Palynology Of Six Species Of *Solanum* (Solanaceae)

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Abstract: This paper presents the palynology of six species of genus *Solanum* (family: Solanaceae) from Saudi Arabia. The species has been investigated by Light microscopy, Scanning and Transmission electron microscopy. Pollen grains are generally radialy symmetrical, isopolar tricolporates zono-aperturates, prolate, subprolate or spheroidal with tectate and columellate exine. Sexine echinates or punctuates. On the basis of amb axis, three distinct pollen types are recognized, viz.; *Solanum* sp.aff. *anguivi* (16-24 µm) type-1, *S. gracileps* (20-26 µm), *S. incanum* (21-26 µm) and *S. villosum* (22-26 µm) type -2 and *S. nigrum* (20-32 µm) and *S. carense* (23-37 µm)) type-3. On the basis of pollen shape class, four distinct pollen types are recognized, viz.; type -1, *S. incanum* (prolate), type-2 *Solanum* sp.aff. *anguivi* (prolate-subprolate), type- 3, *Solanum carens*e (subprolate) and type -4, *S. villosum, S. nigrum* and *S. gracileps* (prolate- spheroidal). On the basis of exine sculptures, five distinct pollen types are recognized, viz.; type-1, echinate-verrucate exine in *S. villosum* and *S. nigrum*, type-2, regulate pislate in *S. incanum* but type-3, *S. gracileps* is crenate, type-4 *Solanum* sp.aff. *anguivi* is regulate scabrates and type-5, *Solanum carens*e is perforate echinates. From the results, the Solanaceae is considering of an europalynous family. The importance of exine sculpture in taxonomy is detailed underlined. The morphological and palynological results obtained above and other taxonomic differences can be used to enhance proper understanding of the species in particular and the genus *Solanum* in general.
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Keyword: Palynology, *Solanum*, LM, SEM, TEM, Saudi Arabia.

1. Introduction

The family Solanaceae is one of the most important families of flowering plants economically, floristically, ethnobotanically and scientifically (Olmstead *et al.,* 1992). *Solanum* is a genus of annuals, perennials, sub-shrubs, shrubs and climbers. It's often had attractive fruit and flowers. Most they are poisonous, but several bear edible fruit or other parts, such as the common foods tomato, potato and eggplant. Synonyms and common names: Nightshade, as well as referring to the related genus *Atropa*, is also used for many members of the genus *Solanum*. The name is also extended to the family Solanaceae. *Solanum, Physalis,* and *Lycium* are widely distributed across both the Eastern and Western Hemisphere (D"Arcy, 1991). The Solanaceae family is composed of 98 genera and about 2300 species belonging to 14 tribes grouped in three subfamilies (Wettstein, 1895; Hunziker, 1979 & D"Arcy 1991). Solanoideae eight tribes, 60 genera and about 1746 species. The subfamily Solanoideae has curved embryos contained in flattened discoid seed and the subfamily Cestoideae with straight or slightly bent embryos in prismatic- subglobose seeds, (Hunziker, 1979 and D"Arcy, 1991). According to Mabberly (1997) the family consists of 94 genera and 2950 species. While, Judd *et al.* (1999) report that the number of the genera in the Solanaceae sense lato (including Nolanaceae) is about 147 genera occurring world wide of which 7 genera and 33 species occur in Saudi Arabia (Migahid, 1978 & Collenette, 1985; 1998). Each of these studies was a certain source of taxonomic criteria. Pollen characters have received more attention in taxonomic and pollen morphology. However, the ultra-structure of the pollen wall, its stratification and internal structure can hardly be studied by light microscope (Zavada, 1990). Therefore Scanning and Transmission Electron microscopy become necessary in examining these characters, El- Ghazaly (1990) and Harky, *et al.,* (2000) reported on the morphology of pollen grains of many plant species. So far, no previous ultra-structural studies of pollen grains of family Solanaceae have been reported in Saudi Arabia except Al-Wadai (2000), he studied the pollen morphology and ultra-structures of *Withania sommnifera*, Al-Wadei (2002), studied the cytomorphology of three species of *Solanum* and Al-Wadei and Lashin (2007), they studied also the palynological and cytological characters of some taxa of *Solanum.* In the other Arabic regions El-Ghazaly (1993, 1999) studied some species of family Solanaceae. The taxonomic significance of pollen morphology in Solanaceae is more or less obscure. Sometimes, different tribes or subtribes have similar type of pollen or vice versa the genera referred to the same tribe or subtribe may have different type of pollen (Erdtman, 1952). However,

in the present work a palynological analysis of a combination of different parameter is believed to serve in a better understanding and evaluation of the relationships between the different taxa of the genus *Solanum.* For this aim the data derived from palynological analysis of the pollen grains by LM, SEM and TEM will serve for contribution to the palynology and taxonomy of the family Solanaceae.

2. Materials and Methods

The pollen from mature anthers was collected from Herbarium specimens deposited at the Department of Biological Sciences, Faculty of Sciences, King Khalid University, Abha, Saudi Arabia (SA.) (Table, I). The exine sculpture as well as shape and size of pollen grains were examined by high resolution Scanning and Transmission electron microscope (Jeol T. 100) pollen grains for each species were measured. The length of polar axis (P) and equatorial axis (E) and length ratio (P/E) were assigned in Table 2. The pollen specimens are investigated under Light microscopy and Scanning and Transmission electron microscope as follow:

Processing of pollen grains for Light microscope (LM): The materials are placed in glacial acetic acid for three minutes acetolysed according the method of Erdtman (1960) and Moor *et al.*, (1991) then mounted in glycerin gel for investigation and photographed by light microscopy using a CH2 Olympus microscope, in the Department of Biological Sciences, Faculty of Sciences King Khalid University, Abha, Saudi Arabia.

