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Biological characteristics of *Alternaria solani* fungal pathogen

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Abstract. The *Alternaria* genus is found in various regions of the world. One economically significant member of this genus is *Alternaria solani*, which causes early blight disease in potatoes and tomatoes. Early blight affects the leaves, stems, and tubers of potatoes. The symptoms on leaves usually appear 5–20 days before flowering as brown or dark brown spots, often surrounded by concentric rings. Studying the biological characteristics of the pathogenic fungus is essential for effective protection of potatoes against early blight. This article presents the results of our laboratory studies on the biological characteristics of *Alternaria* fungi isolated from infected potato plants.

1 Introduction

The continuous increase in the global population has led to a rising demand for food. The tubers of the potato plant (*Solanum tuberosum L.*) are a staple food worldwide and are currently cultivated in over 150 countries. According to FAO data, potatoes are grown on an average of over 20 million hectares annually, yielding more than 375 million tons, making it the fifth most important energy source in human nutrition [1].

Fungi are one of the primary pathogens responsible for plant diseases. Pathogenic fungi use various methods to reproduce, spread, and cause diseases in plants. Some fungi destroy the host plant and feed on dead matter (necrotrophs), while others grow within living tissue (biotrophs). Fungi use various virulence factors to reproduce and spread in host plants, with each virulence factor performing different functions depending on the method of infection. Almost all pathogens disrupt the plant's primary defenses, while necrotrophs produce toxins to kill plant tissue [2].

In recent years, the impact of early blight on potatoes has increased, drawing the attention of phytopathologists and plant protection specialists in many countries. Significant damage has been observed in potatoes even in countries like Sweden, Germany, and the Netherlands, where early blight was not previously considered harmful. This increase is believed to be linked to a reduction in dithiocarbamate usage and rising temperatures, which previously helped control the disease effectively [3, 4].

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In recent years, the quantity and quality of agricultural crop yields have declined due to the effects of harmful organisms. This is attributed to pathogenic microorganisms adapting to climatic conditions and delays in implementing effective control measures [5]. Currently, potato crop losses due to early blight average around 5% annually. In some years, the percentage of infected plants can reach 100%, reducing yields by 5 to 78% [6].

The *Alternaria* genus is found in various regions worldwide. An economically important member of this genus, *Alternaria solani*, causes early blight in potatoes and tomatoes. During epidemics, severe infection and leaf drop can lead to significant crop loss [7-10].

The *Alternaria* genus comprises a morphologically diverse group of taxa and can be found in nearly all ecosystems globally. The estimated number of species ranges from around 150 to several hundred [11]. Diseases caused by *Alternaria* fungi typically manifest as leaf spots and rots but can also lead to seedling blight, stem rot, root rot, and fruit rot [12]. The early blight disease in potatoes is caused by the fungus *A. solani* (Sorauer 1896) [13]. This disease affects the leaves, stems, and tubers of potatoes. Symptoms on leaves typically appear 5–20 days before flowering as brown or dark brown spots, often surrounded by concentric rings. Under favorable temperature and humidity, these spots can develop as early as the second or third day after infection. By the third or fourth day, when the spots reach a diameter of 3 mm, the pathogen's dark gray conidia begin to develop.

2 Materials and methods

For laboratory experiments, we first collected infected plant samples from fields known for widespread early blight in Tashkent Province, specifically from farms in Tashkent District ("Abdusattor agro omad," "Abduazim agro," "Mirqosim Kamola fayz," "Alyor fayz baraka," "Batko agrolyuks"), Qibray District ("Salar agro"), and Zangiota District ("Xamdamjon Akromov," "Jonibek," "Botir," "Oybek-Bilolbek"). The infected plant samples were placed in special bags and transported to the laboratory.

Upon arrival, the samples were washed for 30 minutes in running water, followed by surface sterilization to remove any external microorganisms. The plant surfaces were treated with 75% ethanol (C_2H_5OH) for 30 seconds, then with a 0.5% sodium hypochlorite (NaOCl) solution for 1 minute. The samples were subsequently rinsed three times with sterilized water for 30 seconds and air-dried. These disinfected samples were then placed on filter paper inside Petri dishes in a humid chamber to isolate pure fungal cultures [7]. To prepare the Petri dishes, filter paper was placed in each dish and sterilized in an autoclave at 121°C and 1 atmosphere of pressure for 30 minutes. The filter papers were moistened with distilled water near an alcohol lamp, and plant samples were cut into 5 × 5 mm pieces using a scalpel heated in the flame. Each Petri dish was then inoculated with 3–7 pieces of the plant sample (Figure 1). The dishes were incubated in a thermostat at 25°C [7].

