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REVIEW

A Comprehensive Review of Bunium persicum: A Valuable Medicinal Spice

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ABSTRACT

Bunium persicum (Boiss), B. Fedtsch or Black cumin, is an economically important medicinal spice that is commonly used as a flavour enhancer and preservative in different food systems. It has also been used in Unani, Iranian, and Indian systems of traditional medicine. A member of the Apiaceae family, it possesses myriad phytochemicals, mainly cuminaldehyde, α-terpinene-7-al, γ-terpinene-7-al, γ-terpinene, ρ-cymene, β-Pinene, etc. and endows various proven therapeutic properties including antioxidant, anti-microbial, anti-inflammatory, lipid/glucose lowering activity, anti-carcinogenic activities, etc. This plant grows in the wild in specific areas and is scarcely available and over-exploited; hence, its conservation (both, *in vitro and in situ*) is a major concern. Besides, negligible work has been done for molecular characterisation, identification and development of promising high yielding cultivars/varieties of this valuable plant. With the aim to attract the attention of potential stakeholders towards the immense potential and infinite qualities of black cumin, this review provides an insight in to the phytochemistry, economic importance, including food and therapeutic uses; morphological, biochemical, and molecular characteristics of *Bunium persicum*, along with the efforts towards its conservation and a way forward.

Abbreviations

BPEO: *Bunium persicum* essential oil; BPE: *B. persicum* extract; MPEO: *Mentha piperita* essential oil; DPPH: 2,2-Diphenyl-1-picrylhydrazyl; TBA: Thiobarbituric acid; TPC: Total phenolic content; LD_{50}: Lethal dose; IC_{50}: Half maximal inhibitory concentration; BHT: Butylated hydroxy toluene; BHA: Butylated hydroxy anisole; ABTS: 2,2′-Azino-bis (3-ethyl benzo thiazoline-6-sulphonic acid); MICs: Minimum inhibitory concentrations; BSA: Bovine serum albumin; MS: Murashige and Skoog Medium; MSN: Murashige and Skoog medium with Nitsch Vitamins; B_5: Gamborg's Medium; 2,4-D: 2,4-dichlorophenoxyacetic acid; Kin: Kinetin; JA: Jasmonic acid; IAA: Indole-3-acetic acid; BAP: *6*-Benzylaminopurine; IBA: Indole-3-Butyric Acid; TDZ: Thidiazuron; GA_3: Gibberellic Acid; ABA: Abscisic Acid.

KEYWORDS

Bioactive films; black cumin; *Bunium persicum*; glucose-lowering properties; medicinal plant; spice

Introduction

Bunium persicum (Boiss) B. Fedtsch or Black cumin is an important spice that grows wild in the dry temperate regions of Central Asia, including Iran, Egypt, Pakistan, Afghanistan, Kazakhstan and India. In India, *Bunium persicum* is an important medicinal spice plant growing wild in the Northern Himalayan region.[1] *Bunium persicum* (Boiss) B. Fedtsch is a species from the Apiaceae family commonly known by different names such as kala jeera, siah zeera, and shahi zeera in Jammu &

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Kashmir; Kali zeeri in some patches in Himachal Pradesh and zireh kuhi in parts of Iran. This is very high-valued crop and costs about Indian Rs. 1200–2800/kg.[1,2] Overexploitation of this important spice plant has resulted in a drastic decline in its populations in Jammu & Kashmir, Uttarakhand and Himachal Pradesh regions in India.[3] Owing to its niche-specific nature and properties, Himachal Pradesh state in India has protected it by granting Geographical Indication (GI) under GI number 432.[1] The propagation of this species in its natural habitats through seeds takes 4–6 months for germination and the crop can be harvested only after 3 years of sowing. Such a long life cycle is a serious impediment in commercial cultivation of this plant. Due to its slow growth and long crop duration, farmers show least interest in its cultivation. These factors have resulted in depletion of this economically and medicinally valuable spice plant in its natural habitats at an alarming rate and the plant is now included in the list of endangered species (http://envis.frlht.org). Efficient agronomic strategies, management practices, and conservation strategies such as tissue culture (for mass multiplication and conservation) and cryopreservation need to be devised to conserve this valuable plant species. The species often confused with *Nigella sativa* and *Carum carvi*, has been extensively studied for its phytochemistry and fruit/seed extracts were shown to possess various functional properties including antimicrobial, antioxidant, anti-inflammatory, antidiabetic and other therapeutic properties. It has potential of being used as a natural antioxidant and preservative in different food systems. It has also found a place in novel food applications like formulation of bioactive films/coatings for enhancing the shelf life of food products. Although medicinal properties of *B. persicum* have been reviewed previously by few authors, this review aims to provide comprehensive up-to-date information on morphological, biochemical, molecular characteristics of *B. persicum* along with phytochemistry, novel food, and medicinal applications for consumers, food and pharmaceutical industries and suggest the areas to policymakers that require immediate intervention.

Botanical description

Bunium persicum (Boiss) B. Fedtsch (2 N = 2X = 14) is a member of the family Apiaceae or Umbelliferae. This family consists of about 423 genera, mostly herbs, shrubs, trees and aromatics. There are about 166 species in the genus Bunium including *B. bulbocastenum* L., *B. caroides* (Boiss) Hausskn ex Bornm, *B. chaerophyllocides* (Regel & Schmalh) Drude, *B. cylendricum* (Boiss & Hohen) Drude, *B. elegans* (Fenzl) Freyn, *B. flerulaceum* Sm., *B. kopetdagense* Geld, *B. persicum* (Boiss) B. Fedtsch, etc. that are prevalently found in Central Asia, Caucasus, and Europe.

Bunium persicum is a herbaceous perennial geophyte that can attain a height of 40–80 cm. It is generally cultivated from underground tubers and occasionally from seeds. It may possess one or more stems that branch from middle. The stems are hollow in the internodal region and contain secretory vessels for oils and resins.[3] The leaves are freely pinnate (2–3), finely dissected, and filiform (Fig. 1a). It bears an umbel inflorescence, characteristic of Apiaceae family that has a convex or flat-topped flower cluster (8–20 nos.) arising from a single apex (Fig. 1b-f). The flowers are hermaphrodite with pedicle twice or thrice the flower length (Fig. 1d-f). The petals are free and bifid (Fig. 1g, h). The flowers are zygomorphic containing five white, pink or purple petals and five stamens alternate to petals (Fig. 1g). The stamens arise from an epigynous disc. The anthers are bilobed, introrse and dorsifixed (Fig. 1i). The gynoecium is bicarpelate with an inferior ovary having two styles fused at the base.[3] The flowers are protandrous due to which cross pollination is affected with the help of insects. The fruit is a schizocarp that consists of two sickle-shaped mericarps attached together by a thin axial central stalk (carophore) (Fig. 1j, k). The fruits (commonly but inaccurately called seeds) are brown to dark brown in colour, 3–4 mm in length, and 0.8–1 mm in diameter. The 1,000 seed weight range between 1 and 2 g. Each mericarp usually contains five longitudinal ridges (costae) and furrows (valleculae); oil ducts (vittae) lie underneath the furrows.

