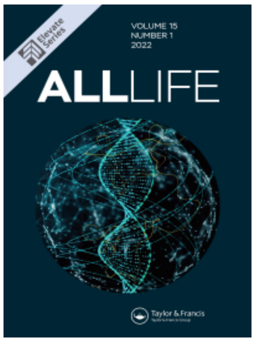

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Spatial repellency effects of Taiwanese plant oils on the biting midge, *Forcipomyia taiwana*

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ABSTRACT

The biting midge, *Forcipomyia taiwana* (Shiraki) (Diptera: Ceratopogonidae), can cause severe allergic reactions. It can pass through typical mosquito netting, so repellents are an important control measure against it. This project sought to identify spatial repellents from plants traditionally used by Taiwanese Aboriginal peoples as insect repellents. Fresh plant leaves were collected, frozen, and powdered, and essential oils extracted using hexane then ethyl acetate as solvents. Some commercial oils were also used. The extracts were tested against lab-reared *F. taiwana* using a Y-tube olfactometer. The composition of the extracts was identified using gas-chromatography mass-spectrometry (GC-MS). Commercial citronella, lavender, and Formosan cypress oils were effective repellents, as were lab-made capillary wormwood and camphor oils. Knockdown was detected in commercial clove leaf, rosemary, common wormwood, and cajuput oils. The oil composition data is the first for many of the Taiwanese plants, many of which contained repellent compounds like caryophyllene, α-pinene, and germacrene D. The results agreed with previous studies on the effects of some plants but differed on others, possibly due differences in plant chemotype, extraction method, and oil concentrations.

Key Policy Highlights

- Botanical oils may have some effect as personal repellents against *Forcipomyia taiwana*.
- Commercially available oils show the most promise.
- Certain endangered trees show promise as sources of repellents, so their conservation should be prioritized.

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Forcipomyia taiwana; insect repellent; biting midge; essential oils; bioprospecting; GC-MS

1. Introduction

The tiny, biting midge *Forcipomyia taiwana* (Shiraki) (Diptera: Ceratopogonidae) is a widely distributed pest in Taiwan (Chuang et al. 2000; Chen et al. 2005). Adult females must blood-feed before laying eggs (Luo 2018). They preferentially feed on human blood, biting exposed skin during daytime hours, especially 13:00-15:00 (Chen et al. 1981; Chuang et al. 2000; Luo 2018). Their bite causes intense itching and inflammation, with a rash that can last for days and, if scratched, scars that last for weeks. They are a significant nuisance, with cases of parks and playgrounds being closed and other tourist attractions negatively affected due to high *F. taiwana* activity (Yu 2019). While not a disease vector, the insect is considered hazardous because approximately 60% of Taiwan's human population are allergic to its salivary proteins, with some requiring medical attention (Lee et al. 2014). Reducing the populations of this pest is challenging, as the larvae are ubiquitous in moist soils (Chen et al. 2016), so bite prevention is seen as the primary defense against the insect.

Insect bite reduction is typically done through protective fabric or use of spatial or topical chemical repellents. As they are extremely tiny (1-1.5 mm), adults *F. taiwana* can fly through window screens and some mosquito nettings, and minimizing exposed skin is not always practical or desirable for all people in all weather, so finding a suitable chemical repellent is essential to prevent *F. taiwana* bites. These

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midges are repelled by diethyltoluamide (DEET), but not as effectively as mosquitoes are (Chang 2016), and other species of biting midges are attracted to some mosquito repellents (Braverman et al. 1999). This reduced efficacy combined with the general public's concerns, unfounded or otherwise, over the safety of synthetic repellents (Shelomi 2020) means demand exists for strong, botanical repellents against *F. taiwana*.

Plant essential oils are a mixture of volatile, secondary plant metabolites primarily containing terpenes, as well as phenols, coumarins, alkaloids, and other compounds (Ríos 2016). Traditionally extracted using hydrodistillation, many authors and lay persons use the term 'essential oil' equally for products extracted using other solvents or via solvent-free extraction processes (Harbone 1998; Younis et al. 2007; Kawase et al. 2013; Li et al. 2013; da Silva et al. 2022). While historically used for their flavors and aromas, or for alleged therapeutic properties, recent interest in their biocidal activities has attracted the attention of entomologists (Isman 2016; Schmidt 2020). Several plant essential oils are known to repel insects, such as *Azadirachta indica* L. oil (Sharma et al. 1993), while other plant-derived substances have insecticidal properties, such as camphor (Jiang et al. 2016) and cajuput oil (Abu Bakar et al. 2012). Essential oils have several present and potential uses in medical entomology, for example as mosquito repellents, larvicides, and adulticides (Al-Mekhlafi et al. 2021; Esmaili et al. 2021; Li et al. 2021; Meisyara et al. 2021; Narayanankutty et al. 2021; Norris and Bloomquist 2021; Spinozzi et al. 2021; Sukkanon et al. 2021; Bhoopong et al. 2022; Corrêa et al. 2022; Luo et al. 2022; Ochola et al. 2022). Some of these are effective as a contact repellent, which is applied to the skin and discourages insects from landing or feeding, while others like citronella (*Cymbopogon* sp. Spreng.) oil are spatial repellents that repel insects from a given area (Revay et al. 2013; Shimomura et al. 2021). Recent studies have examined the use of plant essential oils against bedbugs (Elbanoby 2019), stored-product pests (Qi et al. 2021), cockroaches (Gondhalekar et al. 2021), varroa mites (Alahyane et al. 2022), and root-knot nematodes (Faria and Rodrigues 2021). Essential oils degrade rapidly in the environment, which makes them environmentally friendly, but also limits their duration of effect, especially as a pesticide. Novel techniques like microencapsulation, microemulsions, and oil-loaded nanomaterials are being examined as ways to improve the efficiency of these oils (Menossi et al. 2021; Razola-Díaz et al. 2021; Thakur et al. 2021; Sanei-Dehkordi et al. 2022; Sousa et al. 2022).

