



CITROFOL® AI as Carrier for Liquid Microbial Formulations

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Introduction

Biocontrol and biostimulant products based on living microorganisms are a relevant part of sustainable agriculture. Formulation plays a crucial role in stabilising the viability of microbials and ensuring shelf life and effective application in the field. The most common product formats are wettable powders (WP), aqueous suspension concentrates (SC), oil dispersions (OD) or dispersion concentrates (DC). The latter are liquid product formats which offer benefits such as being dust-free, ready to mix and easy to dose in applications such as spraying, soil drenching or seed coating. To control water activity and thus microbial shelf life, formulations based on non-aqueous, bio-compatible carrier fluids are well suited. In this poster, we present the benefits of Jungbunzlauer's CITROFOL® AI (triethyl citrate) as a novel co-formulant in microbial formulations.

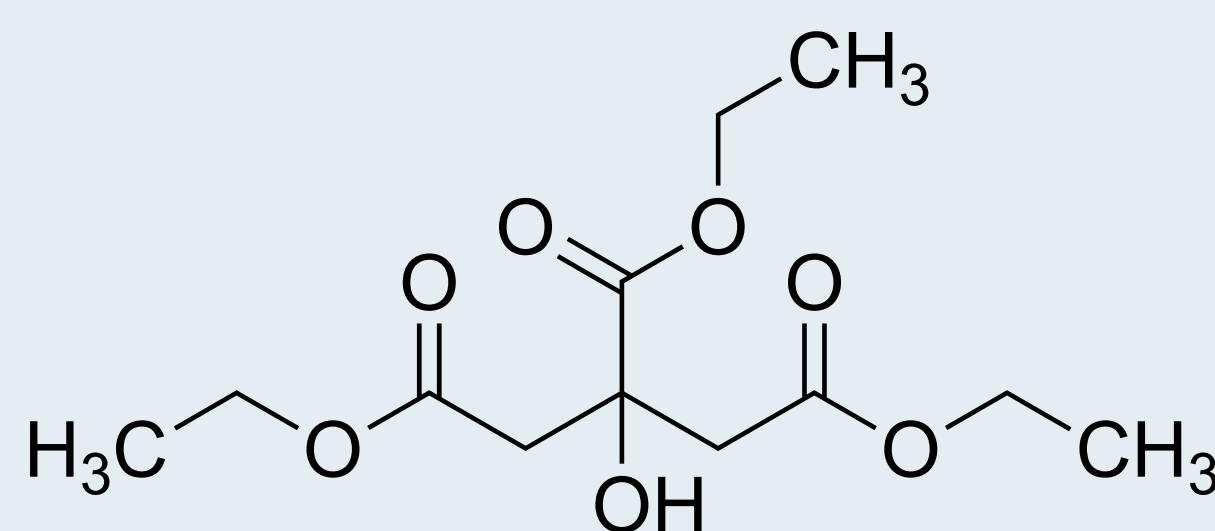
CITROFOL® AI

CITROFOL® is Jungbunzlauer's globally recognised brand of citrate esters. The CITROFOL® range comprises clear, colourless and odourless oily liquids. One of these citrate esters is triethyl citrate, our CITROFOL® AI, which is produced by esterification of raw materials derived from fermentation.



Benefits of CITROFOL® AI in microbial formulation

- Sufficient water solubility, therefore no emulsifier needed
- Water-free, thus no preservative required
- Cold-stable and pourable over a wide temperature range
- Protocols for rheology modification available
- Improved wetting



Physicochemical characteristics

Molecular weight [g/mol]	276
Density [g/ml]	1.14
Boiling point [°C]	287
Flash point [°C]	178
Viscosity [mPas at 25 °C, 100 s ⁻¹]	27
Vapour pressure [mbar at 25 °C]	0.0025
Interfacial tension [mN/m]	32.0
Hansen solubility (δ_D / δ_P / δ_H)	16.5 / 4.9 / 12.0
Water solubility (g/L)	58.1
Hydrolytic resistance (DIN 53402)	610

Safety and regulatory

- Non-hazardous according to EU GHS
- EU-REACH registered
- USDA Certified Biobased (USDA BioPreferred® Program)
- 100% bio-based
- FIFRA listed (Federal Insecticide, Fungicide, and Rodenticide Act)
- Non-flammable, non-volatile

Material and Methods

Shelf life of microorganisms

Commercial biostimulant and biocontrol products were used for viability tests. They contained *Bacillus velezensis* (WP with 1.5×10^9 CFU/g and SC with 1.5×10^{10} CFU/g) or *Trichoderma harzianum* (WP with 1.8×10^9 CFU/g). The amount of CFU in the native products was confirmed by plate counting. The WPs were used to formulate DCs in CITROFOL® AI in a ratio of 1:10. Both the native WPs and the SC served as references. All formulations were subjected to a demanding storage test at 40 °C for 24 weeks. The microbial viability was monitored by plate counting on LB agar (*B. velezensis*) or PDA (*T. harzianum*).

