

Biological Activity of *Bunium persicum* Essential Oil from Western Himalaya



Authors

Iris Stappen¹, Nurhayat Tabanca^{2,3}, Abbas Ali², David E. Wedge⁴, Jürgen Wanner⁵, Velizar Gochev⁶, Vikas Jaitak⁷, Brij Lal⁸, Vijay K. Kaul⁹, Erich Schmidt¹, Leopold Jirovetz¹

Affiliations

- 1 Department of Pharmaceutical Chemistry, University of Vienna, Vienna, Austria
- 2 National Center for Natural Products Research, The University of Mississippi, University, MS, USA
- 3 Department of Entomology and Nematology, Emerging Pathogens Institute, University of Florida, Gainesville, FL, USA
- 4 US Department of Agriculture, Agricultural Research Service, Natural Products Utilization Research Unit, University of Mississippi, University, MS, USA
- 5 Kurt Kitzing Co., Wallerstein, Germany
- 6 Department of Biochemistry and Microbiology, "Paisii Hilendarski" – University of Plovdiv, Bulgaria
- 7 Biodiversity Division, CSIR – Institute of Himalayan Bioresource Technology, Palampur, India
- 8 Centre for Chemical and Pharmaceutical Sciences, Central University of Punjab, Bathinda, India
- 9 Ex-Scientist of CSIR – Institute of Himalayan Bioresource Technology, Palampur, H.P., India

Key words

Bunium persicum, Apiaceae, essential oil, antimicrobial, plant pathogen, larvicidal

received 25.11.2016

revised 08.03.2017

accepted 09.03.2017

Bibliography

DOI <http://dx.doi.org/10.1055/s-0043-106857>

Published online: 2017

Planta Med Int Open 2017; 4: e52–e58

© Georg Thieme Verlag KG Stuttgart · New York

ISSN 2509-9264

Correspondence

Dr. Iris Stappen

Department of Pharmaceutical Chemistry

University of Vienna

Althanstrasse 14

1090 Vienna

Austria

Tel.: +43/1/4277 555 52, Fax: +43/1/4277 9 551

iris.stappen@univie.ac.at

ABSTRACT

The essential oil of *Bunium persicum* collected from cultivated sources in the cold desert area of Lahaul-Spiti is described for its antimicrobial, larvicidal, and biting deterrent activities. Additionally, odor characterization is given. The chemical composition of the essential oil was analyzed by simultaneous GC-MS and GC-FID. γ -Terpinene and p-cymene were found to be the major compounds. Antibacterial testing by an agar dilution assay revealed low activity of the oil against all tested bacteria. Antifungal activity was evaluated against *Candida albicans* as well as three species of the strawberry anthracnose causing plant pathogen *Colletotrichum*. *Bunium persicum* essential oil demonstrated antifungal activity against all four pathogens. Biting deterrent activity against *Aedes aegypti* was greater than the solvent control, but significantly lower than DEET. The essential oil of *B. persicum* exhibited larvicidal activity with an LC₅₀ value of 58.6 ppm against *Ae. aegypti* larvae.

Introduction

Many important pathogens causing severe diseases are transmitted to humans by insects. Malaria, dengue, yellow fever, and the West Nile virus are considered the most important mosquito-borne diseases in Asia, Africa, and South America [1]. However, due to global warming, these infectious pathogens spread to new areas threatening even more people and challenging academic and industrial communities [2]. Although many methods have been developed for mosquito control, the most effective method is the use of pesticides. Besides several synthetic pesticides that are available on the market, plant extracts and essential oils (EOs) have become important to research and industry due to their safety to the environment and non-target organisms [3]. The synergistic effect that has been found between some EOs and synthetic larvicides [1] would additionally offer an opportunity to develop more effective chemical control agents that are more valuable and less toxic to the environment.

Not only has the increasing presence of mosquitoes caused concern in the population, but also resistances against well-established substances have become challenging. Because of widespread indiscriminate use of antibiotics and agricultural pesticides since the 1950s, the development of chemical resistance in many bacteria, fungi, and insects, including mosquitoes, is frequent. Additionally, the use of synthetic food preservatives to prevent the growth of food-borne and spoilage bacteria and fungi is being criticized by customers because of their growing concerns over food safety. Therefore, the search for new natural antibiotics, fungicides, pest management agents, and food preservatives that have activity against multiple target sites and a low chance for the development of chemical resistance has led to an increased interest in EOs. Various studies, summed up and described in detail in a recent review by Reyes-Jurado et al. [4], have confirmed the activity of some EOs against many microbes.

