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Palynological Characterization of Edible and Allergenic Amaranthaceae Species by Scanning Electron Microscopy: Implications for Food Authentication, Allergen Monitoring, and Quality Control in the Food Industry

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ABSTRACT

The Amaranthaceae family includes several economically important food crops (e.g., Beta vulgaris – beet, Amaranthus spp. – amaranth grain, Chenopodium spp. – quinoa relatives) as well as highly allergenic weed species whose pollen can contaminate food production environments and pose health risks to workers and consumers. This study presents a comparative palynological analysis of eight Amaranthaceae species (Amaranthus albus, A. hybridus, A. viridis, Beta vulgaris, Chenopodium album, C. ficifolium, Chenopodiastrum murale, and Nitrosalsola vermiculata) using scanning electron microscopy (SEM). Quantitative and qualitative pollen traits – including polar/equatorial diameters (P/E ratio), exine thickness, aperture type and number, and surface ornamentation – were measured and statistically analysed. Results revealed significant interspecific variation: pollen size ranged from small (14–26 µm in Amaranthus and Nitrosalsola) to large (55–70 µm in Beta vulgaris and Chenopodium album); P/E ratios indicated sub-spheroidal to spheroidal shapes; most taxa exhibited pantoporate apertures (10–40 pores) except A. hybridus (tricolpate); exine thickness varied from 1.0 to 6.0 µm; and ornamentation patterns ranged from psilate-microperforate to densely echinate. These distinctive pollen features can serve as reliable markers for species identification, enabling food manufacturers to authenticate plant-based ingredients, monitor airborne allergen contamination in processing facilities, and develop targeted allergen-control strategies. From a food industry perspective, this palynological approach offers a practical, cost-effective tool for: (i) verifying the authenticity of amaranth, quinoa, and beet-derived ingredients; (ii) detecting contamination by allergenic weed pollen in raw materials; (iii) assessing airborne allergen exposure in processing environments; and (iv) supporting compliance with food safety regulations and allergen labelling requirements. The findings provide a valuable taxonomic and palynological baseline for the food industry, supporting the safety, traceability, and quality assurance of Amaranthaceae-derived food products.

1- INTRODUCTION

The Amaranthaceae family (including the former Chenopodiaceae) comprises approximately 175 genera and over 2,000 species of herbs, shrubs, and small trees distributed worldwide, with high abundance in saline, desert, and semi-desert regions [1, 2]. This family is of considerable economic importance to the food industry, as it includes several staple and specialty food crops:

- ***Beta vulgaris*** – sugar beet (sugar production) and beetroot (vegetable and natural colourant).
- ***Amaranthus* spp.** – amaranth grain, a gluten-free pseudocereal rich in protein, fibre, and minerals, increasingly used in health foods and gluten-free formulations.
- ***Chenopodium* spp.** – quinoa (*C. quinoa*) and other edible species, valued for their complete protein profile and used in salads, flours, and snacks [3, 4].

The increasing consumer demand for gluten-free, plant-based, and functional foods has driven a significant expansion in the cultivation and processing of amaranth, quinoa, and beet products [5]. Amaranth grain, in particular, is gaining popularity as a nutritious alternative to traditional cereals, with a protein content of 13–18% and a balanced amino acid profile [6]. Quinoa, recognised as a complete protein source, has seen a dramatic increase in global production over the past decade [7]. Beetroot and sugar beet are widely used as natural colourants, sweeteners, and functional ingredients in processed foods [8].

However, many Amaranthaceae species are also prolific weed plants that produce large quantities of highly allergenic pollen, which can contaminate food crops during harvesting, processing, and storage, posing a risk of allergic reactions in sensitised individuals [9, 10]. Pollen allergens from *Amaranthus* and *Chenopodium* are recognised as significant aeroallergens worldwide, and their presence in food manufacturing environments can compromise worker health and product safety [11]. Occupational exposure to airborne pollen allergens in food processing facilities, particularly in grain handling and milling operations, is a growing concern for worker health and regulatory compliance [12].

