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Potential of phosphate solubilizing fungi isolated from peat soils as inoculant biofertilizer

DENI ELFIATI[1,♥], DELVIAN[1], HAMIDAH HANUM[2], ARIDA SUSILOWATI[1], HENTI HENDALASTUTI RACHMAT[3]

[1]Faculty of Forestry, Universitas Sumatera Utara, Jl. Tridharma Ujung No. 1. Kampus USU, Medan 20155, North Sumatra, Indonesia
Tel.: +62-61-8220605, ♥email: denielfiati@yahoo.com
[2]Faculty of Agriculture, Universitas Sumatera Utara. Jl. Prof. A. Syofyan No. 3, Kampus USU, Medan 20155, North Sumatra, Indonesia
[3]Forest Research and Development Agency, Ministry of Environment and Forestry. Jl. Raya Gunung Batu, Pasir Jaya, Bogor 16118, West Java, Indonesia

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Abstract. *Elfiati D, Delvian, Hanum H, Susilowati A, Rachmat HH. 2021. Potential of phosphate solubilizing fungi isolated from peat soils as inoculant biofertilizer. Biodiversitas 22: 3042-3048.* Phosphate-solubilizing fungi are the microbes that have the ability to dissolve insoluble phosphate and made it available for plants. Therefore, the purpose of this study was to obtain the phosphate-solubilizing fungi from peat soils. Peat soil samples were taken in a composite at a depth 0-20 cm from the peat ecosystem in Nagasaribu Village, Lintong Nihuta Sub-district, Humbang Hasundutan District, North Sumatra, Indonesia. Soil samples were isolated to obtain phosphate-solubilizing fungi using the Pikovskaya selective medium. The obtained isolates were tested for their ability to dissolve phosphate qualitatively by calculating the dissolution index values and quantitatively by calculating the available phosphorus on Pikovskaya medium by using four phosphate sources, namely $Ca_3(PO_4)_2$, $AlPO_4$, $FePO_4$, and phosphate rock. Total of 12 isolates of phosphate-solubilizing fungi was obtained during the present study. Based on the results of qualitative and quantitative testing, all 12 isolates have the ability to release phosphate from the four tasted phosphate sources. The qualitative test obtains dissolution index values that vary from 2.55 to 4.25, while quantitatively, the isolates were able to dissolve phosphate in the value range from 17.77 ppm to 69.86 ppm. The top five fungal isolates with highest phosphate-solubilizing potential were FG5, FG8, FG9, FG11, and FG12. Based on molecular identification, these five isolates were identified as *Aspergillus niger*.

Keywords: *Aspergillus niger*, peat soil, phosphorus, phosphate- solubilizing fungi, phosphate source

INTRODUCTION

Phosphorus (P) is an essential macronutrient required for plant cell development, the formation of fine roots, flowers, fruits, and seeds as well as the accumulation and energy release (Walpola and Yon 2012). Phosphorus is absorbed by plants in the form of orthophosphate. The availability of P nutrients in soil is generally low due to its binding process or fixation, where it is bound to be Fe-phosphate and Al-phosphate in acidic soils or Ca-phosphate in alkaline soils (Chang and Yang 2009; Oliveira et al. 2009; Malviya et al. 2011; Tallapragada and Seshachala 2012; Sharma et al. 2012; Hou et al. 2018). Phosphorus deficiency can cause disruption of plant root growth, especially fine roots inhibits the absorption of nutrients, thus limiting plant growth.

Plants cannot absorb phosphorus in the bound form, which must be converted into simpler form before make available to plants. Soil microbes such as fungi and bacteria have the ability to change insoluble and bound phosphate into a soluble form that can be absorbed by plants (Rodriguez and Fraga 1999; Chatli et al. 2008; Chang and Yang 2009; Malviya et al. 2011; Das et al. 2013; Sharma et al. 2013). Phosphate-solubilizing fungi of the genus *Penicillium*, *Aspergillus*, *Fusarium*, and *Sclerotium* are commonly known for their ability to dissolve insoluble nutrients including phosphorus with high effectiveness (Whitelaw 2000; Pradhan and Sukla 2005; El-Azouni 2008; Malviya et al. 2011; Elias et al. 2016; Gizaw et al. 2017).

