Frontiers in Bioscience Landmark

Front. Biosci. (Landmark Ed) **2022**; 27(12): 327
https://doi.org/10.31083/j.fbl2712327

Original Research

Essential Oils Land Footprint: A Sustainability Meta-Analysis of Essential Oils Biopesticides

Epameinondas Evergetis[1,*], Serkos A. Haroutounian[1]

[1]Laboratory of Nutritional Physiology and Feeding, Agricultural University of Athens, 11855 Athens, Greece
*Correspondence: epaev@aua.gr (Epameinondas Evergetis)
Academic Editor: Tomasz M. Karpiński
Submitted: 3 October 2022 Revised: 18 November 2022 Accepted: 1 December 2022 Published: 21 December 2022

Abstract

Background: Essential oils (EO) are considered as safe and sustainable alternatives of synthetically produced industrial raw materials. While EO are renewable resources their production is traced to land use, therefore employing nonrenewable resources. This fact is often neglected during market up-take, which is established on EO bioactivity efficacy. **Methods**: Present study is aiming this knowledge gap through an innovative algorithm that employs spatial yield, bioactivity performance and fundamental experimentation details to calculate the land footprint. The proposed methodology is tested upon a concise pool of 54 EO, of which 9 originate from 8 culinary herbs, 27 from 3 juniper *taxa*, and 18 from 6 *Citrus sp.* crops. All 54 EO were subjected to repellent evaluation and 44 of them also to larvicidal, encompassing in the protocol both choice and no-choice bioassays. **Results**: Based on these bioprospecting data the proposed protocol effectively calculated the land footprint for all EO and bioassays. The repellent land footprint indicated as more sustainable the EO from savory, oregano, tarhan, thyme, Greek sage, and juniper berries for which each application corresponds to 3.97, 4.74, 7.33, 7.66, 8.01 and 8.32 m^2 respectively. The larvicidal assessment suggested as more sustainable the EOs from savory, oregano, fennel, thyme, tarhan, and rue with land footprints of 1.56, 1.79, 2.16, 2.89, 3.70 and 4.30 m^2 respectively. **Conclusions**: The proposed protocol managed to calculate the land footprint for each EO and bioactivity and indicated the more sustainable EO per use based on widely available bioprospecting data.

Keywords: sustainability; essential oil; pesticide; land footprint; bioprospecting

1. Introduction

Sustainability was introduced as an international policy priority through the "Rio Declaration" in 1992 and is expected to constitute the leading development principle for the 21st century. During the same year, the sustainable development was unequivocally linked with the natural capital by Costanza and Dally [1]. They, first among many to follow, discriminated the natural capital in two primeval qualities: active or renewable *vs.* inactive or nonrenewable. Moreover, this highly influential work has highlighted as key sustainability target the safeguarding of the natural capital future perspectives. Consequently, numerous scientific efforts were diverted between waste and energy reduction in industrial production [2], but also towards the deployment of renewable resources as a substitute of the nonrenewable assets [3].

Natural products constitute one of the main alternatives for the substitution of nonrenewable resources. They have been proven as an efficient renewable source of raw materials with very promising potentials for the amelioration of various sectors' environmental performance, since they are generally quoted as sustainable [4]. Notable examples of sectoral approaches exploiting the natural products potentials may be traced in pharmaceutical industry since they constitute the primary source for the development of new medicines [5]. Natural products in the form of essential oils (EO) have also been identified as a prominent source for renewable raw materials of chemical industry [6], to produce fine chemicals [7]. Food industry sector also experiences significant impacts by EOs advances either in the form of dietary and health beneficiary compounds [8], or as preservatives and food spoilage control agents [9]. A notable mature example is traced in the agrochemicals industry, where the EOs, in the form of biopesticides, conform a sound and preferable alternative of conventional pesticides, with a plethora of regulatory and best practice documentation [10]. Further justification for the consideration of EOs as preferable natural products is provided by their prospects in cultural heritage artefacts conservation [11], and/or restoration [12].

These promising perspectives for the application of EOs for the sustainable transformation of both economy and society are not unquestioned. The relative discussions, initially focused on legislative and regulatory issues, soon were expanded towards intellectual property rights [13], economic feasibility [14] and ecological sustainability [15]. In specific, Hall and Bawa [16] in their study concerning the trade-offs between economic and ecological issues have indicated their unfavourable impacts on nature conservation caused by the harvesting of wild plant populations. The profound answer of cultivating the herbal material, has also become a matter of vivid conversations within the scientific community, with focus on the competition for land use between food and cash crops [17]. These arguments have initiated the development of high-throughput screening protocols, mostly referred as bioprospecting, aiming to provide a cross assessment of the EO bioactivity efficacy to facilitate market and society uptake of early research results [18].

Essential oils have been occupying a pivotal role on the development of sustainable biocides, including applications as antibacterial [19], antifungal [20], and pesticide agents [21]. Among them their use as pesticides present numerous and diverse targets including preharvest [22], and postharvest pests relating to the aspects of food security and safety [21], special purpose pesticides [11], and disease vectors relating to public health issues that torture earths tropical to temperate zones [23]. In this last case, of the disease vectors, but also in other cases two major mode of activities are evaluated for commercial purposes [21,24]. The repellent when there is a need of low toxicity and selective targeting [24]. The biocidal when there is the need of sterilization that requires high toxicity, but also requires selective targeting [25].

Present study aspires to ameliorate previous advances in bioprospecting through the development and application of an innovative EO sustainability assessment protocol that considers the aspects of yield, bioactivity, and consumption of nonrenewable sources, which are implicated in their production. Protocol development was structured on the case study of essential oils (EO) and their potentials as biopesticides [26], which display significant economic [4], and ecological implications [10]. Moreover, the selection of the present essay's subject was based on the facilitation of both choice and nonchoice bioassays and the consideration of uniform and widely available data sets, ameliorating previous evaluation approaches [27], but also focusing in a versatile and multipurposed natural product in the form of the EO to enhance the proposed methodology's adaptability and replicability [28].

