ISSN: 2790-5306 (Print), 2790-5314 (Online)
IRAQI JOURNAL OF AGRICULTURAL RESEARCH - Ministry of Agriculture
Available online at: www.ijarmoa.gov.iq
VOL. 26 NO. (1) 2022

IJAR
IRAQI JOURNAL OF AGRICULTURAL RESEARCH

TAXONOMIC STUDY ON SALVIA SPECIES GROWING IN IRAQ USING POLLEN GRAINS MORPHOLOGY

Kh. S. Alzawi[1] I. A. M. Aldobaissi[2] A. O. Alfalahi[3]

Keywords: Salvia, Pollen, light microscope, electron microscope
Email: kadersasa@gmail.com

ABSTRACT

The present study carried out to classify Salvia species growing in Iraq using pollen grains morphology. Plants of the seven test species collected from Anbar some of and some of Kurdistan Region between May and June 2021. All species were wild type with the exception of S. *officinalis*, which is cultivated in AR-Ramadi district. Light microscope (LM) and scanning electron Microscope (SEM) examinations revealed significant variations among pollen grains of the test species; the pollen grains of the test species found to be different in size ranged between medium and small; the shapes of pollen grains varied from suboblate to prolate; pollen grains of all test species was hexacolpate.

The surface ornamentation was important in distinguishing some species, as the studied species were divided into two groups according to type of ornamentation, which was either reticulate or bireticulate. The bireticulate was the most common type of surface ornament in mesocolpium, while reticulate was the most common type of surface ornament in apocolpium. The present results provided useful pollen grains properties for species identification.

INTRODUCTION

Salvia has known as sage and it belongs to the Lamiaceae, contains a wide range of species up to 1000 species around the world. The genus possesses remarkable diversity in the form of growth, floral appearance, and pollinating biology (9, 21). It is economically and medically important and has antioxidant, antibacterial, anticancer and antidiabetic effects, is employed in traditional medicine and the pharmaceutical sector worldwide (5, 6, 20). There are 33 species in this genus in the Iraqi flora (1), and a species recently added to become the number 34 species (2).

The taxonomic importance of the pollen grains is due to several main characteristics such as the shape of the pollen grains, its color, its size, the surface ornamentation, the presence of pores or colpus or both together, and the dimensions of these apertures, their number and shapes, which are among the most important micromorphological characteristics (15). Numerous species of Salvia have undergone extensive study of their pollen morphology (8, 10, 19), pollen of Salvia is oblate and elliptic in equatorial and polar views respectively, and hexacolpate sometimes tetra, penta , or-octocolpate, in addition, the pollen has

[1] College of Sciences, University of Anbar, AR-Ramadi, Iraq.
[2] College of Sciences, University of Bagdad, Baghdad, Iraq.
[3] College of Agriculture, University of Anbar, AR- Ramadi, Iraq.
Received: Sep. /2022.
Accepted: Oct. /2022.

six mesocolpia, either two of them are thicker and longer than the remaining four (19) or three of them are broad and the other thin (14).

Moon et al. (13) confirmed from their study using SEM that Salvia species can divided into distinct groups depending on the external ornamentation of the pollen.

Pointed out Aktaş et. al. (3) worked on certain Turkish Salvia and found that palynological traits are crucial for identifying differences among the researched species. Kiliç (13) confirmed that the pollen grains of the majority of Salvia taxa have a hexacolpate aperture and suboblate shape and tectal surface sculpting was a useful factor for identifying certain Salvia taxa.

The present study conducted to test the possibility of using pollen grains morphology as a tool in identifying seven species of the genus Salvia growing in Iraq and evaluate their taxonomic usefulness among species.