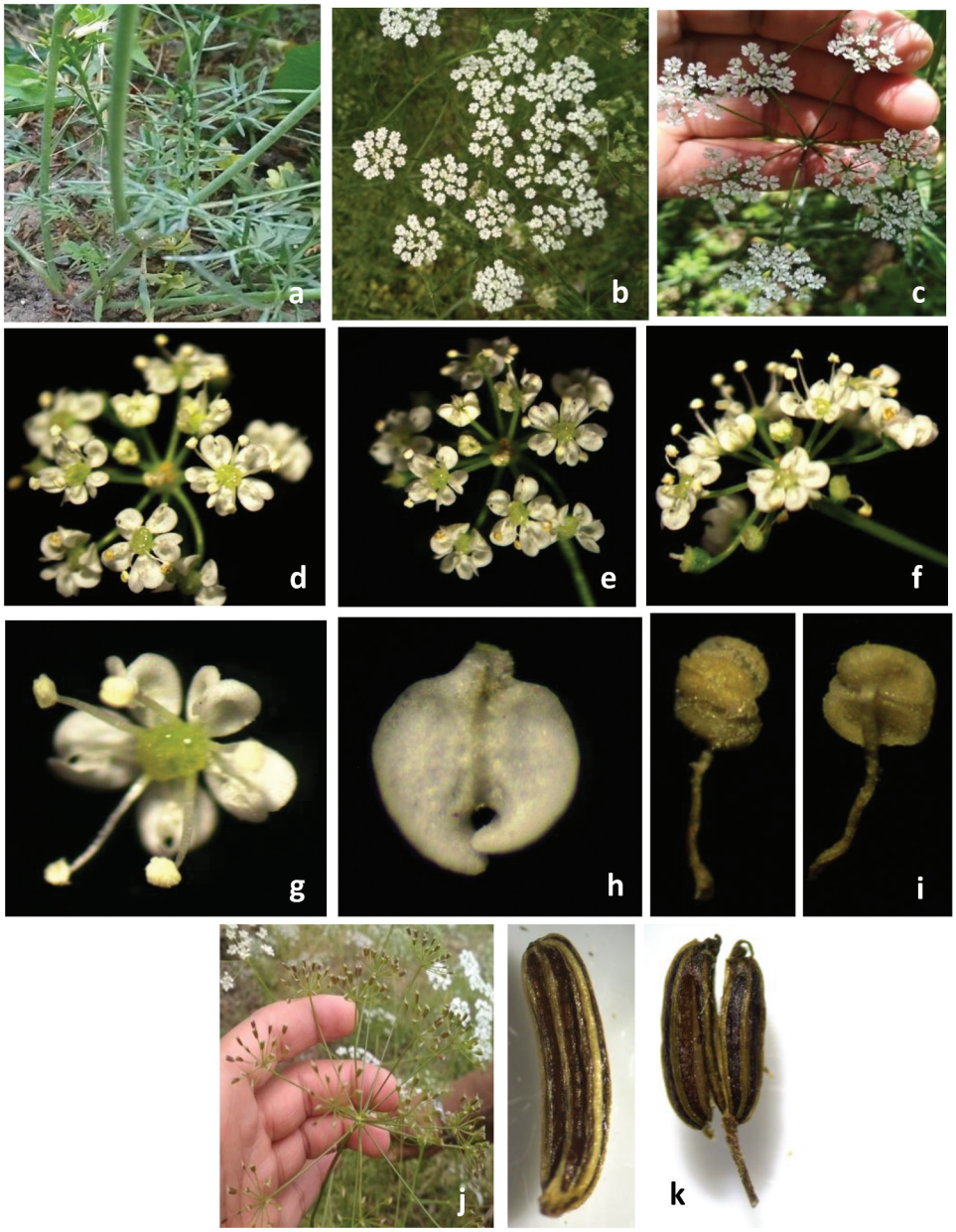

Figure 1. Morphological features of *Bunium persicum*; a) stem and leaves, b, c) umbels containing flowers, d, e, f) magnified view of Inflorescence, g) flower, h) petal, i) stamen, j) fruit bearing umbels, k) fruit.

Traditional uses

Seeds of *B. persicum* have traditionally been used in treatment of urinary and digestive disorders and are well known for their antidiarrheal, anti-asthma, anti-convulsion, anthelmintic, anti-dyspnea, and anti-nociceptive properties in Iranian folk medicine.[4–6] Ethnopharmacological interview of eight nomadic tribes and ten villages of Khabr National Park, Iran revealed that decoction of *B. persicum* fruits is traditionally used for treatment of flatulence, spasm, menstrual pains, and as antimicrobial.[7]

Bunium has been used extensively in Unani medicine for asthma, bronchitis, cough, rheumatism and other inflammatory diseases and is also useful in allergies.[8] A study based on information from old peoples of different localities in Malakand, Pakistan where Unani system of medicine is more prevalent, indicated that *B. persicum* is medicinally used as carminative, stomachic, antiseptic, and lactagogue.[9] In Indian folk medicine, leaves of plant are used to cure abdominal pain and flower heads as carminative. Fruit decoction in water is used to treat cold, headache, stomachache, joint pain, tuberculosis, fever etc.[10]

Phytochemistry

Black cumin seeds are a rich source of phytochemicals that include aliphatic compounds, steroids, terpenes, (monoterpene, oxygenated monoterpenes, sesquiterpene, oxygenated sesquiterpenes), terpenoids, esters, campesterol, stigmasterol, alkaloids, fatty acids, resins, tannins, thymoquinone, saponins, phenolics, and flavonoids. It contains components such as cuminaldehyde, gamma-terpinene -7-al, alpha-terpinene-7-al, γ-terpinene, α-pinene, β-pinene, myrcene, α-terpinene, α-cymene, ρ-cymene, limonene, α-terpinolene, β-sinensal, β-selinene, Germacrene-B, and Dillapiole. Cuminaldehyde's aroma contributes to the pleasant smell of oils, and also makes it a part of perfumes and other cosmetics. The components, γ-terpinene, ρ-cymene, and β-pinene, contribute in reducing the quality of the spice.[11] Cuminaldehyde and ρ-cymene are responsible for strong antifungal activity in *B. persicum*.[11,12]

Chemical constituents of essential oil

The major components of *B. persicum* essential oil are cuminaldehyde, ρ-mentha-1,3-dien-7-al (α-terpinen-7-al), *p*-mentha-1,4-dien-7-al (γ-terpinene-7-al), γ-terpinene, β-pinene, and ρ-cymene. Ripe Bunium seeds may contain 2–9% essential oil rich in monoterpene aldehyde (Table 1). The wild or unripe Bunium seeds contain terpene hydrocarbon such as γ-terpinene, ρ-cymene, β-pinene, limonene that result in low-quality spice.[33] Hydro-distillation for 3 to 4 h using Clevenger type apparatus followed by drying on anhydrous sodium sulphite is the preferable and most commonly used method for extraction of essential oil from Bunium seeds (Fig. 2). In a study, Pourmortazavi et al.[27] have compared the method of supercritical fluid extraction (SFE) with hydro-distillation extraction of essential oil. The GC-MS analysis revealed that the compositions of oil extracted with SFE method and hydro-distillation method was different from each other and hydro-distilled oil had higher concentrations of cuminaldehyde and γ-terpinene thus substantiating the extensive use of hydro-distillation extraction. For separation of individual components of extracted essential oil, GC-MS using HP-5 MS fused silica capillary column (30 m × 0.25 mm i.d. with 0.32 mm film thickness) has been most widely used technique.[27,30] Identification of compounds has been done by comparing the obtained retention indices and mass spectra with those available in literature or with National Institute of Standards and Technology (NIST) library and/or with retention indices of authentic sample on the GC.[12,22]

Maximum phytochemical studies on seed essential oil have been conducted in Iran followed by few reports on Indian samples and one study each on Pakistan/Tajikistan samples (Table 1). The essential oil content in different reported samples ranged from a minimum 0.8% to maximum 9.1%, both in Iranian samples. In most of the samples, major component of essential oil was γ-terpinene (9.77–-46.1%) followed by cuminaldehyde (5.96–37.1%) and γ-terpinen-7-al (2.6–22.3%) (Table 1). Other important components in terms of concentration were ρ-cymene (5.25–20.1%), β-pinene (0.24–-14.9%) and limonene (0.16–10.6%). These differences in essential oil content and composition are attributed to different chemotypes of the species (genetic variation) and environmental factors like geography, altitude, soil, climate, etc.[32] In a report of Sharopov et al., [18] the cluster analysis based on chemical composition of different Bunium samples resulted in at least seven different chemotypes: i) rich in α-terpinen-7-al, ii) high content of γ-terpinene with limonene, *p*-cymene, and cuminaldehyde further divided into two sub-clusters: one with less γ-terpinen-7-al and other with abundant γ-

Table 1. Essential oil content of *Bunium persicum* and its chemical composition.

