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Antimicrobial Activity of Some Essential Oils on Streptococcus Bovis (ES1) Isolated From Rumen Fluid

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Abstract: Different substances are used to either eliminate or decrease the numbers of rumen bacteria to alter their makeup. Essential oils (EO) are one of the substances used for this purpose. The present study was carried out to determine the effects of EO extracted from orange peel (Citrus cinensis), cinnamon (Cinnamomumverum), Laurel (Laurusnobilis), oleaster (Eleagnusangustifolia), garlic (Allium sativum) and thyme (Tymusvulgare) on Streptococcus bovis (ES1). For this purpose, bacterial growth was measured by inoculating stock cultures grown in Hobson's M8 medium with a three-fold increasing series of EO. Essential oil diluted in autoclaved water containing 10% DMSO was added aseptically after the medium was autoclaved to give final concentrations ranging from 50 to 5,000 ppm (0.5 ml to each 6.5 ml of M8). Bacterial growth was measured by reading the optical density at 650 nm hourly until the reading for bacterial growth decreased. Maximal bacterial growth rate was calculated using the MicroFit v 1.0. The results show that the effects of essential oils, doses and dose-oil interactions used in the study are statistically significant. According to the results, garlic and cinnamon essential oil have strong antimicrobial activity on Streptococcus bovis (ES1).

Keywords: Rumen bacteria, Thyme, Orange, Laurel, Streptococcus bovis

Introduction

The removal of antibiotic growth-promoters from animal feeds within the EU has led to an increased interest in alternative means of manipulating rumen fermentation (Wallace, 2004). Essential oils (EO) which are extracted from plants through distillation have been shown to influence both volatile fatty acid production and protein degradation in the rumen. (Newbold, 2004; Busquet et al., 2006; Patra, 2011, Belanche, 2016). Structurally, essential oils can be classified as alcohol, ester or aldehyde derivatives of phenylpropanoids and terpenoids (Greathead, 2003), and the antimicrobial activity of EO has been attributed to the effect of these compounds in disrupting the cytoplasmic membrane of bacteria leading to changes in the microbial population structure within the rumen (McIntosh et al., 2003). However to date the only studies investigating the effect of EO on rumen microbes have used a commercial mixture of essential oils (McIntosh et al, 2003). In studies with non ruminal microorganisms it is known that the antimicrobial spectrum of different EO varies (Dorman and Deans, 2000, Oussalah et al., 2007). However what is not known clearly is how the rumen microbial population responds to individual EO.

Here we have investigated the effects of EO extracted from orange peel (*Citrus cinensis*), cinnamon (*Cinnamomum verum)*, Laurel (*Laurus nobilis*), oleaster (*Eleagnus angustifolia*), garlic (*Allium sativum*) and thyme (*Tymus vulgare*) on Streptococcus bovis (ES1) maximal growth rate.

Method

In this study essential oils were supplied by Doğa Bitki Ürünleri Gıda Limited (Antalya,TURKEY). Samples were stored in dark glass vials at 4 °C prior to use.

In order to characterise the EO used, gas chromatograph-mass spectrometry (GC-MS) analysis was performed using a Hewlett Packard 5973-6890 GC-MS system operating on electron impact (EI) ionisation mode (equipped with a HP 5MS 60 m x 0.25 mm x 0.25 μm film thickness capillary column), using He (1,5 mL min-1) as the carrier gas. The initial temperature of the column was 60 °C and was gradually heated to 250 °C with a 4 °C min–1 rate. Mass spectra were recorded at 70 eV. Mass range was from m/z 35 to 425. Essential oils were identified by comparison of their mass spectral data and retention indices (RI) with spectra from the NIST/NBS Wiley libraries.

S. bovis (ES1) was tested and maintained in Hobson's M8 medium prior to use (Hobson, 1969). The effect of essential oils (EO) on bacterial growth was measured by inoculating stock cultures grown in Hobson's M8 medium with serial three-fold increases in EO. Essential oil diluted in autoclaved water containing 10% DMSO was added aseptically after the medium was autoclaved to give final concentrations ranging from 50 to 5,000 ppm (0.5 ml to each 6.5 ml of M8). Bacterial growth was measured by reading the optical density at 650 nm hourly until the reading for bacterial growth decreased. Maximal bacterial growth rate (μ_{max} [h^{-1}])) and the potential lag time (λ) before growth commenced were calculated using the MicroFit v 1.0 (Institute of Food Research, UK Ministry of agriculture, Fisheries and Food (Food LINK Programme))The concentration of essential oil required to decrease maximal growth rates by 50% IC50μmax and to cause a doubling in the lag before growth commenced IC50 tlag was estimated after plotting μmax and tlag against EO concentration using Curve Expert V1.4 (www.curveexpert.net) fitting a polynomial curve and using the analyse curve function to drive the required value. All measurements were made in triplicate.

Statistical Analyses

Microsoft Excel (version 2013; Microsoft Corp.) was used for compiling the data collected throughout the project and SAS package program (Version 8.0, SAS, 2000) was used for analyses. Repeated measures experimental design and PROC MIXED procedure were used for data analysis. Differences between treatments were declared significant at $P < 0.05$ using the Turkey multiple comparison test.