MATERIALS AND METHODS

Collection of plant samples

Plant samples collected from the fields through the end of March to the end of June of 2021, which is approximately the flowering period of the most types of Salvia (1). S. indica, S. candidissma, S. virgata and S. reuterana were collected from different areas of Kurdistan Region through five field trips, which included Erbil (Mirgasur, Haj Omran), Dohuk (Sarsing), Sulaymaniyah (Dokan), and two field trips to the west of Anbar governorate, which included the city of Haditha and village of Alzawiya, through which were collected S. spinosa and S. lanigera (Figure 1), in addition, one species (S. officinalis) is collected from Al-Ramadi (Aljazira) area and it was cultured after the seedlings taken from the Baghdad arboretum.

The samples diagnosed by the researcher's personal information and classification keys with comparison with the herbal samples kept in the University of Baghdad herbarium (B.U.H).

The collected samples preserved after drying and fixing on cardboard and placing a label, which includes the region, date of collection, and the name of the collector. Then the samples deposited in the Botany Laboratory, College of Science, University of Anbar, for conducting the required tests and measurements.

Preparation of safranin stain

Safranin glycerin stain used for photographing pollen grains of the test species. The stain was prepared by adding one volume of safranin stain 0.5% to six volumes of glycerin (18).

Figure 1: Salvia species, 5= S. spinosa (Anbar- Haditha), 6= S. reuterana (Sulaymaniyah- Dokan), 7= S.virgate (Dohuk-Sarsing).

Pollen grains study:

Preparation of pollen grains for light microscope:

The pollen grains investigation performed using procedure established by several authors (7, 22). The anther of a mature flower taken and placed on a glass slide; a drop of the safranin stain added to it, than the anther opened to extract the pollen grains using a dissecting needle and pointed forceps.

The remains of the anther were removed, then the cover slide was gently placed so that the slide was ready for examination, and then were observed and photographed in a Euromex bscope microscope with a camera, Measurements were taken using an ocular micrometer, at least 20 pollen grains per sample were examined polar view ,polar axis (P) ,equatorial axis (E), P/E,colpus length,colpus width, apocolpium, mesocolpium, wall, shape, size, wall thickness and exine sculpturing, were measured. The maximum and minimum value was calculated and the means of these values.

Preparation of pollen grains for electron microscope:

To determine the external sculpting, the pollen grains were examined by sending a mature flower for each sample to the BPC Analysis Center in Baghdad for photographing the pollen grains by a scanning electron microscope (SEM), the samples were placed in 70% ethanol after drying and kept in the refrigerator until use for examination (18).

RESULTS AND DISCUSSION

Table 1 and figure 2 show the palynomorphological and microscopic shapes of pollen grains of 7 species of Salvia under LM. Results displayed revealed that the pollen grains for the examined species were hexacolpate isopolar-radially symmetric; the size vary between small and medium: polar axis (PA) = 21.2-31.5 in S. reuterana and S. officinalis respectively. The shape was suboblate and prolate: P/E = 0.85-1.50 in S. spinosa and S. officinalis respectively.

Colpus measurements were of different ranges, as the length ranged from 13.1µm in S. reuterana to 21.4µm in S. officinalis, too, width ranged from 3.1µm in S. officinalis to 3.8µm in S. candidissma, apocolpium was ranged from 6.5µm in S. officinalis and S. virgata to 14.5µm in S. candidissma, and mesocolpium was ranged from 12.5µm in S. officinalis to 18.2µm in S. candidissma, it appears as six approximately equal mesocolpia in polar view.

Pollen grains have a variety of ornamentation on their surface (Figure 3), exine sculpturing may be divided into two categories: Reticulate (S. officinalis) and bi-reticulate (S. indica, S. reuterana S. lanigera, S. candidissma, S. spinosa, S. virgata).

In addition, SEM images showed a difference between apocolpium and mesocolpium in some species such as S. candidissma. Mesocolpium walls characterized by bi-reticulate wall- with polygonal primary lumina and irregular circular secondary lumina, which is characterized, by a big semi-central secondary lumen and numerous small lumina concentrate in the primary muri, while apocolpium is characterized by a reticulate wall with lumina smaller than the lumina in mesocolpium walls.

