



Development, phenol and flavonoid content, and allergenicity of *Juniperus polycarpus* pollen

Farkhondeh Rezanejad¹ · Tahereh Sazvar¹ · Elaheh ZamaniBahramabadi¹

Received: 15 July 2021 / Revised: 23 September 2021 / Accepted: 20 October 2021 / Published online: 12 December 2021
© The Author(s), under exclusive licence to Botanical Society of Sao Paulo 2021

Abstract

J. polycarpus K. Koch is a dioecious, wind-pollinated tree of Cupressaceae family. Morphological and anatomical study of male cone development of *J. polycarpus* showed that each cone consists of a central axis, to which sporangiophores are attached, 9–12 on average in each cone, placed in a whorled arrangement. Each sporangiophore has 3–5 microsporangia placed in one row. Male cone development was considered in 5 stages: (1) (October and November) differentiation of shoot apices toward reproductive apices, sporogenous cells in sporangia are surrounded by three wall layers, epidermis, the middle layer, and the tapetum. (2) (December and early January) size of cones increases, middle layer disintegrates, sporogenous cells go through successive mitotic divisions. (3) (late January and February) sporangia become evident between sporangiophores, microspore mother cells undergo meiosis, cytokinesis occurs simultaneously, tetrads are tetragonal and tetrahedral, tapetum shows significant growth and is secretory. (4) (early March) male cones and sporangia dehiscence, tapetum disappears as the pollens mature, only the epidermis with fibrous thickening is seen. (5) (late March) sporangiophores become empty and necrotic and pollen grains are dispersed. The phenolic, flavonoid and protein contents of pollen were 0.45, 0.0076 and 1.3 mg g⁻¹ dry weight, respectively. Allergenicity of *J. polycarpus* pollen was investigated through rat sensitization and immunoblot assay. The serum IgE reaction showed three allergen bands of 36, 41 and 44 kDa. Monocyte level increased due to injection of pollen protein extract, but neutrophils and lymphocytes did not show a significant change.

Keywords Anatomy · Cupressaceae · Light microscopy · Morphology · Pollen allergenicity · Western blot

1 Introduction

Juniperus is the most diverse genus in Cupressaceae and the second most diverse in all conifers after Pinus. Genus *Juniperus* can be divided into three monophyletic sections: *Caryocedrus*, *Juniperus*, and *Sabita*. Section *Juniperus* has 14 species, 12 in East Asia and the Mediterranean, and two distributed in North America. All species of this genus are evergreen trees that grow in rocky and mountainous areas (Perla et al. 2019). *J. polycarpus* is the most prevalent species of this genus in Iran. This tree has a long life and is dioecious and wind-pollinated (Ahani et al. 2013).

In *Juniperus* species, pollen cones are always uniaxial and present in an axillary position at the fertile shoot axis

(Dorken 2019). The structure of pollen cones has been studied in some *Juniperus* species. In *Juniperus drupacea* Labill., each pollen cone has an inflorescence-like arrangement of real pollen cones, high number of microsporangia per microsporangiphore, and microsporangia attached to the stalk in up to three subsequent rows (Dorken 2019). In *Juniperus rigida* Siebold & Zucc., the pollen cone is complex. Microsporangium wall is comprised of epidermis, middle layer and a tapetum. The development of the microspore mother cells is not synchronous. The microsporophylls have their microsporangia on the abaxial surface and axil (Yajuan and Xuezhi 2009).

Wind-pollinated plants provide low-quality pollen, which is due to the lack of food rewards and coloring pigments to attract pollinating insects (Hanley et al. 2008). One determinant of pollen quality is its protein content, which is notably higher in insect- than wind-pollinated pollen (Roulston et al. 2000).

Wind-pollinated plants produce large amounts of pollen and release it to the atmosphere, which can be dispersed

✉ Farkhondeh Rezanejad
frezanejad@uk.ac.ir

¹ Department of Biology, Faculty of Sciences, Shahid Bahonar University of Kerman, Bistodobahman street, 76169-14111 Kerman, Iran

over long distances and infect people with allergic asthma. Pollen allergenicity depends on the presence of hydrophilic proteins or glycoproteins (antigens) with a molecular weight of 10–70 kDa that are resistant to changes in pH and high temperature and can cause strong or weak allergic reactions in individuals. Pollen grains release these proteins upon contact with the respiratory mucosa, interacting with the human immune system to elicit an allergic response in susceptible individuals (Burge and Rogers 2000). Cupressaceae pollen is a major cause of seasonal rhinitis, conjunctivitis and asthma worldwide. Members of this family cause pollen allergy in the Mediterranean regions (*Cupressus sempervirens* L., *Cupressus arizonica* Greene and *Juniperus communis* L.), North America (*Juniperus ashei* J. Buchholz) and Japan (*Cryptomeria japonica* (L.f.) D. Don) (Di Felicea et al. 1994; Sin et al. 2008).

Because there is no published information on the development and allergenicity of *J. polycarpus* pollen, in this study, it was aimed to investigate male cone and pollen development from a morphological and anatomical view, and to determine the molecular weight of putative allergic proteins of this pollen through an immunoblot assay.

