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The Effect of Temperature on the Development of the Ascochyta Pisi Fungus

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Abstract: Mung bean (Vigna radiata L.) is one of the most important leguminous crops, the main producers of which are Asian countries. Its wide adaptability, drought tolerance, ability to absorb atmospheric nitrogen, and valuable nutritional and beneficial properties have made it one of the most important legumes. Ascochyta blight is one of the main diseases affecting leaves, stems, beans and grains of mung bean. The article presents the results of our experiments on isolating a pure culture of the fungus Ascochyta pisi, which causes ascochyta blight, from infected representatives of mung bean plants and studying the effect of temperature on its development.

Key words: plant, mung bean, disease, ascochyta blight, mushroom, Ascochyta pisi, A.phaseolorum, Boeremia exigua, A.phaseolorum, A.bolschhauseri, effect of temperature on the growth of fungal colonies, radial growth.

INTRODUCTION. Mung bean (Vigna radiata (L.) Wilczek) is a short-day plant with a wide range of adaptations, drought tolerance, and ability to fix atmospheric nitrogen, especially through symbiosis with rhizobium bacteria in its root nodules, as well as its valuable nutritional and health-promoting properties, making it one of the most important staple legumes in Asian countries. Annual mung bean production worldwide amounts to 3 million tons of grain on an area of more than 6 million hectares [8; 9].

In recent years, the quantity and quality of agricultural crops have been decreasing due to the influence of harmful organisms. This is due to the adaptation of pathogenic microorganisms to climatic conditions and the lack of timely and effective measures to combat them. [3; 5].

Ascochyta blight is one of the most common diseases of mung bean. The disease is caused by the fungi Ascochyta phaseolorum Sacc. (=Boeremia exigua) [10], A. Phaseolorum [4], A. pisi Lid. [7], A. boltschauseri Sacc. [2].

On the leaves, stems, pods and grains of the affected plant, gray-brown, sometimes dark gray and later blackening, elongated, but more often rounded spots appear, on which pycnidia 0.1-0.2 mm in size are formed. Infected seeds do not germinate when sown, or the sprouts that have subsequently sprouted rot. Plants heavily affected by the disease quickly wither. Plants affected by ascochytosis become sparse, their leaves dry out prematurely and fall off. The plant lags behind in development, produces small, low-vigorous and germinating seeds, which are contaminated. Pycnospores of the pathogen are spread by wind and rain, thus damaging crops. The fungus overwinters in plant debris and seeds [4].

Ascochyta blight is a polycyclic disease that can develop rapidly in humid weather and moderate air temperatures. High relative humidity and temperatures in the range of 20-25 ℃ are favorable for the development of the disease. In the field, the primary source of infection of the disease is ascospores released from mature pseudothecia that develop on infected plant debris in the previous season [7;

Vol. 3 No. 10 (2025)
ISSN: 2995-5483

11]. The source of secondary infection is pycnidiospores, which develop in pycnidia formed in wounds on leaves, stems and pods [7]

Frequent rainfall and temperatures of 20-25 ℃ allow pycnospores and ascospores to spread rapidly in crops and cause severe disease development [4].

The pycnidia of the fungus appear as small black dots 100-200 μm in size. Each pycnidia contains numerous hyaline spores embedded in a slimy matrix. Under the influence of high humidity, the material inside the pycnidia absorbs water, becomes wet and swells, which leads to the release of spores into the slimy mass. It forms a light-colored mycelium on artificial nutrient media. The conidia are hyaline, straight or slightly curved, 1-septate, with rounded ends, 10-16 × 3-4.5 μm in size [13].

Literature Review. Ascochyta disease in mosh is caused by *Ascochyta phaseolorum Sacc.* (=*Boeremia exigua*) [10], *A. phaseolorum* [4], *A. pisi Lid.* [7], *A. boltschauseri Sacc.* [2] is caused by fungi.

