ANTAGONISTIC PROPERTIES OF THE LOCAL STRAIN TRICHODERMA SP 4

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ABSTRACT
The corn root rhizosphere and 23 local microorganisms strain belonging to the Trichoderma family were isolated from other sources. From these strains, during the experiments, Trichoderma sp 4 micromiset strain was selected due to its high antifungal activity. The temperature of 24-28°C for the growth of the fungal strain of Trichoderma sp 4, the nutrient medium was determined to be optimal in the potato dextrose agar with a pH of 5-7. Fusarium oxysporum, Verticillium dahliae and Alternaria alternate were found to exhibit active antagonistic properties against phytopathogenic fungi.

Keywords: Trichoderma sp 4, phytopathogen, Fusarium oxysporum, Verticillium dahliae, Alternaria alternata, antagonist, micromycet.

1. INTRODUCTION
As a result of continuous application of synthetic organic fungicides in agriculture, there has been a decrease in the microflora in the soil and an increase in the adaptation of phytopathogenic microorganisms. Plant diseases play an important role in an extinction of the natural recourses in agriculture. Specially, phytopathogenic fungi in the soil cause significant losses. In the recent years according to the changes carried out in the agriculture, widely dispersed of phytopathogenic fungi which belong to the genus Pitium, Fitoftora, Botrytis, Rhizoctonia and Fusarium groups and negatively affecting to people, environment and as well as to the economic potential of the country (Chet I et al., 1997).

The use of (biological control) micromycetes which displays antagonistic properties against phytopathogenic microorganisms, leads to an increase in resident antagonists and without adverse effects on people as well as the environment. Today, 90% of Trichoderma fungi are used as biological control agents in the cultivation of agricultural crops all around the world (Vyas S, 1995). The bio-products which created on the basis of this generation and the enterprises that produce them are located in America, Europe and Asian countries. Most of them are registered as drugs which with fungicidal properties and only 38 % percent are sold (Sheridan et al., 2014)

The effectiveness of these microorganisms is explained by the fact that it protects plants, enhances vegetative growth, and shows high antifungal properties against pathogenic populations under agricultural conditions, as well as the activation of cellulose-degrading microorganisms in the soil. Also, it ensures sharply decrease of the population of phytopathogen fungus, as well as stabilizes the balance of microorganisms in soil, and prevents diseases which phytopathogen caused. Trichoderma is widespread in nature and is considered as a microbiological object with high biological activity against phytopathogenic fungi. Therefore it is
advisable to widely use the results of modern research on metabolites of the *Trichoderma* which has a mechanism of fungicidal action, especially of plant protection in agriculture (Weidling, 1932).

Preliminary noticeable experiment was made by Weidling (1932) and showed high antifungal activity of fungi belonging to the *Trichoderma* category from vadeuteromycetes to phytopathogenic fungi. The biological activity of *Trichoderma* isolates is due to the formation of primary (proteins, enzymes, amino acids) (Azimova *et al.*, 2020) and secondary metabolites (antibiotics, phytohormones, vitamins and etc.), their manifestation as antagonism and totally dominance over phytopathogens (Turaeva, 2016). Antifungal activity properties of the *Trichoderma* fungus against phytopathogen fungus such as *Rhizoctonia*, *Sclerotium*, *Sclerotinia*, *Fusarium*, *Armillaria*, *Colletotrichum*, *Verticillium*, *Venturia*, *Endothia*, *Pythium*, *Phytophthora*, *Rhizopus*, *Diaporthe*, *Fusicladium* which threatens agricultural crops has been determined (Wells, 1988).

In the scientific sources, the mechanism of antagonistic properties of *Trichoderma* fungi is explained by the loss of nylon tissue coated with lectin or plant lectin secreted by fungal mycelium phytopathogenic micromycetes (Inbar, 1994). A confrontational analysis (analysis of the interaction of cultures in a cup of Petri) was also performed to study the regulation of the synthesis of chitinolytic enzymes in the interaction between the antagonist strain and the phytopathogen (Chet, 1998). The active effect of a combination of enzymes and antibiotics synthesized by *Trichoderma* micromycetes and metabolites on phytopathogens was determined (Lorito, 1998).

