The PRIMA programme is an Art. 185 initiative supported and funded under Horizon 2020, the European Union's Framework Programme for Research and Innovation'

Deliverable title	D4.3 By-products (old leaves and fibrous stems) from the new organic sea fennel crops
Deliverable Lead:	IACKR
Related Work Package:	WP4
Related Task:	Task 4.1 (R). Sustainable production of sea fennel crop in demo field
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Dissemination level	CO
Due Submission Date:	MONTH 12 (30.04.2023)
Actual submission:	01.01.2025
Start date of project	30.05.2022
Duration	36 MONTHS
Summary of Deliverable D4.3 – By-Products from Organic Sea Fennel Crops	Sea fennel cultivation does not only generate edible leaves and tender shoots - it also produces a large share of by-products, such as older leaves and fibrous stems. Deliverable D4.3 focused on quantifying these fractions across the consortium's demo fields in Italy, Croatia, Tunisia, and Türkiye, while also evaluating their potential for valorization. At the Italian sites, biostimulation and irrigation significantly increased overall plant biomass, but also raised the proportion of by-products. On average, more than half of the harvested biomass was classified as "refuse." Interestingly, younger plants had a lower share of fibrous tissues, suggesting that harvest timing can influence the balance between edible yield and by-products. In Croatia, comparisons between the Korčula and Atlantic ecotypes showed that local plants were larger and more productive overall, though both produced substantial amounts of non-edible biomass. Importantly, dry matter content was consistent across plant parts, highlighting a uniform quality that could support further processing. Tunisia faced challenges in 2023 due to high summer heat, but renewed trials in 2024 revealed striking results. While fresh biomass was high, by-products represented up to 80–90% of total plant mass at later harvests, underscoring the need to develop strategies for valorizing this material. In Türkiye, biofertilization boosted both edible and non-edible yields. Second-year harvests showed remarkable increases, with fresh yields exceeding one ton per hectare. Analyses of leaves and flowers revealed rich biochemical profiles, including high levels of antioxidants, flavonoids, carotenoids, phenolics, tocopherols, and vitamin C. These findings confirm that by-products, far

	from being waste, are a valuable source of bioactive compounds with potential applications in food, nutraceuticals, cosmetics, and animal feed.

Versioning and Contribution History

Version	Date	Modified by	Modification reason
v1.0	20/04/2023	Branimir Urlic	First version
v2.0	01/01/2025	Branimir Urlic	Comments after peer reviewing process

Table of Contents

1. By-products (old leaves and fibrous stems) from the new organic sea fennel crops

Objective

Measurement of by-products biomass of sea fennel crop cultivated at demo and open field.

1.1 Italian site

Materials and methods

The epigeal biomass was cut at a height of 5 cm from the ground level, and each by-product of plant was separated from marketable yield (corresponding to edible leaves). then was weighed individually using a laboratory balance to determine the weight. A total of 27 plants were sampled in a randomized manner, with 9 samples taken for each of the 3 replications. These results were calculated with the biomass at the moment of the harvest

Results

Total fresh biomass (g/plant) and marketable yield/fresh biomass (g/plant) for the Camerano experimental site in 2023

Treatment	Total fresh biomass (g/plant)	Marketable Yield (g/plant)	Marketable Yield / Total fresh biomass (g/plant)	% of refuse
CT	523.7	219.95	0.42	58%
BIO	936	439.92	0.47	53%
IRR	820.4	377.38	0.46	54%
Mean	750.75	337.83	0.45	55%

CT = Control

BIO = Biostimulated
IRR = Irrigated

Total fresh biomass (g/plant) and marketable yield/fresh biomass (g/plant) for the Agugliano experimental site in 2024

Treatment	Population	Total fresh biomass (g/plant)	Marketable Yield (g/plant)	Marketable Yield / Total fresh biomass (g/plant)	% of refuse
CT	AT	40.30	27.4	0.68	32%
	MED	45.48	31.8	0.7	30%
BIO	AT	110.25	73.86	0.67	33%
	MED	103.86	73.74	0.71	29%
IRR	AT	89.87	60.21	0.67	33%
	MED	89.11	58.81	0.66	34%
Mean		79.81	54.3	0.68	31%

CT = Control
BIO = Biostimulated
IRR = Irrigated
AT = Atlantic ecotype
MED = Mediterranean ecotype

One-year-old plants have a lower % of refuse due to the lower lignification

1.2 Croatian site

Objective

Measurement of biomass for 2 sea fennel ecotypes (Korčula and Atlantik) cultivated at demo field in summer season

Materials and methods

The whole plant was cut epigeal at a height of 5 cm above the ground level, and each sample was divided on leaves used for determination of marketable yield, flowers and stems with damaged leaves. Each subsample was weighed to get fresh weight and then dried in oven on 70 C for few days to get dry weight. Harvest was done in July 2024 and total of 18 plants were sampled in a randomized manner, with 6 samples taken for each of the 3 replications.