Starting from the third day, fungal growth began to appear in the Petri dishes. On the seventh day, pure cultures of the germinated fungi were isolated by transferring colonies to slant tubes containing sterilized solid agar medium. The tubes were sealed with stoppers and placed back in the thermostat at 25°C. Eleven isolates were obtained from potato cultivars Serxosil, Gala, Rozara, and Aqrab.

To determine the linear growth of *A. solani*, the colony radius was measured in two perpendicular directions (from the inoculation point to the edge of the mycelial growth zone). The radial growth rate of the colony was calculated using the following formula:

$$Kr = (r - r_0) / t$$

Where, *Kr* represents the radial growth rate of the colony in mm/hour; *r* is the colony radius at a specific time in mm; r_0 is the initial colony radius in mm; and, *t* is the time in hours from inoculation until the colony reaches radius *r* [11].

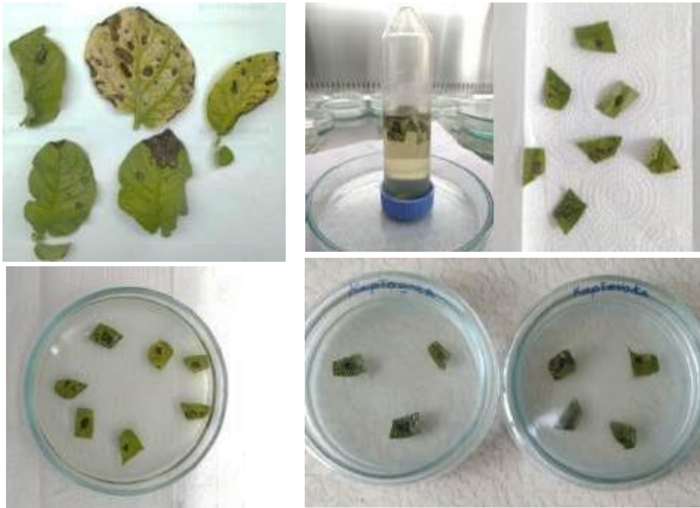

Fig. 1. Process of isolating pure cultures of pathogenic fungi from infected potato plants.

3 Results and discussion

We observed various types of conidia from *Alternaria* fungi, paying special attention to their shape, size, and septation. To identify the species, we used several identification keys and the Mycobank database. Based on the obtained data, the isolated species were identified as *Alternaria solani* (Ellis & G. Martin) L.R. Jones (= *Macrosporium solani* Ellis & G. Martin), *Alternaria alternata* (Fr.) Keissl. (1912) (= *Alternaria fasciculata* (Cooke & Ellis) L.R. Jones & Grout (1897)), and other *Alternaria* sp. species (Table 1).

Table 1. Species and characteristics of *Alternaria* fungi isolated from potato plants

#	Fungal species	Colony color	Conidia shape
1	*A. solani*	Light gray	Long, elliptical, thin, septated
2	*A. alternata*	Black	Short, inverted pear-shaped, thick, up to four septa
3	*A. sp.*	Black	Short, blunt, thick, up to three septa

According to F. B. Gannibal's (2011) data, *A. solani* colonies are gray to greenish-gray, grow rapidly, and have conidia that are mostly solitary, rarely in pairs, and range in color from yellowish to brown. The conidia are long, oval or elliptical, and have between 7 and 11 septa. Additionally, the apical extension of the conidia is typically simple, occasionally bifurcated, and rarely trifurcated, with a length of 60–118 µm [3]. We found that some of our isolated strains share a similar structure. Their colonies are gray, conidia are yellowish, thin and elongated, with five to six septa, and have a long apical extension (Figure 2).

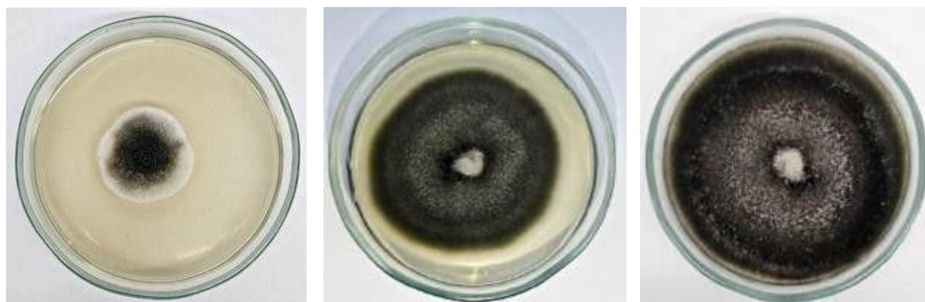

Fig. 2. Colony formation of *A. solani* fungi.