On the leaves, stems, pods and grains of the affected plant, gray-brown, sometimes dark gray and later blackening, elongated, but more often rounded spots appear, on which pycnidia 0.1-0.2 mm in size are formed. Infected seeds do not germinate when sown, or the sprouts that have subsequently sprouted rot. Plants heavily affected by the disease quickly wither. Plants affected by ascochytosis become sparse, their leaves dry out prematurely and fall off. The plant lags behind in development, produces small, low-vigorous and germinating seeds, which are contaminated. Pycnospores of the pathogen are spread by wind and rain, thus damaging crops. The fungus overwinters in plant debris and seeds [4].

Ascochytosis is a polycyclic disease that can develop rapidly in humid weather and moderate air temperatures. High relative humidity and temperatures in the range of 20-25 ℃ are favorable for the development of the disease. The primary source of infection of the disease in the field is ascospores released from mature pseudothecia that develop on plant debris infected in the previous season [7, 11]. The source of secondary infection is pycnidiospores, which develop in pycnidia formed in wounds on leaves, stems and pods [7].

Pycnospores and ascospores spread rapidly in crops under conditions of frequent rainfall and temperatures of 20-25 ℃, causing severe disease development. The minimum temperature for spore development should be 3 ° C, and the maximum temperature should be 33 ° C. The optimal air humidity for disease development should be above 65%, and the temperature should be 18-23 ℃, with a minimum of 8 ℃ and a maximum of 32-33 ℃ [4].

Pycnidia of *A. pisi* , located inside plant wounds, appear as small black dots 100-200 μm in size. They are spherical or pear-shaped. Each pycnidia contains numerous hyaline spores embedded in a slimy matrix. Under the influence of high humidity, the material inside the pycnidia absorbs water, becomes wet and swells, which leads to the release of spores into the slimy mass. It forms a light-colored mycelium on artificial nutrient media. Conidia are hyaline, straight or slightly curved, 1-septate, with rounded ends, 10-16×3-4.5 μm in size [13].

Methodology. To isolate a pure culture of the fungus that causes ascochytosis, samples of infected mung bean plants were initially taken from the fields of the private enterprise "Abdukarim Agro Meva" in the Denov district, placed in packages and brought to the laboratory.

Plant samples were rinsed in clean water for 30 minutes, then disinfected in a solution of 75% ethanol (C_2H_5OH) and 0.5% sodium hypochlorite (NaOCl) for 30 seconds for 1 minute to remove various microorganisms from the outer surface of the samples. Then, they were washed three times in sterilized water for 30 seconds and dried. To isolate pathogenic fungi, filter paper was placed inside the Petri dishes and sterilized in an autoclave at 121 ° C and 1 atmosphere pressure for 30 minutes. Then, the Petri dishes were moistened with distilled water in front of the flame of an alcohol lamp and, using a scalpel heated in the flame, the plant samples were cut into pieces of 5 × 5 mm in size

Vol. 3 No. 10 (2025)
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and 3-4 pieces were placed in each Petri dish and placed in a thermostat with a temperature of 25 ° C for incubation [1].

From the third day, we began to observe the development of pathogenic fungi. On the seventh day of observations, 2x2 blocks were cut from the colonies formed by them to isolate pure cultures of germinated fungi and inoculated onto solid agar medium, which was previously poured into test tubes at an angle. Then, the test tubes were stopped and placed in a thermostat at a temperature of 25 ° C, and after 7 days, they were re-inoculated into Petri dishes with potato dextrose agar (KDA) medium.

A total of 4 isolates were isolated from plant samples in the laboratory. Further studies were conducted on fungi incubated in a nutrient medium for 7 days. Preparations were prepared to study their morphological characteristics, observed under a microscope, and their species were determined.

The isolated fungal isolates were observed to form light-colored mycelium on KDA medium. When we prepared the preparation and observed it under a microscope, it was found that the pycnidia formed by the fungus were small black dots. The conidia were found to be straight, with rounded ends and 1 and 2 septa. (Figure 1).