Effective, botanical repellents for *F. taiwana* may already be known, yet have not been quantitatively evaluated. Namely, several plants have been used as insect repellents by Taiwanese indigenous peoples by rubbing leaves against the skin (Shieh et al. 2009). Some of these and other Taiwanese plants' extracts or their active components have been tested as repellents against adult, non-midge insects like mosquitoes (Cheng et al. 2003; Chio and Yang 2008; Gokulakrishnan et al. 2013), and others have been tested as larvicides against mosquitoes (Cheng et al. 2004; Kuo et al. 2007; Cheng et al. 2008; Zhu et al. 2008; Cheng et al. 2009; Cheng et al. 2009; Cheng et al. 2009; Cheng et al. 2013). One master's thesis found that *Cryptomeria japonica* (Thunb. ex L.f.) D.Don, *Cinnamomum osmophloeum* Kaneh., and *Clausena excavata* Burm.f. are effective repellents against *F. taiwana* at concentrations as low as 1.75%, all superior to 15% DEET, with *C. osmophloeum* working best (Chang 2016). A conference proceedings paper found repellent activities for *Corymbia citriodora* (Hook.) K.D.Hill & L.A.S.Johnson and *Lavandula angustifolia* Chaix ex Vill. Mill., but not for *Eucalyptus globulus* Labill., *Mentha piperita* L., or *Melaleuca alternifolia* (Maiden & Betche) Cheel (Lin et al. 2017). A 2014 paper found that aqueous extracts of *C. osmophloeum*, *Cymbopogon excavatus* (Hochst.) Stapf, *Mentha* sp. L., *Salvia rosmarinus* (L.) Schleid. (syn. *Rosmarinus officinalis* L.), and *Salvia officinalis* L. repel *F. taiwana*, but, again, not *E. globulus* (Lou et al. 2014). Vinegar made from heated *Mikania micrantha* Kunth, the invasive 'mile-a-minute' vine native to Americas but a weed in Taiwan, was also found to repel *F. taiwana* (Hu 2013). The relative paucity of data for *F. taiwana* in peer-reviewed journal papers compared to data on essential oils for mosquitoes is notable, and this project sought to correct this disparity.

Following the principles of biopropsecting, we tested extracts from Taiwanese plants with a history of use as topical insect repellents for spatial repellency activity against *F. taiwana*, with the goal of finding a potent, insect repelling active ingredient that can then be developed for practical use against *F. taiwana*, if not other biting pests. We also identified their chemical composition, as such data for most of these plants' oils is lacking and, as some plants traditionally used as repellents are now considered endangered or threatened (ex: *Chamaecyparis formosensis* Matsum.), identifying the active ingredients to develop a synthetic alternative to the essential oil can prevent the plant from being overexploited.

2. Materials and methods

2.1. Plant collection

Plants were chosen for putative repellency based on prior literature and personal communication with Prof. Chin-Fah Wang, National Chiayi University, on the subject of plants used by some Aboriginal Taiwanese peoples as repellents. The plants are listed in Table 1. For 18 of these plants, commercially-produced essential oils already exist. These oils were purchased from Fusheng Instrument Co., Ltd. (Taiwan) (FSI) or O'ddenio International Company (Taiwan) (OIC) (Table 1). Fresh leaves from 25 other plants were collected from the National Taiwan University (NTU) campus throughout 2021, from the Taipei Botanical Garden (TBG) in April 2021, or from Taipingshan National Forest (TNF) in Yilan in August 2021, after obtaining proper permissions and permits (Table 1). Plants were identified by their identification marker plaques, if present, or by collaborating botanists, and voucher specimens were made and stored in the National Taiwan University Herbarium. Leaves were collected from at least two individual plants per species and mixed together to account for individual variation.

2.2. Essential oil extraction

Plant leaves were immediately brought to the laboratory and kept frozen at −4 °C or −20 °C. To produce essential oils, 50 grams of frozen leaves were weighed and then ground into a fine powder using a motorized Chinese medicine grinder (Yuzako Co., Ltd., Changhua County, Taiwan). Oils were extracted in a custom-fabricated Soxhlet extractor (Sitholé et al. 2010; Redfern et al. 2014). Cotton wool was placed at the bottom of the Soxhlet tube extraction chamber to keep the ground leaves from entering the siphon arm (Ravi et al. 2018). The 50 grams of ground leaves were packed in, and 150 mL of pure hexane was added. The system was run at approximately 70°C for 24 h. The hexane-oil solution was then removed, and 150 mL of pure ethyl acetate was added to the Soxhlet chamber. The extraction was run again at approximately 78°C for 24 h. The ethyl acetate-oil solution was removed, and the remaining leaves discarded. For each plant, two such runs of Soxhlet extraction were performed.

The resulting solvent-oil solutions usually contained two layers: an orange-colored, heavy phase and a green-colored, light phase. The light phase containing the essential oils was separated using a separator

Table 1. Results of the Y-tube olfactometer tests with F. taiwana.

Scientific name	Source	Solvent	Knock	Mean ± SE $RI_{0\mu L}$	Mean ± SE $RI_{10\mu L}$	Mean ± SE $RI_{50\mu L}$	Anova F-stat	Sig.
Acacia confusa Merr. (Fabaceae)	TBG	Hex	0	0.016 ± 0.10	−0.003 ± 0.13	0.004 ± 0.28	F(2,9) = 0.01059	
		EtAc	0	−0.087 ± 0.19	−0.009 ± 0.17	−0.022 ± 0.33	F(2,9) = 0.1183	
Artemisia absinthium L. (Asteraceae)	OIC		4.86%	−0.022 ± 0.11	−0.154 ± 0.07	−0.342 ± 0.13	F(2,9) = 9.2800	**
Artemisia capillaris Thunb. (Asteraceae)	TNF	Hex	0	−0.020 ± 0.14	−0.084 ± 0.17	−0.290 ± 0.10	F(2,9) = 4.1065	•
		EtAc	0	0.073 ± 0.22	0.081 ± 0.12	−0.298 ± 0.20	F(2,9) = 5.6380	*
Azadirachta indica L. A.Juss. (Meliaceae) seed	OIC		0	−0.027 ± 0.12	−0.053 ± 0.09	−0.132 ± 0.22	F(2,9) = 0.5120	
Bidens alba (L.) DC. (Asteraceae)	NTU	Hex	0	−0.001 ± 0.06	−0.001 ± 0.06	−0.036 ± 0.21	F(2,9) = 0.0942	
		EtAc	0	−0.061 ± 0.06	−0.132 ± 0.10	−0.186 ± 0.11	F(2,9) = 1.8063	
Calocedrus formosana (Florin) (Cupressaceae)	NTU	Hex	0	−0.064 ± 0.05	0.037 ± 0.11	−0.005 ± 0.15	F(2,9) = 0.8650	
		EtAc	0	0.066 ± 0.09	0.032 ± 0.16	−0.078 ± 0.10	F(2,9) = 1.5931	
Camellia sinensis L. (Theaceae)	FSI		0	0.117 ± 0.21	−0.064 ± 0.29	−0.284 ± 0.42	F(2,9) = 1.5951	
Chamaecyparis formosensis Matsum. (Cupressaceae) leaves	TNF	Hex	0	0.073 ± 0.14	−0.116 ± 0.23	−0.049 ± 0.12	F(2,9) = 1.2812	
		EtAc	0	0.040 ± 0.12	−0.098 ± 0.20	−0.220 ± 0.31	F(2,9) = 1.3366	
Chamaecyparis formosensis Matsum. (Cupressaceae) wood	OIC		0	−0.004 ± 0.14	−0.135 ± 0.13	−0.513 ± 0.19	F(2,9) = 11.763	**
Cinnamomum camphora (L.) J.Presl (Lauraceae) bark	OIC		0	−0.058 ± 0.08	−0.295 ± 0.19	−0.354 ± 0.22	F(2,9) = 3.2951	•
Cinnamomum camphora (L.) J.Presl (Lauraceae) leaves	NTU	Hex	0	0.058 ± 0.14	−0.114 ± 0.20	−0.459 ± 0.11	F(2,9) = 11.249	**

(*continued*).