Plant compatibility tests

The plant compatibility of CITROFOL® AI was tested in a foliar spray treatment. An aqueous dilution with 0.4 wt% of CITROFOL® AI (corresponding to realistic product dilution) was sprayed on maize at growth stage BBCH* 13. Phytotoxic symptoms were evaluated optically at 7 and 14 days after treatment (DAT). Fresh weight of above ground biomass was determined at 14 DAT.

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Results

Microbial viability

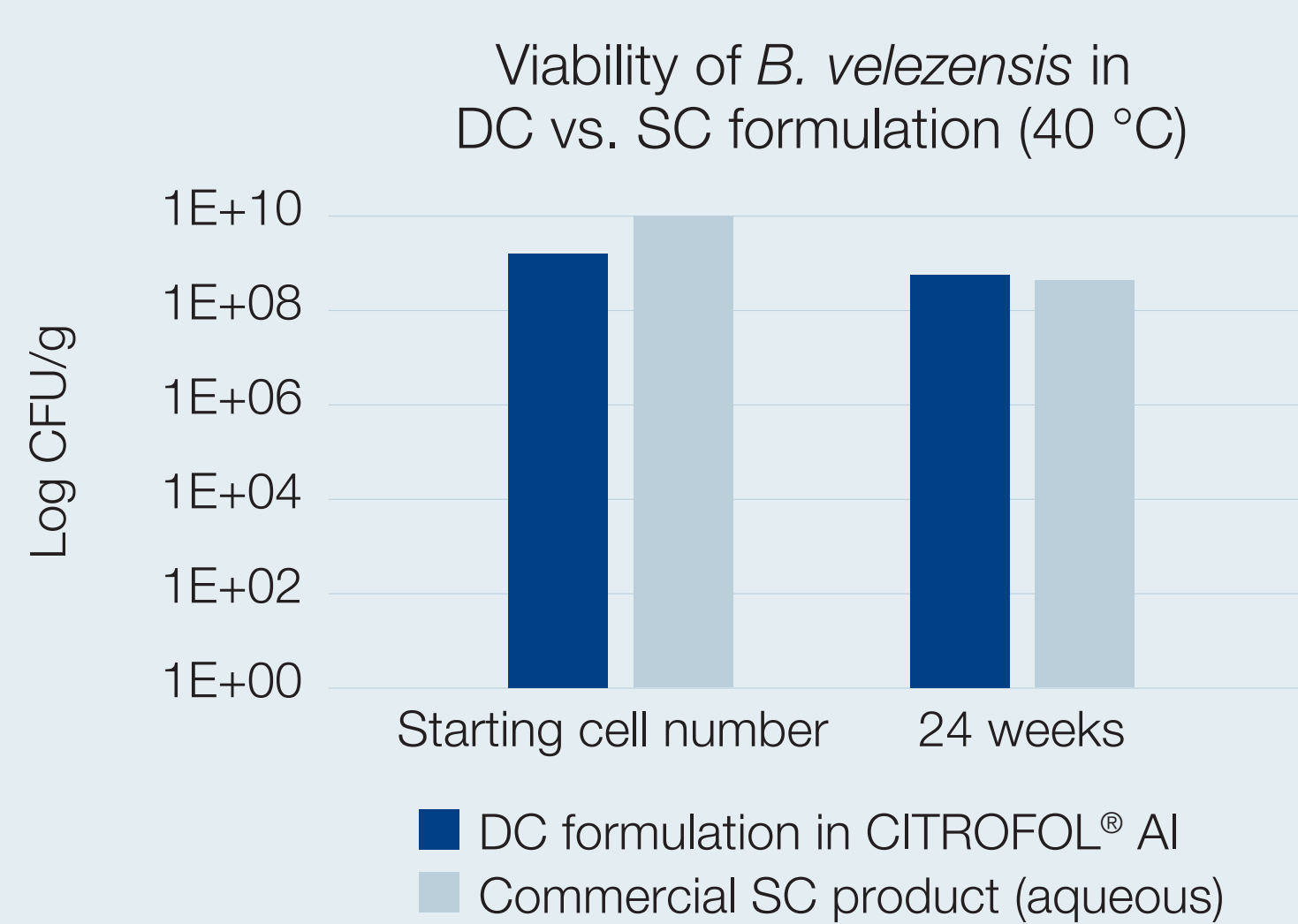


Fig. 1: Comparison of cell viability of Gram positive bacteria in aqueous and non-aqueous liquid formulations