From a food industry perspective, the accurate identification of Amaranthaceae species is critical for three interconnected purposes:

1. **Ingredient Authentication:** With the rising premium price of amaranth, quinoa, and specialty beet products, the risk of adulteration with cheaper species is increasing [13]. Pollen-based authentication offers a rapid, reliable method for verifying the botanical origin of raw materials and detecting fraudulent substitution.
2. **Allergen Risk Assessment:** Airborne pollen from allergenic species can settle on food products during processing and packaging, posing a risk to sensitised consumers [14]. The

ability to identify and quantify specific pollen types in food processing environments enables targeted allergen control measures.

3. **Quality Control:** The presence of weed pollen and seeds in harvested crops can affect product purity, safety, and shelf life [15]. Early detection of contamination through palynological screening supports proactive quality management.

Palynology – the study of pollen morphology – offers a powerful tool for species-level discrimination based on stable, genetically determined pollen traits such as size, shape, aperture type, and exine ornamentation [16, 17]. Scanning electron microscopy (SEM) provides high-resolution surface details that are particularly useful for distinguishing closely related taxa, which may have similar macroscopic features but distinct palynological signatures [18, 19]. SEM-based pollen analysis has been successfully applied in food authentication, honey origin verification, and allergen source identification [20, 21].

Despite the taxonomic, economic, and allergenic significance of this family, comprehensive SEM-based pollen characterisation of Iraqi Amaranthaceae species has been limited. This study therefore aimed to: (i) document the pollen morphological characteristics of eight representative species using SEM; (ii) identify diagnostic traits that can differentiate between edible and allergenic species; and (iii) discuss the practical applications of these findings for food authentication, allergen monitoring, and quality control in the food industry.

2-MATERIALS AND METHODS

2.1. Plant Material Collection

Pollen grains of eight species – *Amaranthus albus* L., *Amaranthus hybridus* L., *Amaranthus viridis* L., *Beta vulgaris* L., *Chenopodium album* L., *Chenopodium ficifolium* Sm., *Chenopodiastrum murale* (L.) S.Fuentes, Uotila & Borsch, and *Nitrosalsola vermiculata* (L.) Theodorova – were collected from mature anthers under natural field conditions from various districts in Iraq between March 2024 and March 2025. Plant identification followed standard taxonomic keys [22–25]. For each species, a minimum of 30 pollen grains were randomly selected and measured to ensure statistical reliability.

2.2. Scanning Electron Microscopy (SEM)

Mature anthers were air-dried, and pollen grains were directly mounted on aluminium stubs with double-sided carbon adhesive tape, without chemical acetolysis to preserve natural exine ornamentation [26]. Samples were coated with gold using a sputter coater and examined under a scanning electron microscope at 20.0 kV accelerating voltage under secondary electron (SE) detection mode. Micrographs were taken at magnifications between 1.00 k× and 5.00 k×. Polar and equatorial views were recorded where possible.

2.3. Palynological Measurements and Statistical Analysis

Quantitative parameters – polar axis (P), equatorial axis (E), P/E ratio, exine thickness, aperture size, and number of

apertures – were measured using digital image analysis software. Data were expressed as mean ± standard deviation (SD). Pollen traits were evaluated for their diagnostic value in species discrimination, with particular focus on traits that could be used for food authentication and allergen identification.

3- RESULTS

3.1. Pollen Morphological Features

The SEM micrographs (Figure 1) and quantitative measurements (Table 1) revealed substantial interspecific variation. These diagnostic pollen traits provide a reliable basis for species identification in food authentication and allergen monitoring applications.