Phosphate-solubilizing fungi can be used as biological fertilizers, but screening process is necessary to obtain effective isolates (Bashan et al. 2013; Sharma et al. 2013). In comparison to bacteria, fungi have been reported to have higher ability to dissolve P (Nahas 1996). Many of the dominant fungal groups grow in acid soils, such as peat soils can be used as a source to obtain phosphate-solubilizing fungi isolates.

The total area of peat lands in Indonesia is estimated about 18.4 million ha, spread over Sumatra, Kalimantan, Irian Jaya, and small portion in Sulawesi. In North Sumatra, there are around 0.325 million ha (Mha) or around 1.8% of the total peat land area in Indonesia (Soekardi and Hidayat 1988; Agus and Subiksa 2008). In North Sumatra, peat areas are spread across several districts, namely Humbang Hasundutan, Langkat and Mandailing Natal.

Peat soils are formed due to high accumulation of dead organic material that has undergone humification but low in mineralization process. The peat ecosystem consists of low pH and anaerobic conditions, which leads to slow of its organic matter. The level acidity of peat is relatively high with a pH range 3-5. Thicker peat horizon having lower bases and the reaction of the soil becomes more acidic. Peat thickness positively correlated with the level of cation exchange capacity but inversely has negative correlation

with base saturation. Thicker peat having higher cation capacity and lower bases caused acidification in the soil. Acidic soils affect the availability of low nutrients. Peatlands is a unique ecosystem (Nurulita et al. 2016) with soils rich in organic matter which is a source of macro and micronutrients for plant and a source of energy and carbon for heterotrophic soil microbes. In various ecosystems, microbes play an active role in determining the availability of nutrients for plant growth. Thus, it is necessary to explore microbes especially phosphate-solubilizing fungi that are beneficial for plant growth. The information of phosphate-solubilizing fungi in peatland soils has not been studied deeply, therefore, the purpose of this study was to obtain phosphate-solubilizing fungi from peat soils that have high potential in dissolving phosphate.

MATERIALS AND METHODS

Study site and soil sampling

The study site used for soil sampling in the present study was within the Nagasaribu Village, Lintongnihuta Sub-district, Humbang Hasundutan District, North Sumatra. Geographically it was located between 2°15'093" N and 98°53'442" E. The peatlands were found fibric in mature and located at 1414 meters above sea level. Soil samples were taken diagonally at a depth of 0-20 cm in six plots measuring 20 m x 20 m for five points. Samples were collected in zipped plastic bags and taken to the laboratory for microbial isolation and analysis of organic C content, available P, and pH values.

Isolation of phosphate-solubilizing fungi

Phosphate-solubilizing fungi were isolated following the method used by Panda et al. (2016). Ten (10) g of soil was taken in 250 mL Erlenmeyer flask containing 90 mL NaCl (0.85%) then shaked for 30 minutes. Serial dilution was made until reaching the concentration of 10^{-5}. One mL of resultant solution was pipetted out from each of 10^{-3}, 10^{-4}, and 10^{-5} serial dilution, poured out in sterilized petri dish containing 12 mL Pikovskaya agar medium (composition per liter distilled water: glucose 10 g; $Ca_3(PO_4)_2$ 5 g; $(NH_4)_2SO_4$ 0,5 g; KCl 0,2 g; $MgSO_4.7H_2O$ 0,1 g; $MnSO_4$ 0,002 g; $FeSO_4$ 0,002 g; yeast extract 0,5 g; agar 20 g) and incubated with upside-down position at 28-30° C for 3 days. The growth of phosphate-solubilizing fungi was indicated by the formation of clear zone surrounding the fungal colony. Fungal purification was conducted in cultivating the fungi into fresh culture medium and transferred to test tubes containing Pikovskaya medium and stored at 4° C prior to further process.

