PLANT-MICROBIAL INTERACTION IN THE SYSTEM *Glycine max* / ENDOPHYTIC FUNGI

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Abstract. Mycocenosis in sod-podzolic soil for soybeans growing had been mainly represented by *Penicillium* Link:Fr, *Fusarium* Link:Fr, *Trichoderma* Hers, *Acremonium* Link, *Alternaria* Nees, *Cladosporium* Corda, *Gliocladium* Corda, *Rhizopus* Ehrenb, *Mortierella* Coem, *Mucor* Mich. Micromycetes biodiversity was different between rhizosphere soil and root surface by its quality and quantity but in both cases the most numerous had been *Fusarium* genera representatives. Endophytic mycobiota of soybean plants had been formed with fungal genera *Penicillium*, *Chaetomium*, *Trichoderma*, *Fusarium*, *Verticillium*, *Phoma*. Taking into attention that representatives of genera *Fusarium*, *Verticillium* and *Phoma* often used to be found as agricultural crops root diseases causative agents, the object of our research were elected the fungi of genera *Penicillium*, *Chaetomium* and *Trichoderma*. Pre-sowing treatment of soybean seeds with spores of the studied endophytic fungi had positive effect on plants growth and symbiotic indicators. The height of inoculated plants increased on 11.9-13.3% according to the control. Above-ground dry mass increased on 12.7 and 14.3% in accordance to inoculation with *Chaetomium* sp. 2061 and *Trichoderma* sp. 2227. Dry roots mass was raised up on 10.5% using *Penicillium* sp. 2221 and *Chaetomium* sp. 2061. A trend to increase the number and mass of nodules on plant roots as well as nitrogenase activity increase on 25.2 - 56.2% using researched endophytic fungi strains has been recorded. The total phosphorus content increased from 0.281% in control to 0.297%, 0.342%, and 0.318% in variants using *Penicillium* sp. 2221, *Chaetomium* sp. 2061, and *Trichoderma* sp. 2227 respectively.

Keywords: endophytes; mycocenosis; phosphorus nutrition; soybean; symbiotic nitrogen fixation.

INTRODUCTION

Soil fungi play an important role in nature. Due to their ability to produce biologically active compounds such as amino acids, proteins, peptides, sugars, organic acids, etc., they create a certain environment in microbiocenoses. In this case there may be favorable conditions for the development of some organisms and, negative for others. In addition fungi form various relationships which can be antagonistic and parasitic as well as symbiotic to plants because fungal genetic and biochemical systems associated with plant relevant systems.

Soybean (*Glycine max* (L.) Merr.) is one of the most widespread and promising legume crops. It is grown all over the world under different climatic conditions. Soybean plays a significant role in global food security as well as agricultural sustainability due to a high seed protein and oil concentration and low reliance on N fertilization [18]. Along with nodule bacteria for biological nitrogen fixation both with free-living bacteria soil fungi actively develop in rhizosphere and on the plant roots. The genus composition and fungi biodiversity in soybean root zone depends on many factors such as cropping system, soil physical and chemical properties, crop rotation, fertilizing, pre-sowing seed inoculation with nodule bacteria [1, 14, 31, 33, 48, 55].

The last decades, researchers considerable attention had been focused on endophytic fungi and their interaction with plants studying. According to the modern ideas endophytic fungi are a phylogenetically diversed. Having a saprophytic, commensal or mutualistic interaction with host plants they asymptomatically colonize plant tissues for some time [44]. Endophytes can provide a number of benefits to plants: produce regulatory substances or induce phytohormones formation, stimulating the accumulation of above-ground biomass and root mass by microsymbiont [3, 19, 57, 61] increase coming to the plant mineral nutrition elements quantity and availability [57-59]; activate photosynthesis process [45]; induce resistance of plants to diseases [32, 42] and pests [12, 49], abiotic stress (drought [20], the heat [22], the cold [39], salting [19], toxic metals [3, 23]). The endophytic soybean fungi strains promising for agriculture can be isolated from sterilized seeds surface, leaves, stems, soybean plant roots of different varieties [6, 10, 40, 42].

The goal of our work was to study the fungi genus composition of soybean root zone, investigate the influence of endophytic fungi on plant growth, nodulation process, symbiotic nitrogen fixation activity and total phosphorus content in soybean plants.

