STUDY OF THE CYTOLOGICAL AND MICRO-MORPHOLOGICAL CHARACTERISTICS OF SOME SPECIES OF THE GENUS *EUPHORBIA* L.BELONG TO EUPHORBIACEAE FAMILY , USING ELECTRON MICROSCOPE IN IRAQ

S.K.Sulaiman **Z.A.Ismail** **S.A.Aliwy ***
Researcher **Assist .Prof.** **Assist. Prof.**
Dept. of Biology,Collage of Eucation ,University of Iraqia **Dept.of Biology,Collage of Science , University of Baghdad**
anaamnoor@yahoo.com

ABSTRACT

This study was aimed to investigate micro-morphological and cytological characteristics for sex species of Euphorbiaceae family in Baghdad during the two growing seasons 2018-2019 . The species are: *Euphorbia granulate* Forssk.,*E.helioscopia* L.,*E. hirta* L.,*E. hypericifolia* L., *E. milli* Des Moul .,*E. puplus* L. Which involved with morphological characters of pollen grains and seeds, as well the chromosomes number, and the study managed to count chromosome number for the above species sequentially:-n=11, n=21, n=9, n=16, n=14,18, n=7,8. The morphological characters of pollen grains surface has been studied by using scanning electron microscope (SEM), shape and size, polar and equatorial axes, spines length, dimensions of ora and colpi, and the ornamentations on pollen surface, and it has been observed that all the species are tri-zono-colporate, spinulose and spinate (echinated), and spinolophate fruite, and importance of the pollen grains in taxonomy. The study includes morphological characteristics of seeds by using (SEM) In terms of size, shape, color, and appearance of the seed surface(configuration). It has been shown that the seeds are significantly different from species to another. Species help to isolate and classify, and through relying on these characteristics, classification limits could be set, whether at the level of species or genus within each family.

Keywords: Euphobiaceae, seeds, pollen grains, chromosomes number, pollen surface.

دراسة الصفات الخلوية والمظهرية الدقيقة لبعض انواع الجنس *Euphorbia* L. من العائلة السوسبية باستعمال المجهر الالكتروني في العراق

سارة خلف سليمان **زبيدة عبد اللطيف اسماعيل** **سكينة عباس عليوي**
باحث **استاذ مساعد** **استاذ مساعد**
قسم علوم الحياة,كلية التربية,الجامعة العراقية **قسم علوم الحياة ,كلية العلوم ,جامعة بغداد**

المستخلص

تناول البحث الحالي دراسة تصنيفية لستة انواع عائدة الى العائلة السوسبية Euphorbiaceae في بغداد لموسمي النمو 2019/2018.والانواع قيد البحث هي *Euphorbia granulate* Forssk.,*E.helioscopia* L.,*E. hirta* L.,*E. hypericifolia* L., *E. milli* Des Moul .,*E. puplus* L. حيث شمل البحث دراسة الصفات المظهرية الدقيقة لحبوب اللقاح (Pollen)والبذور (Seeds)وكذلك حساب العدد الكروموسومي (chromosomal number) للأنواع المذكورة وقد تمكن من معرفة العدد الكروموسومي لكل من الانواع اعلاه حسب التسلسل.n=7,8 , n=14,18 , n=16 , n=9 , n=21 , n=11 وتضمنت الدراسة الصفات المظهرية لسطح حبوب اللقاح بواسطة المجهر الإلكتروني الماسح SEM من شكل وحجم الحبة، طول المحور الاستوائي والقطبي، والزخرفة على سطح حبة اللقاح، وضحت النتائج ان جميع الانواع هي من طرز ثلاثية الأخاديد والثقوب، واتضح ان صفات حبوب اللقاح ذات اهمية تصنيفية مفيدة في عزل وتشخيص الانواع، فضلا عن دراسة جميع الصفات المظهرية الدقيقة للبذرة بواسطة المجهر الالكتروني الماسح، من حيث حجم، شكل، لون، وكذلك المظهر الخارجي لسطح البذرة، اظهرت النتائج ان البذرة مختلفة بشكل كبير من نوع الى اخر حيث ان كل الانواع تساعد على العزل والتصنيف ومن خلال الاعتماد على هذه الصفات يمكن وضع حدود تصنيفية سواء على مستوى النوع او الجنس وضمن كل عائلة.

