Biopesticide potential of the essential oil from a pre-domesticated population of *Satureja montana*

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INTRODUCTION

CITA - Agrifood Research and Technology Centre of ARAGÓN (SPAIN):

- Study the potential of aromatic and medicinal plants (AMPs) to develop products of industrial interest where there is as a whole lack of raw material.

- selection of chemotypes adapted to climatic conditions along with suitable cultivation techniques.

- provide an alternative to dominant local crops, i.e. cereals.

INTRODUCTION

- *Satureja montana* L.- ¨mountain savory¨

- Native flora in Spain. Arid, stony and lime-filled soils, specially in high altitudes and mountain regions (Burillo, 2003; Cunha et al., 2007). Prefers dry climatic conditions.

- *S. montana* has developed several morphological and physiological adaptations, affecting oil yields and composition (Mirjana and Nada, 2004), which are related as well to geographical location and stages of plant development (Cavar, et al., 2008; Slavkovska et al., 2001).

INTRODUCTION

Directive 2009/128/EC

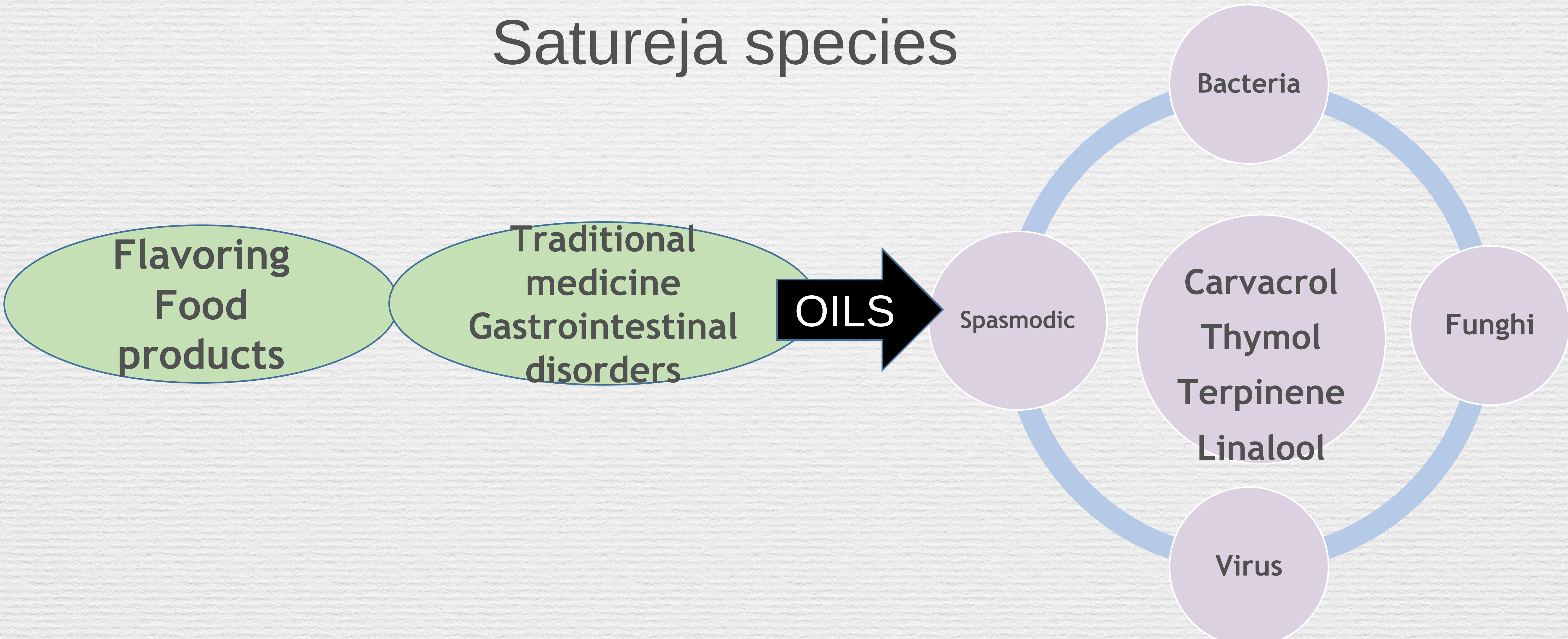
-sustainable use of pesticides in the EU

-alternative approaches or techniques, such as non-chemical alternatives to pesticides.