Table 1. Continued.

Scientific name	Source	Solvent	Knock	Mean ± SE $RI_{0\mu L}$	Mean ± SE $RI_{10\mu L}$	Mean ± SE $RI_{50\mu L}$	Anova F-stat	Sig.
		EtAc	0	−0.057 ± 0.15	−0.180 ± 0.06	−0.214 ± 0.04	F(2,9) = 2.9913	
Cinnamomum osmophloeum Kaneh. (Lauraceae)	TBG	Hex	0	−0.005 ± 0.21	−0.127 ± 0.09	−0.117 ± 0.21	F(2,9) = 0.5694	
		EtAc	0	−0.017 ± 0.16	−0.032 ± 0.17	−0.057 ± 0.13	F(2,9) = 0.0682	
Clausena excavata Burm.f. (Rutaceae)	NTU	Hex	0	−0.022 ± 0.11	0.188 ± 0.11	0.037 ± 0.24	F(2,9) = 1.7185	
		EtAc	0	0.101 ± 0.11	−0.192 ± 0.09	−0.057 ± 0.12	F(2,9) = 7.5759	*
Corymbia citriodora (Hook.) K.D.Hill & L.A.S.Johnson (Myrtaceae)	NTU	Hex	0	0.008 ± 0.09	−0.106 ± 0.05	−0.303 ± 0.31	F(2,9) = 2.7585	
		EtAc	0	0.015 ± 0.05	−0.012 ± 0.08	−0.129 ± 0.17	F(2,9) = 1.8944	
Cryptomeria japonica (Thunb. ex L.f.) D.Don (Cupressaceae)	TNF	Hex	0	−0.083 ± 0.07	−0.081 ± 0.20	−0.074 ± 0.24	F(2,9) = 0.0021	
		EtAc	0	−0.004 ± 0.14	0.075 ± 0.06	0.086 ± 0.16	F(2,9) = 0.5688	
Cunninghamia konishii Hayata (Cupressaceae)	TNF	Hex	0	0.037 ± 0.10	0.024 ± 0.17	0.197 ± 0.14	F(2,9) = 1.8844	
		EtAc	1.79%	0.012 ± 0.13	−0.099 ± 0.22	0.051 ± 0.21	F(2,9) = 0.6735	
Cymbopogon nardus (L.) Rendle (Poaceae)	OIC		0	−0.031 ± 0.22	−0.447 ± 0.21	−0.613 ± 0.22	F(2,9) = 7.5596	*
Dimocarpus longan Lour. (Sapindaceae)	NTU	Hex	0	0.043 ± 0.06	0.073 ± 0.09	−0.007 ± 0.16	F(2,9) = 0.5507	
		EtAc	2.94%	0.003 ± 0.10	0.004 ± 0.19	−0.010 ± 0.14	F(2,9) = 0.0114	
Eucalyptus robusta Sm. (Myrtaceae)	NTU	Hex	0	−0.017 ± 0.03	−0.014 ± 0.10	−0.062 ± 0.13	F(2,9) = 0.3392	
		EtAc	2.17%	−0.014 ± 0.05	0.037 ± 0.07	0.026 ± 0.10	F(2,9) = 0.5172	
Eucalyptus sp. L'Hér (Myrtaceae)	FSI		0	−0.023 ± 0.08	−0.239 ± 0.34	−0.505 ± 0.24	F(2,9) = 3.8868	•
Eupatorium formosanum Hayata (Asteraceae)	TNF	Hex	0	−0.091 ± 0.03	0.001 ± 0.10	0.036 ± 0.16	F(2,9) = 1.4193	
		EtAc	0	−0.160 ± 0.18	−0.006 ± 0.27	−0.107 ± 0.22	F(2,9) = 0.4822	
Ficus septica Burm.f. (Moraceae)	NTU	Hex	0	0.016 ± 0.18	−0.023 ± 0.10	−0.047 ± 0.19	F(2,9) = 0.1557	
		EtAc	0	0.038 ± 0.07	0.078 ± 0.15	0.141 ± 0.13	F(2,9) = 0.7240	
Lagerstroemia indica L. (Lythraceae)	NTU	Hex	0	0.088 ± 0.14	−0.064 ± 0.11	−0.032 ± 0.30	F(2,9) = 0.6417	
		EtAc	0	−0.028 ± 0.12	−0.057 ± 0.13	−0.136 ± 0.21	F(2,9) = 0.5143	
Lantana camara L. (Verbenaceae)	NTU	Hex	0	0.048 ± 0.10	0.063 ± 0.08	0.065 ± 0.18	F(2,9) = 0.0220	
		EtAc	0	−0.006 ± 0.16	−0.063 + 0.22	−0.128 + 0.14	F(2,9) = 0.4631	
Lavandula angustifolia Chaix ex Vill. Mill.(Lamiaceae)	OIC		1.09%	0.061 ± 0.05	−0.301 ± 0.19	−0.518 ± 0.15	F(2,9) = 16.292	**
Leucaena leucocephala (Lam.) de Wit (Fabaceae)	NTU	Hex	0	−0.067 ± 0.14	−0.044 ± 0.31	−0.039 ± 0.26	F(2,9) = 0.0148	
		EtAc	0	−0.034 ± 0.04	0.046 ± 0.16	−0.203 ± 0.40	F(2,9) = 1.0248	
Macaranga tanarius (L.) Muell.Arg. (Euphorbiaceae)	NTU	Hex	0	0.050 ± 0.27	−0.183 ± 0.24	0.022 ± 0.16	F(2,9) = 1.2376	
		EtAc	0	−0.056 ± 0.13	−0.139 ± 0.12	−0.084 ± 0.14	F(2,9) = 0.4109	
Mangifera indica L. (Anacardiaceae)	NTU	Hex	0	0.087 ± 0.05	0.101 ± 0.16	0.051 ± 0.22	F(2,9) = 0.1028	
		EtAc	0	−0.000 ± 0.11	0.018 + 0.17	−0.020 ± 0.30	F(2,9) = 0.0327	
Melaleuca cajuputi Powell (Myrtaceae)	OIC		8.11%	0.053 ± 0.10	−0.027 ± 0.12	−0.303 ± 0.18	F(2,9) = 7.4107	*
Melaleuca leucadendra L. (Myrtaceae)	NTU	Hex	0	0.019 ± 0.09	−0.117 ± 0.23	−0.067 ± 0.06	F(2,9) = 0.9139	
		EtAc	0	−0.066 ± 0.13	−0.088 + 0.09	−0.090 ± 0.02	F(2,9) = 0.0870	
Melissa officinalis L. (Lamiaceae)	FSI		0	−0.001 ± 0.07	−0.154 ± 0.26	−0.505 ± 0.11	F(2,9) = 9.7249	**
Mentha canadensis L. (Lamiaceae)	FSI		0	0.015 ± 0.03	−0.095 ± 0.08	−0.241 ± 0.13	F(2,9) = 7.9800	*
Mikania micrantha Kunth (Asteraceae)	NTU	Hex	0	−0.028 ± 0.03	0.010 ± 0.18	−0.051 ± 0.17	F(2,9) = 0.1804	
		EtAc	1.67%	0.031 ± 0.08	0.024 ± 0.22	0.048 ± 0.03	F(2,9) = 0.0338	
Ocimum basilicum L. (Lamiaceae)	OIC		0	0.035 ± 0.09	−0.237 ± 0.18	−0.352 ± 0.26	F(2,12) = 6.5048	*
Pelargonium sp. L'Her. ex Aiton (Geraniaceae)	FSI		0	0.097 ± 0.08	−0.071 ± 0.26	−0.389 ± 0.29	F(2,9) = 4.5269	*
Pittosporum pentandrum (Blanco) Merr. (Pittosporaceae)	TBG	Hex	0	−0.030 ± 0.08	0.019 ± 0.16	−0.000 ± 0.13	F(2,9) = 0.1493	
		EtAc	1.79%	0.014 ± 0.11	−0.092 ± 0.08	−0.132 ± 0.11	F(2,9) = 2.3314	
Pogostemon cablin (Blanco) Benth. (Lamiaceae)	OIC		0	−0.060 ± 0.10	−0.038 ± 0.14	−0.135 ± 0.21	F(2,15) = 0.6350	
Salvia rosmarinus (L.) Schleid. (Lamiaceae)	OIC		6.23%	0.092 ± 0.06	−0.144 ± 0.13	−0.324 ± 0.20	F(2,9) = 8.6026	**
Santalum album L. (Santalaceae)	OIC		0	0.000 ± 0.00	0.001 ± 0.04	−0.013 ± 0.15	F(2,9) = 0.0296	
Setaria palmifolia (J.Koenig) Stapf (Poaceae)	TNF	Hex	0	0.031 ± 0.14	−0.071 ± 0.12	−0.095 ± 0.12	F(2,9) = 1.1127	
		EtAc	0	0.123 ± 0.12	0.047 ± 0.10	−0.150 ± 0.10	F(2,9) = 6.9506	*
Syzygium aromaticum (L.) Merr. & L.M.Perry (Myrtaceae) bud	OIC		0	0.038 ± 0.10	−0.201 ± 0.12	−0.327 ± 0.29	F(2,8) = 3.4600	•
Syzygium aromaticum (L.) Merr. & L.M.Perry (Myrtaceae) leaf	OIC		17.33%	0.108 ± 0.14	−0.236 ± 0.31	−0.241 ± 0.38	F(2,9) = 1.8802	
Taiwania cryptomerioides Hayata (Cupressaceae)	TNF	Hex	0	0.071 ± 0.10	0.158 ± 0.13	0.081 ± 0.13	F(2,9) = 0.6424	
		EtAc	0	0.063 ± 0.14	−0.142 ± 0.07	−0.271 ± 0.16	F(2,9) = 6.9420	*