Under the influence of the generalized activity of all primary and secondary metabolites synthesized by *Trichoderma* occurs due to the dissolution of the phytopathogenic cell wall of the antagonist strain.

This feature of the antagonist strain limits the development and activity of pathogenic fungi (Harman., 1996). In phytopathogenic hyphae there is a process of disturbance-suppression, antagonist strain hyphae enter the phytopathogenic-fungal mycelium and show dominance (Ridout *et al.*, 1988). *Trichoderma* fungi synthesize stable and non-stable antibiotic components that inhibit the mycelia growth of various fungi. This feature is widely used in *in vitro* screening of producer strains of biopreparations (Ranasinghe, 2006). Dennis and Webster (1971) were the first to determine that the antibiotic production of the fungus *Trichoderma* was associated with an active antagonistic property (Dennis and Webster, 1971). Fungi of the *Trichoderma* family have been found to synthesize more than 100 substances with antibiotic activity (Harman, 2004). According to Rosado *et al.* the key to the success of this type of fungus is its highly active antagonism and affective resistance to plant induced pathogens (Rosado *et al.*, 2007). Peptaibols are a large family of antimicrobial peptides produced by *Trichoderma* sp. The antibiotic mechanism of tricoconin VI peptaibol against plant-pathogenic fungi has been observed to occur through apoptosis (Mei, 2012). Fungi belonging to the genus *Trichoderma* were isolated from the various sources, identified as strains of *T. harzianum*, *T. koningii*, *T. hamatum*, *T. longibrachiatum*, *T. reesei*, *T. viride* and found to produce substances of antibiotic nature (Ghisalberti *et al.*, 1991). Systemic studies have been conducted on the active antagonistic
properties of *T. harzianum* strain against *Botritis cinerea* phytopathogenic fungus, the isolation of antibiotic substances and enzymes formed (Schirmböck et al., 1994). The activity of phytohormones of the fungus *Trichoderma harzianum* 55, i.e., the synthesis of secondary metabolites Gibrelin acid and indolyl sirca acid was determined (Bakhora Turaeva et al., 2020). Indolyl sirca acid activates cell division in the growing part of the plant root and enhances root development, eliminates toxic metabolites produced pathogenic microorganisms and directly controls root pathogens (Harman, 2004).

A number of studies have shown a reduction in diseases of various crops (beans) when treated with pathogenists in soils (Abd-El-Khair et al., 2011, Otadoh et al., 2011). *P.citrinum, T.viride* and *T. harzianum* allow bean plants to be used as a biological control agent to protect against *F.oxysporum* (Hend, 2012).

Rational use of biological agents to protect agricultural crops from phytopathogens, to obtain high as well as quality yield gives effective results.

In this regard, the most commonly used strains are *T.viride, T.lignorum, T.koningi, T.harzianum*. They have broad-spectrum antagonistic properties and synthesize antibiotics such as viridine, gliotopsin, cycleosporin, alamycin, torixopolin (Bukhanova, 2005).

2. MATERIALS AND METHODS

To select the optimal nutrient medium for *Trichoderma* sp 4 fungi (Karimov et al., 2020) isolated from the root rhizosphere of corn, potato dextrose agar (PDA), Eshbi, Saburo, Chapek, Mandels, Starch-ammonia agar (SAA), Wort agar, Shaded casein agar, yogurt whey. Fungi in Petri dishes grown at a temperature of 24-26°C the radius of the colonies was measured using a ruler every 24 hours. The arithmetic mean of the radii of each individual colony was taken at a given time, and the radial velocity was calculated according to this formula:

\[ Kr = \frac{(r - ro)}{(t - to)} \]

\( k \) – radial growth rate, mm/h;
\( to \) – radius of the colony at the time of onset, \( ro \);
\( t \) – the radius of the current colony, (Panikov, 1991)

In a liquid and solid PDA medium of 3,4,5,6,7,8 and 9 to select the optimal pH medium, in a 200 ml medium in 500 ml Erlenmeyer flasks, in a shaker at 180 rpm/min (IKÁ® KS 130 shakers), grown for 20 days at temperatures of 24-26°C.