MATERIALS AND METHODS

Sampling and fungi isolation

The fungi genus composition of soybean root zone was investigated in field conditions. Soil type – sod-medium podzolic silty-sandy (humus content – 1.02%, nitrogen – 54.9 mg/kg; mobile forms of phosphorus – 110-120 mg P_2O_5, exchangeable potassium – 120-130 mg K_2O per 1 kg of soil; pH_{salt} – 5.2, pH_{water} – 6.0). Soybean variety – Legenda (foundation – Institute of Agriculture of the NAAS of Ukraine).

Soil samples between rows and plants (with soil on the roots) had been collected in sterile polyethylene bags. The fungi quantity in rows and rhizosphere (a layer of soil 1-2 mm thick directly on plant roots) was studied using the method of soil dilutions by Waksman [54]. To obtain fungi elements the method of washing from plant root surface was used, followed by dilution and deep sowing in a nutrient medium. Endophytic fungi were isolated from healthy (undamaged) root tissues after surface sterilization with ethanol (96%) for 1.5 min. In all cases, wort agar (4-5° according to Baling) with streptomycin addition (40-50 mg per 1 liter of medium) was used as a nutrient medium to inhibit bacterial growth. Petri dishes were incubated at 26°C for three days, after that the number of colonies had been counted. The number of fungi in the inter-rows, rhizosphere and on plant roots was calculated per 1 g of dry soil or roots. The percentage composition of different genera relative to the total fungi number was detected.

Morphological identification

The cultural and morphological properties of the identified fungi were studied on wort agar, Czapek's agar (CZA) and potato dextrose agar (PDA) in the dark on Petri dishes at a temperature of 26°C. Taxonomic identification by morphology of fungal isolates was mainly based on the identification keys in the 10th edition of Anisworth & Bisby's Dictionary of the fungi [26].

Vegetation experiment

The vegetation experiment was carried on in vegetation house on the same with field experiment type of soil. Soybean seeds of the Arnica variety had been used. Soil moisture was maintained at 60% of full moisture capacity. The duration of the experiment was 35 days. It was used five-fold repetition for the experiment.

In order to obtain fungi spores for pre-sowing seed treatment, endophytic fungi had been growing on wort agar for 10 days. Seeds were treated with spore suspensions at the rate of 1.5% of the seed weight). The inoculation load was 1×10^5 spores per seed.

The vegetation experiment was carried out according to the following scheme:

1. Control (seeds were treated with sterile tap water at the rate of 1.5% of the seed weight);
2. Seed treatment with *Penicillium* sp. 2221;
3. Seed treatment with *Chaetomium* sp. 2061;
4. Seed treatment with *Trichoderma* sp. 2227.

In vegetation experiment, the influence of endophytic fungi *Penicillium* sp. 2221, *Chaetomium* sp. 2061 and *Trichoderma* sp. 2227 on growth (height, above-ground dry mass, root dry mass) symbiotic activity (number and mass of nodules and their nitrogenase activity) and total phosphorus content in soybean plants had been studied.

Determination of plant growth and symbiotic indicators

Growth and symbiotic indicators of soybean plants were determined after 35 days of vegetative experiment at the beginning of the flowering phase of plants. To determine the mass of dry shoots and roots matter, plants had been dried in a drying oven at a temperature of 105°C to a constant mass.

Nitrogenase activity of nodules was determined by the acetylene method [15]. Soybean roots with nodules were placed in vials with a capacity of 120 cm^3, acetylene was introduced in an amount of 10% of the volume of the gas phase of the vial. The samples were incubated with acetylene for one hour, after which samples were taken and analyzed on an Agilent GS System 6850 gas chromatograph (USA). The amount of ethylene formed during incubation was determined.

Determination of total phosphorus content in above-ground plant mass

The total phosphorus content in soybean plants above-ground mass was detected according to Denizhe method modified by Bouvatier [53].

Statistical processing

Statistical processing of results was performed using generally accepted methods. Parametric criteria for average, standard deviation and confidence interval had been applied. To assess the reliability of differences between overlapping it was used Student-t test at a rank of 0.05 by default.

RESULTS

Soybean root zone mold genus composition

According to obtained results the soybean row spacing mycocenosis had been formed with molds of genus *Penicillium* Link:Fr, *Fusarium* Link:Fr, *Trichoderma* Hers, *Acremonium* Link, *Alternaria* Nees, *Cladosporium* Corda, *Gliocladium* Corda, *Rhizopus* Ehrenb, *Mortierella* Coem, *Mucor* Mich and *Dematiaceae* family. *Penicillium* molds were the most numerous (Fig. 1a).

