## Abstract

One of the most important allergens is pollen and a wide range of allergenic plants are in Leguminosae family-.\_In this study; four fresh allergen pollen of this family including Yellow *Melilotus officinalis* (L.) Desr. (Sweet-Clover), *Spartium junceum* L. (Spanish broom), *Robinia pseudoacacia* L. (Black Locust) and *Trifolium repens* L. (White clover) were taken from nature of Kermanshah. The pollen grains were studied by Light Microscopy (LM) for all studied species. In addition, the pollen grains of *S. junceum* were studied by Scanning Electron Microscopy (SEM). Results showed that the pollen grains of these genera were isopolar, spheroidal or subprolate and prolate, medium-size, tricolporate, triangular polar view, oval equatorial view and with microtuberculate or microreticulate ornamentation. Therefore, the genera of Fabaceae family isare very heterogeneous based on pollen grains features.

The morphology of some allergenic pollens of the 1 Leguminosae Family in Kermanshah province (West of Iran)

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*Keywords:* Allergenic plants, exine Surface ornamentation, Fabaceae, Pollen grain, Papilionaceae, Tricolporate.

# 1. INTRODUCTION

15 Kermanshah Province, situated in western Iran, spreads over an area of 25,000 km<sup>2</sup>. It lies 16 between lat. 45.5° and 48° E, long. 33.7° and 35.3° N. The province is bounded on the north 17 by Kurdestan province, on the south by llam province, on the southeast by Lorestan province, on the east by Hamedan province and on the west by Iraq country [<sup>1</sup>]. Several 18 palynological works had done in this area and included: [2] studied five genera of Vicieae 19 20 tribe by using electron microscopy and compared this data the other Fabaceae tribes. The pollen grains of Melilotus indica (Linn.) were investigated by using LM and TEM as allergen 21 pollen [3]. [4] presented an illustrated collection of Iranian plants and allergen pollens. 22 23 Allergenic effects of Spartium junceum L. and Lagerstroemia indica L. were investigated by 24 [5]. The Identification of allergen pollens were done in Kermanshah province by [6] and [7]. 25 Vicia hyrcanica Fisch. et C.A. Mey. from Fabaceae family examined by Light Microscope 26 and the pollen of this species is isopolae and trizonocolporate. Pollen morphology of four 27 species of Lathylus sativus L. were studied by [8] and showed that the pollen grains were

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28 medium to large size, oval and elongated oval with reticulate exine ornamentation. [9] studied pollen analysis of nineteen Chinese honeys. They identified sixty one pollen types 29 30 from thirty three plant families in Natural Honeys. Among the studied species, the pollen of 31 Robinia pseudoacacia L., Sophora japonica L. were Fabaceae type [10] investigated the 32 pollen morphology of sixteen Trifolium taxa in Istanbul by using the data obtained from LM 33 observations which of nine taxa were taken by SEM. The pollen grain Melilotus bicolor as an 34 endemic species from Turkey were investigated by [11]. In this research, the pollen grains 35 are generally trizonocolporate, radially symmetrical, isopolar and subprolate. Sculpturing is 36 usually microreticulate or, rarely, rugulate in the meridional optical section, and 37 microreticulate in the polar optical section [11]. The pollen morphology of Melilotus bicolor Boiss. & Balansa. belongs to the tribe Trifolieae was studied by [11]. According to this study, 38 39 the pollen grains were generally trizonocolporate and microreticulate ornamentation. The 40 obtained list of allergenic plants by [12], the pollen of Melilotus officinalis (L.) Desr. and 41 Trifolium repens L. had a weak allergen, and Black Locust (Robinia pseudoacacia L.) had a 42 moderate allergen effect. The aim of our reseach has been provided to identify a 43 micromorphological survey for four allergen species from Fabaceae family.

### 44 45 2. MATERIAL AND METHODS

Fresh pollen grains were taken from available cultivable plants in Kermanshah nature. The 46 47 pollen grains were acetolyzed according to [13] and mounted in glycerine jelly. The colpi 48 features and exine surface ornamentation studied using Dino capture camera mounted on a 49 Leitz light microscope HM-LUX3 model with a magnification of 400, also at least 25 pollen 50 grains measured in polar and equatorial view. Measurements were recorded using both a 40 51 x objective, and a crossed micrometer evepiece graticule. The pollen data for all species 52 examined are summarized in Table 1. for Light Microscopy. The anther of Spanish broom (Spartium junceum L.) samples were used to study of pollen grain by Scanning Electron 53 54 Microscopy (SEM). Then, the pollen grains located on sampling stanchion and gold sprayed 55 on pollens. At the end, Spanish broom pollen grains studied using Philips scanning electron 56 microscope model XL30 of Netherland. Micrographs with a magnification of 10000 prepared from Spanish broom pollen grains image. Descriptive terminology follows [14], [15] and [15]. 57 58

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### 60 3. RESULTS AND DISCUSSION

## 61 Pollen features of studied species are:

Yellow Sweet-Clover (*Melilotus officinals* (L.) Desr.): Pollen grain, isopolar, prolate, medium
(23.7.±2 μm), tricolporate (Fig. 1A), the general outline is elliptical spheroidal or triangular,
equatorial view elongated oval, polar axis dimensions 30.3-23.6-20.5 μm, equatorial axis

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65 dimensions 27.7 - 18.4 - 14.5 μm, P/E (polar axis/equatorial axis) 1.28, microtuberculate or