The cultivation of *S. montana* can be of environmental importance in semi-arid lands of the Mediterranean

INTRODUCTION

Satureja species



OBJECTIVE

- Evaluate *S. montana* adaptation to the trial area throw a domestication process.
- Gain greater insight into production potential.
- Study yield, quality and bioactivity of its essential oils.

MATERIAL AND METHODS

- <u>Genetic material</u>

The cultivation process began in different experimental plots located in Aragon (Spain). Seeds from selected plants from these preliminary trials (MSAMO-0) have been used for the further trials.

***Seeds MSAMO-0

MATERIAL AND METHODS

<u>Experimental field</u>:

- Ejea de los Caballeros (Aragón, Spain) (42°8’8.73” N, 1°12’31.50” W)
- From 2011 to 2014
- Altitude of 346 m a.s.l.
- Soil: clay-loam texture

MATERIAL AND METHODS

MSAMO-7 population

40 plants with drip irrigation in summer (4 l/hour for 5-6 hours per week)

MATERIAL AND METHODS: production

- *Biomass and essential oils* *(harvesting at 75% of blooming)*

- fresh and dry biomass expressed in kg and %, respectively,

- essential oil yield from hydro-distilled biomass and steam distillation (%).

Clevenger apparatus

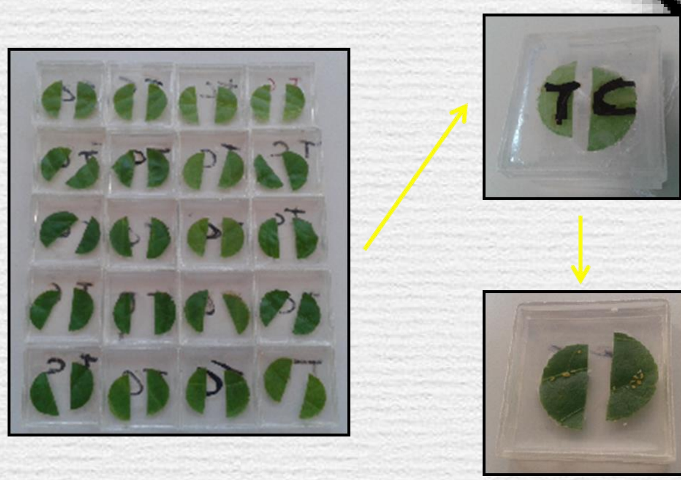
Pilot plant-Steam distillation

MATERIAL AND METHODS: production

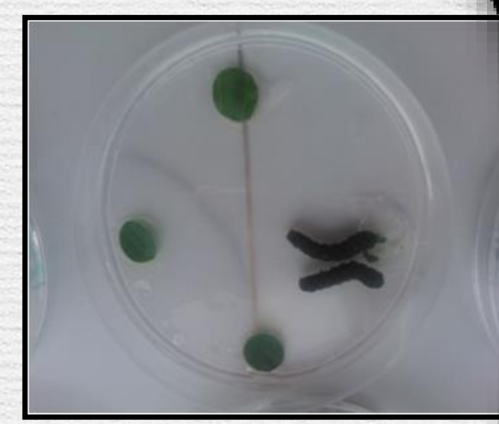
- *Essential oil analysis*

- Gas chromatography mass spectrometry (GC-MS). Individual components were calculated based on the GC peak area (FID response).

MATERIAL AND METHODS: Antifeedant Bioassays



M. persicae: %SI



S. litorallis : %FR

The insect deterrent activity of extracts was evaluated on the settling inhibition (SI) of aphids *Myzus persicae* and on the feeding reduction (FR) of lepidoptera larva *Spodoptera littoralis* (A.L. Ruiz-Jiménez et al., 2017)

MATERIAL AND METHODS: Nematicidal Bioassays

Nematicidal activity of EO – in vitro mortality (J2- Scheider-Orell).