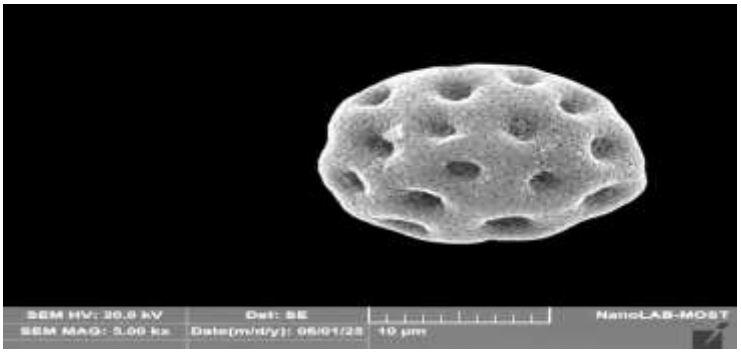
Pollen size: Polar diameter (P) ranged from 14 µm (*N. vermiculata*) to 70 µm (*B. vulgaris*), while equatorial diameter (E) ranged from 15 µm to 65 µm. Overall mean values were P ≈ 29.6 µm and E ≈ 28.6 µm. This wide size range allows clear differentiation between edible species (e.g., *B. vulgaris* with large pollen) and allergenic weed species (e.g., *N. vermiculata* with small pollen), facilitating rapid screening of raw materials.

Shape (P/E ratio): *Amaranthus* species were slightly prolate (P/E ≈ 1.05–1.15), while *Chenopodium*, *Chenopodiastrum*, and *Nitrosalsola* were spheroidal to slightly oblate (P/E ≈ 0.90–1.00). Shape differences, while subtle, provide additional discrimination between closely related taxa.

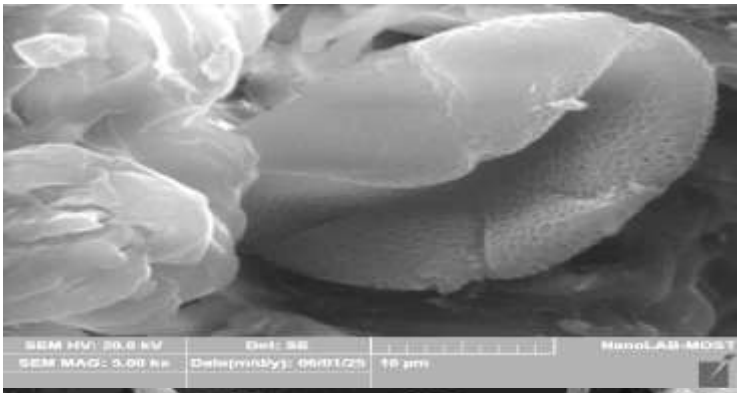
Aperture type: Most taxa were pantoporate with 10–40 pores; *A. hybridus* was distinctly tricolpate, a unique feature. The tricolpate aperture in *A. hybridus* is a rare and distinctive feature that could serve as a specific marker for identifying this allergenic species in mixed pollen samples – particularly useful for allergen source tracking in food facilities.

Exine thickness: Ranged from 1.0 µm (*N. vermiculata*) to 6.0 µm (*B. vulgaris*); larger pollen grains tended to have thicker exine.

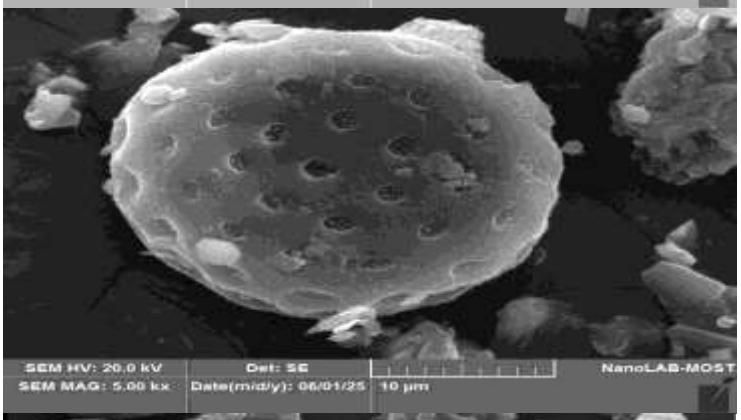
Ornamentation: Varied from psilate–microperforate and granulate to densely echinate. Echinate species (e.g., *B. vulgaris*, *A. albus*, *C. album*) had spines up to ~10 µm, providing clear separation from smooth-ornamented taxa.