2. Materials and Methods

2.1 Materials

2.1.1 Herbal Material Data

A sum of 54 EO in the form of Essential Oils (EO) have been incorporated in the present study. These EOs and their respective details of *taxa* of origin, collection, isolation methodology, yield, composition, and bioactivity have been categorized according to the plant tissues origin and were published in three major clusters.

The first focused on forest plants and includes three species of *Juniperus* of the Cupressaceae Family [29]; *J. phoenicea* L. (juniper) from which 17 EOs were retrieved (J 01 – J 17), *J. drupacea* Labill. (Syrian juniper) with 17 EOs (J 18 – J 34) and *J. excelsa* M.Bieb. (Greek juniper) with 1 EO (J 35).

The second cluster is based on cultivated plants and contains six *Citrus* species of Rutaceae Family [30]; *C. paradisi* Macfad. (grapefruit; C 01 – C 04), *C. limon* (L.) Osbeck (lemon; C 05 – C 08), *C. reticulata* Blanco (tangerine; C 09 – C 12), *C. sinensis* (L.) Osbeck (orange; C 13 – C 16), *C. japonica* Thunb. (kumquat; C 17), and *Citrus* x *auarantium* L. (bitter orange; C 18).

The third cluster is focused on culinary herbs and includes eight *taxa* from various families [24], each contributing one EO apart from tarhan which contributed two. These herbs are *Ruta chalepensis* L. (rue; V 01), *Echinophora tenuifolia* ssp. *sibthorpiana* (Guss.) Tutin (tarhan; V 05 – V 06), *Salvia fruticose* Mill. (Greek sage; V 09), *Thymbra capitata* (L.) Cav. (thyme; V 10), *Origanum onites* L. (oregano; V12), *Foeniculum vulgare* Mill. (fennel; V 14), *Vitex agnus-castus* L. (monk's pepper; V 15) and *Satureja thymbra* L. (savory; V 16). All EOs along with their metadata are included in Appendix Table 2.

2.1.2 Experimental Data

The essential oils of the study were retrieved mostly by hydro distillation (J 01 – J 35, V 01 – V 16, C 04, C 08, C 12, C 16 – C 18), but also through cold press (C 01, C 05, C 09, C 13), and by combination of them (C 02, C 03, C 06, C 07, C 10, C 11, C 14, C 15). This variability of the essential oil retrieval methodology was included to increase the proposed methodology applicability.

The repellent and larvicidal properties of the EOs were assessed against the Asian Tiger Mosquito in bioassays performed uniformly in all EOs [24,29,30].

2.2 Methods

A summary of the proposed methodology is presented as a semantic diagram in Fig. 1 (Ref. [7]), and extensively presented in 3 major stages.

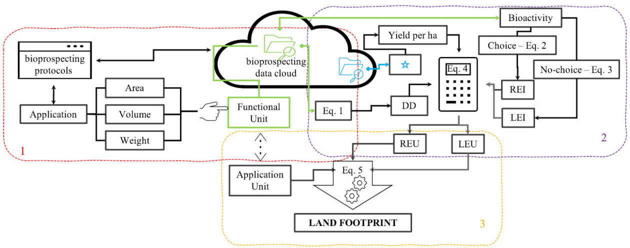

Fig. 1. Semantic diagram (☆ = Evergetis and Haroutounian [7]).

2.2.1 Functional Unit

Starting point of the proposed protocol is the selection of the functional unit, a decision of crucial importance for the indicator's translation. The calculation of the functional unit is based on the available experimental data and incorporates the amount of the EO used for the bioassay and the total area of the experimental application.

The functional unit is calculated for both repellent

IMR Press

and larvicidal experimentation, according to Eqn. 1 and expresses the volume of natural product required for the coverage of 1 m^2.

$$\mathrm{FU} = \left(\mathrm{DD_L} * 10^4\right) / \mathrm{A_L} \tag{1}$$

FU: Functional Unit in mL per m^2.
DD_L: Laboratory discriminative dose in mL.
A_L: Area of Laboratory application in cm^2.

2.2.2 Spatial Yield

The EO's spatial yield constitutes a fundamental figure for structuring the proposed methodology. The calculation of this parameter is based on a methodology recently introduced by authors for the estimation of the essential oil annual crop potentials [7], a methodology that is readily applicable for all classes of natural products. A deviation from this approach is applied for Citrus crops since their prevailing cultivation scheme and the respective plantation density were incorporated instead of the estimated 50% of land coverage.

2.2.3 Bioactivity Coefficient

Next element for the construction of final equation refers to the incorporation of the bioactivity exhibited by the investigated EO. Herein, a binary approach was applied to transform the expression of bioactivity as a clear number ranging from 0 to 1.

In this respect, the repellent coefficient was calculated as a clear number in order to provide a net multiplier of 1 for the optimum result (no landings) and escalate downwards as the number of landings increase. The respective indicator is calculated in accordance with the Eqn. 2.

$$\mathrm{REI} = (1 + \mathrm{L_e})^{-1} \tag{2}$$

REI: Repellent Efficacy Index,
L_e: Number of Landings.

Respectively the larvicidal coefficient was calculated as a single number in order to provide a net multiplier of 1 for the best result (100% mortality) and escalating downwards as the percent of mortality decreases. This indicator is calculated in the terms of Eqn. 3.

$$\mathrm{LEI} = \mathrm{M_e} * 10^{-2} \tag{3}$$

LEI: Larvicidal Efficacy Index.
M_e: Experimental Mortality in %.

2.2.4 Land Equivalent Unit

Finally, all above mentioned figures were incorporated in the calculation of the Land Equivalent Unit indicator. This indicator refers to the annual volume of essential oil production from a land area of 1 hectare and simultaneously incorporates the land use efficacy by each natural product that is evaluated. In specific, this indicator is calculated in the terms of Eqn. 4, reflecting both larvicidal and repellent activities.

$$\mathrm{LEU} = (\mathrm{P_{NP}}/\mathrm{FU}) * \mathrm{B_{CO}} \tag{4}$$

LEU: Land Equivalent Units in m^2 per hectare & year,
P_{NP}: Spatial yield of natural product in mL per hectare and year.
FU: Functional Unit of Repellent or Larvicidal application in mL per m^2,
B_{CO}: Bioactivity coefficient, REI or LEI respectively.

