Türk Tarım ve Doğa Bilimleri Dergisi 11(4): 974–985, 2024

https://doi.org/10.30910/turkjans.1495070

TÜRK
TARIM ve DOĞA BİLİMLERİ
DERGİSİ

TTDB

TURKISH
JOURNAL of AGRICULTURAL
and NATURAL SCIENCES

www.dergipark.gov.tr/turkjans

Araştırma Makalesi

Determination of some Biological Parameters Providing the Basis for the Mass Production of the Phosphorus Solving Fungi *Talaromyces funiculosus**

Fatih ÖLMEZ[1*], Hiranur AKDAŞ[1]

[1]Department of Plant Protection, Faculty of Agricultural Sciences and Technologies, Sivas University of Science and Technology, Sivas, Türkiye

*Sorumlu Yazar: fatih.olmez@sivas.edu.tr

Geliş Tarihi: 03.06.2024 Düzeltme Geliş Tarihi: 04.09.2024 Kabul Tarihi: 05.09.2024

ABSTRACT

Phosphorus is one of the basic nutritional elements that plants need for development. During agricultural production, the phosphorus needs of plants are met with chemical fertilizers. A very significant part of the phosphorus given to the soil as chemical fertilizer binds to other soil elements such as iron, zinc and aluminum in the soil and turns into a form that plants cannot absorb. While this bound phosphorus can be partially dissolved in acidic soils, this dissolution is very slow in regions such as Turkey, where a significant part of the soil is basic. This situation leads to the need for phosphorus fertilizer supplements every year. Some fungi and bacteria help convert the bound phosphorus in the soil into something useful for plants with the chemicals they produce. Within the scope of this study, it was aimed to reveal some biological parameters that will form the basis for the mass production of a *Talaromyces funiculosus* (ST-976) isolate isolated from pistachio roots and known to have the ability to dissolve phosphorus bound in soil. For this purpose, the growth performances of the fungus at different temperatures and water activity values were evaluated. At the end of the experiments, it was determined that the temperature at which the fungus developed best was 25 °C and 99,5% and 99% water activity.

Key words: Fungi, *Talaromyces funiculosus* (ST-976), Phosphorus deficiency, Biofertilizers

Fosfor Çözücü Fungus Talaromyces funiculosus'un Kitlesel Üretimine Temel Teşkil Edecek Bazı Biyolojik Parametrelerin Belirlenmesi*

ÖZ

Fosfor bitkilerin gelişim için ihtiyaç duyduğu temel besin elementlerinden bir tanesidir. Tarımsal üretim sırasında, bitkilerin fosfor ihtiyacı kimyasal gübrelerle kaşılanmaktadır. Kimsayasal gübre olarak toprağa verilen fosforun çok önemli bir bölümü toprakta bulunan demir, çinko, alüminyum gibi diğer toprak elementlerine bağlanarak bitkilerin alamayacağı forma dönüşmektedir. Asidik topraklarda bu bağlı fosfor kısmen çözülebilmekte iken, Türkiye gibi topraklarının çok önemli bir bölümü bazik karakterde olan bölgelerde bu çözünme çok yavaş olmaktadır. Bu durum her yıl tekrar fosforlu gübre takviyesi gereksinimine yol açmaktadır. Bazı fungus ve bakteriler ürettikleri bazı kimysalallar ile toprakta bulunan bağlı fosforun bitkiler için faydalı hale dönüşmesine yardımcı olmaktadırlar. Bu çalışma kapsamında, antepfıstığı köklerinden izole edilen, toprakta bağlı fosforu çözme yeteneğine sahip olduğu bilinen bir *Talaromyces funiculosus* (ST-976) izolatının kitlesel üretimine temel teşkil edecek bazı biyolojik parametlerin ortaya konulması amaçlanmıştır. Bu amaçla fungusun farklı sıcaklık ve su aktivitesi değerlerinde gelişim performansları değerlendirilmiştir. Denemeler sonunda fungusun en iyi gelişim gösterdiği sıcaklık değerinin 25 °C ve %99 su aktivitesi değerleri olduğu belirlenmiştir.

Anahtar kelimeler: Fungus, *Talaromyces funiculosus* (ST-976), Fosfor eksikliği, Biyogübre.

INTRODUCTION

The world population is projected to reach about 10 billion and the demand for agricultural production is expected to increase (by about 50%) (FAO, 2023). Recently, about 5.2 billion hectares of arable land where agricultural production activities are carried out have been increasingly limited by factors such as salinization, drought, and conversion to tourism and residential areas. Due to the limiting factors of production areas and the rapid population growth in 2050, which is predicted to be over 2.4 billion, the principle of getting more product from the unit area has emerged (Baeshen, 2016; Basu et al., 2017; Meena et al., 2017; Anderson and Kim, 2018; Numan et al., 2018). In agricultural production, excessive artificial chemical fertilizers and pesticides are used to minimize losses with the principle of getting more yield and quality products from unit areas. The most important aspect of fertilizer use in high yield and quality is the use of the right product, at the right time, in the right environment and at the right rate (Borkar, 2015).

The application of the three main plant nutrients nitrogen, potassium and phosphorus fertilizers is important and vital for increasing and sustaining productivity in agricultural activities. The use of fertilizers for plant nutrition is as important as the use of seed as a production material to ensure efficient and quality production (Dwivedi et al., 2017). Since industrial substances consisting of nitrogen (N), phosphorus (P) and potassium (K) are chemically synthesized fertilizers, their continuous and excessive use directly or indirectly causes an imbalance in the natural ecosystem (Singh et al., 2019). The agricultural system has been facing major threats in recent years due to dependence on excessive use of agrochemicals, global climate change, population growth and increasing economic and environmental costs of non-renewable resources (Gosal et al., 2020). In addition, there are major problems such as pollution of soil, surface and groundwater due to the current agricultural practices used with the principle of higher productivity and increased use of chemical inputs. These chemical fertilizer and pesticide applications, which are used unconsciously and intensively, bring many health and environmental problems such as birth abnormalities, cancer and damage to the nervous system due to the disruption of the activity of microorganisms in the soil, the formation of resistance in disease and pest agents, eutrophication and nitrate accumulation in water, greenhouse gas effect and heavy metal accumulation, increase in toxic substances, drinking water, animal body, plant roots, air, soil and destruction of beneficial fauna (Sönmez et al., 2008; Tiryaki et al., 2010; Kotan, 2020).