Essential Oil Content (% v/w dry seed wt)	Major Components Concentration (as Percent of Essential Oil)									Country/ Reference
	Cuminaldehyde	γ-Terpinene /α-Terpinene	α-/γ-Terpine-n-7-al	ρ-Cymene	Limonene	α-Pinene/β-Pinene	Cumin alcohol	1-Phellandrene	Sabinene	
2.4	19.8	26.3 0.1	5.2 **22.3**	14.2	6.2	0.6 1.3	–	–	0.7	Iran[13]
–	15.5	**46.1** 1.3	0.2 –	6.7	5.9	2.7 2.5	7.4	–	1.0	Iran[14]
5.51	14.8	39.7 –	0.5 8.3	17.1	6.4	1.6 3.9	–	–	–	Iran, India, Pakistan[15]
1.92	24.1	14.0 –	**37.2** –	8.7	0.5	0.8 14.3	–	–	–	
2.35	**37.1**	13.8 –	7.0 11.7	10.9	0.5	0.8 **14.9**	–	–	–	
1.92	32.81	16.02 0.39	– 8.67	14.07	0.16	1.52 2.72	–	–	–	Iran[16]
2.25	20.49	30.77 0.05	3.77 8.29	**20.1**	5.51	1.14 1.69	–	–	0.85	Iran[17]
–	36.0	10.9 0.1	13.1 15.0	5.3		0.6 9.1	–	0.1	–	Tajikistan, India[18]
–	29.9	10.9 0.1	8.1 17.2	12.5		0.4 7.8	–	0.2	–	
9.1	16.9	44.2 –	10.5 –	8	2.0	1.0 1.6	–	–	1.2	Iran[19]
2.0	11.4	11.37 11.13	–	–	–	– 11.27	–	–	–	Iran[20]
–	22.37	19.36 7.3	11.84 –	6.56	3.01	–	–	–	–	Iran[21]
–	22.08	17.86 –	15.41 2.88	7.99	1.48	0.57 4.68	–	–	–	Iran[22]
–	23.04	14.48 –	–	–	–	0.9 2.27	–	–	–	Iran[23]
–	16.9	44.2 –	0.4 10.5	8.0	2.0	1.0 1.6	–	0.1	1.2	Iran[24]
–	17.28	14.4 –	–	6.21	2.47	–	–	–	–	Iran[25]
5.5	33.6	23.9 –	29.6	16.5	0.41	0.25 0.5	–	–	–	India[26]
3.1	12.7	45.7 –	–	5.6	**10.6**	2.8 3.7	6.4	–	1.2	Iran[27]
2.0	23.9	**46.1** –	4.5 2.6	15.9	4.7	0.6 0.9	–	–	0.3	Iran[5]
–	2.23	14.4 –	–	4.31	4.42	2.37 2.94	–	–	–	Iran[12]
0.8	22.34	9.77 0.1	–	12.04	2.14	– 2.34	–	1.28	–	Iran[28]
8.3	15.5	**46.1** 1.3	0.2 –	6.7	5.9	2.7 2.5	7.4	–	1.0	Iran[29]
2.2	5.96	15.19 0.23	–	5.25	3.62	1.51 2.28	–	–	0.28	Iran[30]
3.8–6.4	11.22–32.62	21.96–41.27 –	–	–	–	0.21–0.34 0.24–0.29	–	17.14–22.48	1.83–2.36	India[31]
3.1–7.9	9.0–18.9	29.2–40.1 0.2–0.4	–	9.4–15.6	3.7–6.4	0.9–1.7 –	**16.4–28.4**	–	0.8–1.2	Iran[32]
9.1	16.9	44.2 –	0.4 10.5	8.0	2.0	1.0 1.6	–	–	1.2 –	Iran[33]

Maximum Concentration: bold font; Minimum Concentration: underlined font.

terpinen-7-al, iii) rich in *p*-cymene, iv) dominated by cuminaldehyde, γ-terpinene, and γ-terpinen -7-al, v) rich in α-pinene, vi) rich in γ-terpinen-7-al, and vii) rich in β-pinene and cuminaldehyde. Extensive studies are required to validate these differences and find out a superior germplasm that can be used as a valuable genetic resource for breeding purpose.

Studies were also conducted to compare the essential oil composition of wild and cultivated samples in order to assess the effect of environmental variations. In one such report by Thappa et al., [26] cultivated plants were found to contain more cuminaldehyde (27.3–34.1%), α-terpinen-7-al, and γ-terpinen-7-al (29.6–36.8%), while in wild collected seeds there were more γ-terpinene (25.6–42.9%) and

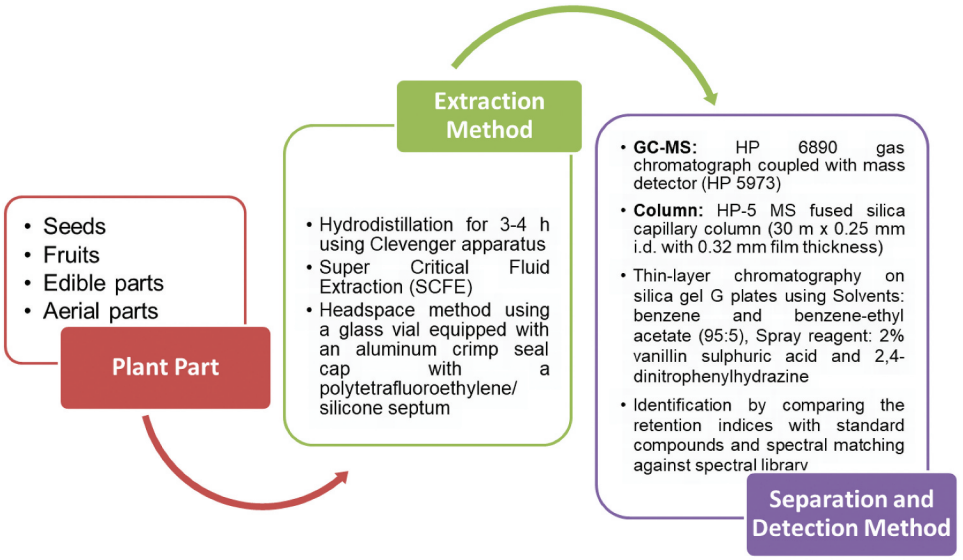

Figure 2. Plant parts, essential oil extraction, separation and identification methods mostly used in phytochemical studies on *Bunium persicum*.

ρ-cymene (24.0–27.8%) and less aldehydes, moreover, the oil obtained from cultivated source was more superior than that from wild sources. Essential oil content was higher in wild seed samples (7–10.2%) as compared to cultivated mature seed samples (5.5%). Similar study by Azizi et al.[33] analysed fruit oils of *B. persicum* from wild type (WT) populations growing at an altitude of 2850 m, fourth (CY1) and fifth year (CY2) cultivars grown at an altitude of 1000 m by GC and GC/MS technique. The essential oil content of the WT (9.1% v/w) was higher than the oil content of the CY1 (6.2% v/w) and CY2 (5.1% v/w). The main constituents reported were γ-terpinene (WT: 44.2%, CY1: 40.8%, CY2: 36.8%), cuminaldehyde (WT: 16.9, CY1: 14.1 and CY2: 11.8%) and γ-terpene-7-al (WT: 16.9, CY1: 10.6, CY2: 18.7%).

Pharmacological activities

The fruits/seeds of black cumin contain numerous phytochemicals that are known to have antioxidant, carminative and anti-flatulent properties and are excellent source of dietary fibres.[34] *B. persicum* seed oil is capable of suppressing the initial stage of an inflammatory process and has been found effective in treatment of gastro-intestinal disorders including diarrhoea[6] (Table 2). Monoterpenic compounds present in the essential oils are responsible anticonvulsant properties of Bunium seeds and are effective in control of severe seizures in the body.[4] Jalilzadeh et al.[23] studied the anti-ulcerative and anti-diarrheal properties of essential oil of this plant. *B. persicum* in combination with *Rhus coriaria* (an old Persian medicine Persumac) was found effective in control of refractory chemotherapy induced nausea and vomiting.[41] Sharififar et al.[29] studied antioxidant activity and medicinal properties of *B. persicum* and reported its usage as anti-spasmodic, carminative, anti-obesity, and lactogage (Table 2).