3. Results and Discussion

3.1 Functional Unit

The enumeration of functional unit is concluded into two distinct values: one for repellent and one for larvicidal bioassay. These values express the amount of natural product required to cover 1 m^2 according to the bioprospecting experimentation protocols. In specific, as repellent discriminative dose was considered the volume of 0.048 mL, which is capable to cover an area of 24 cm^2. This amount corresponds to a functional unit of 20 mL per m^2. On the other hand, the larvicidal discriminative dose was 0.029 mL, which was applied on 10.99 cm^2, resulting in a functional unit of 26.3876 mL per m^2.

The choice of spatial coverage as functional unit has based on the corresponding methodological principle proposed by Wackernagel and Yount [31] and summarizes the so far achievements on sustainability assessment under the footprint heading. This choice enhances the interconnectivity results of the proposed protocol with the National Footprint Accounting, the prominent sustainability assessment methodology [32,33]. The latter incorporates as fundamental figure the regenerative capacity of biosphere in conjunction with the land use productivity.

In addition, this choice also serves the replicability of the proposed methodology, since the required primary data are uniformly obtained through the initial stage of the more prominent bioprospecting protocols for biopesticides and through the plant biology and botanical description. Furthermore, this choice is adaptable by different bioprospecting protocols that incorporate the application of the discriminative dose in volume and/or weight. Furthermore, the functional unit choice also takes into consideration the final product application unit in order to facilitate the expression of the indicator as a clear number that corresponds to the land area required for the production of a single dose of the evaluated natural product.

3.2 Spatial Yield

The annual yield per hectare of the EOs was calculated in accordance with a recently presented methodology [7], which is also proved applicable for differentiated production schemes [34]. These yields are presented in Table 1 and discussed shortly in follow.

As more productive were proven the juniper ripe berry (J 05) and savory (V 16) EOs with annual production of 54.44 L and 44.11 L respectively, followed by the juniper unripe berry (J 04) EO, estimated to 44.04 L. Only 10 more of the EOs were proven to exceed the 10 L yield per hectare and year. In specific, fennel (V 14) and oregano (V 12) were the two EOs that exceeded the 30 L threshold, followed by these from tarhan (V 06), thyme (V 10), Greek sage (V 09), juniper (J 17) and (V 01), which all exceeded the threshold of 20 L.

The remaining three EOs were originated from tarhan (V 05), Juniper (J 11) and Syrian juniper (J 23) with estimated annual production capacity of more than 10 L. The more productive of the *Citrus sp.* EOs were those from orange (C 13, C 16, C 14, C 15) followed by two from grapefruit (C 04, C 01) which were in between 5 and 10 L of projected annual production per hectare and year. In the same range were listed in total 19 EOs mostly originated from juniper leaf and Syrian juniper leaves and berry. Finally, 22 EOs were estimated to be the least productive with annual yield not exceeding the 5 L per hectare. These were originated mostly from the wood of the forest trees and the *Citrus sp.* crops.

It must be noticed here that the above figures may also serve as a sustainability indicator for the finalized products, since there is a specificized content of natural product. This amount can be readily attributed to the corresponding required land for its production through the annual per hectare yield. Nevertheless, since the scope of the study was the *ex-ante* evaluation of the most productive and the most potent EO another critical figure is still missing from the equation.

3.3 Bioactivity Coefficient

The calculation of the two bioactivity coefficients followed the general rule that 0 is translated as no activity and 1 as maximum activity. The calculation algorithms enabled the uniform expression from the results of—both for choice and no-choice experimentation protocols—the performed bioassays for the evaluation of EO bioactivity. The results are presented cumulatively in Table 1, while their distribution in 5 major bioactivity clusters characterized as inactive, mild, moderate, potent, and of high activity is presented and discussed in follow separately for the choice (repellence) and no-choice (larvicidal) bioassays.

3.3.1 Repellence Efficacy Indicator

The distribution of the 54 EOs in the 5 bioactivity clusters is depicted in Fig. 2. In the high activity cluster are included twenty-two EOs, with fifteen of them exhibiting zero landings.

These last EOs belong to various *taxa* and include samples originating from tarhan (V 06), oregano (V 12), thyme (V 10), Greek sage (V 09), juniper leaf (J 02, J 05) and berry (J 16, J 17), orange fruit (C 16), Syrian juniper

Table 1. Calculation results of spatial yield, bioactivity coefficients and land equivalent units according to the proposed methodology.