Processing of pollen grains for scanning electron microscopy (SEM): The dried materials are attached to stubs with double-faced selotape. The stubs are gold- coated in B sputter coater for one minute, and examined. The representative pollen grains are photographed at various magnifications in a Jeol T 100. SEM operated at an accelerating voltage of 15KV. The investigated taxa are illustrated in figures 1-6 and Table, 1- 2. Processing of pollen grains for Transmission electron microscopy (TEM): The materials are fixed for 3 h. in 2.5% glutaraldehyde with 0.05m Cacoldylate buffer at pH 7.4 for 24 h. and post fixed in 1% OsO4 (Osmum tetraoxids) in the same buffer for 2 h. (Cresti *et al.*, 1985). The pollen then dehydrated in graded series of ethanol and embedded in Spurr"s resin (Spurr, 1969) Ultra thin sections are cut using a Diamond Knife on Ultra-microtome, stained with Uranyl acetate followed by lead citrate (Reynolds, 1963). The stained grids are examined and Photographed with a Jeol-Tem 100 B TEM. in the Department of Pathology Faculty of Medicine King Khalid University, Abha, Saudi Arabia. The terminology used for pollen description follow, Faegri, *et al.*, 1989.

Table (1): Sites of collection of the examined species of *Solanum.* Identification of the studied species according to, Migahid (1978) and Collenette (1985-1998)

Herbarium No.	*Species*	Sites of collection
1	*Solanum incanum* L.	Sudd- Samallagi
2	*Solanum nigrum* L.	N.W.Jebel Sawdah
3	*Solanum villosum* (L.) Lam.	N.W.Jebel Sawdah
4	*Solanum carense* Dun. inDc.	Sudd- Samallagi
5	*Solanum* sp.aff. *anguivi* Lam.	Sudd- Samallagi
6	*Solanum gracileps* L.	Abha-Asier

Table (2): Pollen morphology: Dimensions, shape classes and shape (LM, SEM, TEM) among the species of the genus *Solanum*.

Species Characters	Dimensions (LM)			Shape Class		Pollen Class	Sexine sculptures
	Polar axis P (µm)	Equatorial axis E (µm)	P/E	(E view)	P view (Amb)		(IN SEM)
1- *Solanum incanum* L.	32(34)36	21(23)26	1.38-1.52	Prolate	angular	Trizonocolporate	Regulate-scabrate
2-*S. nigrum* L.	20(23)32	20(24)29	1.00-1.10	prolate-spheroidal	angular	Trizonocolporate	Echinate
3-*S. villosum* (L.) Mill.	22(24)26	20(22)25	1.04-1.10	prolate-spheroidal	circular	Trizonocolporate	Echinate
4-*S. carense Dun*. inDc.	23(28)37	20(25)30	1.15-1.23	Subprolate	Rectangular	Trizonocolporate	Perforate-echinate
5-*S.* sp.aff. *anguivi* Lam.	16(17)24	12(15)23	1.04-1.33	prolate-subprolate	circular	Trizonocolporate	Regulate-scabrate
6-*S. gracileps* L.	20(23)26	18(20)25	1.04-1.11	prolate-spheroidal	Semi-angular	Trizonocolporate	Spinulose

3. Results

Pollen grains are generally radialy symmetrical, isopolar tricolporates zono-aperturates, prolate, subprolate or spheroidal with tectate and columellate exine. Sexine echinates or punctuates.

Palynological Description

1- ***Solanum incanum*** L. (Fig. 1, A-I)

LM. (Figs. A-C); Pollen is prolate; P/E: 1.45 (1.38-1.52); pollen axis 27 µm (32(34)36 µm); tricolporate; exine 1.3 µm thick; columella regularly distributed, Pollen grain in different foci is angular rhomboidal with broad ends in equatorial view and angular in polar view. Sculpture is microreticulate and homobrochate.

SEM (Figs. D-F); colpus margins irregular with margo; Ora distinct, lalongate, 2.5 µm.wide; sexine with regulate -scabrate sculptures and faintly perforate; Colpi with tapering ends equatorially constricted.

TEM (Figs G-I); Apertures: pore structures fastigium-like cavity formed by ectoapertural margins. Exine: sexine echinate and thicker than nexine near apertures. columella irregularly distributed and exine tectate.

Comment: Is native to northwestern Africa and the Middle East . Sub-tropical N Africa and SW savanna and *S. incanum L.* sense stricto Asia semi-desert (A common plant in SA. and endemic). The pollen grains are distinguished by their comparatively long polar axis (32(34)36 µm) and rounded amb.

2- *Solanum nigrum* L. (Fig. 2, A-I)

Sy. :S. nodiflorum Jacq.

LM. (Figs. A-C); Pollen prolate-spheroidal; P/E: 1.05 (1.00-1.10); pollen axis 27 µm (20(23)32 µm); tricolporate, exine 1.4 µm thick. Pollen grain in different foci is angular with broad ends equatorial view and angular in polar view. Colpi with tapering ends equatorially constricted.

SEM (Figs. D-F); colpus margins irregular with indistinct margo; pore lolongate (4x5 µm); sexine is microreticulate; homobrochate and echinate.