For these and many other reasons and the sustainability and protection of the existing natural balance, there is a need to search for an effective alternative to chemical fertilizers in agricultural applications, including organic wastes and plant growth-promoting applications for purposes such as increasing productivity, improving the chemical and physical structure of soils, protecting human and environmental health, and supplementing the soil with ecological nutrients (Borkar, 2015). As an alternative to chemical fertilizers and pesticides in agriculture, it is of great importance to use beneficial microorganisms present in nature in natural balance as microbial fertilizers and biopesticides in agriculture and to bring them into agricultural activities. The demand and interest in biological control is increasing day by day due to both the residue risks posed by pesticides in terms of human and environmental health and the resistance problems that occur over time in pests and disease agents against chemical drugs (Kotan 2020).

Soil microorganisms, which exist in natural equilibrium and contain the structure of biological products as an alternative to overused chemicals in modern agricultural practices, play an important role in increasing the availability and access to nutrients through various mechanisms such as the conversion of plant residues into organic matter, solubilization of phosphate into a form that plants can take, mineralization and biological nitrogen fixation (Munns and Tester, 2008). Phosphorus (P) is an important macronutrient that is absolutely essential for plant growth/development. It is a limiting factor for yield and quality parameters in most plant species. Much of the soluble P used as artificial chemical fertilizers can be 'fixed' in the soil by reacting with other trace elements or converted into poorly soluble forms that are not available to the plant (Whitelaw, 1999).

The most widely used fertilizer is the product of acidification of rock phosphates with strong acids. This method also involves an expensive long process with high environmental damage (Vassilev et al., 2006). For all these reasons, it is of great importance to make the bound phosphorus in the soils of our country available for plants. The use of microorganisms that make bound phosphorus in soils useful for plants offers enormous potential for sustainable soils (Mendes et al., 2014). Although bacteria are also capable of solubilizing bound phosphate in soil, fungi are considered to have a higher potential in this regard (Sharma et al., 2013). To date, many phosphorus-solubilizing isolates belonging to the genera *Aspergillus* and *Penicillium* have been identified and it is stated that they are more useful than phosphorus solubilizing bacteria due to the high amount of organic acids they secrete and being less sensitive to subcultivation (Sahoo et al., 2014). There are also studies showing that *Talaromyces* species, which can solubilize phosphate bound in soil, stunt plant growth in some plants (Naraghi et al., 2012).

In this study, it was aimed to determine the maximum values of the development curves of the phosphate solubilizing microorganism *Talaromyces funiculosus* (ST-976) isolate as a microbial fertilizer at temperature and water activity values, which are biological parameters that will form the basis for mass production.

MATERIALS and METHODS

Material

The main material of the study consisted of 1 isolate of *Talaromyces funiculosus* (ST-976), which was isolated from the agricultural fields of Şanlıurfa province and microscopically and molecularly identified previously (Türkölmez, 2022). The study was carried out in the Phytopathology laboratory of the Department of Plant Protection, Faculty of Agricultural Sciences and Technology, Sivas Science and Technology University and the devices in the laboratory constituted the material of the study.

Methods

Adjusting Sports Concentration

A small piece of *T. funiculosus* ST-976 isolate maintained on slant agar medium was inoculated into PDA medium under sterile conditions and incubated at 25°C for 1 week to grow the fungus from the stock culture. After colony development, spore harvesting was performed from the fungal colony. For this purpose, 5 mL of sterile distilled water was added to the petri dish in a sterile cabinet. The density of the spore suspension obtained by scraping the spores on the surface of the colony into the water with the help of a sterile microscope was calculated under a microscope using a Thoma slide. The final spore concentration was adjusted to contain $1x10^6$ spores per mL.

Determination of the effects of different temperature and water activity levels on sporulation of ST-976 isolate

In order to plan the mass production of the fungus, it was aimed to determine at which temperature and water activity (aw) levels it sporulates at the maximum rate. The preparation of media with water activity levels required for maximum sporulation of the fungus was determined according to the methodology put forward by Pitt and Hocking (1977) and Gekas et al. (1998). Accordingly, the basal medium consisted of malt extract (1%), yeast extract (1%), dipotassium hydrogen phosphate (0.1%) and agar (2%) (Table 1).

Table 1. Composition of basal media used for water activity adjustment.

	x	2X
Malt extract	%1	%2
Yeast extract	%1	%2
Agar	%2	%4

250 mL medium (2X) + 250 mL NaCl (2x=1.8%) = 500 mL medium (x) + NaCl (x=0.9%)

Six different NaCl concentrations were used for the water activity experiment. Salt concentrations were adjusted to produce six different water activities (99.5%, 99%, 98%, 96%, 94%, 92%) (Table 2).

Table 2. Water activity NaCl % (Gekas et al., 1998).

Doz	Water activity/Aw (%)	x (NaCl %)	2x (NaCl %)
1	99,5	0,9	1,8
2	99	1,7	3,4
3	98	3,5	7
4	96	7,0	14
5	94	10,0	20
6	92	13,0	26

1.8% NaCl = 1.8 g NaCl in 100 mL distilled pure water.

The medium was prepared separately as a salt solution (2X) and agar-fixing medium (2X) and autoclaved (Table 3). Media were prepared at different aw levels using equal amounts of glucose and fructose according to the methods described by Pitt and Hocking (1977) and Gekas et al. (1998) (Figure 1).

Table 3. Preparation of medium and salt mixture ratio.