Some of the characteristics of our isolated strains were similar to those of the *A. alternata* fungus, as reported in the literature. Specifically, the colonies were black, and upon microscopic observation, the conidia were yellowish-brown, short, inverted pear-shaped, thick, and had up to four septa.

Additionally, another isolate exhibited characteristics distinct from both *A. solani* and *A. alternata*. This isolate also had black colonies, but its conidia were yellowish-brown, short, inverted pear-shaped, thick, and had up to three septa (Figure 3). We designated this strain as *Alternaria sp.*

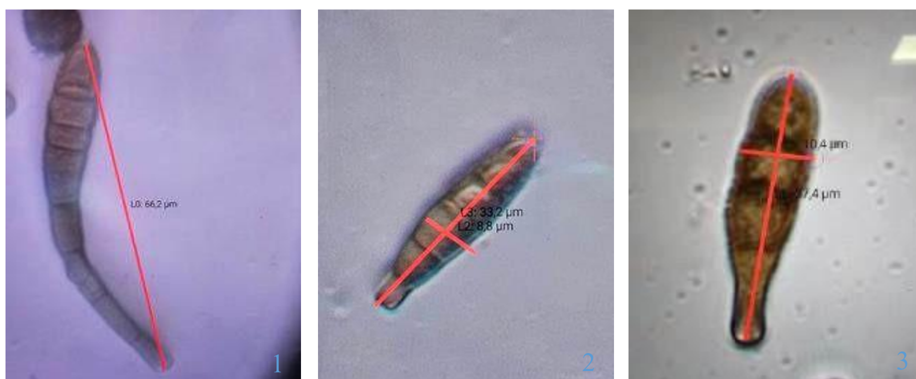

Fig. 3. Microscopic appearance of conidia produced by Alternaria fungi (1-*A. solani*; 2- *A. sp.*; 3-*A. alternata*).

Thus, several fungi responsible for causing alternariosis in potato plants, namely *A. solani*, *A. alternata*, and other *Alternaria* species, were identified and their pure cultures were isolated. Our subsequent research focused on studying some biological characteristics of the *A. solani* fungus, which was the object of our investigation.

Carbohydrates exist in both simple and complex forms in plants, and fungi convert complex carbohydrates into simple, water-soluble carbohydrates with a low molecular weight before utilization. It has been demonstrated that different fungi show significant variations in their development or utilization of various carbohydrate sources depending on specific nutrient elements.

A critical and comprehensive understanding of the nutrient elements and factors influencing fungal growth is essential for any research aimed at understanding plant- pathogen interactions. There has been limited focus on the parameters of the culture and growth environment of pathogens. The *A. solani* fungus requires several specific nutrient elements for its growth and development, even though the fungus is naturally cosmopolitan. However, the nutrients required for the good development of the fungus do not necessarily

ensure its effective sporulation. The morphology of *A. solani* colonies is also influenced by various nutrient media [9].

To develop appropriate strategies for controlling plant diseases, it is essential to study the effects of various nutrient media on the development of fungi, as well as to gain comprehensive information about the physiological state of fungi isolated from infected plant tissues, particularly regarding their sporulation and colony formation characteristics.

In our subsequent experiments, we investigated the growth and development of *A. solani* in different nutrient media. The experiments were conducted in three repetitions using potato dextrose agar (PDA), potato glucose agar (PGA), potato agar (PA), and a control variant in a moist chamber environment.

Cultures of *A. solani* inoculated into various nutrient media were placed in a thermostat and incubated at 25 ºC. On the 3rd, 4th, 5th, and 7th days post-inoculation, the growth and development of fungal colonies, as well as the level of conidia production, were recorded. The following indicators were focused on during the assessment: linear growth of the colonies, the radial growth rate of the colonies, and the formation of conidia.

To determine linear growth, the radius of the colonies was measured in two mutually perpendicular directions (from the inoculation point to the edge of the mycelial growth zone). The linear growth of the fungus on all solid media was evaluated by measuring the diameter of the colonies along the same axis after a 7-day incubation period.

