

NOVEL METABOLITE COMBINATION ENHANCES CROP NUTRITION

Microalgae represent a vast and largely untapped source of bioactive secondary metabolites with the potential to optimize crop growth and resilience (Ördög et al., 2012). TrueAlgae has harnessed this potential in its **TrueSolum**[®] product. TrueSolum is a liquid metabolite mixture produced when growing microalgae. The final product contains no algae biomass, only the valuable metabolites excreted into the water where the algae grow. This unique approach leverages the benefits of algae without the challenges associated with using algal biomass.

Metabolite Characterization and Potential Impacts

Recent research with **Virginia Tech** (Polytechnic Institute and State University), identified specific bioactive metabolites in a novel, consistent, and proprietary combination in TrueSolum using both GC-MS (EC) and LC-MS (RH). Homology searches for compounds with specific peaks were conducted to identify similar structures, probable molecular classes, and/or potential metabolites in various databases. A complex mixture of compounds in the following chemical groups were discovered: 9 small peptides, 4 eicosanoids, 6 octadecanoids, 4 fatty acids, 8 phenolic acids, 2-3 coumarins. In addition, the metabolites that were discovered were consistent across multiple production batches and observed in a consistent ratio between each other. Many of these compounds are known chemical signalers which elicit key microbes in the soil microbiome^{1,2}. This activity is in specific correspondence with the effects of TrueSolum that we have proven through third party research and field trials in agriculture.

1. Novel Compounds (LC-MS Analysis)

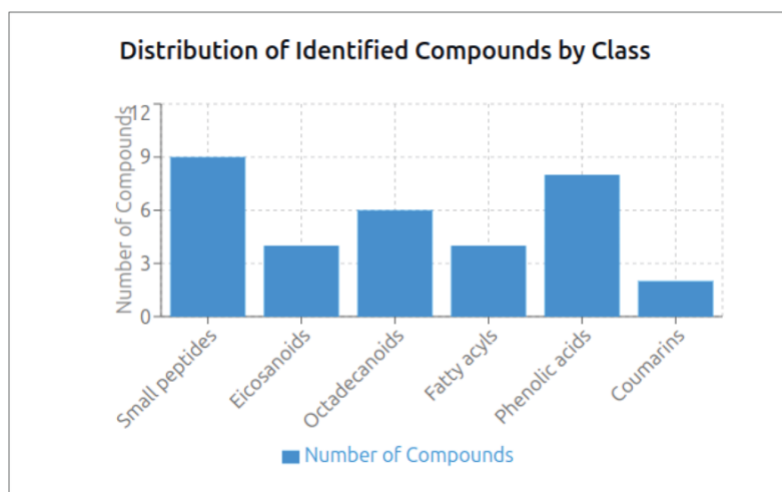


Figure 3: Distribution of identified compounds by their chemical class, highlighting the diversity of metabolites found in the sample.

1 Identified compounds may stimulate beneficial soil microbes (Subramanian et al., 2007; Scharf et al., 2016)
 2 Potential synergistic effects between chemical and biological components (Mathieu et al., 2021)

Compound 1: Flavonoid Glycoside - Quercetin

- Consistent presence across multiple production batches (Collakova & Helm, 2024)
- **Potential roles:**
 - Stress resistance enhancement (Agati et al., 2012)
 - Growth regulation (Winkel-Shirley, 2002)
 - Microbiome signaling (Subramanian et al., 2007)

Compound 2: Linolenic Acid Derivative

- Observed in consistent ratio with the quercetin peak across several batches (Collakova & Helm, 2024)
- **Potential roles:**
 - Microbial communication enhancement (Scharf et al., 2016)
 - Plant defense signaling (Wasternack & Hause, 2013)
 - Soil structure improvement (Chenu, 1989)

2. Additional Components

Polysaccharides

- **Discovery Method:** Identified through freeze-drying analysis (Collakova & Helm, 2024)
- **Characteristics:** Partially insoluble in water/acetonitrile (Collakova & Helm, 2024)
- **Potential roles:**
 - Potential antioxidant and signaling properties (Stirk et al., 2013)
 - May promote microbial growth and plant health (Helm et al., 2022)