In the Himalayan region, many plants and their EOs are economically important and have a history in traditional medicinal use, particularly by the populations living in rural areas where little medical care is available. *Bunium persicum* (Boiss.) B. Fedtsch. (Apiaceae), commonly known as black caraway or black zira, is an aromatic perennial native to the Indian subcontinent (India, Pakistan) and western to middle Asia. *Bunium* seeds are generally black in color, develop a characteristic pleasant aroma, and are widely used as condiments and flavoring agents in local foods [5, 6]. Seeds and the EO are well known for their digestive, anticonvulsive, diuretic, and anthelmintic effects [7, 8]. Due to the wide scope of pharmacological effects, the plants were extensively collected and sold by the Himachal Pradesh people. This led to a highly threatened population status for *B. persicum*. Subsequent attempts at cultivation of the plant demonstrated difficulties in germination because the seeds possess a deep dormancy mechanism. Various techniques were established to meet the complex dormancy requirements suitable for *B. persicum* germination [9]. Today the plants are being successfully cultivated by local farmers in the cold desert of Lahaul-Spiti, an area also with suitable farming conditions for black caraway.

In continuation of our studies on screening EOs from medicinal plants growing wild [10, 11] or cultivated in the western Himalayan region [12], the main purpose of the present investigation was to examine the EO of *B. persicum*. This species was collected from cultivated sources in Lahaul-Spiti at an altitude of 3 500 m, and tested for antimicrobial activities, mosquito biting deterrence, and larvicidal effect against *Aedes aegypti*.

Results and Discussion

B. persicum EO was characterized by olfactory evaluation as aromatic spicy, green herbal, and cumin-like. The results of the quantitative and qualitative oil analyses by simultaneous GC-MS and GC-FID using 2 different columns are listed in ► **Table 1**. Thirty-one compounds were identified from *B. persicum* EO, accounting for 97.7–97.9% of the total oil. γ -Terpinene was present in the highest amount (40.2–40.4%), followed by p-cymene (25.8%), cumin aldehyde (12.8–12.9%), p-mentha-1,4-dien-7-al (9.1–9.2%), and p-mentha-1,3-dien-7-al (4.5–4.7%) (► **Table 1**). Several investigations of *B. persicum* oil samples from plants collected or cultivated in Iran are described in the literature. All showed the same main components, but with different concentrations, e.g., the aromatic monoterpene p-cymene occurred at much lower amounts (5–16%) in the Iranian samples [7, 13–16], whereas cumin aldehyde was higher (24%) compared to our sample from India [13, 17]. An older investigation on *Bunium* fruits collected in Tajikistan reported p-mentha-1,4-dien-7-al (29.0%), γ -terpinene (25.7%), β -pinene (15.6%), and cumin aldehyde (11.7%) as major components [18]. These findings once more demonstrate the influence of climatic and local factors on EO composition [7, 19, 20] and possibly the difference between cultivated and wild growing species [21]. Therefore, plants from different origins or genetic backgrounds should be studied for their chemical composition in order to be able to conclude or link to their biological activities.

In the context of screening *B. persicum* EO for antimicrobial activity, the oil was tested against the Gram-positive bacterium *Staph-*

lococcus aureus, and Gram-negative bacteria *Escherichia coli*, *Salmonella abony*, and *Pseudomonas aeruginosa*. Growth inhibition testing of *Candida albicans* was performed in a liquid agar broth-microdilution assay [10]. Minimum inhibitory concentrations (MICs) in $\mu\text{g/mL}$ are given in ► **Table 2**.

B. persicum EO showed medium to low antimicrobial activity against all tested strains with MICs between 1 000 and 8 000 $\mu\text{g/mL}$ (► **Table 2**). *P. aeruginosa* was the least sensitive bacterium, while *C. albicans* proved to be the only strain sensitive to the tested oil. Essential oils generally show greater antimicrobial activity against Gram-positive bacteria than Gram-negative bacteria, which is probably related to the bacterial cell membrane structure [4, 22]. Literature reports on antibacterial activity of *B. persicum* EO show diverse results. An investigation of Iranian samples attributed very high antibacterial activity against *E. coli*, *P. aeruginosa* (Gram-negative bacilli), and *S. aureus* (Gram-positive cocci) to the oil, reporting MIC values around 15 $\mu\text{g/mL}$ [23]. In another Iranian study, *B. persicum* EO was found to be inactive against *E. coli* [24]. Results of Oroojalian et al. [25] on Iranian *B. persicum* EO showed an antimicrobial effect with an MIC value of 1 500 $\mu\text{g/mL}$ against *E. coli* and were comparable to our outcome. Activity against *S. aureus* (750 $\mu\text{g/mL}$) reported in the same publication was higher. The use of different strains and testing methods is the most probable explanation for these varied findings, but there are differences in EO origin and chemical composition that may play a role. In general, p-cymene and γ -terpene, the main components of *B. persicum* EO, are regarded as monoterpenes with low antimicrobial activity [26, 27]. These two compounds accounted for 66% of the oil tested in the present study. Investigations on pure γ -terpinene against *E. coli*, *S. aureus*, and *P. aeruginosa* revealed no activity against any of these bacterial species [26, 28]. In another study, p-cymene showed growth inhibition zones between 17–21 mm against *C. albicans* and between 9.6–11 mm against *S. aureus*, depending on the specific strains [29]. The authors concluded that none of the single compounds tested were as active as the whole EO. Therefore, the effect of an EO seems to be related to trace compounds or potential synergistic effects of various compound combinations. Using methods and conditions described by Mann et al. [25, 26] and Longbottom et al. [30], p-cymene and γ -terpinene have demonstrated activity against *P. aeruginosa*.