The color of the colonies, growth limits, and mycelial structure were visually observed. To measure the conidia production of the fungus in different nutrient media, a block of 5 mm in diameter was cut from the colonies using a sterile spatula and transferred to 5 ml of sterile distilled water in a test tube, where it was thoroughly mixed. A drop was then taken from the mixture and placed on a microscope slide, and the average number of conidia in three microscopic fields was determined using the small (10X) objective of the microscope.

According to the experimental results, in the control variant, the diameter of the fungal colony was 20.7 mm after 3 days, 30.0 mm after 4 days, 42.3 mm after 5 days, and 54.0 mm after 7 days, with no conidia formation observed (Table 2).

The best growth of the *A. solani* fungus was recorded on potato dextrose agar (PDA) medium. By the fifth day of observation, the diameter of the colonies reached 63.3 mm, and by the seventh day, it reached 82.3 mm. In this variant, a large number of conidia were observed in each microscopic field. This result closely aligns with the findings of S. Koley and S. Mahapatra (2015), where they reported that the diameter of *A. solani* fungal colonies on PDA medium reached 88.67 mm after 7 days of inoculation [9].

Table 2. Study of cultural characteristics of *A. solani* on different nutrient medium.

Name of nutrient medium	Colony diameter (mm) after inoculation				Conidia formation*	Colony color
	3	4	5	7		
Control (moist chamber)	20.7	30.0	42.3	54.0	-	Gray, center is dark
PDA	25.0	43.3	63.3	82.3	++++	Gray
PGA	24.3	34.3	56.0	73.0	+++	Light gray
PA	20.7	30.7	40.3	67.3	++	Dark gray, center is dark
Light Agar	24.3	34.7	54.7	71.0	+++	Dark gray, center is dark

Note: "-" – absent; "+" – present (few); "++" – moderate (several); "+++" – numerous; "++++" – abundant.

In the *Potato Dextrose Agar (PDA)* medium, the fungal colonies showed relatively slow

development. Specifically, the diameter of the colonies was *20.7 mm* on the *3rd day*, *30.7 mm* on the *4th day*, *40.3 mm* on the *5th day*, and *67.3 mm* on the *7th day*, with the number of conidia in one microscopic field being *1-2*.

Good development of the fungus was also observed in the *Potato Glucose Agar (PGA)* medium. According to the measurements taken on the *7th day* of observation, the diameter of the colonies reached *73.0 mm*. Additionally, in the *Light Agar (LA)* medium, the diameter of the fungal colonies was *24.3 mm* on the *3rd day*, *34.7 mm* on the *4th day*, *54.7 mm* on the *5th day*, and *71.0 mm* on the *7th day*, with the number of conidia in a microscopic field being *3-4* after seven days.

In the next experiment, the radial growth rate of *A. solani* fungal colonies was studied. According to the results, the radial growth rate in the control (*moist chamber*) variant was *0.32 mm/s* (Figure 4).

The highest radial growth rate in the experiment was observed in the isolates cultivated in the *Potato Dextrose Agar (PDA)* medium, where the colonies exhibited a radial growth rate of *0.49 mm/s*. In the *Potato Glucose Agar (PGA)*, *Agar (PA)*, and *Light Agar (LA)* media, the radial growth rates were found to be *0.43*, *0.40*, and *0.42 mm/s*, respectively.

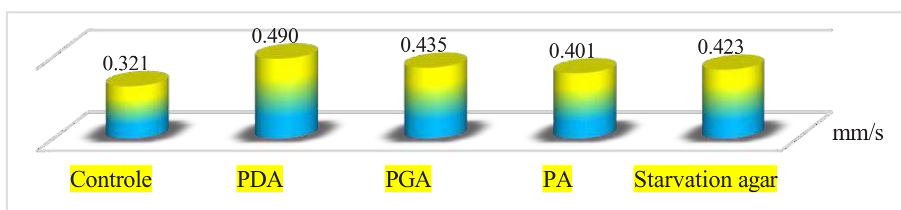

Fig. 4. Radial growth rate of *A. solani* fungal colonies.

Thus, it was determined that the potato dextrose agar medium is the most favorable environment for the development of *A. solani* fungus, with a radial growth rate of 0.49 mm/s for the colonies. Additionally, it was noted that in the PDA medium, *A. solani* produces gray mold and generates a large quantity of conidia.