EO	Spatial Yield (L/ha)	Bioactivity Coefficient		Land Equivalent Unit (m^2/ha)	
		REI	LEI	REU	LEU
V 01	20.46	0.08	0.75	84.30	581.48
V 05	14.78	0.53	0.42	393.09	235.23
V 06	26.99	0.89	0.66	1.194.32	675.01
V 09	21.85	1.00	0.02	1.092.49	16.56
V 10	22.86	1.00	1.00	1.142.86	866.13
V 12	36.89	1.00	1.00	1.844.64	1.397.98
V 14	38.19	0.22	0.80	424.31	1.157.76
V 15	6.67	0.44	0.14	148.25	35.39
V 16	44.11	1.00	0.96	2.205.47	1.604.59
J 01	3.52	0.38	N/A	66.85	N/A
J 02	5.45	1.00	0.67	272.73	138.48
J 03	9.92	0.02	0.04	9.87	15.04
J 04	44.04	0.05	N/A	103.72	N/A
J 05	3.63	1.00	0.02	181.44	2.75
J 06	54.44	0.09	0.08	253.14	165.03
J 09	3.78	0.10	N/A	18.18	N/A
J 10	6.49	0.89	0.18	287.34	44.29
J 11	13.36	0.47	0.26	313.60	131.61
J 14	5.60	0.80	1.00	224.16	212.35
J 15	6.95	0.35	0.90	120.63	236.97
J 16	4.97	1.00	N/A	248.58	N/A
J 17	21.04	1.00	0.02	1.051.84	15.94
J 18	5.45	1.00	0.67	272.29	138.26
J 19	5.00	1.00	0.94	250.16	178.21
J 21	5.80	0.80	0.26	231.97	57.13
J 23	12.84	0.53	0.40	341.52	194.64
J 24	2.91	1.00	1.00	145.30	110.12
J 25	4.80	0.80	0.96	191.81	174.44
J 26	5.11	0.05	0.94	12.08	181.94
J 28	2.53	0.04	N/A	4.88	N/A
J 29	6.30	0.02	0.06	7.62	14.33
J 30	3.09	1.00	N/A	154.27	N/A
J 31	1.64	0.02	N/A	2.04	N/A
J 32	5.92	0.13	0.12	38.82	26.93
J 33	4.58	0.08	0.54	19.07	93.70
J 35	9.11	0.09	N/A	41.39	N/A
C 01	5.12	0.03	0.02	7.55	3.88
C 02	4.25	0.02	0.28	4.12	45.09
C 03	4.10	0.03	0.24	5.24	37.25
C 04	5.73	0.80	0.25	229.08	54.25
C 05	2.52	0.04	0.74	5.25	70.66
C 06	1.61	0.03	0.31	2.01	18.90
C 07	1.37	0.02	0.26	1.69	13.45
C 08	4.19	1.00	0.02	209.30	3.17
C 09	3.36	0.03	0.14	4.54	17.82
C 10	2.42	0.03	N/A	3.59	N/A
C 11	2.26	0.05	0.46	5.95	39.44
C 12	3.14	0.20	0.1	32.21	11.91
C 13	7.59	0.02	0.51	7.52	146.73
C 14	6.07	0.02	0.94	5.83	216.36
C 15	5.06	0.02	0.84	4.76	161.13
C 16	6.41	1.00	0.54	320.60	131.20
C 17	1.38	0.80	0.75	55.23	39.24
C 18	2.90	1.00	N/A	144.76	N/A

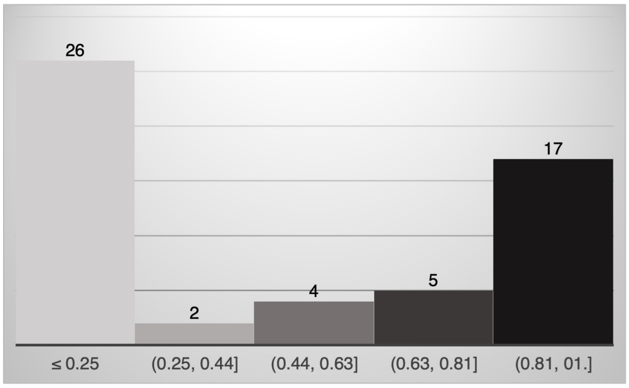

Fig. 2. Repellent Efficacy Indicator Clusters.

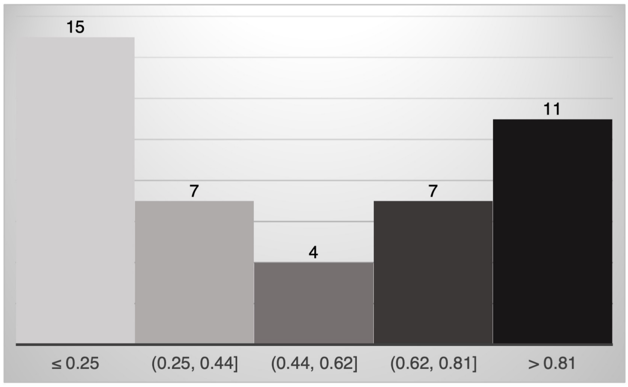

Fig. 3. Larvicidal Efficacy Indicator Clusters.

leaves (J 18, J 19, J 24, J 30) and bitter orange (C 18). In this high activity cluster were also included two more EOs presenting REI higher than 0.80. These were the EOs from tarhan (V 06) and juniper leaves (J 10). In the cluster of potent were assigned 5 EOs, with REI 0.80 and originating from Syrian juniper berry (J 21) and leaves (J 25), juniper leaves (J 14), grapefruit (C 04), kumquat (C 17). In the group of moderate activity were included four EOs, with two of them exhibiting REI of 0.53 (tarhan, V 05; Syrian juniper berries, J 23), while the REI of juniper berries (J 11) EO was 0.47 and that from monk's pepper (V 15) 0.43. Two EOs from the leaves of juniper (J 01, J 15) were found of mild activity exhibiting REI values 0.38 and 0.35 respectively. In the low activity cluster were assigned 26 EOs from which only three presented REI above 0.20 and these were originated from tangerine (C12), fennel (V 14) and Greek juniper (J 35), while all other EOs were assigned REI values close to or lesser to 0.10.

3.3.2 Larvicidal Efficacy Indicator

In the present case not all the investigated EOs were evaluated. This happens because limitations in the laboratory scale production of the EOs did not produce adequate quantities for duplicate testing. In specific 10 EOs were omitted and therefore concluding to 44 samples the distribution of which in the 5 bioactivity clusters is depicted in Fig. 3.

In the high activity cluster are included 11 EOs from which the four that exhibited 100% larval toxicity originate from oregano (V 12), thyme (V 10), juniper leaves (J 14) and Syrian juniper leaves (J 24). In the same group were also assigned seven more EOs presenting LEI values above 0.81. These were in LEI descending order: 0.96 savory (V 16) and Syrian juniper leaves (J 25); 0.94 Syrian juniper leaves (J 19, J 26) and orange (C 14); 0.90 juniper leaves (J 15); 0.84 orange (C 15). In the cluster of potent bioactive EOs were included the EOs from fennel (V 14), rue (V 01), kumquat (C 17), lemon (C 05), juniper leaves (J 02), Syrian juniper leaves (J 18), and tarhan (V 06). The EOs that were found to exhibit moderate activity were these from Syrian juniper berries (J 33), orange (C 16, C 13) and tangerine (C 11). In the mild activity cluster were assigned seven EOs and more specifically these from tarhan (V 05), Syrian juniper berries (J 23), lemon (C 06, C 07), grapefruit (C 02), juniper berries (J 11) ans Syrian juniper berries (J 21). Finally, in the inactive cluster were catalogued fifteen EOs of various origin.