As inoculums, a suspension of \( 10^{6-7} \) spores/ml concentration of *Trichoderma* sp 4 strain grown for 6 days in agar PDA media (solution) was used as planting materials.

*Trichoderma* sp 4 fungal strain was found to produce biomass in an optimal nutrient medium i.e., in PDA. 10 ml of micromycet culture fluid was obtained and 0.45 μм filtered by bacteriological filter. Wet and dry weights of biomass were determined (Ohaus MB 45).
The antagonistic properties of the fungal strain of *Trichoderma* sp 4 against the phytopathogenic fungi *Fusarium oxysporum, Verticillium dahliae, Alternaria alternate* were determined by agar cell and agar block methods (Bilay, 1977).

The mean value of three iterative results from three separate experiments was used for data analysis (±SE) in Microsoft Excel (USA Microsoft Association). In terms of values, the results obtained were analyzed in the ANOVA program for a reliable difference from the control value at P≤0,05.

3. RESULTS AND DISCUSSION

High and efficient yield of agricultural crops and the cultivation of environmentally friendly products require the use of biological preparations. Nowadays, due to the regular use of chemicals, the increased flexibility and high viability of phytopathogenic microorganisms are causing great damage in agriculture. The increase in adverse effects on humans and the environment requires scientific research to identify new microorganisms with antagonistic properties. The antifungal activity of microorganisms is explained by its antibiotic-forming property. According to the scientific sources, micromycetes produce more than 150 antibiotics. In particular, more than 40 species of *Penicillium* and species belonging to the class *Basidiomycetes* have been found to produce 26 antibiotics.

Microbiological and cultural characteristics of the colony of *Trichoderma* sp 4 strain, i.e. growth rate, color and appearance at a temperature of 28-30°C, potato dextrose agar (KDA), Eshbi, Saburo, Chapek, Mandels, Starch-ammonia agar (KAA), Suslo-agar, studied during the growth in solid nutrient media such as shaded casein, agar yogurt whey (table 1).

According to the results of the study, *Trichoderma* sp 4 fungus was found to grow rapidly in the PDA nutrient medium. The center of a 3-day colony was dark green and the color toward the edge of the colony changed to light green and white. The 4-day colony, which covers the surface of the cup, contains mycelium in the form of granules and spores at the same time. By 4-5 days, the colony had a dark green color and the mycelium formed a ring shape.

Table 1 Growth rate of *Trichoderma* sp 4 strain colony in different nutrient media (diameter, mm)

| At the expense of day | Potato dextrose agar (PDA) | Eshbi (mm) | Saburo (mm) | Chapek | Mandels | Starch-ammonia agar (SAA) | Suslo-arap | Soy casein | Agar yogurt whey |
|----------------------|---------------------------|------------|-------------|--------|---------|--------------------------|------------|------------|------------------|
| 2                    | 24±0,3                    | 0          | 14±0,3      | 16±0,7 | 16±0,9  | 8±0,7                   | 25±0,1     | 10±0,9     | 8±0,7            |
| 3                    | 51±0,1                    | 0          | 46±0,1      | 40±0,1 | 43±0,3  | 30±0,3                  | 50±0,3     | 30±0,3     | 19±0,7           |
Saburo, Suslo-agar, Chapek and Mandels environments showed slower growth than PDA. The fungal colony covered the surface of the Petri dish only on day 5. The mycelium on the outer circumference of the colony was white and evenly distributed radially from the center and some places in the form of small granules. In the center of the colony, mycelium was found to be colorless, fluffy and spider-like. Starch ammonia agar (SAA) and shaded casein cultures also showed slower growth than other nutrient media. On day 2 in SAA, it was white in color, slowly growing and spreading in a white granular state. By day 6, it covered the surface of the cup and formed a green color. In the shaded casein nutrient medium, a very slow, white spider web spread. In order to select a relatively inexpensive nutrient medium, an agar yogurt whey nutrient medium was used. On day 6, the colony covered almost the entire surface of the cup. The granular mycelium in the central part was dark and formed a pale yellow circle. The mycelium at the periphery of the colony circle is white, slightly fluffy and the radial rays are not clearly visible due to the formation of granular colonies. On day 3, small and large granular white mycelium is visible around the focal point. On day 6, the granular colonies were observed to be yellow (figure 1).

