

Fungal biostimulants in Agriculture: Towards a sustainable Future



Lobna
Hajji-Hedfi
(TUNISIA)

The 8th National Fungus Day of Egypt

Date: February 24, 2023

Theme: "Mycology by Amateur and Young Mycologists"

✓ **Position:** Senior Researcher of Phytopathology

✓ **Location:** Regional Centre of Agricultural Research of Sidi Bouzid, Tunisia (CARRA-Tunisia)

✓ **Main research interests**

1. Phytosanitary diagnosis of fungal disease infecting strategic agricultural crops;
2. Interaction plant-soil-microorganisms;
3. Microbiological control using fungi and bacteria against phytopathogenic fungi;
4. Impact of regenerative agriculture practice to improve plant resilience towards biotic stress;
5. Impact of climate change on soil microorganisms.

✓ **Work Tasks**

- Research and disseminate research activities on scientific papers, workshop, seminars
- Supervising students in plant pathology studies
- Teaching and Training for professionals, farmers and students
- Fungal diseases inspections and farmers consulting

✓ **Current Records (2022)**

36 publication

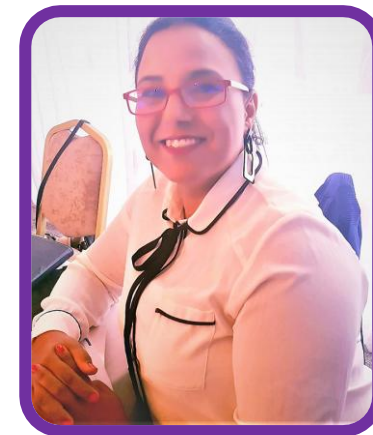
5 book chapters

31 International communications

19 supervision

3 Partnership agreement

Project Membership (8)



ORCID : 0000-0002-3587-4790

Researcher ID : T-3541-2017

Main achievements 2022

**National Coordinator
of PRIMA project**



**Fellow of 7th edition of
women for science
(women for Africa)**



Nuffic Fellow

**The Hague Academy for
Local governance**



**Arab Society for Fungal
Conservation's Award
2022**





Horizon 2020
European Union Funding
for Research & Innovation

Previous H2020 Project

Marie Curie (MSCA-IF-SE-H2020)
2018/2019



Co-Fund programme 2019
(Got Talent Energy programme)



MOBIDOC H2020
R&I (SME and NGO assistance)



PRIMA (2020 -2022)



Created in 2009
 3 governorates: Sidi Bouzid, Kasserine, Kairouan
 4 laboratories: Agronomy, Plant Pathology, Animal Production and Water management and Experimental station (36 ha)



Missions:
 improving technics and transfer;
 Research; production technology
 Networking (Partnership with socio-economic organisms and Universities around);
 Training sessions and hosting students

Cereals

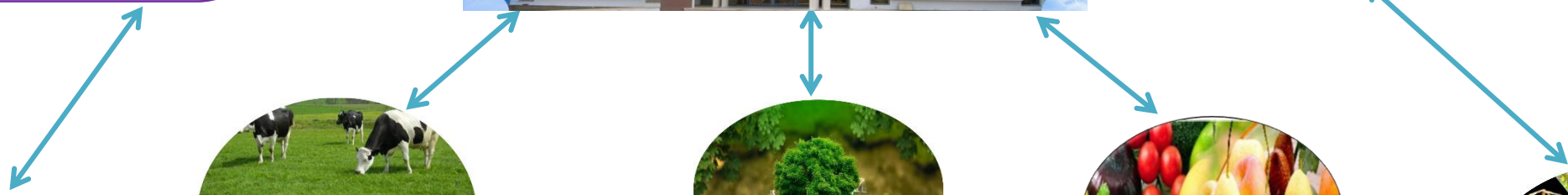
Animal Production

Conservation and management of natural resources

Horticulture

Plant Protection

National and International funding
 Good Publication Record



Regenerative agricultural approaches to improve ecosystem services in Mediterranean vineyards



O. Impact of regenerative methods on pathogens and beneficial microorganisms under Climate change context

Sustainable innovations for Regenerative Agriculture in the Mediterranean area

O.1-The use of microbial biostimulants to enhance plant resilience towards biotic and abiotic stress

O. 2-The impact of using microbial consortia associated with some regenerative approaches



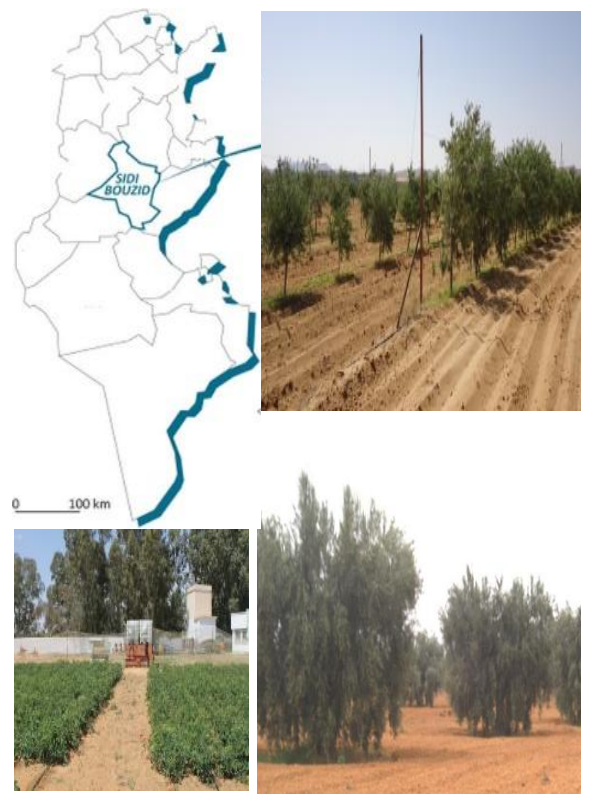
REVINE

REVINE is an innovation project of the *Partnership for research and innovation in the Mediterranean Area (PRIMA)* program, which aims to mitigate the effects of climate change in the Mediterranean, showing that the application of regenerative agriculture is capable of preserving water resources and soil fertility, controlling erosion, and creating physicochemical soil conditions that increase the presence of beneficial microorganisms.