Antioxidant activities

Studies carried out on *B. persicum* extracts and essential oil indicated high antioxidant and free radical scavenging activities.[13,25] Shahsavari et al.[30] have studied chemical composition of essential oil and its antioxidant activity by analysing the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) scavenging and β-carotene bleaching assays. Antioxidant activity of the BPEO oil was also evaluated using crude soybean

Table 2. Nutritional, chemical properties, and food/therapeutic uses of *Bunium persicum*.

Functional Property	Extract Preparation and Test Method	Major Chemical Components	Research Findings and Suggested Food/Therapeutic Uses	Reference
Antioxidant activity	• Essential oil from aerial parts by hydrodistillation • Different concentrations of BPEO (0.02%, 0.04%, 0.06%) added to soybean oil were tested at 60°C by oven method and peroxide value was recorded after 0, 8, 16, 24, 32 days to assess the lipid peroxidation	Caryophyllene (27.81%), γ-Terpinene (15.19%), Cuminyl acetate (14.67%), Cuminaldehyde (5.96%), ρ-Cymene (5.25%)	**Natural antioxidant**: DPPH radical scavenging capacity (IC_{50} value) comparable to synthetic antioxidants BHT and α-tocopherol **Natural antioxidant of soybean oil**: Different concentrations of BPEO (0.02%, 0.04%, 0.06%) was added to Antioxidant activity of BPEO (0.06%) was insignificantly different from activity of BHA (0.02%) in reducing the oxidation rate of soybean oil at 60°C	[30]
	• Essential oil by hydrodistillation and methanol extract of seeds	γ-Terpinene (26.3–30.7%), γ-Terpinen-7-al (22.3–25.6%), Cuminaldehyde (19.8–17.3%), ρ-Cymene (14.2–9.9%), Limonene (6.2–7.3%)	**Natural antioxidant**: Total phenols (8.7–12.8 mg/g), total flavons (4.3–9.5 mg/g), Iron-reduction (6.1–10.5 mg/g) were found to be higher in samples from high altitudes	[13]
	• Essential oil from dried seeds by hydrodistillation • BPEO was added to virgin olive oil at 1000 mg/l concentration and incubated for 42 days at 70 ± 1°C. Observations were recorded weekly	Cuminaldehyde (32.81%) and γ-Terpinene (16.02%), *p*-Cymene (14.07%), *p*-Mentha-1,4-diene 7-ol (8.67%), 2-Caren-10-al (4.28%)	**Natural antioxidant of virgin olive oil**: IC_{50} value of BPEO was significantly higher than synthetic oxidant BHT. In virgin olive oil, BPEO retarded the lipid oxidation process	[16]
	• Essential oil from seeds by hydro-distillation and Solvent extraction (chloroform, methanol, petroleum ether, water)	γ-Terpinene (46.1%) Cuminaldehyde (15.5%), ρ-Cymene (6.7%), Limonene (5.9%)	**Natural antioxidant**: Highest antioxidant activity was demonstrated by BPEO and methanolic extract with IC_{50} value of 23.4 ± 1.6 and 45.7 ± 3.6 µg/mL, respectively in DPPH test and with maximum prevention of β-carotene oxidation and lipid peroxidation.	[29]
	• Essential oil from seeds by hydrodistillation • Corn starch film containing different concentrations of BPEO (1–20 mg/ml) was tested by DPPH and ABTS assays	Cuminaldehyde (22.34%), Carvacrol (19.88%), Anisole (15.19%), o-Cymene (12.04%), γ-terpinene (9.77%)	**Natural antioxidant in corn starch bioactive films**: DPPH and ABTS radical scavenging activity was 71% and 62%, respectively in starch films containing 20 mg/ml BPEO	[28]
Antimicrobial activity	• Essential oil from dried seeds by hydrodistillation • White cheese was prepared from BPEO (1, 2% w/v) supplemented milk and stored at 4°C for 45 days. Bacterial count was recorded on 1, 7, 15, 30 and 45 days	Cuminaldehyde (11.4%), γ-Terpinene (11.37%), β-Pinene (11.27%), α-Pinene (11.27%), α-Terpinene (11.13%).	**Antibacterial in ripening and storage of white cheese**: Significant antibacterial activity against *E. coli* O157:H7 and *L. monocytogenes* in Iranian white cheese	[20]
	• Essential oil by hydrodistillation and methanol extract from powdered fruits • MICs against *S. aureus* ATCC 6538, *E. coli* ATCC 8739, and *Candida albicans* ATCC 10231 were evaluated by agar diffusion method	Cultivated and wild: γ-Terpinene (27.57% and 30.77%), Cuminaldehyde (21.1% and 20.49%), ρ-Cymene (18.32% and 20.1%), γ-Terpinen-7-al (7.84% and 8.29%)	**Antimicrobial activity**: Both extracts demonstrated comparatively low antibacterial potential with MICs raging between 0.375–1.5 mg/ml and 0.75–6.25 mg/ml for wild and cultivated BPEO, but, significant activity against *C. albicans* with MICs of 2.5–5 mg/ml and 5 mg/ml	[17]
	• Essential oil from seeds by hydrodistillation • Effect of whey protein coating containing 5% lactoperoxidase system (LPOS) and BPEO (0.5%) on Gouda cheese's chemical, sensory and microbial profiles was assessed for 90 days storage	–	**Antibacterial activity**: Whey protein coatings containing BPEO and LPOS + BPEO showed inhibition of gram positive bacteria and reduced lipid oxidation and free fatty acids content	[35]

(*Continued*)

Table 2. (Continued).

