# PRELIMINARY STUDIES ON OBTAINING MICROORGANISMS BENEFICIAL TO PLANT DEVELOPMENT AND PROTECTION

**A. GHIGA, Irina GREBENISAN**
***University of Agronomic Sciences and Veterinary Medicine of Bucharest***
***59 Marasti Boulevard, District 1, Bucharest, 011464, Romania***
*Corresponding author: antonghiga@gmail.com*

***Abstract.*** *Phytopathogenic fungi produce significant crop losses annually and to limit their degree of attack we opted for biological control. Although the biological control of plants has been approached by many researchers from different countries, it is important to discover new wild strains native to Romania to combat phytopathogenic fungi and to stimulate plant growth and development. The new wild strains of microorganisms - bacteria and fungi isolated in this experiment have proven their ability to stimulate plant development and combat phytopathogenic fungi that cause seedlings to fall on tomatoes. In vitro experiments were carried out at the laboratory of Ecology and Microbiology at the Faculty of Land Reclamation and Environmental Engineering, U.S.A.M.V. Bucharest starting with March 2020 after soil sampling in Băneasa Forest. In vivo experiments were carried out in a vegetable garden in the village of Tamași, Corbeanca commune, Ilfov county since April 2020. The aim of the experiments was to obtaining microorganisms beneficial to plant development and protection and to highlight the capacity of microorganisms - bacteria and fungi - from forest soil to control phytopathogenic fungus in the complex, causing plant drop at tomatoes, which are frequently occurring in greenhouses and fields. Our research will continue in the direction of rigorous characterization of new wild strains of microorganisms isolated both at the genetic molecular level and of the mechanisms of action in the relationships of plant microorganisms and bioactive compounds secreted by these wild strains of microorganisms. Also, after characterizing the new microbial strains, we aim to obtain on their basis at laboratory and pilot scale growth stimulators and fungicides. For this we will carry out studies to optimize the conditions for growing microbial biomass, to obtain biologically active substances and to formulate ecological fungicides and growth stimulants for plants. After the crisis triggered by the SARS-CoV-2 virus will be overcome and we will be able to have access to the laboratory, we will start the experiments.*

***Keywords:*** *Rhizoctonia solani, tomatoes, biological control, wild strains of soil bacteria and fungi*

## INTRODUCTION

Soil microorganisms - bacteria, yeasts, fungi play many essential roles that support fertility, productivity and health of soils in agricultural ecosystems, but not only. (MATEESCU ET AL., 2004, OLTEANU ET AL., 2007, RASHID ET AL., 2016, FRAC ET AL., 2018) The positive relationships that have been established over time in these ecosystems, between microorganisms and different parts of crop plants - roots, stems, leaves, fruits have led to healthier plants that have gained resistance to phytopathogens or have acquired mechanisms by which to limit the attack of phytopathogens. (GREBENISAN ET AL., 2008, RAHMAN ET AL., 2018) Also, plants could achieve higher yields if, for example, certain types of microorganisms were present in the rhizosphere or rhizoplan that stimulated plant growth by producing phytohormones, enzymes or volatile bioactive compounds. (SOUZA, ET AL., 2015, GAIERO ET AL., 2013).

The FAO website presents some case studies showing the importance of beneficial soil microorganisms, of which we cite: "Beneficial microorganisms include those that create symbiotic associations with plant roots promote nutrient mineralization and availability, produce plant growth hormones, and are antagonists of plant pests, parasites or diseases (biocontrol agents)". (www.fao.org)

Because over time the use of pesticides used to control phytopathogens that cause diseases of crop plants has led to the emergence of resistant strains. For this reason, the current orientation in the control of crop plant diseases must be towards the use of as few chemical pesticides as possible in favor of the use of integrated control in which to use biological control along with cultural techniques and resistance of cultivated plant varieties. (ADESEMOYE ET AL., 2009, HUNGRIA ET AL., 2010, HUNGRIA ET AL., 2013)

Vadakattu says in his work, we quote: "There is an urgent need to harness the multiple beneficial interactions that occur between plants and microorganisms. Some of the commonly promoted and used beneficial microorganisms in agriculture worldwide include *Rhizobia, Mycorrhizae, Azospirillum, Bacillus, Pseudomonas, Trichoderma, Streptomyces* species". (VADAKATTU, 2012)

Hossain et al. presented the importance of fungi associated with plant roots in a review. Researchers highlight some of the properties of fungi considered beneficial for crop development, namely the ability to stimulate germination, seedling vigor, biomass growth, better root development, absorption better of nutrients. They also show the ability of beneficial fungi to induce systemic plant resistance, which allows them to counteract the negative effects produced by phytopathogenic fungi. (HOSSAIN ET AL., 2017)