الكلمات المفتاحية: العائلة السوسبية، البذور، حبوب اللقاح، العدد الكروموسومي.

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INTRODUCTION

Euphorbiaceae considered one of the largest economically important plant family of Anthophyta with about 300 genera and 7500 species, cosmopolitan distribution in the humid tropics and Subtropics of both hemisphers (32). This family distinguished by the milky sap which may caused toxicity and can cause blind when entered to the eyes (12). In Iraq, this family contained about 7 genus and 51 species (34). *Euphorbia* L. is the largest genus in the spurge family with about 2100 cosmopolitan species (14). The genus comprises remarkable life form variability from annual to perennial herbs, shrubs, trees, succulent and xerophyte forms (19,42). All those species, belorgs to this genus characterized by the prescerce of latex and have unique flower structures (1). *Euphorbia* species considered source of many active biological components such as tannins, flavonoids, unsaturated sterole, carbohydrates, diterperoids and triterpenoids (6,49). Due to the presence of these biologically compounds, *Euphorbia* species used for treatment of various ailments such as : skin disease, intestinal parasites and gonorrhea (10). Other studies showed the important of this genus as antitumor , antibacterial , antileshmanial and antiviral (13,23,25,27,41). The first who studied red this family cytologically . Several *Euphorbia* species have basic chromosome number of X=8, wherease other species in clude X=6,7,9 and 10 of chromosome number , which is related to both aneuploidy and polyploidy (21,36). The previous study indicated cytologically various ploidy levels ranging from diploid, tetraploid, hexaploid to actaploid (2n=12-120). This indicate a significant role of polyploidy in evolution and speciation (14,15,21,22). The lowest chromosome number in Euphorbiaceae family is 2n=12 *E.cornuta* , while the highest was 2n=234 recorded in Antides mabuninus (51). Palynology has a strong relationship with taxonomy, it provide a taxonomic evidence which help in separate and identification of many genus and species (18). However , the palynological analysis may provide valuable in formation in further understanding the evolutionary relationship between species. Studies by previous researchers (35,37,43). Provided informations of the pollen of different species belong to *Euphorbia* genus. Seeds feature provide useful characters for the separation of closely related species and in the deduction of phylogenetic relationship. Seed characteristics of subgenera (40) and species (30,33) .In Iraq there are several studies on pollen grains, seeds and chromosome numbers but on another families such as the study of Sadeq (52) about Asteraceae family,as well as (3,4)for Amaranthaceae family ,in addition to study (11) in fabaceae family. This study was focused on characterize six species of Euphorbia (*E.granulata* , *E.helioscopia* , *E.hirta* , *E.hypericifolia* , *E.milli* , *E.puples*). in order to determine their chromosome number, pollen grains and seeds by using light and scanning electron microscopy with the aim of providing additional information as possible about these species with the aim of providing useful taxonomic data that would give further informations into proper classification and identification

MATERIALS AND METHODS

Fresh plant samples were gently collected from different areas 2018,2019 of Baghdad during Jan, Feb and March 2019, randomly between 8:00 am and 1:00 pm. They in cluded all sizes without consideration to the maturation stage, stored in farmens fixative immediately and stored in dark for 24 hr. (17). The taxonomic identification of the plants were characterized by Dr. Sukayna A. Aliwy and Dr. Zubaida A. Ismaeel. For cytological study, the procedure of sadeq (44) was done. The morphology of mature dried seeds and fresh pollen grains were studied by sing SEM. These samples were coated with gdd and examined by SEM at AL-Kufa College Najaf. The size were measured by using the program Imagei. The terminology of pollen grain patterns was adopted by palynology (31), While terminology of cypsela surface patterns adopted from (20,24)