Water activity/Aw (%)	Medium (2X)	NaCl (2X)	Final NaCl concentration
99.5	100 mL	100 mL (2X=1,8)	0,9
99	100 mL	100 mL (2X=3,4)	1,7
98	100 mL	100 mL (2X=7)	3,5
96	100 mL	100 mL (2X=14)	7,0
94	100 mL	100 mL (2X=20)	10,0
92	100 mL	100 mL (2X=26)	13,0

5 different pH levels (4.5, 5.5, 6.5, 6.5, 7.0, 7.5) and 5 different phosphorus sources ($AlPO_4$, $CaHPO_4$, $Ca_3(PO)_4$, $FePO_4$, Phytin ($C_6H_6Ca_6O_24P_6$) were used to adjust the pH of the media to the optimum pH level of 6.5. After sterilization of the media and pouring it into petri dishes, inoculation of the fungal culture was carried out under aseptic conditions (Figure 1). The medium poured into the petri dishes was wrapped with autoclaved cloth to prevent rapid cooling of the medium and to prevent water activity change and water vapor formation, and the edges of the inoculated petri dishes were also covered with parafilm to prevent water loss. A concentration of 10 uL 10^7 spores/mL was placed in the center of each petri dish (105 spore concentration in total), care was taken to ensure that the drop did not slide on the petri dish and that the spores did not splash on the petri dishes photographed at 2-day intervals. Then the containers containing the petri dishes were placed in the incubator and left to incubate for 3 weeks. Fungal growth was monitored at 2-day intervals starting 3 days after sowing in order to obtain the growth curve of the fungus, and the images obtained were measured in cm^2 growth area with IMAGE J program and the data obtained were subjected to statistical analysis.

Figure 1. Preparation of nutrient media with different water activity to be tested in the study.

All experiments were run separately at 25 and 30 °C. Spores were then harvested from the petri dishes using sterile distilled water. A sterile brush was used to pass the spores into the water and the resulting suspension was filtered through sterile triple-layer cheesecloth to remove mycelial fragments. Conidial concentration was determined using a hemocytometer.

Data Analysis and Image Processing

All characters were studied in 5 replications according to the randomized blocks experimental design. In order to analyze the data obtained in the study, ANOVA test and LSD test were used to determine the difference between the groups in case of significant differences between the groups. Statistical analyses were performed using SPSS computer package program. The growth data (in cm^2) of fungi in nutrient media were obtained quickly and precisely, and the images were processed using Image J program after the photographs were taken (Mustafa et al., 2022).

RESULTS and DISCUSSION

Determination of the Effects of Different Temperature and Water Activity Levels on Sporulation of ST-976 Isolate

5 different pH levels (4.5, 5.5, 6.5, 6.5, 7.5, 7.0, 7.5) and 5 different phosphorus sources ($AlPO_4$, $CaHPO_4$, $Ca_3(PO)_4$, $FePO_4$, Phytin ($C_6H_6Ca_6O_24P_6$) at the appropriate pH level (pH 6.5) at different temperatures (25 and 30) and water activity values (99.5, 99, 99, 98, 96, 94 and 92%) (Figure 2).

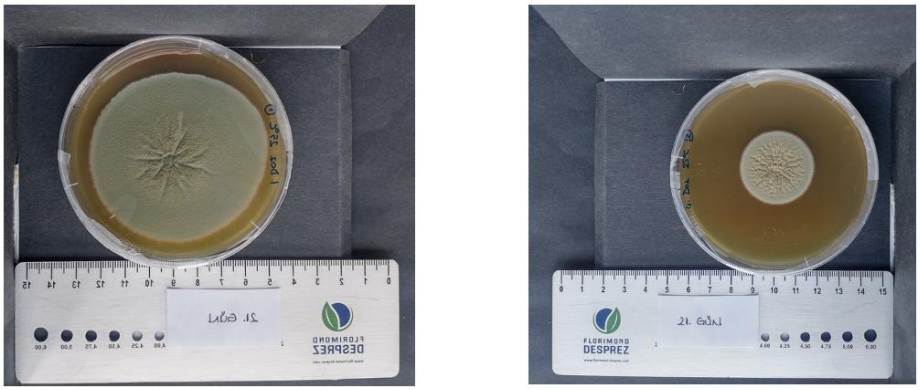

Figure 2. Growth of ST-976 at different water activities (From left to right: 99.5%, 96%).

After three weeks of growth in the incubator at 25 and 30 °C, colony measurements were terminated and spore harvesting was performed and spore counts were performed with the help of a hemocytometer (Table 4).

Table 4. Spore formation of ST-976 at different water activity values.

		ST-976 (average sport/petri)		Average	Average
Water activity	**Repeat**	**25 °C**	**30 °C**	**25 °C**	**30 °C**
99.5	1	185.000.000	72.782.000	587.437.200	127.957.400
99.5	2	987.500.000	148.388.000		
99.5	3	635.400.000	159.934.000		
99.5	4	367.780.000	112.981.000		
99.5	5	761.506.000	145.702.000		
99	1	697.200.000	107.089.000	559.982.800	97.505.200
99	2	822.457.000	43.880.000		
99	3	288.350.000	81.453.000		
99	4	480.507.000	163.214.000		
99	5	511.400.000	91.890.000		
98	1	55.312.000	23.821.000	56.021.900	31.613.200
98	2	83.647.000	28.778.000		
98	3	44.656.000	34.191.000		
98	4	64.435.000	32.677.000		
98	5	32.059.000	38.599.000		
96	1	81.915.000	3.787.000	75.312.000	3.163.000
96	2	91.274.000	2.132.000		
96	3	65.838.000	3.813.000		
96	4	41.812.000	4.042.000		
96	5	95.721.000	2.041.000		

When Table 4 was analyzed, it was revealed that the fungus did not grow at 92 and 94% water activity values, and the water activity value at which it first started to grow was 96%. The best sporulation was obtained at 99.5% and 99% water activity at 25 °C and 99.5% water activity at 30 °C, respectively. ANOVA analyses of the obtained data were carried out with the help of SPSS program. Differences between groups were tested by LSD test. The results are shown in Tables 5 and 6.

Table 5. Analysis of variance of spore count of ST-976 isolate after 21 days at different water activities

SVC	**DF**	**25 °C**		**30 °C**	
		MS	**F**	**MS**	**F**
Water activity (%)	3	4.31E+17	11.97	1.66E+16	20.87
Error	16	3.60E+16	-	7.95E+14	-
General total	19	-	-	-	-

** Significant at p<0.01 level; SVC: Sources of variation; DF: Degrees of freedom; MS: Mean squares; F: F value

As can be seen from Table 6, the highest spore production was obtained at 99.5% and 99% water activity values at 25°C and statistically these two water activity values were in the same group (a) at both temperatures. At the remaining water activity values (98 and 96%), relatively less spore production was realized and these groups were statistically in the same group (b). Again, in the study where the effects of temperature on spore production were tested, statistically 2 groups were formed (A and B), all growth values at 25 °C were in group A, while all growth values at 30 °C were in group B. The results show that temperature is a very important factor in the spore formation of the fungus.