No.	Species	
1	*Amaranthus albus* L.	

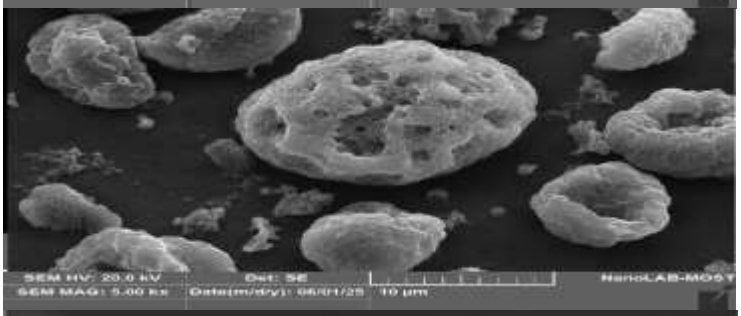
2 *Amaranthus hybridus* L.



3 *Amaranthus viridis* L.



4 *Beta vulgaris* L.



5 *Chenopodium album* L.

6 *Chenopodium ficifolium* Sm.

7 *Chenopodiastrum murale* (L.) S.Fuentes, Uotila & Borsch

8 *Nitrosalsola vermiculata* (L.) Theodorova

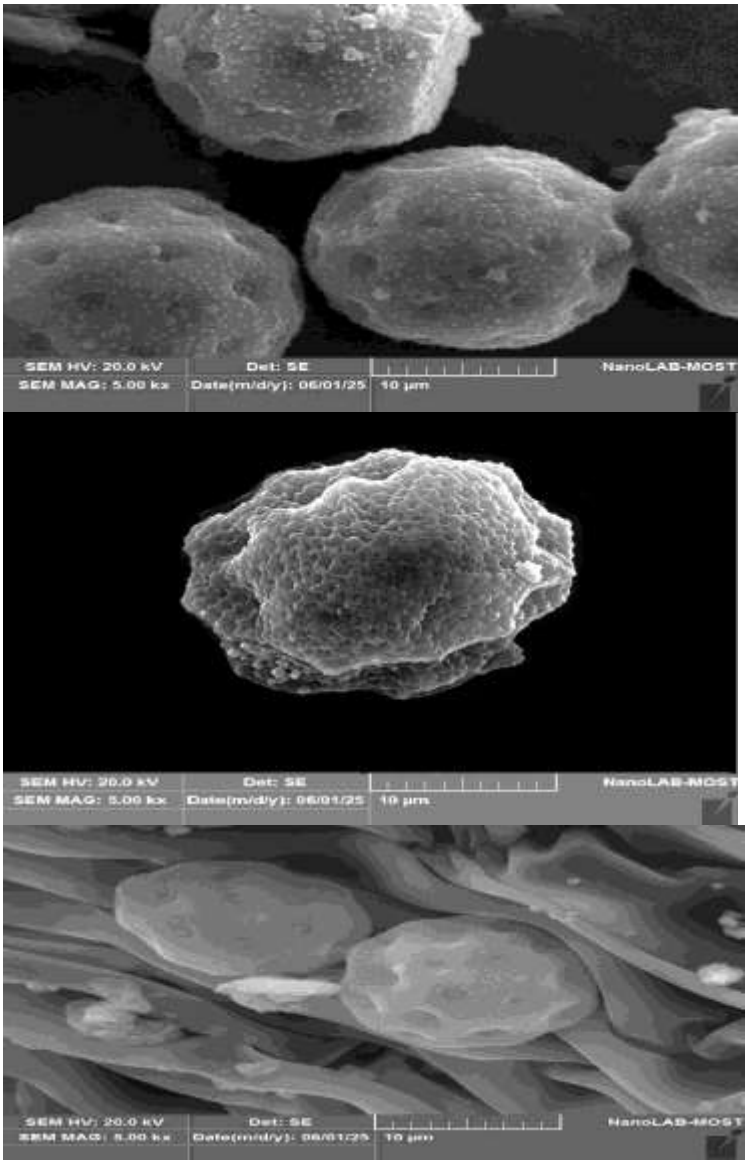


Figure 1: External appearance of pollen grains of the studied species

Table 1. Quantitative and qualitative measurements of pollen grains of the studied species