In their paper Zhang et al., present the capacity of fungi of the genus Trichoderma as being useful in increasing the tolerance of wheat plants to salty soils and resistance to the attack of parasitic nematodes. (ZHANG ET AL., 2016)

Naziya et al. analyzed in an experiment the ability of fungal strains in the soil to stimulate the growth and development of pepper plants and to combat anthracnose. They were able to isolate five strains of beneficial plant fungi from the total of seventy strains they obtained from the soil samples taken. These five strains showed a good ability to stimulate plant growth and induce systemic resistance to pepper plants. (NAZIYA ET AL., 2020)

The study conducted by Hammad and Elbagory over a period of two years shows beneficial fungi of the genus Trichoderma as biocontrolling agents against the common red spider mite in watermelon and as biofertilizers. (HAMMAD ET AL., 2019)

Nakkeeran et al. in the chapter on bioactive molecules secreted by bacteria of the genus *Bacillus* review the mechanisms by which these bacteria contribute to the development and protection of plants. (NAKKEERAN ET AL., 2019)

Timmusk et al. say in their paper, we quote: "The integration of beneficial plant-microbe and microbiome interactions may represent a promising sustainable solution to improve agricultural production". (TIMMUSK ET AL., 2017)

## MATERIAL AND METHODS

In vitro experiments were carried out at the laboratory of Ecology and Microbiology at the Faculty of Land Reclamation and Environmental Engineering, U.S.A.M.V. Bucharest starting with March 2020 after soil sampling in Băneasa Forest. In vivo experiments were carried out in a vegetable garden in the village of Tamași, Corbeanca commune, Ilfov county since April 2020. The aim of the experiments was to highlight the capacity of micro-organisms - bacteria and fungi - from forest soil to control phytopathogenic fungus in the complex, causing plant drop at tomatoes, which are frequently occurring in greenhouses and fields.

### OBTAINING FOREST SOIL MICROORGANISMS FROM BĂNEASA FOREST

For the production of biological control agents for control purposes complex of phytopathogenic fungus, which causes the plants to fall at tomatoes, we collected two samples of forest land from Băneasa forest in the spring of March 2020.

The samples were brought to the laboratory of Ecology and Microbiology of the Faculty of Land Reclamation and Environmental Engineering, U.S.A.M.V. Bucharest to be examined under stereomicroscope and microscope, and to extract live cultures of microorganisms – bacteria and fungi - with the potential for biological control of phytopathogenic fungus agents *Pythium* sp. and *Rhizoctonia* sp. at tomatoes.

Thus, we have microscopically examined the samples using stereomicroscope and microscope in native preparations between slide and slide.

To highlight the existence of fungus that may be used as biocontrol agents in control of phytopathogenic fungus in the plant drop complex at tomatoes, we used the wet camera technique – 50 g of soil have been placed in 120 mm diameter Petri dishes and incubated at room temperature for 10 days. The Petri vessels containing forest soil samples have been wrapped in paper to avoid dehydration. Soil samples were moistened twice in the 10-day interval by spraying sterile distiled water.

**IN VIVO EXPERIMENTS**

Aqueos extracts from the forest soil samples, which contained microorganisms (bacteria, fungi, etc.) that play a role in stimulating growth and plant development by the production of enzymes, growth hormons and substances having an anti-fungic function, were applied to 20 tomatoes seedlings, 50 ml for every seedling at pricked from alveoli in the greenhouse soil at the end of April 2020, and the second treatment after 10 days and the third after another 10 days.

## RESULTS AND DISCUSSIONS

### OBTAINING FOREST SOIL MICROORGANISMS FROM BĂNEASA FOREST

Microscopic examination of the soil extract highlighted the presence of bacteria (cocci and bacilli, but also protozoa) in forest soil samples from Băneasa Forest (fig.1,2,3).

Fig. 1. Stereomicroscopic image of forest soil sample from Băneasa Forest

Fig. 2. Microscopic image of the forest soil sample from Băneasa forest containing PGPB bacteria (Plant Growth Promoting Bacteria) and BCA (Biological control Agent) – 100 x lens magnification

Fig. 3. Microscopic image of the forest soil sample from Băneasa Forest containing PGPB bacteria (Plant Growth Promoting Bacteria) and BCA (Biological control Agent) – 100 x lens magnification

After 5 days of incubating the samples at room temperature in the lab, the surface of the soil samples could be observed for the formation of the felt microeles of mold (fig.4.), and after 10 days, the training of conidia, which gives the characteristic color (fig.5.). Macroscopic and microscopic examination of soil samples allowed us to identify appearance of fungi of genus *Trichoderma*, known as biological control agents.