Table 6. Total number of spores of ST-976 isolate on petridia after 21 days

Water Activity (%)	25 °C	30 °C
99.5	587.437.200±317.354.000 aA	127.957.400±35.437.000 aB
99	559.982.800±206.265.000 aA	97.505.200±43.515.000 aB
98	56.021.900±19.607.000 bA	31.613.200±5.597.000 bB
96	75.312.000±21.948.000 bA	3.163.000±98.8000 bB

* Lowercase letters following the means in the same column indicate that different water activity means are statistically significantly different (Anova p<0.01, Tukey test), ** Uppercase letters following the means in the same column indicate that different temperature means are statistically significantly different (Anova p<0.01, Tukey test).

When the correlation relationship between water activity and temperature in terms of spore development cm2 area was examined, 99.5% water activity at 30 °C for all days and 25 °C for day 3; 99% water activity at 30 °C for days 3, 7, 11 and 13 were the best-developing parameters in the group a. No spore development was observed at 92% and 94% water activity and at all temperatures. Among the parameters, the weakest group in terms of spore development and spore development in cm^2 area was g, but it was 17, 19 and 21 days at 30 and 20 °C at 96% water activity (Tables 7, 8).

Table 7. VK, SD, KO and F value of the total number of spores on the petridia of ST-976 isolate after 21 days

SVC	DF		3		5		7		9			11
			MS	F	MS	F	MS	F	MS	F	MS	F
Water Activity and Temperature	8		0.083021	69.05	0.540963	116.62	1.23395	167.84	1.6267	111.93	2.04474	88.07
Error	21		0.001202		0.004639		0.00735		0.01453		0.02322	
General Total	29											
SVC	DF		13		15		17		19			21
			MS	F	MS	F	MS	F	MS	F	MS	F
Water Activity and Temperature	8		2.42242	70.46	3.52194	88.11	3.65963	85.75	3.75219	72.72	3.70598	73.32
Error	21		0.03438		0.03997		0.04268		0.0516		0.05054	
General Total	29											

* Significant at p<0.01 level; SVC: Sources of Variation; DF: Degrees of Freedom; MS: Mean squares; F: F value

Table 8. Water activity and temperature correlation value of the total number of spores on the petridia of ST-976 isolate after 21 days

Water Activity (%)	Temperature	3rd Day	5th Day	7th Day	9th Day	11th Day	13th Day	15th Day	17th Day	19th Day	21th Day
99.5	20	0.420±0.007c	0.816±0.028d	1.178±0.041c	1.426±0.107d	1.688±0.184c	1.883±0.227d	2.035±0.306d	2.178±0.311de	2.313±0.338de	2.448±0.306de
99.5	25	0.700±0.039a	1.267±0.053b	1.688±0.086b	2.092±0.084bc	2.522±0.076ab	2.940±0.114ab	3.121±0.141ab	3.345±0.119ab	3.473±0.140ab	3.547±0.092ab
99.5	30	0.757±0.026a	1.510±0.061a	2.077±0.077a	2.474±0.145a	2.894±0.255a	3.238±0.238a	3.498±0.207a	3.612±0.211a	3.773±0.111a	3.855±0.061a
99	20	0.427±0.063c	0.760±0.080d	1.135±0.080c	1.356±0.081de	1.592±0.126c	1.802±0.233d	1.922±0.310d	1.997±0.339ef	2.076±0.421ef	2.181±0.445ef
99	25	0.587±0.034b	1.205±0.034bc	1.602±0.003b	2.013±0.056bc	2.457±0.059ab	2.867±0.036abc	3.145±0.049ab	3.342±0.051ab	3.489±0.081ab	3.578±0.067ab
99	30	0.689±0.014a	1.371±0.037ab	1.943±0.178a	2.287±0.314ab	2.736±0.387a	3.047±0.463a	3.207±0.484ab	3.319±0.442ab	3.405±0.441abc	3.484±0.357abc
98	20	0.295±0.043d	0.452±0.016e	0.779±0.032d	1.085±0.028e	1.349±0.062cd	1.584±0.102de	1.734±0.028de	1.873±0.112ef	1.926±0.128efg	2.038±0.115efg
98	25	0.476±0.015c	1.050±0.009c	1.472±0.069b	1.823±0.007c	2.191±0.076b	2.500±0.090bc	2.673±0.117bc	2.821±0.139bc	2.977±0.100bc	3.038±0.112bcd
98	30	0.445±0.008c	1.041±0.161c	1.580±0.161b	1.884±0.185c	2.220±0.125b	2.374±0.132c	2.563±0.141c	2.671±0.187cd	2.848±0.255cd	2.916±0.323cd
96	20		0.304±0.024e	0.354±0.016e	0.419±0.015f	0.627±0.034e	0.828±0.062f	1.038±0.053f	1.204±0.064g	1.367±0.126g	1.436±0.147g
96	25		0.375±0.019e	0.508±0.033e	0.721±0.025f	0.988±0.031de	1.142±0.025ef	1.304±0.043ef	1.452±0.055fg	1.595±0.065fg	1.684±0.093fg
96	30			0.291±0.043e	0.456±0.022f	0.669±0.026e	0.841±0.018f	1.009±0.056f	1.128±0.079g	1.354±0.042g	1.516±0.059g

*Letters following the means in the same column indicate that the means are statistically significantly different (Anova p<0.01, Tukey test)