Qualitative testing of Pikovskaya solid medium

Purified phosphate-solubilizing fungal isolates were tested for their ability to release phosphate on solid Pikovskaya medium with phosphorus source $Ca_3(PO_4)_2$, $AlPO_4$, $FePO_4$, and phosphate rocks. The test medium was put into a petri dish and inoculated with purified fungal isolates, then incubated for five days at room temperature. Qualitative phosphate solubilizing activity of fungi was calculated by using a phosphate solubility index i.e. ratio between the diameter of the clear zone plus the diameter of the colony to the diameter of the colony (Yasmin and Bano 2011; Mardat et al. 2014; Yasser et al. 2014; Elias et al. 2016).

Quantitative testing on Pikovskaya liquid medium

One hundred (100) ml of Pikovskaya liquid medium (adjusted P source ie $Ca_3(PO_4)_2$, $AlPO_4$, $FePO_4$, and phosphate rocks) was inoculated with phosphate-solubilizing fungi and incubated for seven days at 28°-30°C. After that, the culture was centrifuged at 6000 rpm for 10 minutes until filtrate was separated from its spore and hyphae. The clear filtrate was determined to provide P levels by the colorimetric method and measured by a spectrophotometer. The experiment was repeated thrice and the isolates were selected based on the ability of the phosphate-solubilizing fungi to dissolve P on the medium by averaging the three replications. Control was determined as medium without fungi inoculation. The pH of medium was measured to determine the effect of phosphate dissolution by fungi on the pH of medium (Gao et al. 2016).

Analysis of organic acids

Phosphate-solubilizing fungi were inoculated in 100 ml of Pikovskaya liquid medium and incubated at 28°-30°C for three days with a constant shaking of 100 rpm. At the end of the incubation period, the culture was centrifuged at a speed of 1000 rpm at 25°C for 20 minutes. The resultant was then filtered with milipore, and used to determine organic acid levels. Determination was done by High-Performance Liquid Chromatography (Shimadzu Liquid Chromatography LC-3A). Organic acids produced by fungi were compared with standard values from six types of acids namely citric acid, oxalic acid, malic acid, acetic acid, butyric acid, and lactic acid.

Analysis of indole acetic acid

Indole-3-acetic acid (IAA) synthesized by each fungal isolate was measured following the methods used by Bric et al. (1991). Fungal isolates were grown in 100 mL nutrient broth having 0, 50, 100, 200, 400 and 500 µg/mL tryptophan. Fungal cultures were grown for 3 days at 28°C and centrifuged at 3000 rpm for 30 min. The supernatant (2 mL) was mixed with two drops of orthophosphoric acid and 4 mL of the Salkowski reagent. The presence of pink color indicates that the isolate produces IAA. The absorbance of developed pink color is read at 530 nm with the help of spectrophotometer Spectronic 20 D. The IAA concentration was determined using a calibration curve of pure IAA as a standard.

Identification of potential phosphate-solubilizing fungi

The five isolates of phosphate-solubilizing fungi with the high ability to dissolve phosphate quantitatively were identified morphologically and molecularly. Morphological identification was carried out up to genus level by determining the macroscopic and microscopic characteristics (Gilman 1971; Gandjar et al. 1999). Fungi

were grown on potato dextrose agar (PDA) and incubated for 3 days at 28ºC. Molecular identification was done by using ITS 1 rDNA primers and ITS 4 primers according to White et al. (1990). Isolation of fungal genomic DNA followed the technical procedure given by DNeasy Plant Mini kit, amplification of fungal 5.8S rDNA was performed on ITS-rDNA region using ITS1 and ITS4 primers with a reaction mixture containing of Go Taq master mix solution, DNA template, each primer, and nuclease-free water. PCR products were then purified and Sanger-sequenced (First Base Sequencing Service, Singapore). Similarity searches were conducted by using the Basic Local Alignment Search Tool-nucleotide (BLASTn) program in the National Center for Biotechnology Information (NCBI) GenBank database.