Table 1: Pollen morphological data for Salvia L. taxa examined, measurements in µm.

No.	Taxon	Polar view	Equatorial view		Aperture		Apo-colpium	Meso-colpium	Wall	P/E	Shape	Size	Exine sculpturing
			Polar axis	Equatorial axis	Colpus								
					Length	Width							
1	Salvia candidissma	33.1-40.1 (37.5) ±2.2	24.6-28.1 (26.5) ±1.1	26.4-31.5 (30.5) ±1.6	17.3-19.2 (18.2) ±0.7	3.6-4.1 (3.8) ±0.2	12.1-15.5 (14.5) ±1.2	15.9-20.1 (18.2) ±1.4	3.6-4.5 (4.3) ±0.2	0.86	suboblate	Medium	Bireticulate
2	Salvia lanigera	19.3-22.5 (20.5) ±0.9	20.8-25.5 (23.4) ±1.6	24.7-29.5 (27.1) ±1.9	14.5-18.1 (16.3) ±1.5	3.5-3.8 (3.7) ±0.1	9.2-12.1 (11.0) ±1.1	13.1-17.5 (16.1) ±1.3	3.8-4.6 (4.3) ±0.3	0.86	suboblate	Medium	Bireticulate
3	Salvia offianalis	15.7-19.6 (18.5) ±0.8	28.5-33.1 (31.5) ±1.6	19.2-24.3 (21) ±1.7	17.1-22.3 (21.4) ±1.9	2.8-3.5 (3.1) ±0.2	4.6-7.9 (6.5) ±1.0	10.1-14.5 (12.5) ±1.4	3.3-4.1 (3.5) ±0.2	1.50	prolate	Medium	Reticulate
4	Salvia indica	25.5-36.1 (30.7) ±3.3	19.7-24.2 (22.6) ±1.3	24.3-28.6 (26.2) ±1.6	11.1-15.1 (14.0) ±1.2	3.1-3.9 (3.7) ±0.3	7.6-11.3 (10.5) ±1.1	15.1-18.5 (17.1) ±1.0	3.1-3.7 (3.4) ±0.1	0.86	suboblate	Medium	Bireticulate
5	Salvia spinosa	29.5-34.6 (31.4) ±1.4	20.5-25.3 (22.6) ±1.6	23.6-27.8 (26.5) ±1.3	11.3-16.1 (15.0) ±1.8	3.2-3.8 (3.6) ±0.2	7.2-11.1 (9.5) ±1.3	10.3-16.5 (15.5) ±1.4	3.2-3.9 (3.5) ±0.2	0.85	suboblate	Medium	Bireticulate
6	Salvia virgata	19.2-23.3 (21.6) ±1.3	27.5-33.2 (30.5) ±2.1	18.5-23.6 (21.4) ±2.0	16.3-21.1 (19.2) ±1.7	3.0-3.6 (3.3) ±0.1	5.3-8.1 (6.5) ±0.9	11.1-15.5 (13.1) ±1.5	3.7-4.2 (4.1) ±0.1	1.42	prolate	Medium	Bireticulate
7	Salvia reuterana	16.1-20.6 (19.5) ±1.0	19.6-23.5 (21.2) ±1.1	22.6-26.2 (24.5) ±1.4	12.3-15.4 (13.1) ±0.9	3.2-3.9 (3.5) ±0.2	6.3-9.1 (7.3) ±0.9	11.1-15.5 (14.1) ±1.4	3.9-4.9 (4.5) ±0.3	0.86	suboblate	Small	Bireticulate
± standard deviation													

