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EUROPEAN FORUM FOR AGRICULTURAL AND RURAL ADVISORY SERVICES

Collaborative Online Forum For Exchange & Engagement

in collaboration with
EXCALIBUR project

"Tools and knowledge for boosting soil biodiversity and bioinoculant application in agriculture"

speakers:

- Stefano Mocali (CREA)
- Loredana Canfora (CREA)
- Maria Grazia Tommasini (Ri.Nova)

moderator:

- Anita Dzelme (EUFRAS)

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Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria

EXCALIBUR

Exploiting the multifunctional potential of belowground biodiversity in horticolture farming

Stefano Mocali

CREA - Agricoltura Ambiente, Firenze, Italy

EXCALIBUR

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 817946

Funded by the Horizon 2020 Framework Programme of the European Union

EXCALIBUR

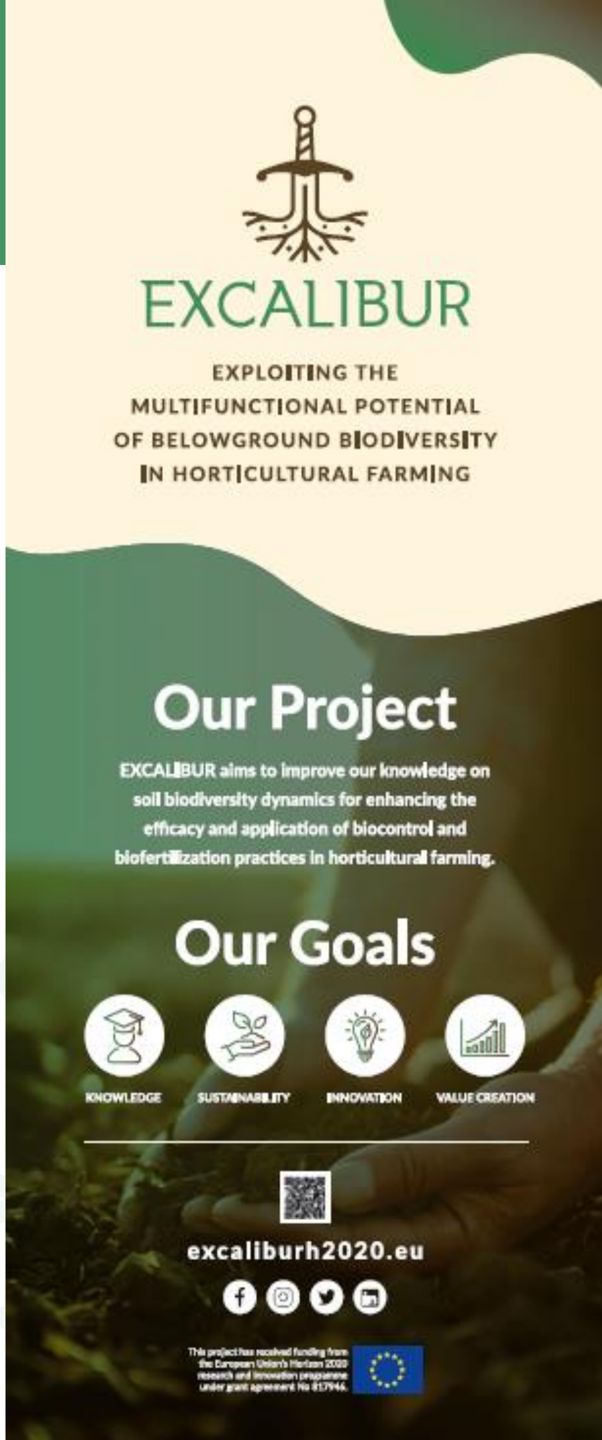
- EXCALIBUR overview (S. Mocali, CREA) (~15min)
- OUTCOMES from the field (M.G. Tommasini, RINOVA) (~20min)
- Main INNOVATIONS (L. Canfora, CREA) (~10min)

MORE INFO https://excaliburh2020.eu

The EXCALIBUR project

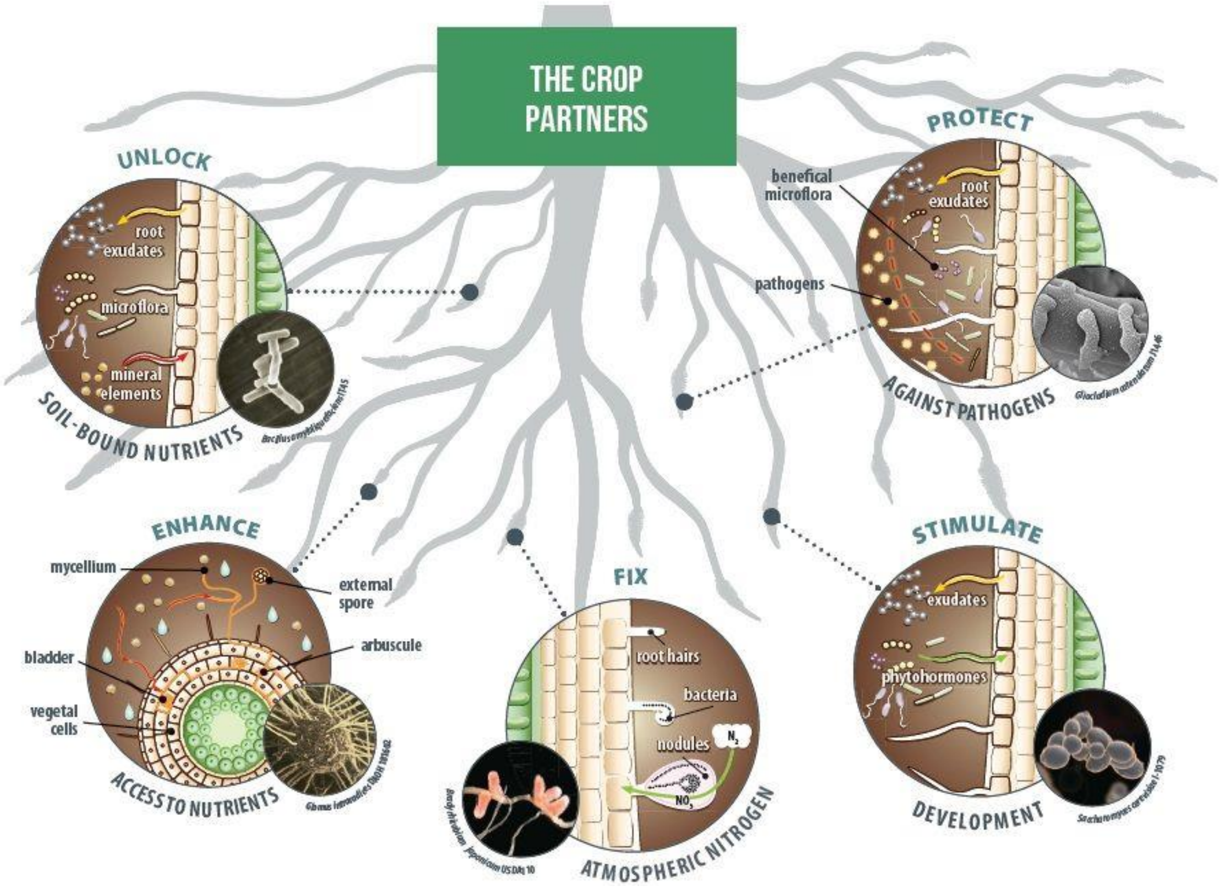
Overall primary objective: the main purpose of the project is to improve the knowledge on soil biodiversity dynamics in relation to different agro-ecological factors, for enhancing the efficacy of biocontrol and biofertilization practices in horticultural farming (tomato, apple, strawberry) by using multifunctional bioinoculants.

Provision of tools and knowledge to boost soil biodiversity and bioinoculants application in horticulture



Microbial inoculants in agriculture

Plant growth-promoting microorganisms (PGPM) are a key group of predominantly bacteria and fungi that contribute significantly to enhancing plant growth through various mechanisms.



Source: https://www.lallemandplantcare.com

Microbial inoculants are defined as bacteria and fungi that are introduced into an environment to perform a specific function such as:

1) **BIOFERTILIZERS** as alternatives to conventional inorganic fertilizers
2) **BIOPESTICIDES** as alternatives to synthetic pesticides
3) **BIOSTIMULANTS** to improve plant resistance to stress, but also to stimulate natural mechanisms to enhance crop yield and quality.

Canfora, L., et al (2021). https://doi.org/10.3390/agriculture11020158

EXCALIBUR's main goals

- Expand the agro-ecological **knowledge** base on the links and dynamics between soil biodiversity and agricultural production
- Enhance the efficacy of biocontrol and biofertilization practices in horticultural farming by using **new multifunctional bioinoculants**
- Value creation: we expect a **reduction on external chemical inputs** of at least 10-30% (depending on crops, soil characteristics and pedoclimatic conditions).
- A **Decision Support System** (DSS) was developed in conjunction with partners and stakeholders to help farmers to adopt a biodiversity-focused soil management.
- Bioindicators and molecular **diagnostic tools** for monitoring the persistence of bio-inocula and their impact on soil and plant-associated biodiversity.
- Development of a molecular **diagnostic kit** to profile soil microbial diversity.

Mentimeter

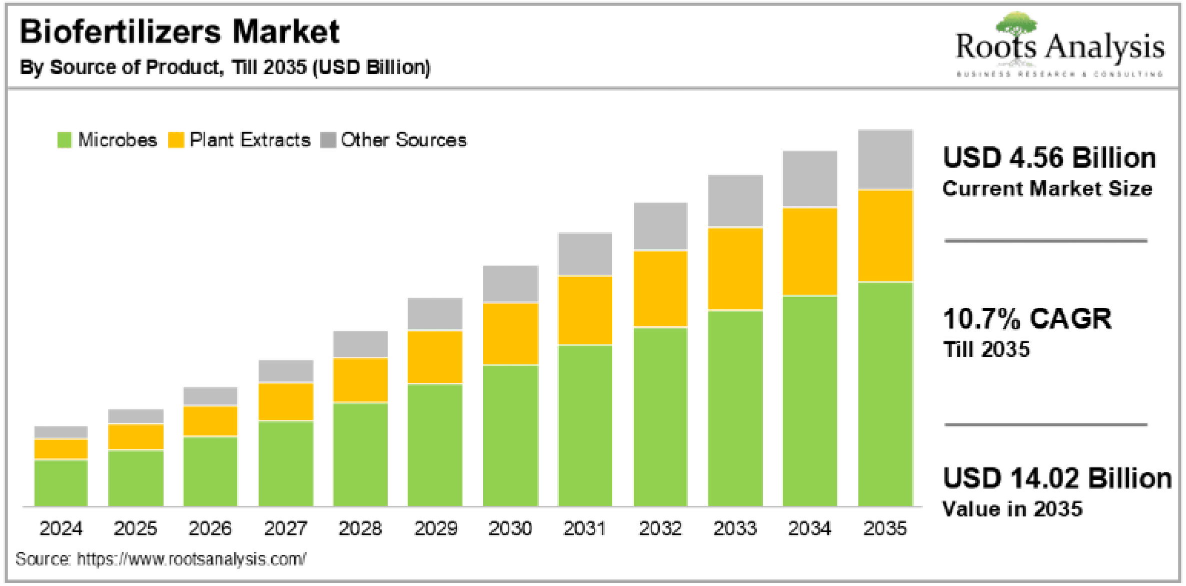
1. Where do you come from?
2. Are you familiar with microbial inoculants?
3. Have you ever recommended the application of any bioinoculant?
4. Indicate which are the main limitations: [high cost, low reliability, low performance, low knowledge, application protocol]
5. Which are the expectations?