RESULTS AND DISCUSSIONS

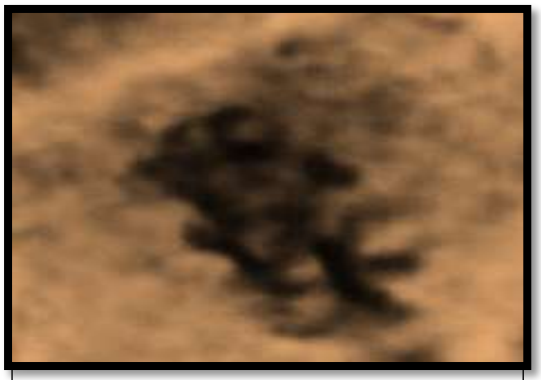
1-Chromosomes number

A total of (120) samples were detected for 6 species that belongs to Euphorbiaceae family and the chromosomes number for each species was calculate as shows in Table 1.

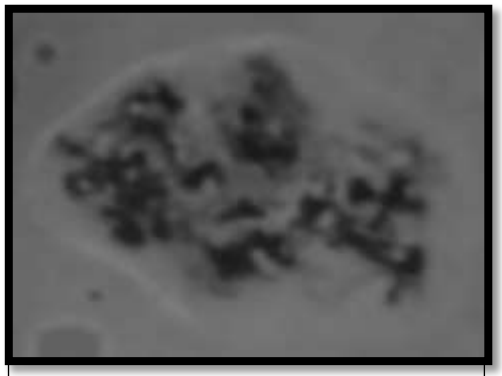
Table 1. Chromosomes number of species

Species	n	2n
E.granulata	**11**	**22**
E.helioscopia	**21**	**42**
E.hypericifolia	**16**	**32**
E.hirta	**9**	**18**
E.milli	**14,18**	**28,36**
E.puples	**7,8**	**14,16**

According to this study *E.granulata* have rerealed n=11,2n=22, this results was supported by the same number. This was ensured that , the chromosomes number of the species was the same although the environmental was differed . Results of *E.helioscopia* agreed with the study of other resarchers (16,45,48) they revealed that chromosomes number was n=21 , 2n=24. Some researchers (26,38,50) pointed out that chromosomes number in *E.hirta* was n=9 , 2n=18. This was supported the results of this scurvy, but it was not agreed with the findings of Alam (2), they revealed the number of chromosomes n=10 ,2n=20 The results of thing study showed that the chromosomes number was n=16 , 2n=32 in *E.hypericifolia* . This result was agreed with the study of Sadeq and Alewy (50). In *E.milli* , chromosomes number was n=14,18, 2n=28,36. The same result was noticed by Soontornchain(46). These results did not other researcher agreement with the study of other researcher(5,47). It was n=20 , 2n=40. This species is cosmopolitan all over the world and to the different of the environmental factors. According to the present study, chromosomes number of *E.puples* was n=7,8 ,2n=14,16. This findings was supported by the study of other's (7,19,28,29).plate.1

