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**Application of
Trichoderma harzianum
in tobacco seedlings
protection**

What is *Trichoderma*

Trichoderma are avirulent soil fungi, always associated with the root and root ecosystem. They are plant symbiotic opportunistic avirulent organisms capable of colonizing roots through mechanisms similar to those of mycorrhizal fungi, developing simultaneously with its growth and producing substances that stimulate plant growth and their defense. They are strong rhizosphere competitors. At the same time, they are plant symbionts that are interactive in root, soil and foliar environments. Colonization of the root with *Trichoderma* results in the rapid establishment of a stable microbial community in the rhizosphere, increasing the crop productivity and resistance to plant pathogens, abiotic factors and stress. *Trichoderma* are characterized by strong bio-control activity on plant pathogens of different origin using a number of mechanisms. They act as parasites and antagonists of many phytopathogenic fungi and participate in plant protection from diseases. They are considered to be the most studied and most effective biocontrol agents of high commercial use as biopesticides. *Trichoderma* strains enhance the growth and development of root system and the absorption of water and nutrients from the soil, positively affecting plant development and creating more vigorous and resistant plants. They have a stimulating effect on plant defense mechanisms.

Biological control

The term biological control (biocontrol) is commonly used as a microbial antagonist for the plant protection from diseases. The organism that control the pathogen is named a biocontrol agent (BCA).

Biological control using biocontrol agents for control of pathogens is a natural, environmentally friendly approach to the management of plant disease that allows reduction the usage of chemical pesticides and incorporation into the IMP system.

The mechanisms of action of the *Trichoderma* species are complex and whatever is defined as a biological fight is the final result of various mechanisms that act synergistically to achieve the effective disease control.



Trichoderma in biological control

Fungi of the genus *Trichoderma* are the most powerful bio-control agents which have been known since 1930s. *Trichoderma* is a "super hero" against numerous phytopathogenic microorganisms. The high effectiveness of these agents in biological control of plant pathogens promotes them as a promising method for protecting plants against diseases. *Trichoderma* is perhaps the best-known mycoparasite that has been suggested as a biocontrol agent against numerous soil pathogens. *Trichoderma* species are widely used in the control of soilborne pathogenic fungi - the causing agents of seed and root rot and damping off diseases in plants. They especially protect the root system from diseases caused by soilborne pathogens of the genera *Pythium*, *Rhizoctonia*, *Fusarium*, *Sclerotinia*, etc. They act against leaf pathogens, too. Their biocontrol potential has been exploited against a many pathogens in vegetable, fruit, field and industrial crops. Due to their confirmed importance in protection of plants, ecology and environment, these agents are used as an active ingredient in a number of biological products. The effect of the one *Trichoderma* species on the certain crop has an advantage in commercialization.



The mechanisms that biocontrol agents incorporate in the control of pathogens are most important for wider application and improvement of the biological control. The knowledge of them (even the limiting factors) make the possibility to develop a way of their isolation, growing, application and the full exploitation of them in plant protection. The greatest potential for enhancing the role of biological control in plant protection from the diseases is not only in the commercial use of biocontrol agents, but also in utilizing our knowledge of the complex interactions between biocontrol agents and plants, as well as the overall biological activity in the ecosystem.

Mechanisms of action of biological control

Competition for food and space

Trichoderma multiplies rapidly in plant's rhizosphere. Its growth is stimulated by the root secretions and it grows together with the root. In this way, it becomes a competitor for food and space to other microorganisms, including pathogens.

Mycoparasitism

Parasitism of one fungus on another is a complex phenomenon or process which involves different degrees of interaction and it is a strong side of the genus *Trichoderma*.

Antibiosis or anatogonism –

is a strong mechanism of action that includes the ability of *Trichoderma* to produce antibiotics, toxins or various antifungal metabolites (volatile and non-volatile).

Induced (acquired) resistance

Trichoderma promotes the formation of various metabolites (phytoalexins, flavonoids, terpenoids, phenol derivatives) which help the plants to resist the attacks and strengthen the defense mechanisms.

Facilitation of nutrients and water absorption / stimulation of plant development

Trichoderma relieves the solubility of certain nutrients, thus increasing their absorption, especially of those in insoluble form such as phosphates and microelements. It enhances the root and thereby increases the nutrients supply, which is reflected on plants vigor.

Predispositions for successful action

- High reproductive material,
- Ability to survive under unfavorable conditions,
- Efficient use of nutrients,
- Higher supply of nutrients for plants,
- Promotion of plants growth and development, , by which vigour becomes a mighty weapon in the control of pathogens,
- Ability to modify the rhizosphere,
- Expressed aggressiveness towards phytopathogenic fungi and development of defense mechanisms in plants,
- Resistance to residues,
- Synergistic effect with some pesticides,
- Compatibility for application with other pesticides.



Application of *Trichoderma*

Fungi of the genus *Trichoderma* especially protect the root system from diseases caused by soilborne pathogens of the genera *Pythium*, *Rhizoctonia*, *Fusarium*, *Sclerotinia*, etc. They act against leaf pathogens, too.

