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Biological Activity of Preparation Gliocladin-SC in vitro to Control Soil Pathogens

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Abstract Particularly dangerous pathogens of crops are fungus of the genus *Sclerotinia* and *Fusarium*. They are known as causal agents of fungal diseases that affect a wide range of cultivated plants: cereals, legumes, beans, vegetables, soy, sunflower, corn, etc. Pathogens cause various pathological phenomena - rot diseases of the roots, stems, seeds, fruits, and general depression and premature aging. Biological control methods that reduce the population of pathogen in the soil appear to be the most practical method. Fungus *Trichoderma virens* Miller, Giddens and Foster is a haploid, filamentous hyphomycete (a subclass of fungi). This fungus is present in most soils throughout the world. The antagonistic activity of *T.virens* showed that it is parasitic on many soil-borne and foliage pathogens. Recent discoveries show that the fungi not only act as biocontrol agents, but also stimulate plant resistance, and plant growth and development resulting in an increase in crop production. The biocontrol activity involving mycoparasitism, antibiotics and competition for nutrients, also induces defense responses or systemic resistance responses in plants. This paper reviews confirmed the antagonistic potential and antifungal ability of biopesticide Gliocladin-SC (active microorganism *Trichoderma virens* strain 3X) against pathogens - agents of white rot and root rot *Fusarium* complex of crops. Gliocladin-SC is recommended as biological product on soy for control the basal and root rot by preplans seed-treatment.

Keywords: *Trichoderma* virens strain 3X, biopesticide Gliocladin-SC, soy, root rot diseases

Introduction

Plant production has a very important place in the national economy all over the world. One of the most important ways of protecting plants and plant products against harmful organisms and diseases, and of improving agricultural production is the use of plant protection products. Many intervention practices (fungicides, organochlorine/organophosphorus pesticides, fumigants, etc.) focus on taking out the pathogen organism after its effects become apparent. Moreover, the use of chemical plant protection products may involve risks and hazards for humans, animals and the environment, especially if incorrectly used. foods and declining energy resources have stimulated elaboration of new alternative forms of

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production such as use of biological control agents.

Root diseases of vegetables, fruits and cereals are known to be highly destructive which can cause a significant economic yield loss in those crops. The species of fungal genus *Fusarium* Lk. ex Fr. cause different types of diseases in a wide range of plant species. The most common diseases are fusarium root rot and fusarium vascular wilt. The most harmful pathogens that cause fusarium in crops cultivated on large areas of the world are *F.graminearum* in wheat and other grains, *F. solani* in soy and *F.moniliforme* in corn (Бёттхер *et al*., 1987). Damage of fusarium root rot is also associated with a possible manifestation of it in the ear and grain. The mold genus *Fusarium* is capable of forming a variety of secondary metabolites, termed as Fusarium toxins due to their adverse effects on higher organisms. The major mycotoxins produced by *F. graminearum* are deoxynivalenol and zearalenone. Fungi *F.graminearum, F.culmorum, F.sporotrichioides* Sherb., *F.avenaceum* (Fr.) Sacc., *F. poae* (Peck) Woll., *F.verticillioides* (syn. *F.moniliforme*) produce fusarium toxins, contaminating the cereal harvests, thereby grain becomes unsuitable for use in food and feed (Бёттхер *et al*., 1987 and Kononenko, Burkin, 2006).

Sclerotinia sclerotiorum (Lib.) de Bary is another of the most widely distributed plant pathogens throughout the world. This pathogen can cause diseases in more than 400 host plant species within 64 families, including many economically important crops such as dry bean, soybean, carrot, lettuce, peanuts and sunflower (Boland and Hall, 1994). Ascospore-initiated infection relies on senescing flower parts as an initial source of necrotic tissue, resulting in common above-ground tissue diseases, while hyphal-initiated infection targets susceptible roots with necrotic tissue, causing few root and crown diseases (Inglis and Boland, 1990; Bardin & Huang, 2001).

The application of many fungicides in order to control root diseases reduce the diversity of soil organisms and select resistant pathogens. Biological control methods that reduce the population of pathogen in the soil appear to be the most practical method (Şesan, Crişan, 1998). Therefore, plant disease control has generally been focused on the use of biological control agents – Microbial Biocontrol Agents (MBCA). There are two types of disease suppression on the use of MBCA: specific and general. Specific suppression results in one organism directly suppressing a known pathogen. General suppression is the result of a high biodiversity of microbial populations that creates conditions unfavourable for plant diseases to develop.