Among the environmental factors that influence the development of microorganisms, temperature holds a leading position. Changes in temperature significantly affect various aspects of the metabolism of mesophilic and thermophilic microorganisms. The impact of temperature on a multi-enzyme system, such as a cell, is very complex, as each component of this system reacts differently to specific environmental factors [11].

In the next experiment, we studied the effect of temperature on the growth of the *A. solani* fungus. In the experiment, Petri dishes containing 20 ml of potato dextrose agar (PDA) were inoculated with 5×5 mm disks taken from a ten-day-old fungal culture. The inoculated cultures were incubated at various temperature ranges of 20 °C, 25 °C, 30 °C, 35 °C, 40 °C, and 45 °C, and the diameters of the colonies were measured on the 8th day after inoculation. According to the results, the growth of *A. solani* was found to be optimal at temperatures between 25 °C and 30 °C. Specifically, at 25 °C, the diameter of the colonies measured 21.0 mm after 3 days, 44.0 mm after 5 days, 62.0 mm after 7 days, and 83.0 mm after 9 days. At 30 °C, the colony diameters were 19.0 mm on the 3rd day, 36.0 mm on the 5th day, 56.0 mm on the 7th day, and 75.0 mm on the 9th day (Table 3).

Furthermore, relatively good growth was observed at 35 °C, with the diameter of the fungal colonies measuring 42.00 mm on the 9th day of observation. When incubated at 15 °C and 40 °C, there was virtually no growth of the fungus. Specifically, at 15 °C, only a growth of 1.00 mm was recorded by the 9th day, while at 40 °C, the growth reached merely 0.50 mm.

Table 3. Effect of temperature on the growth of *A. solani*, mm.

Days \ Temperature	15 ºC	20 ºC	25 ºC	30 ºC	35 ºC	40 ºC
	Colony diameter on measurement day, mm					
1	0.50	0.50	0.50	0.50	0.50	0.50
3	0.60	2.00	21.00	19.00	8.00	0.60
5	0.90	5.00	44.00	36.00	18.00	0.70
7	1.00	9.00	62.00	56.00	27.00	0.70
9	1.50	13.00	83.00	75.00	42.00	1.00

The data obtained from our experiments are similar to those reported [5], who indicated that the maximum growth of *A. solani* occurred at 25 °C, followed by 15 °C, 20 °C, and 35 °C, with the least growth at 40 °C. Similar results were also reported [4], who found that *A. solani* exhibited maximum growth at temperatures ranging from 25 °C to 30 °C [4, 5]. When studying the radial growth rate of *A. solani* colonies, the radial growth rate at 30 °C was found to be 0.34 mm/s (Figure 5). The highest radial growth rate in the experiment was observed when cultivated at 25 °C, where the colonies exhibited a radial growth rate exceeding 0.384 mm/s. Average radial growth rates of 0.347-0.194 mm/s were noted at both 30 °C and 35 °C. The radial growth rate was also low at 20 °C. At 15 °C and 40 °C, the radial growth rates were found to be very low, specifically between 0.005-0.007 mm/s.

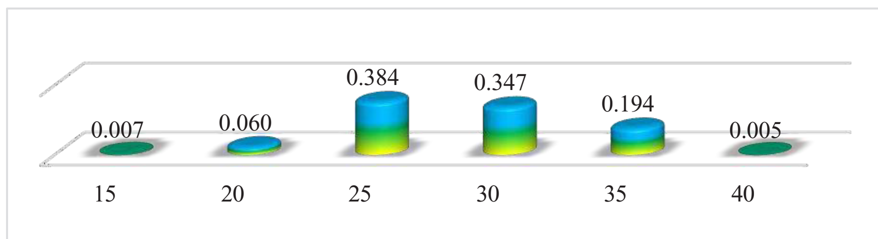

Fig. 5. Effect of temperature on the radial growth rate of *A. solani* colonies, mm/s.

Thus, it was determined that temperature significantly affects the growth of *A. solani*, and the optimal temperature for its growth is found to be between 25-30 °C. Considering that this temperature is very close to the natural air temperature for potato cultivation, it highlights the potential danger of the alternaria disease caused by *A. solani*.

The pH value of the medium is one of the most important conditions for the growth and development of microorganisms. When the pH level of the medium is optimal, it accelerates metabolism in fungi, leading to increased growth.

