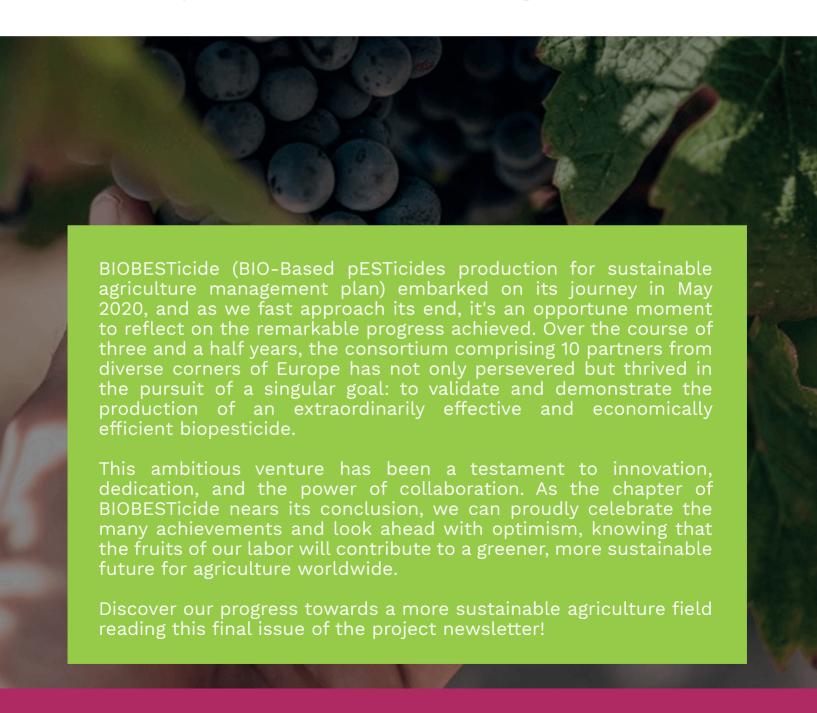


# **BIOBESTicide**

BIO-Based pESTicides production for sustainable agriculture



# **Pythium oligandrum** strain I-5180 growth and scale-up



Greencell, a leader in the field of fermentation, is spearheading the BIOBESTicide project. In WP2, we focus on optimizing the production of *Pythium Oligandrum* using a co-product derived from sugarcane molasses. The goal of this work package is to demonstrate Greencell's capacity for industrial-scale production, for which a dedicated facility has been exclusively constructed for the production of *Pythium Oligandrum* oospores.

Quantifying the oospores in the final product is a crucial step that we have worked on, utilizing cutting-edge technologies such as digital PCR and flow cytometry. This ensures precision and accuracy in determining the quantity of intact and viable oospores in the product. Furthermore, the viability of these oospores has been expertly managed through a combination of techniques. The staining method using Trypan Blue, coupled with plasmolysis, has proven to be a reliable approach. This guarantees that the oospores are not only produced in abundance, but also in their most potent and effective state. This integration of state-of-the-art techniques and sustainable practices positions. Greencell at the forefront of biocontrol solutions.

### Formulation of the biopesticide product

WP3: For WP3 most activities are already concluded, as the bulk of them were to formulate the PO for subsequent field use. For this reason, in the last part of the project we have been focused on providing any specific amount of formulation required by our partners for lab and field trials. Apart from this on-demand task, we have also run a mid-size scale up (circa 5kg) of the liquid P.O. formulation to spot



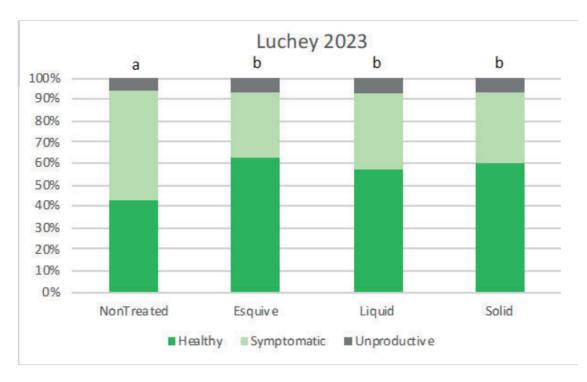
any potential production issue for future formulation manufacturing at plant scale. This has been done to stress the formulated system and check for potential rheological or processing issues that might arise when the liquid formulation is produced at a larger scale. Tests were positive with no problems for the making of the liquid PO formulation, therefore we would be not expecting issues at plant scale too.