<https://www.revine-prima2020.org/>



<https://www.siram-prima.org/>



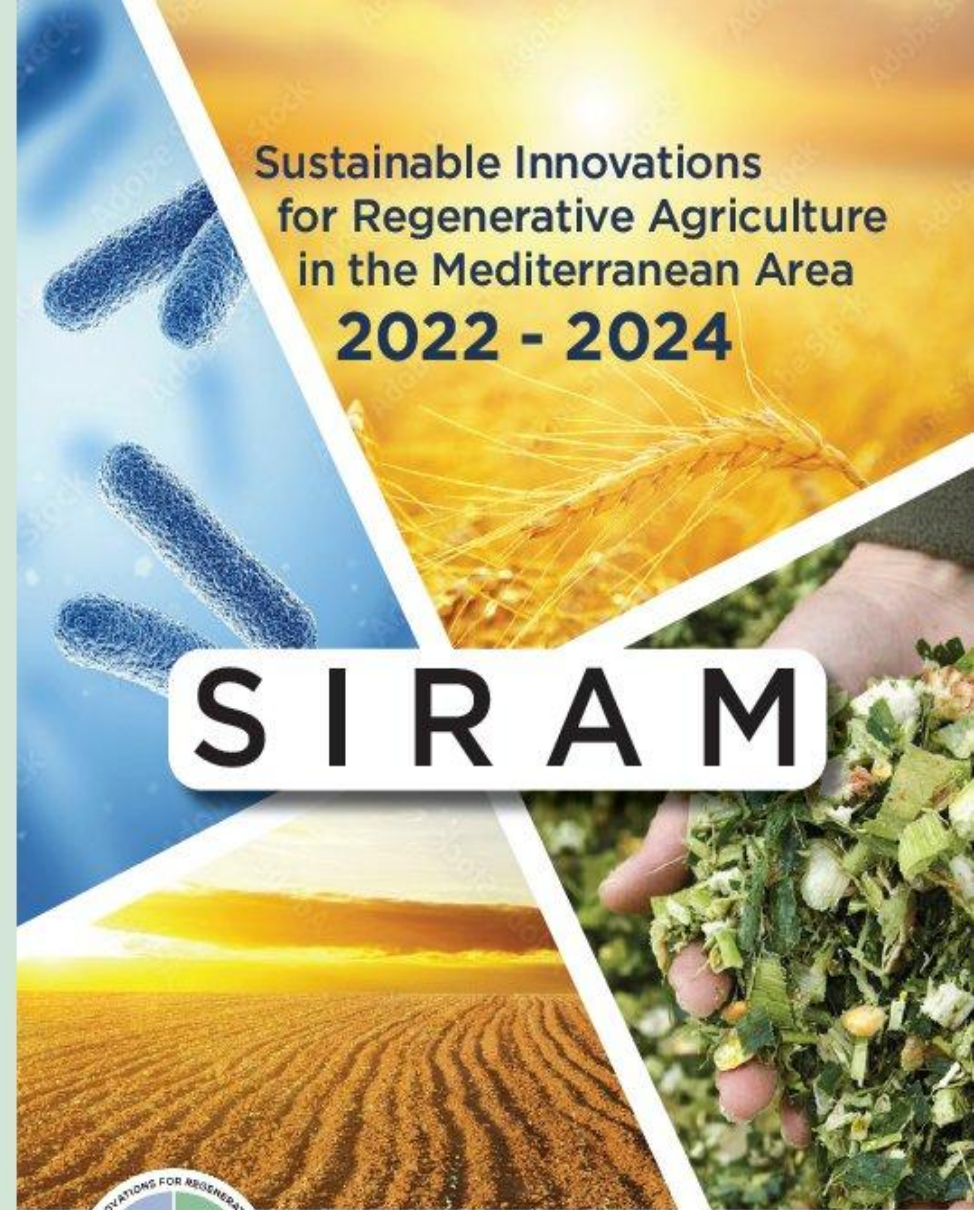
CONSORTIUM

<p>EGYPT</p>  <p>FRANCE</p>  <p>GREECE</p>  <p>ITALY</p>  	<p>MOROCCO</p>  <p>PORTUGAL</p>  <p>SPAIN</p>  <p>TUNISIA</p> 
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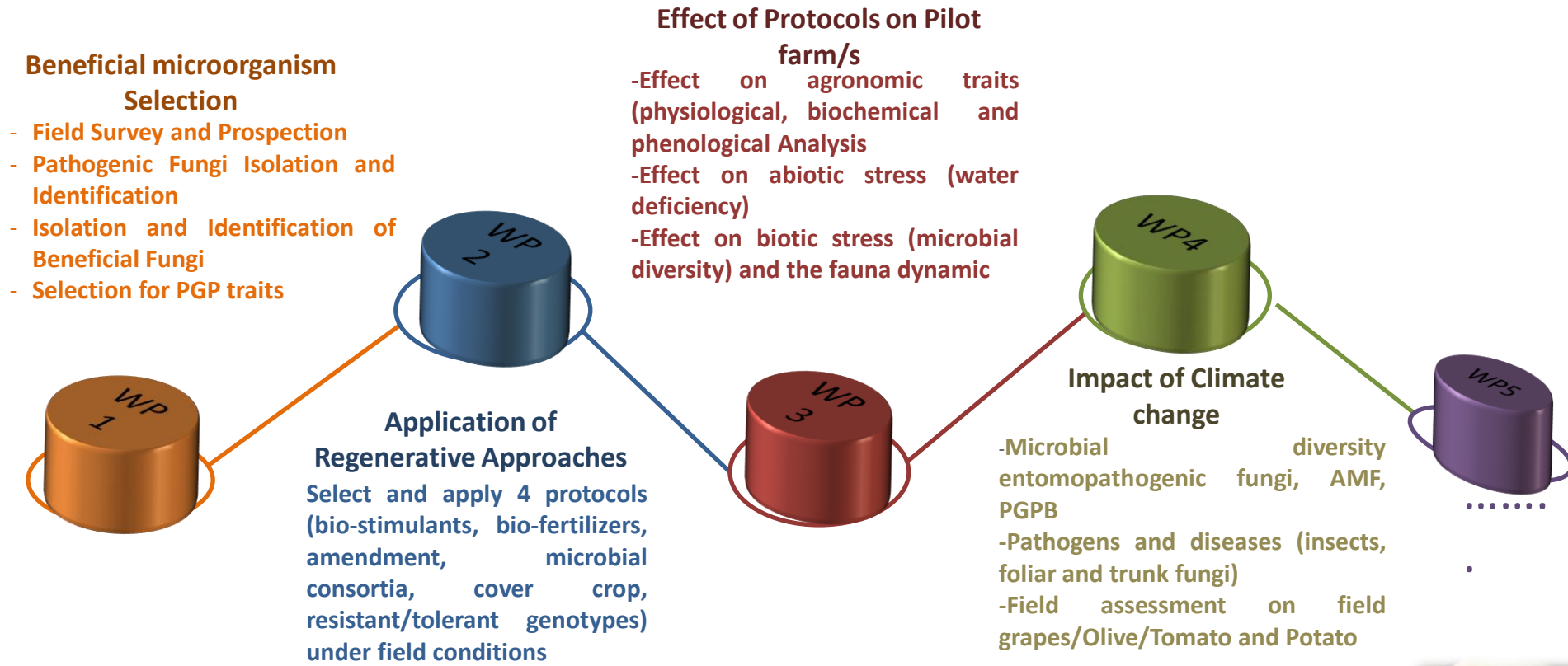
www.siram-prima.org




