

# Supplementary materials: *Thymbra capitata* (L.) Cav. and *Rosmarinus officinalis* (L.) Essential Oils: *in vitro* effects and toxicity on swine spermatozoa.

**Table S1.** Descriptive statistics of the effects of *Thymbra capitata* EO on semen morpho-functional parameters. Data are reported as Mean (standard error of the mean), n=6.

	<i>Thymbra capitata</i> (mg/mL)										
	0	0.2	0.4	0.6	0.8	1	1.2	1.4	1.6	1.8	2
<b>V %</b>	91.9 (1.4)	85.1 (3.4)	18.8 (2.8)	0	0	0	0	0	0	0	0
<b>TotM %</b>	80.2 (2.7)	57.1 (4.9)	0.7 (0.2)	0.8 (0.2)	2.1 (0.6)	0.8 (0.2)	1.9 (0.5)	1.0 (0.2)	1.4 (0.4)	0.5 (0.2)	1.2 (0.2)
<b>ProgM %</b>	46.8 (5.7)	24.0 (5.1)	0.1 (0.1)	0.1 (0.1)	0.1 (0.1)	0	0.1 (0.1)	0.1 (0.1)	0.3 (0.1)	0.1 (0.1)	0.1 (0.1)
<b>AR %</b>	2.1 (0.4)	7.6 (3.0)	69.5 (14.5)	97.8 (1.3)	95.7 (2.5)	95.9 (2.7)	96.0 (2.3)	98.5 (0.7)	100 (0.0)	100 (0.0)	98.5 (1.5)
<b>pH</b>	6.7 (0.0)	6.7 (0.0)	6.7 (0.0)	6.7 (0.0)	6.7 (0.0)	6.7 (0.0)	6.7 (0.0)	6.7 (0.0)	6.7 (0.0)	6.7 (0.0)	6.7 (0.1)

V= Viability; TotM= Total Motility; ProgM= Progressive Motility; AR= Acrosome Reaction.

**Table S2.** Descriptive statistics of the effects of *Rosmarinus officinalis* EO on semen morpho-functional parameters. Data are reported as Mean (standard error of the mean), n=6.

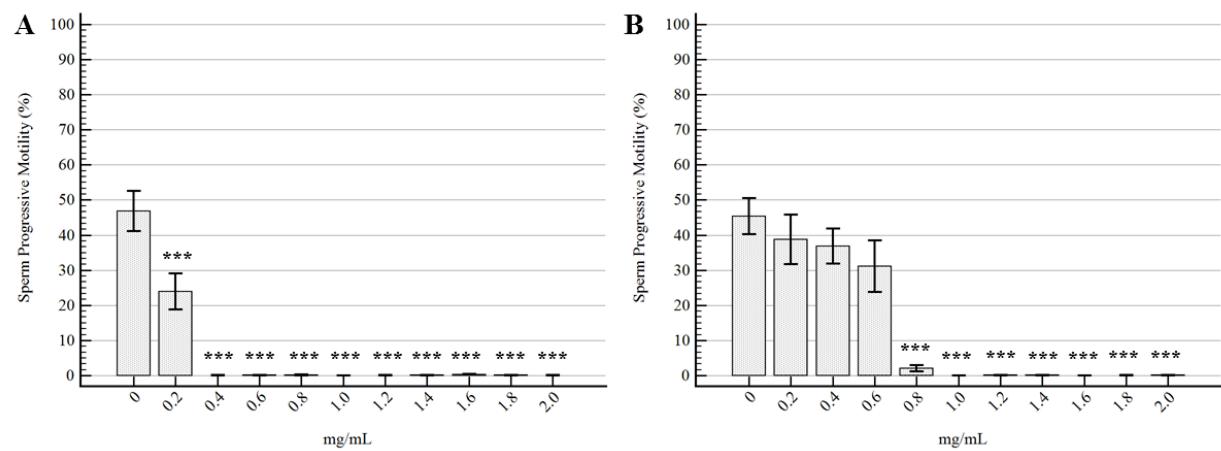
	<i>Rosmarinus officinalis</i> (mg/mL)										
	0	0.2	0.4	0.6	0.8	1	1.2	1.4	1.6	1.8	2
<b>V %</b>	94.3 (1.5)	91.7 (1.6)	89.2 (2.4)	89.4 (2.2)	84.4 (3.4)	74.0 (4.0)	74.5 (5.9)	42.6 (9.5)	36.0 (11.0)	40.4 (11.4)	25 (11.0)
<b>TotM %</b>	83.5 (2.2)	85.1 (2.1)	79.5 (3.6)	67.8 (12.1)	7.2 (4.7)	3.1 (2.5)	1.9 (0.2)	1.2 (0.3)	0.9 (0.4)	0.6 (0.2)	0.9 (0.3)
<b>ProgM %</b>	45.4 (5.2)	38.8 (7.0)	36.8 (5.0)	31.1 (7.3)	2.1 (0.9)	0	0.1 (0.1)	0.1 (0.1)	0	0	0.1 (0.0)
<b>AR %</b>	2.6 (0.7)	2.8 (1.5)	3 (1.4)	3 (1.1)	4.5 (1.8)	5 (2.3)	4.5 (2.0)	5.3 (1.1)	5.6 (1.2)	9.5 (1.8)	11.6 (2.1)
<b>pH</b>	6.7 (0.0)	6.7 (0.0)	6.7 (0.0)	6.7 (0.0)	6.7 (0.0)	6.7 (0.0)	6.7 (0.0)	6.7 (0.0)	6.7 (0.0)	6.7 (0.0)	6.7 (0.0)