# Controlled condition trials, field application and validation of the developed biopesticide

The last year of the project allowed us to obtain the latest results for the BBI project on the efficacy of the biopesticides developed. Different formulations of the biopesticide were tested in greenhouse by INRAE, and the results revealed that 2 formulations, designed by LAMBERTI, reduce pathogen necrosis by up to 80%. These two formulations were tested on nursery by MERCIER, and results obtained confirmed a significant reduction of natural contamination of the pathogen in comparison with non-treated vines. Furthermore, the last fieldtrip highlighted the efficacy of these two formulations in one of the two Bordeaux sites, at Château Luchey-Halde, with a reduction in the expression of Esca symptoms on vines for the last year of treatment, with the same efficacy of the market reference Esquive®.







Percentage of healthy, symptomatic, unproductive vines per treatment at Château Luchey for the year 2023. With non-treated vines, vines treated with the Esquive product (reference product by Agrauxine®) or treated by the liquid or solid Pythium oligandrum biopesticide developed by LAMBERTI. Bars with the same letter are not statistically different ( $\chi$ 2, P < 0.05). These formulations were tested for their viability, and after a dPCR analysis, RNA of P. oligandrum oospores was detected in oldest solid formulation (17 months) but not in the liquid one, perhaps highlighting a better conservation of the solid formulation over time. Finally, no major impact on microbial communities was underlined after the use of these biopesticides on greenhouse experimentations.

## **Products registration at EU level**

For the WP5 of the BIOBESTicide project, EURION Consulting has coordinated and built the approval dossier for *Pythium oligandrum* strain I-5180 according to the data requirements of the Regulation (EC) n°283/2013 (i.e., data requirement on



the active substance) and the Regulation (EC) n°284/2013 (i.e., data requirement on the formulated product), in line with the Regulations (EC) n°1107/2009 concerning the placing of plant protection products on the market. This approval dossier has been sent to the Belgium authorities acting as Rapporteur Member State (RMS) at EU level. After the commenting period and completion of the dossier with further data (managed by EURION Consulting). RMS has edited the final version of the DAR which was sent to EFSA (European Food Safety Authority), in charge of the «quality check» of this DAR prepared by RMS. The public version of the DAR, validated by EURION Consulting and EFSA, was published for the "public consultations" period. Due to questions/remarks highlighted during this public consultation, additional data was sent by EURION Consulting to EFSA. Waiting for the final EFSA conclusions for the approval of Pythium oligandrum strain I-5180 at EU level, EURION Consulting has coordinated and built the registration dossier for the biopesticide which will be sent in EU countries in order to obtain the marketing authorizations, as soon as Pvthium oligandrum strain I-5180 is approved in EU.



#### Environmental, social and economic assessment

In WP6 TECNALIA evaluated the BIOBESTicide formulations from a sustainability point of view, which includes the assessment of the environmental, economic, and social performance. The results show that from the environmental point of view, the use of biopesticides entail lower impacts due to the avoidance of harmful chemicals during the manufacturing and especially due to the impacts potentially avoided during the use stage. These are impacts produced in the human toxicity and ecotoxicity categories. In terms of cost, with the

preliminary information used, it is seen that optimization studies and the economy of scale could improve the performance of the results. However, initial cost-effectiveness and cost analyses are promising. From the social point of view, the avoidance of impacts to the users contributes significantly to the better performance. The potential benefits of biopesticides based on oospores are promising. By promoting the use of these biopesticides, we can reduce the negative environmental impacts associated conventional pesticides, such as water and soil contamination. Furthermore, the improved health outcomes for users contribute to a safer working environment and a healthier society as a whole.



#### **Exploitation and Dissemination**

CiaoTech, as leader of WP7: Exploitation and Dissemination, performed a new market stakeholders analysis to update and expand the results delivered at M24. The analysis carried out led to the identification of new patents and new EU-funded projects in the field of biocontrol agents and biopesticides. The additional potential stakeholders for the BIOBESTicide value chain that were identified from these, expanded the list to a total of 288 stakeholders, including new biomass providers, biopesticide producers and end users. These new insights will be integrated into the Final Exploitation Plan and the Final Business Plan that will be delivered at the end of the project. In addition. CiaoTech also coordinated Dissemination & Communication strategy, aimed at boosting the visibility of the project results.

#### Want to know more about BIOBESTicide?

Visit the project website and follow us on LinkedIn and Twitter!

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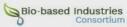


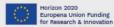
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This project has received funding from the Biobased Industries Joint Undertaking (JU) under the European Union's Horizon 2020 research and innovation programme under grant agreement N° 886776. The JU receives support from the European Union's Horizon 2020 research and innovation programme and the Bio-based Industries Consortium.