The use of predatory fungi of the genus *Arthrobotrys* isolated from soil in the farmlands of Southern Kazakhstan to control nematode infections in tomato plants

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Abstract. In Kazakhstan, parasitic nematodes affect up to 35-40% of the harvest of sugar beets, potatoes and tomatoes. Until recently, chemical nematicides were used to control nematode numbers but posed risks of toxicity to humans and animals, and their effectiveness decreased due to resistant nematodes. There is an urgent need to create alternative non-toxic plant protection products. *Arthrobotrys oligospora* is one of the most studied hyphomycete fungi that is used to create biological products against nematodes. In a laboratory experiment in a pot culture, the activity of *Arthrobotrys* predatory fungi against parasitic nematodes of the genus *Meloidogyne* was assessed when growing tomatoes of the «F1 Russian size» variety in a vermiculite/soil mixture. Physiological indicators of plant growth (stem length, number of leaves, stem and root weight) were used for analysis. To ensure the reliability of the obtained results of the activity of predatory fungi, the number of nematodes and the presence of colonies of native predatory fungi in the soil samples of the used variants post-experiment were taken into account. The length of stems, the number of leaves and the weight of roots and stems during the growing season changed within the experimental error. The most significant result was estimated by the number of live nematodes post-experiment. It was shown that when preparations of predatory fungi were added to the soil, the number of nematodes significantly decreased in all variants. Thus, the nematophagous activity of local predatory fungi of the genus *Arthrobotrys* in tomato cultivation has been proven.

1 Introduction

Plant parasitic nematodes pose a serious threat to the global agricultural economy due to large crop losses [1]. In monetary terms, this is approximately $215.8 billion per year worldwide, based on production and prices for 2010–2013. One of the main crops affected by nematodes are tomato plants, which are grown everywhere and play an important role in a balanced human diet [2]. Parasitic nematodes are difficult to control compared to other pests because they live in the soil and attack underground parts of plants [3]. Despite the fact that chemical nematicides are effective, easy to use and demonstrate a quick effect, the harm they cause to the environment and public health calls into question their continued use. Therefore, it is more important to find new, environmentally friendly alternatives for managing parasitic nematode populations [4]. Nematophagous fungi, which infect nematodes, have recently become an effective component of new biological products that successfully replace traditional chemical nematicides [5]. *Arthrobotrys oligospora* – the first fungus discovered to have nematophagous activity, is being widely studied around the world as a promising effective microbial agent for the control of nematodes. In the presence of nematodes, *A. oligospora* enters a parasitic (predatory) stage, forming complex three-dimensional networks to trap nematodes. Capture initiates a number of processes including adhesion, penetration and immobilization of nematodes [6]. Based on similar studies, based on two species close to *A. oligospora: A. robusta and A. irregularis*, two commercial biological nematicides were developed - Royal 300 and Royal 350; it was shown that applying the drug to the soil before planting tomato plants at a dose of 140 g/ m2 protects them from nematodes [7]. It is also worth noting T.V. Teplyakov's work in Russia, where she managed to not only develop technological regulations for producing nematophagin in biological laboratories and plant protection stations using solid-phase fermentation, but she also proved the possibility of producing preparative forms from deep culture mycelium. Currently, a biological product “nematophagin BL” has already been created in Russia to protect plants from parasitic nematodes based on the *Arthrobotrys oligospora* Fres strain. BKM F – 3062D. Another preparation was obtained using the *Duddingtonia flagrans* Dudd strain. F 882, (RF patent 225367) [8].

However, in Kazakhstan, the listed biological control methods are not used to control nematodes. According to current practice, to create new drugs to combat parasitic plant nematodes, predatory fungi of the local population, which are adapted to certain types of soils and geographic zones, should be used. The use of imported drugs does not give the expected positive effect here in Kazakhstan.

The novelty of the work consists in the work not presented in the Republic of Kazakhstan on the isolation, identification and use of the local nematophagous fungus *Arthrobotrys oligospora* against local parasitic nematodes in tomato culture.