TEM (Figs. G-I); Apertures: Ora lolongate, 4.5 µm wide, Exine: sexine thick as well as nexine and echinate; columella regularly distributed.

Comment: A common endemic plant in SA. The pollen grains are distinguished by their comparatively long polar axis (20(23)32 µm) and rounded wide pore. The distinct protrusion of the colpi margin in polar view fuse to form a bridge-like structure over the ora. Amb very small and mesocolpium very wide.

3- *Solanum villosum* (L.) Mill. (Fig.3, A-H)

LM. (Figs. A-C); Pollen prolate-spheroidal; P/E: 1.07 (1.04-1.10); pollen axis 27 µm (22(24)26µm); tricolporate; exine 1.3um thick; Pollen grain in different foci is circular with tapered ends. Equatorial view is rounded and circular in polar view. Colpi with tapering ends and equatorially constricted.

SEM. (Figs. D-F); colpus margins irregular with granular margo; pore lalongate (4x5 µm); microreticulate; homobrochate and baculate are visible. sexine thicker than nexine and micro-perforate; columella irregularly distributed.

TEM. (Figs. G-H); Apertures: nexine is thicker than sexine near aperture and Ora, 2.7 µm wide. Exine: sexine tectate, irregular columellate and pore distinct , 2.2 µm wide. Exine: 1.7um thick. sexine is thicker than nexine and verrucate sculptures.

Comment: A common in weed plants in SA. and endemic. The pollen grains are distinguished by their comparatively long polar axis (22(24)26 µm) and rounded ends and intine is thicker than exine –margo is granulate and very small apocolpium.

4- *Solanum carense* Dun. inDc. (Fig.4, A-H)

LM. (Figs. A-C); Pollen subprolate; P/E: 1.19 (1.15-1.23); pollen axis 27 µm (23(28)37µm); tricolporate; pore lalongate (4x5 µm); microreticulate; homobrochate. Pollen grain in different foci quadrangular with broad ends and equatorial view is rectangular in polar view. Colpi are with tapering ends.

SEM (Figs. D-F); exine 1.3 µm thick, perforate and columella regularly distributed. colpus margins regular with indistinct margo. microreticulate. Reticulation is heterobrochate.

TEM (Figs. G-H); Apertures: ora indistinct and exine echinates. sexine thicker than nexine.

Comment: A common plant in SA. and endemic. The pollen grains are distinguished by their comparatively long polar axis (23(28)37 µm), perforate tectum, straight colpus and pore indistinct by SEM.

5- *Solanum* sp.aff. *anguivi* Lam. (Fig. 5, A-G)

LM. (Figs. A-C); Pollen prolate-subprolate; P/E: 1.19 (1.04-1.33); pollen axis 27 µm (16(17) 24 µm); tricolporate; colpus margins irregular Pollen grain in different foci is elliptic with broad ends, in equatorial view, rounded and circular in polar view. Colpi with tapering ends equatorially constricted and Ora lolongates, 1.5 µm.

SEM. (Figs. D-E); exine 1.3 µm thick; sexine thicker than nexine and faintly perforate; columella regularly distributed. with indistinct margo; pore lolongate (4x5µm); microreticulate; homobrochate.

TEM. (Figs. F-G); Apertures: pore distinct 1.5 µm wide. Exine: 2µm thick. Sexine thicker than nexine, microreticulate, columella regularly distributed.

Comment: A common plant in SA. but non-endemic (Collenette, 1998). The pollen grains are distinguished by their comparatively short polar axis (16(17)24 µm) and tapering ends the pollen is shed from the anther singly (monads or in multiples

of four (polyads)-Pollinium or massula.

6-*Solanum gracileps* L. (Fig. 6, A-E)

LM. (Figs. A-C); Pollen prolate-spheroidal; P/E: 1.075 (1.04-1.11); pollen axis 27 µm (20(23) 26µm); tricolporate; colpus margins irregular; pore lalongate (4x5 µm); microreticulate; homobrochate nanoverrucae are visible; exine 1.3µm thick; Pollen grain in different foci is elliptic with broad ends equatorial view and semi-angular in polar view. Colpi with tapering ends equatorially constricted.

SEM; (Figs. E-F); sexine thicker than nexine; columella regularly distributed, tectate faintly perforate and pore indistinct. TEM, (Figs. D-E); Exine: 1.4 µm thick. sexine is thicker than nexine and is creinate sculptures. Comment: A common plant in SA. and endemic. The pollen grains are distinguished by their comparatively long polar axis (20(23)26 µm) and rounded amb.

