PRE-DOMESTICATION OF A SPANISH POPULATION OF SATUREJA MONTANA L. AND ESSENTIAL OIL VALORIZATION

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INTRODUCTION

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

- Forest Resources

- 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

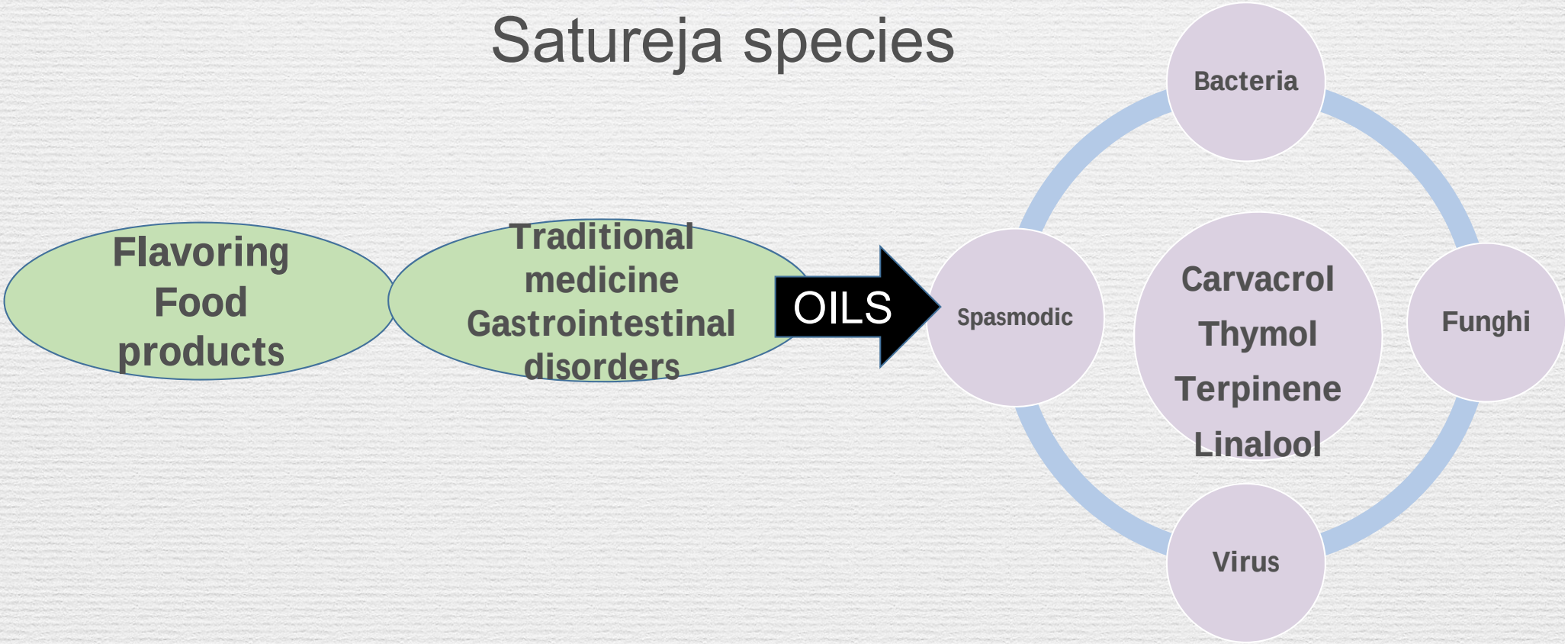
- *Satureja montana* L.- ¨mountain savory¨

- Native flora in Spain. Appears at 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

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

- Genetic material

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

Experimental field:

- 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)