Members of the *Trichoderma* genus occur worldwide and are commonly associated with root, soil and plant debris. Strains of *Trichoderma virens* has frequently been considered as effective biological control agents against variety of soil-borne and other plant pathogens. Since the first application in 1930s, *Trichoderma* species became popular biological agents that protect crops against plant pathogens all over the world.

Trichoderma virens is a haploid, filamentous hyphomycete. This fungus is present in most soils throughout the world. Mechanisms of *T. virens* biological activity include mycoparasitism and antibiosis (direct interaction with the pathogen), induction of host plant resistance, metabolism of pathogen germination stimulants released by seeds, and increase of tolerance to stress by enhancing plant growth (indirect effects). This occurs both through interactions with pathogens and through induced changes in plant chemistry (Howell, 2003, Harman, 2011).

Soybeans are one of the world's most popular crop plants. More than 100 million hectares - an area roughly the size of Germany and France combined - are given over to this crop. Annual production is valued at 100 billion US dollars. Argentina, Brazil and the United States account for almost 80 per cent of soybean production. Each year, around 40 million tons of soybean oil is pressed from the harvest.

In Moldova the cultivated areas of soybeans are increasing. In Republic in 2003 this crop was planted on 18,3 thou. ha, in 2006 – 55,7 thou. ha and in 2010 – 59,0 thou. ha (4.0% of the total cultivated areas). Annual gross harvest of soybeans totaled in 2003 -19.4 thou. tons, in 2006 - 79,8 thou. tons and in 2010 - 110.6 thou. tons; the yield per hectare consisted the 1.21, 1.48 and 1.90 tones, respectively (Statistical Yearbook of the Republic of Moldova, 2013).

The aim of this study is *in vitro* determination of deterrent effect of biopesticide Gliocladin-SC via *Trichoderma virens* strain 3X on the development of white rot and fusarium in soybeans.

Material and methods

Trichoderma virens

Trichoderma virens strain 3X is a naturally occurring soil fungus that was isolated from sclerotia of pathogenic fungus *Sclerotinia sclerotiorum*, gathered in the central regions of Moldova in 1980 by Dr. Steinberg M.E.

Trichoderma virens strain 3X is used as a MBCA in preparation of Gliocladin-SC (suspension concentrate). The suspension of *T.virens* 3X was received by the method of submerged cultivation in Laboratory of Phytopathology and Biotechnology of the Institute of Genetics, Physiology and Plant Protection (Moldova). *T. virens* 3X was found to be of low toxicological concern for mammals, non-infective and non-pathogenic. Acute oral toxicity studies performed with *T. virens* 3X by the oral, intratracheal and subcutaneous administrations did not result in treatment-related adverse effects ($LD/LC_{50} > 1,5 \times 10^8$ cfu/animal).

Gliocladin-SC is a commercial fungicide which is based on living cells of the MBCA (Registration number 08-02-0406, 04/16/2015; 1.0 × 10^8CFU/ml). Gliocladin-SC is approved in Republic of Moldova as a fungicide that is used to protect the roots of plants against root rot fungal pathogens to control *Botrytis cinerea* – on grape (by foliar spray) and *Sclerotinia sclerotiorum, Fusarium sporotrichiella* on sunflower and soy (pre-treatment of seeds).

Morphological characteristics of *Trichoderma virens* strain 3X

The *T.virens* 3X was cultured on potato dextrose agar (PDA) medium and wort agar medium one side of a Petri dish and incubated at 27-29oC for 6-7 days. Within five days the colony forms a 90 × 90 mm round shape with a wavy edge, the surface is rough with concentric circles, the profile is convex. On Czapek's medium and wort agar the profile is flat. Colony has a soft consistency, the mycelium can be easily removed from the surface of the agar. The sporulation is beginning on the third day, the maximum - on the seventh day. Due to the formation of spores, mycelium becomes green; the substrate is not colored.