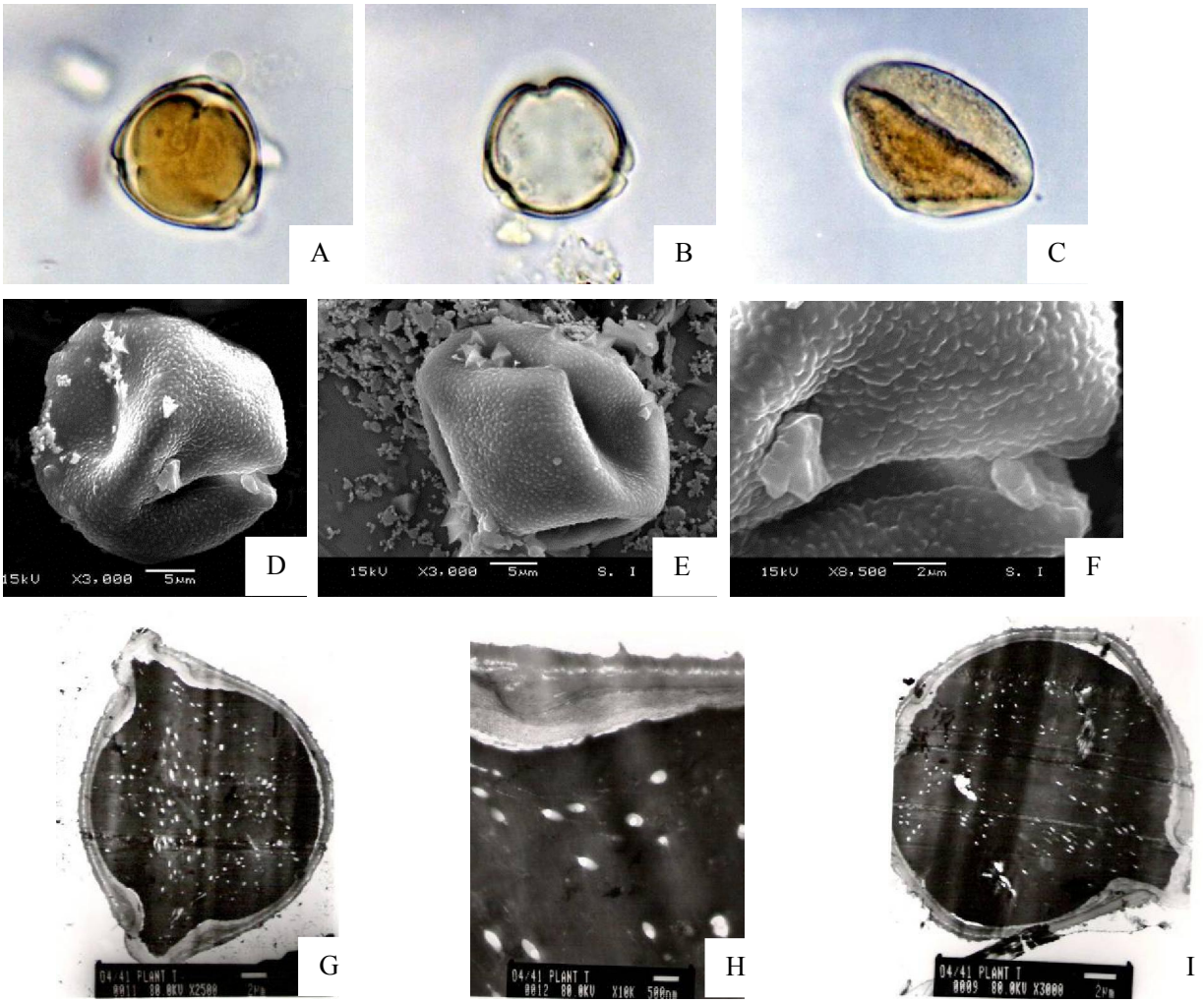

Figure (1): *Solanum incanum;* (L.M. A-Cx1000) A- polar view showing pores structure. B-polar view showing thick exine. C-equatorial view, showing colporate pollen and micro-ornamentation. (SEM, D-F), D- polar view showing tricolporate pollen, x 3000. E-equatorial view showing large mesocolpium of tricolporate pollen, x3000. F-polar view showing lalongate pore and regulate Sexine sculptures, x8500. (TEM, G-I), G- L.S., in pollen grain, showing finely echinate ektexine, pore structures, and fastigium-like cavity formed by ectoapertural margins, x2500. H-L.S., in pollen grains showing echinate Sexine, thick nexine and irregular columella, x10000. I-L.S., in pollen grains showing pore and fastigium-like cavity formed by ectoapertural margins, x3000.