RESULTS: Production

Table 1 – Dry biomass (DB%) and essential oil (EO%) yields, for different extraction methods, pressures and years for *S. montana* (MSAMO-7)

Extraction	Clevenger				Pilot Plant							
Year	2011	2012	2013	2014	2011			2012			2013	2014
Pressure	-	-	-	-	0.5 bar	1.0 bar	1.5 bar	0.5 bar	1.0 bar	1.5 bar	0.8 bar	0.8 bar
DB%	44,4	45,39	44,72	43	44,4			45,39			44,72	43
EO%	0,48	0,39	0,54	0,41	0,01	0,05	0	0,07	0,07	0,01	0,05	0,06

Reference population Year 94: 0,29 (Clevenger)

RESULTS: CG-MS

Table 2. Chemical composition of MSAMO crops

Compound	MSAMO-0*	MSAMO-7					
	Clevenger			Pilot plant			
				0.5 bar	1.0 bar	1.5 bar	1.0 bar
	1994	2012	2013	2012			2013
α-Thujene	0.28	0.76		1.36	1.02	0.49	1.62
(-)-α-Pinene	0.84	0.93		1.25	1.02	0.63	0.92
1-Octen-3-ol	1.13	1.74		1.17	1.04	0.87	1.74
β-Myrcene			0.73				1.96
α-Terpinene	Tr	0.47	1.85	1.34	1.67	1.19	2.23
p-Cymene	10.24	28.08	18.27	33.12	29.38	23.77	22.25
l-Limonene+1,8-Cineol	1.39	1.64	1.39	1.90	1.71	1.40	2.05
ϒ-Terpinene	0.33		9.24				10.83
trans-Sabinene hydrate	0.99	1.22	0.78	1.07	0.68	0.52	0.81
Linalool		0.93	0.98	0.83	0.76	0.72	1.14
Borneol	1.97	1.25	1.58	0.97	0.87	0.77	0.87
Terpinen-4-ol	0.55	0.75	0.88	0.55	0.72	0.70	0.98
Thymoquinone		2.81		1.52	0.91	1.16	
Carvone	1.12						
Thymol		8.30	4.80	4.75	5.16	5.27	5.42
Carvacrol	76.63	49.38	58.32	42.22	48.35	53.86	40.80
trans-Caryophyllene	0.60		0.58	2.09	1.90	1.78	2.15

Reference population MSAMO-O

Higher

Lower

RESULTS: Bioactivity

Table 4. Antifeedant effect of MSAMO extracts in different target insects

MSAMO-7	Antifeedant effect		
	µg/cm[2]	*S. littoralis*	*M. persicae*
	EC_{50}	%FI[3]	%SI
EO94[1]	100	90,4 ± 3	nt[5]
EO12	100	93.5 ± 1.8	nt
	Ec_{50}	28.9 (22.5, 34.1)	nt
EO13	100	77.7 ± 9.8	92.5 ± 2.7
	Ec_{50}	ns[6]	15.2 (8.1, 20.9)
PEO12	100	93.9 ± 1.3	81 ± 6.4
	Ec_{50}	35.7 (10.3, 56.9)	36.6 (26.6, 44.3)
PEO13	100	51.5 ± 13.7	72.7 ± 6.7
	Ec_{50}	ns	46.9 (39.9, 53.3)
Carvacrol [1]	50	55.8 ± 11.8	86.4 ± 3.2
	Ec_{50}	ns	15.5 (11.3, 18.8)

[1] Results are compared with the initial population of plants – Year 1994 and with Carvacrol.
[2] EO : essential oil obtained with Clevenger method; PEO: essential oil obtained with pilot plant.
[3] Percent feeding (FI) / setting inhibition (SI).
[4] Concentration needed to produce 50% feeding / setting inhibition (EC_{50})
[5] Not tested
[6] Not significant

RESULTS: Bioactivity

Table 5. Nematicidal effect of MSAMO extracts against *M. javanica*

MSAMO-7	Concentration	*M. javanica* J2 mortality (%)* 72h
EO94[1]	1 µg/µl	100
EO12	1 µg/µl	nt
EO13	1 µg/µl	100
PEO12	1 µg/µl	100
PEO13	1 µg/µl	99
Carvacrol[1]	0.5 µg/µl	100

[1] Results are compared with the initial population of plants – Year 1994 and with Carvacrol.
[2] EO : essential oil obtained with Clevenger method; PEO: essential oil obtained with pilot plant.
[3] Percent feeding (FI) / setting inhibition (SI).
[4] Concentration needed to produce 50% feeding / setting inhibition (EC_{50})
[5] Not tested
[6] Not significant

Conclusion

* The domesticated population maintained a stable yield of dry material (44,5%) and essential oil (0,45%).

* The oils tested showed little variation in their chemical composition and strong antifeedant and nematicidal effects,

* The selection and pre-domestication process has to continue to have consistent and reliable results on field.

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