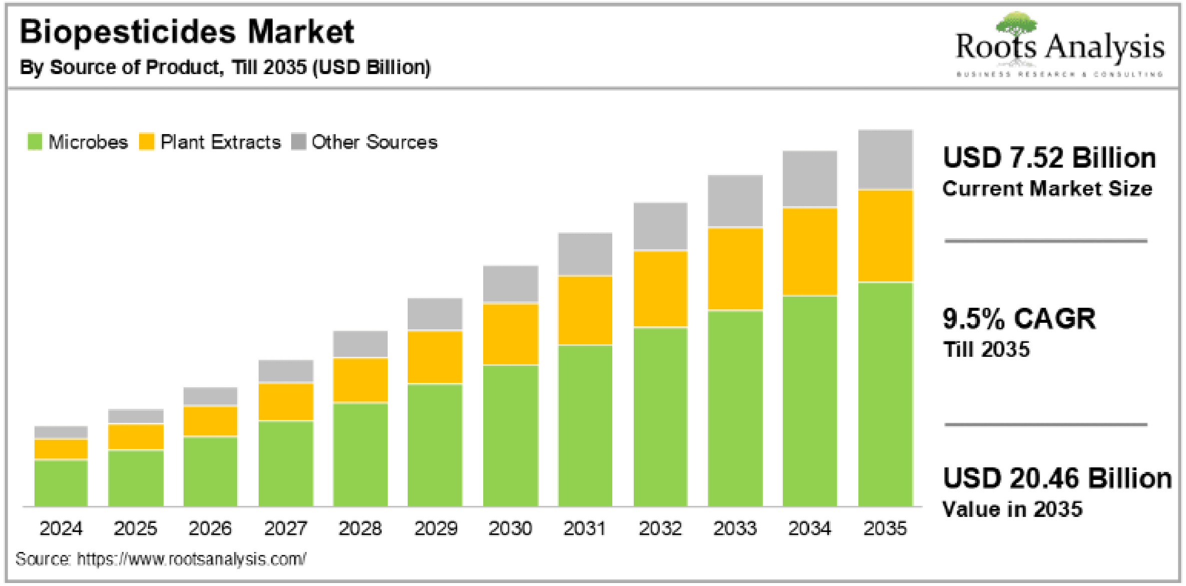
TO BE COMPLETED ONLINE!!!!

https://www.menti.com/alen9qopnq1q

Microbial inoculants in agriculture

The application of **microbial inoculants** in agricolture represents a promising option to reduce chemical inputs.



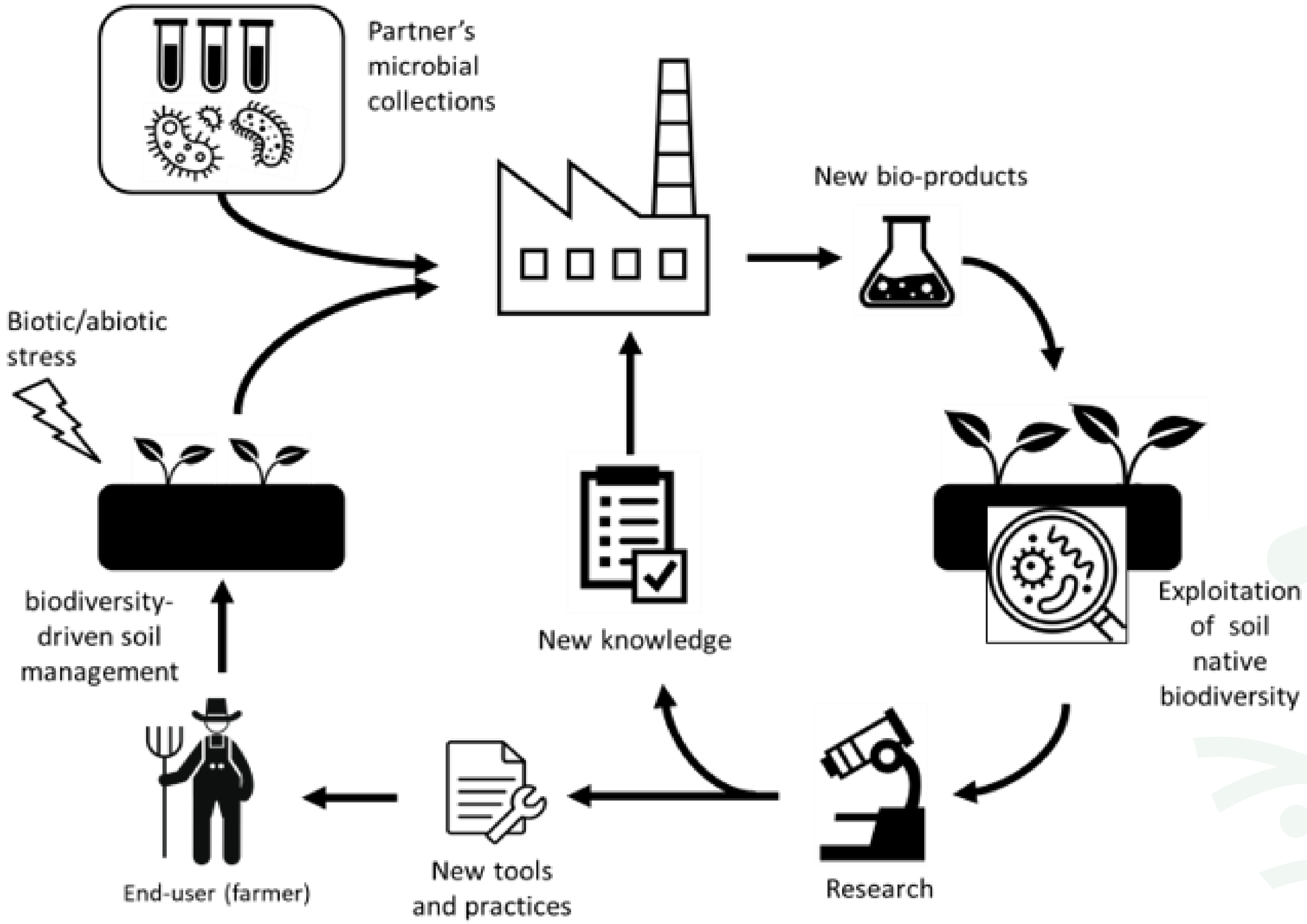


The global **biofertilizers** market size is projected to grow from US$4.56 billion in 2024 to US$14.02 billion by 2035

The global **biopesticides** market size is projected to grow from US$7.52 billion in 2024 to US$20.46 billion by 2035

Their efficacy in the field is still limited and heterogeneous

The strategy



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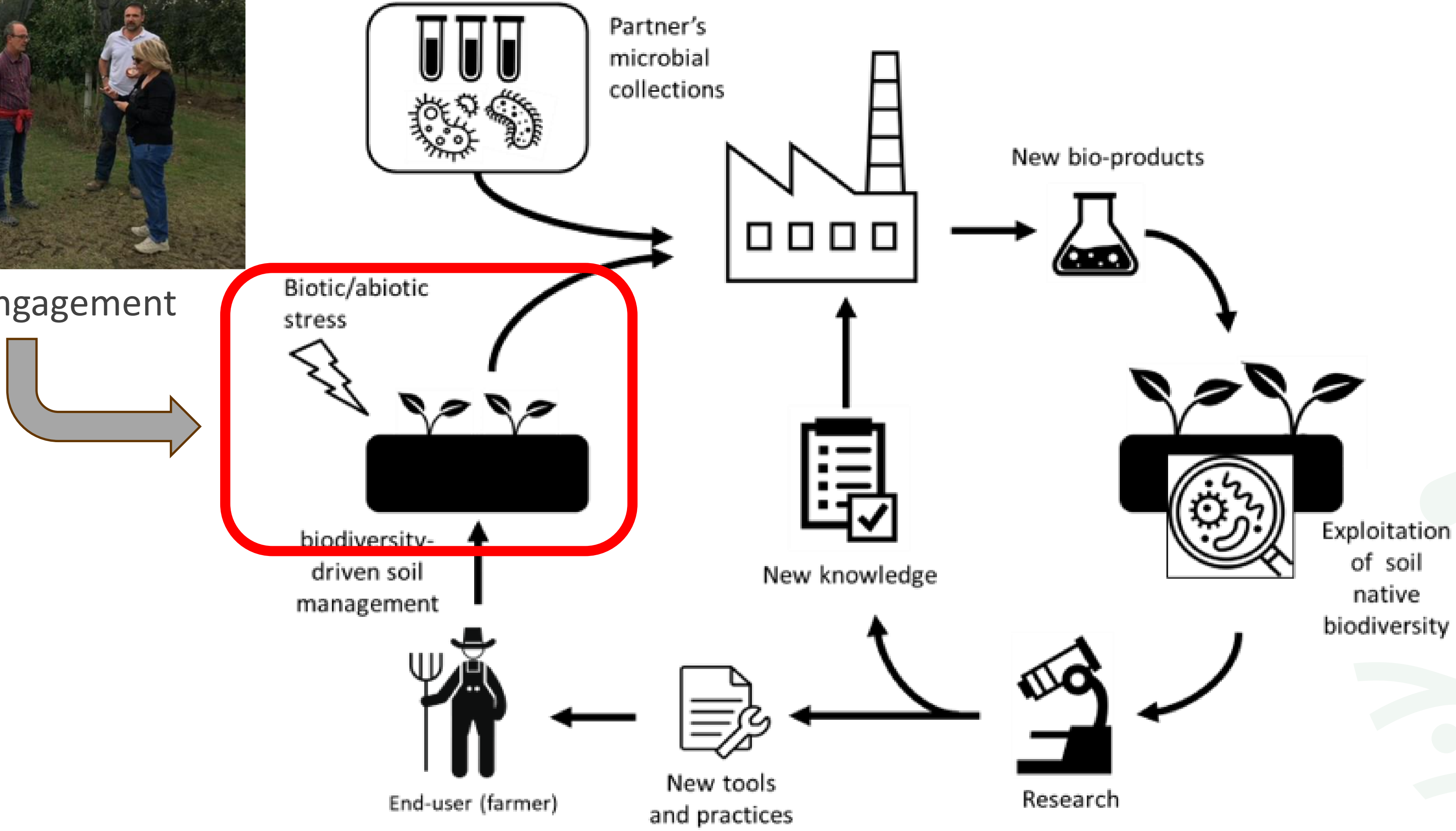
The strategy