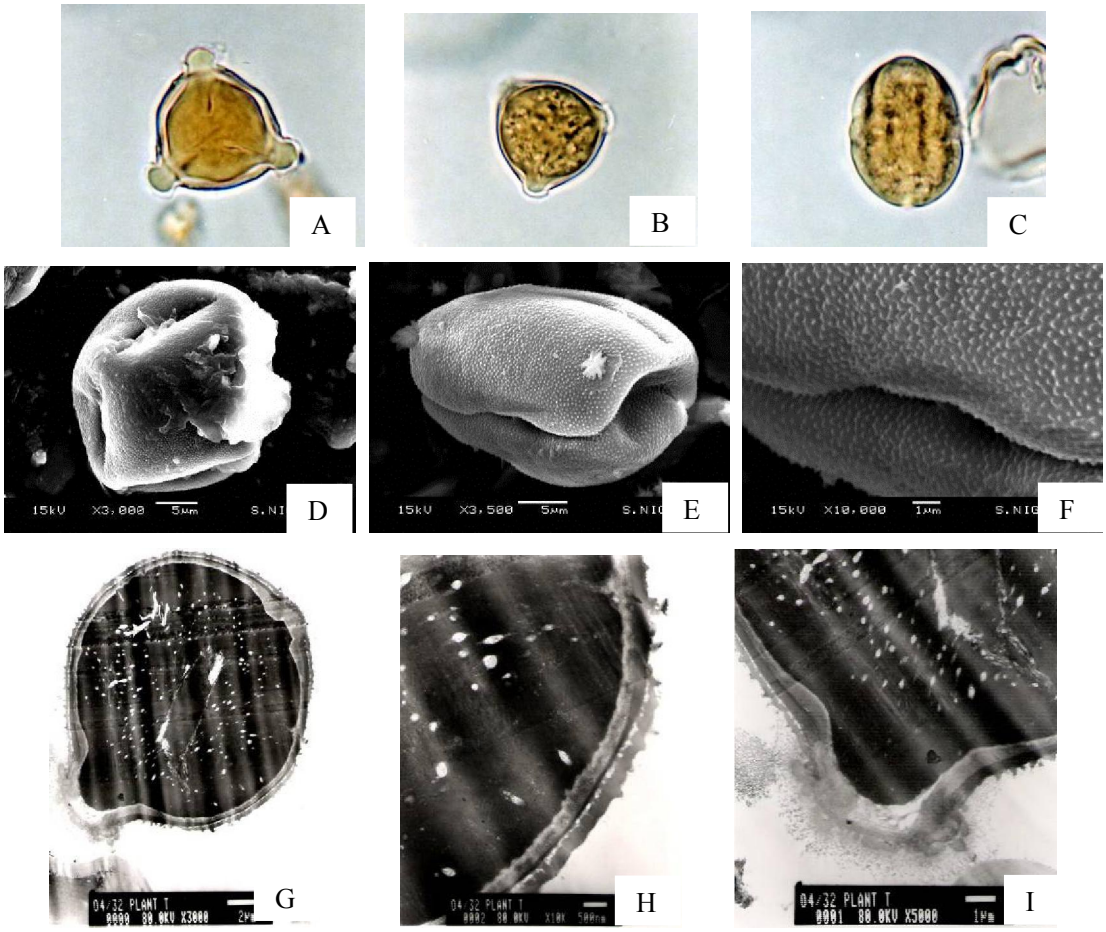

Figure (2): *Solanum nigrum*; (L.M., A-Cx1000), A- polar view showing pores structure, and thick exine. B-polar view showing thick exine and micro-ornamentation. C- equatorial view, showing tricolporate pollen. (SEM, D-F), D- polar view showing colporates pollen, large mesocolpium and small amb, x3000. E-equatorial view showing surfaced furrow and abnormal amb, x3500. F- equatorial view showing colpus margo with the same sculpture of mesocolpium, x10000. (TEM,G-I), G-L.S., in pollen grain, showing pores and exine thickness, x3000. H-L.S., in pollen grains showing spinulose tectum, similar thickness of sexine and nexine with regular columella, x10000. I-L.S., in pollen grains showing circular lalongate pore and fastigium-like cavity formed by ectoapertural margins and very thin sexine near the apertures, x5000.

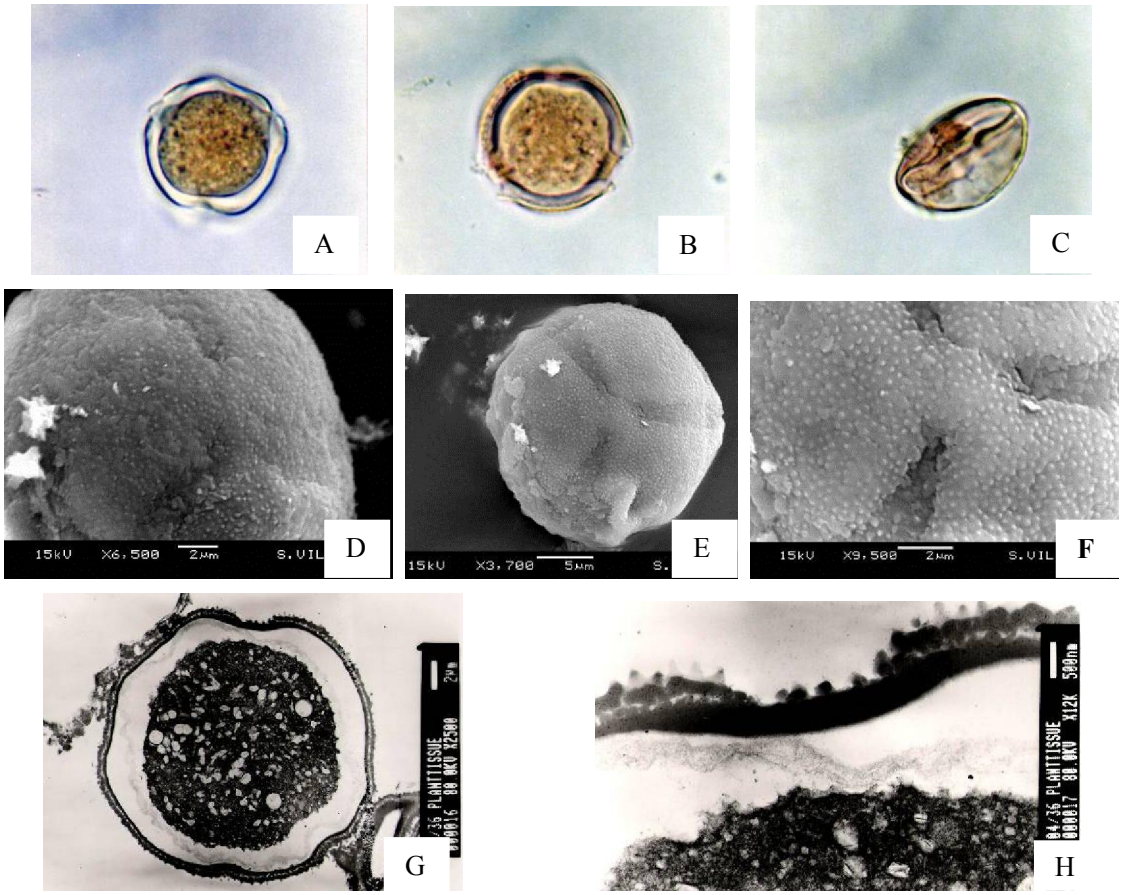

Figure (3): *Solanum villosum*; (L.M. A-Cx1000), A- polar view showing thick exine and pores structure. B-polar view showing micro-ornamentation. C-equatorial view, showing tricolporate pollen. (SEM,D-F), D- polar view, showing surfaced and granulate furrow of colporate pollen,x6500. E-polar view, showing wide mesocolpium, x3700. F-polar view showing very small amb and verrucate margo, x9500. (TEM, G-H), G-L.S., in pollen grain, showing spinulose tectum and thick intine,x2500. H-L.S., in pollen grains showing no tectum and columella at the pore region and verrucate sexine, x12000.