RESULTS AND DISCUSSION

Isolation and qualitative testing of phosphate-solubilizing fungi

The population of phosphate-solubilizing fungi was 2.86 colonies forming unit (CFU) per g of soils. This fungal population is classified as quite high based on the population of phosphate-solubilizing microbes in the soil ranges from 10^4 to 10^6 CFU per g of soil (Gaur et al. 1980). The presence of phosphate-solubilizing fungi is related to the content of organic matter in the soil as an energy source and carbon source, thus affecting the amount and activity. Soil analysis determined that organic C content in peat soil was 38.77% (classified as very high) with acidic pH (4.53), while the occurrence of soluble P was categorized as low (12.69 ppm). Acid soils are suitable habitats for fungi because fungi are dominant in acid soils (Havlin et al. 1999).

The isolation resulted in 12 phosphate-solubilizing fungi isolates. All isolates formed a clear zone around the colony (Figure 1), indicating of solubilization of phosphate source used. Furthermore, twelve isolates were selected qualitatively by calculating the solubility index values (Table 1). All isolates showed their ability to dissolve phosphate from $Ca_3(PO_4)_2$, $AlPO_4$, $FePO_4$, and phosphate rock sources with varying values.

If the solubility index values are higher, qualitatively the ability of isolated fungi to dissolve P will also be higher. The solubility index value varies from 2.55 for dissolving $FePO_4$ compounds to 4.25 for dissolving $AlPO_4$ compounds. Rashid et al. (2004) found that the solubility index varies from 2.16 to 3.3, while Mahamuni et al. (2012) obtained a solubility index phosphate-solubilizing fungi isolated from rhizosphere sugarcane and sugar beet range between 1.13 to 1.59. Elias et al. (2016) reported that solubility index ranging from 1.10 to 3.05. Islam et al. (2019) also reported solubility index ranged between 1.42 to 2.24 for the fungal culture isolated from subtropical soils in Okinawa. The difference in solubility index values may be due to differences in the amount, type, and diffusion rate of organic acids released by the fungal isolates. Phosphate dissolution is related to ability of the fungi to release organic acids. Strong correlation has been reported the correlation between solubility index and organic acids produce (Alam et al. 2002; Das et al. 2013; Islam et al. 2019).

Quantitative testing on Pikovskaya liquid medium

The results of the ability of phosphate-solubilizing fungi to dissolve P derived from $Ca_3(PO_4)_2$, $AlPO_4$, $FePO_4$, and phosphate rocks and the pH of medium can be seen in Table 2. All isolated fungi have the ability to dissolve P from all P sources test, however, the ability of fungi to dissolve phosphate varies among isolates. According to Yadav et al. (2011a), the ability to dissolve P can vary even the same fungi species.

The ability of phosphate-solubilizing fungi to dissolve P was different for each source. The greatest effect of dissolving P was found in the medium containing $Ca_3(PO_4)_2$, followed by $FePO_4$, $AlPO_4$, and phosphate rock. The amount of P dissolved ranged between 36.41 – 69.86 ppm, 22.45 – 58.77 ppm, 25.80 - 59.64 ppm, and 17.77 – 40.77 ppm from $Ca_3(PO_4)_2$, $FePO_4$, $AlPO_4$, and phosphate rock respectively. This is because $FePO_4$, $AlPO_4$, and phosphate rock have complex structure than $Ca_3(PO_4)_2$. Previous studies reported that the solubilization of $Ca_3(PO_4)_2$ was the highest, followed by $AlPO_4$ and $FePO_4$ (Hue et al. 1986; Vassileva et al. 1998; Kang et al. 2002; Gupta et al. 2007; El-Azouni 2008; Zang et al. 2018; Islam et al. 2019).