Within the genus, *T. funiculosus* is one of the less studied species (Kanse et al., 2015). Kanse et al. (2015) described that *T. funiculosus* is able to dissolve phosphate in soil and ameliorate saline soil and is also an agricultural pathogenic fungus that can cause peach pit rot (Mukhtar et al., 2019). *Talaromyces* species grow in a restricted manner, especially in environments with low water activity, and have an extrolyte model quite different from that of *Penicillium* (Houbraken et al., 2014; Yılmaz et al., 2014). According to the results of our study, *T. funiculosus* ST-976 showed better spore production development at 99.5 and 99, which are high water activity ratios. At 98 and 96, relatively little spore production was realized and at 92 and 94, which have low water activity, no spore production development was observed. 5 different pH levels (4.5, 5.5, 6.5, 6.5, 7.0, 7.5) and 5 different phosphorus sources ($AlPO_4$, $CaHPO_4$, $Ca_3(PO)_4$, $FePO_4$, Phytin ($C_6H_6Ca_6O_24P_6$) and pH 6.5. pH is one of the important and determining factors for the metabolism and production of enzymes and thus for the biosynthesis of antimicrobials. Favorable pH has been reported to encourage the fungus to produce antimicrobials (Jain and Pundir, 2011). Merlin et al. (2013) reported that the best pH value for growth and antimicrobial production for the fungus Fusarium solani is pH 6.

Within the scope of the study, the highest spore production was obtained at 99.5% and 99% water activity values at 25 ^oC and statistically all growth values at 25 ^oC were in group A, while all growth values at 30 ^oC were in group B at these two temperature values. The results showed that temperature is a very important factor in the spore formation of the fungus. Sun et al. (2020) used CYA supplemented with 5% NaCl (CYAS), Czapek yeast autolysate agar (CYA), yeast extract sucrose agar (YES), dichloran 18% glycerol agar (DG18), creatine sucrose agar (CREA), oatmeal agar (OA) and malt extract agar (MEA; Oxoid malt) were incubated for 7 days at 25 ^oC, which is the appropriate growth temperature. Temperature directly affects the growth and overall metabolism of the microorganism through its effect on enzymes and protein production and thus the production of antimicrobials, and each microorganism has an optimum temperature at which it achieves the best growth for its growth and the best productivity for all primary and secondary metabolic products (Pereira et al., 2013).

Kanse et al. (2015), in conducted a study on phosphate solubilization of the stress tolerant soil fungus *T. funiculosus* SLS8 isolated from the rhizosphere of a Neem plant. In this study, they used Pikovskaya liquid medium (PLM) containing various nitrogen sources (casein, ammonium sulfate, urea, sodium nitrate or potassium nitrate) and carbon sources (fructose, glucose, sucrose or galactose) to determine the phosphate solubilizing activity of *T. funiculosus* SLS8. After 5 days of incubation, the highest soluble phosphate concentration (187 mg P L^{-1}) was reached in PLM containing glucose and ammonium sulfate. The pH of the PLM culture decreased from 6.5 to 4.2. The pH decrease was caused by organic acid production as determined by HPLC. pH was found to be highly negatively correlated with the amount of phosphate solubilized (r=-0.96) and fungal hyphae produced H+ and organic acids, causing a decrease in pH during phosphate solubilization (Jacobs et al., 2002). The pH was lower in PLM with ammonium due to possible H+ efflux from fungal hyphae during ammonium uptake (Roos and Luckner, 1984). Ammonium has been reported by many researchers as the best nitrogen source in phosphate solubility (Pradhan and Sukla, 2005; Matias et al., 2009; Srividya et al, 2009), but in some mediums nitrate caused the highest phosphate solubility, e.g. *Aspergillus niger, A. tubingensis* and *Penicillium rugulosum* (Isbelia et al., 1999; Seshadri et al., 2004; Relwani et al., 2008). Increasing salinity did not affect phosphate solubility. The maximum tolerance value against systemic fungicides carbendazim, mancozeb and hexaconazole was determined as 12.5 µg mL^{-1}, 2,000 µg mL^{-1} and 250 µg mL^{-1}, respectively, and decreased phosphate solubility by 55%, 37% and 30% at these concentrations, respectively. As a result, *T. funiculosus* SLS8 can be used as a candidate biofertilizer to maintain available phosphate levels in environmentally stressed soils such as saline agricultural soils that have been impacted by systemic fungicide or insecticide treatment and systemic pesticide seed application (Kanse et al., 2015).

T. funiculosus SLS8 maximized phosphate release when glucose was used as the sole carbon source. *Penicillium* spp. prefer glucose for the solubilization of calcium phosphates (Scervino et al., 2011; Yadav et al., 2011), but *P. rugulosum* prefers sucrose (Isbelia et al., 1999). *Aspergillus* spp. showed the highest phosphate release with glucose, arabinose, maltose and mannitol (Narsian and Patel, 2000; Seshadri et al., 2004; Pradhan and Sukla, 2005; Barroso et al., 2006; Srividya et al., 2009; Jain et al., 2012;). *T. funiculosus* SLS8 produces lactic acid and tartaric acid while solubilizing $Ca_3(PO_4)_2$. Similarly, *Aspergillus* sp. produce numerous tartaric acid (Gaur, 1990; Singal et al., 1994). *T. flavus* and *Penicillium janthinellum* produce lactic acid (Scervino et al., 2010).

Penicillium or *Aspergillus* species also produce various other organic acids during phosphate solubilization. *P. purpurogenum* and *P. radicum* produce gluconic acid during P solubilization (Scervino et al., 2010), but Mendes et al. (2013) say that gluconic acid contributes little to P solubilization. An increase in salinity (from 1% to 3% w/v NaCl) that does not reduce phosphate solubility of *T. funiculosus* SLS8 suggests that it can maintain phosphate levels in saline soils.