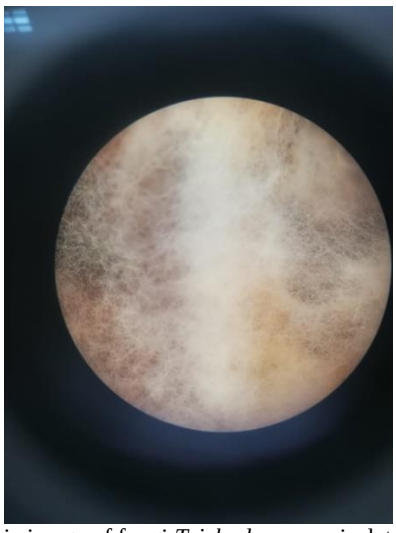

Fig. 4. Stereomicroscopic image of fungi *Trichoderma* sp. isolated from the forest soil originated from Băneasa Forest –hyphae that form the mycelium.

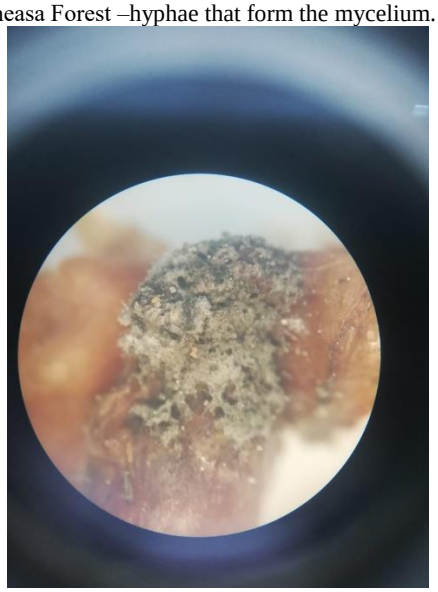

Fig. 5. Stereomicroscopic image of fungi *Trichoderma* sp. isolated from the forest soil originated from Băneasa Forest – mycelyum with spores (green color conditions)

**RESULTS OF IN VIVO EXPERIMENTS**

The blank test tomato plants, which were not treated with the biological control agents, have shown obvious signs of disease. Thus, they were eliminated because compromised plants colapse and die, being infected with phytopathogenic fungal infections *Pythium* sp. and *Rhizoctonia* sp. being lethal (fig. 6.).

Fig. .6. Detail at tomato plant with stem area affected by phytopathogenic fungi *Rhizoctonia* sp.

Fig.7. Detail at tomato plant with parcel area affected by phytopathogenic fungi *Rhizoctonia* sp.

Treatments with biological control agents applied to the tomato plants increased their immunity and ensured their development so they could fructify, the quality of the fruit being unaffected (fig. .8,9,10,11,12,13.).

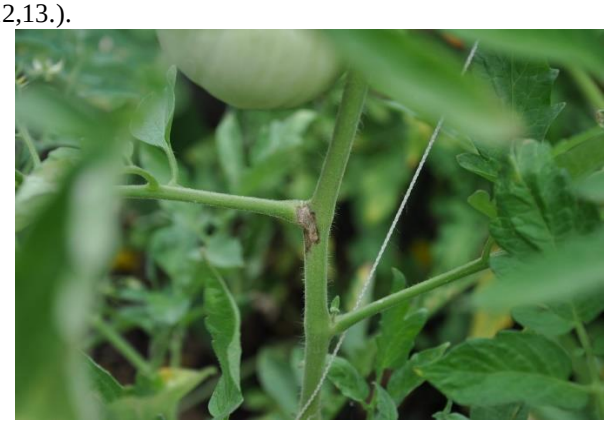

Fig.8. Detail of the mature plant on the affected fruit by *Rhizoctonia* sp.

Fig.9. Detail of the mature plant on fruit with stem area affected by *Rhizoctonia* sp.

Fig.10. Detail of the mature plant on fruit with stem area affected by *Rhizoctonia* sp.

Fig.11. Detail of the mature plant on fruit with stem area affected by *Rhizoctonia* sp.