They are widely used in the control both of soilborne seed and root pathogens - the causing agents of seed and root rot and damping off diseases as well as control of leaf pathogens.

There are various modes of application: treatment (colonization of seeds with the agent), mixing with the substrate, foliar application on seedlings and plants, application of higher amount on the infected place, use of the drop-by-drop system, etc.).

It is of crucial importance to inject the agent in order to promote the reproduction of its population and at the same time to enable activation of all bio-control mechanisms.

Application of herbicide is possible if *Trichoderma* is applied through the most effective application model of this biocontrol agent.

Fungicide should not be used for 3 days before and after treatment with *Trichoderma*.

When applying pesticides, knowledge of the list of compatible active substances is required.

There are some integrated protection strategies based on the combined application of biocontrol agents (with reduced dose of pesticide to sublethal), using the synergistic effect of the biocontrol agent with the certain pesticides.

Trichoderma harzianum – the most effective biocontrol agent in tobacco

The role of the genus *Trichoderma* in plant protection, ecology and the environment is particularly prominent in *Trichoderma harzianum*: it promotes plants development and is highly aggressive towards phytopathogenic fungi but is also effective in the control of a large number of soil and foliar pathogens. It multiplies rapidly and is active in different soil types. It is compatible with a large number of active ingredients.

Some species of the genus *Trichoderma* have an ability to control the causing agents of fungal diseases of tobacco: *T. hamatum*, *T. aureoviride* and *T. harzianum*.

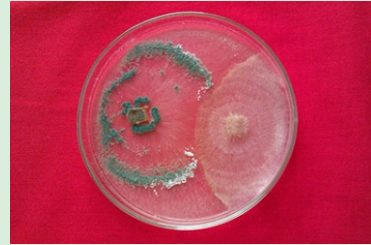
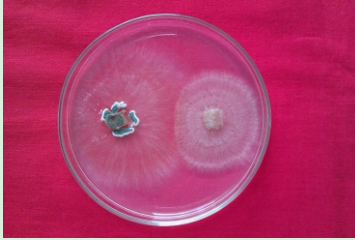
The most effective species, especially in the control of pathogenic fungi *Pythium debarianum* and *Rhizoctonia solani* – the causing agents of root rot and damping off diseases on tobacco seedlings - is *Trichoderma harzianum*. This has been confirmed in *in vitro* studies in biological laboratory, but also in tobacco seedbeds, under real production conditions.

In global frames, one of the most effective strains is *Trichoderma harzianum* T-22. It is the basic ingredient of Trianum P (commercial biofungicide registered in our country) which contains live spores of *T. harzianum* T-22.

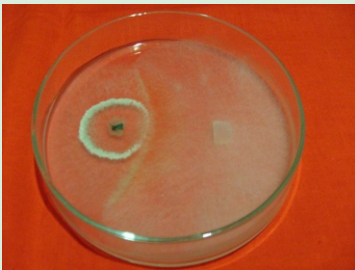
* The mode of application was determined and based on the author's experience in the work with this bio- agent at the Scientific Tobacco Institute -Prilep.



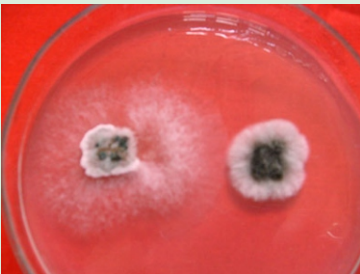
The effect of *Trichoderma harzianum*
Trichoderma harzianum reduces



Rhizoctonia



Pythium



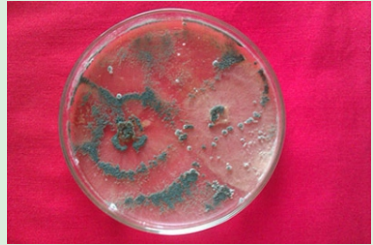
Alternaria



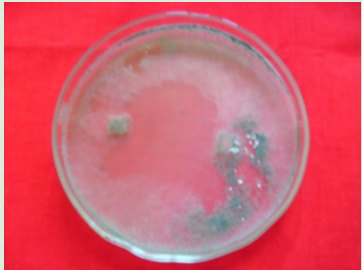
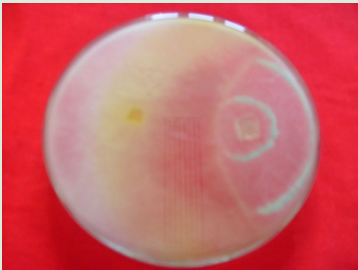
Phytophthora parasitica

In vitro
(Dual culture

on pathogenic fungi in tobacco
the pathogenic effects on tobacco:



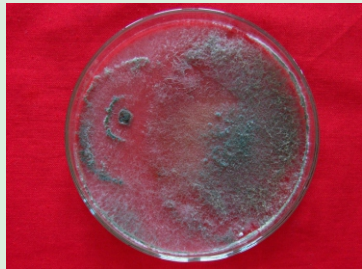
solani



debarianum



alternata



var. *nicotianae*

investigations
method)

Application of *Trichoderma harzianum*

*** Seed treatment before sowing and several successive treatments of tobacco seedlings is the most effective mode of application of this biocontrol agent**

* If the seed is not treated, the first application should be made as soon as possible after sowing

* Additional treatments should not be omitted because they are necessary for maintenance and growth of the agent population and for activation of biocontrol mechanisms.

