

SUPPLEMENTARY DATA

Bio-pesticidal Potential of Nanostructured Lipid Carriers Loaded with Thyme and Rosemary Essential Oils Against Common Ornamental Flower Pests

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Table S1. The 2³ factorial design combinations

Formulation	A: EO (%)	B: T80(%)	C: LL/SL
NLC1	-1	-1	-1
NLC2	1	-1	-1
NLC3	-1	1	-1
NLC4	1	1	-1
NLC5	-1	-1	1
NLC6	1	-1	1
NLC7	-1	1	1
NLC8	1	1	1
NLC0	0	0	0
NLC0	0	0	0
NLC0	0	0	0
NLC0	0	0	0

Table S2. MIG/EO ratio in the NLC formulations of the factorial design

Formulation			MIG/EO ratio	Formulation	
NLC1	NLC3	66.67/33.33	80/20	NLC5	NLC7
			50/50		
			NLC0		
NLC2	NLC4	0/100	40/60	NLC6	NLC8

Table S3. Experimental design of contact toxicity test

Experiment	Formulation	Treatment (Concentration mL/L)
1	Positive control (Insecticide)	1.0 mL/L
2	Negative control (distilled water)	---
3	Acetone (solvent)	0.5 mL/L
4		1.0 mL/L
5		1.5 mL/L
6	REO	0.5 mL/L
7		1.0 mL/L
8		1.5 mL/L
9	TEO	0.5 mL/L
10		1.0 mL/L
11		1.5 mL/L
12	REO-NLC	0.5 mL/L
13		1.0 mL /L
14		1.5 mL/L
15	TEO-NLC	0.5 mL/L
16		1.0 mL/L
17		1.5 mL/L
18	FEO-NLC	0.5 mL/L
19		1.0 mL/L
20		1.5 mL/L

*Rosemary essential oil (REO); Thyme essential oil (TEO); REO loaded Nanostructured lipid carriers (REO-NLC); TEO loaded Nanostructured lipid carriers (TEO-NLC); Free EO Nanostructured lipid carriers (FEO-NLC)