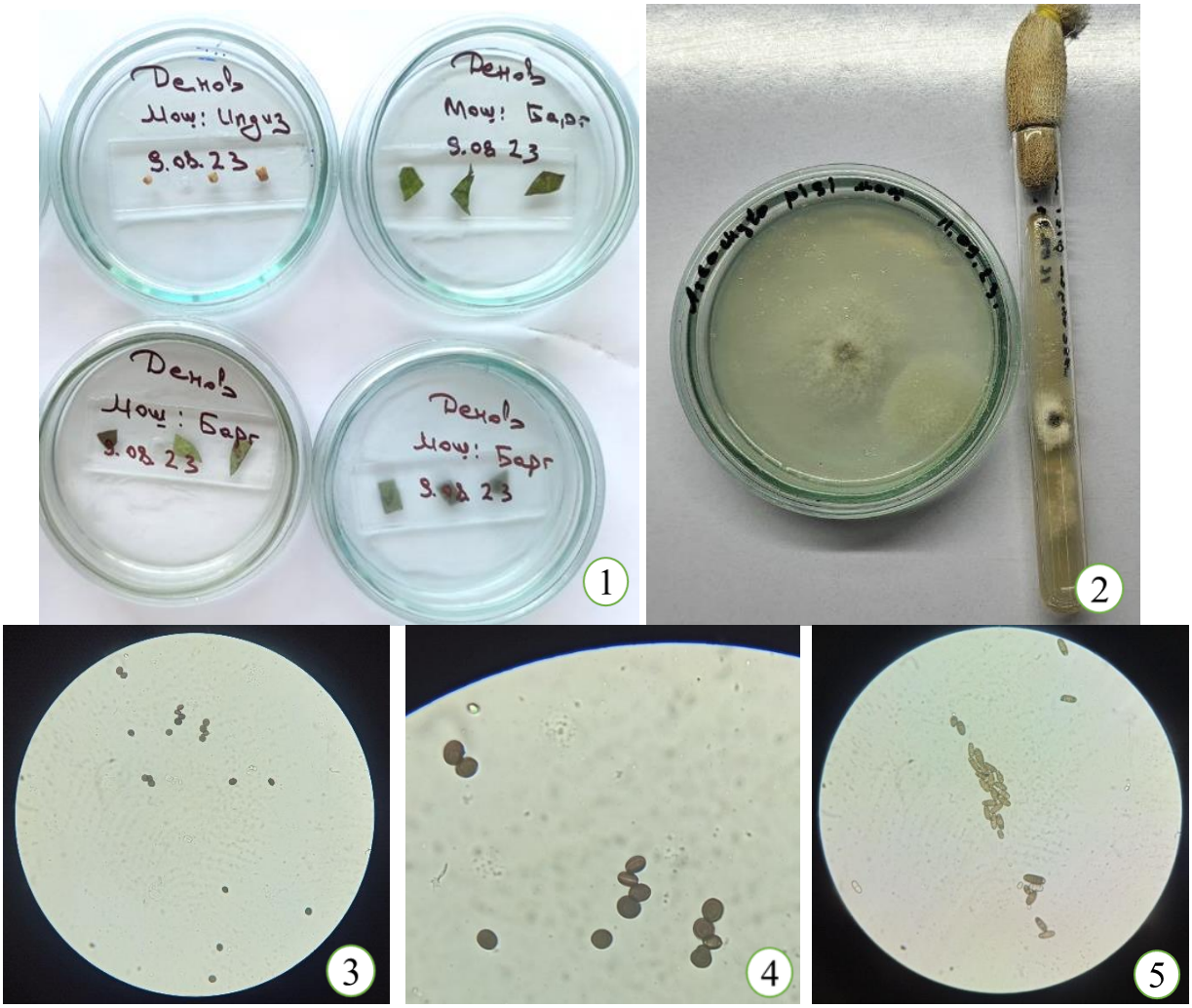

Figure 1. 1- Inoculated samples; 2- Colony formation of the fungus Ascochyta pisi; 3, 4- Pycnidia; 5- Conidia.