Farmers engagement



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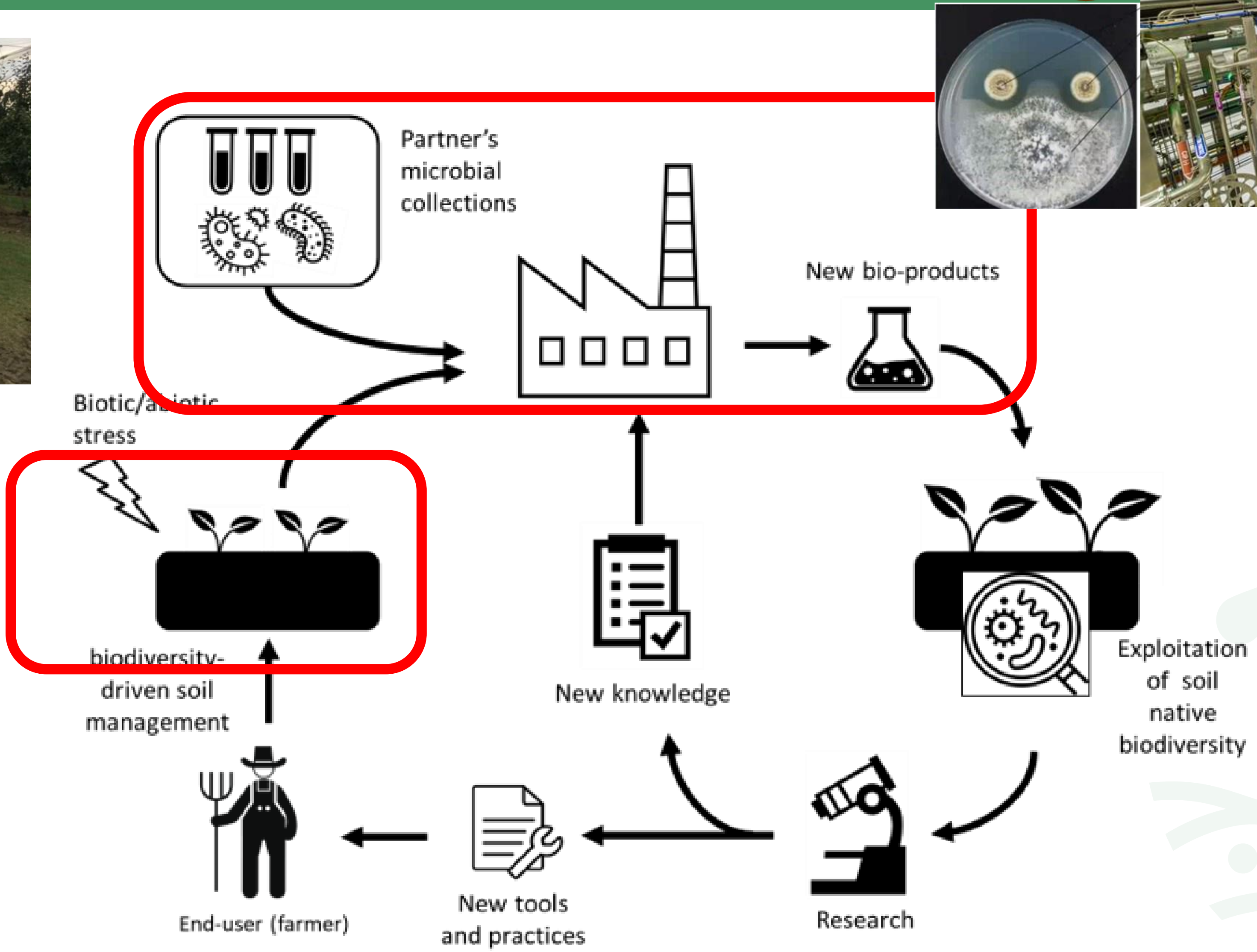
The strategy

EXCALIBUR

1

Farmers engagement



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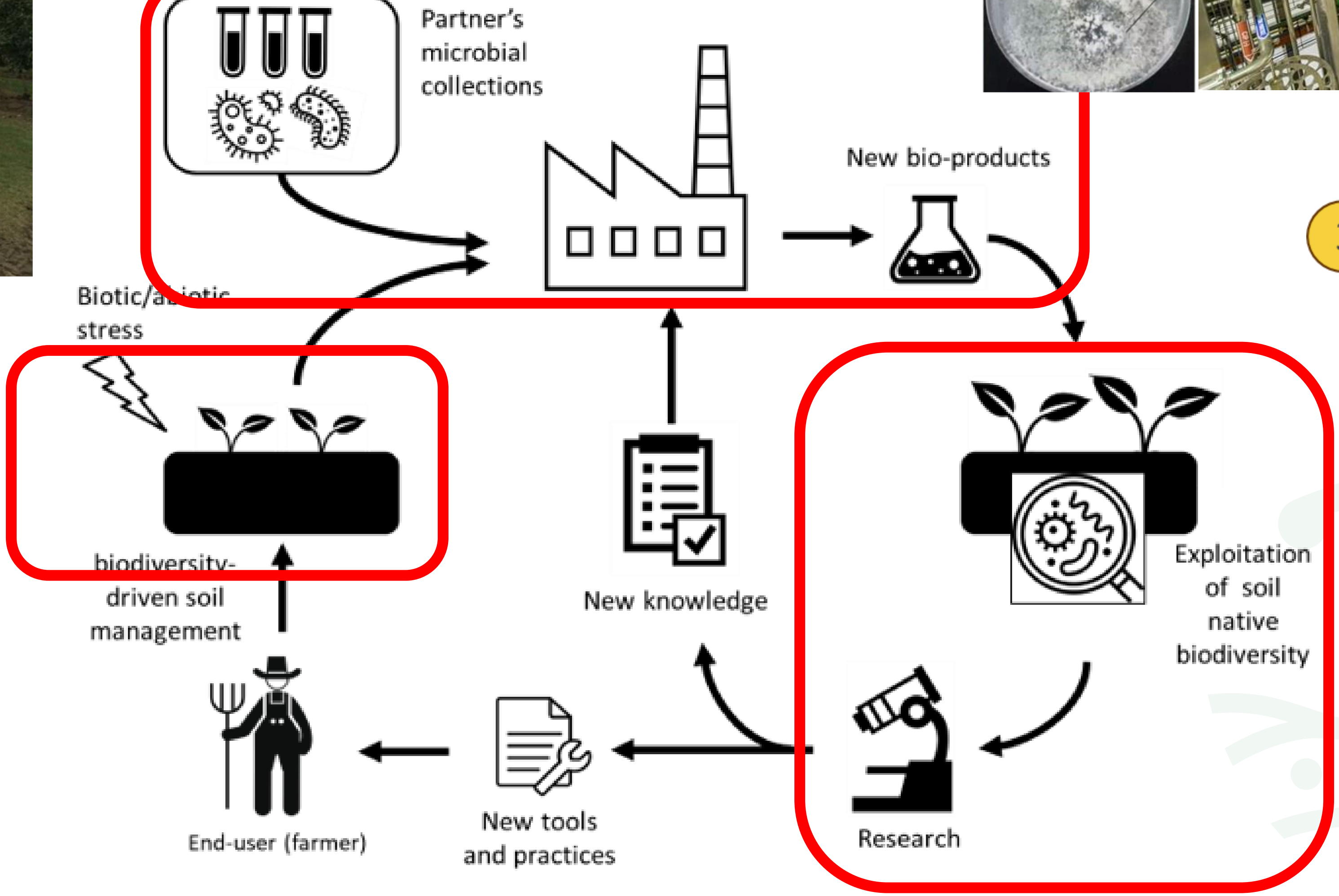
The strategy

1

Farmers engagement



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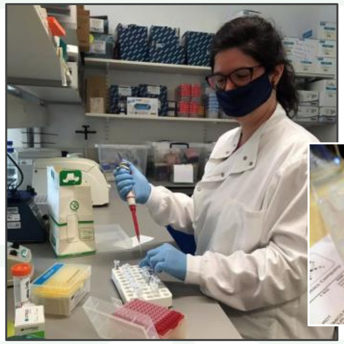

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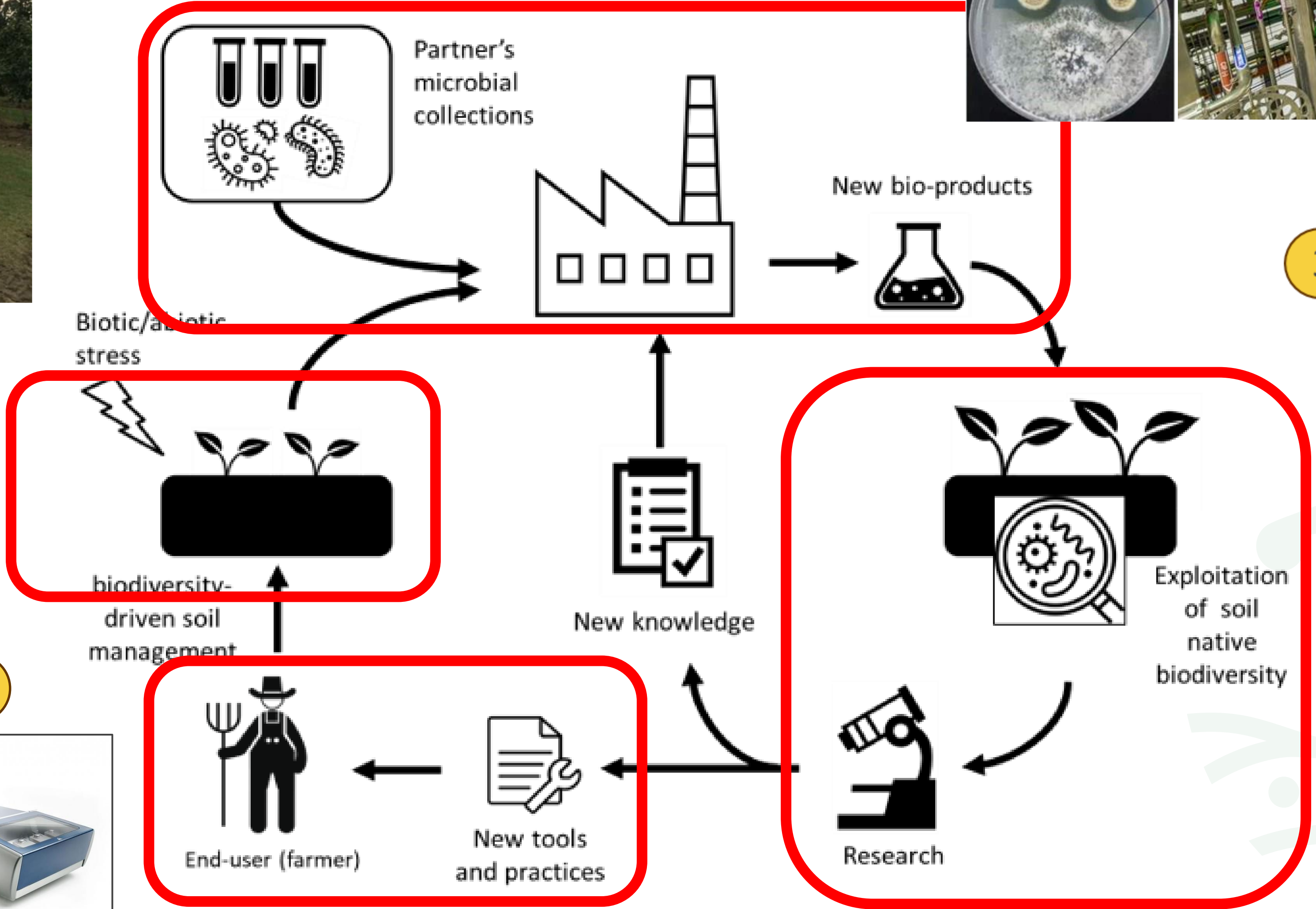
The strategy

EXCALIBUR

1

Farmers engagement

2



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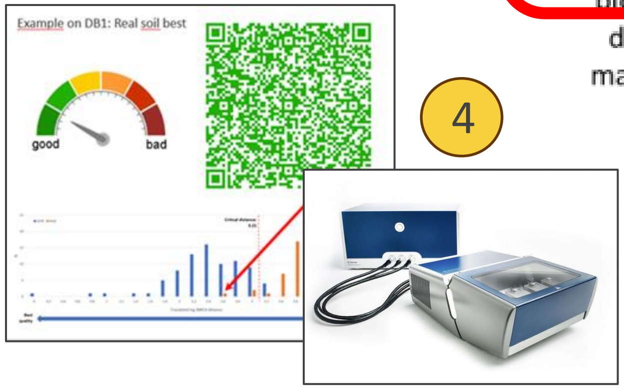