2 Materials and methods

Plant material – Male cones of *J. polycarpus* were collected once a week from October to March in Jiroft region, Iran. Each time almost 200 g male cones were collected from 20 different trees. Pollen of male cones that were collected during the pollination season (early March) were used for biochemical and allergenicity studies.

Morphological and anatomical studies – Fresh cones were observed using stereo and light microscopes (Olympus, Japan) and photographed. Each time, almost 100 fresh cones were fixed in FAA (formaldehyde: acetic acid: 70% ethanol, 5:5:90, v/v/v) for 24 h, washed with distilled water 4 times (each time for 5 min), dehydrated in 30%, 50%, 70%, 80%, 100% ethanol series and embedded in paraffin. 5 µm sections were cut by a rotary microtome (micro Tec, Germany), put on glass slides, stained by hematoxylin and eosin (Johansen 1940), observed by a light microscope (Olympus, Japan) and photographed.

Phenolics, flavonoids and protein content – The content of total phenolic compounds was measured using Folin-Ciocalteu method. To 0.5 g of each sample, 1.5 ml of 96% ethanol was added and the resulting mixture was kept in the dark for 24 h. After centrifugation at 6000 rpm for 10 min, 1 ml of the supernatant was collected, to which 1 ml of 96% ethanol was added, and the volume was reached to 5 ml with distilled water. 0.5 ml of 50% Folin reagent

and 1 ml of 5% sodium carbonate were added, and after 1 h of storage in the dark, the absorption intensity was read with a spectrophotometer at 725 nm. Gallic acid was used for preparing the standard curve (Bijami et al. 2020). The content of flavonoids was measured using aluminum chloride colorimetric method. 0.1 g of each sample was thoroughly ground in 10 ml of methanol and stored overnight. The extract was centrifuged at 6000 rpm for 10 min and the supernatant was collected. 0.5 ml of each extract was reached to 5 ml with distilled water. The solution, 0.3 ml of 5% NaNO₂ and after 5 min, 0.6 ml of 10% AlCl₃ were added. Then 2 ml of 1 M NaOH and 2 ml of distilled water were added. The absorption was measured with a spectrophotometer at 510 nm. Quercetin was used for preparing the standard curve (Bijami et al. 2020). For protein extraction, 0.5 g of pollen was poured to 3 ml of 0.1 M phosphate buffered saline (PBS) pH 7.4 and kept overnight in 4 °C. The mixture was centrifuged at 4 °C for 40 min at 6600 rpm, the resulting solvent was separated and centrifuged again at 6600 rpm for 5 min. The concentration of protein was obtained by Bradford method and albumin was used for preparing the standard curve (Bradford 1976). The protein extract was used as antigen. Biochemical measurements were done with 4 replications.

Rat sensitization and serum collection – All experiments were carried out in accordance with the national ethical requirements for animal research and approved by the Ethics Committee of Shahid Bahonar University (Ethics Code: EC/105). Twelve male Wistar rats, 4–6 weeks old, were purchased from the animal center of the Shahid Bahonar University of Kerman, Iran. They were randomly divided into control and treatment groups and housed in cages, six rats per cage, at 24 ± 2 °C with the altering 12 h:12 h light–dark cycle and fed with laboratory diet and water ad libitum. The rats were sensitized by injection of 75 µg protein of pollen extracts with alum or PBS as control, on six occasions at weekly intervals through the intraperitoneal route (Khajooie et al. 2013). The signs in injection sites were observed after 1 h of injection. After the injection period, the animals were nasally treated with pollen, 1 mg in 20 µl of PBS or 20 µl PBS alone for six consecutive days in the first and second weeks and four consecutive days in the third week (days 0–5, 7–12 and 14–17). The breathing mode of animals was determined clinically for 2 h after every nasal treatment (Khajooie et al. 2013). After the last week of nasal pollen application, the animals were decapitated and their blood was collected. A portion of blood was used to count white blood cells using Sysmex XN1000. From the rest of the blood, serum was obtained through centrifugation at 6600 rpm and stored at –20 °C until usage.