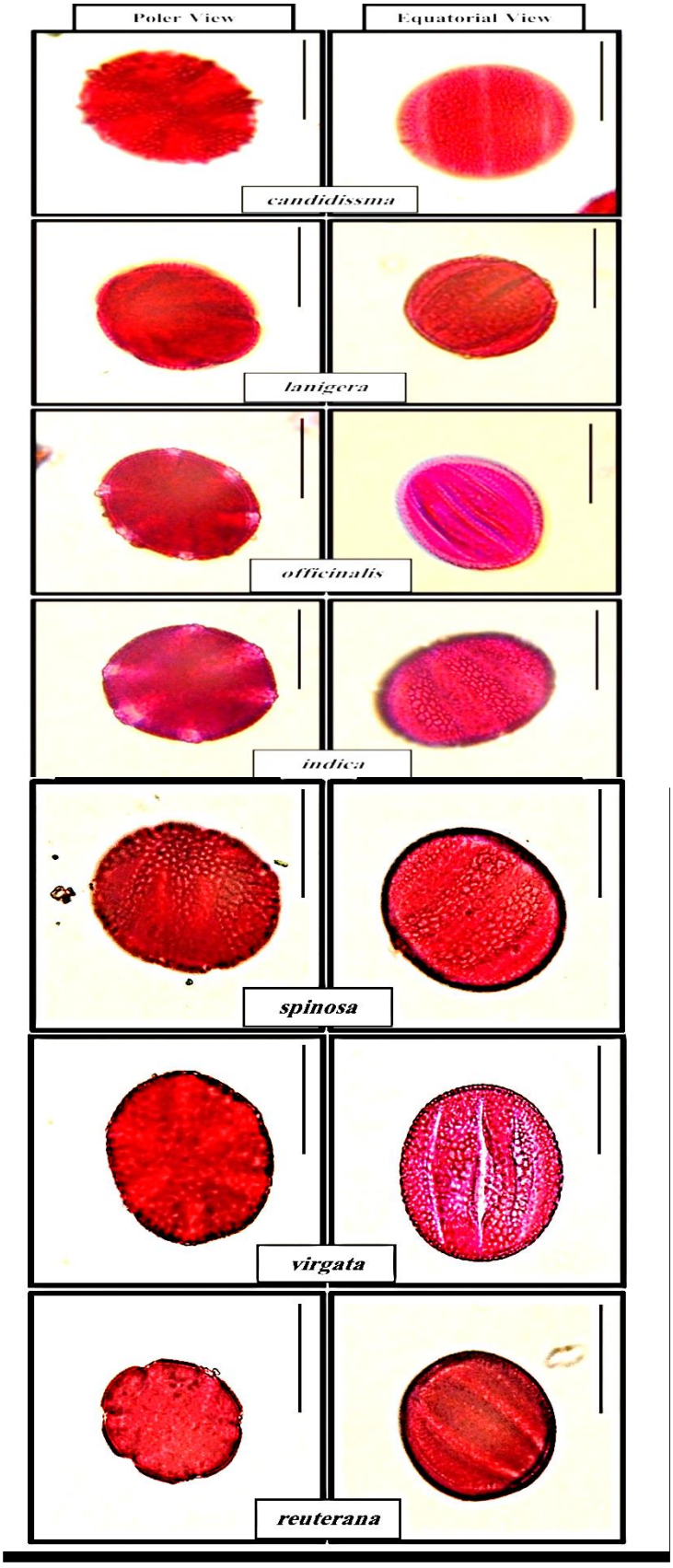

Figure 2: Light microscope micrographs of pollen grains of test *Salvia* species. Scale bar = 20 µm.

S. lanigera mesocolpium walls are characterized by bi-reticulate walls- with polygonal primary lumina and numerous circular secondary lumina, while apocolpium is characterized by micro foveolate walls. In S. officinalis apocolpium and mesocolpium walls are characterized by reticulate configuration with polygonal primary lumina.