Commercial oils were tested with ethanol as negative control. Because the flies did not always distribute themselves perfectly randomly in control Y-tubes, repellency index values are given at 0 [control], 10, and 50 μL applications. Means are of ≥ four independent Y-tube per application amount. Sources: FSI = Fusheng Instrument Co., Ltd. [commercially-produced essential oil]; NTU = National Taiwan University campus [collections in summer of 2020 or 2021]; OIC = O'ddenio International Company [commercially-produced essential oil]; TBG = Taipei Botanical Garden [collected 8 April 2021]; TNF = Taipingshan National Forest [collected 6 August 2021]. EtAc = Ethyl acetate. Hex = Hexane. SE = Standard Error. RI = Repellency index. Knock = Mean knockdown percentage at 50 μL doses. Sig. = Significance (*p*-values) of the ANOVA test comparing the three concentrations: • - $p < 0.1$ * - $p < 0.05$ ** - $p < 0.01$ *** - $p < 0.001$

funnel, and the heavy phase containing waxes, water, and protein discarded. The light phases were each dehydrated in a beaker by adding enough anhydrous sodium sulfate and swirling until the sodium sulfate could swirl freely, indicating all water had been absorbed (Liu et al. 2012). The remaining dry solvent-oil mixture was decanted into amber jars, and stored at 4 °C. Once two Soxhlet runs per plant were complete, the extracts were concentrated using a rotary evaporator, which separates the solvent from the oil. The solvent can be re-used, and the material that that remained in the evaporator flask was re-dissolved in approximately 5 mL of hexane or ethyl acetate. These extracts were stored in amber vials at 4 °C. For analysis, equal volumes of extracts from the two replicate Soxhlet runs were mixed together, such that each plant had one vial of hexane extract and one vial of ethyl acetate extract used for assays.

2.3. *Insect behavioral assay.*

The behavioral assays were performed using lab-reared *F. taiwana* in the Department of Biotechnology, Chia-Nan University of Pharmacy and Science (Luo 2018), using a custom-made Y-tube olfactometer (Geier and Boeckh 1999; Lin et al. 2017). The Y-tube had a starting arm (A-arm) 25 cm long with an 8.5 cm long insect-holding chamber at the end. The two end arms (B and C arms) were also 25 cm long with 8.5 cm long chambers to hold the test samples. The diameters of the arms were 4 cm.

Measured (0, 10, or 50 µL) amounts of commercial oils or the hexane or ethyl acetate extracts were dripped onto filter paper. The volumes chosen are modified from Lin et al. (2017), whom used 1, 10, 100, and 200 µL amounts of essential oil against *F. taiwana*. Zero microliter tests were used as blanks, to confirm the Y-olfactometer was clean of contaminating odorants. The solvent was allowed to evaporate from the paper, and the paper was placed at the end of one of the B or C arms of the tube. In the other arm was put filter paper impregnated with an equal amount of solvent (hexane or ethyl acetate) for ethanol (for the commercial oils) as a control. Two ICAF 2X6 Inline Clean Air Filters (Sigma Scientific LLC., Micanopy FL, USA) were placed at the ends of arms B and C to purify the incoming air. Experiments were conducted at room temperature. After allowing the odors to diffuse inside the tube for 5 min, a small container of approximately twenty adult *F. taiwana* was connected to the A-arm such that the flies could fly freely into chamber. To encourage the flies to leave the A arm, a lamp was set over the A arm and the B and C arms were placed in shade, as the flies are photophobic and would theoretically leave the well-lit arm. After five minutes the numbers of flies in the starting, control, and test arms of the tube were recorded. The gender of the flies was determined based on their antennae (males have distinctively larger and fuzzier antennae), and the sex ratio of males and females in each arm was measured. Knockdown, or flies dying or falling unconscious during the test, was also recorded. This process was repeated for four replicates for each extract, flipping the Y-tube in between trials to account for any potential differences between the left and right sides of the experimental arena. The Y-tube olfactometer was washed thoroughly in detergent and methanol between oils.