B. persicum EO was evaluated for its antifungal activity against the strawberry anthracnose, causing fungal plant pathogens *Colletotrichum acutatum*, *Colletotrichum fragariae*, and *Colletotrichum gloeosporioides*. Testing was performed with two concentrations of EO at 80 μg and 160 μg applications against three *Colletotrichum* species in the direct overlay bioautography assay (► **Table 3**). At the highest tested concentration of 160 $\mu\text{g/spot}$, *B. persicum* EO demonstrated better activity against all three *Colletotrichum* species with clear zones of 9.0–10.0 mm. A recent study showed growth inhibition activity against the soilborne phytopathogenic fungus *Fusarium osysporum* [31]. In another investigation, high antifungal activity of *B. persicum* EO against the same fungus was determined. The authors attributed this high inhibitory activity to cumin aldehyde and p-cymene [32]. In our previous antifungal studies, we demonstrated that EOs rich in non-oxygenated mono- or sesquiterpenes did not show good antifungal activity [33, 34]. Therefore, the antifungal effect of *B. persicum* oil might either be

► **Table 1** Chemical composition of *B. persicum* EO (in % peak area) determined by GC-FID and GC-MS.

No.	Compound	RI#	%	RI##	%
1	cumene	928	tr.	1151	tr.
2	α -thujene	931	0.3	1018	0.3
3	α -pinene	941	0.1	1015	0.2
4	sabinene	980	0.7	1109	0.7
5	β -pinene	986	0.1	1100	0.1
6	myrcene	992	0.7	1142	0.7
7	δ -3-carene	1018	0.1	1134	0.1
8	α -terpinene	1023	0.2	1163	0.2
9	p-cymene	1032	25.8	1252	25.8
10	limonene	1036	0.3	1184	0.3
11	β -phellandrene	1038	0.2	1196	0.2
12	1,8-cineole	1039	0.3	1202	0.2
13	(E)- β -ocimene	1049	tr.	1216	tr.
14	γ -terpinene	1067	40.4	1233	40.2
15	cis-sabinene hydrate	1070	tr.	1444	tr.
16	terpinolene	1096	0.3	1261	0.4
17	fenchone	1097	0.3	1376	0.3
18	linalool	1102	tr.	1523	tr.
19	trans-sabinene hydrate	1106	tr.	1530	tr.
20	fenchol	1124	0.1	1555	0.1
21	terpinen-4-ol	1188	0.2	1579	0.2
22	p-cymen-8-ol	1192	0.1	1820	0.1
23	α -terpineol	1201	0.1	1675	0.1
24	p-menth-3-en-7-al	1203	0.5	1540	0.4
25	(E,E)-nona-2,4-dienal	1209	tr.	1549	0.1
26	neral	1243	tr.	1661	0.1
27	carvone	1251	tr.	1708	0.1
28	cumin aldehyde	1254	12.9	1751	12.8
29	p-mentha-1,3-dien-7-al	1299	4.7	1767	4.5
30	p-mentha-1,4-dien-7-al	1304	9.1	1772	9.2
31	cuminic acid	1374	0.3	2350	0.4
	Sum		97.9		97.7

#50 m \times 0.25 mm \times 1.0 μ m SE-54; ##60 m \times 0.25 mm \times 0.25 μ m CW20M

► **Table 2** Antimicrobial activity of *B. persicum* EO (agar dilution assay, MIC given in μ g/mL).

Tested compounds	Test microorganism				
	<i>S. aureus</i> ATCC 6538	<i>E. coli</i> ATCC 25922	<i>S. abony</i> ATCC 6017	<i>P. aeruginosa</i> ATCC 27853	<i>C. albicans</i> ATCC 10231
<i>B. persicum</i> EO	4000	2000	2000	8000	1000
Ciprofloxacin	0.25	0.15	0.25	1	–
Cefazolin	0.50	2	2	4	–
Amphotericin B	–	–	–	–	0.25
Fluconazole	–	–	–	–	0.25

correlated to p-mentha-1,4-dien-7-al, p-mentha-1,3-dien-7-al, or cumin aldehyde in accordance with [32], or attributable to possible synergistic effects of one or more compounds present in the oil. Further research is needed to conduct bioassay-guided fractionation to separate and isolate the active compounds against *Colletotrichum* species.