Species	P (µm)	E (µm)	P/E Ratio	Exine (µm)	Aperture/Pore (µm)	No. of Apertures	Ornamentation	Mean P ± SD	Mean E ± SD
Amaranthus albus	18–22	16–20	1.05–1.15	1.5–2.5	2.5–4.0	Pantoporate (~20–40)	Echinate	20 ± 2	18 ± 2
Amaranthus hybridus	22–26	20–24	1.05–1.10	1.2–2.0	2–4 (colpus width)	Tricolpate	Reticulate	24 ± 2	22 ± 2

Amaranthus viridis	20–24	18–22	1.05–1.10	2.0–3.0	1.5–2.5	Pantoporate (~25–40)	Microperforate	22 ± 2	20 ± 2
Beta vulgaris	55–70	50–65	1.05–1.10	4.0–6.0	4–8	Pantoporate	Strongly echinate	62.5 ± 7.5	57.5 ± 7.5
Chenopodium album	45–60	42–58	1.03–1.08	3.5–5.5	3–6	Pantoporate	Densely echinate	52.5 ± 7.5	50 ± 8
Chenopodium ficifolium	18–24	20–26	0.90–1.00	1.5–2.5	1.0–2.0	Pantoporate (~20–35)	Granulate	21 ± 3	23 ± 3
Chenopodiastrum murale	16–22	18–24	0.90–1.00	1.2–2.2	1.0–2.0	Pantoporate (~18–30)	Microverrucate	19 ± 3	21 ± 3
Nitrosalsola vermiculata	14–18	15–20	0.90–1.00	1.0–1.8	0.8–1.5	Pantoporate (~10–20)	Psilate–microperforate	16 ± 2	17.5 ± 2.5

Table 2. Summary of pollen parameters across all species

Parameter	Minimum	Maximum	Overall Mean
Polar diameter (P)	14 µm	70 µm	~29.6 µm
Equatorial diameter (E)	15 µm	65 µm	~28.6 µm
Exine thickness	1.0 µm	6.0 µm	~2.6 µm
Aperture size	0.8 µm	8.0 µm	~2.9 µm

3.2. Ecological and Distribution Data

Tables 3–5 provide distribution coordinates, phenology, habitats, and altitudes for each species. These data are relevant to food industry risk assessment, as they indicate the proximity of allergenic pollen-producing species to agricultural and processing areas. For instance, *A. hybridus* and *A. viridis* are widespread in central Iraq, including urban areas near food processing facilities, while *B. vulgaris* and *C. album* occur in both arid and cultivated environments. This spatial information can guide allergen monitoring strategies in food manufacturing regions.

Table 3. Longitude and latitude lines of distribution of the studied species

No.	Species	Region	Coordinates (N, E)
1	*Amaranthus albus* L.	Suwaira	32°56′17.1″ N, 44°47′18.6″ E
		Tarmiya	33°39′32.9″ N, 44°22′29.7″ E
2	*Amaranthus hybridus* L.	Jadiriyah (Baghdad)	33°16′07.9″ N, 44°23′20.7″ E
3	*Amaranthus viridis* L.	Dora (Baghdad)	33°17′04.4″ N, 44°25′15.2″ E
		Zafaraniya (Baghdad)	33°15′06.6″ N, 44°31′38.7″ E
4	*Beta vulgaris* L.	Rutba	33°02′16.4″ N, 40°16′54.2″ E
		Ali ash Sharqi (Amara)	32°06′29.0″ N, 46°44′40.8″ E
5	*Chenopodium album* L.	University of Baghdad	33°16′48.5″ N, 44°23′07.3″ E
		Falluja	33°20′22.8″ N, 43°45′50.8″ E
6	*Chenopodium ficifolium* Sm.	Abu Ghraib	33°17′00.9″ N, 44°05′07.9″ E
		Baghdad	33°17′24.2″ N, 44°25′42.2″ E
		Ali Al-Garbi (Amara)	32°27′54.0″ N, 46°41′16.9″ E
7	*Chenopodiastrum murale* (L.) S. Fuentes, Uotila & Borsch	Baghdad	33°18′52.7″ N, 44°24′32.8″ E
		Ramadi	33°26′39.8″ N, 43°15′24.6″ E
8	*Nitrosalsola vermiculata* (L.) Theodorova	Rutba	33°02′07.5″ N, 40°16′59.5″ E
		Amara	31°51′58.4″ N, 47°06′41.2″ E