**Figure 1.** Optimal nutrient media for *Trichoderma sp 4* strains:
A) Potato dextrose agar; B) Saburo; C) Chapek; D) Mandels;
E) Starch ammonia agar; F) Suslo agar; G) Soy casein; H) Agar with yogurt whey.

According to the study, *Trichoderma sp* 4 fungus, is a fast-growing fungus, and in most nutrient media studied, its colony completely covers the cup surface after 4 days. In the colony of *Trichoderma sp* 4, the formation of granular mycelium and conidia in the form of green pigment, radial light and ring-shaped growth is a morphological character property of this fungus (Sharma et al., 2014, Srinivasa et al., 2014, Azimova, 2018, Turaeva, 2019).

To study the effect of different pH environments on *Trichoderma* sp 4 strains, PDA was grown in a pH medium with a pH of 3 to 9. The biomass formation in a liquid medium and growth rate in solid agar medium were measured for 20 days. The observations showed that the growth dynamics were high in a pH environment of 5 to 7.

Due to the high alkalinity of the medium, the biomass formation rate of *Trichoderma* sp 4 strains at pH 8 and 9 was observed to be low. At pH environments 3 and 4, however, the growth rate due to the highly acidic environment resulted in significant low biomass accumulation. On day 3 of the developmental period high levels of biomass is produced (figure 2).

![Figure 2 The influence of pH environment on biomass formation of Trichoderma sp 4 strain (in wet state)](image)

When determining the amount of biomass under the influence of different pH environments in the PDA nutrient medium, it was found that the wet biomass accumulated more in 3 days, while this biomass was more in 10-14 days in the dry state (figure 3).
Figure 3. The effect of pH on biomass formation of *Trichoderma* sp 4 strain (in the dry state)

Studies have shown that the biomass accumulation of *Trichoderma* sp 4 strain in the dry state decreases after 14 days. In the studied sources, *Trichoderma* family fungi produce large amounts of biomass when the optimal pH of growth is in the range of 5-7, primary metabolities on days 1-4 and secondary metabolities on days 8-14. The temperature of pH,

Table 2 Antagonistic properties of *Trichoderma* sp 4 against pathogenic strains grown in liquid medium at different pH (in the groove method)