Working with whole EOs is difficult because they are a complex oily matrix of chemistry, and meaningful biological conclusions will require specific bioassay methods and approaches. Direct bioautograph with fungal pathogens should identify the number of active compounds in the EO. Purification and subsequent testing of single entity chemicals at μ molar concentrations in a dose-re-

► **Table 3** Antifungal activity of *B. persicum* EO against 3 *Colletotrichum* species using direct bioautography assays.

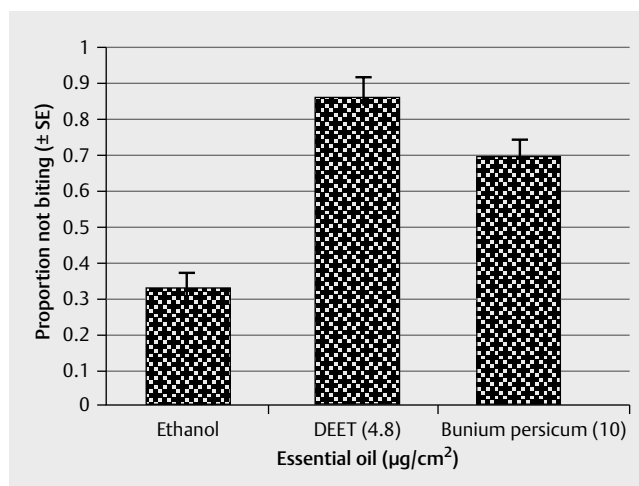
Tested compounds	Mean fungal growth inhibition [§] (mm) ± SD					
	<i>C. acutatum</i>		<i>C. fragariae</i>		<i>C. gloeosporioides</i>	
	80 µg/spot	160 µg/spot	80 µg/spot	160 µg/spot	80 µg/spot	160 µg/spot
<i>B. persicum</i> EO	5.0 ± 0.0	9.5 ± 0.7	5.5 ± 0.7	10.0 ± 0.0	5.0 ± 0.0	9.0 ± 0.0
Standard fungicides *						
Benomyl	Diffuse		21.5 ± 0.7		Diffuse	
Captan	12.0 ± 0.0		18.5 ± 0.7		19.5 ± 0.7	
Cyprodinil	Diffuse		Diffuse		Diffuse	
Azoxystrobin	Diffuse		24.5 ± 1.4		Diffuse	

[§]Mean inhibitory zones ± SD; * Technical grade of internal standards. Technical grade agrochemical fungicides (without formulation) were applied 2 µL from the 2 mm concentration

sponse format using liquid microdilution broth assays may help determine effects on spore germination and mycelial growth, and may suggest potential structure activity relationships.

Mosquito biting deterrent activity was determined against the yellow fever mosquito, *Ae. aegypti*. *B. persicum* EO showed activity greater than the solvent control (ethanol), but it was significantly less active than DEET, a standard biting deterrent, which was used as a positive control (► **Fig. 1**). Toxicity of *B. persicum* EO was determined in mosquito larvicidal bioassays [35]. *B. persicum* EO showed medium to low activity against 1-d old *Ae. aegypti* larvae with an LD₅₀ of 58.6 ppm. The LD₅₀ value of permethrin, which was used as a positive control, was 0.0034 ppm. Cheng et al. [36] reported that essential oils showing LC₅₀ values < 50 ppm can be considered as very active, whereas results > 100 ppm indicated an inactive EO. Making a valid scientific conclusion of results from the literature is difficult because of the variability in testing methods and conditions used by different authors. Since there was no universally accepted guideline to assess larvicidal activity, the use of positive and negative controls is necessary to compare results and make conclusions. Recently, the EO of *B. persicum*, collected in the Kerman region in Iran, was investigated for its larvicidal effects against *Anopheles stephensi* and *Culex pipiens* larvae. Results indicated high toxicity against adult insects with LC₅₀ values of 27.72 and 20.61 ppm after 24 h, respectively [17]. However, we report for the first time in this paper on the mosquito biting deterrent and larvicidal activity of *B. persicum* EO against *Ae. aegypti*. Studies using larvicidal activity to drive bioassay-guided fractionation should determine the active compounds. This method will also help determine the number of active compounds in the EO.

In conclusion, it appears that the EO of *B. persicum* from cultivated sources from western Himalaya had antifungal activity against *Colletotrichum* species, and future studies need to be conducted with active components using 1D-TLC bioautography to identify active individual compounds. The active compounds could be subsequently evaluated in 96-well microdilution broth assays against a broader range of important plant pathogenic fungi. The larvicidal activity of *B. persicum* EO against *Ae. aegypti* could be considered as medium to high. Additionally, the oil exhibited good biting deterrent activity. Based on these results, Himalayan black caraway oil (*B. persicum*) may be a promising potential source for new naturally derived agrochemicals or biopesticides. This research needs to be further investigated to study single entity



► **Fig. 1** Proportion of not biting values of *B. persicum* EO at 10 µg/cm² against female *Ae. aegypti*. DEET at 4.8 µg/cm² was used as a positive control. Ethanol was used as a solvent control.

active molecules and possible synergistic effects of chemical combinations.