Design: 0,5 m between plants

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

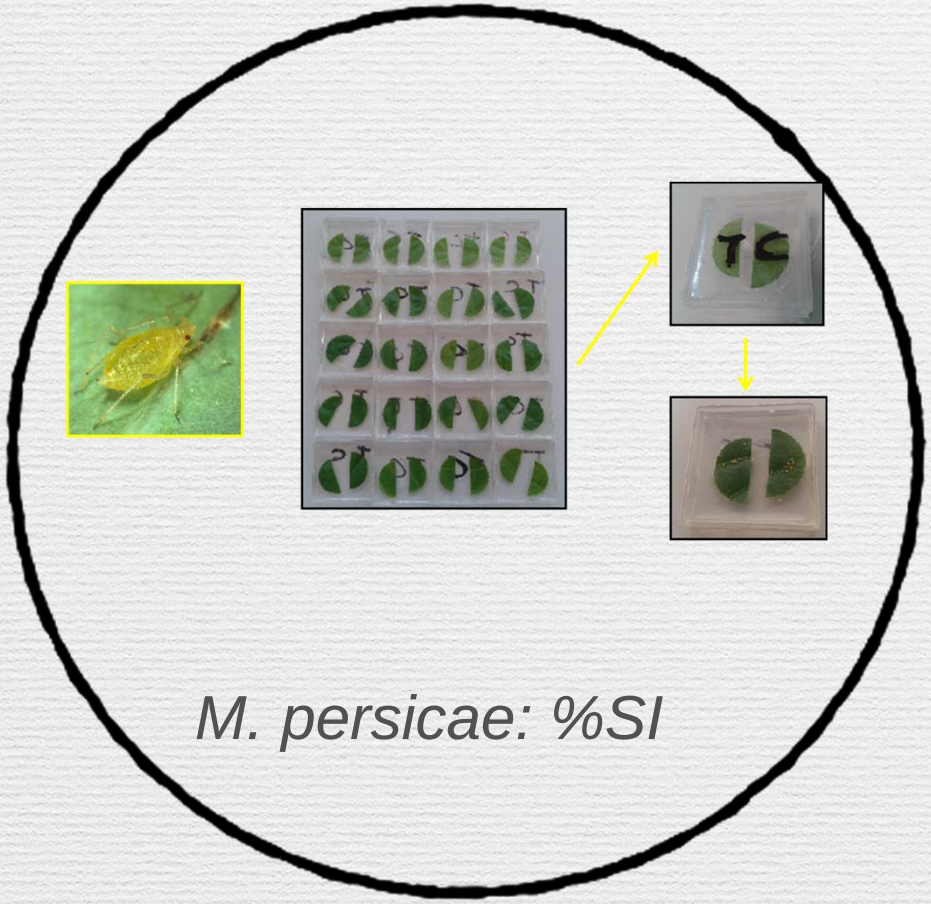
- *Germination rate*

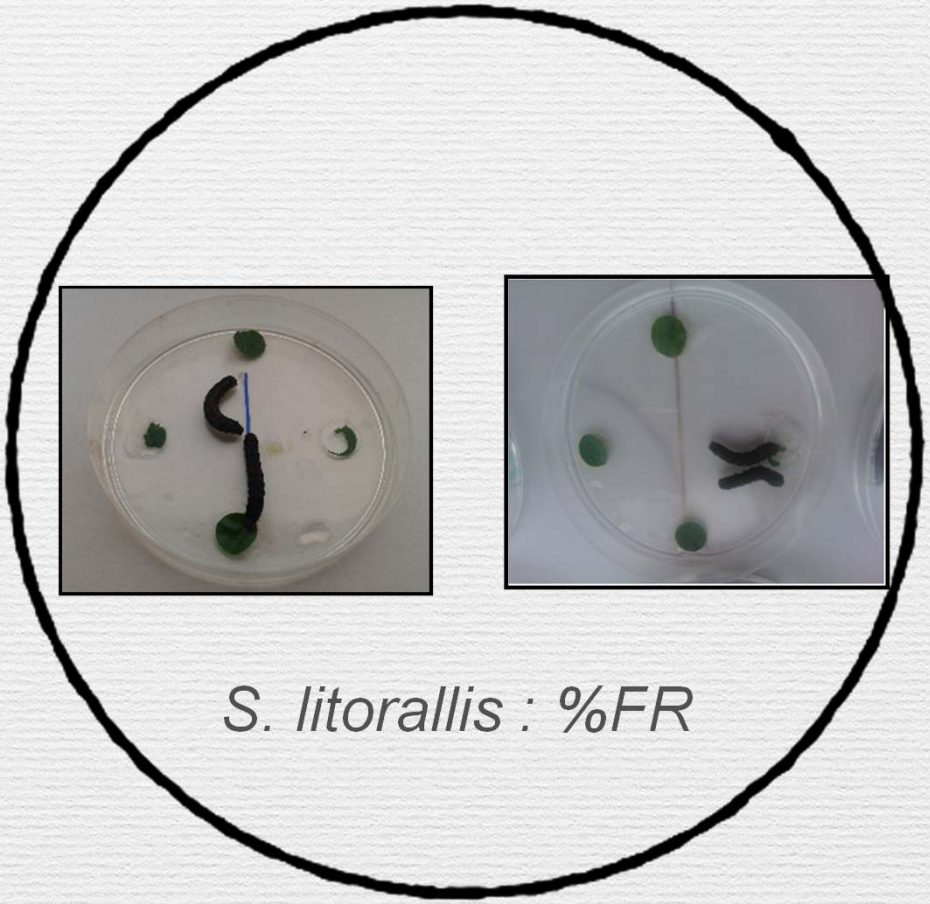
- Seeds for the last 2 years of the study (2013 and 2014).

