Supporting Information

Nanoformulations from olive pomace to enhance the efficacy of hydroxytyrosol as natural pest control agent.

Sara Falsini^a, Marzia Cristiana Rosi^{b*}, Silvia Urciuoli^c, Annalisa Andreani^b, Alessio Papini^a, Cristina Gonnelli^a, Sandra Ristori^{d*}

^a Dipartimento di Biologia, Università degli studi di Firenze, via P.A. Micheli 1-3, 50121, Firenze, Italy;

^b Dipartimento di Scienze e Tecnologie Agrarie, Alimentari, Ambientali e Forestali, Università degli Studi di Firenze, Via Maragliano 77, 50144 Firenze, Italy;

^c Laboratorio PHYTOLAB (Pharmaceutical, Cosmetic, Food supplement Technology and Analysis), DiSIA,
Dipartimento di Statistica, Informatica, Applicazioni "Giuseppe Parenti", Università degli Studi di Firenze,
Polo Scientifico e Tecnologico via U. Schiff, 6-Sesto Fiorentino (FI) 50019, Italy;
^d Dipartimento di Chimica "Ugo Schiff" and CSGI, Università di Firenze, Via della Lastruccia 3, 50019

Sesto Fiorentino, Firenze, Italy.

*Correspondence to Sandra Ristori E-mail: <u>sandra.ristori@unifi.it</u> and Marzia Cristiana Rosi E-mail: <u>marziacristiana.rosi@unifi.it</u>

Oviposition activity data were analysed using Multiple Factor Analysis (MFA), which can comprehensively explore complex data sets and provide insight into combined effects. The complexity of the oviposition deterrence experiment on *C. capitata* can be attributed to the number of variables involved and the highly adaptive behaviour of Medflies females. Oviposition activity was assessed by counting at two-time intervals (5 hours and 72 hours) several parameters: the number of egg clusters (EC), the number of eggs (E), and the average number of eggs per cluster (E/EC) from both treated and untreated areas of the net. These parameters have been considered similar to the natural condition of females who prefer to lay eggs on favorable substrates.

In natural oviposition behavior, *C. capitata* is highly adaptive; females encountering unfavorable substrates may reduce egg stinging and the total number of released eggs. Medflies release marking pheromones that induce other females to redistribute egg-laying to less favorable areas to reduce competition ^{1,2}. Medflies release marking pheromones that cause other females to redistribute egg-laying to less favorable areas to reduce competition. When competition is high, females may lay fewer egg clusters but more eggs per cluster ³. Multiple Factor Analysis (MFA) was performed using the FactoMineR and FactoExtra packages for Rstudio 4,5. For the MFA analysis, data were organized into three groups of quantitative variables: the number of eggs (E), the number of egg clusters (EC), and the average number of eggs per cluster (E/EC); each group included data from the treated and untreated areas of the net. The variables "time" and treatments were treated as qualitative.

Dimensions 1 (Dim. 1) and Dimension 2 (Dim. 2) summarized the majority of the information, exceeding the eigenvalue criterion of 1.73 ⁶. Analysis of group contributions to total variance (Table S1 and Figure S1) revealed that Dim. 1 was primarily determined by Eggs (32.37%) and Egg Clusters (32.85%). Both groups also contributed to Dim. 2 (37.22% and 27.58%, respectively). Eggs and Egg Clusters were well-projected for both treated and untreated areas (Figure S2), exhibiting a strong internal relationship (RV = 0.90).

These findings suggest that the dataset variability stemmed primarily from the number of eggs and egg clusters, which convey similar information. Furthermore, the Eggs group displayed the closest association with the overall MFA configuration (RV = 0.75) (Table S2), suggesting it best represents the observed variability. Conversely, the Eggs/Egg Clusters group minimally contributed to both dimensions (Dim.1 = 6.58%, Dim.2 = 18.02%) and exhibited poor representation in both treated and untreated areas (Figure S2), indicating a weak association with the overall MFA (RV = 0.44) (Tables S1 and S2). These findings suggest that E/EC does not significantly vary with the treatment conditions and time intervals. Possibly due to the relatively constant number of eggs per cluster across different conditions or the insignificant variation compared to other variables.