The differences between mycocenosis in soybean rhizosphere soil and row spacing were qualitative and quantitative (Fig. 1b). As far as *Penicillium* species were more numerous in row spacing (41.6 %), *Fusarium* fungi had a higher number in rhizosphere soil (71.7%). The relative number of *Penicillium* species decreased (from 41.6 to 8.9%) as well as *Trichoderma* (from 4.1 to 1.0%), *Rhizopus* (from 1.2 to 0.5%), *Gliocladium* (from 6.1 to 0.7%), *Mortierella* (from 6.1 to 0.1%). Instead, the relative amount of *Fusarium* species raised up almost 13 times (from 5.7 to 71.7%). The relative number of *Acremonium* fungi also increased slightly (from 4.5 to 5.8%). The single *Verticillium* molds were noted (0.3%), which had not been observed in soil without plants. Simultaneously *Alternaria*, *Cladosporium* and *Mucor* species being represented in row spacing could not been found in rhizosphere soil.

The soybean root surface mycocenosis was comparatively depleted and consisted of *Fusarium* (48.6%) and *Gliocladium* (32.9%) species above all. The relative amount of fungi *Penicillium* species decreased down to 4.0% compared to rhizosphere soil. Instead, the relative number of *Verticillium, Rhizopus* and *Trichoderma* fungi slightly increased (Fig. 1c).

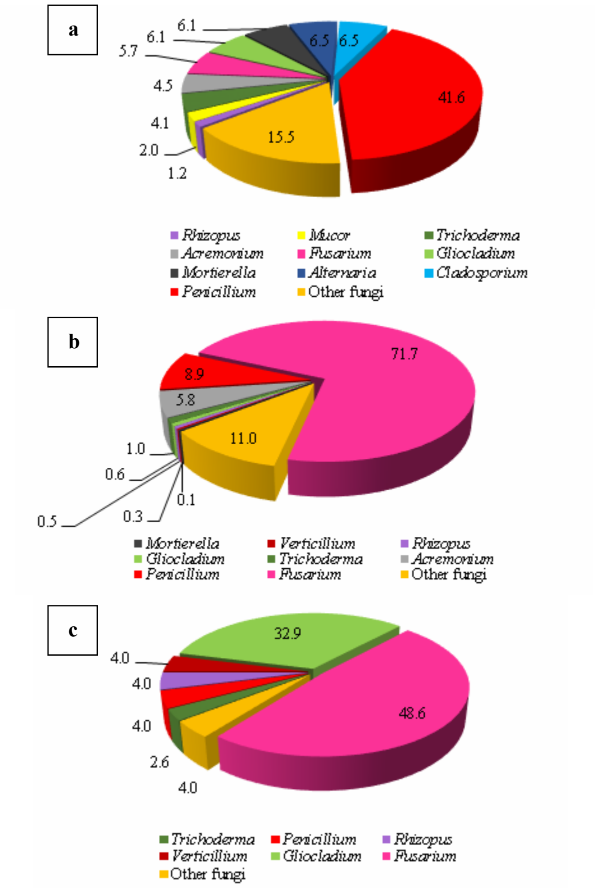

Figure 1. Soybean fungi genus composition in row spacing and root zone, total amount percents (field experiment, 2021): a) - row spacing, b) -rhizosphere soil, c) - washed roots of plants

Fungi capable for endophytic in the soybean internal plants roots tissues

During the research, special attention was paid to molds isolated from soybean histosphere because it's known the ability to penetrate into the internal plant roots tissues is common to certain species and strains of micromycetes and interaction with endophytic fungi can expand the adaptive capabilities of plants giving them new metabolic functions which provides a significant increase agrocenoses productivity. According to the results we obtained the endophytic mycobiota of soybeans was formed by micromycetes of the genera *Penicillium*, *Chaetomium*, *Trichoderma*, *Fusarium*, *Verticillium*, *Phoma*. The isolates of *Penicillium* sp. 2221, *Chaetomium* sp. 2061, *Trichoderma* sp. 2227 were selected to study the effect on soybean plants because the literature data state that the fungi of the mentioned genera are characterized by their positive effect on soybean plants and other crops [3, 9, 23, 25, 30, 42, 52, 62, 64] while the fungi of the genera *Fusarium*, *Verticillium*, *Phoma* are described mainly as phytopathogens of many types of culture plants [2, 5, 7, 43, 47, 50, 51, 56, 63].