- controlled temperature/light (21◦C, 16 h light /8 h dark - 2.960 LUX).

Germination cabinet

MATERIAL AND METHODS: Bioassays





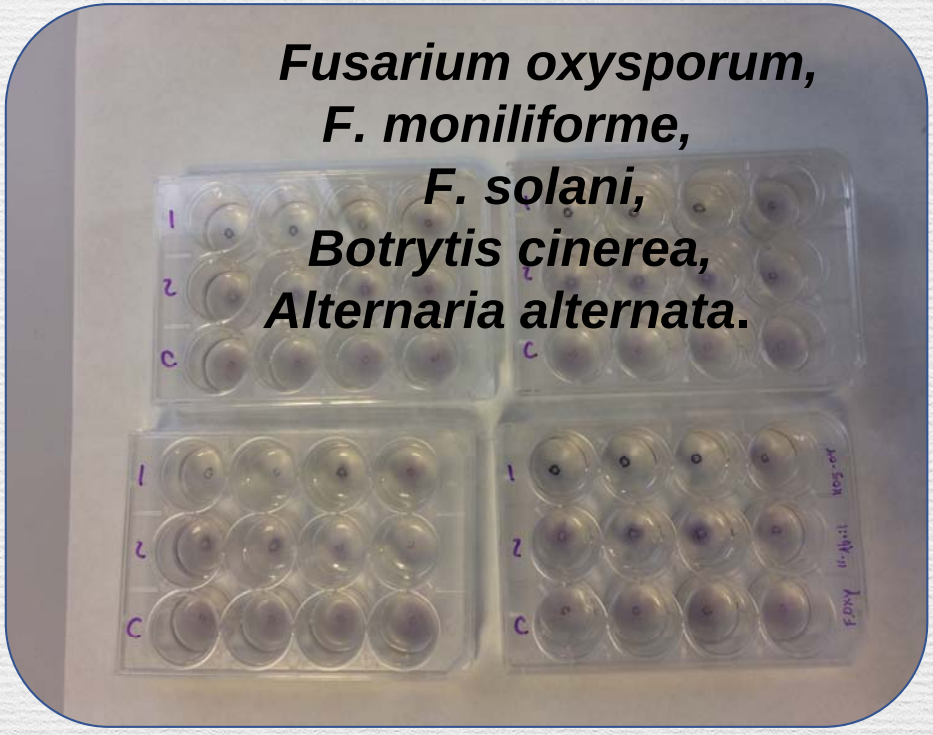
The insect deterrent activity of extracts was evaluated on the settling inhibition (SI) of aphids *Myzus persicae* and on the feeding reduction (FR) of lepidoptera larva *Spodoptera littoralis (*A.L. Ruiz-Jiménez et al., 2017)

MATERIAL AND METHODS:
Antifungal activity

Extracts of *S. Montana*

Antifungal activity

Phytopathogenic fungi



extracts was measured by percentage of growth inhibition in the spot-

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

MSAMO-0=0,29 (Burillo, 2003)

- Germination: 2013: 76,25 ± 2,63
  2014: 71,25 ± 1,89

RESULTS: CG-MS

Table 2. Chemical composition of MSAMO crops

Compound	MSAMO-0*	MSAMO-7					
	Clevenger			Pilot plant			
	1994	2012	2013	0.5 bar	1.0 bar	1.5 bar	1.0 bar
				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 3. Antifungal activity (and EC**50** values) of MSAMO extracts against *Fusarium oxysporum, F. moniliforme, F. solani, Botrytis cinerea and Alternaria alternata*.