Materials and Methods

Plant material and isolation procedure

B. persicum, cultivated by local farmers, was harvested from Lahulspiti—a cold desert area of western Himalaya, at an altitude of 3500 m, in October/November 2009. The samples were identified by the taxonomist (Brij Lal) of the Institute of Himalayan Biore-source Technology (IHBT Palampur, India). A voucher specimen (PLP 2845) was deposited at the Herbarium-PLP of the IHBT Palampur. Black seeds were air-dried at room temperature (about 25 °C) in the shade. One kilogram of seed material was hydrodistilled in a clevenger-type apparatus as described in [12], yielding light yellow colored oil (0.52% on dry wt. basis).

Essential oil analysis

Using a Finnigan ThermoQuest TraceGC with 2 split/splitless injectors, a FID detector, and a Finnigan Automass quadrupole mass spectrometer, GC-MS-FID analyses were carried out in one instru-

ment on a 50 m × 0.25 mm × 1.0 μm SE-52 fused silica column (CS Chromatographie Service) and a 60 m × 0.25 mm × 0.25 μm CW20M (J&W Scientific) column, respectively. Splitting of the column effluent was done with a quartz Y connector, one outlet connected to the MS interface via a short (ca. 20 cm) 0.1 mm ID fused silica capillary flow restrictor. The other outlet was attached to a 1 m × 0.25 mm deactivated fused silica capillary as a transfer line to the FID.

The use of this configuration resulted in an FID and an MS chromatogram with identical chromatographic separation and nearly the same retention times, thus facilitating peak assignments in the FID chromatogram with respect to the MS. The carrier gas was helium 5.0 with a constant flow rate of 1.5 mL/min, the injector temperature was 230 °C, FID detector temperature 250 °C, GC-MS interface heating 250 °C, ion source 150 °C, EI mode at 70 eV, scan range 40–500 amu, and scan rate of 500 μs. The following temperature program was used: 60 °C for 1 min isotherm, then increased at a rate of 3 °C/min to 230 °C. Identification of the compounds was performed as described in [10].

Olfactory evaluation

For olfactory evaluation, one droplet of *B. persicum* EO was applied onto commercially available paper blotters. Each sample was examined by a panel consisting of a professional perfumer and 2 aroma chemists over 90 min to control odor progression. Odor descriptions were compared to our own database of referenced aroma compounds.

Antimicrobial testing

The antimicrobial effects of *B. persicum* EO were tested against the Gram-negative bacteria *E. coli* (ATCC 25922), *S. abony* (ATCC 6017), and *P. aeruginosa* (ATCC 27853) and the Gram-positive bacterium *S. aureus* (ATCC 6538), as well as *C. albicans* (ATCC 10231). The strains were purchased from the National Reference Laboratory of Mycology at the National Center of Infectious and Parasitic Diseases, Sofia; Department of Microbiology and Immunology at the Medical University of Plovdiv and Clinical Laboratory Chronolab Ltd., Plovdiv. They were deposited in the Microbial Culture Collection of the Department of Biochemistry and Microbiology (University of Plovdiv, Bulgaria) and bacterial strains were stored on Nutritional Agar (NA, HiMedia Laboratories Ltd.). The fungal strain was stored on Sabouraud Dextrose Agar (SDA, HiMedia Laboratories Ltd.). Antimicrobial activity of *Bunium* EO was evaluated by broth microdilution tests described earlier [10]. Minimal inhibition concentration (MIC) was defined as the lowest concentration of EO that resulted in an absorbance reduction of >90% compared to the observed absorbance of control samples without EO. For positive controls, standard antibacterial antibiotic HiComb™ MIC test strips of Ciprofloxacin and Cefazolin and antifungal HiComb™ MIC test strips of Amphotericin B and Fluconazole (HiMedia Laboratories Ltd., 100% purity) were evaluated. All tests were performed in duplicate.

Antifungal testing

Pathogen production and bioautography procedures of Stappen et al. [10] were used to evaluate antifungal activity against fungal plant pathogens. The sensitivity of each fungal species to each test compound was determined by comparing the sizes of the inhibi-