The influence of endophytic fungi on soybean plants growth parameters

As evidenced by vegetation experiment results (Table 1), pre-sowing treatment of soybean seeds with endophytic fungi spores had positive effect on plant growth indicators (height, above-ground mass and root mass). All investigated strains contributed to a reliable increase of inoculated plants height up to 11.9-13.3% compared to the control variant. The usage of *Chaetomium* sp. 2061 and *Trichoderma* sp. 2227 for seeds treatment allowed to increase dry aerial plant mass in accordance on 12.7 and 14.3%. The dry roots weight increased on 10.5% by using *Penicillium* sp. 2221 and *Chaetomium* sp. 2061.

The influence of endophyte fungi on *Glycine max – Bradyrhizobium japonicum* symbiotic system

Since the studied endophytic fungi contributed to the growth of soybean plants it was the interest to investigate their effect on *Glycine max* – *Bradyrhizobium japonicum* symbiotic system functioning. The soil for vegetation experiment contained soybean nodule bacteria population owing to previous years agricultural crop cultivation. That's why the soybean had in average 15 nodules per plant for control variant (Fig. 2a). During pre-sowing seeds treatment with endophytic fungi spores it was noted a tendency to increase both the number and mass of nodules on plant roots surface (Fig. 2a, 2b). The most important indicator for effective symbiotic system functioning is the nodules to fix molecular nitrogen. The obtained results proved that nitrogenase activity of nodules increased on 25.2-56.2% using investigated endophytic fungi strains (Fig. 2c).

Along with studying endophytic fungi effect on soybean plants growth and symbiotic indicators, it was important to assess their influence on phosphorus plants nutrition after inoculation. As we know, phosphorus plays a key role in almost all major metabolic processes, in particular, photosynthesis, respiration, energy metabolism, macromolecular biosynthesis [24] and biological nitrogen fixation in legumes [41]. According to obtained results, the total phosphorus content was 0.281% in the above-ground mass in control variant. Pre-sowing treatment of seeds with endophytic fungi spores raised up the phosphorus content to 0.297%, 0.342% and 0.318% in variants with *Penicillium* sp. 2221, *Chaetomium* sp. 2061 and *Trichoderma* sp. 2227 (Fig. 3).

Table 1. The influence of endophytic fungi on soybean plant growth (vegetation experiment)

Variants	Height (cm)	Above-ground dry mass (g)	Root dry mass (g)
Control (without inoculation)	45.2±0.9	1.26±0.02	0.38±0.01
Inoculation with *Penicillium* sp. 2221	50.7±1.7*	1.37±0.03	0.42±0.01*
Inoculation with *Chaetomium* sp. 2061	51.2±2.1*	1.42±0.03*	0.42±0.01*
Inoculation with *Trichoderma* sp. 2227	50.6±1.8*	1.44±0.07*	0.40±0.01