3.4 Land Equivalent Unit

The cumulative results of LEU calculation for the repellence and toxicity sustainability assessments are presented in Table 1. These results present for first time an estimation for EO land productivity corrected upon their bioactivity. The sustainability evaluation of both repellent and larvicidal properties of the EOs studied highlighted in both cases oregano (V 12) and savory (V 16) as most potent with repellent LEU values 1397.98 and 1604.59 m^2/ha and larvicidal LEU values 1844.64 and 2205.47 m^2/ha. In general, the culinary herbs were proven the more sustainable source of EOs in both choice and no-choice experiments.

In the repellent sustainability assessment juniper and Syrian juniper EOs presented significant records. In specific, juniper (J 17) and Syrian juniper berries (J 23) berries EOs exhibited repellent LEU of 1050.84 and 341.52 m^2/ha respectively, while from the *Citrus sp.* EOs, only orange (C 14) presented a relatively sustainable profile with repellent LEU 320.60 m^2/ha. In the larvicidal sustainability assessment next to the culinary herbs were listed the EOs from juniper leaves (J 15, J 14) with LEU 237.96 and 212.35 m^2/ha, while as more sustainable *Citrus sp.* EO, was proven the orange (C 14) with LEU 216.36 m^2/ha.

These values which are capable for outlining the land utilization efficacy of each EO, may be further translated on the base of the functional unit refinement. In specific the adjusting of LEU, in respect with the covered area per application can provide a land footprint projection for each of the natural products, EOs in the present case, under consideration. The numerical calculations were performed according to Eqn. 5.

$$\mathrm{LF} = 10^4/(\mathrm{LEU}/\mathrm{OU}) \quad (5)$$

LF: Land Footprint in m^2.

LEU: Land equivalent unit in m^2/ha.

OU: Operational Unit in m^2.

This final step of the proposed methodology was omitted from the description of the protocol as it is highly depended upon the nature of the EO utilization. It was deemed more appropriate, since these calculations are established upon case specific figures, to be presented for each purpose separately. Thus, the land footprint projections are described, presented, and discussed in follow for the repellence and larvicidal bioassays.

3.4.1 Repellent Land Footprint

Based on the fact the final application of any repellent scopes to protect the exposed human skin, as operational unit for the repellent land footprint was chosen the coverage of the 50% of the average human skin's surface. Considering that the area of the human skin varies between individuals from 1.5 to 2.0 m^2 an average of 1.75 m^2 was considered for the calculation of the repellent operational unit to 0.875 m^2 [35]. This operational unit was utilised for the transformation of repellent LEU from m^2/ha to number of applications per hectare. Then the number of applications per hectare was transformed to land area per application concluding thus to the land footprint per application for each EO. These results are presented in Fig. 4 and shortly discussed in follow.

The sustainability assessment of repellent usage of EOs, highlighted as best of the best these from savory (V 16), oregano (V 12), tarhan (V 06), Greek sage (V 10), thyme (V 09) and juniper (J 17) exhibiting a land footprint per application less than 10 m^2. All these EOs exhibited REI value of 1, while were also include in the top ten of spatial yield. This group of EOs constitutes the primary target for further research elaborating on the minimum landing inhibition EO concentration which will greatly improve their land footprint. Under this perspective the utility of the proposed methodology maybe expanded beyond the *ex-ante* assessment and include also more advanced research results. This expansion precludes the replacement of the discriminative dose with the indicated minimum EO concentration, which requires extensive research resources. Therefore, the present assessment is most valuable for the decision of further experimentation and the selection of its subjects. In this respect as most viable targets, maybe considered these from juniper leaf (J 02, J 05) and berry (J 16), orange fruit (C 16), Syrian juniper leaves (J 18, J 19, J 24, J 30) and bitter orange (C 18). In addition, the slope of Fig. 4 indicates that the repellent discriminative dose of the initial screening maybe significantly lowered to highlight bioactivity differences among the various EOs.

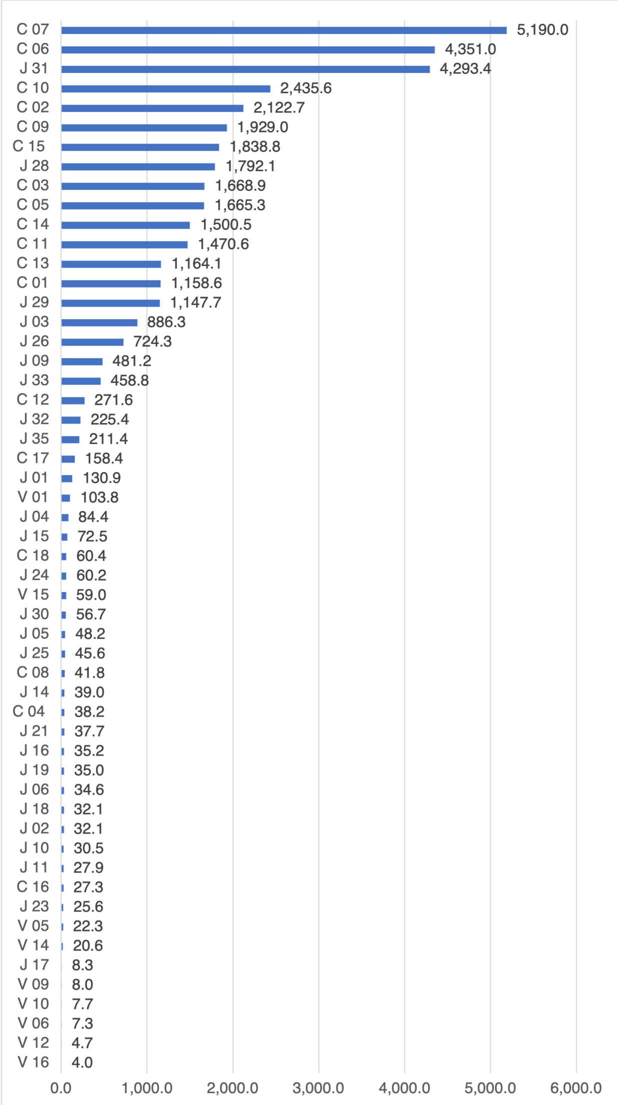

Fig. 4. Land footprint of repellent EOs in m^2 per application.