<p>Coordination: prof. Edoardo Puglisi Università Cattolica del Sacro Cuore edoardo.puglisi@unicatt.it</p>	<p>Communication & dissemination: Dr. Gabriele Sacchetti OpenTEA gabriele.sacchetti@opentea.eu</p>
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Roles in REVINE and SIRAM





CLIMATE CHANGE

AGRICULTURE

Climate change, defined by the United Nations Framework Convention on Climate Change as “a change of climate which is attributed directly or indirectly to human activity that alters the composition of the global atmosphere and which is in addition to natural climate variability observed over comparable time periods”

Create environmental pressures that result in new diseases caused by fungi



Emergence-

Environmental disruptions due to climate change such as floods, storms, and hurricanes can disperse and aerosolize fungi or implant them via traumatic wounds, resulting in infections by previously very rare or unknown fungal species.



Diversity-

Climate change can increase the geographic range of pathogenic species or their vectors, leading to the emergence of diseases in areas where they have not previously been reported



with consequences for health, biodiversity, and food security



BENEFICIAL FUNGI

AGRICULTURE



Plant-associated fungi harbor enormous potential to provide economical and sustainable solutions to current agricultural challenges.

The main goal is to devise a practical effective eco-friendly strategy for the integrated use of bio-control agents (BCAs) for the safe crop production.

Finding new microorganisms that can sustainably support plant development, nutrition, fitness, disease control, and productivity in dynamic and stressful environments therefore depends on developing strategies to manage **phytomicrobiomes**

1. Biofertilization

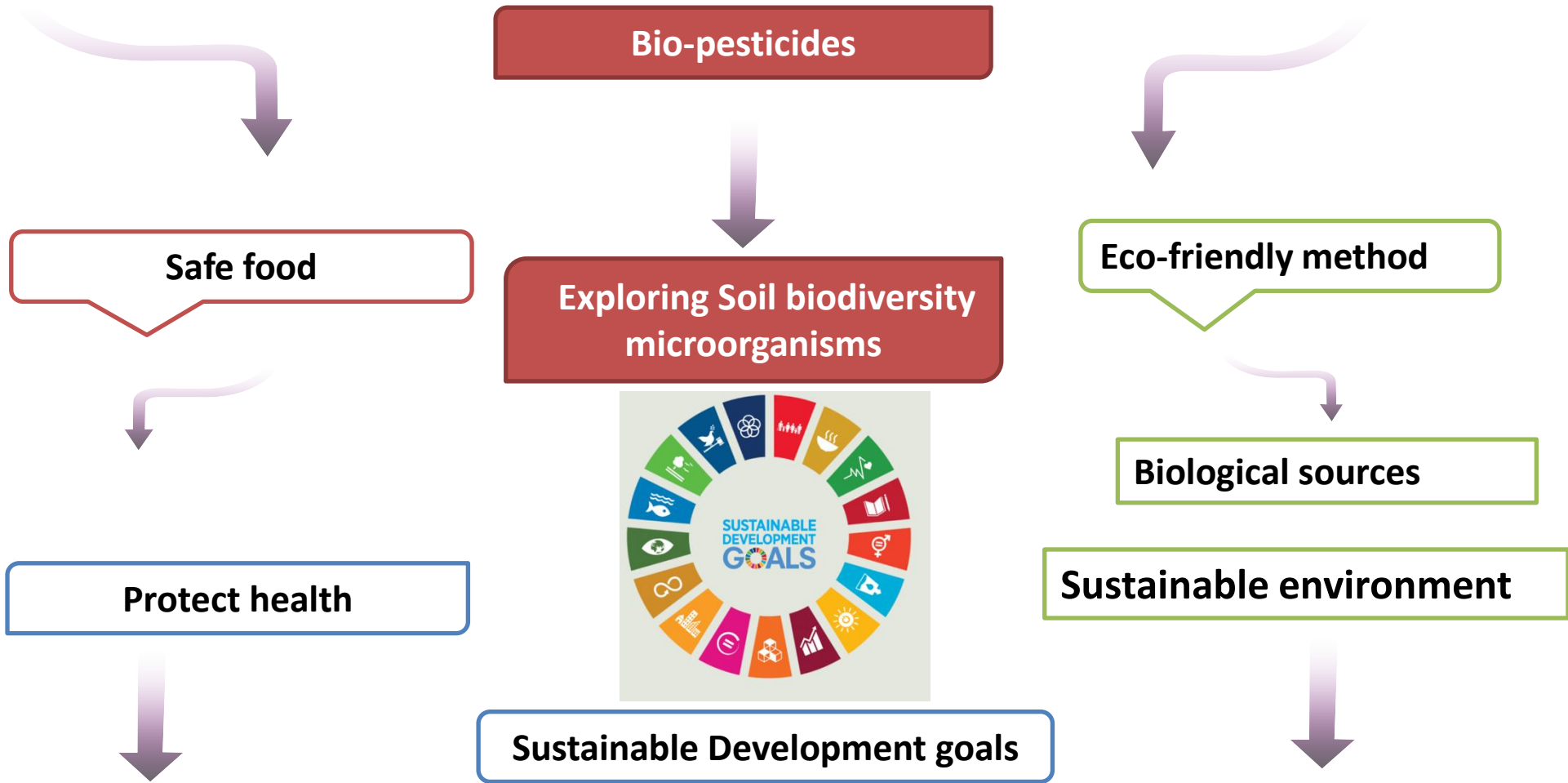
Plants require macronutrients such as nitrogen (N) and phosphorus (P). In nature, plants source N from ammonia produced by nitrogen-fixing fungi