66 psilate ornamentation

Black Locust (*Robinia pseudoacacia* L.): Pollen grain isopolar, spheroidal, elongated oval
(Fig. 1B), medium (27.8 ± 1.9 μm), tricolporate, the general outline; polar view triangular
(Fig. 1C), equatorial view elongated oval, polar axis dimensions 31.9 – 27.8 -23.0 μm,
equatorial axis dimensions 29.7 - 26.3 - 22.9 μm, P/E 1.05, with tubercular or microechinate
surface ornamentation.
Spanish broom (*Spartium junceum* L.): Pollen grain isopolar, subprolate, medium (35.2 ± 2.1
μm), tricolporate, the general outline; prolate spheroidal or triangular (Fig. 1D), equatorial

- 74 view oval (Fig.1E), polar axis dimensions 30.6-35.2-40.2 μm, equatorial axis dimensions
- 75 24.4-30.8-36.8 μm, P/E 1.14, with microreticulate surface ornamentation (Fig. 2A). Muri solid
- 76 with  $0.2 1.0 \mu m$  wide. The Lumina are  $0.1 0.9 \mu m$  in diameter (Fig. 2B).

77 White clover (*Trifolium repens* L.): Pollen grain, isopolar, subprolate, medium (21.7  $\pm$  1.7 78  $\mu$ m), tricolporate, the general outline; prolate spheroidal or triangular (Fig. 1G), equatorial

79 view broad oval (Fig. 1F), polar axis dimensions 17.7-21.7-22.0  $\mu$ m, equatorial axis

- dimensions 16.2-18.4-22.0 μm, P/E 1.17 with microtubercular or psilate ornamentation.
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### 83 DISCUSSION

- 84 [17] considered Papilinaceae as the subfamily in Fabaceae family and following features 85 noted for studied pollen grains (450 genera of Europe); pollen grains tricolporate, poro-86 colporate and porate. Also they studied another species of Melilotus pollen grain in which is 87 similar to M. officinalis in aspect of size and colpi type (tricolporate). [2] demonstrated that 88 Fabaceae family pollen grains in studied genera are like as follow: In Arachis L., Onobrychis 89 Adans, Hedysarum L. tricolpate, and in Genera Galega L., Sophora L., Thermopsis, Lupinus L., Genista L., Laburmum Medic. tricolporate ,and in Ononis L., Medicago L., Trifolium L., 90 Melilotus Lotus L., Colutedea L., Chesneya Lind., Oxytropis DC., Glycyrrhiza L., triporate. In 91 92 majority of genera have reticulate ornamentation, and perforate ornamentation observed in 93 Lathyrus, Medicago, Colutea, and striate ornamentation in Coronilla, Glycyrrhiza. 94 The exine surface ornamentation in studied genera is similar to [2] investigation. According 95 to Gapotchka; the pollen of Trifolium, Ononis, Melilotus in Trifolieae tribe are similar each 96 other; on the other hand, the pollen grain of Medicago is almost similar to Lotus in Loteae 97 tribe. However, M. officianalis in tudied species has microtuberculate and psilate ornamentation; in spite of [2] who introduced it as reticulated one. In addition, results 98
- 99 obtained pollen grain of Spartium junceum L. using SEM from Kermanshah region scanning

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100 electron microscopy showed that pollen grain is spheroid, tricolpate and microreticulate 101 ornamentation.

102 M. bicolor as an endemic species from Turkey with microreticulate or, rarely, regulate

103 ornamentation were seen [11]. In our research, microtuberculate or psilate ornamentation

104 was observed in M. officinals. Thus, this character in this genus is varied and specified in 105 species level.

- 106 Both R. pseudoacacia and T. repens had a moderate allergen effect [12]. In our research R.
- 107 pseudoacacia had specific pollen micromorphological characters include: spheroidal shape
- 108 and with tubercular or microechinate surface ornamentation. Also, prolate spheroidal or
- triangular shapes with microtubercular or psilate ornamentation were seen for T. repens. 109
- 110 S. junceum L. had identified by by [5] as major allergen pollen type in west of Iran. The
- 111 microreticulate surface ornamentation and subprolate shape as qualitative characters were
- 112 distinct for this species.
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#### 114 **COMPETING INTERESTS**

115 The authors of this manuscript declare that they have no conflict of interests.

#### **AUTHORS' CONTRIBUTIONS** 117

- The authors designed this research, conducted the laboratory work, and wrote the 118
- 119 manuscript. All of the authors read and approved the final manuscript.

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	Specific name	Dollon aiza	polar avia (D)	oquatorial	D/E	Form	-
NU.	Specific name	Polien size (μm)	polar axis (P) (µm)	diameter (E) (µm)	P/E	FOIM	
1	Melilotus officinalis (L.) Desr.	23.6±2	20.5-23.6-30.3	14.5-18.4-27.7	1.28	prolate	_
2	Robinia pseudoacacia L.	27.8±1.9	23.0-27.8-32.0	23.0-26.3-29.7	1.05	spheroidal	
3	Spartium junceum L.	35.2±2.1	30.6-35.2-40.2	24.4-30.8-36.8	1.14	Subprolate	
1	Trifolium repens L.	21.7±1.7	17.7-21.7-22.0	16.2-18.4-22.0	1.17	Subprolate	_
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Fig. 2. Scanning electron micrographs of pollen grain in *Spartium junceum* L.: A) Polar view;

B) microreticulate ornamentation exine in detail.