The time variable predominantly contributes to Dim. 1 (26.09%), while the treatment variable shaped Dim. 2 (15.67%). Both variables exhibited moderate correlations with the MFA configuration (RV = 0.49 and RV = 0.57, respectively). Interestingly, no significant correlation was observed between time and treatment (RV = 0.00), underlining the distinct contributions of temporal dynamics and treatment effects.

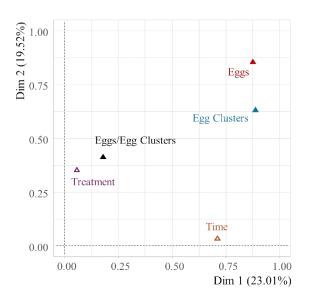
Table S1 Relative contribution and correlation of the active and supplementary groups of variables for the first two principal dimensions of the analysis. E = number of eggs, EC = Egg clusters, E/EC = Eggs / Egg clusters, Contr. = contribution to the dimension construction, Corr. = correlation

	Dim.1		Dim.2		
	Contr.	Corr.	Contr.	Corr.	
E/EC	6.58	0.42	18.02	0.65	
E	32.37	0.96	37.22	0.92	
EC	32.85	0.94	27.58	0.88	
Time	26.09	0.84	1.51	0.19	
Treatments	2.11	0.24	15.67	0.60	

Correlation refers to the square of the correlation ratio; it was reported when the value was significantly different from 0 (p < 0.05).

Table S2 Coefficients correlation (RV) between the groups of variables of the two first dimensions of the MFA Analysis

	E/EC	Е	EC	Time	Treatments
Eggs/Egg clusters	1.00	-	-	-	-
Eggs	0.16	1.00	-	-	-
Egg cluster	0.08	0.90	1.00	-	-
Time	0.02	0.37	0.44	1.00	-
Treatments	0.08	0.05	0.03	0.00	1.00
MFA	0.44	0.75	0.73	0. 49	0.57



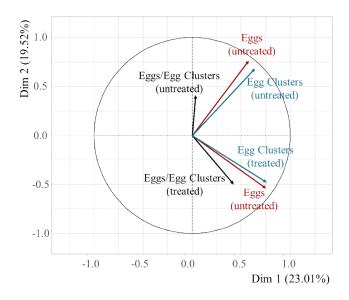


Figure S1 Graph of the groups showing the correlation to Dim. 1 and Dim. 2 between both quantitative (solid triangle) and qualitative variables (empty triangles)

Figure S2 Correlation circle between the quantitative variables and the first two MFA dimensions

References

- 1. Arredondo J., Díaz-Fleischer F. Oviposition deterrents for the Mediterranean fruit fly, *Ceratitis capitata* (Diptera: Tephritidae) from fly faeces extracts. *Bull Entomol Res* 2006;**96**(1):35–42. Doi: 10/d9nbnq.
- 2. Prokopy RJ., Ziegler JR., Wong TTY. Deterrence of repeated oviposition by fruit-marking pheromone inCeratitis capitata (Diptera: Tephritidae). *J Chem Ecol* 1978;**4**(1):55–63. Doi: 10.1007/BF00988260.
- Papanastasiou SA., Ioannou CS., Papadopoulos NT. Oviposition-deterrent effect of linalool a compound of citrus essential oils – on female Mediterranean fruit flies, Ceratitis capitata (Diptera: Tephritidae). *Pest Manag Sci* 2020;**76**(9):3066–77. Doi: 10.1002/ps.5858.
- 4. Lê S., Josse J., Husson F. FactoMineR: An R Package for Multivariate Analysis. *J Stat Softw* 2008;**25**(1):1–18. Doi: 10.18637/jss.v025.i01.
- 5. Pagés J., Husson F. Multiple factor analysis with confidence ellipses: a methodology to study the relationships between sensory and instrumental data. *J Chemom* 2005;**19**(3):138–44. Doi: 10.1002/cem.916.
- Karlis D., Saporta G., Spinakis A. A Simple Rule for the Selection of Principal Components. *Commun Stat Theory Methods* 2003;32(3):643–66. Doi: 10.1081/STA-120018556.