To define repellency, Repellency Index (RI) (Nentwig et al. 2016; de Lara de Souza et al. 2019; Ganassi et al. 2020) was calculated as follows: If T is the number of flies in the test arm, C is the number of flies in the control arm, and S is the number in the starting arm, then the RI = (T-C)/(T + C + S). The RI values thus range from −1 to 1, with −1 being strongly repellent and 1 being strongly attractive. The RI's among the different amounts of oil applied were compared using a one-way ANOVA (https://goodcalculators.com/one-way-anova-calculator/), and these results are listed in Table 1. Knockdown is listed in Table 1 as the mean percentage of flies knocked down at 50 µL applications.

2.4. *Gas chromatography mass spectrometry*

The composition of the essential oils and the relative abundances of the constituent parts was analyzed using an Agilent Technologies 7890A-5975C gas chromatography mass spectrometer (GC-MS) at NTU. The GC was equipped with a DB-5MSUI fused silica capillary column (30, 0.25 mm i.d., 0.25 μm film thickness, Agilent Technologies Inc., CA, USA). Helium was used as the carrier gas with a constant flow rate of 1.0 mL/min. The injector temperature was 250 °C. The initial GC oven temperature was held at 30 °C for 2 min and then increased to 300 °C at 10 °C/min. Mass spectra were scanned in full scan mode from 50 to 500 m/z. Kovats indices were calculated based on the retention time in relation to a serious of *n*-alkane mixture (C7-C30) under the same analyzing conditions. The MS library identifications produced by an NIST library search were compared to published Kovats retention indices for these compounds to find matches for the Kovats indices produced by the GC (Shu et al. 2021). This data is summarized in Supplementary Data Set S1, and the complete raw data is available at http://doi.org/10.5281/zenodo.7030377.

3. Results

Table 1 summarizes the Y-olfactometer results. For each sample (species and solvent, or just species for commercial oils), the mean RIs and results of the ANOVA test comparing the RIs at 0, 10, and 50 µL applications are listed, along with % knockdown at 50 µL. Table 2 specifically shows the mean percentages of flies that made decisions to leave the starting chamber and the results of the ANOVA comparing this at the different applications. With no exceptions, flies were more likely to leave the starting chamber during tests with essential oil in one arm than the control tests, suggesting the olfactometer assay was effective and reliable.

Commercial oils were more successful at repelling *F. taiwana* than the extracts produced in the laboratory. Commercial citronella oil from *C. nardus* had the strongest repellency effect ($\mathrm{RI}_{50\mu\mathrm{L}}$: -0.613 ± 0.22), as did commercial oils of *L. angustifolia*, *C. formosensis* wood, *Cinnamomum camphora* (L.) J.Presl bark, *Eucalyptus sp.* L'Hér, *Melaleuca cajuputi* Powell, *Melissa officinalis* L., and *Pelargonium* sp. L'Her. ex Aiton. Among the laboratory-made oils, the most strongly repellent was the hexane oil of *C. camphora* ($\mathrm{RI}_{50\mu\mathrm{L}}$: -0.459 ± 0.11), followed by the hexane oil of *C. citriodora* ($\mathrm{RI}_{50\mu\mathrm{L}}$: -0.303 ± 0.31), both oils of *Artemisia capillaris* Thunb., and hexane oil of *Taiwania cryptomerioides* Hayata.

Significant knockdown (mean 8.74% at 10 µL and 17.33% at 50 µL) was reported for commercial clove leaf oil (*Syzygium aromaticum* (L.) Merr. & L.M.Perry, syn. *Eugenia caryophyllus* (L.) Baill.), but not clove bud oil, which had a stronger repellent effect. Knockdown above five percent at 50 µL was also found for commercial oils of *M. cajupti* (8.11%) and *S. rosmarinus* (6.23%), both of which also had repellency effects. Commercial oil of *Artemisia absinthium* L. had mean knockdown of 4.86% at 50 µL and significant repellency effects. Knockdown effect between 1-3% was reported for the ethyl acetate oils of *Cunninghamia konishii* Hayata, *Dimocarpus longan* Lour., *Eucalyptus robusta* Sm., *M. micrantha*, and *Pittosporum pentandrum* (Blanco) Merr., all of which were non-repellent along with their hexane oils.

Supplementary Data Set S1 summarizes the top twenty constituents for the extract samples analyzed. The lists include some chemicals that are not volatile organic compounds, such as oleamide, which is known to leak out of laboratory plasticware and contaminate data (McDonald et al. 2008); phytol, a component of chlorophyll; and the fatty acid 2-palmitoylglycerol and its functional parent, hexadecenoic acid. For this reason, the concentrations of sesquiterpenes and other compounds of interest given in Supplementary Data Set S1 are likely underestimates of the true values. The ethyl acetate extractions contained relatively fewer compounds of interest than the initial hexane extracts, and frequently contained compounds like toluene, 1-ethoxybutane (also known as butyl ethyl ether), and butyl acetate.

Germacrene D, a common plant essential oil sesquiterpene and known insecticidal compound, appeared commonly (Pavela and Govindarajan 2017; Liu et al. 2021), as did the known repellents caryophyllene (Nararak et al. 2019) and α-pinene (Haselton et al. 2015). Plants whose oils had particularly diverse complements of aromatic compounds were *A. capillaris*, *C. osmophloeum*, *C excavata*, and *M. micrantha*. Plants with particularly high percentages of aromatic compounds in the hexane oils included *C. camphora* (45.65% linalool), *C. citriodora* (47.23% citronellal and 7.38% citronellol), and *Melaleuca leucadendra* L. (27.82% ledol camphor and 20.72% eucalyptol).

4. Discussion

Frequently, less than half of *F. taiwana* made decisions to leave the starting tube in the Y-tube olfactometer, but this is not surprising: *F. taiwana* typically do not move except to mate and to feed, and are generally much less active than mosquitoes (Wei 2012; Luo and Jian 2020) or parasitoids (Vet et al. 1983). In Lin et al.'s (2017) study using a Y-olfactometer to test the effects of essential oils on *F. taiwana*, flies flew to the control arm in tests with 1 µL essential oil, but with higher volumes they did not leave the starting arm at all. The RI formula chosen for this study

Table 2. Mean percentage of F. taiwana that made decisions in the Y-olfactometer tests.