2. Biostimulation

Many plant-associated fungi can synthesize plant hormones such as auxin, ethylene, and cytokinins that have crucial multifaceted roles in plants

3. Biocontrol

Engineered fungi might be useful to diagnose plant physiological changes caused by biotic stresses and to deliver desired traits



3 GOOD HEALTH AND WELL-BEING

11 SUSTAINABLE CITIES AND COMMUNITIES

13 CLIMATE ACTION

15 LIFE ON LAND

Applied studies on beneficial fungi in sustainable agriculture

1. Effect of *Verticillium leptobactrum* and *Purpureocillium lilacinum* to control Root-knot and Potato cyst nematodes and growth –promoting Potato
2. Fungal diversity associated with tomato wilt disease complex in Tunisia
3. The use of Phytomicrobiome (fungi) in controlling wilt disease complex (*Meloidogyne javanica* and *Fusarium* f.sp. *lycopersici*) on tomato crop
4. Beneficial fungi against early blight and grey mold disease of tomato
5. Screening and application of Beneficial fungi in Tunisian vineyards

Study 1

1. Effect of *Verticillium leptobactrum* and *Purpureocillium lilacinum* to control Root-knot and Potato cyst nematodes and growth –promoting Potato

Evaluation of biocontrol potential of indigenous strains of *V. leptobactrum* and *P. lilacinum* in individual and combined with *M. javanica* et *G. pallida* (PCN) and their effect on potato growth

Soil application of each isolate of *P. lilacinum* and *V. leptobactrum* reduced significantly PCN development on root and soil.

Application of two fungi increased potato growth (agronomic traits) and yield and reduced the combined infection by both nematodes



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Am. J. Potato Res. (2017) 94:178–183
DOI 10.1007/s12230-016-9554-0

SHORT COMMUNICATION

Biocontrol Potential of *Verticillium leptobactrum* and *Purpureocillium lilacinum* Against *Meloidogyne javanica* and *Globodera pallida* on Potato (*Solanum tuberosum*)

Lobna Hajji¹ · Wassila Hlaoua¹ · Hajer Regaieg¹ · Najet Horrigue-Raouani¹

Published online: 23 December 2016
© The Potato Association of America 2016

Abstract Pot experiment was conducted in a greenhouse to assess the biocontrol potential of *Purpureocillium lilacinum* and *Verticillium leptobactrum* against single or concomitant infestations of *Meloidogyne javanica* and *Globodera pallida* in potato cv. Spunta. The incorporation of each fungus alone into the soil significantly increased the growth parameters. Fresh weight of shoots, roots and tubers were lower ($P \leq 0.05$) in the untreated control than in plants treated with having the above-mentioned fungi treatments. Control efficacy achieved by soil application of *P. lilacinum* was 73% and 76% in terms of root/g of roots and soil population/g of soil, respectively and that of *V. leptobactrum* was 73% and 55% 117 days after inoculation. The results revealed also that the application of *P. lilacinum* and *V. leptobactrum* decreased significantly the development of potato cyst-nematode in roots by 76% and 83% and in the soil by 61% and 66% respectively. Combined infection by the two pathogens had also a significant reduction in case by introducing *V. leptobactrum* or *P. lilacinum* in soil.

Resumen Se condujo un experimento en macetas en invernadero para analizar el potencial de biocontrol de *Purpureocillium lilacinum* y *Verticillium leptobactrum* contra infestaciones simples o combinadas de *Meloidogyne javanica* y *Globodera pallida* en papa var. Spunta. La incorporación de cada hongo individual en el suelo aumentó significativamente los parámetros de crecimiento. El peso fresco de los tallos, raíces y tubérculos fue más bajo ($P \leq 0.05$) en el testigo no tratado que en plantas que tuvieron los tratamientos fúngicos mencionados. La eficacia en el control alcanzada mediante la aplicación al suelo de *P. lilacinum* fue de 73 y 76% en términos de raíz/raíz y población del suelo/g de suelo, respectivamente, y la de *V. leptobactrum* fue de 73% y 55%, a 117 días después de la inoculación. Los resultados revelaron también que la aplicación de *P. lilacinum* y *V. leptobactrum* disminuyó significativamente el desarrollo del nemátodo de quiste de la papa en las raíces en un 76% y 83% y en el suelo fue de 61% y 66%, respectivamente. La infección combinada por los dos patógenos tuvo también reducción significativa mediante la introducción de *V. leptobactrum* o *P. lilacinum* en el suelo.

Keywords *Solanum tuberosum* · Potato cyst nematode · Root-knot nematode · Antagonistic fungi

Introduction
Potato (*Solanum tuberosum* L.) is one of the most important Solanaceae crops in worldwide and in Tunisia (Fabeiro et al. 2001; Graham et al. 2001). Several species of plant-parasitic nematodes have been found associated with potato cropping systems worldwide. The potato cyst nematode (PCN) *Globodera pallida* and the root-knot nematode (RKN) (*Meloidogyne javanica*) are the major pests, which can cause a major yield losses (Hlaoua and Horrigue Raouani 2007; Castillo et al. 2001). PCN induced losses in Potato on Tunisian production areas were estimated by 45% (B'Char 1990). RKN species found associated to Potato in Tunisia were *M. javanica* and *M. incognita* and combined infection by *Meloidogyne spp.* and *Globodera spp.* were revealed (Hlaoua 2011).

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Springer

Study 2

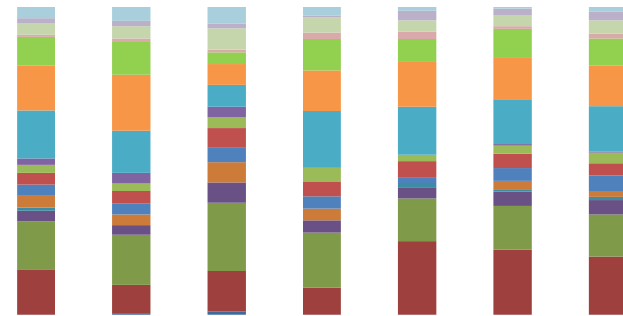
2. Fungal diversity associated with tomato wilt disease complex in Tunisia

135 prospected parcels and Samples collected (soil and root) during 3 years and screened for their Oomycota and fungi diversity

Study the potential correlations between fungal diversity and abiotic factors

31 fungal species and 17 genera

Fusarium oxysporum (11%)
dominance followed by
Fusarium solani (6%)



Fungi Frequency



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Symbolis
<https://doi.org/10.1007/s13199-019-00629-x>