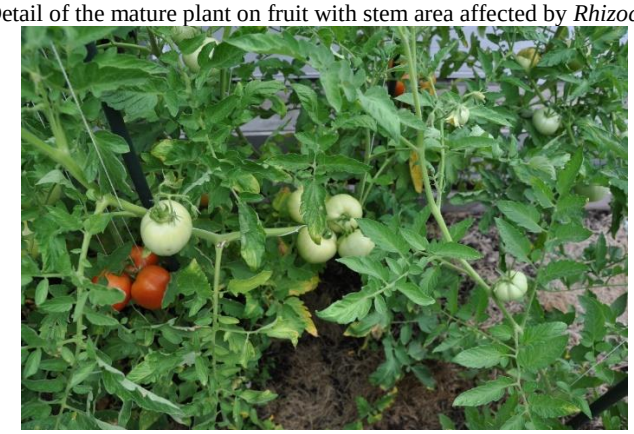

Fig.12. Detail of the mature plant on fruit with branches affected by *Rhizoctonia* sp.

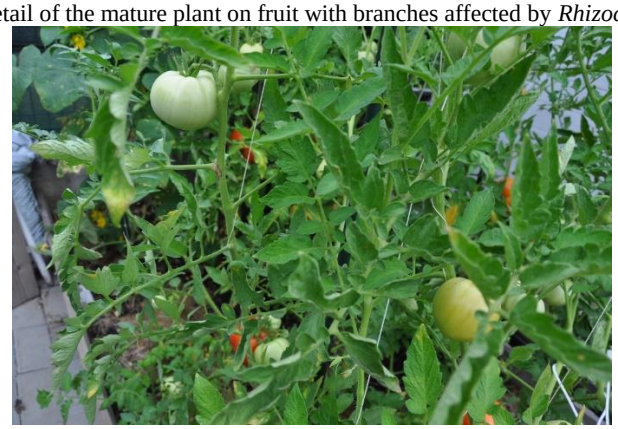

Fig.13. Detail of the mature plant on fruit fruit with branches affected by *Rhizoctonia* sp.

## CONCLUSIONS

The preliminary results obtained in this experiment allow us to draw the following conclusions: the soil samples taken from Baneasa Forest showed a great diversity of microorganisms; isolated wild-type microorganisms showed the characteristics of microorganisms included in the category of biological control agents and those that stimulate the development of plants; treatments with biological control agents applied to the tomato plants increased their immunity and ensured their development so they could fructify, the quality of the fruit being unaffected.

Our research will continue in the direction of rigorous characterization of new wild strains of microorganisms isolated both at the genetic molecular level and of the mechanisms of action in the relationships of plant microorganisms and bioactive compounds secreted by these wild strains of microorganisms. Also, after characterizing the new microbial strains, we aim to obtain on their basis at laboratory and pilot scale growth stimulators and fungicides. For this we will carry out studies to optimize the conditions for growing microbial biomass, to obtain biologically active substances and to formulate ecological fungicides and growth stimulants for plants.

## BIBLIOGRAPHY

ADESEMOYE, A.O., TORBERT, H.A., KLOEPPER, J.W., 2009, Plant growth-promoting rhizobacteria allow reduced application rates of chemical fertilizers, Microb Ecol. 58(4):921-9, https://link.springer.com/article/10.1007/s00248-009-9531-y

FRĄC, M., HANNULA, S.E., BEŁKA, M., JĘDRYCZKA, M., 2018 - Fungal Biodiversity and Their Role in Soil Health, Front. Microbiol., 9:707. doi: 10.3389/fmicb.2018.00707 https://www.frontiersin.org/articles/10.3389/fmicb.2018.00707/full

GAIERO, J.R., MCCALL, C.A., THOMPSON, K.A., DAY, N.J., BEST, A.S., DUNFIELD, K.E., 2013 – Inside the root microbiome: bacterial root endophytes and plant growth promotion, Am J Bot., 100(9):1738-50, https://pubmed.ncbi.nlm.nih.gov/23935113/

GREBENISAN, I., CORNEA, P., MATEESCU, R., CIMPEANU, C., OLTEANU, V., CAMPENU, G.H., STEFAN, L.A., OANCEA, F. AND LUPU, C., 2008 - *Metschnikowia pulcherrima*, a new yeast with potential for biocontrol of postharvest fruit rots, Acta Hortic. 767, 355-360 DOI: 10.17660/ActaHortic.2008.767.38 https://doi.org/10.17660/ActaHortic.2008.767.38

HAMMAD, R., ELBAGORY, M., 2019 - Using Plant Growth-promoting Fungi (PGPF), as a Biofertilizer and Biocontrol Agents against Tetranychus cucurbitacearum on Nubian Watermelon (Citrullus lanatus L.). *Journal of Advances in Microbiology*, *16*(2), 1-15. https://doi.org/10.9734/jamb/2019/v16i230119