Figure S1: Chromatogram of Thyme essential oil

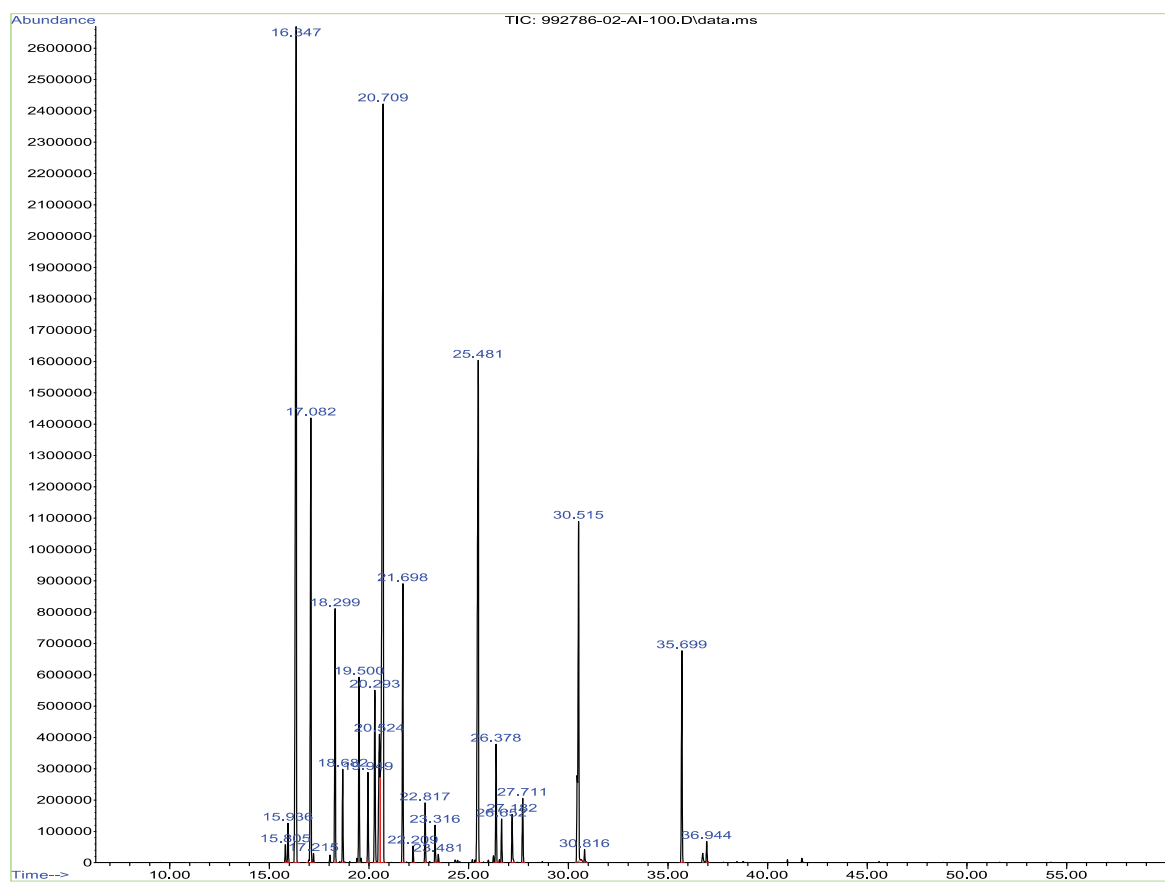


Figure S2: Chromatogram of Rosemary essential oil

Table S4. Coefficients of the model and ANOVA table for particle size

PARTICLE SIZE								
Coefficients:								
	Estimate	Std. Error	t value	Pr(> t)				
(Intercept)	817.789	18.295	44.701	< 2e-16 ***				
A	347.158	22.406	15.494	2.9e-15 ***				
B	-47.642	22.406	-2.126	0.042434 *				
C	-89.675	22.406	-4.002	0.000418 ***				
A:B	43.917	22.406	1.960	0.060016 .				
A:C	-85.433	22.406	-3.813	0.000692 ***				
B:C	45.633	22.406	2.037	0.051244 .				
A:B:C	5.142	22.406	0.229	0.820169				
Signif. codes:	'***'	0.001	'**'	0.01	'*'	0.05	'.' 0.1	' ' 1
Residual standard error: 109.8 on 28 degrees of freedom								
Multiple R-squared: 0.91, Adjusted R-squared: 0.8875								
F-statistic: 40.45. on 7 and 28 DF, p-value: 5.415e-13								
Analysis of Variance Table								
Response: Particle size								
	Df	Sum Sq	Mean Sq	F value	Pr(>F)			
A	1	2892454	2892454	240.0549	2.896e-15 ***			
B	1	54473	54473	4.5209	0.0424340 *			
C	1	192999	192999	16.0176	0.0004177 ***			
A:B	1	46288	46288	3.8416	0.0600162 .			
A:C	1	175173	175173	14.5382	0.0006924 ***			
B:C	1	49978	49978	4.1478	0.0512442 .			
A:B:C	1	634	634	0.0527	0.8201693			
Residuals	28	337376	12049					
Signif. codes:	'***'	0.001	'**'	0.01	'*'	0.05	'.' 0.1	' ' 1