Pathogens

The objects of study were pure cultures of pathogens *Sclerotinia sclerotiorum* and *Fusarium sporotrichiella* (Билай, 1977), isolated from infected soy plants. Isolation of pathogenic fungi was carried out by conventional methods in microbiology (Бёттхер *et al.*, 1987)

Inhibitory activity on the pathogen

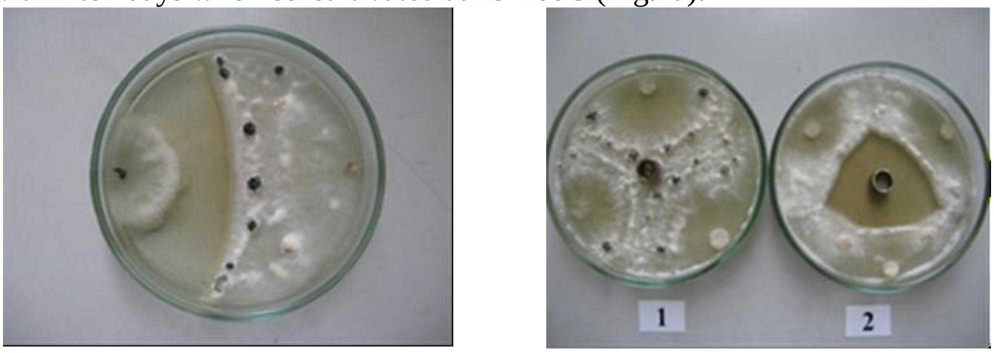
The antagonistic activity of *T.virens* 3X was assayed by dual cultures test (Comporota, 1985) on potato dextrose agar (PDA) medium and wort agar medium. Antifungal activity of *T.virens* 3X has been evaluated against the pathogen *Sclerotinia sclerotiorum*, the causative agent of white rot, pathogens *F.oxysporum, F.culmorum, F.solani and F.sporotrichiella*, agents of fusarium root rot complex.

Experiments were carried out in Petri dishes by the method of horizontal diffusion in agar with the use of paper filters and metallic cylinders (Hudzicki, 2009). The agar plates were inoculated with a suspension of the spores of pathogen. The sterile paper disc containing the bioproduct Gliocladin-SC was then placed in the center of the plat. In the control plates the paper disc was saturated with sterile water. In cylinder method we used small metal cylinders (outside diameter - 10 mm, internal diameter - 8 mm, height - 10 mm). Sterile metal cylinder was placed at the center of the agar plat. Then Gliocladin-SC was pipetted into cylinder in volume equal to 0.5 ml. Cell suspension of *T.virens 3 X* was adjusted to 1 x 10^5 cfu/ml. In control plates into cylinders were pipetted aliquot volume of sterile water. Five Petri dishes (9-cm) were used for each treatment. Plates were incubated at 25-26oC and 13-15oC (optimal temperatures for *S. sclerotiorum*) and at 25oC (optimal temperatures for fungi *Fusarium*) for 5 days (Егоров, 2004 and Гагкаева *et al.*, 2011).

The diameters of the inhibition zone of the pathogens growth were recorded and calculated at 4-5 and 10 days after incubation and compared in experiment and control. If the bioproduct shows the antifungal activity, the inhibition zones are formed between of the filter (cylinder) and grown pathogen culture.

Results

The antagonistic effect of the fungus *T. virens* 3 X against the pathogen *S. sclerotiorum* showed the full colonization of the pathogen within ten days when co-cultivated at 25-26oC (Fig.1a).

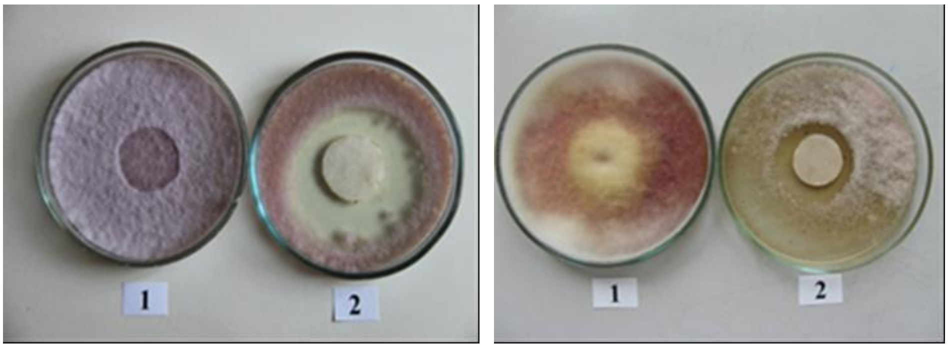


a) b)

Figure 1. Inhibitory effects of *T.virens* 3X on *S.sclerotiorum* on agar medium (orig.): a) method of dual cultures (antibiosis); b) method of diffusion in agar. 1- untreated control, 2 - experiment

Another mechanism of biocontrol - antibiosis was showed by fungus-antagonist when cultivated at 13-15oC. In this case, the sterile zones (lack of growth) were equal to 20-25 mm (Fig.1b).