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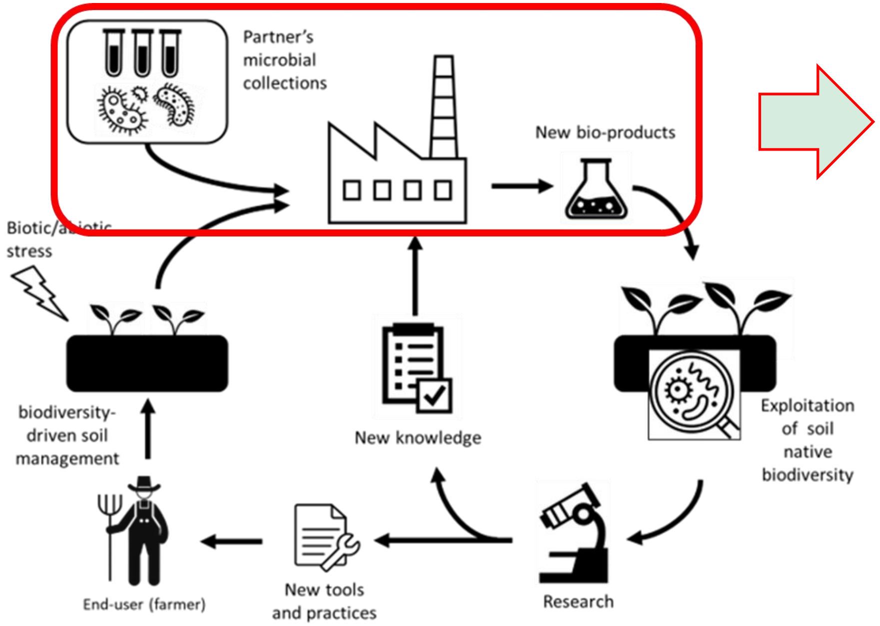
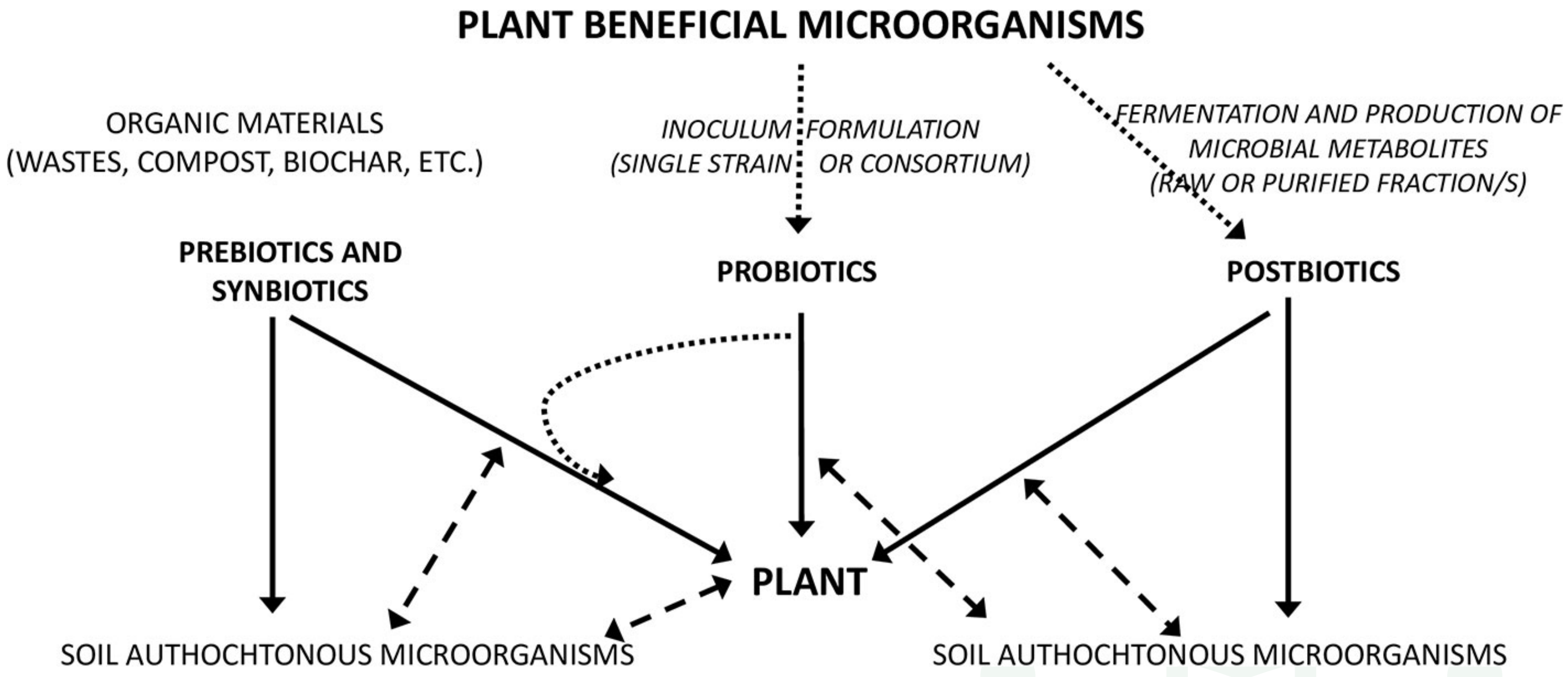
New microbial-based bioinoculants





We 'artificially' promoted soil biological functions and diversity integrating management practices with newly developed formulations containing beneficial microbial bio-inocula ('**probiotic approach**') and bio-effectors ('**prebiotic approach**')

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New microbial-based bioinoculants

The application of **microbial inoculants** in agriculture represents a promising option to reduce chemical inputs.
Their efficacy in the field is still limited and heterogeneous.

- Developed **novel microbial bio-products** to embed benefits of soil biodiversity into farming practices by improving **fermentation and formulation processes.**
- Selected bioproducts were tested under controlled conditions on **tomato, strawberry, apple.**
- Bioproducts that proved to be effective in field conditions were taken to **higher TRL** with the support of industrial partners.

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Overview of bioproducts's application on representative cropping systems in Europe

Maria Grazia Tommasini
Sara Turci

RINOVA gricoltura mbiente limentazione

EUFRAS Coffee break, 27/02/2025

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Main objectives

Evaluate the effect of proposed formulations on agronomic performance under field conditions in both conventional and organic systems

46 bioproducts were tested in the laboratory and **16** were used in field trials.

11 Fungi

- ✓ AMF (Rhea, Asteria, Micosat,…)
- ✓ *Trichoderma* spp.
- ✓ *Metarhizium brunneum*
- ✓ *Clonostachys rosea*
- ✓ *Fusarium oxysporum*
- ✓ *Beauveria brongniartii*

1 Bacteria

Paenibacillus polymyxa

5 Non-microbial biostimulants

- ✓ Stronger
- ✓ GHI SN837
- ✓ Vinassa
- ✓ Bactim
- ✓ Compost

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Plant-soil-microorganism interaction

Field studies on plant responses to the effects of bioinoculants in different pedo-climatic conditions

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3 crops

8 EU countries

Type of trial		Management	Poland INHORT (PL)	IT CRPV (IT)	IT UNITO (IT)	Au TU-GRAZ (AT)	DE FOEKO (DE)	DE KOB (DE)	UK EMR-NIAB (UK)	SI KIS (SI)	DK UCPH (DK)	FR INPlus (FR)	TOT	Tot. f - p	TOT crop
Apple	Biofertilizer (f)	ORGANIC		4AOFf-CRP		2AOFf-TUG	6AOFf-FOE 7AOFf-FOE	8AOFf-KOB					5	8	14
		IPM	1AIFf-INH	5AIFf-CRP		3AIFf-TUG							3		
	Biopesticides (p)	ORGANIC	9AOFp-INH				12AOFp-FOE 13AOFp-FOE	14AOFp-KOB					4	6	
		IPM							10AIFp-NIA 11AIFp-NIA				2		
Strawberry	Biofertilizer (f)	ORGANIC											0	2	11
		IPM		16SIGf-CRP		15SIGf-TUG							2		
	Biopesticides (p)	ORGANIC	18SOFp-INH	25SOGp-CRP						20SOGp-KIS	21SOFp-UCP		4	9	
		IPM	17SIFp-INH		23SIGp-UNI 24SIGp-UNI					19SIGp-KIS	22SIFp-UCP		5		
Tomato	Biofertilizer (f)	ORGANIC										28TOFf-INP 29TOFf-INP	2	4	7
		IPM	26TIFf-INH	27TIFf-CRP									2		
	Biopesticides (p)	ORGANIC		32TOFp-CRP									1	3	
		IPM	30TIFp-INH		31TIGp-UNI								2		
			6	6	3	3	4	2	2	2	2	2	32	32	32

32 trials

14 (Biofertilizers)

18 (Biocontrol)

Biostimulants: Microbials and non microbials

SOIL APPLICATIONS

LEAF APPLICATIONS

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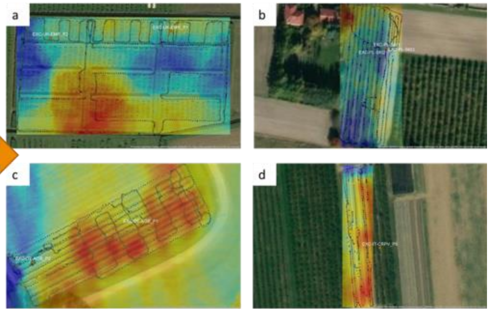
Set-up and rationale of the experimental field trials

Farmers’ engagement

Trials selection



Gradient analysis to guarantee homogenisation

Comparative analysis: meaning and interpretation of results

Evaluation of the effect on soil:

- ✓ Physico-chemical analysis
- ✓ Biochemical analysis
- ✓ DNA-based analyses
- ✓ AMF-root based analysis
- ✓ Analysis of the soil fauna (nematodes, QBS, earthworms)
- ✓ Soil microbial diversity analysis

Analysis of the agronomic performace, looking at:

- ✓ **Agronomics parameters**
- ✓ **Physiological parameters**
- ✓ **Plant health**

Bio-based products application

Trials selection; **baseline** analysis

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In-field trial application: a look at three-years results (2021-2023)

- E.g. the results on the agronomic performance → YIELD



- E.g. the results on the physiological parameters → CROWN and VEGETATIVE DEVELOPMENT



- E.g. the results on the physiological parameters → PLANT BIOMASS



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Efficacy of the biofertilizers (BF) and biopesticides (BP) after 2 years of trials (2021-2022)

Crop	Type of trial	Management	INHORT	RINOVA	UNITO	TUGRAZ	FOEKO	KOB	NIAB	KIS	UCPH	INplus
Apple	BF	Org		😐		😐	😐 😐	😐				
		IPM	😐	😐		😐						
	BP	Org	n.d.				🙂 🙂	😐				
		IPM							😐 😐			
Strawberry	BF	Org										
		IPM		😐		🙂						
	BP	Org	😐	😐						😐	😐	
		IPM	😐		🙂 🙂					😐	😐	
Tomato	BF	Org										😐 🙂
		IPM	😐	😐								
	BP	Org										
		IPM	😐		🙂							