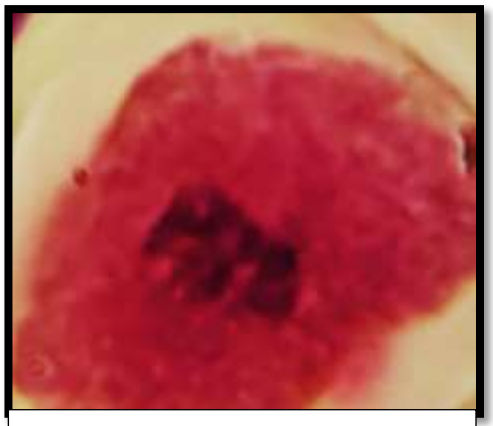


Metaphase I of *E.helioscopia* at .1000X

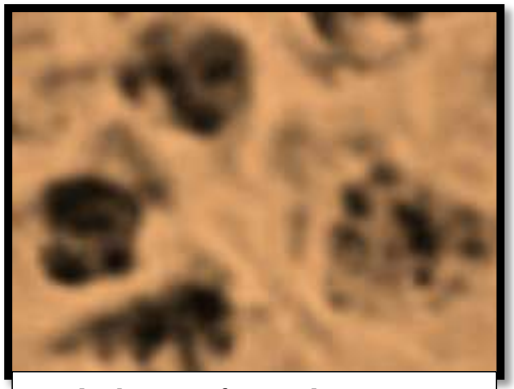


Multiplying phase of *E.granulata* May at 1000x

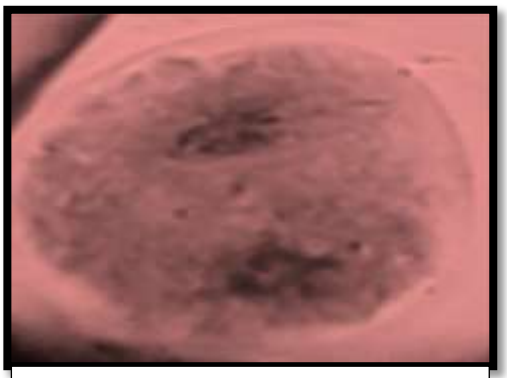
Anaphase I of *E.hirta.* at 1000X



Metaphase I of *E.hyericifolia* May at 1000x



Telophase II of *E.puplus* at 1000X



Anaphase I of *E.milli.* at 1000X

Plate 1. Phases of chromosomal division of the studied species (1000X)

2-Pollen grains

The results of this study show the important of the surface sculpturing which was found to be Reticulate. This finding was agreed Park (35). Measurment of the dimensions of the pollen grains is very important in differentiate between species and added a new important in formation in Taxonomy (39). A great number in this family have Zonocolporate. Grains have 3 colpi (Trizonocolporate) in *E.puples*, *E.hirta*, *E.heloscopia*, and *E.granulata*. According to the results, studied species can be classified into 3groups:

1-First group: including pollen that have elongated shapers in *E.puples* and *E.helioscopia*

2-Second group: including grains have flat spherical shape. This group included only *E.hirta*

3-Third group: including grains which have elongated spherical shapes as in *E.granulata*.

All these characters have an important taxonomic value (9). Results were showed that grains were isopolar, have simple apertures, trizonocolporate except in *E.granulata*. which was zonocolporate. According to the results of LM , all the grains have yellowish color. In polar view, 1-have circular to sub circular shape as in *E.granulata*, *E.helioscopia*, *E.hirta*, and *E.puples*. 2-Have flat to ovate shape as in *E.milli*. 3-Have ovate to width ovate as in *E.hypericifolia*.In equatorial view: 1-Long to circular in *E.hirta*, *E.granulata* and *E.puples*

2-Flat spherical in *E.milli* and *E.helioscopia*.

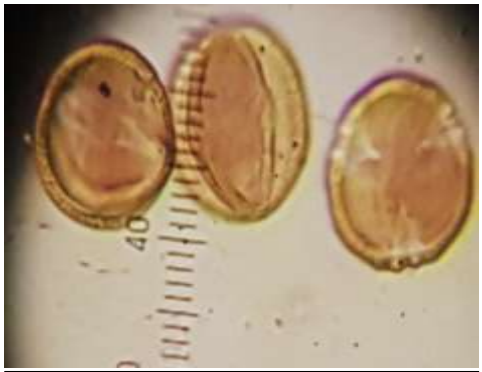
The sculptured was reticulate in *E.hirta*, pitted in *E.granulata* and *E.milli*, accurate reticulate in *E.helioscopia*, *E.puples* and *E.hypericifolia*.Table2.3