Seafennel plants harvested in July 2024 - fresh and dry weight, and dry weight percentage (data from unfertilized plots)
Means are shown as +/- standard error

Ecotype	Plant	Leaves	Stem	Flover
	Fresh weight (g/plant)			
Atlantik	212 ± 22,1	85,3 ± 8,46	67,4 ± 9,1	59,4 ± 7,1
Korčula	374 ± 29,0	189 ± 18,2	159 ± 11,9	24,5 ± 4,8
	Dry weight (g/plant)			
Atlantik	58,1 ± 5,7	23,5 ± 2,1	18,8 ± 2,4	15,7 ± 1,6
Korčula	97,5 ± 7,5	48,1 ± 4,5	42,9 ± 3,4	6,5 ± 1,3
	Dry weight percentage (%)			
Atlantik	27,7 ± 0,4	28,1 ± 0,5	28,3 ± 0,5	26,9 ± 0,5
Korčula	26,1 ± 0,3	25,6 ± 0,3	26,8 ± 0,4	27,1 ± 0,6

First harvest of full developed plants was done in July 2024, because in summer 2023 plants were small for harvesting due to late planting. No differences were found between unfertilized or fertilized plots.

Ecotype Korčula had bigger plants than ecotype Atlantic and all plant parts had simillar dry weight percentage for both ecotypes.

1.3 Tunisian sites

The cultivation of sea fennel for last year (2023) failed due to high temperatures, the cultivation was renewed in the year 2024.

Materials and methods

Two harvests of sea fennel aerial parts were conducted; September 2024 and January 2025. Sampled plants were separated into "edible portion" and "refuse" categories. A total of 24 plants were harvested randomly with 6 samples taken in every treatment*ecotype combination. The plant material was dried in an oven until reaching a constant weight for the determination of the dry matter content, which was expressed in % of fresh weight (FW).

Results

Averages of Fresh-Biomass (g/plant) of refused parts of sea fennel populations

Ecotype	Treatment	Harvest period	Total plant fresh biomass (g)	Refused parts mass (g)	% of refuse
Tunisia	NPK	Sep-24	15.38±10.80	12.02±10.62	52%±39%
Tunisia	Control	Sep-24	8.03±9.60	5.43±7.53	51%±28%
Atlantic	NPK	Sep-24	3.36±1.63	0.00	0%
Atlantic	Control	Sep-24	1.92±1.17	0.00	0%
Tunisia	NPK	Jan-25	179.11±148.83	162.90±137.65	91%±3%
Tunisia	Control	Jan-25	103.36±98.33	90.02±86.76	86%±6%
Atlantic	NPK	Jan-25	24.78±7.20	22.22±6.70	89%±2%
Atlantic	Control	Jan-25	4.06±3.38	3.45±2.99	81%±8%

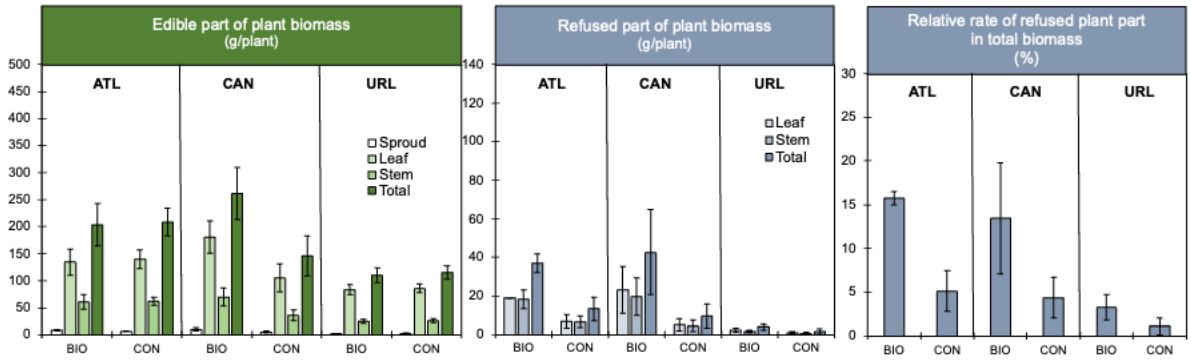
1.4 Turkish sites

Materials and methods

Sea fennel aerial parts were separated into "edible portion" (sprouts, tender young leaves, and stems) and "refuse" (older leaves and fibrous stems) categories, which were both stored at +4°C immediately after harvesting, prior to their analysis/use. Liquid nitrogen were used to store some of the sea fennel plant samples for further chemical analysis. Then the samples were stored in -70 °C. For each sample, a portion of the plant material was dried in a forced draft oven at 105 ◦C until reaching a constant weight for the determination of the dry matter content, which was expressed as g 100 g−1 fresh weight (FW). Leaf area (m2) and CIE Lab colour was performed and the CIE L (lightness) a* (redness) b* (yellowness) color scale.