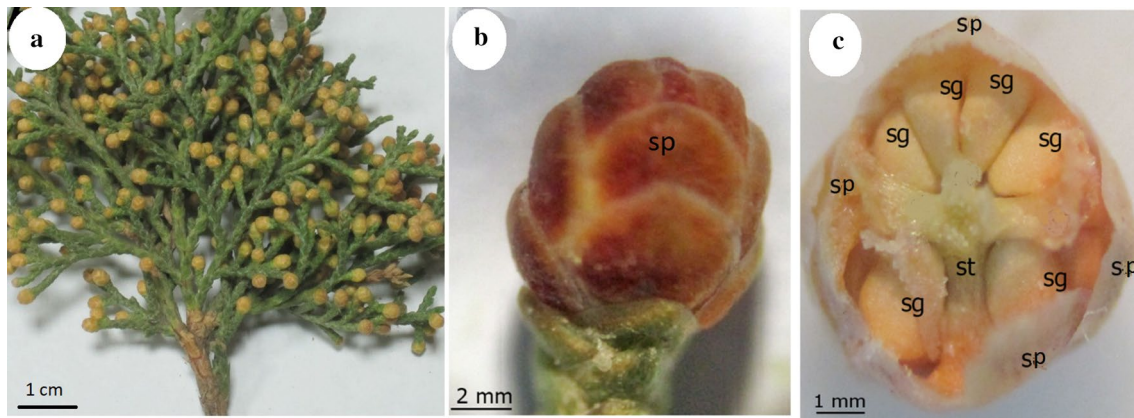


Fig. 1 Male cone structure of *J. polycarpus*. A stem with a number of male cones (a), exterior of a semi-mature male cone (b), internal view of a semi-mature male cone (c). *sg* sporangium, *sp* sporangiophore, *st* stem

SDS-PAGE and western blot assay – Pollen proteins were separated by 12.5% sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) (Laemmli 1970) and stained by Coomassie blue, or electro-transferred onto a 0.45 μ m pore size polyvinylidene difluoride (PVDF) membrane. A prestained protein ladder (Fermentase) was also used. Immunoblot analysis of IgE binding protein fractions was carried out by the method of Towbin et al. (1979). The membranes were blocked for 1 h at 4 °C in blocking buffer containing 5% non-fat milk powder in Tris Buffered Saline Tween 20 (TBST). The membranes were washed 3 times for 5 min in TBST and incubated for eight hours with 1:20 diluted serum in TBST. The membranes were washed and incubated for 1 h with 1:10,000 dilution of an anti-rat IgE, tagged with horse radish peroxidase in TBST. The membrane was washed and then treated by ECL chemiluminescence substrate (Amersham, UK). The allergen bands were revealed on X-OMAT Kodak film after 2 min of contact with membranes (Khajooie et al. 2013). The assays were repeated 4 times.

Statistical analysis – Data on the amount of white blood cells are presented as mean of repeats $\pm$ standard error of means. Duncan multiple range test was performed using SPSS software to compare the means at 0.05 significance level.

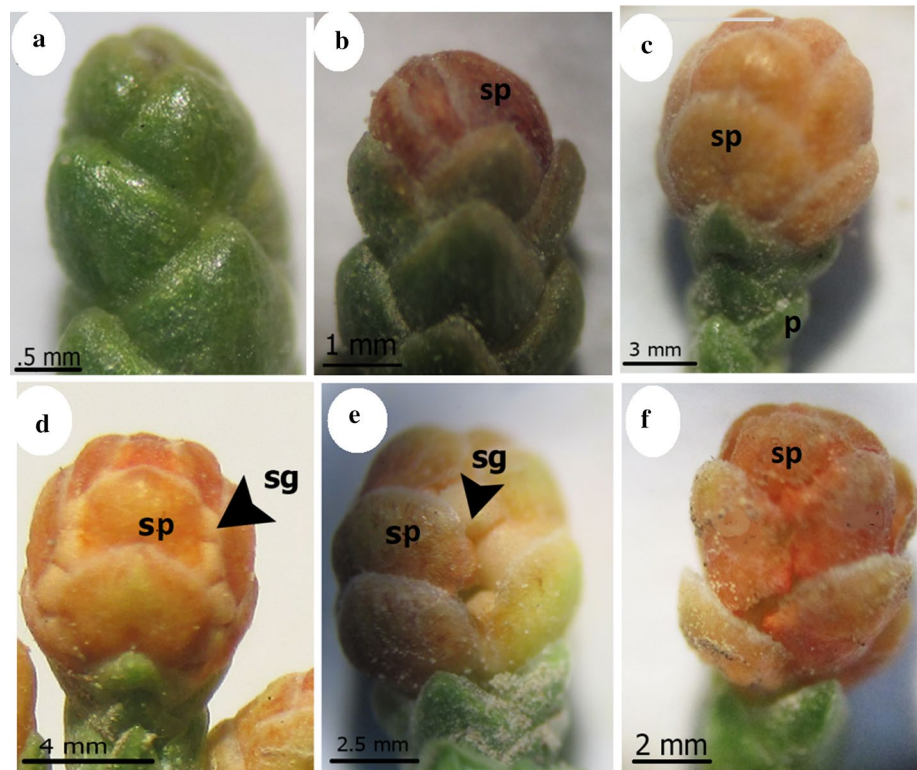
3 Results

General morphology of male cones of *J. polycarpus* – In early autumn, male cones begin to develop at the end of the branches. Their growth is limited and they do not become woody (Fig. 1 a). Each male cone consists of a central axis or stem that does not branch. To this axis, reproductive scales (microsporophyll or sporangiophore) are

attached. The number of scales was 9–12 on average, placed in a whorled arrangement. Each scale has 3–5 microsporangia or pollen sacs placed in one row. In the upper part of the cones, the size and number of scales are greatly reduced and they often lack pollen sacs (Fig. 1 b, c).