Fungal diversity in rhizosphere of root-knot nematode infected tomatoes in Tunisia

Lobna Haji-Hedfi¹ · Naima M'Hamdi-Boughaleb¹ · Najet Horrigue-Raouani¹

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© Springer Nature B.V. 2019

Abstract

This research explores the occurrence and diversity of fungi associated with root-knot nematodes (*Meloidogyne* spp.) infestations on tomato crops in bioclimatic zones of Tunisia. One hundred and thirty five tomato samples (roots and soil) collected between 2011 to 2013 from tomato fields were screened for Oomycetes and other fungi. A high level of fungal diversity was found in the presence of *Meloidogyne* spp. A total of 31 fungal species belonging to 17 different genera were recovered from roots and soil samples collected in fields infested with root-knot nematode. The most frequent fungal species associated with the nematode was *Fusarium oxysporum* (11%) followed by *Fusarium solani* (6%). The species composition was dependent on environmental conditions. Temperature seems to be important as the rhizosphere microflora in the Kebili and Tozeur areas with 'saharien' bioclimatic stages was different from other localities. Our findings may be valuable for predicting this disease complex.

Keywords Diversity · Fungal communities · Root knot nematodes · Tomato · Environment

1 Introduction

Root-knot nematodes, *Meloidogyne* spp., are among the most harmful pests of cultivated crops worldwide and control is difficult (Koenning et al. 2004; Sharma et al. 2008). Yield losses caused by plant-parasitic nematodes on tomato crop are between 28 and 70% (Ibrahim et al. 2000). *Meloidogyne* species cause major production losses on tomato (Ebrahim et al. 2015).

The *Meloidogyne* spp., are sedentary endoparasitic root-feeding nematodes. The infectious juveniles (J2), migrate through the soil, enter the host root near the tip, and establish feeding sites near the vascular system. The root damage resulted from the root-knot nematode infestation is often associated a host predisposition to secondary colonization by pathogenic or beneficial microorganisms (Kerry 2000). Root-knot nematodes interact with the rhizospheric mycoflora and can cause a root decay complex in association with other microorganisms (Davies 2005). The resultant plant diseases, are

often caused by multispecies synergistic interactions (Lamichhane and Venturi 2015). Disease complexes, can cause even greater damage to the host plant than a single pathogen or parasite (Haji et al. 2016a).

On the other hand, antagonistic interactions between nematode and some rhizospheric microorganisms can provide a plant defense against soil-borne plant pathogens and parasites, including *Meloidogyne* spp. Such interaction if understood may provide phytonematode control (Kerry 2000; Hallmann et al. 2009).

The rhizosphere is a zone characterized for the first time by Hiltner (1904) that involves a complex of biological and ecological processes (Lynch 1990; Brimacombe et al. 2001; Bais et al. 2006). Microorganisms in plant rhizosphere, especially fungi, play an important role in plant ecology. Fungi colonize all matrices and they contribute to maintaining equilibrium in the ecosystem (Anastasi et al. 2009, 2013; Vofřiková and Baldrian 2013). Studies on fungal diversity are needed to understand the functional role of fungi in the rhizosphere (Chen et al. 2017).

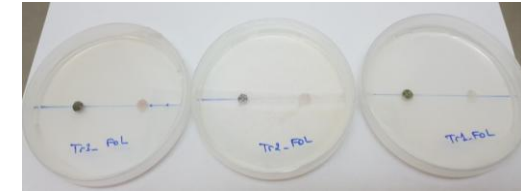
The present study aimed at assessing 13 root-knot nematodes...

Species composition is dependent on environmental factors in particular the **temperature**

Study 3

3. The use of Phytomicrobiome (fungi) in controlling wilt disease complex (*Meloidogyne javanica* and *Fusarium f.sp. lycopersici*) on tomato crop

- ❑ Screening of potential antagonistic of indigenous fungi
- ❑ Observation or parasitism by scanning electron microscopy (SEM)
- ❑ Biological control of Wilt disease complex



Paecilomyces, *Lecanicillium*, *Penicillium*, *Pochonia* and *Trichoderma* showed interesting **nematicide activity**; 3 *Trichoderma* isolates and *Penicillium*: **fungicide** potential

Trichoderma reduced **wilt disease incidence** in particular *T. longibrachiatum*.

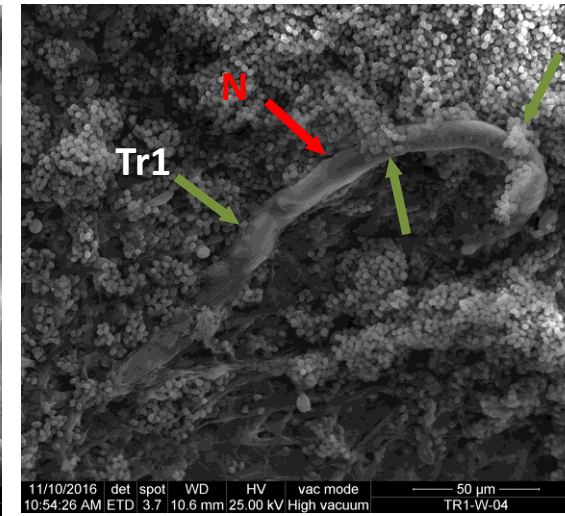
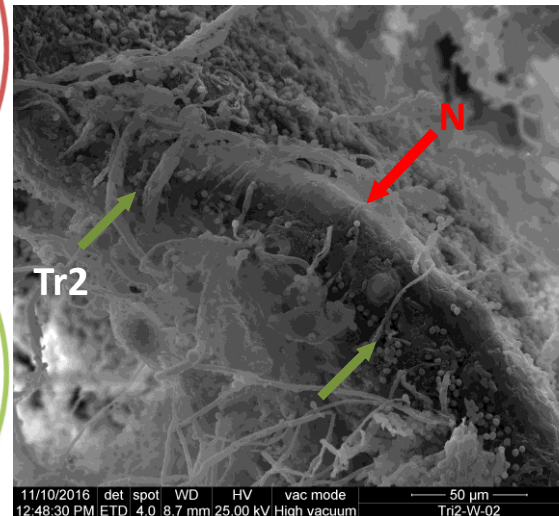
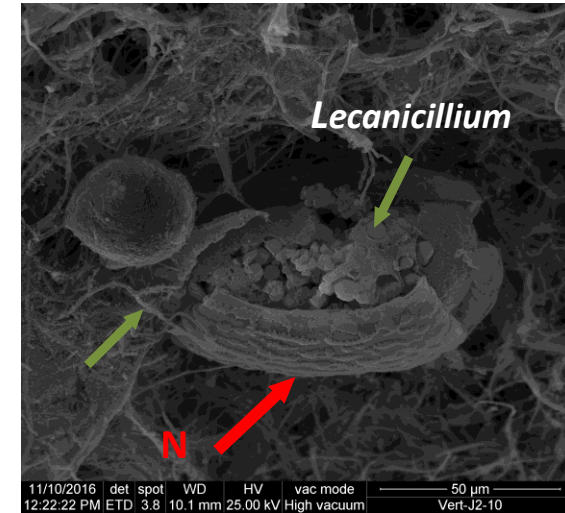
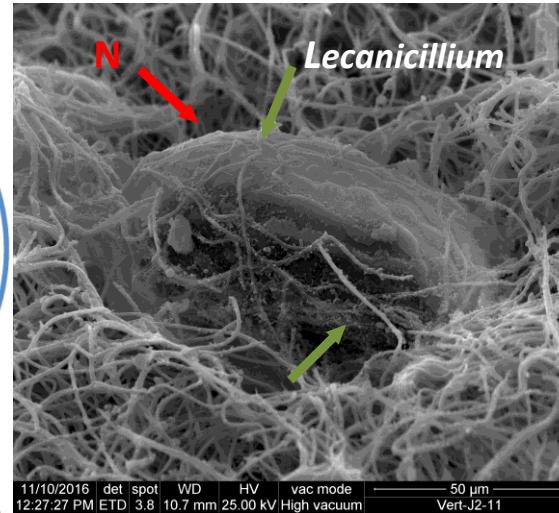