Khan et al. (2011) found that *T. funiculosus* LHL06 did not affect soybean growth from salinity stress. *T. funiculosus* SLS8 isolate maintained phosphate solubilization activity even at high levels of fungicides present in soil from agricultural fields. This suggests that the fungus can maintain existing phosphate levels in agricultural soils affected by systemic fungicides. Although experiments in liquid media are widely used to determine phosphate solubility by microorganisms in soil, they cannot directly determine phosphate solubility in soil. Liquid media experiments utilize sorption/desorption processes of P and interactions with other microbes in the soil, such as competition or facilitation. They also use high concentrations of C and N (Wang et al., 2012). Furthermore, plants inoculated with a mixed concentrate inoculum containing free-living phosphate solubilizers and mycorrhizal fungi benefited more from rock phosphate or soil P than plants inoculated with only one of the microorganisms. (Osorio and Habte, 2001; Zaidi and Khan, 2007; Matias et al., 2009). The success of these synergistic interactions depends on the efficiency of mycorrhizal fungi to take up P in solution and transport it to the roots. This prevents phosphate released by isolates of free-living phosphate-solubilizing fungi from being remobilized by the soil (Osorio and Habte, 2001). Therefore, according to

Türkölmez et al. (2022), isolated *T. funiculosus* ST-976 from pistachio (*Pistacia vera* L.) rhizosphere. They investigated the effect of ST-976 on phosphorus solubility in soils with different physicochemical properties. Seventy-eight *Talaromyces* isolates were obtained from pistachio rhizosphere heavily infested with *Neoscytalidium* spp. The phosphorus solubilization capacity of ST-976 isolate was tested on six different soil textur collected from different parts of Şanlıurfa province. The pH values of the soil samples ranged from 7.21 to 7.88. The analysis of ST-976 isolate applied to different clay and clay-loam soil structures showed that it solubilized 109-311% more phosphorus than the control sample. In the study, isolate ST-976 can solubilize phosphorus without adding any additives to the soil solution.

CONCLUSION and RECOMMENDATIONS

Talaromyces funiculosus ST-976 showed the highest spore production at 99.5% and 99% water activity values at 25°C and statistically these two water activity values were in the same group (a) at both temperature values. At the remaining water activity values (98 and 96%), relatively less spore production was realized and these groups were statistically in the same group (b). At 98 and 96% water activity ratio, relatively little spore production was realized and spore production did not develop at low water activity ratios 92 and 94. Again, in the study where the effects of temperature on spore production were tested, statistically 2 groups were formed (A and B), all development values at 25 °C were in group A, while all development values at 30 °C were in group B. As a result, it shows that temperature is a very important factor in spore formation of the fungus.

Phosphorus, one of the macronutrients essential for plant growth and development, is usually bound to other elements in the soil and plants cannot utilize this bound phosphorus. The low amount of soluble phosphate necessitates that the phosphorus needed by plants should be supplied to the soil regularly every year with chemical fertilizers. However, phosphate mineral resources are very limited in our country and Turkey is dependent on foreign sources for phosphate. The increasing use of chemical fertilizers brings with it negative environmental impacts such as pollution of soil and drinking water. At this rate, the world's phosphate resources are projected to be completely depleted in the next 50-100 years (Heppell et al., 2016). The decline in phosphorus resources makes it difficult to supply phosphorus in chemical fertilizers. Turkey has imported an average of 80-100 million dollars of phosphate in the last five years and a large part of it is used in chemical fertilizer production (Anonymous, 2023). For plants to utilize phosphorus, phosphorus in inorganic and organic phosphorus compounds must be broken down into phosphate anions. Some fungi can transform the phosphorus bound in the soil into a form that can be taken up by plants with some organic acids they secrete. The use of such microorganisms alone or in combination with existing organomineral fertilizers not only reduces the need for phosphorus in fertilizers but also allows the use of less phosphorus fertilizers by making the unusable bound

phosphorus in the soil useful for plants. *T. funiculosus* ST-976 single spore isolate isolated from pistachio production areas was found to solubilize bound phosphorus in soil.

When the examples of the use of beneficial fungi as microbial fertilizers are examined, it is seen that studies on mycorrhizal fungi are mostly found in the world. When it comes to the mass production of these fungi, the realization of a standard production at large scales requires the optimization of highly sensitive processes. In this study, it was aimed to determine the maximum values of the development curves at temperature and water activity values, which are the biological parameters that will form the basis for the mass production of *T. funiculosus* ST-976 isolate, a promising biologically phosphorus-degrading microorganism that is environmentally friendly and does not threaten the health of humans and other living things.

Authors contribution statement
All authors contributed equally to this work. The manuscript was reviewed by all authors.

Ethical approval
Not applicable.

Declaration of competing interest
The authors report no declarations of interest.

Acknowledgments
This work has been funded by TAGEM AR&GE project TAGEM/20/AR-GE/27, granted by GDAR (General Directorate of Agricultural Research and Policies, Türkiye Ministry of Agriculture and Foresty) and HEKTAŞ T.A.Ş. We would like to express our special thanks to Mr. Şahimerdan TÜRKÖLMEZ who provided the *Talaromyces funiculosus* (ST-976) isolate used in this study.

AUTHOR ORCHID NUMBERS

Fatih ÖLMEZ https://orcid.org/0000-0001-7016-2708
Hiranur AKDAŞ https://orcid.org/0009-0007-6008-2270

REFERENCES

Anderson, A. J., & Kim, Y. C. (2018). Biopesticides produced by plant-probiotic Pseudomonas chlororaphis isolates. Crop Protection, 105, 62-69.

Baeshen, A.A. (2016). "Use of Pseudomonas aeruginosa as Fertilizer in Eruca sativa, International Journal of Current Microbiology and Applied Sciences, 5(10): 301-312 pp.

Barroso, C. B., Pereira, G. T., & Nahas, E. (2006). Solubilization of $CaHPO_4$ and $AlPO_4$ by *Aspergillus niger* in culture media with different carbon and nitrogen sources. Brazilian Journal of Microbiology, 37, 434-438.

Basu, S., Rabara, R., & Negi, S. (2017). Towards a better greener future-an alternative strategy using biofertilizers. I: Plant growth promoting bacteria. Plant Gene, 12, 43-49.

Borkar, S. G. (2015). Microbes as bio-fertilizers and their production technology. Woodhead Publishing India Pvt, Ltd., 218p.

Dwivedi, B. S., Singh, V. K., & Meena, M. C. (2017). Efficient nitrogen management under predominant cropping systems of India. In The Indian Nitrogen Assessment (pp. 95-115). Elsevier.

Food and Agriculture Organization of the United States (FAO), www.faostat.org (04.10.2023).

Gaur, A. C. (1990). Phosphate solubilizing micro-organisms as biofertilizer. Omega scientific publishers.