☹️ negative effects compared to CTRL

😐 no difference with CTRL

🙂 positive effects compared to CTRL

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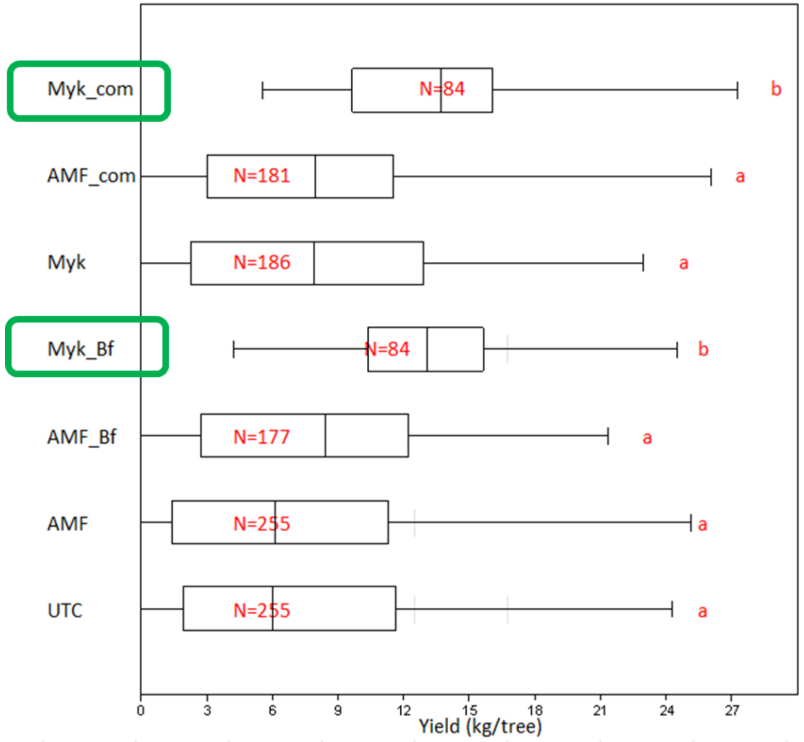
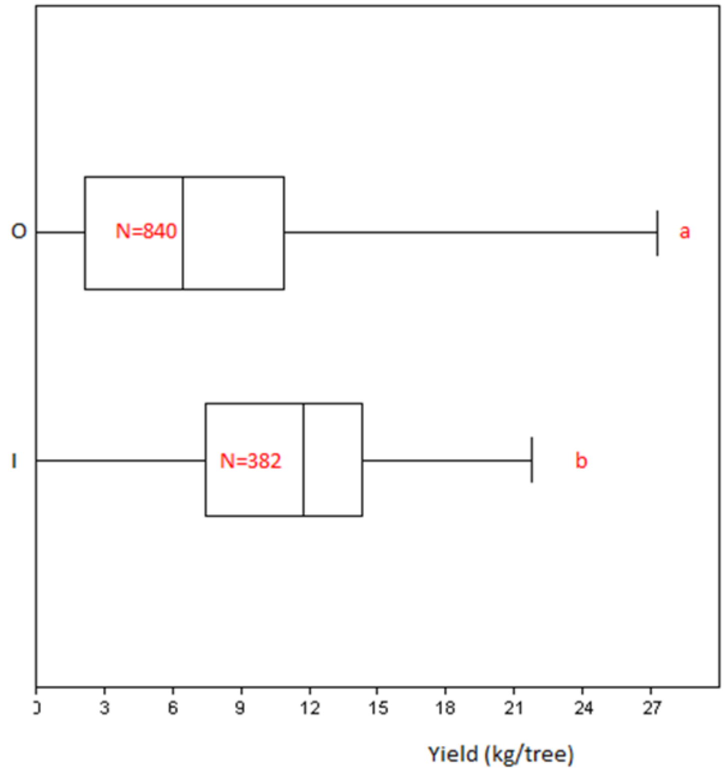
To what extent does the bio-based products affect the yield (Kg/plant)? The showcase of the apple field trial





Myk combined with biostimulants (compost, Stronger, vinassa), has a more significant effect on the **yield**

BUT, **the management** as well has a significant effect: indeed, **the trials in IPM shows a higher yield compared to Organic trials**

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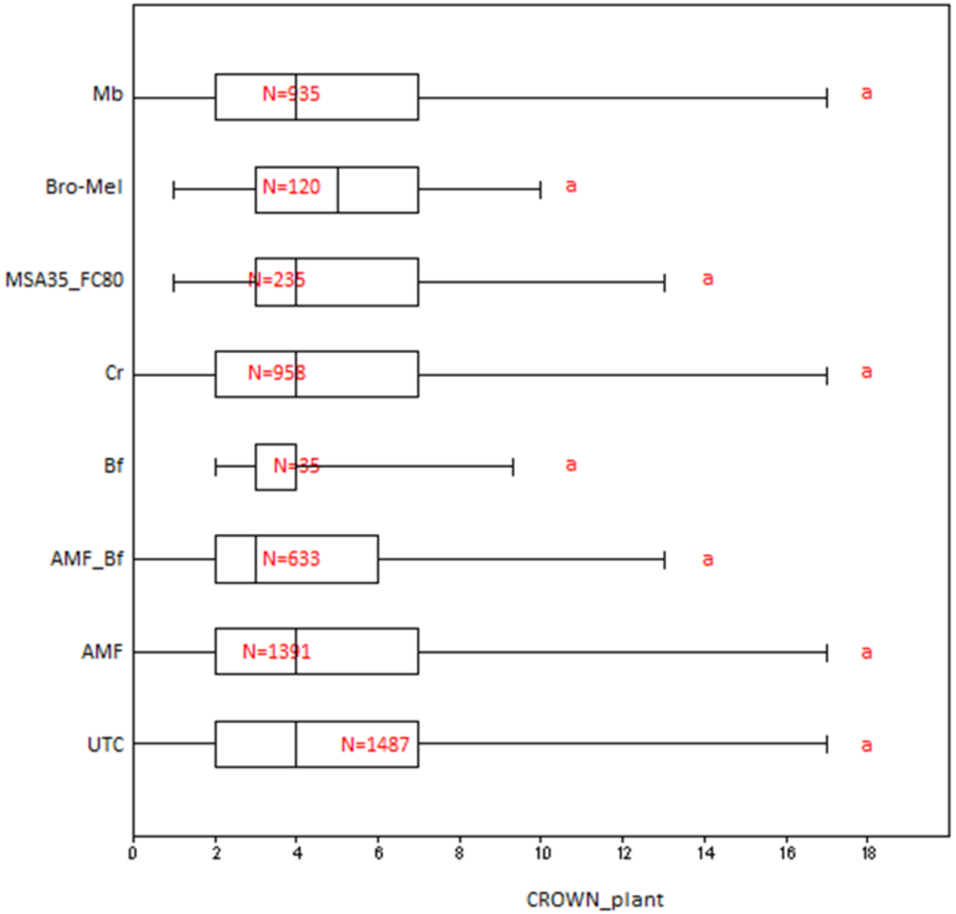
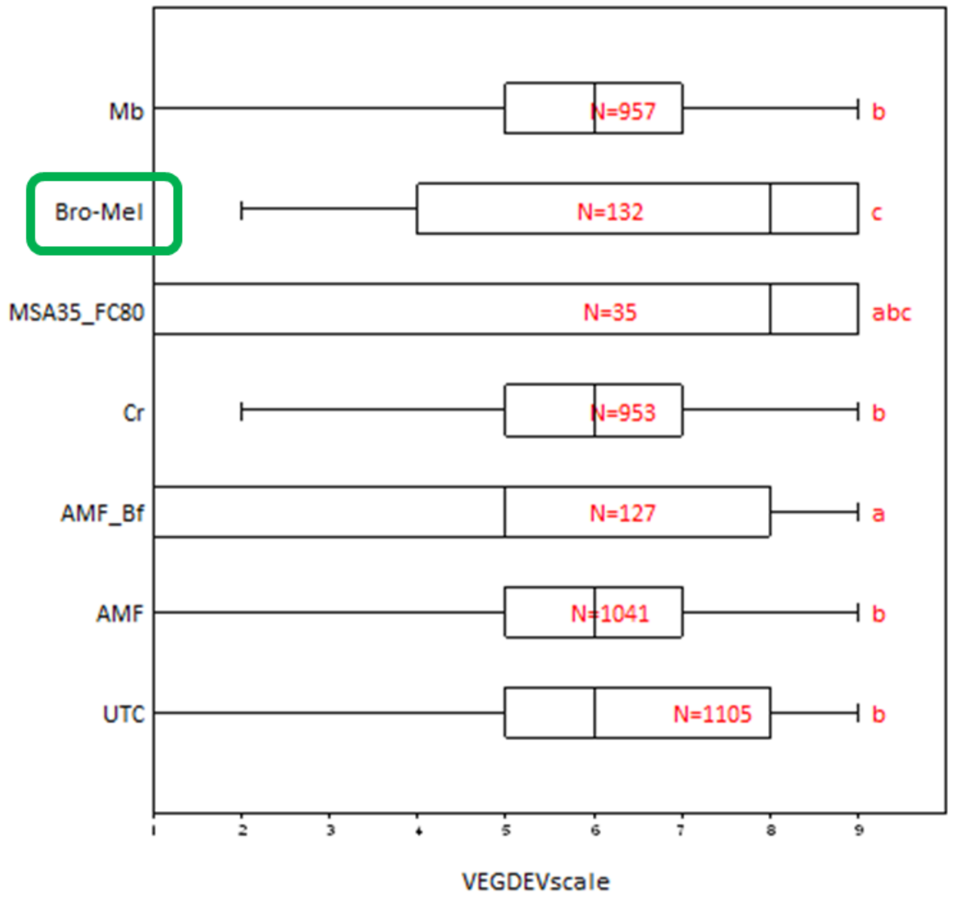
And what about the effect of the strawberry field trial? Look at CROWN and vegetative development (VEGDEV)!





There are no differences on the number of CROWN

Bauveria brongniartii showed a better effect on vegetative development (VEGDEV) than other products and the control

However, the effect of location, management (IPM vs. BIO) and experimental conditions (open field vs. greenhouse) are also significant.