* – significant differences at the level of 0.05

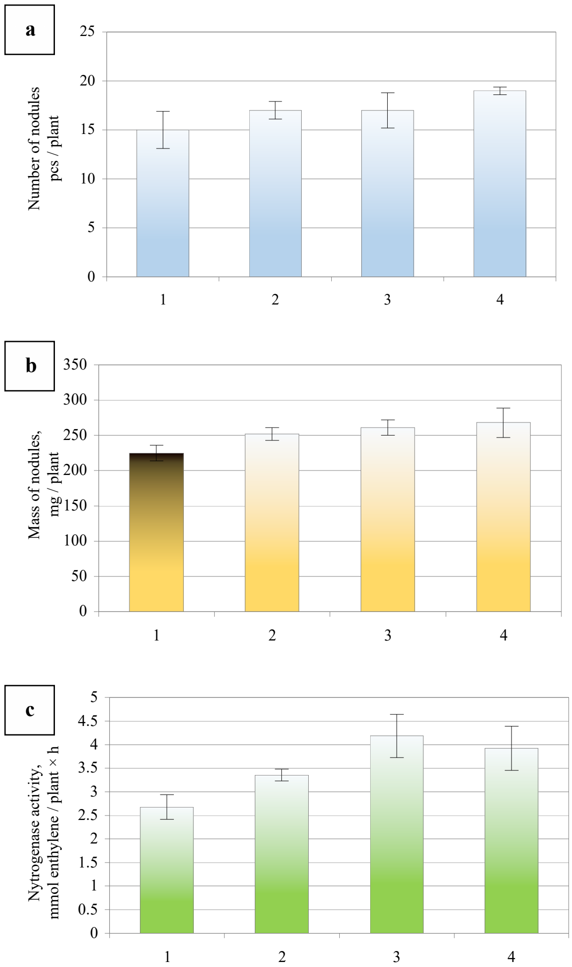

Figure 2. The influence of endophytic fungi on soybean symbiotic indicators (a - number of nodules, b - mass of nodules, c - nitrogenase activity in nodules) in vegetation experiment: 1 - Control (water), 2 - *Penicillium* sp. 2221, 3 - *Chaetomium* sp. 2061, 4 - *Trichoderma* sp. 2227.

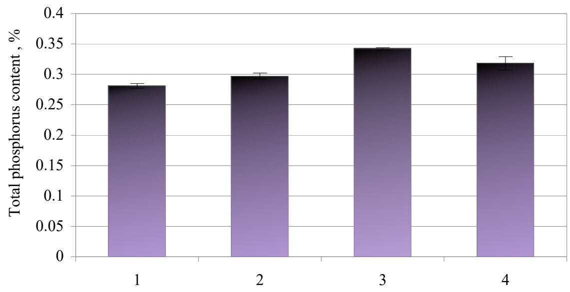

Figure 3. The influence of endophytic fungi on total phosphorus content in above-ground part of soybean plants (vegetative experiment): 1 - Control variant (water), 2 - *Penicillium* sp. 2221, 3 - *Chaetomium* sp. 2061, 4 - *Trichoderma* sp. 2227.

DISCUSSION

According to literature sources there are a lot of soybean endophytes fungi genera representatives such as: *Acremonium* [37, 64], *Alternaria* [17, 37, 60], *Ampelomyces* [6, 10], *Aspergillus* [37, 64], *Cladosporium* [10, 17, 37], *Chaetomium* [6, 9, 37], *Colletotrichum* [10, 37], *Fusarium* [10, 17, 37, 40, 60, 64], *Penicillium* [19, 25, 37, 64], *Phoma* [10, 17, 60], *Scopulariopsis* [37, 40], *Trichoderma* [17, 32, 42, 64]. The most positive effect on plants was revealed among *Chaetomium*, *Penicillium* and *Trichoderma* genera representatives that had been isolated by us capable for endophyte with soybean plants.

The influence of endophytic fungi on plants is multifunctional. Fungi are able to produce biologically active metabolites with phytohormonal, antimicrobial, antiviral, insecticidal, antioxidant, antagonistic activity. Thus in a review article Toghueo & Boyom [52] report about more than 280 biologically active substances produced by *Penicillium* endophyte fungi. A special attention has to be paid to their ability for producing phytohormones such as auxin (indolyl-3-acetic acid) [21, 61], gibberellin (GA_1, GA_3, GA_4, GA_7 and GA_9) [3, 19, 21], cytokinin (zeatin) [61] which impacts to plant growth and productivity enhancement. It had been shown a positive effect of *Penicillium* endophytes on soybean germination [21], growth characteristics of plants (the shoots length [21, 62], aerial and root mass [19, 62], leaf area [19] and chlorophyll content [19]).

Harzianic acid (HA), 6-pentyl-α-pyrone (6PP), and hydrophobin1 (HYTLO1) had been discovered among biologically active metabolites affecting on plants growth, nutrients absorption and soybean productivity produced by *Trichoderma* spp. [35]. Plant growth stimulation is an important research and usage aspect for *Trichoderma* fungi genus [4, 35, 46]. Senger *et al.* revealed that *Trichoderma* fungi usage contributed not to only soybean plants germination, preservation and increasement of shoots and roots biomass but a significant nodules number and mass increasement according to control variant [46]. A significant soybean growth stimulation (up to 39-41%) with the influence of *Trichoderma* spp. had been noted by another authors [4, 35]. *Trichoderma* fungi are able not only to rise up soybeans productivity increasement [35, 46] but they may improve the grain quality, in particular, it was mentioned a boosting of fatty acids (oleic, linolenic, 11-eicosenoic, and stearic) content in grain [35].