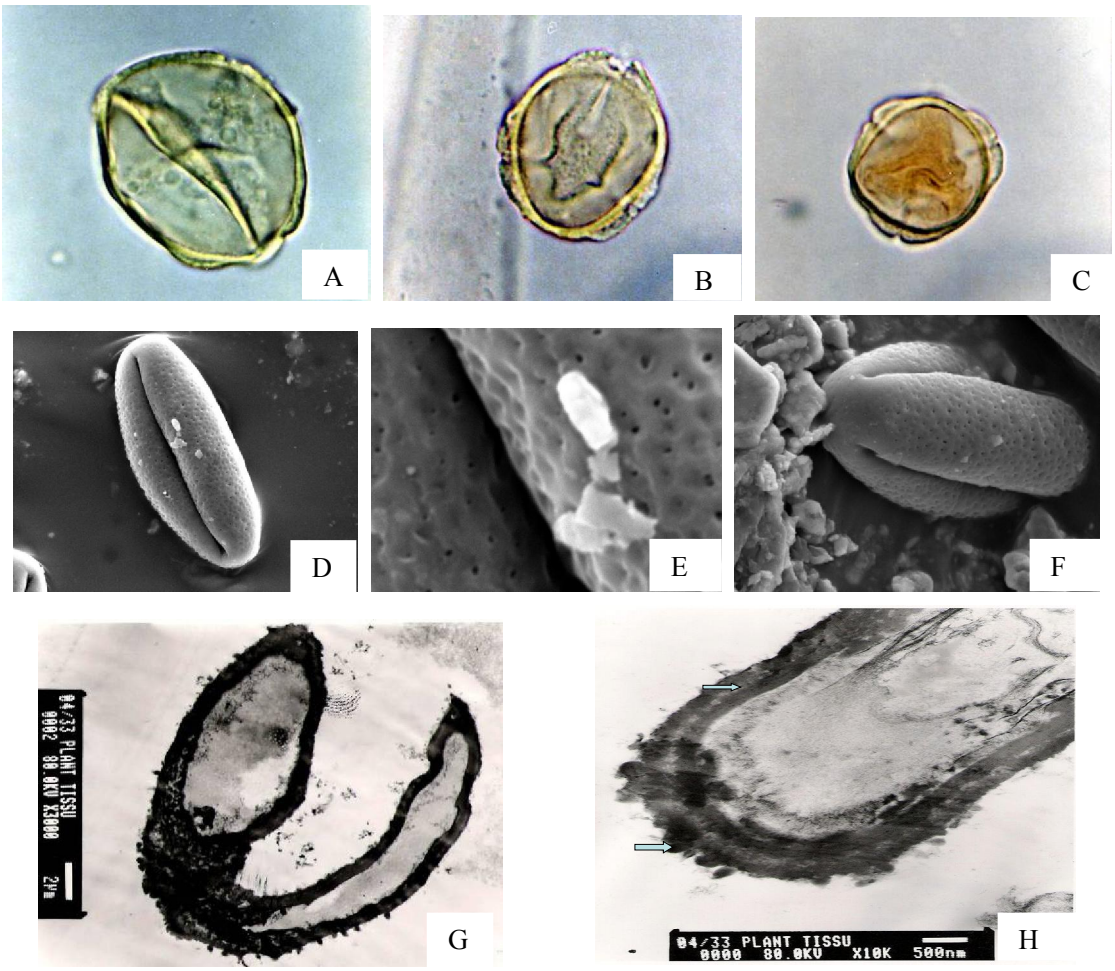

Figure (4): *Solanum carense;* (L.M., A-Cx1000), A-equatorial view, showing large colpus and thick exine of the pollen. B-polar view, showing micro-ornamentation of the exine. C-polar view showing pores structure. (SEM, D-F),D-equatorial view showing straight colpus and mesocolpium, x3300.E-polar view, showing furrow and margo in the same sculpture, x20000. F-equatorial view, showing amb and mesocolpium, x5000. (TEM,G-H),G-L.S., in pollen grain, showing indistinct pore and spinulose tectum, x3000. H-L.S., in pollen grains, showing exine and tectum, x5000.