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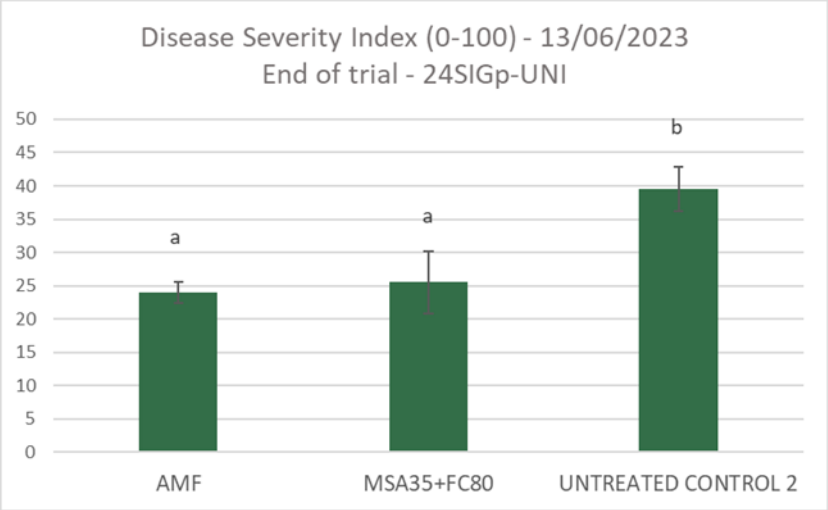
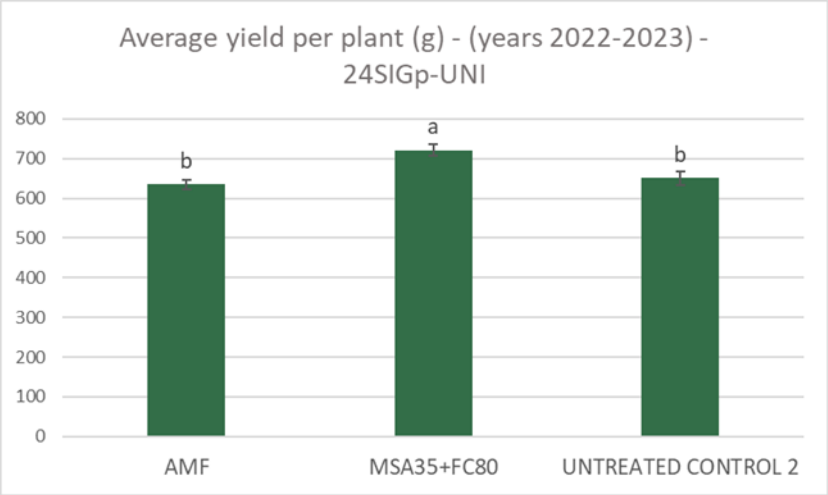
An interesting showcase: Italy (UNITO)





(Tukey p>0.05: different letters correspond to statistically different values)

In 2 trials the **bioinoculants showed a significant reduction** of the **disease severity** of the main pathogen responsible of crown and root (*Rhizoctonia solani*). These results confirm the **efficacy and efficiency** of the **microbial control agent.**

Product	Product Provider
Excalibur consortium AMF Rhea	IN+
Fusarium oxysporum MSA35 + Trichoderma asperellum FC80 (1:1 v/v)	Agroinnova (UNITO)

Plants treated with the **coinocula MSA35+FC80** showed **higher root colonisation rates** by AMF fungi (more than those treated with AMF-based products).

These results seem to suggest that **MSA35+FC80 treatments have an effect in increasing the recruitment of AMF fungi into the root system.**

In addition, it is possible to assume a positive correlation between higher percentage of root mycorrhization and plant health/yield.

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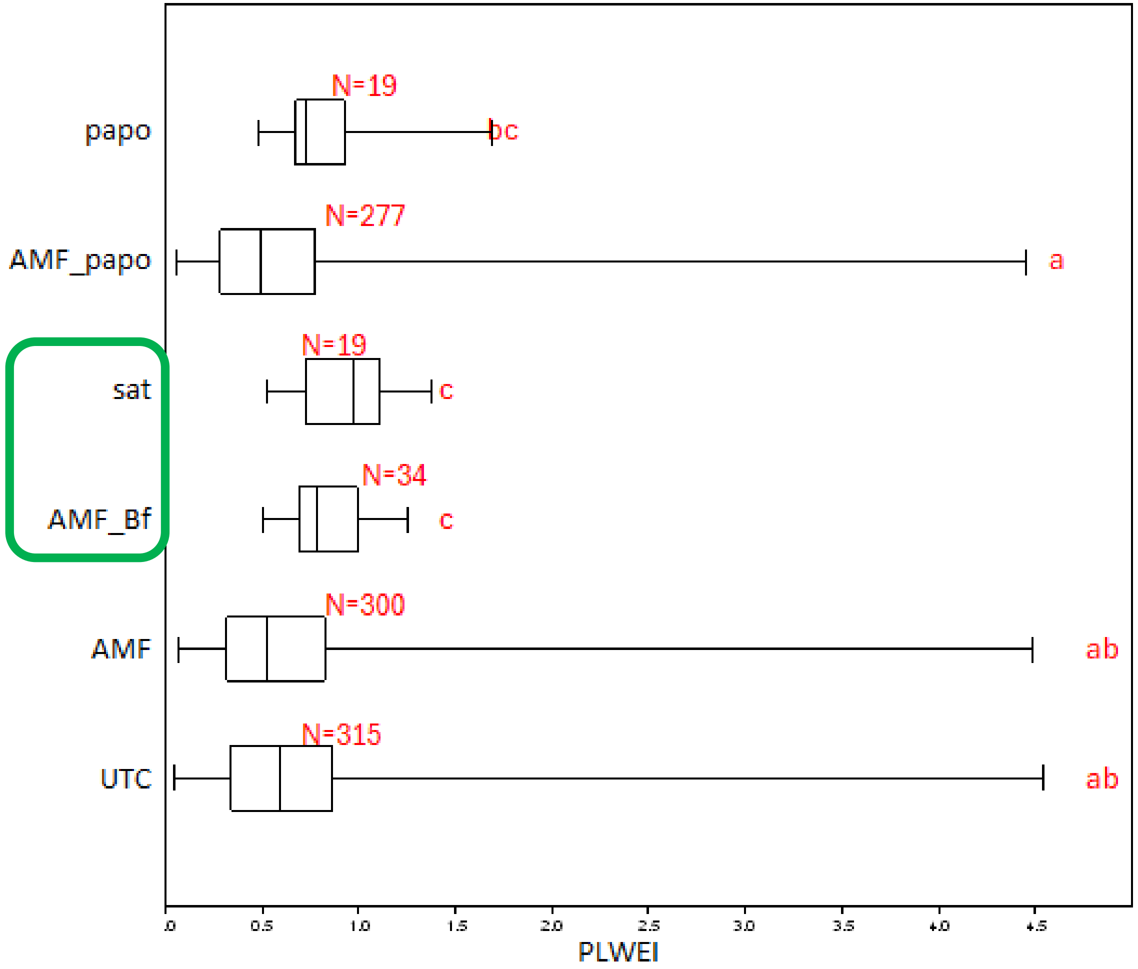
To what extent does the bio-based products affect the plant biomass of tomato?



- ✓ **Effective Biomass (PLWEI): Micosat** and **AMF+Bf** clearly showed significant differences compared to control, as well as to AMF+ *Paenibacillus polymixa* (but not to *P. polymyxa* alone*)*
- ✓ Also in this case, **we recorded the effect of several other variables**

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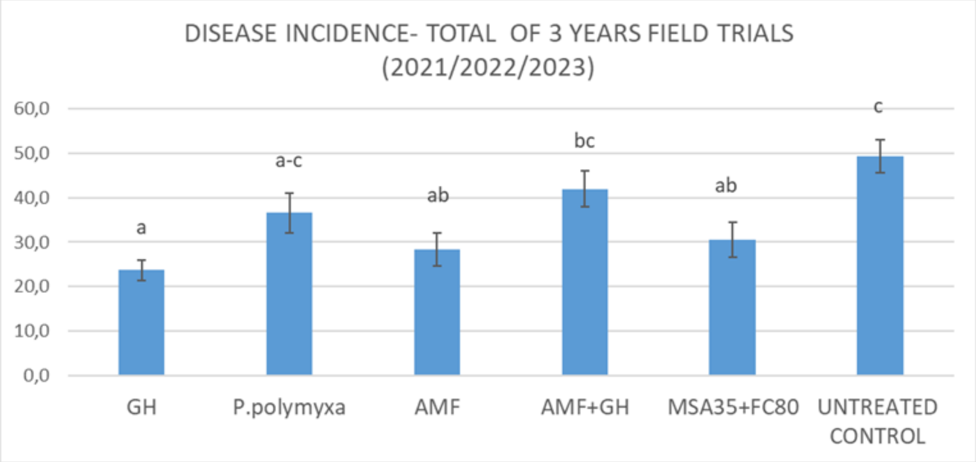
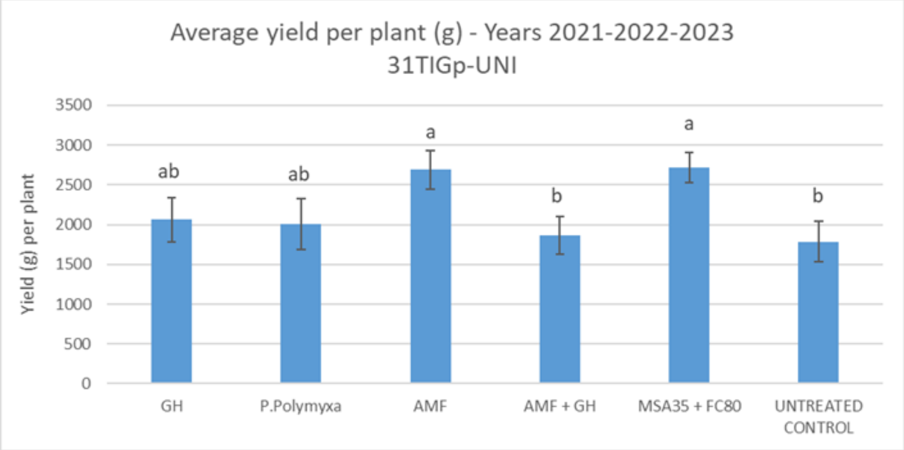
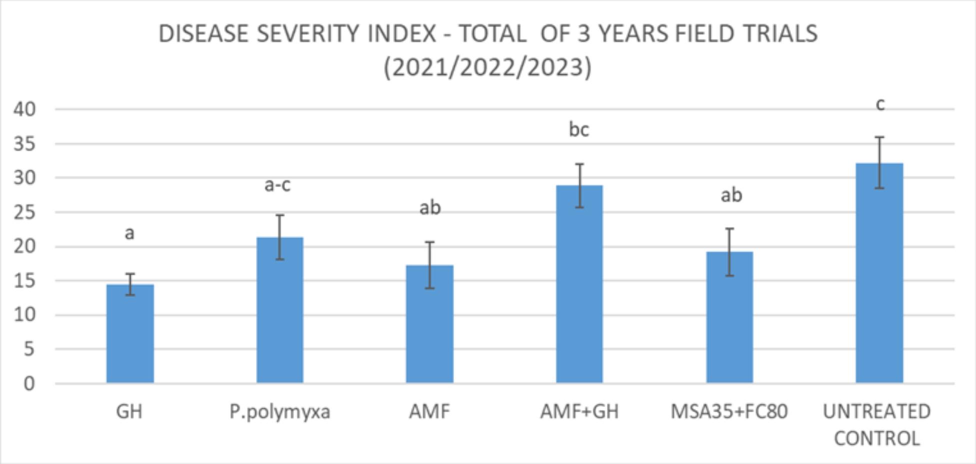
The italian Case study (UNITO)





Product	Product Provider
Bio-effector GH	GreenHas
Paenibacillus polymyxa	INHORT
Excalibur consortium AMF Rhea	IN+
Excalibur consortium AMF Rhea +GH	IN+ and GreenHas
***Fusarium oxysporum* MSA35 + *Trichoderma asperellum* FC80 (1:1 v/v)**	Agroinnova

(Duncan p>0.05: different letters correspond to statistically different values)



(Tukey p>0.05: different letters correspond to statistically different values)

The results highlight the **potential use of combined bioinocula and bioeffectors** as an effective measure **to control soilborne pathogens** (*Rhizoctonia solani*, *Fusarium oxysporum*) on tomatoes under field conditions.

AMF and MSA35+FC80 showed a **positive effect** in both **disease management** and **yields improvement,** resulting in a **promising solution** to be commercially exploited.