SEM observations of nematicide effect

Immobilization
of larvae /eggs

Degradation of
nematode
cuticle

Germination
and
reproduction
inside
nematode



Micrographes by (SEM) of eggs and juveniles
parasitized by *Lecanicillium* and 2 *Trichoderma*
species

SEM observations of fungicide effect

Biological control of wilt disease complex on tomato crop caused by *Meloidogyne javanica* and *Fusarium oxysporum* f.sp. *lycopersici* by *Verticillium leptobactrum*Lobna Hajji-Hedfi¹ · Hajer Regaieg¹ · Asma Larayedh¹ · Noura Chihani¹ · Najet Horrigue-Raouani¹Received: 6 January 2017 / Accepted: 14 September 2017
© Springer-Verlag GmbH Germany 2017

Abstract The efficacy of *Verticillium leptobactrum* isolate (HR1) was evaluated in the control of root-knot nematode and *Fusarium* wilt fungus under laboratory and greenhouse conditions. Five concentrations of *V. leptobactrum* (HR1) isolate were tested for their nematicidal and fungicidal activities against *Meloidogyne javanica* and *Fusarium oxysporum* f.sp. *lycopersici* in vitro. Laboratory trials showed that mycelium growth inhibition of *Fusarium* wilt fungus was correlated to the increase of the concentration of culture filtrate. All dilutions showed efficiency in reducing the growth of *Fusarium oxysporum* f.sp. *lycopersici*. The greatest nematicidal activity was observed at 50, 75, and 100% filtrate dilutions. The egg hatching percentage reached 42%, and the juvenile's corrected mortality registered 90% for the above treatments. In greenhouse experiment, the biocontrol agent fungus enhanced significantly tomato growth components (height and weight of plant and root). The multiplication rate of root-knot nematode and the *Fusarium* wilt disease incidence declined significantly

with soil application of *V. leptobactrum* as with chemical treatments. The isolate HR1 was efficient to control wilt disease complex caused by *M. javanica* and *Fusarium oxysporum* f.sp. *lycopersici*.

Keywords Biocontrol · *Verticillium leptobactrum* · Root-knot nematode · *Fusarium* wilt fungus · Tomato

Introduction

The root-knot nematodes (*Meloidogyne* spp.) are one of the major biotic constraints facing vegetables crops distributed worldwide and could infect more than 2000 plant species (Park et al. 2014). Tomato (*Lycopersicon esculentum* Mill.) is an important vegetable crop worldwide and in Tunisia (Causse et al. 2003; FAO 2003). The *Meloidogyne* spp. infection cause severe damage on both tomato yield and quality (Horrigue-Raouani 2003; Netscher and Sikora 1990; Moens et al. 2009) and lead to secondary attack by root pathogenic fungi such as *Fusarium oxysporum* (Taylor 1990). The simultaneous infection by root-knot nematode and *Fusarium* wilt (*Fusarium oxysporum* f.sp. *lycopersici*) results in greater and

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Article

Comparative Effectiveness of Filamentous Fungi in Biocontrol of *Meloidogyne javanica* and Activated Defense Mechanisms on TomatoLobna Hajji-Hedfi^{1,*}, Wassila Hlaoua², Awatif A. Al-Judaibi³, Abdelhak Rhouma¹, Najet Horrigue-Raouani² and Ahmed M. Abdel-Azeem^{4,*}¹ Regional Centre for Agricultural Research of Sidi Bouzid, CRRA, B.P. 357 Gafsa Road Km 6 Sidi Bouzid, Sidi Bouzid 9100, Tunisia² Higher Agronomic Institute of Chott-Mariem 4042, Sousse University, Sousse 4000, Tunisia³ Department of Biology, Faculty of Science, University of Jeddah, Jeddah 23218, Saudi Arabia⁴ Department of Botany and Microbiology, Faculty of Science, University of Suez Canal, Ismailia 41522, Egypt

* Correspondence: elhajjilobna@yahoo.fr (L.H.-H.); ahmed1_abdelazeem@science.suez.edu.eg (A.M.-A.); Tel.: +20-1006344462 (A.M.-A.)