Functional Property	Extract Preparation and Test Method	Major Chemical Components	Research Findings and Suggested Food/Therapeutic Uses	Reference
	• Essential oil from dried seeds by hydrodistillation • PLA films containing BPEO (0.5%, 1% v/v) and cellulose nanoparticles (0 or 1% w/v) prepared by solvent casting technique were tested against gram positive and gram negative bacteria by disk diffusion method	2-Methyl-3-phenyl Propanal (34.08%), Cymene (18.23%), Myrtenal (12.37%)	**Antibacterial in polylactic acid films**: 1.5% BPEO in PLA films exhibited significant antibacterial activity against *Bacillus cereus* ATCC 11778, *Staphylococcus aureus* ATCC 65138 and *Vibrio parahaemolyticus* ATCC 43996 whereas cellulose nano-particles showed no activity	[36]
	• Essential oil from dried seeds by hydrodistillation • Triplicates of 108 combinations having varied BPEO concentrations (0, 0.08, 0.16, 0.24%), pH levels (5, 6, 7), incubation temperatures (15, 25, 35°C), and inoculum size (102, 103, 104 CFU/ml) were tested for *S. aureus* inhibition. Bacterial growth was observed daily for 30 days	γ-Terpinene (44.2%), Cuminaldehyde (16.9%), γ-Terpinen-7-al (10.5%), ρ-Cymene (8.0%)	**Use in Hurdle technology**: Combinations containing BPEO showed increased time to detection for *S. aureus* growth and it was higher with high concentrations of BPEO but low levels of temperature, pH, and inoculums	[24]
	• Commercial hydrodistilled BPEO • Antibacterial effects of BPEO (0.05, 0.2 and 0.4%) along with other hurdles including smoking, salting, storage at freezing temperatures were tested in fish model systems (kutum broth and cold smoked kutum broth)	Cuminaldehyde (22.37%), γ-Terpinene (19.36%), γ-Terpinene -7-al (11.85%), α-Terpinene (7.3%), ρ-Cymene (6.56%)	**Use in Hurdle technology**: BPEO was highly effective in inhibiting *L. monocytogenes* without any undesirable sensory effect	[21]
	• Essential oil from dried seeds by hydrodistillation • Broth microdilution method was used to estimate the MIC	γ-Terpinene (44.2%), Cuminaldehyde (16.9%), γ-Terpinen-7-al (10.5%), ρ-Cymene (8%)	**Activity against food-borne pathogens**: Minimum inhibitory concentration of BPEO was found to range between 0.18–3 mg/ml when tested on various pathogens (*Staphylococcus aureus, Bacillus cereus, Escherichia coli* O157:H7, *Salmonella enteritidis, Listeria monocytogenes*). Besides, BPEO showed synergistic effects with *Cuminum cyminum* EO in inhibiting the pathogens	[19]
	• Essential oil by headspace method • Dish pack method was used for antifungal assays	γ-Terpinene (14.4 µg/g), Limonene (4.42 µg/g), ρ-Cymene (4.31 µg/g), β-Pinene (2.94 µg/g), Cuminaldehyde (2.23 µg/g)	**Antifungal activity**: Volatile oil showed strong anti-fungal activity against *F. oxysporum*. Cuminaldehyde and ρ-cymene the main components responsible for antifungal activity aslo exhihbited the activity aginst other fungi *Verticillium dahliae, Botrytis cinerea* and *Alternaria mali*	[12]
Antiparasitic activity	• Essential oil from plant materials by hydrodistillation • Prophylactic effect of BPEO (0.05, 0.1 ml/kg) administration for 14 days was analyzed on NMRI mice (8 per treatment). Toxoplasmosis was induced on 15th day by intraperitoneal inoculation of tachyzoite (10^4) of *T. gondii* RH strain	γ-Terpinene (46.1%), Cuminaldehyde (15.5%), Cuminyl alcohol (7.4%), ρ-Cymene (6.7%), Limonene (5.9%)	**Anti-toxoplasmosis activity**: Oral administration of BPEO at a concentration of 0.1 ml/kg showed prophylactic effects against protozoan parasite Toxoplasma gondii in mice	[14]
	• Essential oil from seeds by hydrodistillation • BPEO (3.125–50 µl/ml) was used for 5–30 min on protoscoleces extracted from sheep livers having hydatid cysts and protoscoleces viability was assessed using eosin exclusion test	γ-Terpinene (46.1%), Cuminaldehyde (15.5%), Cuminyl alcohol (7.4%), ρ-Cymene (6.7%)	**Scolicidal effect against protoscoleces**: BPEO at concentrations 25 and 50 mcL/mL killed 100% protoscoleces within 5 min	[37]

(*Continued*)

Table 2. (Continued).

Functional Property	Extract Preparation and Test Method	Major Chemical Components	Research Findings and Suggested Food/Therapeutic Uses	Reference
Antinociceptive and anti-inflammatory activities	• Hydroalcoholic (Ethanol: water 8:2) extract of fruits • Essential oil from fruits by hydrodistillation • Paw edema was induced in rats by 0.1 ml of 1% (w/v) Carrageenan injection. BPEO (1% v/v) and hydroalcoholic extract were given orally 1 h and 30 min before injection. Paw volume was recorded before and 4 h after injection. Croton oil induced ear edema was also used to assess the anti-inflammatory activity	γ-Terpinene (46.1%), Cuminaldehyde (23.9%), ρ-Cymene (15.9%), Limonene (4.7%), 1,4 – *p* – menthe dien-7-al (4.5%)	**Antinociceptive and anti-inflammatory in animals**: Hydroalcoholic, polyphenolic extracts and BPEO showed pain reducing effects in animal models tested by acetic acid-induced writhing and formalin tests and anti-inflammatory effects tested using carrageenan-induced paw edema test and Croton oil-induced ear edema test in rats	[5]
Anticonvulsant activity	• Methanolic extract and BPEO from seeds • BPEO (0.25, 0.75, 1, 1.25 and 1.5 ml/kg) and methanolic extract (4 and 5 g/kg) was tested against pentylenetetrazole (110 mg/kg) and maximal electroshock induced convulsions in NMRI mice	–	**Anticonvulsant activity in animals**: Methanolic extract of seeds and BPEO extended the onset time of seizures (clonic and tonic) in both pentylenetetrazole and maximal electro shock induced convulsions in mice	[4]
Hypolipidemic activity	• Aqueous extract of fruits • Hypercholesterolemic mice were given BPE (20 mg/Kg/day) along with endurance exercise (18 m/min speed, 40 min/day, 5 days/week) for 6 weeks. All mice were assessed for 5 successive days for their running capacity	–	**Hypolipidemic activity**: Oral administration of aqueous extract of *B. persicum* significantly reduced the total cholesterol, low density lipoprotein c, triglycerides with simultaneous increase in high density lipoprotein in hypercholesterolemic mice	[38]
Antidiabetic activity	• *B. persicum* seed powder • A group of 30, type 2 diabetic persons were orally given one 1000 mg BP capsule twice a day for 8 weeks and physical activity, diets, anthropometric/blood biomarker measurements were recorded	–	**Antidiabetic activity in type 2 diabetes**: BP capsule (1 g) supplementation in obese/overweight, type 2 diabetic patients for 8 weeks led to significant improvement in serum glucose and basal metabolic indices	[39]
	• Hydroalcoholic extract of seeds • Effect of hydroalcoholic extract (5, 10, 15, 30 µg/ml) on glucose induced BSA glycation, aggregation and thiol group oxidation was tested *in vitro*	–	**Antidiabitic activity**: TPC of BPE was 122.41 mg gallic acid equivalents/g. Glycation of BSA, BSA aggregation and thiol group oxidation was significantly reduced with increasing concentration of BPE (10, 15, and 30 µg/ml) significantly inhibited the formation of GA in a concentration-dependent manner	[40]

oil by monitoring peroxide and thiobarbituric acid values of the oil substrate under accelerated condition at 60°C. The results showed that BPEO was able to reduce the oxidation rate of soybean oil. Differential extracts and essential oil of black cumin evaluated using β-carotene bleaching, DPPH and ammonium thiocyanate methods revealed highest antioxidant activity in essential oil and methanolic extracts with IC_{50} values of 23.4 ± 1.6 µg/ml and 45.7 ± 3.6 µg/ml in DPPH assay, respectively.[29] In daily diets, *B. persicum* can be used as an efficient and cost-effective natural alternative of synthetic antioxidants as suggested by the results of peroxide value (PV) and thiobarbituric acid (TBA) tests done by Zangiabadi et al.[34] Differential extracts of *B. persicum* possess anti-oxidative and antitoxic activities and could prevent reactive oxygen species (ROS) induced hematotoxicity in leukemic blood rats.[42]

Hypolipidemic and antidiabetic effects

The hypoglycaemic, antiobesity, and antidiabetic effects of *B. persicum* extracts were studied and confirmed through inhibition of glycoside hydrolase activity.[43] Another study by Seri et al.[40] revealed a significant decrease in albumin glycation, thiol group oxidation, and aggregation due to high concentration of polyphenolic compounds in hydroalcoholic extract of *B. persicum*. Aqueous extract of *B. persicum* has showed significant hypolipidemic effects on animals.[38] Administration of *B. persicum* aqueous extract significantly reduced the total cholesterol, triglycerides, and low-density lipoproteins with a simultaneous increase in high-density lipoprotein concentrations. The aqueous extract was found to be more beneficial than exercise in improvement of lipid profile in hyperlipidaemic animals (Table 2). In combination with endurance exercise, its administration also led to increased cardiorespiratory capacity.[38]

Antimicrobial properties

Antibacterial effects of different extracts and BPEO tested against different strains of bacteria showed that essential oil has higher inhibitory effect on gram-positive bacteria than gram-negative bacteria.[19] The activity is due to the presence of phenolic compounds, namely cuminaldehyde, γ-terpinene, and ρ-cymene, [44] which being lipophilic, creates instability in the cell membrane. Permeability of the bacterial membrane is increased by the interactions between essential oil compounds with polysaccharides, fatty acids, and phospholipids and loss of ions and cellular content results in cell death.[45] Essential oil can cause the death of bacteria through denaturation of cytoplasmic proteins, inactivation of cellular enzymes, adverse effects on proton pump, membrane clotting, and busting out of cell contents.[46]

Inhibitory effects of *Bunium persicum* essential oil have been demonstrated on several food-borne pathogens, such as *Bacillus cereus, Bacillus subtilis, Escherichia coli* O157:H7, *Klebsiella pneumonia, Listeria monocytogenes, Proteus vulgaris, Pseudomonas aeruginosa, Salmonella enteritidis*, and *Staphylococcus aureus*.[11,19–21,24,47,48] Bunium extracts (aqueous and hydroalcoholic) have also shown inhibitory effects on growth of *Acinetobacter baumannii*.