In in vitro experiments, we first studied the effect of temperature on the growth and growth rate of A. pisi . In this study, 5×5 mm discs of A. pisi isolates were cut and inoculated into Petri dishes containing KDA medium. The inoculated cultures were grown at different temperatures: 18ºC, 22ºC, 25ºC, 28ºC, 30ºC, and 40ºC for 9 days.

According to the results of the experiment, it turned out that the optimal temperature for the growth of A. pisi fungus is 22-25ºC. In particular, at a temperature of 22ºC, the diameter of the fungal colonies was 16.7 mm after 3 days, 26.30 mm after 5 days, 56.30 mm after 7 days, and 69.70 mm after 9 days. At a temperature of 25ºC, the colony diameter increased by 19.43 mm on the 3rd day, 31.83 mm on the 5th day, 60.30 mm on the 7th day, and 80.73 mm on the 9th day (Table 1).

Table 1 the effect of temperature on the growth of the fungus Ascochyta pisi

Temperature / Days	18 ºC	22 ºC	25 ºC	28 ºC	30 ºC	40 ºC
	Colony diameter on the day of counting,mm					
1	0,50	0,50	0,50	0,50	0,50	0,50
3	2,30	16,70	19,43	14,13	7,50	0,66
5	6,70	26,30	31,83	26,03	15,70	0,74
7	12,37	56,30	60,30	37,93	20,27	0,80
9	23,53	69,07	80,73	57,83	32,40	1,02

At 40°C, the growth of A. pisi was not observed, the colony diameter reached only 1.02 mm in 9 days. In addition, at 28°C, *A. pisi* developed slightly better, that is, on the 9th day of observation, the diameter of the fungal colonies was 57.83 mm.

When grown at 18 o C and 30 o C, the growth of fungal colonies was observed to be very slow. At 18 o C, it was 12.37 mm in 7 days and 23.53 mm in 9 days. At 30 o C, it was 20.27 mm in 7 days and 32.40 mm in 9 days. When calculating the radial growth rate of *A. pisi* , the highest radial growth was observed at 25 o C. At this temperature, the radial growth of colonies was observed at a rate of more than 0.186 mm/s (Figure 2).

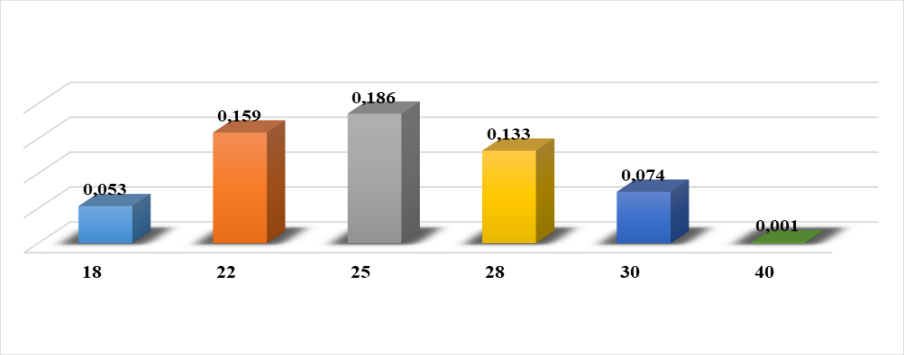

Figure 2. Effect of temperature on the radial growth rate of A. pisi fungus colonies, mm/s.

The lowest rate in the experiment was observed when incubated at 40ºC (0.001 mm/s). Average radial growth rates were observed at 22 and 28ºC. At 28 oC, colonies grew at 0.133 mm/h, and at 28ºC, they grew at 0.159 mm/h.

Conclusion. It was found that temperature significantly affects the growth of A. pisi fungus, and the most favorable temperature for the growth of fungal colonies was found to be 22-25ºC. At this temperature, the radial growth rate of colonies reached 0.159-0.186 mm/s. Based on the above data, taking into account air temperature in mung bean cultivation and disease control allows controlling ascochyta blight.

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Vol. 3 No. 10 (2025)
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