On the other hand, another group of EOs that presented land footprint less than 40 m^2 without being among the most efficient and productive in terms of spatial yield were these from Syrian juniper (J 23, J 21), juniper (J 11, J 10, J 19, J 14) and grapefruit (C 04), while in the present group was also included the most productive EO (J 06) exhibiting a land footprint of 34.57 m^2. This former group includes EOs that may be considered as viable targets for complementary research in terms of their production potentials. This group though less sustainable is also of significant importance because of the market characteristics of the end-products in which a series of other values like odour, persistence, allergies, irritation *etc*. must be considered. It is thus profound that although valuable the proposed methodology it must be complemented by expansion of the research subjects to ameliorate market up-take of preliminary bioprospecting results.

3.4.2 Larvicidal Land Footprint

The fundamental assumption for the larvicidal application unit was defined by the large-scale interventions, which in municipal level are mostly focussed on catch-basins treatment. The size of them varies between countries but also in relation to the drainage system grade; primary catch-basins dimensions escalate from 0.25 by 0.25 m to 0.5 by 0.7 m. For the purposes of the study as application unit was selected an intermediate size rectangle catch-basin 0.5 by 0.5 m the surface of which was calculated to 0.25 m^2. Same as for the repellent footprint this application unit was translated in quantity of EO suggested and correspondingly to the respective land footprint. These results are presented in Fig. 5 and shortly discussed in follow.

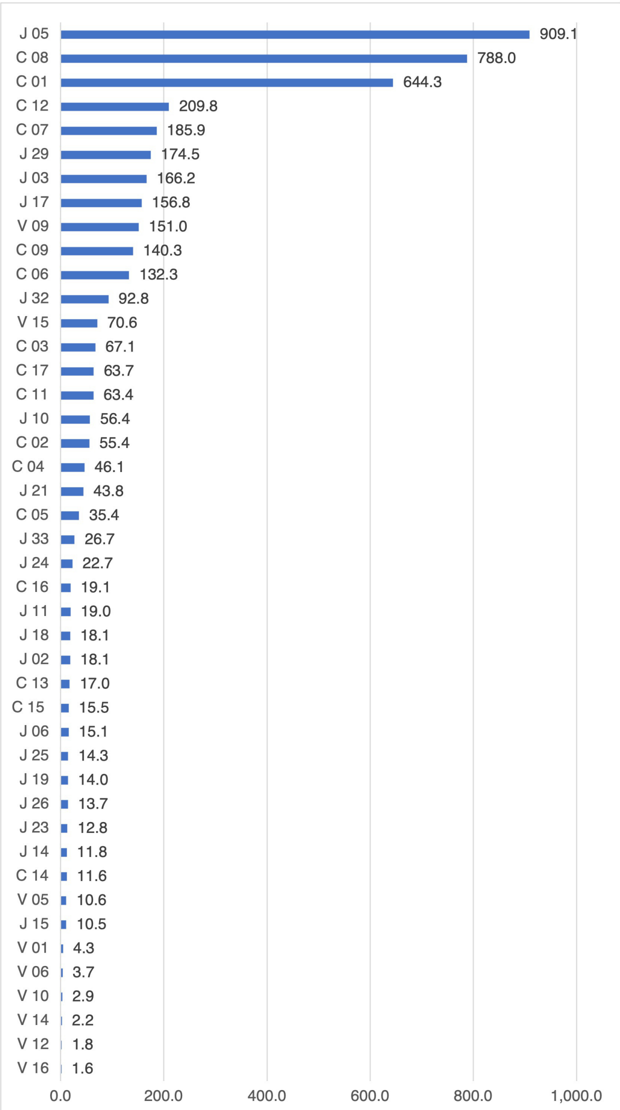

Fig. 5. Land footprint of larvicidal EOs in m^2 per application.

A first remark on the larvicidal land footprint of the EOs reflects the comparison in between the two uses considered in the present study, which indicates larvicidal use as more sustainable than repellent, since in all the EOs from oregano (V 12), Greek sage (V 10), juniper (J 14) and Syrian juniper (J 24) that presented LEI and REI value of 1, as more sustainable was proven the larvicidal use which in oregano presented a larvicidal land footprint of 1.79 m^2 less than half of the repellent (4.74 m^2). The same land footprint gap in between larvicidal and repellent use was found for the EOs from Greek sage and Syrian juniper in which the repellent use required 2.65 more land than the larvicidal, while the EO from juniper with larvicidal and repellent land footprints of 11.77 *vs.* 39.03 m^2, required 3.35 more land in the case of the repellent use.

Another significant difference is observed in the bioactivity coefficient of the six more sustainable EOs that presented land footprint less than 10 m^2. In specific, savory (V 16) the more sustainable of the EOs presented LEI value of 0.96, while among the rest fennel's (V 14) LEI was 0.8, tarhan's (V 06) 0.66 and rue's (V 01) 0.75, and only oregano (V 12) and Greek sage (V 10) presented LEI value of 1. These results advocated by the distribution of the EOs larvicidal land footprint distribution presented in Fig. 5 suggest that the larvicidal discriminative dose was more efficient in the bioactivity evaluation of the EOs than the repellent.