Results and Discussion

Results of the experimental factors are given in Tables 1-3. It was determined that the effect of essential oils, doses and dose-oil interaction on the specific growth rate (μ_{max} [h^{-1}]), which is one of the parameters measuring the growth rate of the bacteria, was statistically significant (P<.0001). When Table 2 demonstrating the effects of essential oils is observed, it is seen that the most powerful of oils in terms of antimicrobial effect are garlic, cinnamon, laurel, orange peel, thymus and oleaster respectively. In the study, the most powerful antimicrobial effect against S. bovis was that of garlic oil. That result is compatible with the study of Busquet et al.(2006) The effects of oils on the specific growth rate of bacteria was found to be important both in quadratic and linear terms. Analyzing Table 3 which shows the effect of doses on the specific growth rate (μ_{max} [h^{-1}]) of Streptococcus bovis, it can be seen that all of the oils demonstrated antimicrobial effect at the 5000 ppm dose.

Table 1. Effect of different doses of orange peel (*Citrus cinensis*), cinnamon (*Cinnamomum verum),* Laurel (*Laurus nobilis*), oleaster (*Eleagnus angustifolia*), garlic (*Allium sativum*) and thyme (*Tymus vulgare*) oil on Maximal growth rate of Streptococus bovis (ES1)

Essantial Oil (EO)	**Doses(ppm)**	**µmax** [h^{-1}]	**Essantial Oil (EO)**	**Doses (ppm)**	**µmax [h^{-1}]**
Cinnamon	0	0.83±0	Oleaster	0	1.443±0.497
Cinnamon	50	0.743±0.067	Oleaster	50	2.777±0.43
Cinnamon	100	0.823±0.11	Oleaster	100	2.597±0.091
Cinnamon	200	0.92±0.137	Oleaster	200	3.015±0.361
Cinnamon	300	0.843±0.023	Oleaster	300	3.13±0.465
Cinnamon	400	0.847±0.072	Oleaster	400	2.927±0.305
Cinnamon	600	0.823±0.095	Oleaster	600	3.157±0.725
Cinnamon	800	0.843±0.055	Oleaster	800	2.663±0.309
Cinnamon	1000	0.827±0.087	Oleaster	1000	2.517±0.08
Cinnamon	5000	0.777±0.04	Oleaster	5000	1.393±0.047
Garlic	0	1.067±0.021	Orange peel	0	1.25±0.072
Garlic	50	0.653±0.04	Orange peel	50	1.23±0.062
Garlic	100	0.647±0.057	Orange peel	100	1.207±0.035
Garlic	200	0.65±0.017	Orange peel	200	1.553±0.055
Garlic	300	0.6±0.125	Orange peel	300	1.63±0.125
Garlic	400	0.597±0.074	Orange peel	400	1.63±0.082
Garlic	600	0.425±0.021	Orange peel	600	1.633±0.569
Garlic	800	0.5±0.042	Orange peel	800	1.28±0.184
Garlic	1000	0.635±0.007	Orange peel	1000	1.863±0.185
Garlic	5000	0.533±0.015	Orange peel	5000	1.26±0.042
Laurel	0	1.207±0.012	Thyme	0	1.287±0.076
Laurel	50	1.317±0.11	Thyme	50	2.113±0.42
Laurel	100	1.533±0.11	Thyme	100	2.097±0.114
Laurel	200	1.26±0.325	Thyme	200	2.227±0.006
Laurel	300	1.613±0.067	Thyme	300	2.25±0.141
Laurel	400	1.737±0.172	Thyme	400	1.68±0.099
Laurel	600	1.607±0.283	Thyme	600	1.563±0.488
Laurel	800	1.565±0.148	Thyme	800	1.76±0.198
Laurel	1000	1.245±0.064	Thyme	1000	0.8±0
Laurel	5000	0.14±0.014	Thyme	5000	0.227±0.101
Effects (P<)	EO	0.0001	Effects (P<)	EO	0.0001
	Doses	0.0001		Doses	0.0001
	EO *Doses	0.0001		EO *Doses	0.0001

Table 2. Effect of essantial oils (EO) on Maximal growth (µmax [h^{-1}]) rate of Streptococus bovis (ES1)

Essantial Oil (EO)	**µmax [h^{-1}]**
Cinnamon	0.83±0.07 d
Garlic	0.64±0.17 e
Laurel	1.36± 0.42 c
oleaster	2.54±0.7 a
orange peel	1.46±0.28 c
Thymus	1.59±0.68 b

Each letter (a,b,c,d,e) shows that the EO are different from each other at p <0.0001

Table 3. Effect of doses on Maximal growth rate of Streptococus bovis (ES1)

doses	**μmax [h^{-1}]**
0ppm	1.18±0.26 d
50ppm	1.47±0.80 bc
100ppm	1.48±0.71 bac
200ppm	1.53±0.80 bac
300ppm	1.64±0.90 a
400ppm	1.56±0.80 bac
600ppm	1.6±0.94 b
800ppm	1.48±0.77 bac
1000ppm	1.39±0.72 c
5000ppm	0.72±0.48 e

Each letter (a,b,c,d,e)) shows that the doses are different from each other at $p < 0.0001$

Conclusion

S. bovis plays an important role in cases of tympany in feeder cattle resulting from feeding with high levels of grain. Thus, essential oils can be used in those cases in order to decrease their number in rumen. Garlic and cinnamon oils can be suggested to this end.

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