* The application mode is adapted to the real process of seedling production, i.e. with a use of herbicide. In order to reduce the adverse effects, treatments should be made at shorter intervals, especially in the early stages of development.

* The preparation should be suspended in adequate amount of water for a good and balanced intake of the biocontrol agent.

* Attention should be paid to temperature at the time of application. Like every living organism, this fungus is sensitive to high temperatures, which complicates its application in foliar diseases. At temperatures below 100 C⁰, however, its activity is significantly reduced.

* When using pure culture, it is recommended to use min. 6 Petri-dishes with a well-developed culture at 10m², but there is no restriction because the larger population (number of colonies/g soil) has a greater effect in reducing the disease.

* The application model as well as the effects of the application of this bio-agent have been confirmed in pure culture. For easier application, a model with application of commercial preparation is also shown, using the units of measurement for powder formulations. (Norms - dose, preparation and water amount are in accordance with the preparation Trianium P).

Mode of application with pure culture

| Procedure | Dose (amount of water) |
|---|--|
| Keep the seed 24-48 h in Petri dish with a pure culture of the biocontrol agent The other Petri dishes (spores and mycelium) are added to those in which the seeds were kept * it is mixed with sand, but sowing with water is more favorable | min 6 Petri dishes/10 m² 1/2 pack seed in 1 Petri dish – *the amount of cultures is adjusted to the amount of seed |
| it is mixed with sand, but sowing with water is more favorable | min. 6 Petri dishes/10 m² (watering with ≈ 30 l water) |
| + 2-3 additional treatments – watering of seedling in each stage starting from 4-leaf stage | Min. 6 Petri dishes / 10 m ² for each application |

Mode of application (commercial preparation)

| Procedure | Dose (amount of water) |
|---|---|
| Dissolve required measure of the preparation in 5 measures of water | 15 g preparation / 10 m² (15 g suspended in ≈ 100 ml water) |
| Treatment of seed after sowing: 4,5 g in 15 g preparation for 10m ² (prepared as recommended) and min. 24 h storage *48 h is suggested | 15 g preparation / 4,5 g seed / 10 m ² *Sowing with water is recommended |
| + 2-3 additional treatments – watering of seedling in each stage starting from 4-leaf stage | 15 g / 10 m ² applied with min. 25 l water with each application/watering |
| *Treatment prior to pulling is recommended because of its positive effect on rooting and development in field | |



Benefits of the application of *Trichoderma harzianum* in tobacco

Provides plant protection from soil and foliar diseases, ensuring healthy and good quality seedlings and, ultimately, raw material without pesticide residues.

Improves the root system of the seedlings. Well developed root system means better rooting of tobacco and higher tolerance to drought.

Provides better absorption of water and nutrients, strengthens the plants and, with proper growth and development, increases the predispositions for cultivation of healthy tobacco plants.

Provides long-term protection. When properly used, *Trichoderma harzianum* grows in symbiosis with plant roots and thereby protects the plants from various soilborne diseases during the growth season.

It is effective in different soil types and substrates, at wide temperature range and different pH values.

It does not develop resistance - one of the many positive traits that put this fungus above other protection chemicals

The correct model of application allows its use along with other herbicide

The effects of *Trichoderma* application in protection of tobacco seedlings from root rot disease



Damping off in tobacco seedlings – different intensity of attack



Application of *Trichoderma* - healthy seedlings

The application of *Trichoderma* in biological control of the causing agents of root rot disease in tobacco seedlings is an effective, eco-friendly and long term measure of protection



Safe and eco-friendly application of *Trichoderma*

The application of *Trichoderma* is an important and effective ecological measure in agriculture:

- * Completely safe for humans, animals and plants.
- * Provides effective disease control, thus minimizing the harmful effects of pesticides.
- * *Trichoderma* strains do not induce formation of residues.
- * No phytotoxic effects
- * Higher number of treatments has no adverse effects
- * Reproduction of *Trichoderma* in plant rhizosphere provides long-lasting positive effects
- * *Trichoderma harzianum* plays an important role in the bioremediation of environment contaminated with pesticides and harmful substances

Biological control is a necessary and perspective measure of sustainable agriculture.

The application of biocontrol agents in the control of pathogens is a natural and ecological approach in protection from diseases which enables reduced use of chemicals and incorporation into the integral tobacco protection system.

Biocontrol agents - fungi of the genus *Trichoderma* are the alternative to the synthetic pesticides without negative impact on the environment.

The application of *Trichoderma* is characterized by the highest level of safety for both producers and the environment. It provides long-term protection against diseases and has multiple stimulating effects.

The use of *Trichoderma harzianum* in the biological control against the causing agents of root rot diseases in tobacco seedlings is an effective, safe, environment-friendly and long-term measure in modern agriculture.

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