Table 4. Flowering and fruiting periods, common name, and synonyms

No.	Species	Flowering & Fruiting Period	Local Name	Synonyms
1	*Amaranthus albus* L.	Flowering: Jun.–Sep.; Fruiting: Aug.–Nov.	Rutha	*Euxolus albidus* (L.) E.H.L. Krause; *Galliaria albida* (L.) Bubani; *Glomeraria alba* (L.) Cav.
2	*Amaranthus hybridus* L.	Flowering & Fruiting: Sep.–Dec.	Smooth pigweed	*Galliaria hybrida* (L.) Nieuwl.; *Galliaria patula* (Bertol.) Bubani; *Amaranthus aureus* Moq.
3	*Amaranthus viridis* L.	Flowering & Fruiting: Mar.–May & Aug.–Oct.	Pigweed	*Albersia caudata* (Jacq.) Boiss.; *Glomeraria viridis* (L.) Cav.; *Pyxidium viride* (L.) Moq.
4	*Beta vulgaris* L.	—	—	—
5	*Chenopodium album* L.	Flowering & Fruiting: May–Sep.	Goosefoot	*Atriplex alba* (L.) Crantz; *Botrys albus* (L.) Nieuwl.; *Botrys pagana* (Rchb.) Lunell
6	*Chenopodium ficifolium* Sm.	Flowering & Fruiting: May–Aug.	Fig-leaved goosefoot	*Chenopodium trilobatum* Ják.
7	*Chenopodiastrum murale* (L.) S. Fuentes, Uotila & Borsch	Flowering & Fruiting: Apr.–Aug.	Nettle-leaved goosefoot	*Anserina muralis* (L.) Montandon; *Rhagodia congesta* (Hook.fil.) Moq.; *Vulvaria trachisperma* Bubani
8	*Nitrosalsola vermiculata* (L.) Theodorova	Flowering: Jun.–Sep.; Fruiting: Aug.–Nov.	Wormleaf salsola	*Caroxylon vermiculatum* (L.) Akhani & Roalson; *Salsola buxifolia* Dum.Cours.; *Salsola rodinii* Botsch.

Table 5. Habitat description and altitude for species

No.	Species	Habitat Description	Altitude (m a.s.l.)
1	*Amaranthus albus* L.	Mountainsides; gardens; sandy waste land along riverbanks in steppe; fields; ditch sides; silty alluvial plains in desert regions	Up to 750
2	*Amaranthus hybridus* L.	Mountain valleys; cultivated by streams; irrigated gardens on plains	Up to 1100
3	*Amaranthus viridis* L.	Orchards and date gardens; fields; shady ditches; muddy banks of receding rivers and tidal estuaries	Up to 150

4	*Beta vulgaris* L.	Irrigated cultivated fields; occasionally saline soils; sandy loam; riverbanks; rocky ground; roadsides; saline alluvial soils by lakes	2–260
5	*Chenopodium album* L.	Waste places; cultivated land	0–1500
6	*Chenopodium ficifolium* Sm.	Waste places; cultivated gardens; other anthropogenic habitats	0–500
7	*Chenopodiastrum murale* (L.) S. Fuentes, Uotila & Borsch	Waste places; roadsides; seashores; riversides; fields and gardens; fine gravel soils mixed with organic matter (sheep manure)	0–300
8	*Nitrosalsola vermiculata* (L.) Theodorova	Rocky, gravelly, and sandy-clay hillsides; silty soils	0–650