BPEO has shown significant antifungal properties against different types of food spoilage, pathogenic and phytopathogenic fungi including Aspergillus spp., *Saccharomyces cerevisiae, Candida albicans, Penicillium chrysogenum, Alternaria mali, Botrytis cinerea, Colletotrichum lindemuthianum, Fusarium oxysporum*, and *Verticillium dahliae*.[11,12,17,49] Cuminaldehyde and ρ-cymene, two major components of BPEO showed highest antifungal activities against different phytopathogenic fungi.[12] BPEO inhibited the growth of 15 common food-borne moulds and mycotoxigenic fungi including *Aspergillus flavus* and aflatoxin B1 secretion in stored masticatories.[45]

Antiparasitic effects

Essential oil and extracts of *B. persicum* have shown antiparasitic potential. BPEO nano-liposomes are a promising option for treatment of *Trichomonas vaginalis*, a flagellated protozoan parasite that causes sexually transmitted diseases.[50] In the study, BPEO (51.64 ± 1.24% loading rate) was loaded on liposomal vesicles made from phosphatidylcholine and cholesterol in 70:30 ratio. IC_{50} value of BPEO loaded liposomes against *T. vaginalis* (10^5) cultured on TYI-S-33 medium was found to be 45.19 μg/mL and 14.41 μg/ml after 12 and 24 hours, respectively. It has shown to possess significant anti-toxoplasmosis effects[37] and substantial scolicidal activity with no significant toxicity.[37]

Food applications

Common food uses: The underground tuberous roots (basically taproot) of *B. persicum* are edible and consumed either raw or cooked as vegetable. Seeds are the most valuable part of this species that are

commonly used as spice and flavouring agent. The seeds are similar in shape to cumin seeds but relatively smaller and dark brown in colour and are highly aromatic. *B. persicum* seeds have astringent, pine like, bitter flavour. To enhance flavour, seeds are used in food and beverages[25] due to their strong piney less earthy aroma. It is used in bread, rice, yoghurt, cheese, confectionery products, and cosmetics industry for its aroma.[3,25,28,29,51] Several reports are available about its therapeutic and functional properties[52,53] (Fig. 3).

Food preservation: *Bunium persicum* seeds have good antimicrobial and antioxidant properties due to which it can be used in foods to prevent microbial and chemical spoilage and food-borne diseases as well (Table 2). Besides use as natural flavour, the seed essential oil can also contribute to good health and can be used as a preservative against microbial contamination in foods like masticatories, fish, edible oils, Gouda cheese, Iranian white cheese, etc.[20,21,35,45,54–56] Comparative studies on the effect of BPEO on maintenance and oxidative stability of various types of edible oils such as olive, linseed and soybean oil have shown that it can be used as a natural antioxidant to increase the shelf life and oxidative stability of oils either alone or in combination with other natural or synthetic antioxidants.[16,30,34,57]

Bioactive films and coatings: Due to its high antioxidant activity and inhibitory effect on various food pathogens, BPEO can also be used as an active ingredient in biodegradable film/nano-emulsion formulations, to improve the shelf life of various foods/food products.[28,36,45] Antioxidant potential of BPEO has been demonstrated in biodegradable corn starch films.[28] To prepare the film, corn starch (3%) solution containing glycerol (1.8%) was made and gelatinized with continuous stirring at 90°C for 10 minutes. Varied concentrations (1, 2.5, 5, 10, 15, 20 mg/ml) of BPEO were added and homogenized (Ultra-turrax) at 2000 rpm for 2 minutes. The films were casted on Teflon petriplates and allowed to dry. Total phenolic content of starch films increased with increase in BPEO concentrations and maximum TPC (20 mg gallic acid/g film) and antioxidant activity was observed at BPEO concentration of 20 mg/ml (Table 2).

BPEO containing polylactic acid (PLA) films may be useful for packaging of foods in order to increase their shelf life and safety.[36] PLA films can be made by solvent casting technique.[36] In this method, polylactic acid solution (1% w/v) was prepared in chloroform by continuous stirring at room temperature for 8 h. Essential oils (0.5% and 1%) of *B. persicum* and *Mentha piperita* (MPEO) and nano-cellulose particle (1%) were added and stirred for 20 minutes. The solution was homogenized at 12000 rpm for 2 minutes and casted on glass petriplates and dried. In this study, [36] BPEO was found to be more effective than MPEO in inhibiting bacteria especially gram positive (*Staphylococcus aureus* ATCC 65138, *Bacillus cereus* ATCC 11778) whereas cellulose nano-particles showed no antibacterial activity.

B. persicum essential oil (BPEO) and its nano-encapsulated formulation (in chitosan) are a potential natural preservative for stored plant masticatories, as nano-encapsulated BPEO completely inhibited the fungal growth and mycotoxin production.[45] In this study, [45] BPEO loaded chitosan nano-emulsion (CS-NP-BPEO) is formulated as per details given in Fig. 4. Xeta potential of BPEO loaded nanoparticles was analysed by dynamic light scattering (DLS) along with morphological and structural analysis by scanning electron microscopy (SEM) and X-ray diffraction (XRD). The size of formulated nano-particles ranged from 80 to 300 nm as observed in SEM analysis with mean particle size of 291.7 nm and Xeta potential of +291.7 nm as analysed by DLS. Loading capacity (3.04 ± 0.16%) was maximum in BPEO loaded chitosan (CS) nano-particles containing CS:BPEO in ratio of 1:1 whereas encapsulation efficiency was maximum in CS:BPEO ratio 1:0.2 formulation. Maximum antifungal activity (83.64%) and anti-aflatoxigenic activity (76.56%) of CS-NP-BPEO were observed at concentration of 0.8 µL nano-emulsion/mL of media.

Chitosan incorporated with 1% BPEO nano-emulsion coating could significantly reduce the growth of *E. coli* O157:H7 in vacuum packaged rainbow trout fillets.[58] Two percent (w/v) chitosan (LMW 1.03×10^5 and 91% deacetylation degree) solution was prepared in 1% (v/v) acetic acid by mixing at 40° C for 10 minutes. To this transparent solution, 0.75 ml/g glycerol was added as a plasticizer and BPEO

(1%) with emulsifier Tween 80 (0.2 g) was added and stirred for 30 minutes. After that nano-emulsion was formulated and rainbow trout fillets were immersed in this solution and vacuum packaged.

Storage efficacy and safety of BPEO: In *Phyllanthus emblica*, BPEO demonstrated significant efficacy against aflatoxin B1 (AFB1) contamination up to 12 months of storage.[45] No AFB1 was observed in BPEO fumigated samples whereas in control inoculated and uninoculated samples AFB1 concentration was 46.33 and 40.19 μg/Kg, respectively.

In toxicity studies on mice, Lethal Dose$_{50}$ (LD_{50}) of BPEO was found to be 14584.54 μg/Kg body weight.[45] Similarly, no mortality up to a BPEO dose of 4 g/kg was observed in another study on mice.[4] This comparatively high LD_{50} value and long history of traditional use makes BPEO a safe natural food additive and preservative having significant antioxidant and antimicrobial activities (Table 2).