Table 2. Shows measurements of electron microscopy for pollen grains of species of Euphorbiaceae family

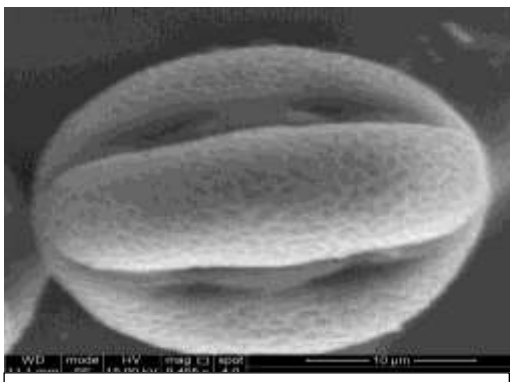
Pollen shape	E/P Um	Equatorial um /axis	Polar um /axis	Species
Oblong	1.36	3.574	4.885	*E. granulate*
Oblong spherical	1.14	23.295	26.647	*E. helioscopia*
Flat spherical	0.99	8.975	8.910	*E. hirta*
Elongated spherical	1.13	4.285	4.854	*E. puples*

Table 3.Quantitative and qualitative traits of *Euphorbia* species pollen grains types of measured was micrometer by light microscope

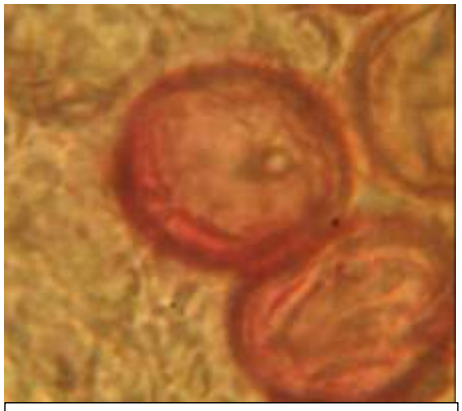
Surface configuration	Pollen grain in polar view	Pollen grain shape in equilateral view	Wall thickness rate	E/P	Equilateral axis	Polar axis	Species
Pitted	Spherical	Elongated spherical	1.7	0.94	18(21-16)	17(18-15)	*E.granulate*
Accurate reticulate	Spherical	Flat spherical	1.6	1.14	41(42-38)	47(53-45)	*E.helioscopia*
Reticulate	Spherical	Elongated spherical	1.3	0.92	13(14-11)	12(13-10)	*E.hirta*
Accurate reticulate	Ovate-Width ovate	Semi-spherical	0.9	1.06	15(16-13)	16(17-12)	*E.hypericifolia*
Pitted	Flatted ovate	Flatted spherical	2	0.96	31(33-29)	30(31-29)	*E.milli*
Accurate reticulate	spherical	Elongated spherical	1	0.43	29(31-25)	27(28-25)	*E.puples*