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Conclusions

- ✓ In general**, the application of bioinoculants did not show significant effects on the plants** (apple, strawberry and tomato), **BUT the results hold the same effect as the common practice,** and **no adverse effects on plant growth and productivity** have been observed.
- ✓ The significant effect of other variables (besides treatment) should also be considered e.g. different soil-climatic conditions (different countries), management (IPM vs. BIO), cropping system (open field vs. greenhouse) and soil biodiversity to optimise bioinoculant application.
- ✓ When high minerals (e.g., P, N, K) are available in the soil, lower bio-inoculum activity on plants has generally been observed (this means that is recommended monitoring of the soil quality!)
- ✓ The effect of repeated applications over time is to be investigated further.
- ✓ The application of bioinoculant can boost the reduction of chemical inputs: with lower chemical fertilisation input (< 30-50%) and the application of bioinoculant, the plant agronomic performance doesn't show significant changes.
- ✓ **The application of bioinoculants can significantly improve soil quality and biodiversity, thereby increasing the ecosystem service provided by the soil.**

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Some take-home messages

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- ✓ Based on the EXCALIBUR results, **it is not possible to identify a bioproduct that is suitable/effective in all situations**
- ✓ The selected microbial strains might not necessarily find the conditions to establish and proliferate in all the pedo-climatic conditions
- ✓ The effect of native community, cannot be excluded

Success stories: the superiority of native soil microorganisms in supporting plant growth and soil-borne pathogens control

The most significant results on biocontrol were obtained when the specific bioinoculants (microorganisms) applied were from a selection made in the soils of the same area, and the results of the antagonistic effect on soil pathogens were positive!!

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Costs of innovation

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Example: Apple (Italy)

	TYCPICAL	CASE 1	CASE 2
A - Explicit Direct Cost	**14.698,86**	**14.963,86**	**15.198,86**
Machinery direct Costs	1.832,16	1.832,16	1.832,16
Labor	9.339,00	9.339,00	9.339,00
Inputs (fertilizer, phitosanitary prod, etc)	3.527,71	3.792,71	4.027,71
B - Esteemed Direct Cost	**825,00**	**825,00**	**825,00**
Insurance	50,00	50,00	50,00
Taxes	425,00	425,00	425,00
Land improvement	200,00	200,00	200,00
Overheads	150,00	150,00	150,00
C - Depreciation	**4.765,96**	**4.765,96**	**4.765,96**
Plant depreciation	3.865,47	3.865,47	3.865,47
Machinery depreciation	900,49	900,49	900,49
D - Opportunity Cost	**2.987,59**	**2.987,59**	**2.987,59**
Palnt Interests	1.739,46	1.739,46	1.739,46
Machinery Interests	254,02	254,02	254,02
Interest on working capital	244,11	244,11	244,11
Land Cost	750,00	750,00	750,00
Totale complessivo	**23.277,41**	**23.542,41**	**23.777,41**

Typical:
- Cost of a typical farm

Case 1:
- Reduction in fertilizer use (-50%)
- Addiction of bioinoculants (estimated cost = 500€)

Case 2:
- Addiction of bioinoculants (estimated cost = 500€)

Final consideration: **the impact of bioinoculants application on production costs is not significant.**

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"Tools and knowledge for boosting soil biodiversity and bioinoculant application in agriculture"

Loredana Canfora

Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria
Centro di ricerca Agricoltura e Ambiente

Loredana.canfora@crea.gov.it

crea Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria

February, 27 2025

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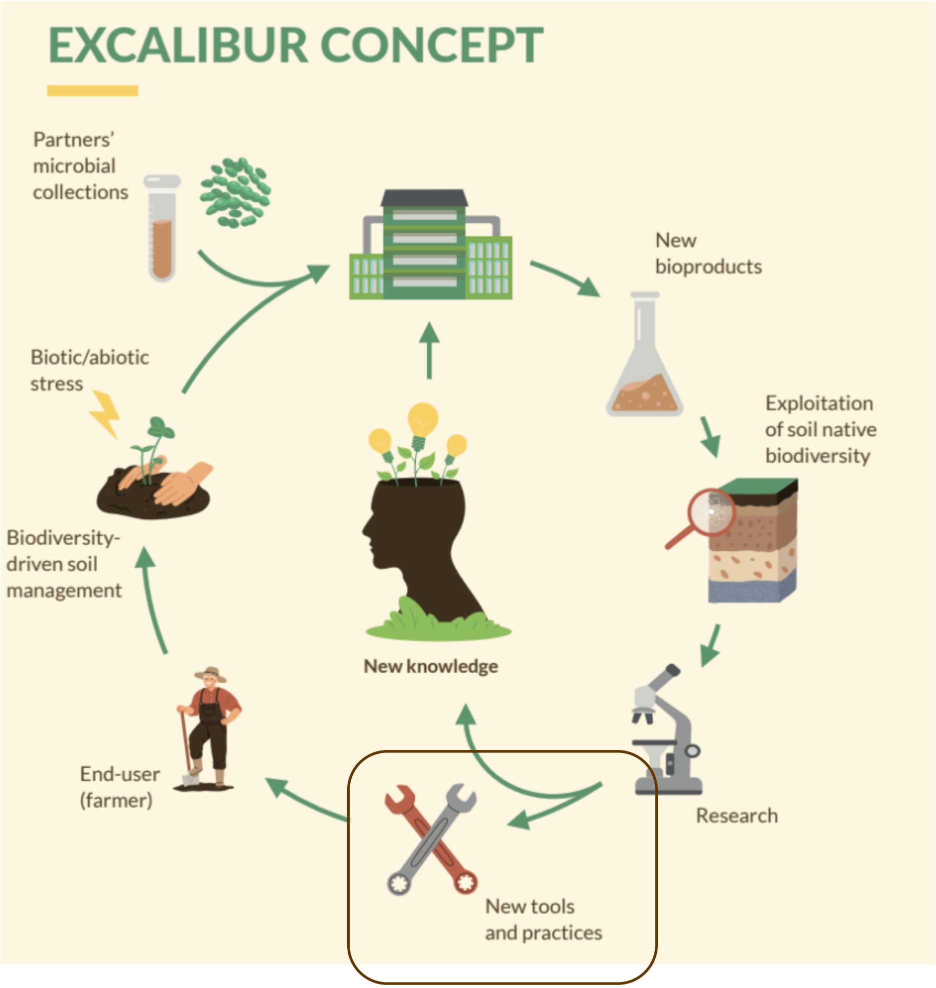
A snapshot on the tools developed to support the optimization and the application of bioinoculant in soil



- The inoculation of the soil with bioinoculants may affect its native microbial populations, with effects that depend on the soil's chemical and physical characteristics and the environmental conditions
- Changes occurring to the soil microbial structure may affect the overall soil health status, impacting crop productivity, quality, and human health

Thus, the field application of such products requires their registration at the EU and national levels, together with an indication by the manufacturer of various specifications and analytic methods, making it possible to trace their destiny in the environment and prove their medium- and long-term effectiveness.

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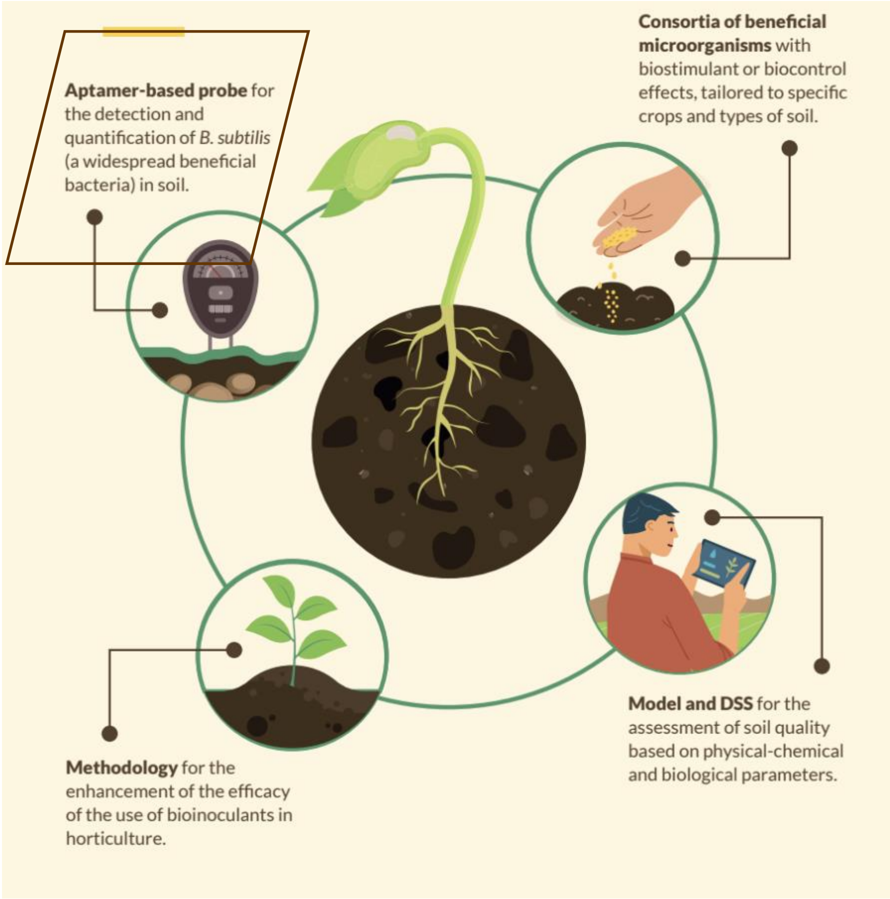
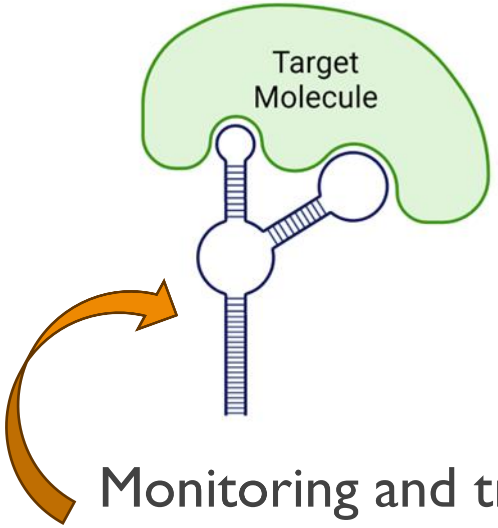
Innovative molecular tools for tracking microbial inoculants in soil





Bacillus subtilis (PCM/B00105) is being tested as a plant growth-promoting rhizobacteria (PGPR) and represent a key component in some biofertilizers for plant protection. Important role in advancing sustainable bio-farming practices.

Monitoring and tracking *Bacillus subtilis* is key to optimizing bioinoculum application, ensuring the right amount of beneficial bacteria for maximum plant growth and protection.