Cytomorphology and genetics

Little information is available regarding cytomorphology and genetics of *B. persicum*. For effective management, future domestication and breeding for superior germplasm, knowledge about cytogenetics, molecular biology and genetic diversity of Bunium populations and specific markers need to be developed. Diploid nature of black cumin with 14 chromosomes and karyotype formula = 1–2 M + 2–3 Sm = 3–4 St has been reported[59] (Table 3). Similarly, haploid (meiotic) and diploid (mitotic) chromosome number of various ecotypes of Kashmir valley in India revealed that the ecotypes present are diploid having 2 n = 14 chromosomes and haploid number of n = 7, confirming that only *B. persicum* is prevalent at the hot spots of diversity instead of *Carum carvi* or *Bunium bulbocastanum*.[60] In another study, [65] physical location of 18S-5.8S-26S (45S) and 5S ribosomal RNA (rDNAs) on metaphase chromosomes was identified using fluorescent *in situ* hybridisation (FISH). The study[65] confirmed that seven pairs of homologous chromosomes (1 to 7) and 18S-5.8S-26S rRNA gene was located on two sites at telomeric region of chromosomes 1 and 2. Similarly, chromosomes 5 and 7 possess 5S rRNA gene at sub-telomeric regions. Few molecular studies related to *Bunium persicum* have been conducted to assess the genetic diversity of selected samples using random amplified polymorphic DNA (RAPD) markers (Table 3). The genetic relationships amongst 20 wild black cumin populations collected from Iran (15), India (2), Afghanistan (2), and Europe (1) was analysed using 26 RAPD primers.[64] The genetic similarity among the population ranged from 0.37 to 0.95. European population clustered separately from the rest of populations and in the second sub-group, Iranian population and non-Iranian populations formed different clusters. Pezhmanmehr et al.[62] have used RAPD and amplified fragment length polymorphism (AFLP) markers to analyse the genetic diversity of 20 Iranian populations of *Bunium persicum*. RAPD primers yield more polymorphic bands (86%) than AFLP primers (75%) and the similarity coefficients ranged from 0.4 to 0.82 for RAPD and 0.39 to 0.96 for AFLP markers. The relative genetic distances did not correlate with geographical distribution and several populations clustered as different groups, showing a high level of genetic diversity and unique genetic background.

A distinct molecular study to detect cumin adulteration in *B. persicum* using DNA barcoding and cumin-specific marker revealed that DNA barcode psbA-trnH was best in authentication of black cumin.[66] The study is useful in food authentication, as *B. persicum* being expensive is generally mixed with other resembling low-value spices like *Cuminum cyminum*. Besides, the species is often confused with *Carum carvi* (morphologically similar) and *Nigella sativa* (black cumin, similar common name); therefore, molecular methods have proven useful in specific discrimination of different species.[66]

Propagation and conservation of *B. persicum*

B. persicum is generally propagated through tubers and sometimes from seeds. The major problem in propagating *B. persicum* is its long juvenile period and the effects of seasonal fluctuations. Due to seed dormancy and lack of hypocotyl growth after cotyledon emergence,

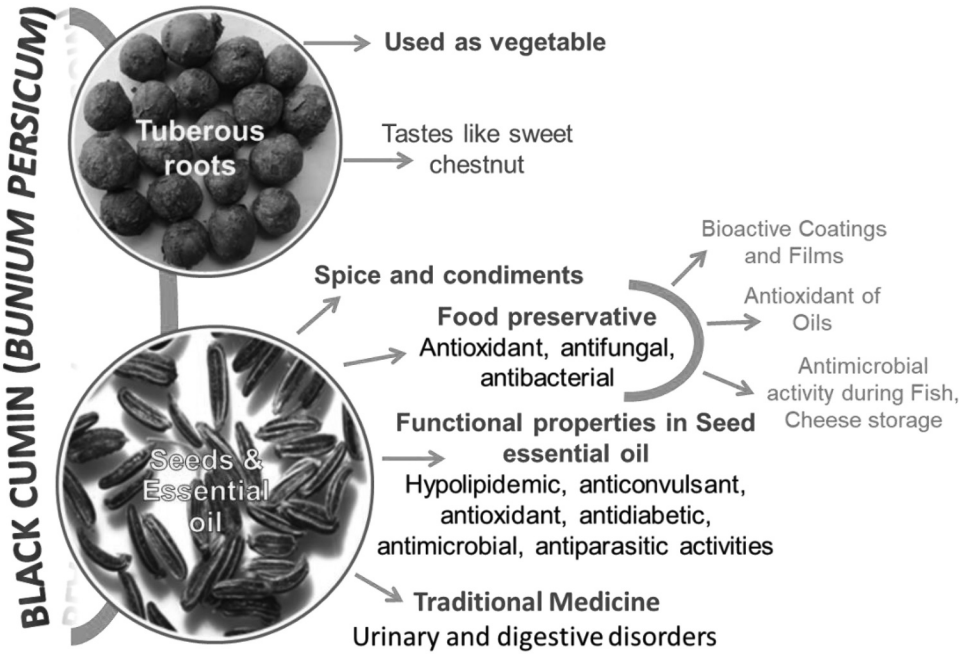

Figure 3. Food applications and functional properties of *Bunium persicum*.

in vivo cultivation of *B. persicum* has not succeeded.[67] Few studies undertaken for breaking seed dormancy and effective seed germination revealed that cold stratification for about 8 weeks at 4–5°C, with or without growth hormones, is necessary for effective seed germination in Bunium[68–70] (Table 4). Tissue culture strategies have also been tried to provide an alternate mean of propagation, but to a certain extent, the findings remain undifferentiated with low rates of responses.[71–73,77] Different explants used for microproapagation of *B. persicum* gave varied responses but all resulted in callusing and somatic embryogenesis and no successful report is available on direct regeneration of *B. persicum* (Table 4).

In vitro conservation of *B. persicum* was tried by Majeed.[61] In the study, callus induction was achieved from stem and leaf explants on MS medium containing 4 mg l^{-1} Kin + 2 mg l^{-1} 2,4-D and shoots were induced by supplementing MS medium with 0.25 mg l^{-1} TDZ and 0.05 mg l^{-1} IBA followed by rooting on MS medium supplemented with 0.3 mg l^{-1} IBA. Conservation of plantlets was achieved at low temperature (9°C) and highest survival of micro-shoots was found on MS medium supplemented with 3% sucrose and sorbitol each and 0.5 mg l^{-1} Kin.

Efforts were also made for *in vitro* microtuber induction in *B. persicum* in order to substantiate its vegetative propagation.[74,76,78] Grewal[78] reported induction of microtubers by using MS medium with different levels of kinetin and sucrose. One-half MS medium could induce more conversion of somatic embryos (55%) into longer and tubering shoots, even though tuber size was only one third of a full-grown medium size tuber. Mardani et al.[74] used seed explants of *B. persicum* for microtuberization and successfully worked out combination of MS + 5 mM Jasmonic acid and incubation at 15°C for microtuber formation.

Although several reports are available on tissue culture but limited success has been achieved in development of micropropagation protocols for this species with meagre field applicability. No effective strategy is available till date to reduce the propagation cycle for cultivation of *B. persicum*. Besides, the plant is highly niche specific that is a major constraint in domestication of this valuable plant species.