| Pathogenic fungi | pH medium | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|------------------|-----------|---|---|---|---|---|---|---|
| **3 days (mm)**  |           |   |   |   |   |   |   |   |
| *A. alternata*   |           | 14±0,9 | 18±0,3 | 28±0,3 | 44±0,9 | 38±0,9 | 18±0,6 | 10±0,8 |
| *F. oxysporum*   |           | 8±0,3 | 34±0,3 | 40±0,3 | 42±0,6 | 42±0,6 | 38±0,9 | 22±0,8 |
| *V. dahliae*     |           | 4±0,3 | 42±0,3 | 42±0,3 | 44±0,8 | 38±0,6 | 32±0,9 | 20±0,8 |
| **4 days (mm)**  |           |   |   |   |   |   |   |   |
| *A. alternata*   |           | - | 30±0,3 | 4±0,3 | 4±0,3 | 44±0,6 | 40±0,9 | 36±0,8 |
| *F. oxysporum*   |           | - | 8±0,3 | 10±0,3 | 8±0,3 | 34±0,7 | 30±0,6 | 28±0,5 |
|       | 6 days (mm)                      | 8 days (mm)                      | 10 days (mm)                      | 12 days (mm)                      | 14 days (mm)                      | 16 days (mm)                      |
|-------|----------------------------------|----------------------------------|----------------------------------|----------------------------------|----------------------------------|----------------------------------|
|       | 20±0,7                           | 44±0,7                           | 50±0,7                           | -                                | 46±0,6                           | 42±0,9                           | 34±0,3                           |
| V. dahliae |                                |                                  |                                  |                                  |                                  |                                  |                                  |
| A. alternata |                              |                                  |                                  |                                  |                                  |                                  |                                  |
| -      | 38±0,9                           | 28±0,9                           | 30±0,6                           | 40±0,9                           | 38±0,9                           | 6±0,9                            |
| F. oxysporum |                              |                                  |                                  |                                  |                                  |                                  |                                  |
| -      | 4±0,6                            | 32±0,6                           | 38±0,9                           | 42±0,7                           | 38±0,7                           | 6±0,3                            |
| V. dahliae | 8±0,3                            | 44±0,6                           | 34±0,6                           | 36±0,9                           | 44±1,2                           | 42±1,2                           | 14±0,3                           |
|       | 12±0,7                           | 46±0,6                           | 20±0,6                           | 18±0,6                           | 40±0,9                           | 38±0,6                           | 26±0,8                           |
| A. alternata |                              |                                  |                                  |                                  |                                  |                                  |                                  |
| -      | 38±0,6                           | 40±0,6                           | 38±0,9                           | 42±0,9                           | 40±0,6                           | 40±0,8                           |
| F. oxysporum |                              |                                  |                                  |                                  |                                  |                                  |                                  |
| -      | 8±0,3                            | 6±0,3                            | 8±0,6                            | 34±0,6                           | 28±0,3                           | 30±0,8                           |
| V. dahliae | 4±0,6                            | 44±0,9                           | 38±0,9                           | 42±0,6                           | 28±0,9                           | 22±0,3                           | 32±0,8                           |
|       | 12±0,6                           | 28±0,7                           | 30±0,7                           | 32±0,7                           | 42±0,6                           | 38±0,9                           | 38±0,5                           |
| A. alternata |                              |                                  |                                  |                                  |                                  |                                  |                                  |
| -      | 38±0,6                           | 40±0,6                           | 38±0,9                           | 42±0,9                           | 40±0,6                           | 40±0,8                           |
| F. oxysporum |                              |                                  |                                  |                                  |                                  |                                  |                                  |
| -      | 4±0,6                            | 12±0,6                           | 12±0,3                           | 24±0,3                           | 20±0,6                           | 12±0,8                           |
| V. dahliae | 12±0,6                           | 28±0,7                           | 30±0,7                           | 32±0,7                           | 42±0,6                           | 38±0,9                           | 38±0,5                           |
|       | 4±0,3                            | 34±0,3                           | 28±0,3                           | 36±0,3                           | 28±0,6                           | 40±0,9                           | 32±0,5                           |
| A. alternata |                              |                                  |                                  |                                  |                                  |                                  |                                  |
| -      | 20±0,9                           | 28±0,9                           | 34±0,9                           | 20±0,9                           | 26±0,9                           | 10±0,5                           |
| F. oxysporum |                              |                                  |                                  |                                  |                                  |                                  |                                  |
| -      | 16±0,3                           | 4±0,3                            | 32±0,6                           | 8±0,9                            | 24±0,9                           | 14±0,3                           |
| V. dahliae | 12±0,9                           | 14±0,9                           | 10±0,3                           | 14±0,9                           | 48±1,2                           | 40±0,6                           | 18±0,9                           | 22±0,6                           | 28±0,2                           |
|       | 8±0,9                            | 40±0,9                           | 26±0,9                           | 22±0,9                           | 18±0,3                           | 12±0,3                           | 8±0,3                            |
| A. alternata |                              |                                  |                                  |                                  |                                  |                                  |                                  |
| -      | 28±0,9                           | 24±0,3                           | 26±0,6                           | 22±0,6                           | 26±0,7                           | 26±0,3                           |
| F. oxysporum |                              |                                  |                                  |                                  |                                  |                                  |                                  |
| -      | 14±0,9                           | 48±1,2                           | 40±0,6                           | 18±0,9                           | 22±0,6                           | 28±0,2                           |
| V. dahliae | 10±0,3                           | 14±0,9                           | 14±0,9                           | 38±0,7                           | 44±0,3                           | 30±0,6                           | 40±0,6                           |
|       | 14±0,3                           | 24±0,9                           | 34±0,9                           | 38±0,7                           | 44±0,3                           | 30±0,6                           | 40±0,6                           |
| A. alternata |                              |                                  |                                  |                                  |                                  |                                  |                                  |
| -      | 18±1,2                           | 26±0,6                           | 34±0,3                           | 28±0,3                           | 26±0,7                           | 18±0,3                           |
nutrient medium are important sources for the development of micromycetes strain isolated from nature (Anuradha et al., 2014).