Scientific name	Source	Solvent	Mean ± SE % dec. 0 µL	Mean ± SE % dec. 10 µL	Mean ± SE % dec. 50 µL	ANOVA F stat	sig
Acacia confusa (Fabaceae)	TBG	Hex	31.81 ± 13.60	45.39 ± 15.36	53.82 ± 18.78	F(2,9) = 1.91388	
		EtAc	25.94 ± 4.45	36.19 ± 12.14	55.62 ± 10.06	F(2,9) = 4.78984	*
Artemisia absinthium (Asteraceae)	OIC		23.61 ± 11.75	38.54 ± 18.94	64.56 ± 10.82	F(2,9) = 8.39474	**
Artemisia capillaris (Asteraceae)	TNF	Hex	22.28 ± 7.41	46.38 ± 14.25	55.75 ± 7.06	F(2,9) = 11.62051	**
		EtAc	35.20 ± 10.25	53.86 ± 10.92	46.89 ± 7.84	F(2,9) = 3.73435	•
*Azadirachta indica (*Meliaceae) seed	OIC		19.06 ± 5.64	47.25 ± 7.02	65.84 ± 8.00	F(2,9) = 45.88594	***
Bidens alba (Asteraceae)	NTU	Hex	3.45 ± 3.99	20.83 ± 9.92	38.10 ± 14.00	F(2,9) = 11.60787	**
		EtAc	27.11 ± 4.47	35.88 ± 8.25	43.05 ± 11.09	F(2,9) = 3.62061	•
Calocedrus formosana (Cupressaceae)	NTU	Hex	18.76 ± 8.64	23.00 ± 13.74	29.95 ± 11.97	F(2,9) = 0.94225	
		EtAc	15.72 ± 16.68	16.57 ± 11.31	29.23 ± 6.73	F(2,9) = 1.52049	
Camellia sinensis (Theaceae)	FSI		40.22 ± 19.73	67.63 ± 13.87	69.39 ± 10.39	F(2,9) = 4.65248	*
Chamaecyparis formosensis (Cupressaceae) leaves	TNF	Hex	31.84 ± 7.51	51.52 ± 10.63	51.16 ± 15.53	F(2,9) = 3.70696	•
		EtAc	31.10 ± 13.17	41.20 ± 16.09	48.01 ± 9.58	F(2,9) = 1.65647	
Chamaecyparis formosensis (Cupressaceae) wood	OIC		29.78 ± 12.42	55.87 ± 26.48	72.65 ± 19.76	F(2,9) = 4.49554	*
Cinnamomum camphora (Lauraceae) bark	OIC		39.17 ± 25.44	54.24 ± 23.34	67.36 ± 12.50	F(2,9) = 1.7718	
Cinnamomum camphora (Lauraceae) leaves	NTU	Hex	23.21 ± 12.41	42.76 ± 10.85	50.48 ± 7.64	F(2,9) = 7.18381	*
		EtAc	32.00 ± 10.86	34.01 ± 5.65	39.86 ± 7.39	F(2,9) = 0.9779	
Cinnamomum osmophloeum (Lauraceae)	TBG	Hex	30.10 ± 17.46	41.36 ± 14.24	44.97 ± 24.21	F(2,9) = 0.66054	
		EtAc	32.57 ± 20.69	35.42 ± 17.80	54.36 ± 12.43	F(2,9) = 1.87097	
Clausena excavata (Rutaceae)	NTU	Hex	26.15 ± 20.03	48.40 ± 11.77	55.98 ± 1.94	F(2,9) = 5.31015	*
		EtAc	23.18 ± 14.87	33.08 ± 8.85	47.28 ± 13.29	F(2,9) = 3.70282	•
Corymbia citriodora (Myrtaceae)	NTU	Hex	32.64 ± 22.62	51.06 ± 21.65	56.52 ± 18.26	F(2,9) = 1.42786	
		EtAc	9.97 ± 8.62	12.47 ± 5.73	21.74 ± 10.07	F(2,9) = 2.21509	
Cryptomeria japonica (Cupressaceae)	TNF	Hex	36.34 ± 18.73	40.81 ± 10.27	44.75 ± 12.28	F(2,9) = 0.35042	
		EtAc	34.14 ± 21.54	40.55 ± 9.62	55.46 ± 8.75	F(2,9) = 2.26678	
Cunninghamia konishii (Cupressaceae)	TNF	Hex	36.34 ± 18.73	40.81 ± 10.27	44.75 ± 12.28	F(2,9) = 0.35042	
		EtAc	39.29 ± 9.22	50.24 ± 12.82	60.42 ± 12.95	F(2,9) = 3.21481	•
Cymbopogon nardus (Poaceae)	OIC		40.78 ± 19.69	54.15 ± 20.43	67.22 ± 15.67	F(2,9) = 1.99614	
Dimocarpus longan (Sapindaceae)	NTU	Hex	22.11 ± 18.61	28.33 ± 15.46	30.09 ± 22.26	F(2,9) = 0.33679	
		EtAc	25.09 ± 10.88	33.33 ± 16.67	38.67 ± 20.84	F(2,9) = 0.67576	
Eucalyptus robusta (Myrtaceae)	NTU	Hex	19.36 ± 13.65	26.18 ± 20.14	33.25 ± 17.68	F(2,9) = 0.64033	
		EtAc	27.28 ± 22.80	32.40 ± 8.29	46.66 ± 10.38	F(2,9) = 1.73751	
Eucalyptus sp. (Myrtaceae)	FSI		26.21 ± 21.73	37.10 ± 26.69	50.54 ± 23.86	F(2,9) = 1.01642	
Eupatorium formosanum (Asteraceae)	TNF	Hex	18.51 ± 12.19	31.55 ± 13.23	37.16 ± 27.19	F(2,9) = 1.03311	
		EtAc	38.69 ± 14.28	61.15 ± 5.39	53.12 ± 10.99	F(2,9) = 4.39045	*
Ficus septica (Moraceae)	NTU	Hex	22.94 ± 7.37	46.42 ± 16.16	60.62 ± 22.44	F(2,9) = 5.30642	*
		EtAc	9.90 ± 4.82	22.85 ± 11.26	22.40 ± 12.16	F(2,9) = 2.17562	
Lagerstroemia indica (Lythraceae)	NTU	Hex	29.32 ± 12.69	58.29 ± 29.79	53.32 ± 11.89	F(2,9) = 2.42157	
		EtAc	17.45 ± 4.46	38.43 ± 12.30	48.08 ± 19.37	F(2,9) = 5.38382	*
Lantana camara (Verbenaceae)	NTU	Hex	35.46 ± 18.36	49.87 ± 13.95	54.82 ± 14.94	F(2,9) = 1.6101	
		EtAc	28.43 ± 13.79	54.11 ± 17.37	66.03 ± 14.01	F(2,9) = 6.43507	*
Lavandula angustifolia (Lamiaceae)	OIC		15.63 ± 11.14	46.42 ± 13.08	66.45 ± 10.23	F(2,9) = 19.68179	***
Leucaena leucocephala (Fabaceae)	NTU	Hex	33.85 ± 18.94	44.87 ± 6.34	58.72 ± 11.49	F(2,9) = 3.50955	•
		EtAc	14.61 ± 14.04	29.95 ± 11.59	59.15 ± 14.11	F(2,9) = 11.57994	**
Macaranga tanarius (Euphorbiaceae)	NTU	Hex	32.75 ± 9.58	57.51 ± 22.83	73.56 ± 14.86	F(2,9) = 6.08192	*
		EtAc	23.06 ± 6.36	28.04 ± 8.58	44.26 ± 7.44	F(2,9) = 8.71042	**
Mangifera indica (Anacardiaceae)	NTU	Hex	14.55 ± 5.55	22.21 ± 12.78	29.75 ± 23.34	F(2,9) = 0.93809	
		EtAc	13.29 ± 3.23	35.03 ± 5.14	42.23 ± 5.98	F(2,9) = 37.5355	***
Melaleuca cajuputi (Myrtaceae)	OIC		38.32 ± 16.46	56.31 ± 19.29	70.55 ± 12.11	F(2,9) = 3.96452	•
Melaleuca leucadendra (Myrtaceae)	NTU	Hex	22.34 ± 14.15	35.08 ± 17.82	40.05 ± 1.98	F(2,9) = 1.92014	
		EtAc	17.81 ± 10.59	30.79 ± 13.57	24.97 ± 14.57	F(2,9) = 0.99854	
Melissa officinalis (Lamiaceae)	FSI		26.35 ± 14.16	38.44 ± 13.75	53.65 ± 10.36	F(2,9) = 4.51872	*
Mentha canadensis (Lamiaceae)	FSI		4.80 ± 6.33	20.05 ± 17.92	39.99 ± 18.56	F(2,9) = 5.26250	*
Mikania micrantha (Asteraceae)	NTU	Hex	24.11 ± 17.77	42.41 ± 16.78	47.40 ± 18.96	F(2,9) = 1.88617	
		EtAc	9.11 ± 3.07	24.51 ± 9.76	26.18 ± 14.88	F(2,9) = 3.25933	•
Ocimum basilicum (Lamiaceae)	OIC		13.69 ± 8.32	27.93 ± 23.74	45.56 ± 18.24	F(2,12) = 3.95898	*
Pelargonium sp. (Geraniaceae)	FSI		15.79 ± 3.68	29.71 ± 11.95	49.89 ± 23.64	F(2,9) = 4.93377	*
Pittosporum pentandrum (Pittosporaceae)	TBG	Hex	19.38 ± 12.02	29.54 ± 4.77	39.71 ± 11.03	F(2,9) = 4.29317	*
		EtAc	13.54 ± 2.08	14.76 ± 11.67	31.72 ± 7.85	F(2,9) = 6.13291	*
Pogostemon cablin (Lamiaceae)	OIC		18.68 ± 20.35	28.22 ± 20.88	33.05 ± 20.14	F(2,15) = 0.76712	
Salvia rosmarinus (Lamiaceae)	OIC		26.14 ± 21.82	45.62 ± 22.89	64.95 ± 18.53	F(2,9) = 3.36429	•
Santalum album (Santalaceae)	OIC		8.33 ± 10.64	24.63 ± 13.62	36.84 ± 17.83	F(2,9) = 3.98342	•
Setaria palmifolia (Poaceae)	TNF	Hex	46.36 ± 12.92	56.11 ± 12.95	57.78 ± 10.31	F(2,9) = 1.03635	
		EtAc	33.35 ± 7.77	49.95 ± 18.96	49.51 ± 13.11	F(2,9) = 1.815	
Syzygium aromaticum (Myrtaceae) bud	OIC		63.51 ± 8.38	60.75 ± 15.81	59.88 ± 18.15	F(2,8) = 0.06689	
Syzygium aromaticum (Myrtaceae) leaf	OIC		47.05 ± 16.69	86.23 ± 7.24	92.84 ± 5.00	F(2,9) = 20.65571	***
Taiwania cryptomerioides (Cupressaceae)	TNF	Hex	26.03 ± 10.14	46.54 ± 16.02	54.82 ± 18.85	F(2,9) = 3.68589	•
		EtAc	34.52 ± 15.15	56.60 ± 5.24	56.78 ± 4.54	F(2,9) = 7.07732	*