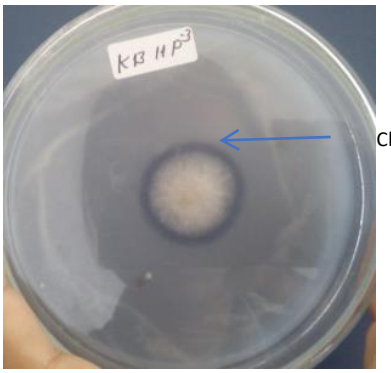

Figure 1. Clear zone around phosphate-solubilizing fungi colony

Table 1. Solubility index of phosphate-solubilizing fungi with four phosphate source

Isolate code	Phosphate source			
	$Ca_3(PO_4)_2$	$AlPO_4$	$FePO_4$	Phosphate rock
FG1	3.69	2.93	2.77	2.77
FG2	3.30	3.17	2.75	2.81
FG3	2.90	3.14	3.12	2.89
FG4	3.18	3.56	2.55	2.80
FG5	3.08	4.25	3.08	3.38
FG6	3.34	2.81	3.50	2.90
FG7	3.26	3.38	3.13	3.21
FG8	2.87	3.22	2.86	3.34
FG9	2.88	3.56	3.35	3.22
FG10	3.24	3.04	2.65	2.76
FG11	3.32	4.24	3.21	2.98
FG12	3.63	3.18	3.77	3.81

Note: F: Fungi; G: Gambut

Table 2. Dissolution of phosphate (ppm) by phosphate-solubilizing fungi from four phosphate source

Isolate code	Phosphate source							
	$Ca_3(PO_4)_2$	pH of medium	$FePO_4$	pH of medium	$AlPO_4$	pH of medium	Phosphate rock	pH of medium
Control	6.37	6.81	2.26	6.66	3.74	6.73	3.27	6.81
FG1	36.41	3.85	32.66	2.35	44.91	2.79	29.64	3.76
FG2	46.00	4.20	42.50	3.06	27.02	2.82	27.05	5.24
FG3	46.89	4.16	57.77	2.73	36.23	2.66	31.27	4.40
FG4	56.00	4.03	31.98	2.32	42.18	2.69	17.77	3.74
FG5	57.59	3.71	58.59	2.47	40.77	2.61	34.39	3.70
FG6	53.05	3.81	46.61	2.10	33.95	2.64	33.91	4.35
FG7	55.32	3.71	22.45	2.90	25.80	2.59	32.02	3.08
FG8	69.86	4.05	52.50	2.24	59.64	2.74	33.95	2.95
FG9	58.95	3.89	26.30	2.37	50.25	2.61	29.00	2.38
FG10	40.77	3.52	41.82	2.20	37.32	2.49	27.77	3.00
FG11	64.02	3.46	48.07	2.23	39.93	2.68	40.52	2.31
FG12	64.02	3.80	50.25	2.45	36.59	2.81	28.73	3.37

Meanwhile, research by Zang et al. (2018) showed that the fungi isolated from the bamboo rhizosphere were higher at dissolving P from $AlPO_4$ and $FePO_4$ than $Ca_3(PO_4)_2$. The isolate FG8 showed highest potential to dissolve P from $Ca_3(PO_4)_2$ and $AlPO_4$ sources. Similarly, isolate FG5 showed the highest solubility of P from $FePO_4$ and isolate FG11 from phosphate rock. Studies on phosphate dissolving ability were carried out on phosphate rock source because direct application of phosphate rock showed less effective result in short period of time to most perennial plant (Goenadi et al. 2000). Gyaneshwar et al. (2002) determined that acid-producing microbes as that of phosphate-solubilizing fungi can increase the phosphate solubility in phosphate rock.

