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PP-266 [Beekeeping Technology and Quality]

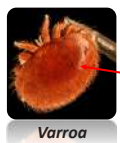
The influence of Varroa mite treatments on the quality of propolis in Greece

Freideriki Papakosta [1], Konstantia Graikou [1], Leonidas Charistos [2], Fani Hatjina [2] and Ioanna Chinou [1]

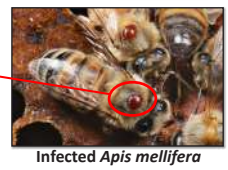
[1] Laboratory of Pharmacognosy and Chemistry of Natural Products, Faculty of Pharmacy, National and Kapodistrian University of Athens, Panepistimiopolis, Zografou, 15771, Athens, Greece
[2] Department of Apiculture, Hellenic Agricultural Organisation 'DIMITRA', Nea Moudania, 63 200, Greece

INTRODUCTION

As part of our ongoing research on beekeeping products [1,2], this study examines the chemical composition of **15 propolis** samples collected from the Department of Apiculture in Chalkidiki (Northern Greece), along with an assessment of the potential glycerol residue percentage resulting from different treatments used to control the ectoparasitic honey bee mite ***Varroa destructor***. In Greece, *Varroa* represents a major threat to apiculture, with its management relying on insecticides and acaricides approved by the European Medicines Agency (EMA). These treatments include **amitraz-impregnated strips**, **glycerin-impregnated strips with oxalic or formic acid**, and **oxalic acid** applied via **instillation** or **sublimation** [3]. The increasing use of non-toxic, organic acaricides has emerged as a crucial approach, driven by the need to address mite resistance to synthetic treatments and minimize residue contamination in beekeeping products.



Varroa destructor

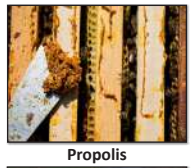


Infected *Apis mellifera*

MATERIALS AND METHODS

Extraction and Silylation: Propolis samples were obtained from five different beehives at the Department of Apiculture, Institute of Animal Science, in Chalkidiki, Greece. Each beehive was subjected to a distinct treatment method for the control of *Varroa destructor*.

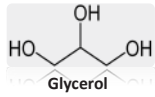
Samples were collected on days 7, 21, and 65 following treatment application (Table 1). The collected propolis was extracted using an ethanol/water solution (70:30), and the extract was subsequently evaporated to dryness. Each dried extract (5 mg) was silylated and analyzed using GC-MS [1]. Compound identification was conducted through mass spectral comparison with reference libraries (Wiley Registry of Mass Spectral Data) and bibliographic sources.



Propolis

Sublimation of oxalic acid

Oxalic/glycerin strips



Glycerol

Table 1: Propolis samples according to the treatment used against to *Varroa*.

Beehives	Propolis Samples	Anti-*Varroa* treatments
Beehive A	A-1 / A-2 / A-3	4 Strips with oxalic acid & glycerin (500 g/L glycerin)
Beehive B	B-1 / B-2 / B-3	Instillation of oxalic acid (80 g oxalic acid in a 1:1 solution of sugar: water)
Beehive C	C-1 / C-2 / C-3	2 Strips with amitraz (500 mg/ strip)
Beehive D	D-1 / D-2 / D-3	Sublimation of oxalic acid (2.5 g/beehive)
Beehive E	E-1 / E-2 / E-3	4 Strips with formic acid & glycerin (50% formic acid: 40% water: 10% glycerin)
Beehive F	CON1/CON2/CON3	No treatment

1/2/3: samples collected at days **7, 21** and **65** after the application of treatment

RESULTS AND CONCLUSIONS

Impact of Varroa Treatments on Glycerol Residues in Propolis

- **Glycerin-Impregnated Strips**
  Caused **increased glycerol residues**:
  From **20.51% to 24.30% (A)** or minor drop (from **9.25% to 8.91% for sample E**)
  -Negative effects on propolis quality:
  Alters **organoleptic** properties
  Possible **physiological effects** (e.g., laxative action)
- **Instillation (B)**
  Moderate decrease in glycerol: **14.48% → 12.60%**
- **Amitraz (C)**
  Glycerol levels ranged: **5.09%–9.19%**
  -Not recommended due to:
  Risk of **mite resistance**
  Unfavorable **chemical profile**
- **Oxalic Acid Sublimation (D)**
  Significantly minimized glycerol residues:
  Day 7: 9.98%
  Day 21: 6.07%
  Day 65: 5.12%
  -Considered safe and effective.

❖ **Seasonal variation** affected chemical composition: **fall** samples showing similar profiles and higher resin collection, while early **winter** samples exhibit increased sugars, reduced diterpenes, and altered glycerol levels due to reduced foraging and reliance on stored food.