Source abbreviations as in Table 1. Commercial oils were tested with ethanol as negative control. Means are of ≥ four independent Y-tube per application amount. % dec. = percentage of flies from the total that made a decision to leave the starting arm and enter the control or test arm. EtAc = Ethyl acetate. Hex = Hexane. SE = Standard error. Sig. = Significance (p-values) of the ANOVA test comparing the three concentrations: • - $p < 0.1$ * - $p < 0.05$ ** - $p < 0.01$ *** - $p < 0.001$

accounted for the sedentary aspect of their biology and produced realistic results (Nentwig et al. 2016; de Lara de Souza et al. 2019; Ganassi et al. 2020). Table 2 shows that the likelihood of decision making increased with higher strength applications of oils, and that oils with stronger RI values (Table 1) were more likely to induce significantly more flies to make a decision. The data all supports the validity of the methods used, in addition to their extensive history of use in prior literature.

Several commercial oils had statistically significant and/or strong spatial repellency effects against *F. taiwana*, such as oils of *C. nardus* (citronella), *L. angustifolia* (common or English lavender), *C. formosensis* (Formosan cypress) wood, and *M. officinalis* (lemon balm). Citronella oil is a well established spatial repellent (Lindsay et al. 1996; Müller et al. 2008; Lou et al. 2014) while relatively fewer studies have been done on lavender (Mauchline et al. 2008) or lemon balm (Koliopoulos et al. 2010). Oil of *C formosensis* is less well-studied than the other commercial oils, as the tree is endemic to Taiwan and listed as endangered on the IUCN Red List (Zhang and Christian 2013). Commercial oils from *C. formosensis* are typically produced from the wood, not the leaves, and have known insecticidal activity (Kuo et al. 2007). The commercial oil used in this study had a repellency effect, while the leaf oils made in the lab did not, nor did they contain large amounts of the aromatic compounds previous studies identified in *C. formosensis* wood oils (Wang et al. 2005; Ho et al. 2012) (Supplementary Data Set S1). This suggests the wood, not the leaves, are the best sources for this plant's effective essential oils.