S. indica mesocolpiun walls are characterized by a bi-reticulate wall- with polygonal primary lumina and tinny secondary lumina, apocolpium characterized by reticulate configuration smaller than the configuration in mesocolpium walls, while in S. spinosa apocolpium and mesocolpium walls are characterized by a bi-reticulate wall- with irregular primary lumina and numerous tinny circular secondary lumina.

S. reuterana mesocolpiun walls are characterized by a bi-reticulate wall- with polygonal primary lumina and irregular circular secondary, apocolpium characterized by the reticulate wall. Also S. virgata bi-reticulate wall- with polygonal primary lumina and numerous small circular secondary lumina, apocolpium characterized by the reticulate wall.

Discussion:

The result of this study is in agreement with several previous studies that indicated that pollen grains in the Salvia are isopolar, many of them are hexacolpate, with different sizes and shapes, pollen in most species have a range of size from small to medium and have various forms of suboblate and prolate (4,9,11,16).

The difference in size and shape of pollen grains of the same species found in several studies may be due to the differences in type of samples used whether it is fresh or dry which is taken from herbariums. The variation attributed to different methods of preparation could not be excluded, Reitsma (16) who pointed out That the size or shape of the pollen varies according to the acetolysis method used.

Results revealed that the surface ornamentation of pollen grains varied and different between species and it could be divided into two main types, namely reticulate and bi-reticulated, and there are differences between apocolpium and mesocolpium for some species. Some of these traits have been pointed out by many researchers (4,9,11,16).

Conclusion:

This study showed that Pollen grains morphology can occasionally serve as supplementary evidence to highlight species differences and that it can enhance phenotypic categorization by acting as a tool for species differentiation.

Scanning Electron microscopy (SEM) results were very useful in distinguishing species, due to the diversity of the external ornamentation of pollen, where it divided the studied species into two sections according to the type of external ornamentation.

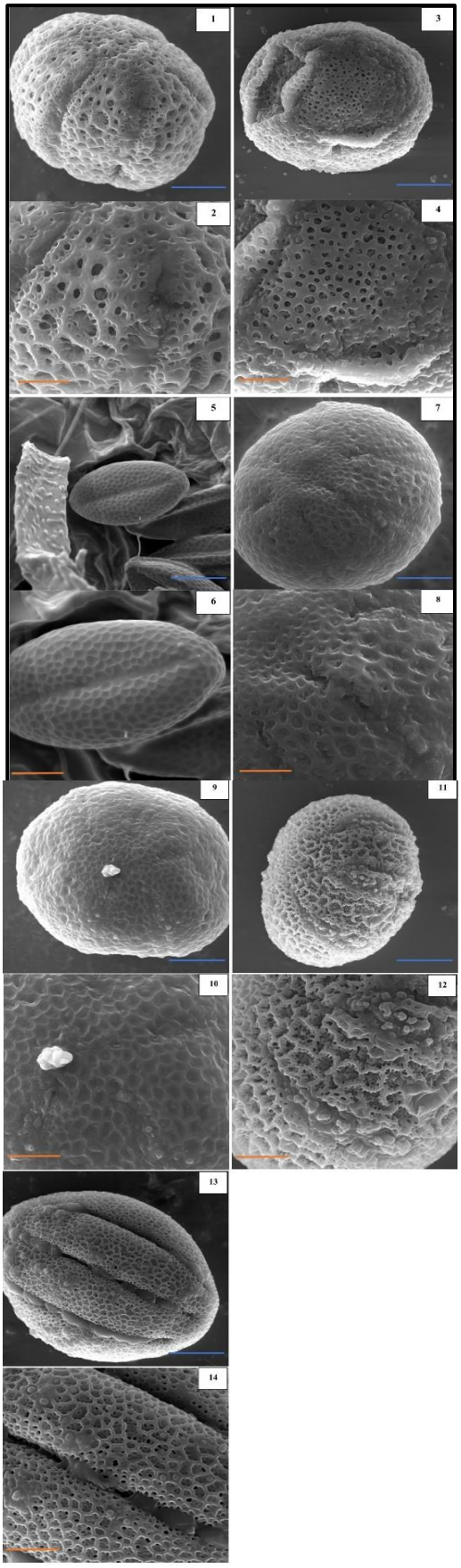

Figure 3: SEM micrographs of pollen grains in the *Salvia* taxa examined. (1-2) S. candidissma. (3-4) S. lanigera (5-6) S. officinalis. (7-8) S. indica. Scale bars: (1, 3, 5, 7) = 10 µm (2, 4, 6, 8) =10 µm.