MSAMO-7	Mycelial growth inhibitition (%)					
	mg/ml / EC_{50}	*Fusarium*			*Botrytis*	*Alternaria*
		oxysporum	*moniliforme*	*solani*	*cinerea*	*alternata*
EO94	0.5	93.5±0.5	93.6±0.4	92.4±0.9	93.9±0.7	nt
	EC_{50}	0.25 (-0.43,0.94)	0.29 (-0.24,0.84)	0.33 (-0.07,0.73)	0.14 (-0.93,2.21)	
EtOH	0.5	2.0±4.7	2.56±4.2	31.6±3.4	8.2±2.5	nt
EO12	0.5	95.5±0.6	90.9±1,1	92.9±0.6	64.5±0.6	66.1±1.2
		0.12(-0.5,0.7)	0.18(-0.3,0.7)	0.17(-0.3,0.6)		
PEO12-0.5	0.5	89.35±1.1	89.7±1.9	93.2±0.5	70.1±0.6	68.6±0.5
	EC_{50}	0.079(0.06-0.09)	0.14(0.12-0.17)	0.08(0.06-0.09)	0.07(0.05-0.1)	
PEO12-1.0	0.5	88.6±1.1	88.9±1.1	94.3±0.9	70.2±0.9	67.5±0.8
	EC_{50}	0.070(0.05-0.08)	0.14(0.12-0.17)	0.07(0.05-0.08)	0.13(0.09-0.2)	
PEO12-1.5	0.5	92.3±0.8	91.7±0.5	90.1±0.7	58.6±1.5	56.1±1.4
	EC_{50}	0.07(0.06-0.09)	0.106(0.08-0.12)	0.11(0.08-0.14)		
EO13	0.5	95,6±0,3	92,3±1,3	95,1±2,1	76,2±0,4	63,4±1,0
	EC_{50}	0.06(-0.77,0.89)	0.2(-0.39,0.79)	0.25(-0.24,0.74)	0.27(-0.5,1.06)	
PEO13	0.5	92.2±0.7	76.9±1.7	86±0.9	77.4±0.3	54,9±1,2
	Ec50	0.19 (-0.43,0.94)	0.47(0.17,0.76)	0.37(0.1,0.6)	0.51(0.06,0.95)	
Carvacrol	0.5	90.30±1.3	93.7±0.7	90.8±0.7	62.3±0.7	64.4±0.6
	EC_{50}	0.38(-5.56,1.32)	nc	nc		

RESULTS: Bioactivity

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

MSAMO-7	µg/cm²	*S. littoralis*	*M. persicae*
		%FI	%SI
EO94	100	94,31 ± 1	90,4 ± 3
EtOH	100	66.7 ± 11.9	44.9 ± 8.5
EO12	100	84.9 ± 10.3	93.5 ± 1.8
	Ec_{50}	39.46 (13.56, 63.32)	28.93 (22.55, 34.08)
EO13	100	77.7 ± 9.8	92.5 ± 2.7
	Ec_{50}	ns	15.24 (8.07, 20.93)
PEO12-0.5	100	80.5 ± 11.2	93.6 ± 2.2
	Ec_{50}	ns	46.83 (42.98, 50.65)
PEO12-1.0	100	93.9 ± 1.3	81 ± 6.4
	Ec_{50}	35.74 (10.34, 56.93)	36.59 (26.62, 44.27)
PEO12-1.5	100	88.7 ± 9.1	90.5 ± 2.8
	Ec_{50}	42.2 (21.45, 64.39)	27.78 (19.74, 33.97)
PEO13	100	51.5 ± 13.7	72.7 ± 6.7
	Ec_{50}	ns	46.98 (39.9, 53.33)
Carvacrol	50	55.8 ± 11.8	86.4 ± 3.2
	Ec_{50}	ns	15.53 (11.32, 18.84)

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 biocidal (antifungal and antifeedant) effects,

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

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