2 Materials and methods

2.1 Objects of research.

Soil samples for isolating predatory fungi and nematodes were taken from the experimental field of the Institute of Potato and Vegetable Farming (Almaty region, “Pervomaika” village). Predatory fungi - *Arthrobotrys oligospora*, nematodes (*Meloidogyne sp*), tomatoes variety "F1 Russian size".

2.2 Obtaining pure strains of carnivorous fungi.

Samples of *A.oligospora* from the collection of the Laboratory of “Ecological Biotechnology” of the Faculty of Biology and Biotechnology of Kazakh National University al-Farabi, propagated on potato dextrose agar (PDA).

2.3 Isolation of nematodes for screening the activity of predatory fungi.

To isolate nematodes, the generally accepted Berman method is used [9].

2.4 Growing plants.

Tomato plants of the "F1 Russian size" variety were used in the experiments, 5 plants in each variant and in the control in 3 replicates.

The seeds were previously disinfected in a bleach solution and grown in pots with a vermiculite/sterilized soil mixture in a 1:1 ratio. Experimental plants were grown indoors at a temperature of 20°C, illumination of 1600 lux, and a 16-hour photoperiod. Predatory fungi and nematodes were added to pots after the formation of full leaves in experimental plants [10].

2.5 Assessment of the action of the predatory fungi Arthrobotrys oligospora.

Control – soil mixture without the addition of nematodes and nematophagous fungi. (K - Control). Experiment.

Variant 1 – influence of predatory fungi. Predatory fungi *Arthrobotrys oligospora* were added to the soil at the rate of 55 g. soil containing *Arthrobotrys oligospora* per pot, without adding nematodes.

Variant 2 – the influence of nematodes on the growth of tomatoes. 860 nematodes were added to each pot, without adding predatory fungi.

Variant 3 – assessing the impact of simultaneous addition of predatory fungi and nematodes. Live nematodes in the amount of 860 individuals and predatory fungi *Arthrobotrys oligospora* at the rate of 55 g were simultaneously added to the soil, soil with predatory fungi for one pot.

Variant 4 – Live nematodes were added to the soil of tomatoes in the amount of 860 individuals; the predatory fungi *Arthrobotrys oligospora* were added to the soil only 2 weeks after the addition of nematodes, to assess the effect of predatory fungi on plants that had already been exposed to the influence of nematodes for some time.

2.6 Physiological indicators of plant growth.

Measurements were made: length of plants with roots (cm); number of leaves (pcs); root mass (g); stem weight (g). The analysis was carried out after 65 days of plant growth.

The results of measurements of physiological parameters were analyzed using Microsoft Excel. To compare data means at significance levels ($p \leq 0.05$ and $p \leq 0.01$). ANOVA method was used.

2.7 Determination of the presence of micromycetes in soil samples.

A soil sample from each variant was placed in a Petri dish with potato-dextrose agar (PDA) and incubated in a thermostat at 25°C for 7 days.

The presence of micromycetes was determined using a binocular magnifier MBS – 10.

2.8 Determination of the number of nematodes.

10 g of soil from each variant was used to isolate nematodes using the Berman method. The number of nematodes in the total volume of soil was determined using proportions.

3 Results and discussion

Physiological indicators: plant length, number of leaves, stem weight, root weight.

All measurements were carried out after 65 days of plant growth. Figure 1 shows a general view of the experiment with tomato plants.

Fig. 1. General view of the experiment with tomato plants.

The results of measuring the growth parameters of tomato plants are shown in Table 1.

Table 1. Growth parameters of tomato plants when infected with nematodes (*Meloidogyne sp.*) and predatory fungi (*Arthrobotrys oligospora*). Standard deviation ($p \leq 0.05$).

№	Variants	Stem length, (cm)	Number of leaves (pcs)	Weight of stems, (g)	Weight of roots, (g)
1	Control	21,33±2,33	33±4,36	2,27±0,71	1,54±0,63
2	PF	22,5±4,25	40±4,16	2,80±0,1	2,24±0,76
3	Nematodes	18±1,00	26,67±2,96	1,87±0,27	1,14±0,3
4	Nematodes + PF	19±3,06	36,67±1,33	2,11±0,69	1,31±0,33
5	Nematodes + PF 2 weeks	14,67±1,59	35±2,08	1,66±0,49	0,69±0,24

Note: PF - predatory fungi;

Estimation of the number of nematodes in soil samples after completion of the experiment.