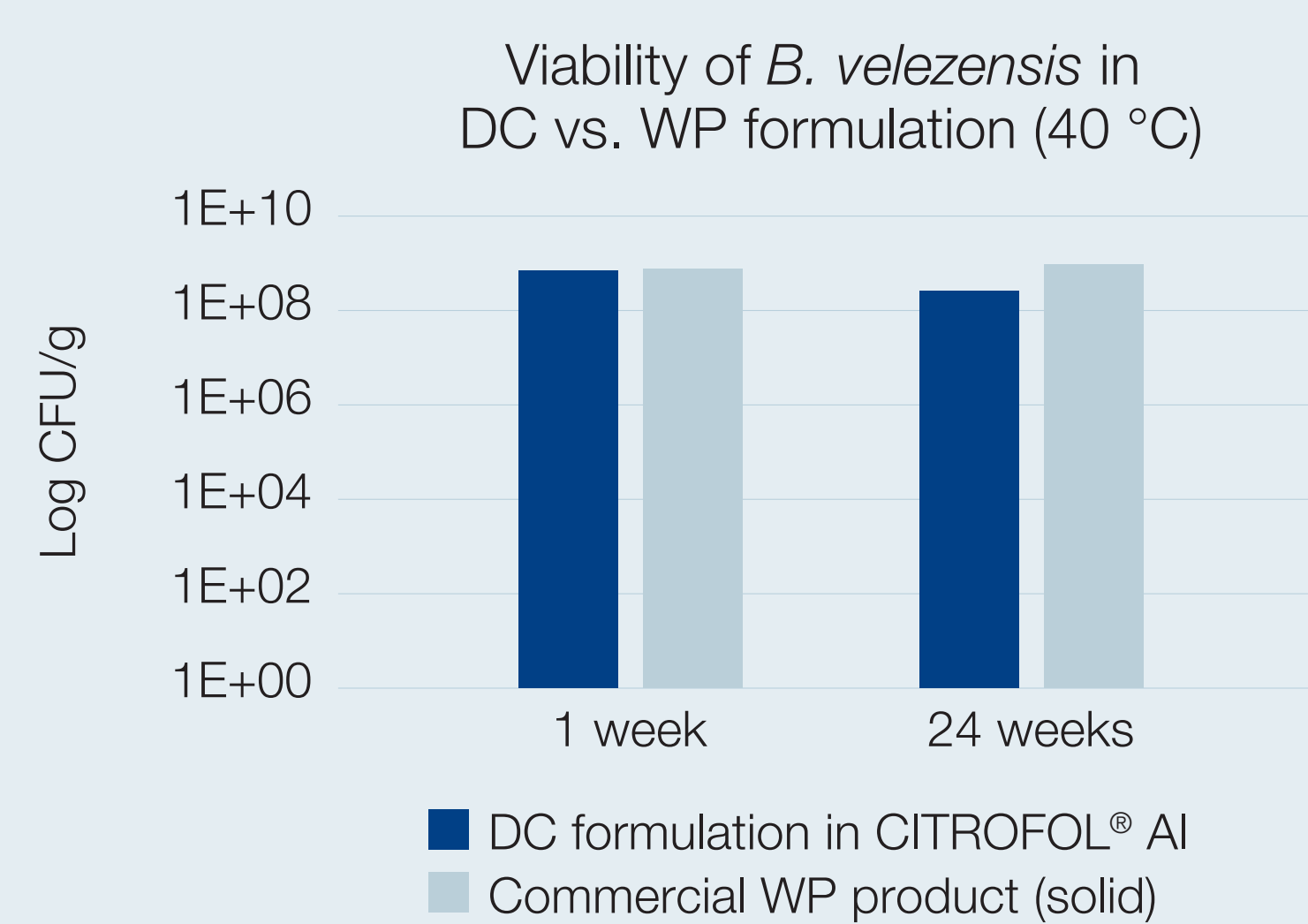


Fig. 2: Comparison of cell viability of Gram positive bacteria in non-aqueous liquid and solid product format