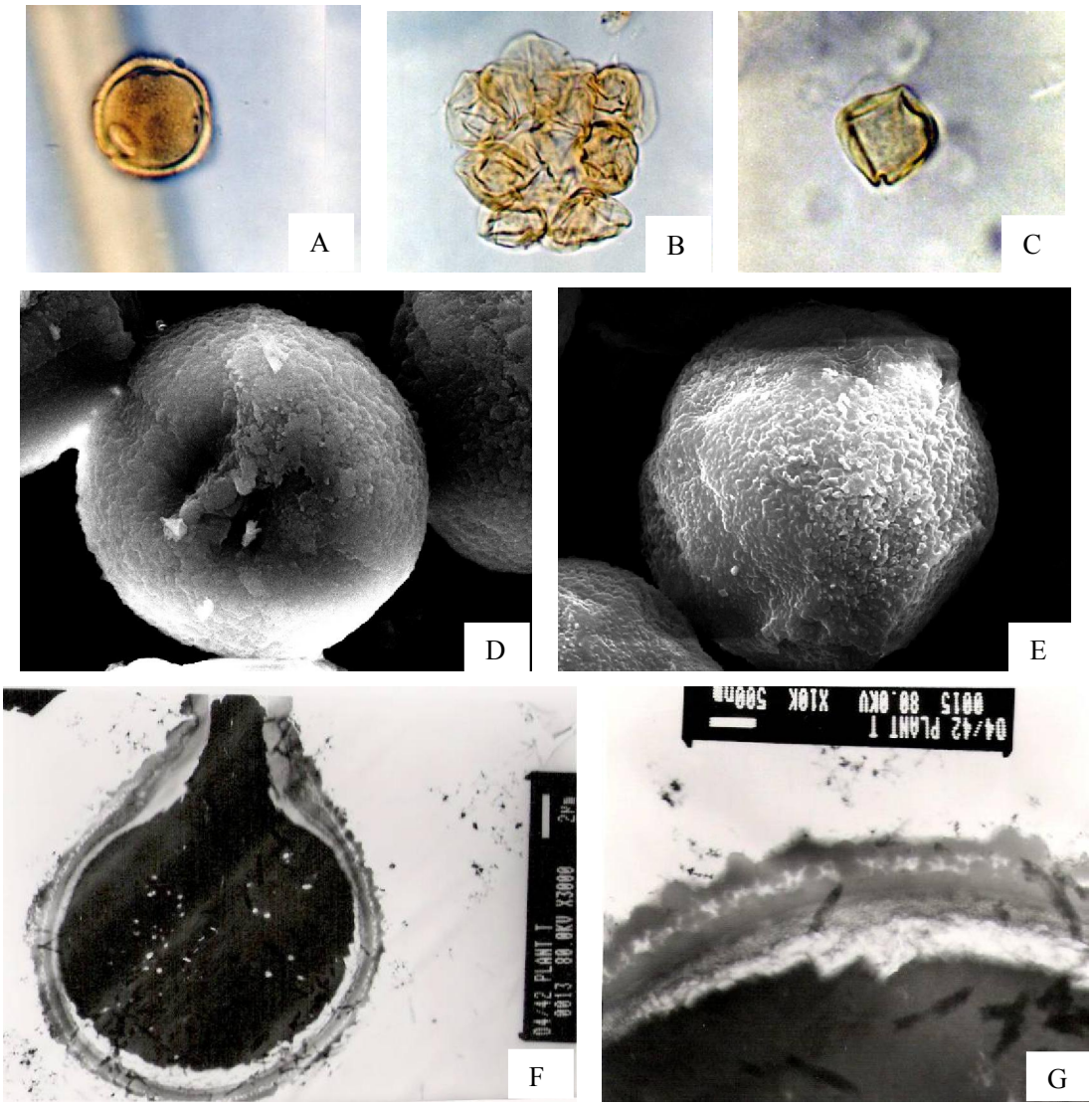

Figure (5): *Solanum* sp.aff. *anguivi*; (L.M., A-Cx1000), A-polar view showing thick exine and micro-ornamentation. B-polyads view, showing tricolporate pollen, x400. C-equatorial view showing of monad, pores structure, x1000. (SEM, D-E), D-equatorial view showing pore bridge and indistinct colpi, x4500. E-polar view, showing surfaced furrow and scabrate sexine, x4300. (TEM, F-G), F-showing aperturate lalongate pore, x3000. G-L.S., in pollen grain, showing crenate sexine and thick nexine, x10000.