Table S5. Coefficients of the model and ANOVA table for PDI

PDI									
Coefficients:									
		Estimate		Std. Error		t value		Pr(> t)	
(Intercept)		0.26236		0.02022		12.973		2.32e-13 ***	
A		0.06158		0.02477		2.486		0.01914 *	
B		0.02092		0.02477		0.844		0.40556	
C		-0.04250		0.02477		-1.716		0.09723 .	
A:B		-0.08867		0.02477		-3.580		0.00128 **	
A:C		0.07575		0.02477		3.058		0.00486 **	
B:C		-0.14675		0.02477		-5.925		2.24e-06 ***	
A:B:C		-0.07200		0.02477		-2.907		0.00706 **	
Signif. codes: '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1									
Residual standard error: 0.1213 on 28 degrees of freedom									
Multiple R-squared: 0.7296, Adjusted R-squared: 0.662									
F-statistic : 10.79 on 7 and. 28 DF, p-value: 1.595e-06									
Analysis of Variance Table									
Response: PDI									
		Df		Sum Sq		Mean Sq		F value	Pr(>F)
A		1		0.09102		0.09102		6.1819	0.019141 *
B		1		0.01050		0.01050		0.7132	0.405557
C		1		0.04335		0.04335		2.9443	0.097230 .
A:B		1		0.18868		0.18868		12.8150	0.001280 **
A:C		1		0.13771		0.13771		9.3533	0.004860 **
B:C		1		0.51685		0.51685		35.1038	2.241e-06 ***
A:B:C		1		0.12442		0.12442		8.4501	0.007063 **
Residuals		28		0.41226		0.01472			
Signif. codes: '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1									

Table S6. Coefficients of the model and ANOVA table for zeta potential

ZETA POTENTIAL					
Coefficients:					
	Estimate	Std. Error	t value	Pr(> t)	
(Intercept)	-34.4972	0.9124	-37.810	< 2e-16 ***	
A	5.6167	1.1174	5.026	2.58e-05 ***	
B	-3.8250	1.1174	-3.423	0.001925 **	
C	5.0667	1.1174	4.534	9.90e-05 ***	
A:B	4.6917	1.1174	4.199	0.000246 ***	
A:C	0.2667	1.1174	0.239	0.813122	
B:C	2.1083	1.1174	1.887	0.069603 .	
A:B:C	-2.1083	1.1174	-1.887	0.069603 .	
Signif. codes:	'***' 0.001	'**' 0.01	'*' 0.05	'.' 0.1	' ' 1
Residual standard error: 5.474 on 28 degrees of freedom					
Multiple R-squared: 0.7462, Adjusted R-squared: 0.6828					
F-statistic: 11.76 on 7 and 28 DF, p-value: 6.907e-07					
Analysis of Variance Table					
Response:	Zeta Potential				
	Df	Sum Sq	Mean Sq	F value	Pr(>F)
A	1	757.13	757.13	25.2643	2.58e-05 ***
B	1	351.13	351.13	11.7169	0.0019248 **
C	1	616.11	616.11	20.5587	9.90e-05 ***
A:B	1	528.28	528.28	17.6281	0.0002462 ***
A:C	1	1.71	1.71	0.0569	0.8131224
B:C	1	106.68	106.68	3.5598	0.0696031 .
A:B:C	1	106.68	106.68	3.5598	0.0696031 .
Residuals	28	839.11	29.97		
-					
Signif. codes:	'***' 0.001	'**' 0.01	'*' 0.05	'.' 0.1	' ' 1

Table S7. Coefficients of the response surface model for particle size at low EO concentration

PARTICLE SIZE				
Coefficients:				
	Estimate	Std. Error	t value	Pr(> t)
(Intercept)	508.4083	3.6523	139.2004	7.933e-15 ***
Surfactant	-91.5583	3.6523	-25.0683	6.862e-09 ***
LL_SL_ratio	-4.2417	3.6523	-1.1614	0.279
Surfactant:LL_SL_ratio	40.4917	3.6523	11.0865	3.910e-06 ***
Signif. codes:	***' 0.001	**' 0.01	'* 0.05	' 0.1
Multiple R-squared: 0.9895, Adjusted R-squared: 0.9855				
F-statistic: 250.9 on 3 and 8 DF, p-value: 2.998e-08				

Table S8. Coefficients of the response surface model for particle size at high EO concentration