Development of male cones of *J. polycarpus* – Male cone development was considered in 5 stages morphologically and anatomically. In the first stage (October and November), the vegetative shoot apices begin to differentiate toward the reproductive apices, which are characterized by a change in color (Fig. 2 a, b). Pollen sacs are forming as masses of cells, in some of which the sporogenous cells are surrounded by three wall layers, including the epidermis, the middle layer, and the tapetum layer. Sporogenous tissue has cells with dense cytoplasm and high staining capacity (Fig. 3 a). In the second stage (December and early January), the size of the cones and the number of reproductive scales increase gradually. Cone size increment indicates the growth of pollen sacs. Cone maturation begins at the bottom and progresses to the top. At this stage, the scales are firmly attached to each other (Fig. 2 c). As the pollen sacs mature, the tapetum cells separate and the middle layer disintegrates. Sporogenous cells become microspore mother cells through successive mitotic divisions and differentiation. Microspore mother cells have large nuclei that indicate their readiness to begin meiotic division (Fig. 3b). In the third stage (late January and February), scales and internodes between them grow, and pollen sacs are placed between them (Fig. 2 d). Microspore mother cells produce dyad cell (a cell with two nuclei) and tetrads during meiosis. Cytokinesis occurs simultaneously and tetrads are tetragonal and tetrahedral. The tapetum layer shows significant growth at this stage and is of the secretory type (Fig. 3 c, d). In the fourth stage (early March), the male cones and pollen sacs dehiscence gradually and the pollen is released. Dehiscence starts from the bottom

Fig. 2 Developmental stages of the male cone of *J. polycarpus*. A vegetative shoot apex with a slight color change at the peak (a), the first stage in October and November (b), the second stage in December and early January with increasing number of reproductive scales (c), the third stage in late January and February with the growth of scales and internodes between them and the emergence of pollen sacs (d), the fourth stage in early March with the gradual dehiscence of cones and pollen sacs acropetally (e), the fifth stage in late March with empty pollen sacs (f), sg sporangium, sp sporangiophore



and progresses to the top of the cone (Fig. 2 e). The tapetum layer is decomposed gradually at this stage and disappears completely as the pollens mature, and only the epidermal layer with fibrous thickening is seen (Fig. 3 e, f). Stage 5 (late March) is the final stage. The scales open completely and the pollen sacs become empty and necrotic. Most of them stay on the tree for two to three weeks and eventually fall off the tree (Fig. 2 f). Pollen grains are dispersed (Fig. 3 g, h).

Amount of phenolic, flavonoid and protein compounds in pollen extract – The phenolic, flavonoid and protein compounds of pollen extract were 0.45 mg g^{-1} , 0.0076 mg g^{-1} and 1.3 mg g^{-1} dry weight, respectively.

Allergenicity of pollen extract *Skin tests.* After 12–24 h of intradermal injection of pollen protein extract, swelling and stiffness were observed at the injection site, but no redness (erythema) was observed. The diameter of the swelling was about 1.8 cm. No obvious changes were seen in the control group (Fig. 4).

Blood tests. Monocyte level increased due to injection of pollen protein extract, but neutrophils and lymphocytes did not show a significant difference comparing control at 0.05% level (Table 1).

Detection of pollen allergens. SDS-PAGE of pollen protein extract showed five distinct bands of 28, 35, 39, 43 and 68 kDa (Fig. 5). The serum IgE reaction of rats sensitized

by pollen protein extract showed three allergen bands with molecular weights of about 36, 41 and 44 kDa. No band was observed in control (Fig. 5).

4 Discussion

J. polycarpus tree is an anemophilous, dioecious species of Cupressaceae family. As we observed, the male cone of this tree is terminal and has an unbranched axis, containing hyposporangiate sporangiophores (scales, homologous to leaves), and has a number of microsporangia or pollen sacs. It has been reported that in the genus *Juniperus*, the sporangiophores are arranged either whorled (Section *Juniperus*) or decussate (Section *Sabina*), with a varying number of abaxial microsporangia, but mostly within just a single row (Dorken 2019). In this study, the number of scales in male *J. polycarpus* cones was observed to be 9–12, with 3–5 pollen sacs under each of them. This high number of pollen sacs provides enough pollen for wind pollination. It is known that the number of male cones per tree, the number of scales per cone, the number of pollen sacs per scale and the number of pollen grains per pollen sac are four characteristics important in determining the amount of pollen grains of a tree. Conifers release large amounts of pollen into the air, and the release of this high

Fig. 3 Stages of *J. polycarpus* pollen development. Sporogenous cells are surrounded by three wall layers including the epidermis, middle layer and tapetum layer (**a**), the tapetum cells separate and the middle layer disintegrates; sporogenous cells are transformed into microspore mother cells through mitotic divisions (**b**), microspore mother cells produce dyad cell (a cell with two nuclei) and tetrads through meiosis. Cytokinesis occurs simultaneously and tetrads are tetragonal and tetrahedral; tapetum is of the secretory type (**c, d**), the tapetum layer decomposes and disappears gradually, only the epidermal layer with fibrous thickenings is obvious (**e, f**), pollen grains are completely dispersed (**g**), hydrated pollen grains with thick intine and distinct exine, the arrowhead indicates a separated exine (**h**), *dc* dyad cell, *ep* epidermis, *ex* exine, *in* intine, *ml* middle layer, *mmc* microspore mother cell, *tc* tetrad cells, *tp* tapetum