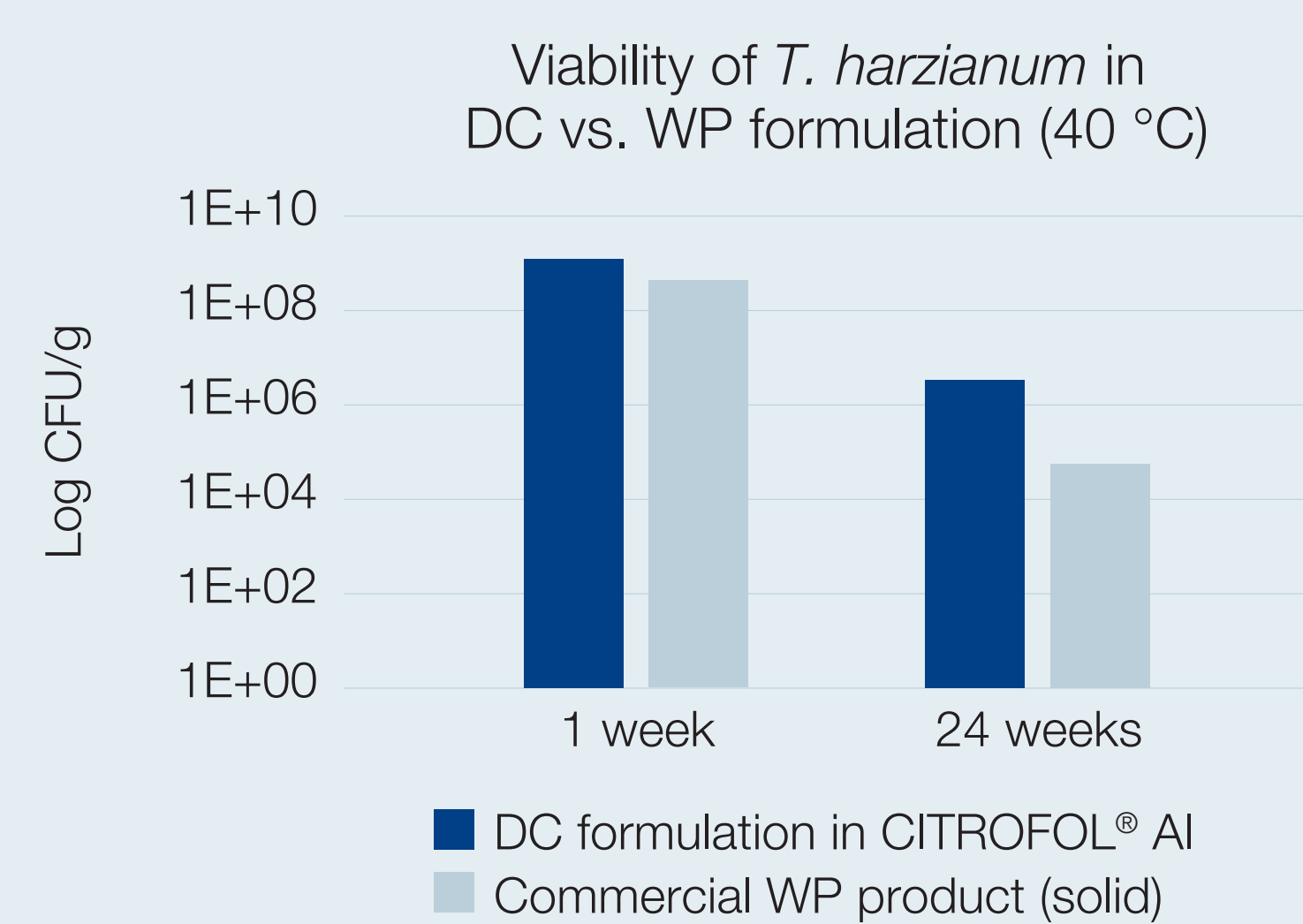


Fig. 3: Comparison of cell viability of fungi in non-aqueous liquid and solid product format

B. velezensis generally showed a high robustness during storage. Comparing liquid product formats, the decline in cell viability was approximately one order of magnitude higher in the SC compared to the DC formulation in CITROFOL® AI (Fig. 1). The cell viability in the WP was most stable (Fig. 2).

T. harzianum generally was highly susceptible during storage. However, the cell viability after 24 weeks was two orders of magnitude higher when formulated as DC in CITROFOL® AI compared to the WP (Fig. 3).

Plant compatibility

No phytotoxic symptoms (discolouration, necrosis) nor a significant reduction in fresh biomass were observed in maize sprayed with CITROFOL® AI solution.

Conclusion

- Jungbunzlauer's CITROFOL® AI (triethyl citrate) has favourable characteristics as a co-formulant for liquid microbial products
- CITROFOL® AI shows excellent bio-compatibility with microbial agents and plants
- CITROFOL® AI improves the shelf life of liquid formulations of *B. velezensis* and *T. harzianum*

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