

Evaluating the impact of essential oils on bovine mammary alveolar tissue cells *in vitro*C. Chylinski¹, O. Tavares², C. Experton², A. Fauriat³ and S. Athanasiadou¹¹SRUC, Easter Bush, EH25 9RG, Midlothian, Scotland, United Kingdom, ²ITAB, Rue de Bercy, 75595 Paris, France, ³FEVEC, St Symphorien sur Coise, Rhone, France; caroline.chylinski@sruc.ac.uk

The increasing resistance of bacteria to antibiotics and their regulated use in organic production systems has prompted research into the identification of new nutraceuticals with broad activity to reduce the use of contentious inputs in organic systems of production. Essential oils (EO) contain a wide variety of metabolites that are capable of inhibiting or slowing the growth of bacteria and offer a promising alternative for the control of mastitis; one of the most common infections requiring antibiotic treatments in both organic and conventional livestock farming. There is anecdotal evidence that French organic farmers use EO to minimise mastitis by applying it topically onto the bovine mammary gland, however scientific quantification of their efficacy and evaluation of their potential toxicity is lacking. The focus in H2020 RELACS project is to identify EO which can safely be used to reduce antibiotic input in organic systems of dairy production. This study aimed to test *in vitro* the toxicity of 12 EO, reportedly used by French organic farmers. The impact of each of the EO on the growth and proliferation of bovine mammary alveolar epithelial cells (MAC-T) was monitored using the xCELLigence® real time cell analyser (RTCA) system over 48 hours and compared against a control (sunflower oil). All 12 EO were screened for cytotoxic effects at a concentration of 0.1%. The results showed that 10/12 EO had an impact on cell growth. Of these, two EOs had slightly higher cell indices than the controls, indicating that they may promote cell growth. The eight remaining EO had lower cell indices compared to the controls, indicating they may promote cell death. In conclusion, the results identify potential cytotoxic risks associated with the use of EO when tested *in vitro* on a monolayer of MAC-T cells. Cytotoxicity results may vary if used on the complete mammary organ *in vivo*, however our *in vitro* data provide clear indication for potential cytotoxic risks of some EO. *In vivo* toxicity tests and/or close monitoring of the application site should always accompany the use of EO for mastitis control in organic dairy animals to ensure their welfare.

Selecting botanicals for mastitis control: efficacy and safety characterization of essential oilsO. Tavares¹, C. Experton¹, A. Fauriat², M. Guideur³, D. Belenor⁴, E. Chemin⁵, P. Sulpice² and B. Lemaire⁴¹ITAB, 9 rue André Brouard, 49100 Angers, France, ²FEVEC, Domaine GIRAUD, 42130 Boen sur Lignon, France, ³IDELE, Monvoisin, 35650 Le Rheu, France, ⁴ITEIPMAI, 3, Belle Tête, 49120 Chemille-en-Anjou, France, ⁵ADAGE 35, rue du Bas Village, 35577 Cesson-Sevigne, France; marlene.guideur@idele.fr

Organic Farming principles for health care and welfare give preference to preventive methods. When prevention is not enough, the EC Organic Regulation states that organic livestock should be treated preferably using phytotherapeutics. However, there is a lack of methodology to assess the assumed effects and the safety for human consumption of these non-antibiotic alternatives. In France, farmers' groups have been using essential oils to control mastitis on dairy cows to explore alternatives to the use of antibiotics. A RELACS multi-actor panel developed an innovative approach to evaluate essential oil's effectiveness, safety, main effects on animal health, and final product quality. Based on a selection of 10 essential oils reported to be efficient by farmers to control mastitis, their effects in several major mastitis pathogens were quantified using aromatograms. Cytotoxicity tests using mammalian epithelial cells were performed. Chromatograms provided a chemical characterization of the active molecules thus linking them to presumed effects according to their composition. Bibliographic reference research on each essential oil for mastitis control and/or presenting antibacterial activity and bibliography about its potential risk for humans and animals completed this work. These results were compared with the regulatory status and availability of the essential oils. As a result, two essential oils were selected to be further evaluated on commercial farms. The second round of tests analysed the effects on milk sensory and technical quality, using milk samples of animals treated with essential oils. Tests included: detection of markers of the essential oils in the milk, their potential impacts on acidification and sensory properties of milk, and verifying the absence of reaction in a test to detect antibiotic residues. Funded by EU H2020 No 773431 – RELACS.