Antimicrobial activity of essential oils against Paenibacillus larvae

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INTRODUCTION

Fig. 1.a. Signs of American foulbrood in field

American foulbrood is a serious bacterial disease that affects *Apis mellifera* colonies; the causative agent is *Paenibacillus larvae* [1].

THE AIM OF THE STUDY was to evaluate the *in vitro* antimicrobial activity of 32 essential oils against *P. larvae.*

Fig. 1.b. Field test for American foulbrood

MATERIALS AND METHODS

Chromatographic analysis

Oils from 21 botanical species were analyzed by gas chromatography (CG and CG/EM). All essential oils were classified according to the composition of their main components in two groups: benzene ring compounds (BRC) and terpene compounds (TC).

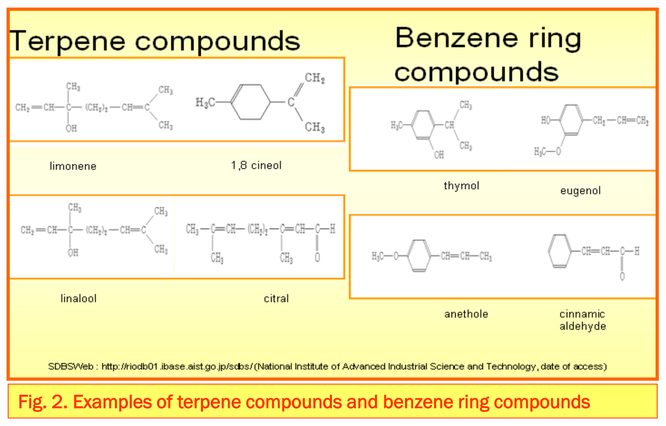


Fig. 2. Examples of terpene compounds and benzene ring compounds

Antimicrobial activity

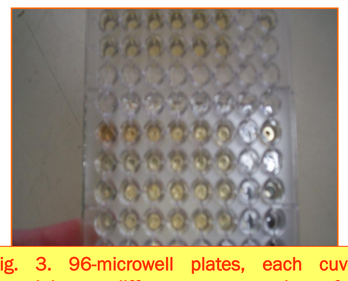


Fig. 3. 96-microwell plates, each cuvette containing a different concentration of the essential oil.

Minimal inhibitory concentration (MIC) in MYT broth [2] was assessed by the microdilution method. Final serial dilution concentrations of the essential oils ranged between 2,000-12.5 µg/mL. The bacterial isolates were collected from different Argentina's region.

The data obtained from the CIM were statistically analyzed using multivariate analysis by means BioEstat 5.0 program.

RESULTS

Chromatographic analysis

The chromatographic analysis showed that a 67% of the essential oils contained predominately terpene compounds, while the remaining 33% included mainly compounds with benzene rings.