tory zones. Bioautography experiments were performed multiple times using both dose- and non-dose-response formats. Fungicide technical grade standards benomyl (>98%), cyprodinil (>98%), azoxystrobin (>98%), and captan (>98%; Chem Service, Inc.) were used as controls at 2 mM in 2 μL of 95% ethanol. *B. persicum* EO was spotted with 80 and 160 μg/spot in hexane. To detect biological activity directly on the TLC plate, silica gel plates were sprayed with one of the 3 spore suspensions adjusted to a final concentration of 3.0×10^5 conidia/mL with liquid potato dextrose broth (PDB, Difco) and 0.1% Tween-80. Using a 50-mL chromatographic sprayer, each TLC plate with a fluorescent indicator (250 μm, silica gel GF Uniplate; Analtech, Inc.) was sprayed lightly (to a damp appearance) 3 times with the conidial suspension. Inoculated plates were then placed in a 30 × 13 × 7.5 cm moisture chamber (39 °C, 100% relative humidity; Pioneer Plastics, Inc.) and incubated in a growth chamber at 24 ± 1 °C and a 12-h photoperiod under $60 \pm 5 \mu\text{mol} \cdot \text{m}^{-2} \text{s}^{-1}$ light. The inhibition of fungal growth was measured 4 days after treatment. The sensitivity of each fungal species to each test compound was determined by comparing the size of the inhibitory zones on the TLC plate.

Mosquito biting testing

Mosquitoes: *Ae. aegypti* larvae used in these studies were from a laboratory colony maintained at the Mosquito and Fly Research Unit at the Center for Medical, Agricultural and Veterinary Entomology, USDA-ARS, Gainesville, Florida, using standard rearing practices [37]. For biting deterrence bioassays, pupae were maintained in the laboratory at 27 ± 2 °C and 60 ± 10 % RH in a photoperiod regimen of 12:12 h (L:D). For larvicidal bioassays, the eggs were hatched and the larvae were maintained at the above temperature.

Mosquito biting bioassays: Experiments were conducted by using a 6-celled *in vitro* Klun and Debboun (K&D) module bioassay system developed by Klun et al. [38] for quantitative evaluation of biting deterrent properties of candidate compounds. Briefly, the assay system consists of a 6-well reservoir with each of the 3 × 4 cm wells containing 6 mL of blood. As described by Ali et al. [39], a feeding solution consisting of CPDA-1 and ATP was used instead of blood. A green fluorescent tracer dye (www.blacklightworld.com) was used to determine the feeding by the females. Treatments of the EO of *B. persicum* were applied at 10 μg/cm², and DEET (97%, N, N-diethyl-*meta*-toluamide; Sigma Aldrich) at 25 nmol/cm² was used as a positive control, while ethanol served as a solvent control. All treatments were conducted as described in [10].

Larvicidal bioassays

Bioassays were conducted to test the EO of *B. persicum* for their larvicidal activity against *Ae. aegypti* by using the bioassay system described by Pridgeon et al. [40]. Further methods and statistical analyses are described in [35]. DMSO was used as a solvent to prepare the treatments and was also used as a negative control. Permethrin (95.7%; Chem Service, Inc.) was used as a positive control.

Acknowledgements

This study was supported in part by USDA/ARS grant No. 56-6402-1-612 and the Deployed War-Fighter Protection research program grant funded by the U.S. Department of Defense through the

Armed Forces Pest Management Board. We thank Ms. J. L. Robertson and Ms. R. Pace for their help in the antifungal bioassays and Dr. James J. Becnel, Mosquito and Fly Research Unit, Center for Medical, Agricultural and Veterinary Entomology (CMAVE), USDA-ARS, Gainesville, Florida, USA, for supplying *Ae. aegypti* eggs. Authors Vijay K. Kaul, Brij Lal, and Vikas Jaitak are thankful to the Director CSIR-IHBT Palampur for providing necessary facilities to carry out the plant survey/collection of plant materials as well as for hydro-distillation and analytical work. We would also like to thank Alden S. Estep and William Reid, USDA-ARS, CMAVE, Gainesville, Florida USA, for internal peer review of the manuscript and their suggestions.

Conflicts of Interest

The authors declare no conflict of interest.