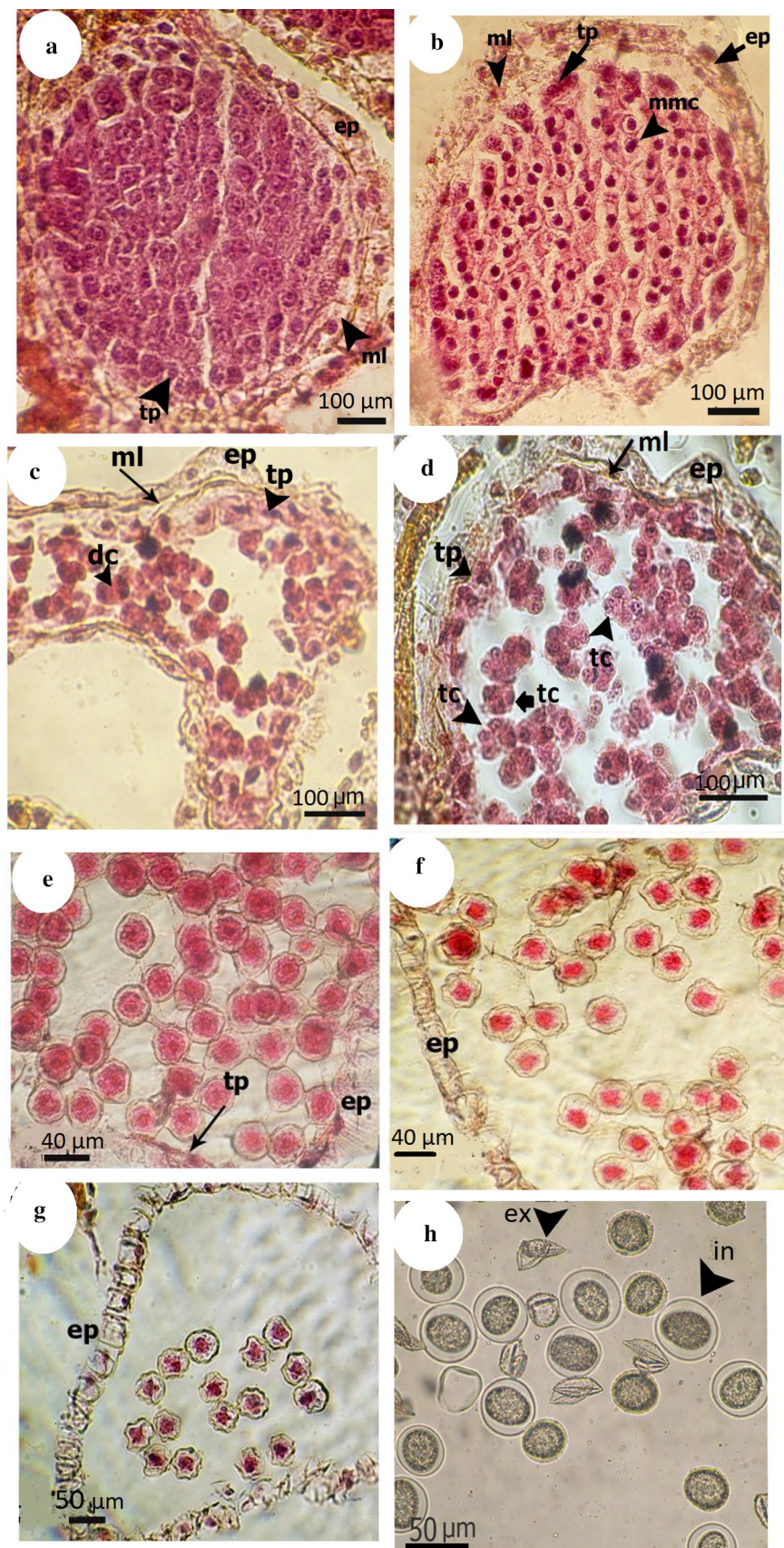


Fig. 4 Swelling due to injection of *J. polycarpus* pollen protein extract. Injection of pollen extract (a), control (b)

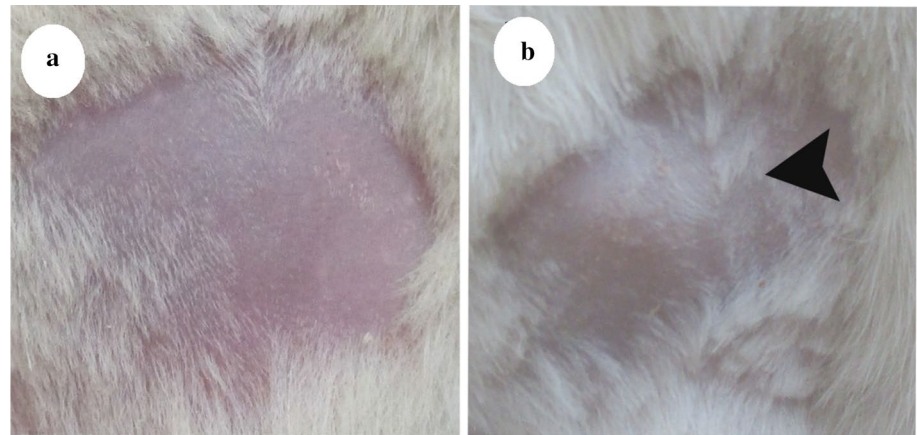


Table 1 Effect of *J. polycarpus* pollen protein extract injection on white blood cell count in rat blood