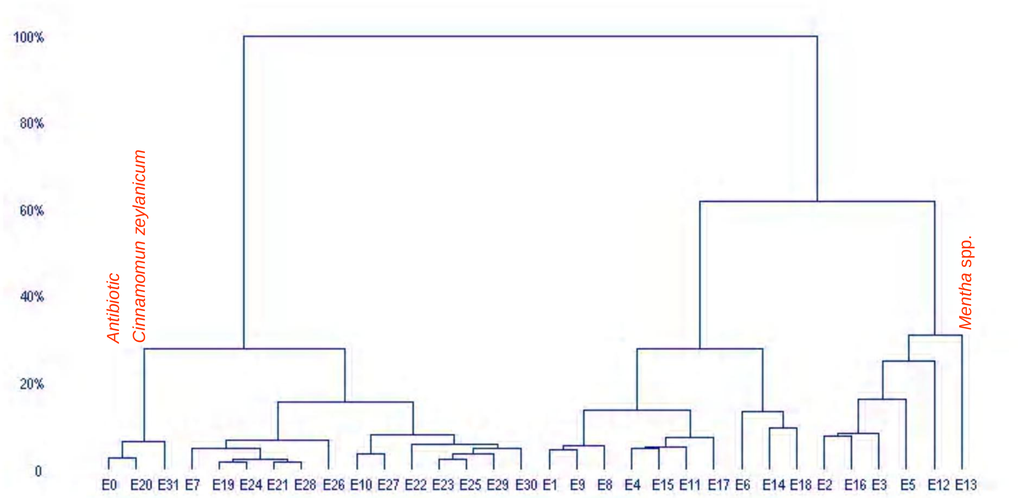
Antimicrobial activity

Antimicrobial agent		*Paenibacillus larvae* isolates							
		P.l Cb	P.l SdP	P.l LP	P.l V	P.l Tp	P.l Cy	P.l Vr	P.l MdP
OTC...	CIM	3,125-5	2,5-3,125	0,5-0,625	2,5-3,125	5-6,25	0,1	5	6,25
Bergamota	CIM	700	500-600	6					
	EB	1200-1400	1000-1200	12					
Burrito	CIM	1400	1000	12					
	EB	>2000	1800	>2					
Cajeput	CIM	1200	1000	1					
	EB	>2000	>2000	>2					
Eucalipto 1	CIM	600	600	7					
	EB	1200	>1200	1					
Eucalipto 2	CIM	1200	600-700	1					
	EB	>1600	900	1600-1					
Laurel	CIM	800	800	800-1					
	EB	1600	1600-1800	1800-2					
Lavanda 1	CIM	200-400	300	4					
	EB	900	800-900	9					
Lavandin 2	CIM	500	450	6					
	EB	1200-1400	800-1000	14					
Lavandin gros	CIM	600	500	500-6					
	EB	1400	1000	1200-1					
Lemongrass	CIM	150	200	2					
	EB	450	400	2					
Menta 0	CIM	600	700	600-7					
	EB	1000	700	1					
Menta 1	CIM	1800	1400	12					
	EB	2000	1800	18					

Higher CIM

Lower CIM

Antimicrobial agent		*Paenibacillus larvae* isolates							
		P.l Cb	P.l SdP	P.l LP	P.l V	P.l Tp	P.l Cy	P.l Vr	P.l MdP
Anis	CIM	300	300	300	300	300	300	300	300
	EB	600	600	600-700	600	600	500-600	600	600
Canela 1	CIM	50-100	25-50	50	25	50	50	25-50	50
	EB	150-200	100-150	150	150	150-200	150	150-200	150
Canela 2	CIM	350	300-350	300	350	300	300	350	300-350
	EB	700	600-700	600	700	700	600-700	700	600-700
Canela 3	CIM	200	250	300	300	350	300-350	200-300	200-250
	EB	450	500	500-600	600	600-700	600-700	400-600	400-500
Clavo 1	CIM	250-300	250-300	200-300	250	300	200-250	250-300	300
	EB	600	700	600	600	600	500-600	600	700
Clavo 2	CIM	300	300	300	300	350	300-350	300	350
	EB	600	600	600	600	600-700	600-700	600	700
Hinojo	CIM	250	250	250	250	250	250	250	250
	EB	500	500		450-500	500	500	500-600	600
Orégano	CIM	400	400	400	350-400	400	350	400	400
	EB	700	700-800	800	800	700	700-800	800	800
Tomillo 1	CIM	150	150-200	150	200	200	150	150-200	200
	EB	350	350-400	350	400	350-400	300	400	400
Tomillo S...	CIM	50-100	50-100	<50	50-100	<50	<50	50	50-100
	EB	250	200	100	200	100	100	100-150	100-200
Tomillo 2	CIM	300	300	300-350	350	300	300	350	300-350
	EB	800	800-900	800-1000	900	800-1000	800	900	900-1000
Tomillo C	CIM	300	200-300	300	300	200	200	300	300
	EB	1000	800-1000	1000	800	600	800	900	900-100
Tomillo 3	CIM	200-300	200	300	200	150-200	250-300	200	200-300
	EB	600	400-500	600	450	300	600	400-450	600



Benzene ring compounds (BRC group) | Terpene compounds (TC group)

Fig. 4. Dendrogram grouping of essential oils in relation to their antimicrobial activity against 16 isolates of *P. larvae* analyzed.

From the TC group, *Cymbopogon citratus* essential oil showed the best antimicrobial activity against *P. larvae* with MIC values between 150 and 250 µg/ml. The essential oils from *Aloysia polystachya* and *Mentha* spp. had the lowest inhibitory activity. Among the oils from the BRC group, one of the lowest MIC values was found with cinnamon essential oil (*Cinnamomun zeylanicum*) being between 25 and 50 µg/ml; *Origanum vulgare* showed the highest MIC values (350-400 µg/ml).

CONCLUSION

Essential oils, especially those with BRC in their composition, presented inhibitory capacity against *P. larvae* strains.

The present experience, in which essential oils were tested *in vitro* against *P. larvae,* promotes

their use for American fouldbrood management in honey bee colonies.

REFERENCES

1. E Genersch (2010) J Invertebr Pathol. 103: 10–19.
2. LB Gende, MJ Eguaras, R Fritz (2008) Rev Argent Microbiol. 40:147-150.

ACKNOWLEDGMENTS

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