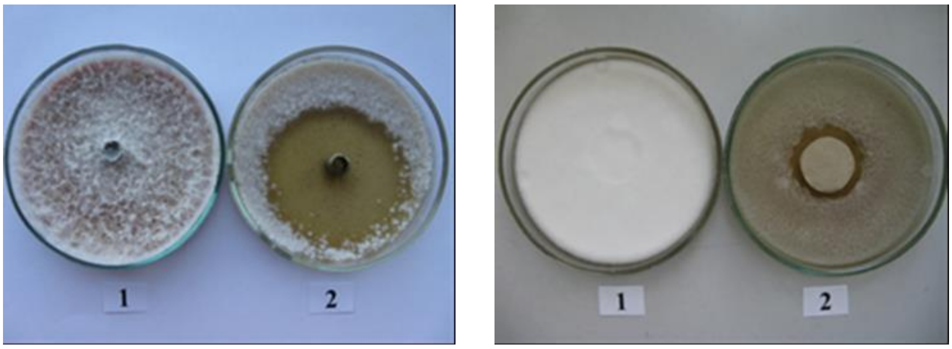
Co-cultivation of antagonist *T.virens* and fungi *Fusarium* in a dual culture at 25oC (the optimum temperature for pathogen) showed the high antagonistic activity of *T.virens*.



a) *F.oxysporum* b) *F.culmorum*

Figure 2. Inhibitory effects of *T.virens* 3X on pathogens (orig.): a) *F.oxysporum*; b) *F.culmorum*. 1- untreated control, 2 – experiment.

Fungus *T. virens* colonized each of pathogens within 6-8 days. Zone of growth inhibition of the pathogen *F.oxysporum* was equal to 42 mm (Fig. 2a). The zone of growth inhibition of pathogen *F.culmorum* was equal to 21 mm. **(**Fig. 2b**).**



a) *F.sporotrichiella* b) *F.solani*

Figure 3. Antifungal effects of *T.virens* 3X on pathogens (orig.): a) *F.sporotrichiella*; b) *F.solani*. 1-untreated control, 2 – experiment.

In laboratory incubations the pathogen fungus *F.sporotrichiella*, extracted from the roots of soybeans, affected the soybean sprouts on the 100%. While under the influence of the bioproduct Gliocladin-SC the beginning of the mycelial growth was observed at 5-6 days later than in the control; the zone of growth inhibition was equal to 60 mm (Fig. 3a). The size of the delay zone of the pathogen *F.solani* growth, extracted from soybean seeds, was equal to 11.5 mm (Fig. 3**b**).

Discussion

An early planting date, which is recommended for soybean production, can increase soybean yield potential. However, soybean yield potential can be compromised by early environmental stresses and a complex of soil-borne pathogens, which negatively affect root health and seedling vigor. Cool (less than 15oC) and moist soil conditions, can slow germination and establishment of soybean seeds, making them more susceptible to soil-borne seed and seedling pathogen attacks such as *Fusarium* and *Sclerotinia.* In this study was found that the use of the bioproduct Gliocladin-SC, on the basis of the active microorganism - fungus *Trichoderma virens,* can reduce diseases and protect seeds from infection.

The obtained results showed that biopesticide Gliocladin-SC, based on live cells of the fungus *T.virens* strain 3X, exhibited antifungal effects against fungi genus Sclerotia and Fusarium. Biologically active substances, components of the biological products Gliocladin-SC, inhibit the development of pathogens, forming sterile inhibition zone. The diameter of the zones of inhibition of *S. sclerotiorum* reached 45 mm. The size of the zones of inhibition of the fungus *F.sporotrichiella* was equal to 60 mm. It was experimentally established that Gliocladin-SC is also active against other species of Fusarium, causing diseases of many crops. the zones of inhibition *F.oxysporum* Schl. reached 42 mm, *F.solani* Ap. et Woll – 11,5 mm, *F.culmorum* Sacc. – 21 mm, *F.graminearum* Shwabe – 20 mm, *F.verticillioides* – 18,8 mm.

The study confirmed the antagonistic potential and antifungal ability of biopesticide Gliocladin-SC against pathogens - agents of white rot and root rot-*Fusarium* complex. Gliocladin-SC is recommend as biological product on soy for control of the basal and root rot by preplans seed-treatment.

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