Nematodes were isolated using the Berman method from soil samples in variants 2,3,4. The data obtained are presented in Table 2. The number of nematodes in 10 g of soil from each option was assessed. Then the number of nematodes contained in 10 g was multiplied by the total mass of the soil mixture in each variant and the total number was obtained.

Table 2. The number of nematodes contained in the total soil mass after the experiment.

№	Variant	Number of nematodes per 10g of soil (pcs)	Total mass of soil mixture (g)	Total number of nematodes (pcs)
1	Nematodes	17±2,78	685	1164±11,62
2	Nematodes + PF	2±0,83	880	176±7,29
3	Nematodes + PF 2 weeks	5±1,37	1170	585±6,89

According to the data from Table 2, the expected largest number of nematodes was noted in the “Nematodes” variant. It should also be noted that in the “Nematodes + PF 2 weeks” variant there are more living nematodes than in the “Nematodes + PF” variant.

Assessment of the presence of predatory fungi in soil samples after completion of the experiment.

In subsequent experiments, the presence of predatory fungi in the soil was assessed in variants 1,3,4. The microscopy results are shown in Figure 2.

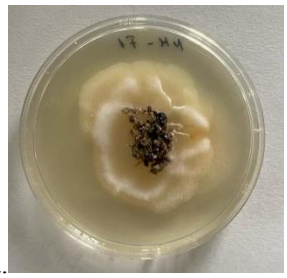
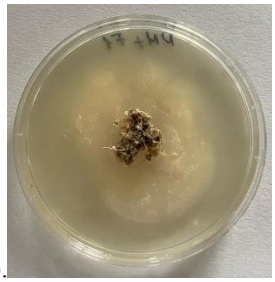
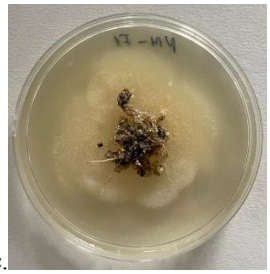
a.  b.  c. 

Fig. 2. a – PF colony on PDA substrate obtained from soil. (variant 1 - predatory fungis); b – PF colony on PDA substrate obtained from soil (variant 3 - predatory fungi + nematodes); c – PF colony on PDA substrate obtained from soil (variant 4 - predatory fungi + nematodes for 2 weeks).

When analyzing the data obtained in Table 1, it should be noted that the length of the stems varies from 21,33±2,33 in the control to 14,67±1,59 in variant 4. The decrease in the length of the stems in variant 4 may be due to the impact of nematodes in the first 2 weeks of growth without predatory fungi. The number of leaves increases slightly in variants 3 and 4. The decrease in the mass of stems and roots in variants 3 and 4 may be associated with the presence of nematodes. However, this statement requires additional experiments. Based on the results of physiological indicators of tomato plants after 65 days of growth, no reliable data were obtained on the influence of nematodes and predatory fungi on growth processes, which does not contradict the data obtained by other authors [10].

In addition to the physiological indicators of tomato plant growth, to confirm the activity of predatory fungi, the remaining number of living nematodes in variants 2,3,4 was counted. Based on the data in Table 2, the decrease in the number of nematodes in all variants, compared to the control, is a reliable indicator of the activity of predatory fungi. When assessing the presence of predatory fungi in soil samples after completion of the experiment, (Figure 2) the presence of colonies of predatory fungi *Arthrobotrys oligospora* in all studied samples in the rhizosphere was noted, which determined the decrease in the number of nematodes in variant 3, variant 4.

4. Conclusion

In conclusion, it should be noted that the isolated and identified local fungi of the genus *Arthrobotrys* sufficiently exhibit nematophagous activity in the presence of local parasitic nematodes of the genus *Meloidogyne* in experiments on growing tomatoes. The results obtained show the prospects of research in this direction for the future production of biologics based on local strains of nematophagous hyphomycetes of the genus *Arthrobotrys*. In the future, it is planned to conduct similar studies on other crops such as sugar beet and potatoes.

As a result, biologics will be created to combat parasitic nematodes for use on farmland in Kazakhstan.

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