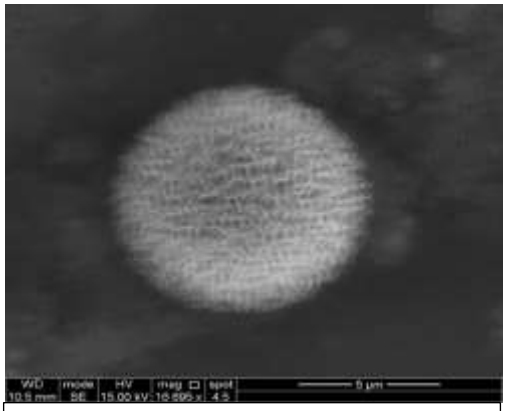
E.helioscopia



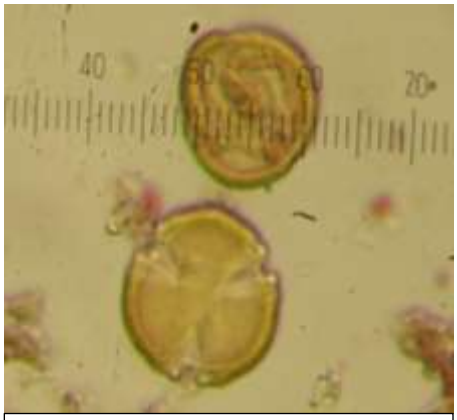
E. helioscopia



E. hirtia

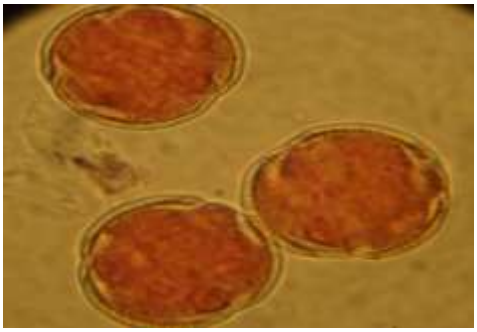


E.hirta

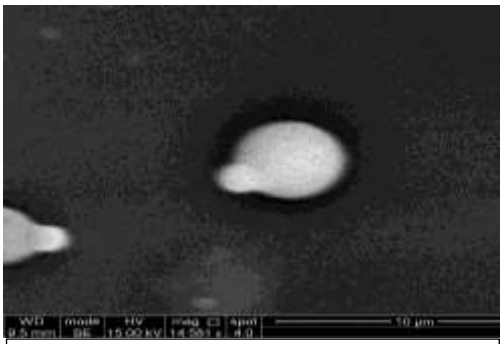


E. hypericifolia.

E. milli .

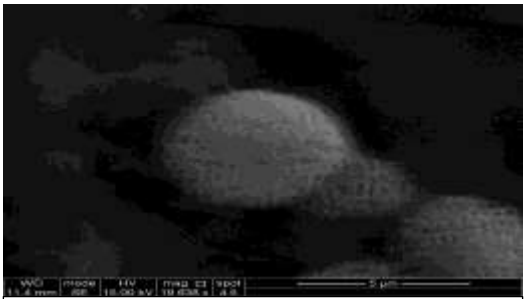


E.granulata



E.granulate

E. puples



E. puples

Plate 2. Scanning electron micrographs and Light micrographs (300X.)For pollen grains of species.

3-Seeds

SEM results showed two different shape

1-Oblong ovate and different in dimensions in *E.puples* , *E.hirta* and *E.helioscopia*

2-Three sided oblong pyramidal in *E.granulata*

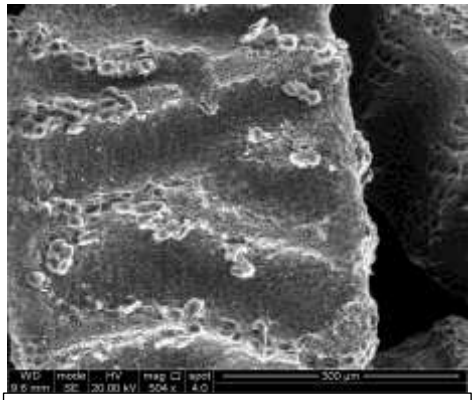
The sculpture was undulated with concave as aribs in *E.helioscopia* and undulating zigzag in *E.hirta* while in *E.puples* , the sceptered was concave papillae widespread on the external surface of the seed. Theresults showed that dimensions ranged between 889.919 mm in *E.granulata* and 1.713 in *E.puples*. According to LM,all the studied species have oblong ovate seeds with undulating in *E.granulata* and reticulate undulating in *E.hirta* and have different shape in *E.helioscopia* . While in *E.puples*, it was circular concave like pits. Al-Bukhati (8). Showed that seeds sculptured was different for the species that he studied. All these results were first show in Iraq and revealed the important taxonomic value of the studied characters.Table4,5