REFERENCES

1- Abbas, A. F.; A. H. Al-Mousawi and A. H. E. Al-Musawi (2013). The Ecology and geographical distribution for the species of the genus Salvia L. of labiatae in Iraq. Baghdad Sci. J., 10(4):1082-1087.

2- Ahmad, S. A. (2016). Salvia ali-askaryi (Lamiaceae), a new species from Kurdistan, Iraq. Harvard Papers in Botany, 21(2):227-229.

3- Aktaş, K.; C. Özdemir; M. Özkan and P. Baran (2020). Pollen Morphology of Some Turkish Salvia L. (Lamiaceae: Mentheae) Species. Planta daninha, 38.

4- Al-Watban, A. A.; A. R. Doaigey, and M. El-Zaidy (2015).Pollen morphology of six species of subfamily Stachyoideae (Lamiaceae) in Saudi Arabia. African Journal of Plant Sci., 9(5):239-243.

5- Baylac, S. and P. Racine (2003). Inhibition of 5-lipoxygenase by essential oils and other natural fragrant extracts, Int. J. Aromather., 13:138-142.

6- Chalchat, J. C.; A. Michet and B. Pasquier (1998). Study of clones of Salvia officinalis L. yields and chemical composition of essential oil, Flavour Fragr. J., 13: 68-70.

7- Erdtman, G. (1952). Pollen Morphology and Plant Taxonomy. Angiosperms. Chronica Botanica Co., Waltham, Massachusettes.

8- Firat, M.; Başer, B. and Aziret, A. (2017). Pollen and nutlet micromorphology of a rare species Salvia kurdica (Lamiaceae) from Turkey. Pakistan Journal of Botany 49: 617−621.

9- Gürcan, H.; I. Potoglu and N. Öztürk (2016). Anatomical and palynological studies of three Salvia L. species in and around Eskisehir, Turkey. Bangladesh Journal Bot., 45:269-275.

Hassan, N.; Osman, A. K. and I. A. El-Garf, (2009). Pollen types of the Egyptian species of the genus Salvia (Lamiaceae). Feddes Repertorium 120: 394–404.

Kahraman, A.; M. Doğan, and F. Celep, (2011). Salvia siirtica sp. nov. (Lamiaceae) from Turkey. Nordic Journal of Botany, 29:397-401.

Kahraman, A.; Doğan, M. and Celep, F. (2009). Morphology, anatomy and palynology of Salvia indica L. (Labiatae). World Appl. Sci. J., 289-296.

Kiliç, F. M. (2021) Pollen morphological investigations of Salvia L. in southeastern of turkey and its taxonomic implication. Bangladesh J. Plant Taxon. 28(2): 395–403.

Moon, H. K.; S. Vinckier; J. B. Walker; E. Smets and S. Hysmans (2008). A search for phylogenetically informative pollen characters in the subtribe Salviinae (Mentheae: Lamiaceae). International Journal of Plant Sci., 169: 455–471. https://doi.org/10.1086/526463

Nasrallah, E. K. (2007). A comparative taxonomic study of the wild species Phlomis L and Sideritis L of Labiatae. Ph.D. thesis, University of Baghdad, College of Education, Ibn Al-Haytham, 453 pages.