According to our experience the fungus *Chaetomium cochliodes* 3250 is able to penetrate into soybean plants inner tissues [28] and to have positive influence on growth indicators and crop yield [30]. Pre-sowing treatment of seeds with *C. cochliodes* 3250 influenced affirmatively on symbiotic system of soybean plants [29]. Thus, the number of nodules on roots per plant increased up to 3.4 times compared to control variant. The nodulation process had happened at the expense of nodule bacteria local population formed in soil as a result of crop cultivation in previous years. Mass of nodules per plant and their nitrogenase activity raised up during inoculation with *C. cochliodes* 3250 respectively on 22.2 and 66.2%.

An important property of soil fungi is the ability to convert phosphorus from mineral and organic compounds into a form available to plants, which improves their phosphorus nutrition. The most described PSF (phosphate-solubilizing fungi) in scientific literature are representatives of *Penicillium*, *Aspergillus*, *Rhizopus*, *Fusarium*, *Trichoderma* and *Sclerotium* genera [11]. Information about endophytic fungi influence on phosphate-mobilizing ability is not significantly enough. In the article Khan *et al.* [21] there is a mention that soybean endophyte *P. funiculosum* LHL06 dissolves tricalcium phosphate. We revealed an increase of total phosphorus content in soybean above-ground mass after the action of endophytic strains *Penicillium* sp. 2221, *Chaetomium* sp. 2061 and *Trichoderma* sp. 2227.

It was revealed the ability of endophytic fungi to alleviate plant abiotic stress. A number of experiments according to *Penicillium* endophytic fungi influence on soybean plants had been carrying out in salt stress conditions [19, 21, 62] or in soil contaminated with heavy metals [3, 23]. Via abiotic stress in tissues of plant treated with endophytic fungi of *Penicillium* genus the level of abscisic acid being a plant stress marker is reduced and also isoflavones biosynthesis intensifies [3, 19, 21]. Endophytic fungi, in particular *Chaetomium* genus fungi, also has demonstrated a positive effect on host plants improving biotic stress tolerance level which was evident via higher production of resistance markers such as salicylic acid and polyphenols content with further antioxidant status improvement [9].

The systemic resistance of plants is getting more because of endophytic fungi. *Chaetomium cochliodes*, using to penetrate in soybean roots tissues, produces 2,4-epibrasinolide which has an important role in plant resistance to pathogens. In addition the fungus synthesizes phytohormonal substances: indole-acetic and gibberellic acids, as well as ergosterol. Indol-3-acetic acid and ergosterol synthesized by the fungus can be the basic mediator molecules in forming of endophytic symbiotic systems between fungus and plants [8, 27]. While forming such systems the activity of succinate dehydrogenase (SDH) in plant roots may be increased up to 2.9 times [28]. It was also established that systematic plant resistance caused by pre-sowing soybean treatment with *Trichoderma* fungi may be associated with such changes in plants as increase of abaxial leaf surface stomatal index, root cortex thickness, adaxial epidermis thickness, vascular cylinder diameter, mesophyll thickness and spongy parenchyma thickness among soybean plants [36].

Trichoderma fungi are widely known and described in many works primarily as soybean phytopathogens biocontrol agents [13, 32, 34, 35, 38, 46, 64]. Representatives of various *Trichoderma* species are effective against series of soybean pathogen such as *Sclerotium rolfsii* [64], *Fusarium virguliforme* [38], *Macrophomina phaseolina* [32] and *Sclerotinia sclerotiorum* [34]. Mycoparasitism is an essential biocontrol mechanism of *Trichoderma* spp. which are able to secrete chitinases, degrade the cell wall of another fungi and lyse their cells contents [16]. It should be noted that investigations of *Trichoderma* fungi interaction with soybean plants had been carrying out not only with endophytic representatives of the genus but also with saprotrophic *Trichoderma* strains. There are scientific data of *Penicillium* endophytic fungus isolated from soybean seeds which is able to limit the development of *Septoria glycines* and *Diaporthe eres* phytopathogens up to 60-80% [25]. *Chaetomium* genus endophytic fungus also had limited the development of phytopathogenic fungi known as soybean root rot causative agents [9]. Along with this it had been shown high nematocidal activity of the mentioned fungus which led to 100% death of root knot nematode after 48 hours of exposition [9].

We obtained the results evidently for *Penicillium*, *Trichoderma* and *Chaetomium* endophytic fungi to have positive influence on soybean symbiotic apparatus, molecular nitrogen fixation and total phosphorus content in above-ground biomass of plants. It complements the experimental data of many researchers regarding the multifunctional effect of these fungi on plants.

Conflict of interest. There is no actual or potential conflict of interest in relation to this article.

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