Table 4. Showing the characteristics and dimensions of seeds in an electron microscope

Seeds shapes	Surface configuration	Dimensions		Species
		Seeds width	طول البذرةssss	
Oblong pyramidal	Reticulate undulated	462.237	889.919	*E. granulate*
Oblong ovate	Reticulate undulated	1.482	2.238	*E. helioscopia*
Oblong ovate	reticulate undulating	1.082	2.074	*E. hirta*
Oblong ovate	circular concave	1.940	1.713	*E. puples*

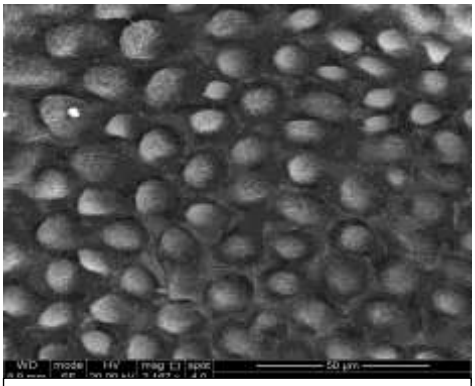
Table 5. Showing the characteristics and dimensions of seeds by light microscopy(LM.)

Seeds shapes	Surface configuration	Seeds dimensions		Species
		Seed W.	Seed L.	
oblong ovate with undulating	Reticulate undulated	0.75	0.95	*E. granulate*
Oblong sharp ovate	Reticulate	1.35	2.45	*E. helioscopia*
Oblong ovate with differenced ends	Reticulate zigzag	1.15	1.9	*E. hirta*
Little oblong sharped ovate	circular concave like pits	1.1	1.8	*E. puples*



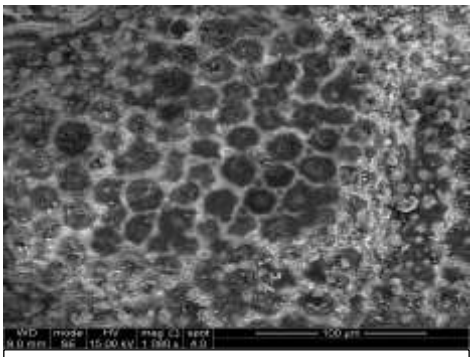
E.granulata. SEM

E.granulata. SEM



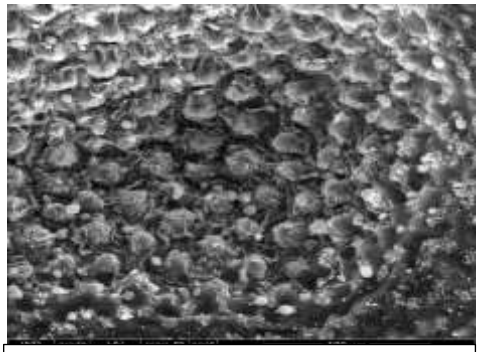
E.helioscopia SEM

E.helioscopia SEM

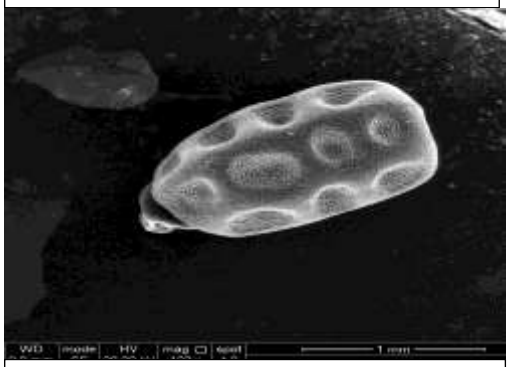


E.hirta SEM

E.hirta SEM



E.puples SEM



E.puples SEM

Plate 3. Scanning electron micrographs showing seeds configuration for the species

E. hileoscopia

E. granulate.

E. puples

E. hirta.

Plate 4. showing seeds of species by Dissect light microscope

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