The most effective of the laboratory-made oils was the hexane extract of *C. camphora* leaves. Camphor – the name of the tree, its oil, and a particular chemical within the oil used as an insecticidal fumigant (Fu et al. 2015) – comes in five chemotypes: borneol, camphor, eucalyptol, linalool, and nerolidol. The GC-MS data confirms the trees samples in this study were the linalool chemotypes. From camphor oil, camphor crystals are filtered away and the rest is typically separated by fractional distillation into a light fraction (white camphor), and heavier brown, yellow, and blue camphor oils. The heavy oils are high in safrole, which is carcinogenic, and so these oils are typically not commercially available or suitable for therapeutic use (Tisserand and Young 2014). While camphor has a centuries long history of use as a fumigant, perfume, and embalming fluid, it is a skin penetration enhancer and highly toxic if consumed, so its utility as a topical insect repellent is low (Chen et al. 2013). One should also note extremely high levels of safrole in this study were found in the extracts of *C. excavata*, and safrole also detected in *Bidens alba* (L.) DC. and *Acacia confusa* Merr. extracts.

The other laboratory-produced oils with strong repellency activities in the Y-olfactometer assays were the hexane and ethyl acetate extracts of *A. capillaris* (capillary or mosquito wormwood). Both contained confirmed arthropod repellents or mosquito oviposition deterrents like linalool (Mauchline et al. 2008), caryophyllene (Nararak et al. 2019), humulene (da Silva et al. 2015), and nerolidol (Lwande et al. 1999). They also contained insecticidal compounds like γ-terpinene (Gong and Ren 2020) and D-limonene (Karr and Coats 1988). As the alternative common name 'mosquito wormwood' suggests, *A. capillaris* has long been recognized as a mosquito repellent, though typically by burning the wood to make spatially repellent smoke (Martínez et al. 2012; Shu et al. 2021). Note, however, that the commercial oil of the related *A. absinthium* (common wormwood) was a stronger repellent.

Besides spatial repellency, knockdown was observed from commercial oils of *S. aromaticum* leaves, *M. cajuputi*, *S. rosmarinus,* and *A. absinthium*. *S. aromaticum* (clove) had effects at 10 μL, including weak and variable repellency ($RI_{10\mu L}$: -0.236 ± 0.31) that did not become much stronger at higher concentrations ($RI_{50\mu L}$: -0.241 ± 0.38). The possibility that it could be used as an adulticide merits deeper research. *M. cajuputi* (cajuput) oil is commonly produced commercially and is high in eucalyptol (Tisserand and Young 2014), a well-known insect repellent and toxicant (Klocke et al. 1987; Southwell et al. 2003), although it is also toxic to humans if consumed or inhaled (Tisserand and Young 2014). The predominant constituents of *S. rosmarinus* (rosemary) oil vary with the chemotype grown, but include eucalyptol, borneol, camphor, α-pinene, bornyl acetate, camphene, and verbenone (Tisserand and Young 2014). *A. absinthium* oil is a known mosquito larvicide (Govindarajan and Benelli 2016), whose components (Supplementary Data Set S1) include a known insect repellent (Avé et al. 1987).

This work is comparable to the limited number of other Y-tube olfactometer studies on the effectiveness of essential oils against *F. taiwana* (Lin et al. 2017; Lou et al. 2014). While many results were similar, the most striking difference was that it found no repellent effect for *C. osmophloeum* (indigenous cinnamon), which other researchers identified as a strong repellent (Lou et al. 2014; Chang 2016). The negative results for *C. osmophloeum* may be due to the time of year the leaves were collected (April), or due to the specific chemotype grown at the Taipei Botanical Garden (Cheng et al. 2004), meaning this study's oils were relatively lacking a component found in the other studies' oils that showed repellency. Common components of *C. osmophloeum* oils from the literature, which vary by chemotype, include eucalyptol, spathulenol (Chao et al. 2005), cinnamaldehyde, linalool (Fang et al. 1989), camphor, cinnamyl acetate, eugenol, and anethole (Cheng et al. 2004). From these, the GC-MS (Supplementary Data Set S1) found $< 2\%$ cinnamaldehyde and only trace levels of linalool in the hexane and ethyl acetate oils. *C. osmophloeum* is native to Taiwan, and is considered vulnerable according to the IUCN Red List (Pan 1998). Exploiting this species may thus be ill advised, but synthesizing a substitute from its essential oil constituents may be a better alternative.

The commercial oils were overall far more likely to produce statistically significant results than the oils produced in the lab. One possible explanation is that the plants with extant oil production are those with more or stronger volatile organic compounds: the plants for which no commercial oil is available are those whose oils would not be worth commercializing in the first place. A second possibility is that the laboratory Soxhlet extraction method produced lower concentrations of active ingredients compared to the industrial steam extraction used to make the commercial oils. A third is that the lab-produced oils were overly diluted in solvent compared to the commercial oil, and could be more effective at greater concentrations, but quantifying the concentration of the oils or reducing the losses was not possible with the available tools.

A final caveat is that 'natural' does not mean safe. Whether most of these oils pose unacceptable inhalation or dermal toxicity to humans and pets needs to be tested. As contact repellents, essential oils typically have shorter repellency durations than DEET, and most have far stronger topical toxicities to humans than DEET, which can be safely used on human skin at 100% concentrations (Sudakin and Trevathan 2003; Leal 2014; Shelomi 2020). By contrast, lavender essential oil has a dermal maximum of 0.1% (Tisserand and Young 2014), camphor oil is a known convulsant with relatively high dermal toxicity (Tisserand and Young 2014), and oil of *M. officinalis* (balm or lemon balm) has a dermal maximum of 0.9% or lower, is teratogenic, and interacts negatively with diabetes medication (Tisserand and Young 2014). While some of the plants in this study show promise as sources of repellents, there is no reason to believe *a priori* that their essential oils are safer than DEET, which remains the gold standard for both efficacy and safety.

5. Conclusions

To summarize, this study found several essential oils with statistically significant spatial repellent effects against *F. taiwana*, most of which are already commercially available: citronella, lavender, camphor, Formosan cypress, and capillary wormwood. The results from the novel oils match most previous findings, while differences may be due to differences in oil volumes used or in the chemotype of the plants sampled, which illustrates the importance of combining GC-MS identification of constituent compounds with any essential oil assay. The data set on the chemical components of essential oils from Taiwanese plants that had to this point not yet been analyzed is a new and welcome contribution. The oils produced in this study can be tested in the future as contact repellents and as larvicides against *F. taiwana* (Chang 2016) and mosquitoes such as *Aedes aegypti* (L.) (Shu et al. 2021). Powders from the original plants and remaining oils produced in this lab are still available, so collaborators wishing to test them further are encouraged to contact the corresponding author.

Author contributions

Conceptualization, M.S.; methodology, L-D.G, Y-P.L., M.S.; formal analysis, M.S.; investigation, L-D.G., P-Y.L., K.C–C.C., M.S.; resources, Y-P.L., P-Y.L., K.C–C.C., M.S.; data curation, M.S.; writing – original draft preparation, M.S.; writing – review and editing, Y-P.L., M.S.; supervision, Y-P.L., M.S.; project administration, M.S.; funding acquisition, M.S. All authors

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Disclosure statement

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