The performed *ex-ante* sustainability assessment results indicated in this case also potential directions and targets for further research. Beside the already mentioned EOs as potent active ingredients deserving more focus were indicated 15 more EOs that presented land footprint less than 20 m^2. Prominent among them were the orange (C 13, C 14, C 15) EOs, which being crude and processed industrial by-products present intriguing aspects encompassing the circular economy and fostering the bio-based industrial solutions. Then the numerous juniper (J 15, J 14, J 06, J 02, J 11) and Syrian juniper (J 23, J 26, J 25, J 18) EOs along with these from tarhan (V 05) and orange fruit (C 16) maybe further evaluated in terms of ecotoxicity along with their land footprint to delineate this crucial characteristic for field application that will be also environmentally neutral.

4. Conclusions

The proposed methodology has been proven able to provide a sustainability assessment of natural product in the form of EOs based on broadly available early experimentation data. This assessment was performed through the respective land footprint calculation, which produced comparable results for both choice and no-choice bioassays. Spatial yield and bioactivity were effectively combined and corrected each other producing a cross-checked sustainability performance figure. Moreover, the initial stage of functional unit selection also serving the operational unit definition manages to increase the adaptability of the proposed methodology in all types of bioprospecting screening. Finally, the cumulative results provided insights on the validity of the initial experimentation design and were proven capable to discriminate on the sustainability of different

Table 2. Herbal material metadata.

Code	*Taxon*	EO origin	EO Yield (mL/kg)
V 01	*Ruta chalepensis* L.	whole plant	4.71
V 05	*Echinophora tenuifolia* ssp. *sibthorpiana* (Guss.) Tutin	whole plant	3.13
V 06			5.71
V 09	*Salvia fruticosa* Mill.	aerial parts	4.46
V 10	*Thymbra capitata* (L.) Cav.	aerial parts	5.33
V 12	*Origanum onites* L.	aerial parts	6.72
V 14	*Foeniculum vulgare* Mill.	aerial parts	5.35
V 15	*Vitex agnus-castus* L.	aerial parts	2.05
V 16	*Satureja thymbra* L.	aerial parts	13.11
J 01	*J. phoenicea* L.	leaf	0.86
J 02			1.33
J 03		berry unreap	3.12
J 04			13.84
J 05		berry reap	1.05
J 06			15.76
J 09		leaf	0.92
J 10			1.59
J 11		berry unreap	4.20
J 14		leaf	1.37
J 15			1.70
J 16		berry unreap	1.56
J 17			6.61
J 18	*J. drupacea* Labill	leaf	1.47
J 19			1.36
J 21		berry unreap	1.84
J 23		berry reap	3.79
J 24		leaf	2.91
J 25			1.30
J 26			1.38
J 28		berry unreap	0.80
J 29		leaf	1.71
J 30			0.84
J 31		berry unreap	0.52
J 32			1.88
J 33			1.45
J 35	*J. excelsa* M. Bieb	aerial parts	2.55
C 01	*C. paradisii* MacFab	CPEO	0.50
C 02		CPEO Vol. Fr. 1	0.42
C 03		CPEO Vol. Fr. 2	0.40
C 04		fruit	0.56
C 05	*C. limon* (L.) Osbeck	CPEO	0.70
C 06		CPEO Vol. Fr. 1	0.45
C 07		CPEO Vol. Fr. 2	0.38
C 08		fruit	1.16
C 09	*C. reticulata* Blanko	CPEO	0.60
C 10		CPEO Vol. Fr. 1	0.43
C 11		CPEO Vol. Fr. 2	0.40
C 12		fruit	0.56
C 13	*C. sinensis* (L.) Osbeck	CPEO	0.70
C 14		CPEO Vol. Fr. 1	0.56
C 15		CPEO Vol. Fr. 2	0.47
C 16		fruit	0.59
C 17	*C. japonica* Thunb.	fruit	0.87
C 18	*C.* x *auarantium* L.	fruit	0.38

uses for the same EO. Thus, the proposed methodology maybe proven a valuable tool for the valorisation of numerous broadly available experimental data, enhancing this way the EO market up-take.

Availability of Data and Materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Author Contributions

EE and SH—designed the research study. EE—performed the research and analyzed the data. SH—provided help, guidance, and advice on method development. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

Ethics Approval and Consent to Participate

Not applicable.

Acknowledgment

The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript. We also thank the project coordinator and colleague Dr. Antonios Michaelakis for sharing the data included in the present study.

Funding

This research was funded by EU Environmental Funding Programme Life+ Environment Policy And Governance and Greek National Funds And Emilia-Romagna Regional Funds, grant number LIFE12 ENV/GR/000466.

Conflict of Interest

The authors declare no conflict of interest.

Appendix

See Table 2.

References

[1] Costanza R, Daly M. Natural Capital and Sustainable Development. Conservation Biology. 1992; 6: 37–46.
[2] Ulgiati S, Brown MT. Monitoring patterns of sustainability in natural and man-made ecosystems. Ecological Modelling. 1998; 108: 23–36.
[3] Parris TM, Kates RW. Characterizing a sustainability transition: Goals, targets, trends, and driving forces. Proceedings of the National Academy of Sciences. 2003; 100: 8068–8073.
[4] Lubbe A, Verpoorte R. Cultivation of medicinal and aromatic plants for specialty industrial materials. Industrial Crops and Products. 2011; 34: 785–801.
[5] Newman DJ, Cragg GM. Natural Products as Sources of New