The antagonistic properties of Trichoderma sp 4 strain, which exhibits antagonistic properties among soil microorganisms were studied against phytopathogenic fungi for use in the fight against plant diseases. Phytopathogenic fungi of the Trichoderma sp 4 strain grown in media with different pH A. Alternata, F. oxysporum, V. Dahliae antagonistic properties were studied. During the study, this strain, grown in PDA nutrient medium, formed a ring of 42 mm at a pH of 6-7 against F. oxysporum on day 3 of the growth period. A. Alternata on the 16th day of the growth period relative to the alternate formed a 38-44 mm ring at 6-7 pH. V. Dahliae, on the 14th day of the growth period, a 48-40 mm ring was formed at an environment of 5-6 pH (table 2)

Growth restriction was observed when different pH environments were studied affected, when created (3,4,8,9 pH medium), favorable pH environments (5, 6, 7 pH environment) well growth and rapid development was observed. Although the antagonism against F. oxysporum manifested rapidly in the images, it did not last long to retain its properties. A Alternata and V. dahlia slowly showed complete antagonism, gradually covering the surface of the Petri dish completely. Studies on V. Dahliae have shown the separation of a pink pigment from itself (figure 4)

| V. dahliae | 14±0.9 | 22±0.9 | 34±0.9 | 40±0.6 | 18±0.6 | 20±0.3 | 32±0.3 |
|------------|--------|--------|--------|--------|--------|--------|--------|
| 18 days (mm) |        |        |        |        |        |        |        |
| A. alternata | -   | 8±0.9 | 42±0.6 | 34±0.7 | 38±0.9 | 12±0.3 | 18±0.8 |
| F. oxysporum | -   | 16±0.3 | 24±0.9 | 34±0.9 | 34±0.9 | 16±0.6 | 16±0.8 |
| V. dahlia | - | 32±0.3 | 36±0.9 | 38±0.3 | 36±0.6 | 28±0.9 | 22±0.8 |
| 20 days (mm) |        |        |        |        |        |        |        |
| A. Alternata | - | 34±0.7 | 32±0.6 | 36±0.3 | 38±1.2 | 10±0.3 | 12±0.5 |
| F. oxysporum | - | 12±0.3 | 12±0.9 | 14±0.3 | 14±0.7 | 6±0.3 | 6±0.8 |
| V. dahlia | 16±1.2 | 44±0.3 | 26±0.3 | 34±0.3 | 32±0.6 | 18±0.3 | 28±0.3 |
Figure 4. Antagonistic properties of *Trichoderma* sp 4 strain grown in different pH medium against pathogenic strains (in the groove method)

A) *Alternaria alternata*; B) *Fusarium oxysporum*; C) *V. dahliae*

*Trichoderma sp* 4 strain phytopathogenic fungi grown in PDA nutrient medium with pH environment 4-9. *A. Alternata, F. oxysporum, V. dahlia* studies have been conducted. During the study, high antagonism compared to *A. Alternata* formed a 41 mm ring at 5 and 6 pH in 3 days. With respect to *F. oxysporum*, it formed a 42-44 mm ring at 6 and 7 pH in 4 days. In the case of *V. dahlia*, it was found to form a 44-46 mm ring at 4 and 7 pH in 4 days.
### Table 3  Antagonistic properties of *Trichoderma* sp 4 against pathogenic strains grown in different pH media in PDA nutrient medium (block method)

| Pathogenic fungi | pH medium | 4     | 5 | 6     | 7     | 8     | 9     |
|------------------|-----------|-------|---|-------|-------|-------|-------|
|                  |           | 3 days (mm) |   | 4 days (mm) |   | 6 days (mm) |   | 8 days (mm) |   | 10 days (mm) |   | 12 days (mm) |   |
|                  |           | 6 days (mm) |   | 8 days (mm) |   | 10 days (mm) |   | 12 days (mm) |   |               |   |               |   |
|                  |           | 10 days (mm) |   | 12 days (mm) |   |               |   |               |   |               |   |               |   |