Neutrophil	Control	Pollen extract
Monocyte	13.33 ± 4.09a	18.00 ± 0.57a
Lymphocyte	1.00 ± 0.00b	3.33 ± 0.33a
	82.33 ± 2.60a	78.33 ± 0.88a

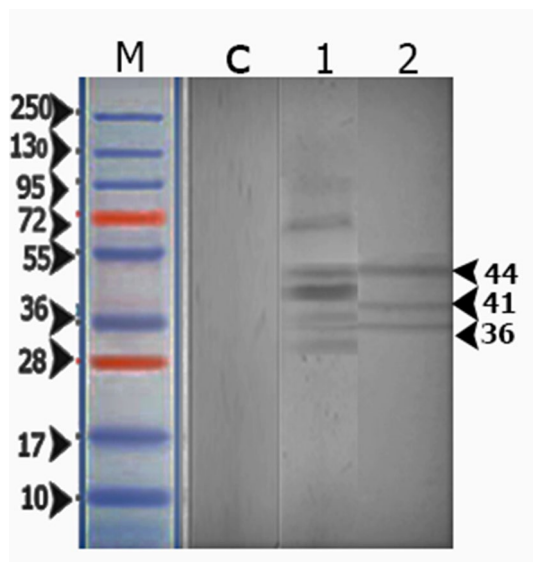


Fig. 5 Pollen protein electrophoresis profile (1) and pollen allergens (2) of *J. polycarpus* extract, M marker, C control

amount of pollen is important to ensure successful pollination (Hidalgo et al. 2003).

The pollen sac development in *J. polycarpus* is very slow. Autumn is spent only on the development of microspore mother cells. In early winter, meiosis and pollen development, and in late winter, pollination take place. Herein, pollen sacs appeared yellowish white in the last stage of

development, which is similar to *J. drupacea* (Dorken 2019). Pollen sac color at pollination time is dark yellow in *C. arizonica* and tend to turn white in *C. macrocarpa* (Hartw.) and *C. sempervirens* (Schulz et al. 2014). The pre-meiotic pollen sac contains bulky sporogenous tissue surrounded by three wall layers. In wind-pollinated plants, the epidermal layer is the only layer that remains in the adult anther and acts as a mechanical layer (Pacini 2000). Cupressaceae members produce small, spherical, monoporous, intectate pollen grains with a separate annulus ring (Hidalgo et al. 2003). These properties facilitate their dispersion by wind. These pollens do not have air sacs, their intine is hydrophilic and elastic, but exine is inelastic. Upon contact with water, pollen grains absorb water and the flexible intine swells, but the exine, which is not flexible, separates from the intine and the pollen (Takaso and Owens 2008). The morphological characteristics of pollen grains are very common and not sufficiently distinct in Cupressaceae and do not allow determination at subfamily level (Hidalgo et al. 2003).

Pollen allergens belong to a limited number of plant protein families. The pollen of Cupressaceae members is a major cause of pollinosis in Mediterranean regions, and its allergy prevalence has increased in the past few decades (Charpin et al. 2019). The highly dominant major allergens in Cupressaceae pollen, to which more than 90% of Cupressaceae pollen allergic patients are sensitized, belong to the pectate lyase protein family (Ehrenberg et al. 2020). Plants contain both allergens and antiallergy compounds, among which phenolic compounds are considered to have high antioxidant and antiallergic properties (Tanaka and Takahashi 2013). It is reported that some phenolic compounds are capable of inhibiting pectate lyase activity (Zaidi-Yahiaoui et al. 2008). In addition, flavonoids are able to inhibit the release of chemical mediators of cytokine production by mast cells and basophils (Kawai et al. 2007). However, our results revealed low concentrations of total phenolics and flavonoids in *J. polycarpus* pollen. The reason may be the wind pollination property of this pollen, as they do not need

coloring and animal-attracting substances. Protein content is usually low in most wind-pollinated plants, as well. The pollen of Cupressaceae family has been found to have a low protein content of about 2–3% of dry weight (Barletta et al. 1998).

Neutrophils are present in the airways and release mediators such as metalloproteins and elastases, which are found in the sputum or nasal secretions of allergic individuals. In severe cases of allergic asthma, blood neutrophils increase, but in people with mild to moderate asthma, or in healthy people, the level of these cells is low (Giudice et al. 2010). It seems that the injection of pollen extract did not cause severe asthma in the rats. Monocytes produce cytokines that activate lymphocytes and stimulate the inflammatory process (Furukawa and Altman 1978). Monocytes increased in treated rats, although the content of lymphocytes did not show a significant change.