PARTICLE SIZE				
Coefficients:				
	Estimate	Std. Error	t value	Pr(> t)
(Intercept)	1202.725	27.118	44.3520	7.372e-11 ***
Surfactant	-3.725	27.118	-0.1374	0.8941373
LL_SL_ratio	-175.108	27.118	-6.4573	0.0001968 ***
Surfactant:LL_SL_ratio	50.775	27.118	1.8724	0.0980459 .
Signif. codes:	***' 0.001	**' 0.01	'* 0.05	' 0.1
Multiple R-squared: 0.8497, Adjusted R-squared: 0.7933				
F-statistic: 15.07 on 3 and 8 DF, p-value: 0.001178				

Table S9. Coefficients of the response surface model for PDI at low EO concentration

PDI				
Coefficients:				
	Estimate	Std. Error	t value	Pr(> t)
(Intercept)	0.26375	0.02287	11.531	2.9e-06 ***
Surfactant	0.10958	0.02287	4.791	0.001371 **
LL_SL_ratio	-0.11825	0.02287	-5.170	0.000853 ***
Surfactant:LL_SL_ratio	-0.07475	0.02287	-3.268	0.011391 *
Signif. codes:	***' 0.001	**' 0.01	'* 0.05	' ' 0.1
Residual standard error: 0.07924 on 8 degrees of freedom				
Multiple R-squared: 0.883, Adjusted R-squared: 0.8391				
F-statistic: 20.12 on 3 and 8 DF, p-value: 0.0004395				

Table S10. Coefficients of the response surface model for PDI at high EO concentration

PDI				
Coefficients:				
	Estimate	Std. Error	t value	Pr(> t)
(Intercept)	0.38692	0.02179	17.755	1.04e-07 ***
Surfactant	-0.06775	0.02179	-3.109	0.0145 *
LL_SL_ratio	0.03325	0.02179	1.526	0.1656
Surfactant:LL_SL_ratio	-0.21875	0.02179	-10.038	8.25e-06 ***
Signif. codes:	***' 0.001	**' 0.01	'* 0.05	' ' 0.1
Residual standard error: 0.07549 on 8 degrees of freedom				
Multiple R-squared: 0.9337, Adjusted R-squared: 0.9089				
F-statistic: 37.58 on 3 and 8 DF, p-value: 4.613e-05				

Table S11. Coefficients of the response surface model for zeta potential at low EO concentration

ZETA POTENTIAL				
Coefficients:				
	Estimate	Std. Error	t value	Pr(> t)
(Intercept)	-37.6000	0.5775	-65.112	3.44e-12 ***
Surfactant	-8.5167	0.5775	-14.748	4.39e-07 ***
LL_SL_ratio	4.8000	0.5775	8.312	3.31e-05 ***
Surfactant:LL_SL_ratio	4.2167	0.5775	7.302	8.37e-05 ***
Signif. codes:	***' 0.001	***' 0.01	*' 0.05.	' ' 0.1
Residual standard error: 2 on 8 degrees of freedom				
Multiple R-squared: 0.977, Adjusted R-squared: 0.9684				
F-statistic: 113.3 on 3 and 8 DF, p-value: 6.816e-07				

Table S12. Coefficients of the response surface model for zeta potential at low EO concentration

ZETA POTENTIAL				
Coefficients:				
	Estimate	Std. Error	t value	Pr(> t)
(Intercept)	-26.3667	0.1173	-224.856	< 2e-16 ***
Surfactant	0.8667	0.1173	7.391	7.69e-05 ***
LL_SL_ratio	5.333	0.1173	45.483	6.03e-11 ***
Surfactant:LL_SL_ratio	-1.458e-15	0.1173	0.000	1
Signif. codes:	***' 0.001	***' 0.01	*' 0.05	' ' 0.1
Residual standard error: 0.4062 on 8 degrees of freedom				
Multiple R-squared: 0.9962, Adjusted R-squared: 0.9948				
F-statistic: 707.8 on 3 and 8 DF, p-value: 4.878e-10				