Results

Effect of bio-fertilizer application versus no fertilization on the biomass distribution of sea fennel in 2023, including edible and refused (leaf, stem, and total) plant fractions, as well as the relative proportion of refused plant parts in the total biomass of three populations, are shown in the figure below. Considering the average biomass values of the edible parts of all three populations, a 19% increase was observed in the plots with biological fertilizer application, yielding 191 g/plant, compared to the 160 g/plant from the control plots. When considering the refused parts of the plant, the effect of biological fertilization was more pronounced, resulting in a 3.5-fold increase compared to control plots. The biomass of the refused parts was 28 g/plant in the biologically fertilized plots, compared to 8 g/plant in the unfertilized plots.

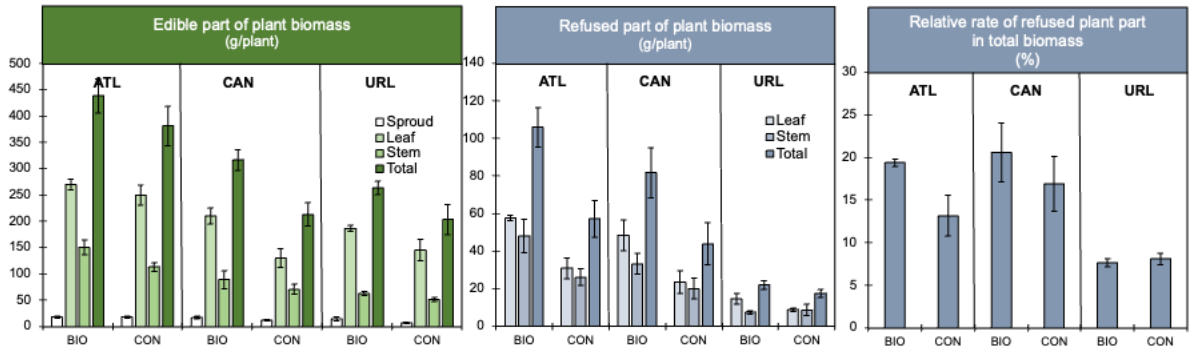


Effect of bio-fertilizer application versus no fertilization on the biomass distribution of sea fennel (Crithmum maritimum L.) in 2023, including edible (sprout, leaf, stem, and total) and refused (leaf, stem, and total) plant fractions, as well as the relative proportion of refused plant parts in the total biomass of three populations (ATL: Atlantis, CAN: Çandarlı, and URL: Urla).

The highest biomass values were recorded in the ATL and CAN populations, with 206 g/plant and 204 g/plant, respectively, followed by the URL population with 130 g/plant. The lowest proportion of refused parts was observed in the URL population (2%). Moreover, biological fertilization increased the ratio of refused plant parts in the total biomass by an average of 7%.

In the second year of the field trial (2024), an average 68% increase in the edible biomass was recorded. The effect of biological fertilizer application was further enhanced, with 340 g/plant of edible biomass obtained in fertilized plots and 266 g/plant in unfertilized plots, resulting in a 28% increase due to fertilization. On the other hand, the biomass of the refused plant parts increased by 75% in the fertilized plots, reaching 70 g/plant, compared to 40 g/plant in the unfertilized plots.

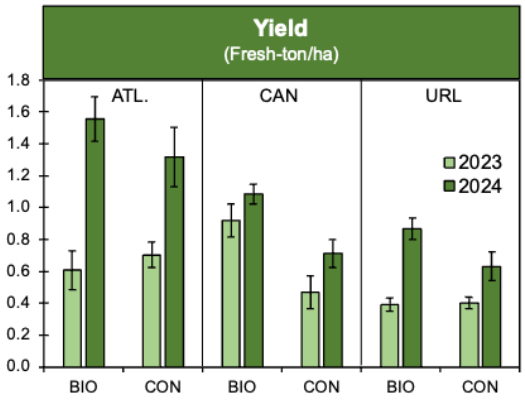
The highest biomass value in 2024 was recorded in the ATL population, with 410 g/plant, followed by the CAN and URL populations with 265 g/plant and 234 g/plant, respectively. The lowest proportion of refused parts was observed in the URL population (8%). Additionally, biological fertilization increased the ratio of refused plant parts in total biomass by an average of 3%.