HOSSAIN M.M., SULTANA F., ISLAM S., 2017 - Plant Growth-Promoting Fungi (PGPF): Phytostimulation and Induced Systemic Resistance. In: Singh D., Singh H., Prabha R. (eds) Plant-Microbe Interactions in Agro-Ecological Perspectives. Springer, Singapore. https://doi.org/10.1007/978-981-10-6593-4_6

HUNGRIA, M., CAMPO, R.J., SOUZA, E.M., PEDROSA, F.O., 2010 - Inoculation with selected strains of *Azospirillum brasilense* and *A. lipoferum* improves yields of maize and wheat in Brazil, Plant Soil, 331:413–425, https://link.springer.com/article/10.1007/s11104-009-0262-0

HUNGRIA, M., NOGUEIRA, M.A., ARAUJO, R.S., 2013 - Co-inoculation of soybeans and common beans with rhizobia and azospirilla: Strategies to improve sustainability. Biol Fertil Soils, 49:791–801, https://link.springer.com/article/10.1007/s00374-012-0771-5

MATEESCU, R., CORNEA, C.P., GREBENISAN, I., BABEANU, N., CAMPEANU, G., 2004 - In vivo

biocontrol activity of *Bacillus* spp. strains on *Alternaria tenuis*, Roumanian Biotechnological Letters, Vol. 9, No. 3, 2004, pp 1713-1721, https://e-repository.org/rbl/vol.9/iss.3/9.pdf

NAKKEERAN, S., VINODKUMAR, S., RENUKADEVI, P., RAJAMANICKAM, S., JOGAIAH, S., 2019, Bioactive Molecules from Bacillus spp.: An Effective Tool for Plant Stress Management, Bioactive Molecules in Plant Defense Signaling in Growth and Stress**,** Print ISBN: 978-3-030-27164-0, Electronic ISBN: 978-3-030-27165-7, https://www.springerprofessional.de/en/bioactive-molecules-from-bacillus-spp-an-effective-tool-for-plan/17190160?fulltextView=true

NAZIYA, B., MURALI, M., AMRUTHESH, K. N., 2020, Plant Growth-Promoting Fungi (PGPF) Instigate Plant Growth and Induce Disease Resistance in Capsicum annuum L. upon Infection with Colletotrichum capsici (Syd.) Butler & Bisby, Biomolecules, 10, 41, www.mdpi.com

OLTEANU, V., GREBENISAN, I., CAMPEANU, G., 2007 – New biological compound with application in plant protection, Bulletin of University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca. Agriculture, ISSN: 1843-5246

RAHMAN, S.F.S.A., SINGH, E., PIETERSE, C.M.J., SCHENK, P.M., 2018, Emerging microbial biocontrol strategies for plant pathogens, Plant Science, Volume 267, ISSN 0168-9452, https://doi.org/10.1016/j.plantsci.2017.11.012., https://www.sciencedirect.com/science/article/abs/pii/S016894521730540X?via%3Dihub

RASHID, M.I., MUJAWAR, L.H., SHAHZAD, T., T., ALMEELBI, T., ISMAIL, I.M.I., OVES, M., 2016 – Bacteria and fungi can contribute to nutrients bioavailability and aggregate formation in degraded soils, Microbiological Research, Volume 183, ISSN 0944-5013, https://doi.org/10.1016/j.micres.2015.11.007, http://www.sciencedirect.com/science/article/pii/S0944501315300288)

SOUZA, R. D., AMBROSINI, A., PASSAGLIA, L. M., 2015 - Plant growth-promoting bacteria as inoculants in agricultural soils. Genetics and molecular biology, *38*(4), 401–419. https://doi.org/10.1590/S1415-475738420150053

TIMMUSK, S., BEHERS, L., MUTHONI, J., MURAYA, A., ARONSSON, C., 2017 - Perspectives and Challenges of Microbial Application for Crop Improvement, Frontiers in Plant Sciences, https://doi.org/10.3389/fpls.2017.00049)

VADAKATTU, G., 2012 - Beneficial microorganisms for sustainable agriculture, Microbiology Australia 33(3):113-115, DOI: 10.1071/MA12113

ZHANG S, GAN Y AND XU B, 2016 - Application of Plant-Growth-Promoting Fungi *Trichoderma longibrachiatum* T6 Enhances Tolerance of Wheat to Salt Stress through Improvement of Antioxidative Defense System and Gene Expression. *Front. Plant Sci.* 7:1405. doi: 10.3389/fpls.2016.01405

*** http://www.fao.org/agriculture/crops/thematic-sitemap/theme/spi/soil-biodiversity/case-studies/soil-biological-management-with-beneficial-microorganisms/en/