- **A. alternata**
- **F. oxysporum**
- **V. dahliae**
|                      | A. alternata | F. oxysporum | V. dahliae |
|----------------------|--------------|--------------|------------|
| 14 days (mm)         |              |              |            |
| A. alternata         | 24±0,6       | 14±0,6       | 22±0,8     |
|                      | 28±0,7       | 20±0,6       | 20±0,3     |
|                      | 38±0,3       | 20±0,3       | 22±0,8     |
|                      | 42±0,6       | 28±0,6       | 28±0,3     |
|                      | 18±0,9       | 18±0,9       | 24±0,6     |
|                      | 10±0,3       | 16±0,3       | 20±0,9     |
|                      |              |              |            |
| 16 days (mm)         |              |              |            |
| A. alternata         | 38±0,6       | 10±0,3       | 44±1,2     |
|                      | 42±0,3       | 18±0,9       | 28±1       |
|                      | 32±0,3       | 36±0,3       | 40±0,6     |
|                      | 36±0,6       | 40±0,9       | 42±0,9     |
|                      | 32±0,3       | 16±0,3       | 44±0,3     |
|                      | 26±0,9       | 18±0,9       | 34±0,9     |
|                      |              |              |            |
| 18 days (mm)         |              |              |            |
| A. alternata         | 40±0,9       | 24±0,9       | 28±0,3     |
|                      | 8±0,6        | 28±0,3       | 40±0,9     |
|                      | 32±0,6       | 28±0,3       | 42±0,3     |
|                      | 28±0,9       | 24±0,3       | 40±0,3     |
|                      | 16±0,3       | 20±0,3       | 36±0,6     |
|                      | 20±0,3       | 22±0,3       | 26±0,9     |
|                      |              |              |            |
| 20 days (mm)         |              |              |            |
| A. alternata         | 14±0,3       | 12±0,6       | 28±0,6     |
|                      | 22±0,3       | 24±0,9       | 32±0,3     |
|                      | 40±0,7       | 30±0,9       | 42±0,9     |
|                      | 38±0,3       | 26±0,7       | 38±1       |
|                      | 38±0,7       | 22±0,6       | 32±0,9     |
|                      | 28±0,3       | 14±0,9       | 30±0,9     |

It was found that antagonism to phytopathogens occurs more rapidly in solid media than in liquid media. It was observed that the strain of *Trichoderma* sp 4 has a high antagonism against phytopathogenic fungi *A. alternata* and *V. dahliae*. 

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Figure 5. Antagonistic properties of *Trichoderma* sp 4 strain grown in different pH environment against pathogenic strains (agar block method)

A) *Alternaria alternata*; B) *Fusarium oxysporum*; C) *Verticillium dahliae*

4. CONCLUSION

The effects of 9 different nutrient media on the growth of *Trichoderma* sp.4 micromycetes were studied during the conducted research. The most active growth of micromycetes was observed in the PDA nutrient medium, and on the 4\textsuperscript{th} day it was found to completely cover the surface of the Petri dish. The effect of the initial pH value on the antagonistic activity of this strain against phytopathogens was studied. It was determined that micromycetes manifested to grow well at pH ranges from 4.0 to 9.0. The highest biomass formation and antagonistic properties showed high activity in media with a pH of 5.0 to 7.0. According to the results of studies on the effect of antagonistic properties of *Trichoderma* sp.4 micromycetes on growth duration, the highest antagonistic activity was detected on the 3\textsuperscript{rd} day of growth.
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