V= Viability; TotM= Total Motility; ProgM= Progressive Motility; AR= Acrosome Reaction.

**Table S3.** Effects of *Thymbra capitata* and *Rosmarinus officinalis* EOs on spermatic kinematic parameters. In the table are only reported the samples with a total motility  $\geq 20\%$ . Data are reported as Mean (standard error of the mean),  $n=6$ . Differences were calculated by means of Dunnett PostHoc test (\* =  $p<0.05$ ; \*\* =  $p<0.01$ ; \*\*\* =  $p<0.001$ ).

	<i>Tc</i> (mg/ml)			<i>Ro</i> (mg/mL)		
	0	0.2	0	0.2	0.4	0.6
<b>TotM (%)</b>	80.2 (2.7)	57.1** (4.9)	83.5 (2.2)	85.1 (2.1)	79.5 (3.6)	67.8 (12.1)
<b>VAP (μm/s)</b>	90.0 (5.4)	48.8** (7.2)	86.4 (10.0)	77.1 (11.8)	84.0 (9.3)	73.9 (12.2)
<b>VCL (μm/s)</b>	197.4 (11.3)	106.6** (16.5)	189.0 (22.7)	170.7 (26.5)	189.4 (21.3)	167.7 (27.1)
<b>VSL (μm/s)</b>	49.7 (4.5)	26.7** (3.9)	47.4 (4.7)	43.2 (6.07)	42.5 (4.7)	37.9 (5.4)
<b>DAP (μm)</b>	52.3 (2.4)	29.3** (4.9)	49.9 (5.6)	42.0 (6.4)	48.6 (5.4)	43.7 (6.4)
<b>DCL (μm)</b>	117.1 (5.6)	65.7** (11.4)	111.5 (13.0)	95.6 (15.2)	112.6 (12.6)	101.6 (14.4)
<b>DSL (μm)</b>	27.1 (2.0)	15.2** (2.7)	25.7 (2.1)	21.8 (2.9)	22.5 (2.5)	21.0 (2.4)
<b>LIN (%)</b>	25.7 (1.7)	25.1 (0.6)	26.2 (1.4)	25.8 (0.9)	23.0 (0.7)	24.3 (1.0)
<b>STR (%)</b>	54.9 (2.5)	52.8 (1.6)	55.4 (2.2)	54.9 (1.4)	50.7 (1.2)	52.8 (1.8)
<b>WOB (%)</b>	45.6 (1.0)	45.8 (0.6)	46.1 (0.6)	45.2 (0.6)	44.2 (0.3)	44.6 (0.7)
<b>ALH (μm)</b>	9.6 (0.5)	7.3 (0.9)	9.6 (0.8)	9.1 (1.1)	9.4 (0.7)	9.1 (1.0)
<b>BCF (Hz)</b>	36.9 (0.5)	36.9 (3.4)	36.0 (1.0)	36.7 (1.4)	35.6 (1.4)	36.4 (1.8)

TotM= Total Motility; VAP= velocity average path; VCL= velocity curved line; VSL= velocity straight line; DAP= distance average path; DCL= distance curved line; DSL= distance straight line; LIN= linearity (VSL/VCL); STR= straightness (VSL/VAP); WOB=wobble (VAP/VCL); ALH= amplitude of lateral head displacement; BCF= beat cross frequency.



**Figure S1. Effects of the EOs on Progressive Motility.** (A) *Thymbra capitata*. (B) *Rosmarinus officinalis*. Data are expressed as mean  $\pm$  standard error of the mean ( $n=6$ ). 0 mg/ml represents the control sample (only emulsifiers). \* =  $p<0.05$ ; \*\* =  $p<0.01$ ; \*\*\* =  $p<0.001$ .