Effect of bio-fertilizer application versus no fertilization on the biomass distribution of sea fennel (Crithmum maritimum L.) in 2024, including edible (sprout, leaf, stem, and total) and refused (leaf, stem, and total) plant fractions, as well as the relative proportion of refused plant parts in the total biomass of three populations (ATL: Atlantis, CAN: Çandarlı, and URL: Urla).

According to the results from the field trials, the average fresh yield in 2023 was recorded at 0.58 tons/ha, with a 76% increase in 2024, reaching 1.02 tons/ha. When considering the average yields over both trial years, the ATL population exhibited the highest yield at 1.05 tons/ha, followed by the CAN and URL populations with yields of 0.80 tons/ha and 0.57 tons/ha, respectively.

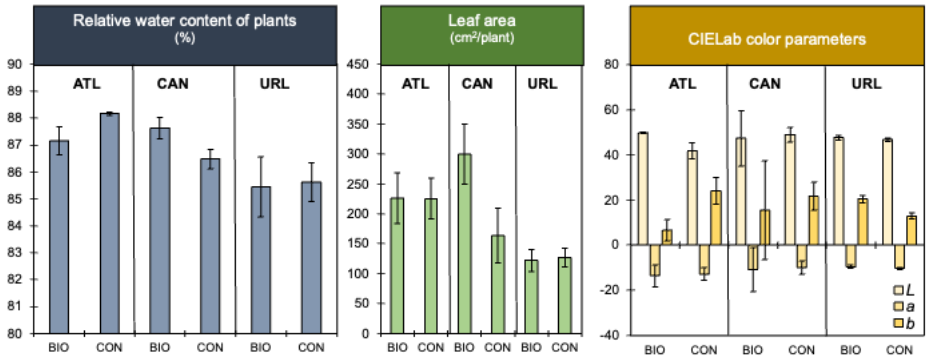
In 2023, biological fertilization resulted in a significant yield increase of 96% in the CAN population, whereas no notable effect was observed in the other populations. In 2024, biological fertilization positively impacted all populations, with an average yield increase of 32%.



Effect of bio-fertilizer application versus no fertilization on the fresh yield of three sea fennel (Crithmum maritimum L.) populations (ATL: Atlantis, CAN: Çandarlı, and URL: Urla) during the 2023 and 2024 growing seasons.

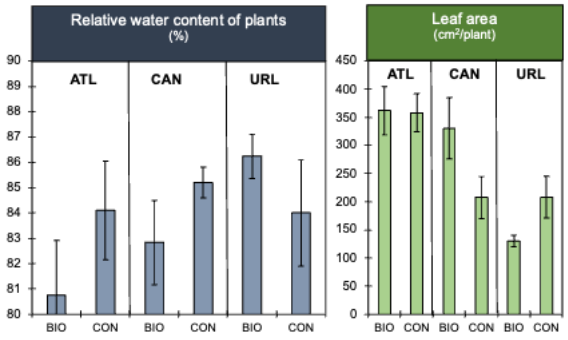
The average relative water content (RWC) of the harvested sea fennel plants in 2023 was 87%, with no statistically significant differences observed between the populations in terms of RWC. In the same year, the average leaf area did not exhibit significant variation between the ATL and CAN populations, which measured 226 cm² and 232 cm², respectively. However, the leaf area of the URL population was significantly lower than the other two, at 125 cm². Additionally, biofertilization led to an 83% increase in the leaf area of the CAN population. According to the results obtained from the CIELab color analysis, the average L (lightness) value of sea fennel plants was recorded as 47.1. The highest L value was

observed in the ATL population treated with biological fertilizer (BIO), at 49.9, while the lowest value was obtained from the ATL population under control conditions (CON), at 41.9.



Effect of bio-fertilizer application versus no fertilization on relative water content of plants, leaf area and CIELab color of three sea fennel (Crithmum maritimum L.) populations (ATL: Atlantis, CAN: Çandarlı, and URL: Urla) in 2023.

Regarding the a (redness/greenness) values, the ATL population with BIO treatment exhibited the lowest value (-13.5), indicating greater greenness, whereas the URL population with BIO treatment showed the highest value (-9.5). As for the b (yellowness) values, the highest measurement (24.1) was obtained from the ATL population under control conditions (CON), whereas the lowest measurement (6.7) was recorded for the ATL population under BIO treatment. Overall, biological fertilizer application did not result in consistent or significant changes across the CIELab color parameters in sea fennel populations.