Gekas, V., Gonzalez, C., Sereno, A., Chiralt, A., & Fito, P. (1998). Mass transfer properties of osmotic solutions. I. Water activity and osmotic pressure. International Journal of Food Properties, 1(2), 95-112. http://dx.doi.org/10.1080/10942919809524570

Gosal, S. K., Kaur, J., & Kaur, J. (2020). Microbial biotechnology: a key to sustainable agriculture. Phyto-Microbiome in Stress Regulation, 219-243.

Houbraken, J., de Vries, R. P., & Samson, R. A. (2014). Modern taxonomy of biotechnologically important *Aspergillus* and *Penicillium* species. Advances in applied microbiology, 86, 199-249.

Isbelia, R., Louis, B., Simard, R.R., Phillipe, T., Hani, A. (1999). Characteristics of phosphate solubilization by an isolate of a tropical *Penicillium rugulosum* and two UV-induced mutants. FEMS Microbiology Ecology, 28(3), 291-295. https://doi.org/10.1016/S0168-6496(98)00118-4

Jacobs, H., Boswell, G. P., Ritz, K., Davidson, F. A., & Gadd, G. M. (2002). Solubilization of calcium phosphate as a consequence of carbon translocation by *Rhizoctonia solani*. FEMS Microbiology Ecology, 40(1), 65-71.

Jain, P., & Pundir, R. K. (2011). Effect of fermentation medium, pH and temperature variations on antibacterial soil fungal metabolite production. J Agric Technol, 7(2), 247-269.

Jain, R., Saxena, J., & Sharma, V. (2012). Solubilization of inorganic phosphates by *Aspergillus awamori* S19 isolated from rhizosphere soil of a semi-arid region. Annals of microbiology, 62, 725-735.

Kanse, O. S., Whitelaw-Weckert, M., Kadam, T. A., & Bhosale, H. J. (2015). Phosphate solubilization by stress-tolerant soil fungus *Talaromyces funiculosus* SLS8 isolated from the Neem rhizosphere. Annals of Microbiology, 65(1), 85-93. https:// doi.org/10.1007/s13213-014-0839-6.

Khan, A. L., Hamayun, M., Kim, Y. H., Kang, S. M., & Lee, I. J. (2011). Ameliorative symbiosis of endophyte (*Penicillium funiculosum* LHL06) under salt stress elevated plant growth of Glycine max L. Plant Physiology and Biochemistry, 49(8), 852-861.

Kotan, R. (2020). Tarımda Biyolojik Çözümler. Harman Yayıncılık, İstanbul, ISBN: 978-605-68060-4-9. Haziran 2020. s.158.

Matias, S. R., Pagano, M. C., Muzzi, F. C., Oliveira, C. A., Carneiro, A. A., Horta, S. N., & Scotti, M. R. (2009). Effect of rhizobia, mycorrhizal fungi and phosphate-solubilizing microorganisms in the rhizosphere of native plants used to recover an iron ore area in Brazil. European Journal of Soil Biology, 45(3), 259-266.

Matias, S. R., Pagano, M. C., Muzzi, F. C., Oliveira, C. A., Carneiro, A. A., Horta, S. N., & Scotti, M. R. (2009). Effect of rhizobia, mycorrhizal fungi and phosphate-solubilizing microorganisms in the rhizosphere of native plants used to recover an iron ore area in Brazil. European Journal of Soil Biology, 45(3), 259-266.

Meena, V. S., Meena, S. K., Verma, J. P., Kumar, A., Aeron, A., Mishra, P. K., Bisht, J. K., Pattanayak, A., Naveed, M., & Dotaniya, M. L. (2017). Plant beneficial rhizospheric microorganism (PBRM) strategies to improve nutrients use efficiency: a review. Ecological Engineering, 107, 8-32.

Mendes, G.D.O., Freitas, A.L.M., Pereira, O.L., Silva, I.R., Vassilev, N.B., & Costa, M.D. (2014). Mechanisms of phosphate solubilization by fungal isolates when exposed to different P sources. AnnMicrobiol., 64(1): 239–249. https://doi.org/10.1007/s13213-013-0656-3

Merlin, J. N., Christhudas, I. V. S. N., Kumar, P. P., & Agastian, P. (2013). Optimization of growth and bioactive metabolite production: *Fusarium solani*. Asian J Pharm Clin Res, 6(3), 98-103.

Mukhtar, I., Quan, X., Chou, T., Huang, Q., Yan, J., Chen, B., ... & Xie, B. (2019). First report of *Talaromyces funiculosus* causing fruit core rot of peach (*Prunus persica*) in China. Plant disease, 103(8), 2124. https://doi.org/10.1094/PDIS-11-18-2050-PDN.

Munns, R., & Tester, M. (2008). Mechanisms of salinity tolerance. Annu. Rev. Plant Biol., 59, 651-681.

Mustafa, Z., Akkaya, M., & Olmez, F. (2022). Use of ImageJ Image Processing Software in Fungal Growth Tracking. IV. Balkan Agricultural Congress, 31 August – 02 September 2022, Edirne, Turkey.

Naraghi, L., Heydari, A., Rezaee, S., & Razavi, M. (2012). Biocontrol agent *Talaromyces flavus* stimulates the growth of cotton and potato. Journal of Plant Growth Regulation, 31, 471-477.

Narsian, V., & Patel, H. H. (2000). *Aspergillus aculeatus* as a rock phosphate solubilizer. Soil Biology and Biochemistry, 32(4), 559-565.

Numan, M., Bashir, S., Khan, Y., Mumtaz, R., Shinwari, Z. K., Khan, A. L., ... & Ahmed, A. H. (2018). Plant growth promoting bacteria as an alternative strategy for salt tolerance in plants: a review. Microbiological research, 209, 21-32.

Osorio, N. W., & Habte, M. (2001). Synergistic influence of an arbuscular mycorrhizal fungus and a P solubilizing fungus on growth and P uptake of *Leucaena leucocephala* in an Oxisol. Arid Land Research and Management, 15(3), 263-274.