References

- [1] Pohlit AM, Lopes NP, Gama RA, Tadei WP, de Andrade Neto VF. Patent literature on mosquito repellent inventions which contain plant essential oils – a review. *Planta Med* 2011; 77: 598–617
- [2] Chen TH, Aure WE, Cruz EI, Malbas FF Jr., Teng HJ, Lu LC, Kim KS, Tsuda Y, Shu PY. Avian Plasmodium infection in field-collected mosquitoes during 2012–2013 in Tarlac, Philippines. *J Vector Ecol* 2015; 40: 386–392
- [3] Vatandoost H, Dehkordi AS, Sadeghi AMT, Davari B, Karimian F, Agai MR, Sedaghat MM. Identification of chemical constituents and larvicidal activity of *Kelussia odoratissima* Mozaffarian essential oil against two mosquito vectors *Anopheles stephensi* and *Culex pipiens* (Diptera: Culicidae). *Exp Parasitol* 2012; 132: 470–474
- [4] Reyes-Jurado F, Franco-Vega A, Ramirez-Corona N, Palou E, Lopez-Malo A. Essential oils: Antimicrobial activities, extraction methods and their modeling. *Food Eng Rev* 2015; 7: 275–297
- [5] Phani KG, Gupta S, Pal MM, Singh SB. Ethnobotanical studies of Nubra Valley – A cold arid zone of Himalaya. *Ethnobot Leaflets* 2009; 13: 752–765
- [6] Ali H, Sannai J, Sher H, Rashid A. Ethnobotanical profile of some plant resources in Malam Jabba valley of Swat, Pakistan. *J Med Plant Res* 2011; 5: 4676–4687
- [7] Azimzadeh M, Amiri R, Assareh MH, Bihanta MR, Forootan M. Genetic diversity of Iranian *Bunium persicum* germplasm by morphological markers and essential oil components. *J Med Plant Res* 2012; 6: 1119–1129
- [8] Mandegary A, Arab-Nozari M, Ramiar H, Shafifar F. Anticonvulsant activity of the essential oil and methanolic extract of *Bunium persicum* (Boiss.) D. Fedtsch. *J Ethnopharm* 2012; 140: 447–451
- [9] Sharma RK, Sharma S. Effect of storage and cold-stratification on seed physiological aspects of *Bunium persicum*: A threatened medicinal herb of trans-Himalaya. *Int J Bot* 2010; 6: 151–156
- [10] Stappen I, Wanner J, Tabanca N, Wedge D, Ali A, Khan IA, Kaul VK, Jaitak B, Gochev V, Girova T, Stovanova A, Schmidt E, Jirovetz L. Chemical composition and biological effects of *Artemisia maritima* and *Artemisia nilagirica* essential oils from wild plants of western Himalaya. *Planta Med* 2014; 13: 1079–1089
- [11] Stappen I, Wanner J, Tabanca N, Wedge DE, Abbas A, Kaul VK, Lal J, Jaitak V, Gochev VK, Schmidt E, Jirovetz L. Chemical composition and biological activity of essential oils of *Dracocephalum heterophyllum* and *Hyssopus officinalis* from Western Himalaya. *Nat Prod Comm* 2015; 10: 133–138
- [12] Stappen I, Ali A, Tabanca N, Khan IA, Wanner J, Gochev VK, Singh B, Lal B, Jaitak V, Kaul VK, Schmidt E, Jirovetz L. Antimicrobial and repellent activity of the essential oils of two Lamiaceae cultivated in Western Himalaya. *Curr Bioact Comp* 2015; 11: 23–30
- [13] Hajhashemi V, Sajjadi SE, Zomorodkia M. Antinociceptive and anti-inflammatory activities of *Bunium persicum* essential oil, hydroalcoholic and polyphenolic extracts in animal models. *Pharm Biol* 2011; 49: 146–151
- [14] Shariffar F, Yassa N, Mozaffarian V. Bioactivity of major components from the seeds of *Bunium persicum* (Boiss.) Fedtch. *Pakistan J Pharm Sci* 2010; 23: 300–304
- [15] Azizi M, Davarenejad G, Bos R, Woerdenbag HJ, Kayser O. Essential oil content and constituents of black zira (*Bunium persicum* [Boiss.] B. Fedtsch.) from Iran during field cultivation (domestication). *J Ess Oil Res* 2009; 21: 78–82
- [16] Shahsavari N, Barzegar M. Antioxidant activity and chemical characterization of essential oil of *Bunium persicum*. *Plant Foods Hum Nutr* 2008; 63: 183–188
- [17] Sanei-Dehkordi A, Vatandoost H, Abaei MR, Davari B, Sedaghat MM. Chemical composition and larvicidal activity of *Bunium persicum* essential oil against two important mosquitoes vectors. *J Ess Oil Bear Plants* 2016; 19: 349–357
- [18] Baser KHC, Özek T. Composition of the essential oil of *Bunium persicum* (Boiss.) B. Fedtsch. from Tajikistan. *J Ess Oil Res* 1997; 9: 597–598
- [19] Fathiazad F, Mazandarani M, Hamedeyazdan S. Phytochemical analysis and antioxidant activity of *Hyssopus officinalis* L. from Iran. *Adv Pharm Bull* 2011; 1: 63–67
- [20] Omidbaigi T, Arvin MJ. Effect of growing locations of the essential oil content and chemical compositions of *Bunium persicum* Boiss. wild growing in Iran. *J Ess Oil Bear Plants* 2009; 12: 34–40
- [21] Nemeth E, Bernáth J, Tarján G. Quantitative and qualitative studies of essential oils of Hungarian *Achillea* populations. *JHSMP* 2007; 13: 57–69
- [22] Tongnuanchan P, Benjakul S. Essential oils: Extraction, bioactivities, and their uses for food preservation. *J Food Sci* 2014; 79: R1231–R1249
- [23] Talei GR, Mosavi Z. Chemical composition and antibacterial activity of *Bunium persicum* from west of Iran. *Asian J Chem* 2009; 21: 4749–4754
- [24] Aliakbarlu J, Sadaghiani SK, Mohammadi S. Comparative evaluation of antioxidant and anti-food-borne bacterial activities of essential oils from some species commonly consumed in Iran. *Food Sci Biotech* 2013; 22: 1487–1493
- [25] Oroojalian G, Kasa-Kermanshahi R, Azizi M, Bassami MR. Phytochemical composition of the essential oils from three Apiaceae species and their antibacterial effects on food-borne pathogens. *Food Chem* 2010; 120: 765–770
- [26] Mann CM, Cox SE, Markham JL. The outer membrane of *Pseudomonas aeruginosa* NCTC 6749 contributes to its tolerance to the essential oil of *Melaleuca alternifolia* (tea tree oil). *Lett Appl Microbiol* 2000; 30: 294–297
- [27] Papadopoulos CJ, Carson CF, Chang BJ, Riley TV. Role of the MexAB-OprM efflux pump of *Pseudomonas aeruginosa* in tolerance to tea tree (*Melaleuca alternifolia*) oil and its monoterpenes terpinen-4-ol, 1,8-cineole, and α -terpineol. *Appl Environ Microbiol* 2008; 74: 1932–1935
- [28] Aridogan BC, Baydar H, Kaya S, Demirci M, Oezbasar D, Mumcu E. Antimicrobial activity and chemical composition of some essential oils. *Arch Pharm Res* 2002; 25: 860–864
- [29] Filipowicz N, Kaminski M, Kurlenda J, Asztemborska MA, Ochocka JR. Antibacterial and antifungal activity of juniper berry oil and its selected components. *Phytother Res* 2003; 17: 227–231