Abstract: The nematicidal potential of five filamentous fungi as biological control agents (BCAs) against the root-knot nematode (RKN), *Meloidogyne javanica*, infecting tomato was assessed in vitro and in pot experiments. The five promising native taxa, namely *Trichoderma longibrachiatum*, *T. harzianum*, *T. asperellum*, *Lecanicillium* spp., and *Metacordyceps chlamydosporia*, were selected to compare their effectiveness against both chemical (Mocap, 10% ethoprophos) and biological (abamectin) nematicides on *M. javanica* reproduction indices and plant growth parameters. The stimulation of defense mechanisms was assessed by monitoring changes in the enzymatic activities of the polyphenol oxidase (PPO), peroxidase (POD), ascorbate peroxidase (APX), catalase (CAT), lipid peroxidation (MDA), phenols, and proteins content of tomato roots. The laboratory assays revealed that *T. longibrachiatum*, *M. chlamydosporia*, and *Lecanicillium* spp. seemed to be the most effective under laboratory conditions, with more than 60% of juvenile mortality. The egg infection rate was above 62%, and the egg hatching rate was below 32%. The direct parasitism by the five taxa was confirmed by scanning electron microscope observation. The results of this study found a similar parasitism mechanism for *T. longibrachiatum*, *T. harzianum*, and *M. chlamydosporia*, where their hyphae and spores adhered to the *M. javanica* juveniles cuticle layer and formed trapping rings around them. The pot experiment

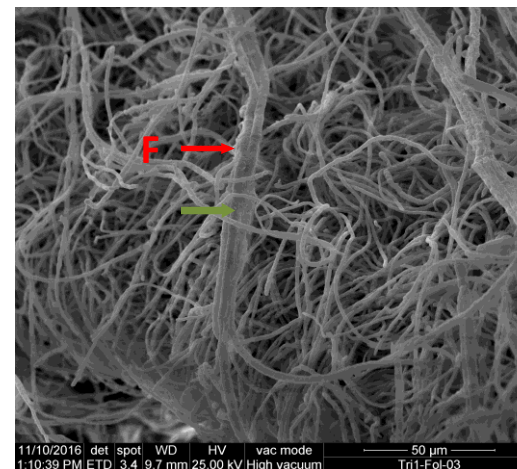
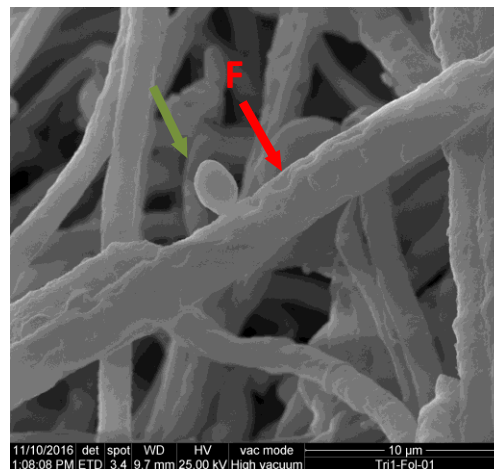
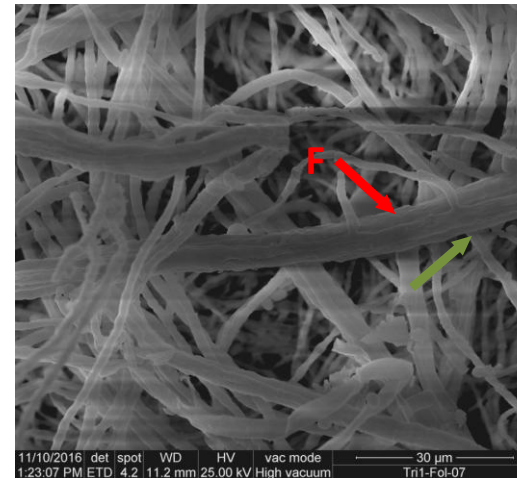
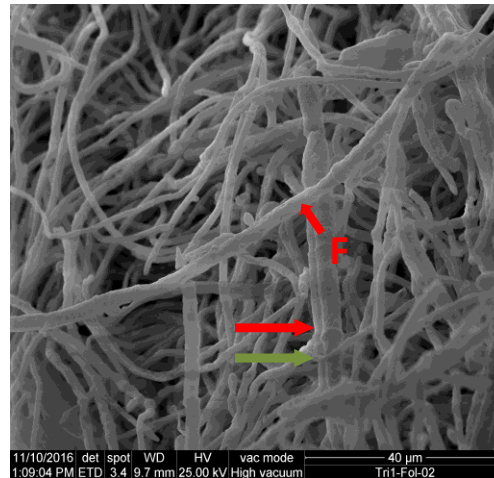


Citation: Hajji-Hedfi, L.; Hlaoua, W.; Al-Judaibi, A.A.; Rhouma, A.; Horrigue-Raouani, N.; Abdel-Azeem, A.M. Comparative

Transformation of hyphae into cords

Coiling of pathogen mycelium

Emergence of Tr1 conidia from FOL mycelium

Micrographies by (SEM) of FOL parasitized by *Trichoderma longibrachiatum*

4. Beneficial fungi against grey mold disease of tomato



1. Antifungal activities of BCFs against *B. cinerea*
2. Biochemical characterization of soil microorganisms
3. Potential of plant defense mechanism stimulation (SDP)
4. Effect on tomato growth and quality criteria

Botrytis cinerea

Light brown spots on the leaves



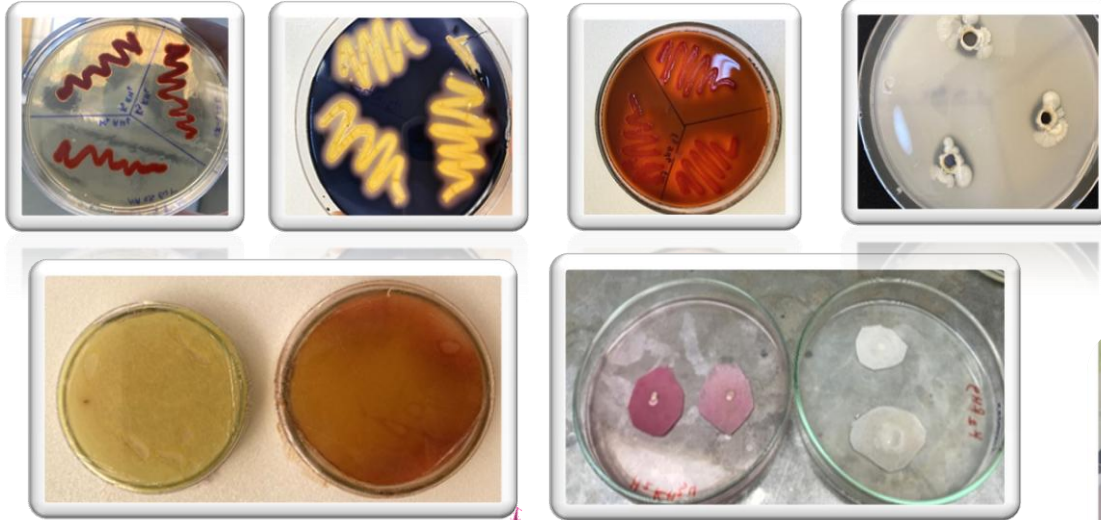
A whitish to light brown canker on the stem