Effect of bio-fertilizer application versus no fertilization on relative water content of plants and leaf area of three sea fennel (Crithmum maritimum L.) populations (ATL: Atlantis, CAN: Çandarlı, and URL: Urla) in 2023.

In the second year of the study (2024), the relative water content (RWC) of sea fennel plants decreased by 3.5% compared to the first year, reaching 84%. However, greater variation was observed between populations and treatments. The highest RWC value was recorded in the URL population treated with biological fertilizer at 86%, while the lowest RWC was observed in the ATL population under the same treatment at 81%. Leaf area measurements showed a similar trend to the first year, with the highest values recorded in the ATL and CAN populations, reaching 360 cm² and 270 cm², respectively. The leaf area of the URL population was lower, measured at 169 cm².

According to the biochemical analyses of sea fennel populations, total antioxidant activity (FRAP) values ranged from 112 to 177 µmol FRAP/g DW. The highest antioxidant activity was recorded in the flowers samples of URL population (URL-F) (177 µmol FRAP/g DW), while the lowest was in leaf samples of CAN population (CAN-L) (112 µmol FRAP/g DW). Total flavonoid content varied significantly among samples, with the highest value observed in leaf samples of ATL population (ATL-F) (11.2 mg/g DW) and the lowest in CAN-L (2.71 mg/g DW).

Antiradical activity (DPPH) ranged from 66 to 265 µg/mL. The lowest IC50 value, indicating the highest antiradical activity, was observed in leaf samples of URL population (URL-L) (66 µg/mL), while the highest IC50 was recorded in URL-F (265 µg/mL). Total carotenoid content was highest in URL-L (113.1 mg/g DW) and lowest in flower samples of CAN population (CAN-F) (28.6 mg/g DW).

Total antioxidant activity, total flavonoids, antiradical activity, total carotenoidsi total phenolic compoundsi total tochoperol and vitamin C content of leaves (L) and flowers (F) of three sea fennel (Crithmum maritimum L.) populations (ATL: Atlantis, CAN: Çandarlı, and URL: Urla).

Ecotypes	[1] Total antioxidant activity µmol FRAP/g DW	[2] Total flavonoids mg/g DW	[3] Antiradical activity DPPH as IC_{50}, µg/ml	[4] Total carotenoids mg/g DW	[5] Total phenolic compounds mg/g DW	[6] Total tochoperol mg/kg DW	[7] Vitamin C mg/g DW
ATL-L	172.5 ±28.2	2.89 ±0.88	136.0 ±23.3	86.2 ±22.5	6.12 ±2.13	369 ±19.4	1.72 ±0.19
ATL-F	146.5 ±32.1	11.2 ±1.66	243.8 ±40.6	47.7 ±13.6	16.2 ±3.18	23.9 ±8.8	1.80 ±0.09
CAN-L	112.3 ±24.1	2.71 ±1.04	87.7 ±18,2	48.3 ±17.4	2.10 ±0,98	218 ±15.7	1.19 ±0,24
CAN-F	154.0 ±17.0	6.03 ±1.12	220.1 ±27.0	28.6 ±12.7	10.8 ±2.44	44.0 ±3.45	0.92 ±0.16
URL-L	163.8 ±18.8	3.44 ±1.29	66.3 ±12.4	113.1 ±33.6	2.84 ±0,34	143 ±16.6	2.04 ±0.37
URL-F	177.2 ±27.3	3.82 ±0.78	265.1 ±31,5	50.4 ±12.7	8.79 ±2.05	14.9 ±3.02	1.86 ±0.11

[1]AO-Benzie and Strain (2009), [2]FLV-Zhishen et al. (1999), [3]AR-Brand-Williams et al. (1995), [4]TC-Arnon and Copper (1949), [5]TPC-Singleton et al.(1999), [6]TTC-Biswas et al (2011), [7]VC-Asghari et al. (2015)

Total phenolic compounds ranged from 2.10 to 16.2 mg/g DW. ATL-F had the highest phenolic content (16.2 mg/g DW), whereas CAN-L had the lowest (2.10 mg/g DW). Tocopherol content varied significantly, with ATL-L showing the highest level (369 mg/kg DW) and URL-F the lowest (14.9 mg/kg DW). Vitamin C content ranged from 0.92 to 2.04 mg/g DW. The highest vitamin C content was detected in URL-L (2.04 mg/g DW), while the lowest was in CAN-F (0.92 mg/g DW).

Overall, biochemical profiles varied notably across ecotypes and plant parts, indicating both genetic and treatment-related differences in the accumulation of bioactive compounds.