- [30] Longbottom CJ, Carson CH, Hammer KA, Mee BJ, Riley TV. Tolerance of *Pseudomonas aeruginosa* to *Melaleuca alternifolia* (tea tree) oil is associated with the outer membrane and energy-dependent cellular process. *J Antimicrob Chemother* 2004; 54: 386–392
- [31] Behtoei H, Amini J, Javadi T, Sadeghi A. Composition and in vitro antifungal activity of *Bunium persicum*, *Carum copticum* and *Cinnamomum zeylanicum* essential oils. *J Med Plants Res* 2012; 6: 5069–5076
- [32] Sekine T, Sugano M, Majid A. Antifungal effects of volatile compounds from black zira (*Bunium persicum*) and other species and herbs. *J Chem Ecol* 2007; 33: 2123–2132
- [33] Tabanca N, Demirci B, Gurbuz I, Demirci F, Becnel JJ, Wedge DE, Baser KHC. Essential oil of five collections of *Achillea biersteinii* from central Turkey and their antifungal and insecticidal activity. *Nat Prod Comm* 2011; 6: 701–706
- [34] Tabanca N, Gao Z, Demirci B, Techen N, Wedge DE, Ali A, Sampson BJ, Werle C, Bernier UR, Khan IA, Baser KHC. Molecular and phytochemical investigation of *Angelica dahurica* and *A. pubescentis* essential oils and their biological activity against *Aedes aegypti*, *Stephanitis pyrioides* and *Colletotrichum* species. *J Agric Food Chem* 2014; 62: 8848–8857
- [35] Ali A, Tabanca N, Demirci B, Baser KHC, Ellis J, Gray S, Lackey BR, Murphy C, Khan IA, Wedge DE. Composition, mosquito larvicidal, biting deterrent and antifungal activity of essential oils of different plant parts of *Cupressus arizonica* var. *glabra* (Sudw.) Little ('Carolina Sapphire'). *Nat Prod Comm* 2013; 8: 257–260
- [36] Cheng SS, Chang HT, Chang ST, Tsai KG, Chen WJ. Bioactivity of selected plant essential oils against the yellow fever mosquito *Aedes aegypti* larvae. *Biores Technol* 2003; 89: 99–102
- [37] Pridgeon JW, Meepagala KM, Becnel JJ, Clark GG, Pereira RM, Linthicum KJ. Structure-activity relationships of 33 piperidines as toxicants against female adults of *Aedes aegypti* (Diptera: Culicidae). *J Med Entomol* 2007; 44: 263–269
- [38] Klun JA, Kramer M, Debboun M. A new in vitro bioassay system for discovery of novel human-use mosquito repellents. *J Am Mosq Control Assoc* 2005; 21: 64–70
- [39] Ali A, Cantrell CL, Bernier UR, Duke SO, Schneider JC, Khan I. *Aedes aegypti* (Diptera: Culicidae) biting deterrence: structure-activity relationship of saturated and unsaturated fatty acids. *J Med Entomol* 2012; 49: 1370–1378
- [40] Pridgeon JW, Becnel JJ, Clark GG, Linthicum KJ. A high throughput screening method to identify potential pesticides for mosquito control. *J Med Entomol* 2009; 46: 335–341