Drugs over the 30 Years from 1981 to 2010. Journal of Natural Products. 2012; 75: 311–335.
[6] Eissen M, Metzger JO, Schmidt E, Schneidewind U. 10 Years after Rio-Concepts on the Contribution of Chemistry to a Sustainable Development. Angewandte Chemie International Edition. 2002; 41: 414–436.
[7] Evergetis E, Haroutounian SA. Exploitation of apiaceae family plants as valuable renewable source of essential oils containing crops for the production of fine chemicals. Industrial Crops and Products. 2014; 54: 70–77.
[8] Kuszak AJ, Hopp DC, Williamson JS, Betz JM, Sorkin BC. Approaches by the us National Institutes of Health to support rigorous scientific research on dietary supplements and natural products. Drug Testing and Analysis. 2016; 8: 413–417.
[9] Roller S. The quest for natural antimicrobials as novel means of food preservation: Status report on a European research project. International Biodeterioration and Biodegradation. 1995; 36: 333–345.
[10] Chandler D, Bailey AS, Tatchell GM, Davidson G, Greaves J, Grant WP. The development, regulation and use of biopesticides for integrated pest management. Philosophical Transactions of the Royal Society B: Biological Sciences. 2011; 366: 1987–1998.
[11] Ebadollahi A, Ziaee M, Palla F. Essential Oils Extracted from Different Species of the Lamiaceae Plant Family as Prospective Bioagents against Several Detrimental Pests. Molecules. 2020; 25: 1556.
[12] Gatti L, Troiano F, Vacchini V, Cappitelli F, Balloi A. An In Vitro Evaluation of the Biocidal Effect of Oregano and Cloves' Volatile Compounds against Microorganisms Colonizing an Oil Painting—A Pioneer Study. Applied Sciences. 2021; 11: 78.
[13] David B. New regulations for accessing plant biodiversity samples, what is ABS? Phytochemistry Reviews. 2018; 17: 1211–1223.
[14] Polski M. The institutional economics of biodiversity, biological materials, and bioprospecting. Ecological Economics. 2005; 53: 543–557.
[15] Rombaut N, Tixier AS, Bily A, Chemat F. Green extraction processes of natural products as tools for biorefinery. Biofuels. Bioproducts and Biorefining. 2014; 8: 530–544.
[16] Hall P, Bawa K. Methods to assess the impact of extraction of non-timber tropical forest products on plant populations. Economic Botany. 1993; 47: 234–247.
[17] Hellmann F, Verburg PH. Impact assessment of the European biofuel directive on land use and biodiversity. Journal of Environmental Management. 2010; 91: 1389–1396.
[18] Dedeurwaerdere T. From bioprospecting to reflexive governance. Ecological Economics. 2005; 53: 473–491.
[19] Shen C, Chen W, Li C, Aziz T, Cui H, Lin L. Topical advances of edible coating based on the nanoemulsions encapsulated with plant essential oils for foodborne pathogen control. Food Control. 2022; 145: 109419.
[20] Hamad GM, Mehany T, Simal-Gandara J, Abou-Alella S, Esua OJ, Abdel-Wahhab MA, *et al*. A review of recent innovative strategies for controlling mycotoxins in foods. Food Control. 2022; 144: 109350.
[21] Papanikolaou NE, Kavallieratos NG, Iliopoulos V, Evergetis E, Skourti A, Nika EP, *et al*. Essential Oil Coating: Mediterranean Culinary Plants as Grain Protectants against Larvae and Adults of Tribolium castaneum and Trogoderma granarium. Insects. 2022; 13:165.
[22] Mishra B, Chandra M. Evaluation of phytoremediation potential of aromatic plants: a systematic review. Journal of Applied Research on Medicinal and Aromatic Plants. 2022; 31: 100405.
[23] Evergetis E, Michaelakis A, Haroutounian SA. Essential oils of Umbelliferae (Apiaceae) family taxa as emerging potent agents for mosquito control. Integrated Pest Management and Pest Control. 2012; 26: 613–637.
[24] Evergetis E, Bellini R, Balatsos G, Michaelakis A, Carrieri M, Veronesi R, *et al*. From bio-prospecting to filed assessment: The case of carvacrol rich essential oil as a potent mosquito larvicidal and repellent agent. Frontiers in Ecology and Evolution. 2018; 6: 1–12.
[25] Giunti G, Benelli G, Palmeri V, Laudani F, Ricupero M, Ricciardi R, *et al*. Non-target effects of essential oil-based biopesticides for crop protection: Impact on natural enemies, pollinators, and soil invertebrates. Biological Control. 2022; 176: 105071.
[26] 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 Medica. 2011; 77: 598–617.
[27] Adorjan B, Buchbauer G. Biological properties of essential oils: an updated review. Flavour and Fragrance Journal. 2010; 25: 407–426.
[28] Takakura KI. Reconsiderations on Evaluating Methodology of Repellent Effects: Validation of Indices and Statistical Analyses. Journal of Economic Entomology. 2009; 102: 1977–1984.
[29] Evergetis E, Michaelakis A, Papachristos DP, Badieritakis E, Kapsaski-Kanelli VN, Haroutounian SA. Seasonal variation and bioactivity fluctuation of two Juniperus sp. essential oils against Aedes (Stegomyia) albopictus (Skuse 1894). Parasitology Research. 2016; 6: 2175–2183.
[30] Kapsaski-Kanelli VN, Evergetis E, Michaelakis A, Papachristos DP, Myrtsi ED, Koulocheri SD, *et al*. "Gold" Pressed Essential Oil: An essay on the volatile fragment from Citrus Juice Industry By-products chemistry and bioactivity. BioMed Research International. 2017; 1–9.
[31] Wackernagel M, Yount JD. Footprints for Sustainability: the next steps. Environment, Development and Sustainability. 2000; 2: 21–42.
[32] Steen-Olsen K, Weinzettel J, Cranston G, Ertug EA, Hertwich EG. Carbon, Land, and Water Footprint Accounts for the European Union: Consumption, Production, and Displacements through International Trade. Environmental Science & Technology. 2012; 46: 10883–10891.
[33] Borucke M, Moore D, Cranston G, Gracey K, Iha K, Larson J, *et al*. Accounting for demand and supply of the biosphere's regenerative capacity: The National Footprint Accounts' underlying methodology and framework. Ecological Indicators. 2013; 24: 518–533.
[34] Misyri V, Tsekouras V, Iliopoulos V, Mavrikou S, Evergetis E, Moschopoulou G, *et al*. Farm or lab? Chamazulene content of Artemisia arborescens (Vill.) L. essential oil and callus volatile metabolites isolate. Industrial Crops and Products. 2021; 160: 113114.
[35] Wilkinson PF, Millington R. Skin. 1st edn. Cambridge University Press: Cambridge. 2009.