4- DISCUSSION

4.1. Palynological Traits as Diagnostic Markers for Food Authentication

The SEM analysis clearly differentiated the eight species based on pollen size, shape, aperture type, and exine ornamentation. These traits are genetically stable and environmentally robust, making them ideal for species authentication in food products and environmental monitoring [16, 17]. For example, the large, strongly echinate pollen of *Beta vulgaris* (beet) can be easily distinguished from the small, psilate pollen of *Nitrosalsola*, allowing rapid identification of beet-derived ingredients or detection of cross-contamination by weed pollen in beet sugar or flour processing [27].

The tricolpate aperture in *A. hybridus* is a rare and distinctive feature within the family, which could serve as a marker for identifying this species in mixed pollen samples – potentially useful for allergen source tracking in food facilities [18]. This unique trait provides a clear diagnostic signal that can be detected even in complex pollen mixtures, supporting accurate identification in environmental monitoring programs.

4.2. Food Industry Relevance: Authentication, Allergen Control, and Quality Assurance

4.2.1. Ingredient Authentication and Fraud Prevention

Amaranth grain, quinoa, and beet products are increasingly used in gluten-free, functional, and clean-label foods. However, adulteration with cheaper or allergenic species (e.g., weed *Amaranthus* species) can occur, compromising product quality, safety, and consumer trust [13]. The distinct pollen traits documented here can support microscopic authentication of ingredients, ensuring label accuracy and consumer safety. For instance, the large pollen size of *B. vulgaris* (55–70 µm) and the small, psilate pollen of *N. vermiculata* (14–18 µm) provide clear diagnostic markers that can be detected in mixed samples using SEM or light microscopy, enabling rapid screening of raw materials for adulteration.

The global market for amaranth, quinoa, and specialty beet products has grown significantly, with rising prices attracting counterfeiters. Pollen-based authentication offers a relatively low-cost, reliable method for verifying the botanical origin of ingredients, protecting brand integrity and consumer trust [28]. This is particularly important for premium gluten-free and organic products, where authentication claims must be substantiated.

4.2.2. Allergen Monitoring in Processing Environments

Pollen from *Amaranthus*, *Chenopodium*, and *Salsola* species are recognised as potent aeroallergens. In food processing facilities, airborne pollen can settle on surfaces and contaminate products, posing a risk to sensitised workers and consumers [11, 12]. The ability to identify and quantify these pollen types using SEM-based palynology can help food manufacturers implement targeted air-filtration and cleaning protocols, reducing allergen exposure and complying with food safety regulations (e.g., EU Food Information Regulation, FDA Food Allergen Labeling requirements) [14, 29].

Occupational exposure to airborne pollen allergens in grain handling and processing facilities is a growing concern. Regular monitoring of indoor air quality using palynological methods can provide early warning of elevated allergen levels, enabling proactive interventions [30]. The wide distribution of allergenic species (Table 3) in central Iraq, including areas near food processing facilities, underscores the importance of routine allergen monitoring in food manufacturing environments.

The integration of palynological data with food safety management systems (e.g., HACCP) can help manufacturers identify critical control points for allergen contamination, implement targeted control measures, and demonstrate due diligence in allergen management [31].

4.2.3. Quality Control in Raw Materials

The wide ecological amplitude of these species means they can co-occur with crops in fields. Harvesting and storage can introduce weed pollen and seeds, affecting product purity, shelf life, and safety. Palynological screening of raw materials (e.g., amaranth grain, beet slices) can detect contamination early, enabling corrective actions and maintaining product quality [32].

Early detection of contamination through palynological screening supports proactive quality management. For example, the presence of high levels of *A. hybridus* pollen in amaranth grain could indicate co-harvesting with allergenic weeds, triggering additional cleaning or processing steps to ensure product safety and quality [33].

4.2.4. Supporting Allergen-Free Product Claims

For manufacturers producing allergen-free or low-allergen products, the ability to verify the absence of allergenic pollen contamination is essential for substantiating claims and building consumer trust. Palynological analysis can provide direct evidence of the pollen-free status of products, supporting marketing claims and regulatory compliance [34].