Ozler, H.; Pehlivan, S.; Celep, F.; Doğan, M.; Kahraman, A.; Fişne, A. Y.; Başer, B. and S. Bagherpour (2013). Pollen morphology of Hymenosphace and Aethiopis sections of the genus Salvia (Lamiaceae) in Turkey. Turk J Bot 37: 1070-1084.

17- Reitsma, T. (1969). Size modification of recent pollen grains with different treatment. – Review of Palaeobotany and Palynology, 9:175-202.

Sass, J. E. (1958). Botanical Microtechnique, the Iowa State University Press, Ames.

ISSN: 2790-5306 (Print), 2790-5314 (Online)
مجلة الزراعة العراقية البحثية -وزارة الزراعة
متاح على الانترنت: www.ijarmoa.gov.iq
مجلد 26 العدد (1) 2022

IJAR
IRAQI JOURNAL OF AGRICULTURAL RESEARCH

19- Trudel, M. C. G. and J. K. Morton (1992). Pollen morphology and taxonomy in North American Labiatae. Canadian Journal of Botany, 70: 975–995.

20- Ulubelen, A. (2003). Cardioactive and antibacterial terpenoids from some Salvia species, Photochemistry, 64:395-399.

21- Walker, J. B. and K. J. Sytsma (2007). Staminal evolution in the genus Salvia (Lamiaceae): Molecular phylogenetic evidence for multiple origins of the staminal lever, Ann. Bot., 100:375-391.

22- Walker, J. W. and J. A. Doyle (1976). The basis of Angiosperm phylogeny, Palynology. Ann. Mo. Bot. Grad., 62:666-723.

دراسة تصنيفية باستخدام الصفات المظهرية الدقيقة لحبوب اللقاح لأنواع Salvia في العراق

خضر صكر الزاوي[1] اسراء عبد الرزاق مجيد الدبيسي[2] أيوب عبيد الفلاحي[3]

الكلمات الدالة: **Salvia**، حبوب اللقاح، المجهر الضوئي، المجهر الكهربائي

Email: kadersasa@gmail.com

الملخص

أجريت الدراسة الحالية باستخدام المجهر الضوئي والإلكتروني الماسح على حبوب لقاح سبعة أنواع من نبات **Salvia** التي تنمو في العراق، اذ تم جمع الأنواع من محافظة الأنبار والعديد من مناطق كردستان بين شهري مايو ويونيو من عام **2021**. كانت الأنواع جميعها تنمو برياً باستثناء نوع واحد تم زراعته وهو **S. officinalis** في الرمادي. اظهرت فحوص المجهر الضوئي (**LM**) والمسح المجهري الإلكتروني (**SEM**) اختلافات كبيرة بين حبوب اللقاح، وكانت حبوب اللقاح بأحجام مختلفة بين (المتوسطة والصغيرة)، أيضاً تباينت اشكال حبوب اللقاح من متطاول **prolate** الى شبه مفلطح **suboblate**. وكانت حبوب اللقاح في الأنواع كافة التي تمت فحصها هي **hexacolpate** ومتساوية الأقطاب **isopolar**، كانت الزخرفة السطحية مهمة في تمييز بعض الأنواع، حيث قسمت الأنواع المدروسة الى مجموعتين حسب نوع الزخرفة التي كانت اما **reticulate** او **bireticulate**. على الرغم من أن **bireticulate** كانت أكثر أنواعاً لزخرفة السطح شيوعاً في **mesocolpium** بينما كانت **reticulate** أكثر شيوعاً في **apocolpium**، مع هذا فأن النتائج الحالية قدمت بعض خصائص حبوب اللقاح المفيدة لتحديد الأنواع.

[1] كلية العلوم، جامعة الانبار، الرمادي، العراق.
[2] كلية العلوم، جامعة بغداد، بغداد، العراق.
[3] كلية الزراعة، جامعة الانبار، الرمادي، العراق.

تاريخ تسلم: أيلول/ 2022.
تاريخ القبول: تشرين اول/ 2022.