Italian Patent Application n. 102022000022590, 3.11.2022

European Patent Application No. 23207337.9 filed on 2/11/2023

Loredana Canfora; Andrea Manfredini – CREA-AA

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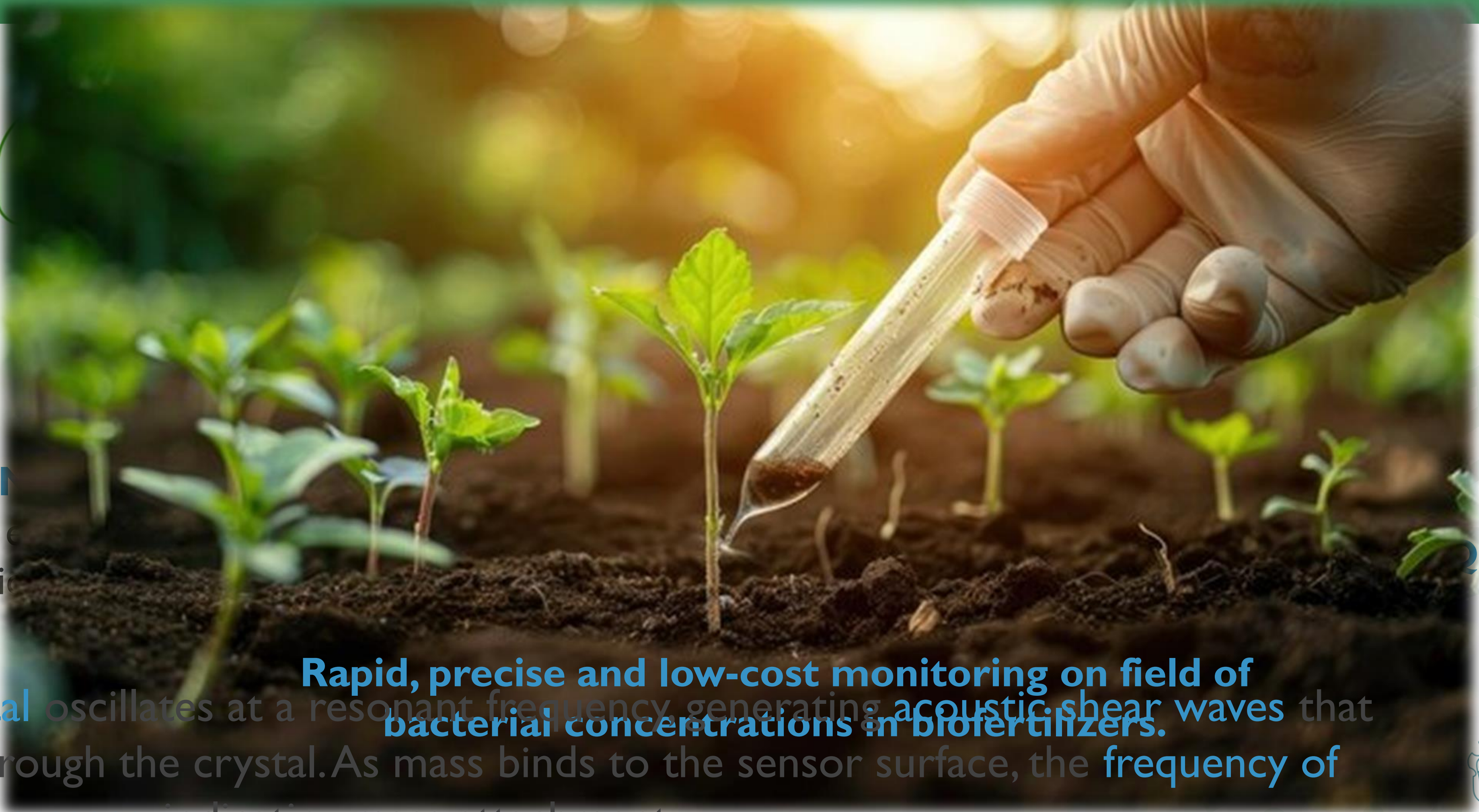

Two **ssD**
Manfredini
specifi

uartz

Quartz crystal oscillates at a resonant frequency generating acoustic shear waves that propagate through the crystal. As mass binds to the sensor surface, the frequency of oscillation decreases, indicating mass attachment.

SCUOLA NORMALE SUPERIORE
CNRNANO PISA NEST
NeuroSens
INTA INTELLIGENT ACOUSTICS SYSTEMS

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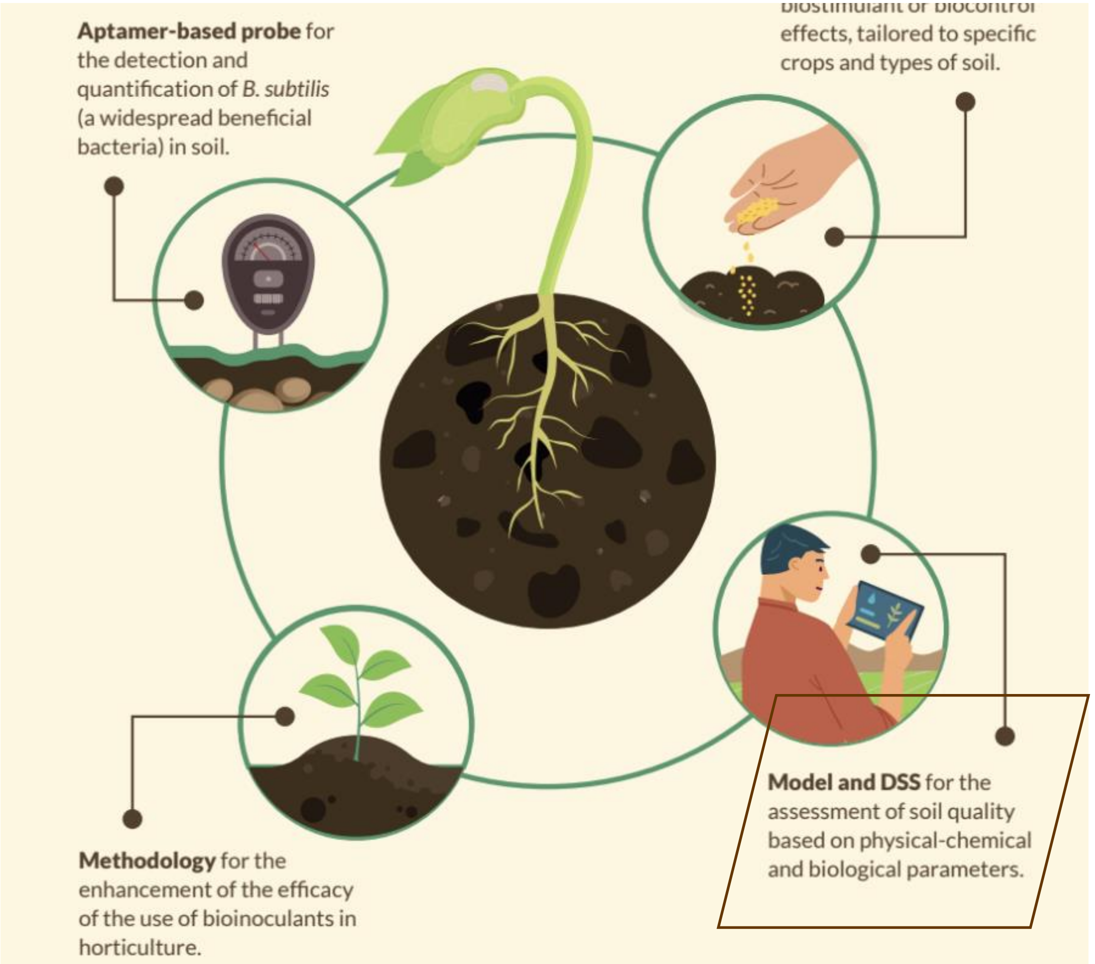
DSS SIMCA platform for soil quality

- Generally, the role of soil derives from its multifunctionality, which is based on providing different ecosystem services, including soil processes such as nutrient cycling and biodiversity protection (Lehmann et al., 2020).
- In literature, to develop soil quality indexes able to assess the impact of different agronomic practices, different groups of parameters have been used approaching simple statistical approaches (Obriot et al., 2016; Bünemann et al., 2018).

In the context of soil quality, a DDS based on a class-modelling approach was developed in the Excalibur project to construct soil quality indices based on basic physicochemical parameters.



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DSS SIMCA platform for soil quality

SIMCA (Soft Independent Modelling of Class Analogy) algorithm - Based on Principal Component Analysis (PCA)

Input variables

Soil Quality Index

Physical Chemical parameters

Year: 2022 **Sample name:** 2AOF_TUG

Texture

Clay: 35.00 % **Sand:** 33.00 % **Silt:** 32.00 %

Category: Loamy **Class:** 2 **USDA:** Clay Loam

Chemical parameters

pH: 6.88 **OM:** 22.00 g/Kg **P:** 788.80 ppm **K:** 1651.00 ppm

Mg: 4309.00 ppm **Ca:** 2957.00 ppm **K/Mg:** 0.38 **CEC:** 50.01

SIMCA model value & positioning in the quality ranges

SIMCA quality metric bar (SIMCA combined rule value)

0.98

Very good | Good | Discrete | Sufficient | Bad

QR-code with the same colour of the SIMCA quality range

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EXCALIBUR

The web interface of the model was developed using "HTML" technology for the variables' input mask and "PHP" technology for preparing the variables entered by the user, executing the model, and displaying the output on the web page.

https://agritechlab.crea.gov.it/model/formSE.html

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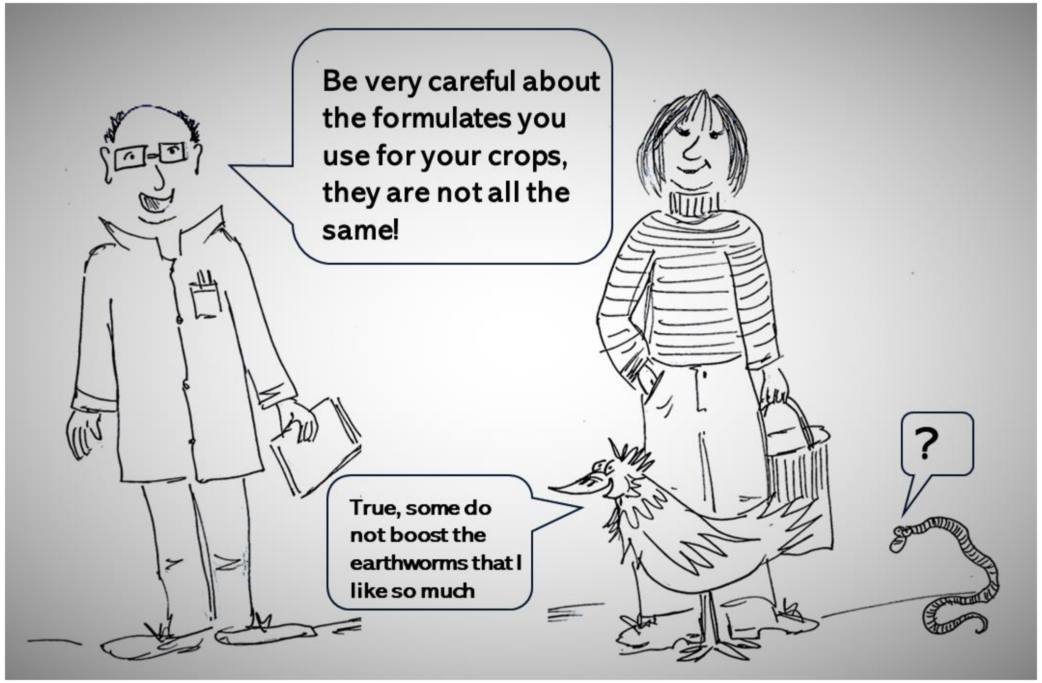
Conclusions

Despite the challenges posed by the soil complex matrix, the successful implementation of modern **methods** for traceability and **monitoring of microbial inoculants in soil**, and the efforts put in place for developing a **soil quality index are** a crucial step towards a better understanding of ecological systems and the correct adoption of practices involving the use of microbial-based products



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EXCALIBUR

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THANK YOU!