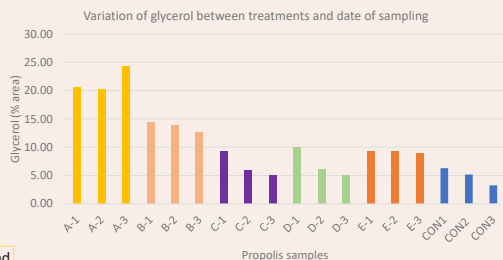


Figure 1. Variation of glycerol percentage between different treatments and date of sampling.

➢ Careful *Varroa* management is essential to preserve propolis quality; **avoid glycerin-based treatments and prefer oxalic acid sublimation**.
➢ Beekeepers and authorities should **promote residue-free practices** to ensure the chemical integrity of propolis.

Table 2: Categorization of components detected by GC-MS in propolis samples.

	Area %																
Chemical category	**A-1**	**A-2**	**A-3**	**B-1**	**B-2**	**B-3**	**C-1**	**C-2**	**C-3**	**D-1**	**D-2**	**D-3**	**E-1**	**E-2**	**E-3**	**CON1**	**CON2**	**CON3**
Aliphatic acids	3.05	3.15	3.38	3.38	3.88	1.67	4.48	3.57	3.22	4.56	4.23	3.60	3.59	3.41	3.04	3.22	2.84	5.25
Aromatic acids	1.78	1.36	0.27	1.33	1.28	0.16	3.01	0.88	1.02	0.97	0.73	<0.1	1.02	1.04	1.35	1.54	1.21	0.56
Aromatic acid esters	2.17	2.06	0.95	2.72	2.11	0.81	3.94	2.25	1.48	2.16	1.48	<0.1	2.19	2.61	1.86	1.72	1.77	2.53
Diterpenes	31.41	32.27	18.07	23.59	22.16	11.50	34.04	38.02	22.32	32.13	25.34	5.21	31.09	35.12	27.00	41.65	41.87	30.24
Sesquiterpenes	0.26	0.21	0.23	0.27	0.28	0.30	0.38	0.39	0.49	0.26	0.32	0.77	0.38	0.31	0.40	0.25	0.24	-
Flavonoid & Chalcones	2.19	2.25	1.66	3.10	3.66	2.64	7.27	3.39	3.02	3.46	2.99	0.98	3.21	4.23	4.41	1.30	1.26	2.16
Glycerol	**20.51**	**20.23**	**24.30**	**14.48**	**13.78**	**12.60**	**9.19**	**5.95**	**5.09**	**9.98**	**6.07**	**5.12**	**9.25**	**9.30**	**8.91**	**6.30**	**5.18**	**3.27**
Unidentified Sugars	37.72	37.63	47.70	49.23	50.59	63.89	36.19	44.41	58.36	45.17	55.51	66.39	48.21	42.69	50.06	41.28	44.10	52.86
Unidentified compounds	0.51	1.16	3.69	2.28	2.51	3.46	1.03	1.39	5.29	1.92	3.80	14.22	1.41	1.70	3.26	2.30	2.47	1.69

REFERENCES
[1] Pyrgioti, E. et al., Molecules 2022, 27(20):7018; **[2]** Graikou, K. et al., LWT-Food Science Technol 2016, 65:261; **[3]** Kagiali, E. et al., Insects 2023, 14(2): 193; **[4]** Simone-Finstrom, M., & Spivak., Apidologie 2010, 41(3), 295-311.

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The honeybee (Apis mellifera L.) is a crucial agricultural pollinator, producing valuable beekeeping products such as: honey and propolis. Honey bees are significantly threatened by various diseases and pests, the most severe being the ectoparasitic mite Varroa destructor. In Greece, beekeepers apply acaricide treatments, such as amitraz strips, glycerin-impregnated oxalic or formic acid strips and oxalic acid administered through sublimation or instillation, in order to mitigate Varroa infestations; however, the impact of these treatments on the quality of propolis remain unstudied. In the current study, propolis samples were collected over a 65-day period from five stable beehives, where all -mentioned treatments were applied. Propolis samples were extracted with ethanol/water (70:30) and analyzed by GC-MS, showing the predominance of diterpenes. Furthermore, the analysis demonstrated that both oxalic acid sublimation and amitraz treatments resulted in significantly lower levels of glycerol residues in propolis, whereas the use of glycerol-impregnated strips caused a substantial increase in glycerol concentrations, when compared to all other treatment methods. Our findings strongly suggest that the application of glycerol-impregnated strips should be discouraged, when propolis is going to be collected from these same colonies later on, as they can have a detrimental effect on the quality of harvested propolis, which may affect its suitability for subsequent uses in nutritional or cosmetic products. Considering these results, it is recommended that National Authorities and Beekeeping Associations issue formal guidelines advising beekeepers to avoid the use of glycerol-impregnated strips in Varroa control practices before the collection of propolis in order to ensure the preservation of propolis quality. Further studies are needed to demonstrate for how long glyserin residues remain in the treated colonies.