A greyish down on the fruits



Biochemical characterization (PGP)



In vitro tests



In vivo tests



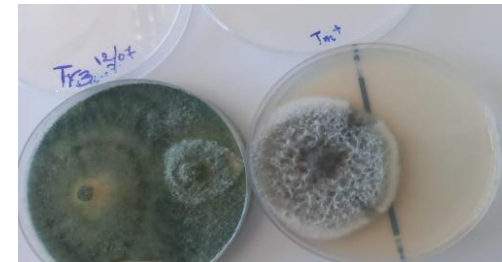


In vitro tests

Antifungal activity

Trichoderma viride: 79.6%

Direct method

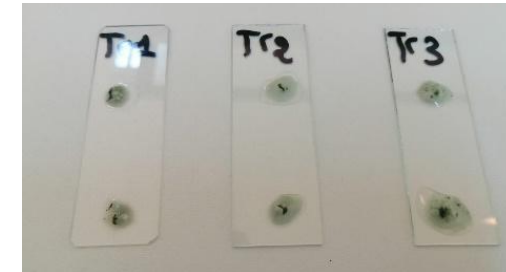


Indirect method (VOC)



Culture filtrate

Fungus growth **spore germination**





In vivo tests

**Promoting plant growth
Reduction disease incidence (DI)**



Reduction of grey mold rate on fruit



Treatments	MDA ($\mu\text{mol/g}$)	Total protein content (mg/g)	Total phenolic content ($\mu\text{g/g}$)
TR1	3.32 \pm 0.004a	19.46 \pm 0.02b	0.91 \pm 0.009b
TR2	2.73 \pm 0.006b	12.71 \pm 0.007b	0.7 \pm 0.003c
TR3	2.32 \pm 0.006c	31.24 \pm 0.02ab	0.78 \pm 0.01c
AS	2.11 \pm 0.007cd	50.85 \pm 0.1a	0.74 \pm 0.003c
TM+	1.86 \pm 0.01d	15.5 \pm 0.02b	0.59 \pm 0.01d
TM-	2.04 \pm 0.02cd	14.88 \pm 0.05b	1.04 \pm 0.01a
P-value	<0.01	<0.01	<0.01



Study 5

5. Screening and application of Beneficial fungi in Tunisian vineyards

Soil fungal diversity of vineyards on The Centre of Tunisia

Correlation Soil physio-chemical proprieties-microbial biodiversity

Soil parameter	method
soil texture	Sedimentation analysis (e.g. pipette method, hydrometer)
pH	Potentiometric determination in H ₂ O and in KCl / CaCl ₂
SOC	Wet oxidation (potassium dichromate) - Walkley and Black
total N	Kjeldahl
available P	Olsen (Na bicarbonate 0.5 M, pH 8.5)
exchangeable K	Ba chloride, pH 8.1
CEC	Ba chloride, pH 8.1
EC	Electrical resistance of a 1:2 soil:water suspension
water-stable aggregates	Elliot et al., 1986
bulk density	Cylindrical Core Method

*Selection of fungi with PGP traits
and antifungal activity*



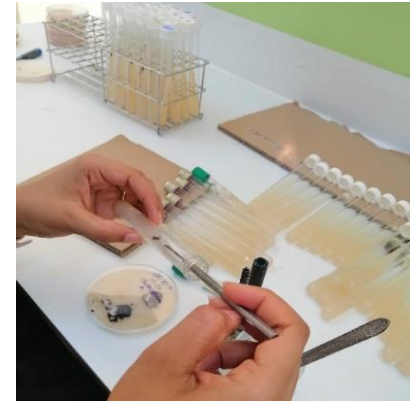
Isolation
Identification

Diversity Analysis

Correlation (CPA)



Field application on pilot
parcel

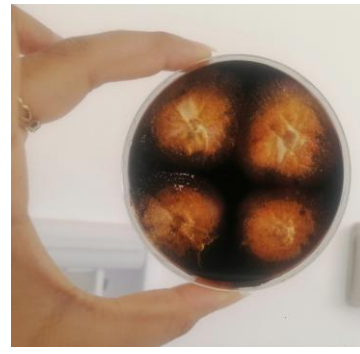




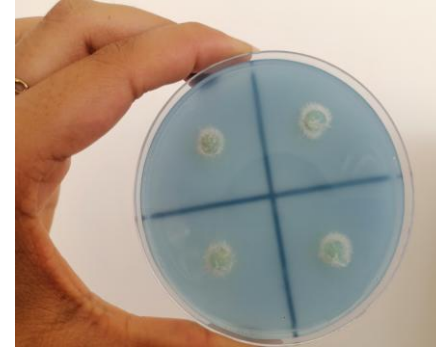
Protease activity



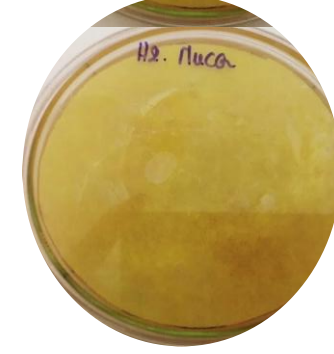
Azote fixation



Amylolytic activity



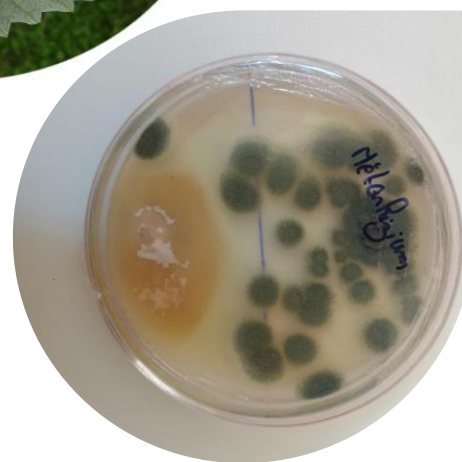
Phosphate solubilisation



Production of HCN antibiotic



Antifungal activity



These studies are interesting for improving plant health and productivity and give new insight and significant consequences in agriculture in global.



The crop management and sustainability could be assessed by Knowledge of the interactions within a phytobiome.



Understand plant-fungi interaction at molecular and genomic level



Discover new isolates; Fungi Kingdom richness of fungi with high interest to sustainable agriculture



Thank You for your attention

ANY QUESTIONS?

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