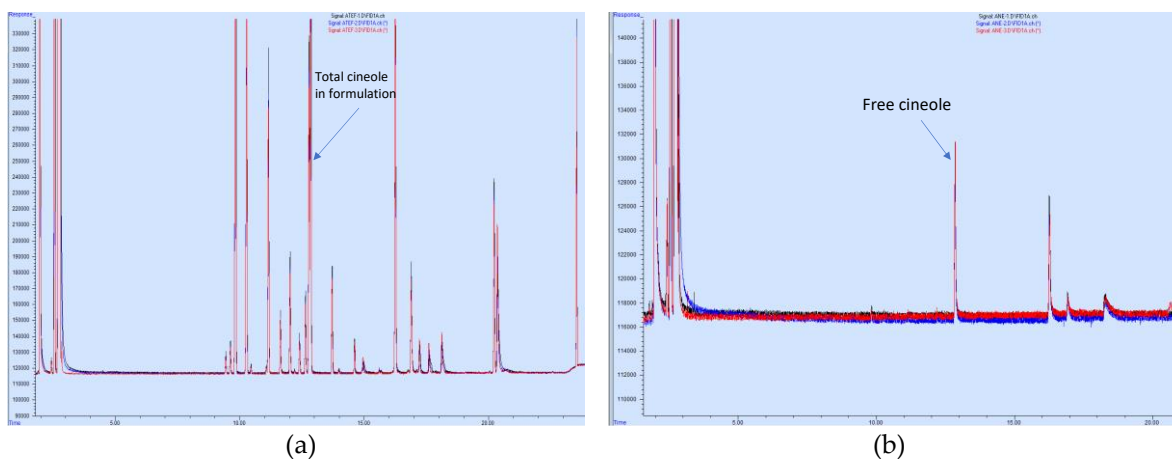


Figure S3. Chromatograms of (a) total rosemary essential oil extracted from the formulation. The chromatographic peak of total cineole (marker compound) in the formulation is highlighted; (b) unencapsulated rosemary essential oil. The chromatographic peak of unencapsulated cineole is highlighted

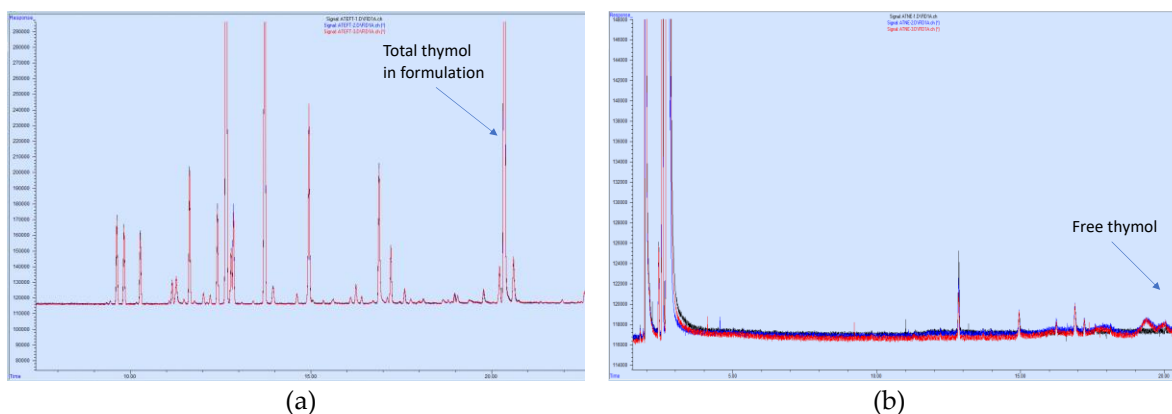


Figure S4. Chromatograms of (a) total compounds of thyme essential oil extracted from the formulation. The chromatographic peak of total thymol in the formulation is highlighted (b) unencapsulated compounds of thyme essential oil. The chromatographic peak of unencapsulated thymol is highlighted