGEFLCPVCRGLANSVLPALPAE
TKRSTPSLSTDPSDAVGLPTLRFQEVLFLLQSAADVAGSREILQSLPVQQFG
QMRVNLDYVVRILCEMYFPDKDKISESGRLSHSLILFDTLKYSLISTEIAARS
GNTSLAPNYSLGALYKELKSTNCFILALLLSIVQSTRSKDSLTVLLRLRGIQL
FVKSICSDISADEYPDSPIVGGNMQDILEFSETELQYPDIQFWKRCSDPVLAH
DAFSSLTWVLYCLPCQFLSCEKSFLCLVHLFYVVTITQIVITYSRKLQSSLSM
SGCSDSLVTDIYRIIAENGVAYKDFDSNHIETHDVKDAIRSLSFPYLRRCALL
WKLVRSSVSAPFSGGSNILDGLPYSMGETMECGGNIPVEFNEIEKLEKLFKI
PPLDDVISDETVRFVVPSWLRRFSKQFEARMLNGAMYSSPAVPFKLMLLPH
LYQDLLQRYIKQNCPDCGVVLEEPALCLLCGRLCSPNWKPCCRESGCQTH
AMACGAGTGVFLLIKKTTVLLQRSARQASWPSPYLDAFGEEDSGMNRGKP
LYLNEERYAALTHMVASHGLDRSPKVLHQTNIGNFFVL