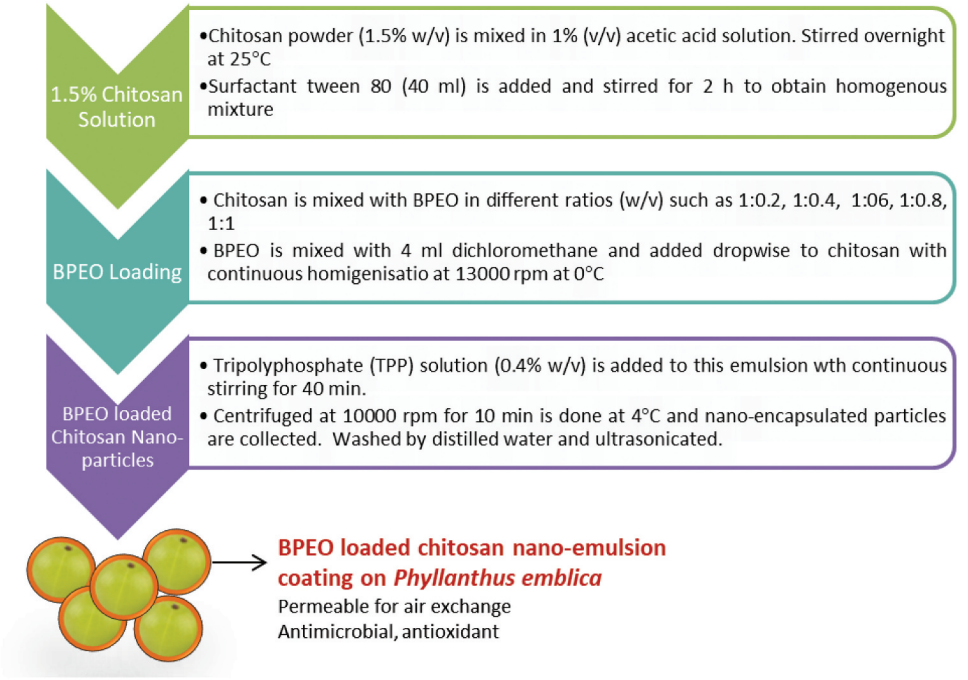

Figure 4. Preparation of *Bunium persicum* essential oil (BPEO) loaded chitosan nano-emulsion coating formulation[45].

Table 3. Cyto-morphological and genetic divergence studies in *B. persicum*.

S. No.	Study	Inference	Reference
1.	Cytomorphological studies	Chromosome no. = 14, Karyotype formula = 1–2 M + 2–3 Sm = 3–4 St, Total chromosome length = 28–35.9, Largest chromosome = 5.0–6.2, smallest chromosome = 3.0–4.0	[59]
2.		Diploid with 2 n = 14 and haploid number of n = 7, Mitotic divisions and chromosomes disjunction were regular and complete.	[60]
3	Genetic divergence studies	RAPD markers to assess the genetic diversity of Bunium plants collected from 15 locations of Himachal Pradesh and J&K.	[61]
4.		RAPD (15) and AFLP (17) markers were used to study the genetic relationships amongst 20 Iranian populations	[62]
5.		Genetic variation in germplasm of Persian Cumin collected from 43 different locations of Kerman province using RAPD (27) molecular markers	[63]
6.		RAPD (15) markers was used to study the genetic relationships amongst 20 wild Persian Zira populations from Iran (15), India (2), Afghanistan (2) and Europe (1)	[64]
7.	Karyotyping and chromosomal localization	Bicolour FISH technique involving 18S-5.8S-26S and 5S ribosomal RNA genes as probes to assign physical localization and measurement of rDNA sites on homologous chromosomes	[65]
8.	Biological adulterant detection	Four DNA barcoding loci namely ITS2, rbcL-a, mat K and psbA-trnH and a specific locus Cum were used for adulterant detection and authentication of *B. persicum*	[66]

Conclusion

Bunium persicum is a valuable seed spice and medicinal plant that grows wild in specific niche areas. It is widely used in people's diet due to its flavouring and various functional properties. In addition, its tuberous roots are consumed as vegetable by local populations. Its seeds possess high amounts of essential oils that have anti-inflammatory, antimicrobial and antioxidant as well as lipid and glucose-

Table 4. Propagation and tissue culture studies in *B. persicum*.

Explant	Method	Best Treatment/Medium	Result	Reference
Seed Germination Studies				
Seed	Stratification and chemical treatment	Cold treatment at 4°C and BA (10^{-5} M)	92.2% seed germination	[68]
Seed	Stratification and plant hormones treatment	GA_3 (100 µmol/L) and TDZ (6.3 µmol/L) treatment with moist chilling at 5°C	93.7% seed germination rate	[69]
Seed	Stratification	Cold treatment at 5°C	Reactive oxygen species (ROS) induced from abiotic stress stimulated GA biosynthesis and ABA catabolism	[70]
Seed	Plant hormones treatment	Kin (0.01 mM) with moist chilling at 4°C	77.33% seed germination was observed	[71]
Tissue Culture Studies				
Root, hypocotyl and cotyledon (leaf)	Somatic embryogenesis	MS + 0.5 mg l^{-1} NAA + 0.5 mg l^{-1} Kin + 4 mg l^{-1} NAA or MS + 2 mg l^{-1} 2, 4-D + 0.5 mg l^{-1} Kin	Callogenesis	[67]
		MS + 2 mg l^{-1} 2, 4-D, 0.5 mg l^{-1} BAP or MS + 1 mg l^{-1} BAP + 0.2 mg l^{-1} Kin	Root explants gave highest indirect embryogenesis	
Hypocotyl	Regeneration via indirect embryogenesis	B_5 + 0.1 mg l^{-1} 2,4-D + 2 mg l^{-1} Kin or B_5 + 1 mg l^{-1} NAA + 2 mg l^{-1} Kin	Callus initiation	[72]
		B_5 + 2 mg l^{-1} 2,4-D	Somatic Embryogenesis	
		B_5 + 0.1 mg l^{-1} NAA + 4 mg l^{-1} Kin	Shoot and root regeneration	
Hypocotyl	Regeneration via indirect embryogenesis	MS + 0.1 mg l^{-1} 2,4-D or MS + 1 mg l^{-1} 2,4-D or MS + 2 mg l^{-1} 2,4-D + 0.5 mg l^{-1} kin	Callus induction	[73]
		MS + 2 mg l^{-1} 2,4-D	Somatic embryogenesis	
		MS + 1 mg l^{-1} 2,4-D	Shoot regeneration	
Leaves, hypocotyl, inflorescence,	Regeneration	MSN + 0.4 mg l^{-1} 2,4-D	Callus initiation and establishment	[60]
		MSN + 2.5 mg l^{-1} Kin	Shoot induction	
		MSN + 6% sucrose + 1 mg l^{-1} IBA	Tuberized microshoot formation	
		MSN + 0.05% activated charcoal	Somatic embryogenesis	
Stem and leaf explants	Regeneration	MS + 4 mg l^{-1} Kin + 2 mg l^{-1} 2,4-D	Callus induction	[61]
		MS + 0.25 mg l^{-1} TDZ + 0.05 mg l^{-1} IBA	Shoot induction	
		MS+ 0.3 mg l^{-1} IBA	Root induction	
	Conservation	MS + 3% sucrose and sorbitol		
Seed	Microtuberization	MS + 5 mM JA and 15°C temperature	Microtubers	[74]
Cotyledon, hypocotyl and nodal explants	Callusing	MS + 2 mg l^{-1} BAP	Cotyledon explants gave best callus production (90%)	[75]
		MS + 2 mg l^{-1} BAP + 2 mg l^{-1} IAA	Shoot regeneration (70%)	
		½ MS + 1.5 mg l^{-1} IBA	Rooting	
Corm	Regeneration via indirect embryogenesis	MS + 90 g/L sucrose	Corm fresh weight (164.9 mg)	[76]
		MS medium + 1.0 mg l^{-1} 2,4-d	Somatic embryogenesis callus (74.9%)	
		½ MS medium + 20 g/l banana powder	Plantlet regeneration (65.8%)	

lowering effects. This plant can be exploited by different food and pharmaceutical industries to enhance the quality and shelf life of food products and develop functional foods/nutraceuticals, respectively. The species is threatened in its natural habitats; therefore, conservation of this valuable germplasm, development of efficient propagation methods, screening and breeding for superior germplasm should be taken up before it becomes extinct.

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Disclosure statement

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Author contribution statement

The author SB prepared outline of the review and collected the literature with authors KS and V. Authors SB, AAL, and EVM contributed to compilation of data and manuscript preparation. Authors SK and RS provided their critical inputs and edited the manuscript.

Data availability statement

This manuscript has no associated data.

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