Pereira, E., Santos, A., Reis, F., Tavares, R. M., Baptista, P., Lino-Neto, T., & Almeida-Aguiar, C. (2013). A new effective assay to detect antimicrobial activity of filamentous fungi. Microbiological research, 168(1), 1-5.

Pitt, J. I., & Hocking, A. D. (1977). Influence of solute and hydrogen ion concentration on the water relations of some xerophilic fungi. Microbiology, 101(1), 35-40.

Pradhan, N., & Sukla, L. B. (2006). Solubilization of inorganic phosphates by fungi isolated from agriculture soil. African Journal of Biotechnology, 5(10).

Relwani, L., Krishna, P., & Sudhakara Reddy, M. (2008). Effect of carbon and nitrogen sources on phosphate solubilization by a wild-type strain and UV-induced mutants of Aspergillus tubingensis. Current microbiology, 57, 401-406.

Roos, W., & Luckner, M. (1984). Relationships between proton extrusion and fluxes of ammonium ions and organic acids in *Penicillium cyclopium*. Microbiology, 130(4), 1007-1014.

Sahoo, H. R., & Gupta, N. (2014). Phosphate-solubilizing fungi: impact on growth and development of economically important plants. Phosphate Solubilizing Microorganisms: Principles and Application of Microphos Technology, 87-111.

Scervino, J. M., Mesa, M. P., Della Mónica, I., Recchi, M., Sarmiento Moreno, N., & Godeas, A. (2010). Soil fungal isolates produce different organic acid patterns involved in phosphate salts solubilization. Biology and fertility of soils, 46, 755-763.

Scervino, J. M., Papinutti, V. L., Godoy, M. S., Rodriguez, M. A., Della Monica, I., Recchi, M., ... & Godeas, A. M. (2011). Medium pH, carbon and nitrogen concentrations modulate the phosphate solubilization efficiency of *Penicillium purpurogenum* through organic acid production. Journal of applied microbiology, 110(5), 1215-1223.

Seshadri, S., Ignacimuthu, S., & Lakshminarasimhan, C. (2004). Effect of nitrogen and carbon sources on the inorganic phosphate solubilization by different *Aspergillus niger* strains. Chemical Engineering Communications, 191(8), 1043-1052.

Seshadri, S., Ignacimuthu, S., & Lakshminarasimhan, C. (2004). Effect of nitrogen and carbon sources on the inorganic phosphate solubilization by different Aspergillus niger strains. Chemical Engineering Communications, 191(8), 1043-1052.

Sharma, S. B., Sayyed, R. Z., Trivedi, M. H., & Gobi, T. A. (2013). Phosphate solubilizing microbes: sustainable approach for managing phosphorus deficiency in agricultural soils. SpringerPlus, 2, 1-14. https://doi.org/10.1186/2193-1801-2-587 PMID: 25674415

Singal, R., Gupta, R., & Saxena, R. K. (1994). Rock phosphate solubilization under alkaline conditions by *Aspergillus japonicus* and A. foetidus. Folia microbiologica, 39, 33-36.

Singh, D. P., Prabha, R., & Gupta, V. K. (2019). Microbial inoculants for sustainable crop management. Microbial Interventions in Agriculture and Environment: Volume 2: Rhizosphere, Microbiome and Agro-ecology, 1-35.

Sönmez, İ., Kaplan, M., & Sönmez, S. (2008). Kimyasal gübrelerin çevre kirliliği üzerine etkileri ve çözüm önerileri. Derim, 25(2), 24-34.

Srividya, S., Soumya, S., & Pooja, K. (2009). Influence of environmental factors and salinity on phosphate solubilization by a newly isolated *Aspergillus niger* F7 from agricultural soil. African Journal of Biotechnology, 8(9).

Sun, B. D., Chen, A. J., Houbraken, J., Frisvad, J. C., Wu, W. P., Wei, H. L., ... & Samson, R. A. (2020). New section and species in *Talaromyces*. MycoKeys, 68, 75–113.

Tiryaki, O., Canhilal, R., & Horuz, S., 2010. Tarım ilaçları kullanımı ve riskleri. Erciyes üniversitesi fen bilimleri enstitüsü fen bilimleri dergisi, 26(2), 154-169.

Türkölmez, Ş., Abdullah, E. R. E. N., Göksel, Ö. Z. E. R., & Derviş, S. (2022). The effect of *Talaromyces funiculosus* ST976 isolated from pistacia vera rhizosphere on phosphorus solubility in soil samples with different physicochemical properties. Kahramanmaraş Sütçü İmam Üniversitesi Tarım ve Doğa Dergisi, 25(5), 1077-1085.

Vassilev, N., Vassileva, M., & Nikolaeva, I. (2006). Simultaneous P-solubilizing and biocontrol activity of microorganisms: potentials and future trends. Applied microbiology and biotechnology, 71, 137-144.

Wang, M., Li, Z., Fang, X., Wang, L., & Qu, Y. (2012). Cellulolytic enzyme production and enzymatic hydrolysis for second-generation bioethanol production. Biotechnology in China III: biofuels and bioenergy, 1-24. https://doi.org/10.1007/10_2011_131.

Whitelaw, M. A. (1999). Growth promotion of plants inoculated with phosphate-solubilizing fungi. Advances in agronomy, 69, 99-151.

Whitelaw, M. A., Harden, T. J., & Helyar, K. R. (1999). Phosphate solubilisation in solution culture by the soil fungus *Penicillium radicum*. Soil biology and biochemistry, 31(5), 655-665.

Yadav, J., Verma, J. P., & Tiwari, K. N. (2011). Solubilization of tricalcium phosphate by fungus *Aspergillus niger* at different carbon source and salinity. Trends in Applied Sciences Research, 6(6), 606.

Yilmaz, N., Visagie, C. M., Houbraken, J., Frisvad, J. C., & Samson, R. A. (2014). Polyphasic taxonomy of the genus *Talaromyces*. Studies in Mycology, 78, 175-341. https://doi.org/10.1016/j. simyco.2014.08.001

Zaidi, A., & Khan, M. S. (2007). Stimulatory effects of dual inoculation with phosphate solubilising microorganisms and arbuscular mycorrhizal fungus on chickpea. Australian Journal of Experimental Agriculture, 47(8), 1016-1022.