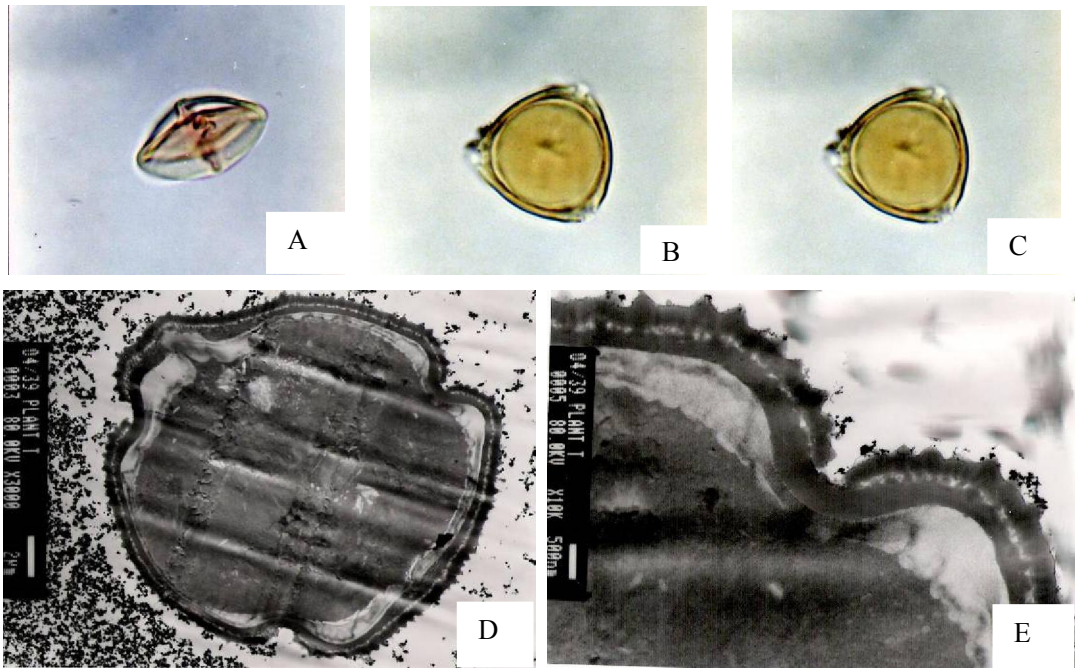

Figure (6): *Solanum gracileps*; (L.M.A-Cx1000), A-equatorial view, showing tricolporate pollen.x1000. B-polar view showing thick exine and psilate-ornamentation. X1000.C- polar view showing pores structure. x1000. (TEM, D-E), D-L.S., in pollen grain, showing crenate tectum, x3000. E-L.S., in pollen grains showing serrate -like, thick ektexine, regular columellae, and the pore intectates, x10000.

4. Discussion and Conclusion

The pollen morphology of the family is quite heterogeneous (Erdtman, 1952). However, tricolporate grains are universally present. Pollen grains are usually radialy symmetrical, isopolar, prolate-spheroidal to sub-prolate or prolate rarely oblate-spheroidal. The work investigates the ultra-structures and comparative structures of the pollen grains in six species of the genus *Solanum*. Palynological data of the species of *Solanum* indicated that all the pollen morphology is isopolar, symmetrical trizoncolporates. While the size measurement of the six species showed an overlapped in most cases. The pollen grains of *Solanum* sp.aff. *anguivi* are the smallest and are classified according to Erdtman, 1952 as (sporae minutae, M1) while spores of the three species *S. carense*, *S. incanum* and *S. nigrum* are similar size and classified as large (sporae Long, M2). However, the two other species *S. gracileps* and *S. villosum* are of similar size classified as medium (sporae mediae, M3). This variation in size may be due to indiscriminate mating leading to hybridization which may be operating in this complex. This is not surprising since previous workers have made similar observations in other groups of angiosperm (Moore & Webb, 1978; Bonnefille & Riollet, 1980; Agwu & Beug, 1982 Agwu & Osibe, 1992; Luna–Cavazos & Gara- Moy, 2002).

The pollen shape expressed as the ratio of the length of polar axis to that of equatorial dimension (Erdtman, 1952), is found to be more or less quinquangular in polar view and elliptic in equatorial view in both species. According to the estimated P/E ratio, pollen grains of *S. incanum* classified as prolate and the other three species of *S. gracileps, S. villosum* and *S. nigrum* as prolate- spheroidal. While the other two species, *S. carense* and *S.* sp.aff. *anguivi* as subprolate (most often prolate-subprolate). The observed differences in pollen morphology between the studied species agree with the observations of Gbile & Sowunmi (1979) that conducted a palynological study in *Solanum* species and found highly significant differences in pollen size and shape within the studied groups (Shrma, 1974; Bonnefille & Riollet; 1980 & El-Ghazali, 1993). However, tricolporate pollen with scabrate tectum is more commonly found within the family. The exine sculpturing provided features for taxonomic distinction. *S. carense* is distinct from the

species by its perforate sexine patterns. Both *S. villosum* and *S. nigrum* are characterized sexine patterns with clearly echinate at *S. nigrum* and verrucate *in S. villosum*. However, the genera and species of this pollen grains type are easily separated on the basis of pollen shape class. Pollen grains of *Solanum nigrum* is recognized by its scabrate sexine. The *S. incanum* pollen had regulated -scabrate sexine and non perforates exine. Although there is much overlap in exine sculptures types, pollen grains size may be significant in delimiting some taxa within the family (Hesse, 1981& Meltsov *et al.,* 2008). The data derived from palynological and characters of the examined taxa could contribute to the taxonomy of Solanaceae and it is found that the Solanaceae is an europalynose family and not stenopalynous as previous authors reported (Erdtman, 1952; Gbile & Sowunmi, 1979; El-Ghazaly, 1991; Moore *et al.*, 1991 & Al-Wadei & Lashin, 2007;). Moreover, *S.* sp.aff. *anguivi* characteristics by poylads pollen dispersal, that character supported by Guinet & Ferguosen (1989) who's suggested that the higher polyads pollen grains number increases the reproductive capacity of the species. The results of the morphology and palynology of the species of *Solanum* studied have proved to be of immense assistance in interpreting problems related to plant taxonomy. The results could therefore be utilized with information from other discipline in clarifying taxonomic relationships of these taxa with other genera and species or subspecies.

Key of the Species:

1-Tricolporates pollen grains, sexine crenate and columella is distinct in SEM and pollen shape is prolate –spheroidal ----------*Solanum gracileps* L.

2-Densly columellate, verrucate sexine and granulate margo……..*S. villosum* (L.) Mill.

3-Columella distinct and wide mesocolpium and ora lolongate .....……..... *S. nigrum* L.

4-Columella distinct, short echinates or scabrate sexine and prolate shape…….. *S. incanum* L.

5-Columella indistinct, perforates tectum and subprolate shape ……*S. carense* Dun. inDc.

6-Columella indistinct, scabrate or regulate sexine and prolate – subprolate ….*S.* sp.aff. *anguivi* Lam.

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