Wind-pollinated plants release large amounts of small pollen into the air. The pollen of these plants contains certain proteins in its walls that cause allergies in sensitive individuals (Ehrenberg et al. 2020). Cupressaceae is known as the most important cause of winter allergy, and its symptoms are similar to the common cold, which can be confused with seasonal illnesses. In many parts of the world, especially in the Mediterranean, 30% of patients with pollen allergies suffer from this type of allergy (Ariano et al. 2006). Identifying pollen allergens of these plants is the first step in the correct diagnosis and treatment of this type of allergy. So far, several allergens have been identified from the pollen of Cupressaceae plants. These allergens belong to a small family of proteins, including pectate lyases, EF hand, Glycohydr 28, and Thaumatin (Radauer and Breiteneder 2006). The first group of these allergens are pectate lyase enzymes, the role of which is to promote pollen germination. In this group, Cup a 1, 43 kDa (Di Felicea et al. 1994), Cup s 1, 42 kDa (Ford et al. 1991), Jun a 1, 43 kDa (Midoro-Horiuti et al. 1999), cha o 1, 40 kDa (Kimura et al. 2008), and Cry j 1, 41–46 kDa (Fujimura et al. 2007) have been identified and purified from *C. arizonica*, *C. sempervirens*, *J. ashei*, *Chamaecyparis obtusa* (Siebold & Zucc.) Endl., and *C. japonica*, respectively. There is no report on the putative allergens of *J. polycarpus*. Our results revealed three allergens with molecular weights of about 36, 41 and 44 kDa. The molecular weights of these three allergens are very close to the previously identified pollen allergens of Cupressaceae family and may be homologues to them.

In conclusion, the development of the male cone of *J. polycarpus* continues from early autumn to late March. The male cone consists of a central axis and a number of scales (9–12), under each of which are microsporangia (3–5). Sporangia have three wall layers including epidermis, middle layer and tapetum. Tapetum is of secretory type. Mature pollens are small and spherical, which facilitates their

dispersion by wind. After water absorption, the flexible intine swells, but the exine, which is not flexible, separates from the intine and the pollen. Low concentrations of total phenolics and flavonoids in *J. polycarpus* pollen may be due to the wind pollination property of this pollen, as they do not need coloring and animal-attracting substances. Three protein allergens were detected in rats sensitized by pollen protein extract with molecular weights close to previously identified pollen allergens in Cupressaceae. Since this species is wind-pollinated and its pollen can be transferred for long distances, it can be the case of seasonal rhinitis, conjunctivitis and asthma.

Author contribution FR planned the experiment. TS conducted the lab experiments. EZB prepared the manuscript.

Declarations

Conflict of interest All authors agree on the content of the paper and have no conflict of interest.

References

- Ahani H, Jalilvand H, Nasr SMH, Kouhbanani HS, Ghazi MR, Mohammadzadeh H (2013) Reproduction of juniper (*Juniperus polycarpus*) in Khorasan Razavi. Iran for Sci Pract 15:231–237
- Ariano R, Mistrello G, Mincigrucci G, Bricchi E, Iannotti O, Frenquelli G, Passalacqua G, Panzani RC (2006) In vitro and in vivo biological activities of old and fresh *Cupressus arizonica* pollen. J Investig Allergol Clin Immunol 16:177–182
- Barletta B, Tinghino R, Corinti S, Afferni C, Iacovacci P, Mari A, Pini C, Di Felice G (1998) Arizona cypress (*Cupressus arizonica*) pollen allergens. Identification of crossreactive periodate-resistant and sensitive epitopes with monoclonal antibodies. Allergy 53:586–593
- Bijami A, Rezanejad F, Oloumi H, Mozafari H (2020) Minerals, antioxidant compounds and phenolic profile regarding date palm (*Phoenix dactylifera* L.) seed development. Sci Hortic 262:109
- Bradford M (1976) A rapid and sensitive method for the quantitation of quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–254
- Burge HA, Rogers CA (2000) Outdoor allergens. Environ Health Perspect 108:653
- Charpin D, Pichot C, Belmonte J, Belmonte J, Sutra JP, Zidkova J, Chanez P, Shahali Y, Senechal H, Poncet P (2019) Cypress pollinosis: from tree to clinic. Clin Rev Allergy Immunol 56:174–195
- Di Felicea G, Caiaffab MF, Barilettob G, Affernia C, Di Paolab R, Maria A, Palumbo S, Thinghino R, Sallusto F, Tursi A, Macchiaia L, Pini C (1994) Allergens of Arizona cypress (*Cupressus arizonica*) pollen: characterization of the pollen extract and identification of the allergenic components. J Allergy Clin Immunol 94:547–555
- Dorken VM (2019) Are the male reproductive units of *Juniperus drupacea* Labill. (Cupressaceae s.str.) a compound, polyaxial inflorescence or a dense cluster of simple, uniaxial pollen cones? Feddes Repertorium 0:1–8
- Ehrenberg AE, Klingebiel C, Östling J, Larsson H, Mattsson L, Vitte J, Lidholm J (2020) Characterization of a 7 kDa pollen allergen