In our next experiments, we studied the effect of hydrogen ion concentration (pH) on the growth of *A. solani*. In the experiment, 20 ml of potato dextrose agar (PDA) medium was placed in Petri dishes. Then, disks cut into 5×5 mm from a ten-day-old fungal culture were inoculated into the dishes. The pH level of the medium was measured using a digital pH meter and adjusted to 3.5, 4.5, 5.5, 6.5, 7.5, and 8.5 by adding a few drops of hydrochloric acid (HCl) and sodium hydroxide (NaOH).

According to our experimental results, the hydrogen ion concentration (pH) level of 5.5- 7.5 is considered a good range for the growth of *A. solani*. However, it was found that a slightly acidic medium, specifically at pH 6.5, is the most optimal for the growth of the fungus. In particular, when the pH of the medium was 6.5, the diameter of the fungal colonies reached 84.5 mm by the 9th day of observation (Table 4).

Table 4. Effect of hydrogen ion concentration (pH) on the growth of *A. solani.*

Days \ pH	3.5	4.5	5.5	6.5	7.5	8.5
	Diameter of colonies (mm) on the measurement day					
1	0.50	0.50	0.50	0.50	0.50	0.50
3	0.65	1.55	11.50	22.50	18.00	5.50
5	0.75	3.65	18.00	48.50	24.00	11.00
7	0.85	8.50	26.00	63.50	36.50	19.50
9	0.90	14.50	35.00	84.50	45.00	21.00

When the medium is slightly alkaline, i.e., at pH 7.5, the diameter of the fungal colonies reached 45.0 mm by the 9th day of observation. In an alkaline medium, where pH is 8.5, the growth of the colonies was only 21.0 mm by the 9th day.

It can also be observed that the growth of the fungal colonies slows down when the pH of the medium is acidic. At pH 3.5, the diameter of the colonies reached only 0.9 mm by the 9th day of incubation. Additionally, at pH 4.5 and 5.5, the diameters of the colonies on the 9th day were 14.5 mm and 35.0 mm, respectively. When studying the radial growth rate of *A. solani* fungal colonies, the fastest growth rate was recorded at pH 6.5, measuring 0.391 mm/s (Figure 6).

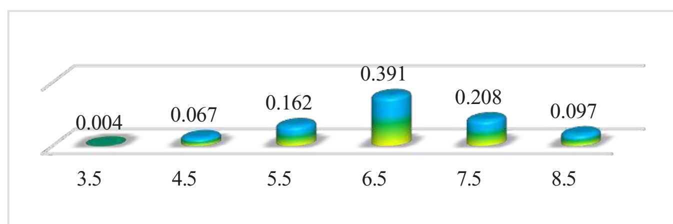

Fig. 6. Effect of hydrogen ion concentration (pH) on the radial growth rate of *A. solani* fungal colonies, mm/s.

Additionally, at pH 3.5, the radial growth rate was 0.004 mm/s, at pH 4.5 it was 0.067 mm/s, at pH 5.5 it was 0.162 mm/s, at pH 7.5 it was 0.208 mm/s, and at pH 8.5 the growth rate of the fungal colony was 0.097 mm/s.

Thus, the level of hydrogen ion concentration (pH) has a significant impact on the growth of *A. solani* fungus. It has been determined that slightly acidic conditions, specifically a pH level of 6.5, are optimal for the good growth and development of the fungus.

4 Conclusions

Thus, several fungi that cause alternariosis disease in potato plants, namely *A. solani*, *A. alternata*, and other species of *Alternaria*, have been identified and their pure cultures isolated. In our subsequent research, we studied some biological properties of the *A. solani* fungus, which was the object of our investigation.

It has been established that potato dextrose agar (PDA) is the most favorable medium for the development of *A. solani*, with colonies exhibiting a radial growth rate of 0.49 mm/s in this medium. Additionally, it was observed that in the PDA medium, *A. solani* produces gray mold and a large quantity of conidia.

The impact of temperature on the growth of *A. solani* was also significant, with the optimal growth temperature identified as being between 25-30 °C. Considering that this temperature range is very close to the natural air temperature for potato cultivation, it underscores how dangerous the alternariosis disease caused by *A. solani* can be.

Furthermore, it has been determined that the level of hydrogen ion concentration (pH) significantly affects the growth of *A. solani*. It was found that slightly acidic conditions, specifically a pH level of 6.5, are optimal for the good growth and development of the fungus.

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