4.2.5. Supply Chain Traceability and Food Fraud Prevention

The distinct palynological signatures of different Amaranthaceae species can be used to trace the origin and authenticity of ingredients, supporting supply chain transparency and food fraud prevention [35]. For example, the presence of *N. vermiculata* pollen in a beet-based product could indicate contamination during processing or adulteration with cheaper ingredients, triggering further investigation and corrective action.

4.3. Taxonomic and Ecological Insights with Industrial Implications

The observed variation in pollen size and ornamentation correlates with habitat and life-history traits. Larger pollen grains with thicker exine (e.g., *Beta vulgaris*, *Chenopodium album*) are often associated with cultivated or nutrient-rich environments, while smaller, thin-exined grains (e.g., *Nitrosalsola*) occur in xeric, stressed habitats [36]. This ecological profiling can inform predictive models for pollen dispersal and allergen exposure in different agricultural settings [37].

Understanding the ecology and distribution of allergenic species can help food manufacturers anticipate seasonal peaks in pollen production and implement targeted control measures. For example, harvesting and processing activities during peak flowering periods of allergenic species may require additional cleaning and air-filtration measures to maintain product purity and worker safety.

4.4. Limitations and Future Research Directions

While this study provides a comprehensive palynological reference for Amaranthaceae species of food and allergenic importance, several limitations should be acknowledged:

1. **Quantitative morphometric analysis:** Future studies should apply principal component analysis (PCA) and discriminant analysis to quantitatively validate the diagnostic power of individual pollen traits for species identification in food authentication applications [18].
2. **Molecular validation:** DNA barcoding (e.g., ITS, *rbcL*, *matK*) of the studied species would provide independent validation of taxonomic identification and support the development of integrated authentication strategies [24].
3. **Pollen degradation:** The effects of food processing (e.g., heating, grinding, fermentation) on pollen morphology and diagnostic traits should be assessed to confirm the reliability of palynological authentication in processed foods [32].
4. **Large-scale screening:** The feasibility of integrating palynological screening into routine quality control workflows should be evaluated in collaboration with food manufacturers, assessing cost, time, and equipment requirements.
5. **Cross-validation with other techniques:** The sensitivity and specificity of pollen-based authentication should be compared with other analytical methods (e.g., DNA barcoding, HPLC, FTIR) to

develop optimal authentication strategies.

5- CONCLUSION

This SEM-based palynological study provides a comprehensive reference for the pollen morphology of eight Amaranthaceae species of food and allergenic importance. The significant interspecific variation in pollen size, shape, aperture type, and exine ornamentation offers reliable markers for species identification, with direct applications in food authentication, allergen monitoring, and quality control.

From a food industry perspective, these findings support:

1. **Ingredient Authentication:** Accurate labelling of amaranth, beet, and quinoa products through pollen-based verification.
2. **Allergen Monitoring:** Detection and management of airborne pollen allergens in processing environments through routine environmental monitoring.
3. **Quality Control:** Enhanced raw material purity assessment through early detection of contamination.
4. **Regulatory Compliance:** Supporting compliance with food safety regulations and allergen labelling requirements.
5. **Supply Chain Transparency:** Enabling traceability and food fraud prevention through distinct palynological signatures.

The integration of palynological data with food safety management systems can help reduce allergen risks, ensure regulatory compliance, and build consumer trust in clean-label and specialty food products. Further quantitative morphometric analyses (e.g., principal component analysis) and molecular validation are recommended to refine these diagnostic tools for routine industrial use. The wide distribution of allergenic species (Table 3) in central Iraq, including areas near food processing facilities, underscores the importance of routine allergen monitoring in food manufacturing environments.

Ethical Approval

This study did not involve human participants or animals. All experimental procedures were conducted in accordance with institutional and laboratory biosafety guidelines.

Conflict of Interest

The authors declare that they have no conflict of interest.

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Data Availability

All data supporting the findings of this study are available within the manuscript.

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