Hydroxy-octadecatrienoic acids (HOTrE derivatives)

- **Discovery Method:** Identified through mass spectral analysis (Collakova & Helm, 2024)
- **Characteristics:** Multiple isomers (Collakova & Helm, 2024)
- **Potential roles:**
 - Lipid-based signaling molecules (Scharf et al., 2016)
 - Microbial quorum sensing modulators (Stirk et al., 2013)

3. Soil and Microbiome Interactions

- **Microbial Growth Stimulation:** Metabolites like flavonoids and polysaccharides enhance beneficial microbial populations, fostering soil health.
- **Nutrient Uptake:** Compounds produced by algae improve the bioavailability of essential nutrients like iron, zinc, and manganese, supporting robust plant development (Mathieu et al., 2021).

Conclusion

The discovery of the specific metabolites contained in TrueSolum through this comprehensive analysis revealed multiple bioactive components. The novel combination of quercetin glycoside and a derivative of linolenic acid seem to work synergistically with each other and the other biological elements found in the product to enhance soil biological activity and plant health. Previous research has shown TrueSolum's impact on the soil microbiome through signaling specific bacteria to proliferate, creating the appropriate environment to help release nutrients from the soil for uptake by the crop. Both quercetin and linolenic acid are known to aid in microbial communication and signaling. The presence of polysaccharides and other bioactive compounds aligns with current understanding of algal metabolites' roles in plant growth promotion (Ördög et al., 2012; Stirk et al., 2013).

References

- Agati, G., Azzarello, E., Pollastri, S., & Tattini, M. (2012). Flavonoids as antioxidants in plants: Location and functional significance. *Plant Science*, 196, 67-76.
- Chenu, C. (1989). Influence of a fungal polysaccharide, scleroglucan, on clay microstructures. *Soil Biology and Biochemistry*, 21(2), 299-305.
- Collakova, E., & Helm, R. F. (2024). Identification of Active Metabolites in TrueSolum. Virginia Tech Research Report, Department of Biochemistry.
- Helm, R. F., Koenig, R. L., & Stratton, J. (2022). The use of an algae-based biostimulant for enhancing soybean growth and yield. *Frontiers in Plant Science*, 13, 856243.
- Mathieu, S., Cusant, L., Leborgne-Castel, N., Herouart, D., & Gianinazzi-Pearson, V. (2021). A seaweed extract-based biostimulant promotes maize growth and yield by modulating the soil microbiome and nutrient availability. *Frontiers in Plant Science*, 12, 730011.
- Ördög, V., Stirk, W. A., Lenobel, R., Bancířová, M., Strnad, M., van Staden, J., ... & Szabó, K. (2012). Screening microalgae for some potentially useful agricultural and pharmaceutical secondary metabolites. *Journal of Applied Phycology*, 25(3), 943-955.
- Scharf, B. E., Hynes, M. F., & Alexandre, G. M. (2016). Chemotaxis signaling systems in model beneficial plant-bacteria associations. *Plant Molecular Biology*, 90(6), 549-559.
- Stirk, W. A., Bálint, P., Tarkowská, D., Novák, O., Strnad, M., Ördög, V., & Van Staden, J. (2013). Hormone profiles in microalgae: Gibberellins and brassinosteroids. *Plant Physiology and Biochemistry*, 70, 348-353.
- Subramanian, S., Stacey, G., & Yu, O. (2007). Arabidopsis plants overexpressing a novel transcription factor, GmNAC6, are hypersensitive to stress. *Plant Physiology*, 144(4), 2054-2068.
- Treutter, D. (2006). Significance of flavonoids in plant resistance and enhancement of their biosynthesis. *Plant Biology*, 8(6), 706-711.
- Wasternack, C., & Hause, B. (2013). Jasmonates: biosynthesis, perception, signal transduction and action in plant stress response, growth and development. An update to the 2007 review in *Annals of Botany*. *Annals of Botany*, 111(6), 1021-1058.
- Winkel-Shirley, B. (2002). Biosynthesis of flavonoids and effects of stress. *Current Opinion in Plant Biology*, 5(3), 218-223.