- belonging to the gibberellin-regulated protein family from three Cupressaceae species. *Clin Exp Allergy* 50:964–972
- Ford SA, Baldo BA, Panzani R, Bass D (1991) Cypress (*Cupressus sempervirens*) pollen allergens: identification by protein blotting and improved detection of specific IgE antibodies. *Int Arch Allergy Immunol* 95:178–183
- Fujimura T, Futamura N, Midoro-Horiuti T, Togawa A, Goldblum RM, Yasueda H, Saito A, Shinohara K, Masuda K, Kurata K, Sakaguchi M (2007) Isolation and characterization of native Cry j 3 from Japanese cedar (*Cryptomeria japonica*) pollen. *Allergy* 62:547–553
- Furukawa CT, Altman LC (1978) Defective monocyte and polymorphonuclear leukocyte chemotaxis in atopic disease. *J Allergy Clin Immunol* 61:288–293
- Giudice MM, Pedulla M, Brunese FP, Capristo AF, capristo C, (2010) Neutrophilic cells in sputum of allergic asthmatic children. *Eur J Inflamm* 8:151–156
- Hanley ME, Franco M, Pichon S, Darvill B, Goulson D (2008) Breeding system, pollinator choice and variation in pollen quality in British herbaceous plants. *Funct Ecol* 22:592–598
- Hidalgo PJ, Galan C, Dominguez-Vilches E (2003) Male phenology of three species of Cupressus: correlation with airborne pollen. *Trees* 17:336–344
- Johansen DA (1940) Plant microtechnique. McGraw-Hill, New York
- Kawai M, Hiranol T, Higa S, Amrimitsu J, Maruta M, Kuwahara Y, Ohkawara T, Hagihara K, Yamadori T, Shima Y, Ogata A, Kawase I, Tanaka T (2007) Flavonoids and related compounds as anti-allergic substances. *Allergol Int* 56:113–123
- Khajooie A, Rezanejad F, Esmaeili-Mahani S, Badoei-Dalfard A (2013) Study on allergenicity of *Thuja orientalis* pollen grains in rat. *Aerobiologia* 29:413–417
- Kimura Y, Kuroki M, Maeda M, Okano M, Yokoyama M, Kino K (2008) Glycoform analysis of Japanese cypress pollen allergen, Cha o 1: a comparison of the glycoforms of cedar and cypress pollen allergens. *Biosci Biotechnol Biochem* 72:485–491
- Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 227:680–685
- Midoro-Horiuti T, Goldblum RM, Kurosky A, Goetz DW, Brooks EG (1999) Isolation and characterization of the mountain cedar (*Juniperus ashei*) pollen major allergen, Jun a 1. *J Allergy Clin Immunol* 104:608–612
- Pacini E (2000) From anther and pollen ripening to pollen presentation. *Plant Syst Evol* 222:19–43
- Perla F, Oriane H, Thierry R, Sonja SY, Leitch Ilia J, Adams Robert P, BouDagher-Kharrat M (2019) Polyploidy in the conifer genus *Juniperus*: An unexpectedly high rate. *Front Plant Sci* 10:676
- Radauer C, Breiteneder H (2006) Pollen allergens are restricted to few protein families and show distinct patterns of species distribution. *J Allergy Clin Immunol* 117:141–147
- Roulston TH, Cane JH, Buchmanns L (2000) What governs protein content of pollen: pollinator preferences, pollen–pistil interactions, or phylogeny? *Ecol Monogr* 70:617–643
- Schulz C, Klaus KV, Knopf P, Mundry M, Dorken V, Stutzel T (2014) Male cone evolution in conifers: Not all that simple. *Am J Plant Sci* 5:2842
- Sin AZ, Ersoy R, Gulbahar O, Ardeniz O, Gokmen NM, Kokuludag A (2008) Prevalence of cypress pollen sensitization and its clinical importance in Izmir, Turkey, with cypress allergy assessed by nasal provocation. *J Investig Allergol Clin Immunol* 18:46–51
- Takaso T, Owens JN (2008) Significance of exine shedding in Cupressaceae-type pollen. *J Plant Res* 121:83–85
- Tanaka T, Takahashi R (2013) Flavonoids and asthma. *Nutrients* 5:2128–2143
- Towbin H, Staehelin T, Gordon J (1979) Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: Procedure and some applications. *Proce Natl Acad Sci USA* 76:4350–4354
- Yajuan CZSFL, Xuezhi FJW (2009) Microsporogenesis and male gametophyte development of *Juniperus rigida*. *Scientia Silvae Sinicae* 1:1–10
- Zaidi-Yahiaoui R, Zaidi F, Bessai A (2008) Influence of gallic and tannic acids on enzymatic activity and growth of *Pectobacterium chrysanthemi* (*Dickeya chrysanthemi* bv. *chrysanthemi*). *Afr J Biotech* 7:482–486

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.