Phosphate dissolution followed by a decrease in pH of the medium was observed in the range 6.81 - 2.20. The decreasing pH of medium has also been reported from previous studies. The *Aspergillus* genus showed the highest decrease in pH compared to other genera (Pradhan dan Suklan 2005; Khan et al. 2009; Das et al. 2013; Yasser et al. 2014; Acevedo et al.2014; Elias et al. 2016; Islam et al. 2019). The decrease in pH was caused by the production of organic acids during metabolic process of fungi. Fungi have been known to secrete various types of organic acids such as citric, oxalic, formic, and others (Illmer and Schinner 1995). Organic acids convert tricalcium phosphate to mono and dicalcium phosphate so that phosphorus nutrients became available to plants. Nahas (1996); Mardad et al. (2014); and Anand et al. (2016) showed that organic acids produced by fungi dissolve insoluble phosphate with a decrease in pH, chelation of cations, and competition with phosphate on sorption sites in the soil. The research result of Omar (1998); Whitelaw (2000); and Chatli et al. (2008) who used bacteria and phosphate solubilizing fungi show that organic acids produce by microbial metabolism bound or chelate cations that bound P, so that P solubility increases. According to Mahidi (2011) and Wei et al. (2018), the ability of organic acids to chelate a metal cation is strongly influenced by its molecular structure, especially by the number of carboxyl and hydroxyl groups. Besides the strength of the acid, the type and position of the ligand also determine its effectiveness in the dissolution process.

Based on the ability to dissolve phosphate compounds, five isolates were selected namely, FG5, FG8, FG9, FG11, and FG12 were the highest dissolving compound P. The five selected isolates consistently dissolved P from the four P sources used, including the high criteria (more than 26 ppm).

The ability of isolates to produce organic acids

Phosphate-solubilizing fungal isolates produce organic acids with different amounts and types (Table 3). Results of all the isolates indicated that butyric and oxalate acids were two major acids produce by all the tested phosphate-solubilizing fungi. The different amount and types of organic acids produce affect the ability of fungi to dissolve P. All fungi isolates produce butyric acid, eight fungi isolates produce citric acid, ten isolates produce oxalate acid, five isolates produce malic acid, eight isolates produce acetate acid, and four isolates produce lactate acid. Result of the present study is supported by the research of Rashid et al. (2004) who studied the production of organic acids by phosphate-solubilizing fungi. Five selected isolates (FG5, FG8, FG9, FG11, and FG12) produce citric, oxalate, and butyric acid. FG12 isolates also produce malic, acetate, and lactate acid, isolates FG5 and FG9 also produce malic and acetic acid, while isolates FG8 and FG11also produce acetic acid. The ability of organic acids to release P bonds is not the same as one another. Ryan et al. (2001); Hou et al. (2018) reported that citric acid dissolved P higher than oxalic and malic acid. Organic acids that are able to form a more stable complex with metal cations will be more effective in releasing aluminum and iron soil minerals so that they will release greater phosphorus. According to Whitelaw (2000), the ability of phosphate-solubilizing fungi to produce organic acids was ten times higher than that of bacteria. The pH value of the medium can decrease to pH 1 or 2. The ability to produce organic compounds is basically determined by genes, but

also be influenced by environmental factors such as carbon. A high C/P ratio increases organic acid production (Wang et al. 2004).

According to Whitelaw (2000) and Yadav et al. (2011b), the effectiveness of phosphate-solubilizing microbes is not only caused by their ability to increase the availability of phosphorus but also because of their ability to produce growth-regulating agents such as indole acetic acid (IAA). Indole acetic acid is a growth regulator that functions in cell enlargement and division, to lengthen roots, resistance to stress factors, biosynthesis of various metabolites, stimulation of nitrogen fixation, and increase rate of xylem formation (Khan et al. 2014). All phosphate-solubilizing fungi isolates produce IAA with varying amounts between isolates, of which five selected isolates were the most IAA producers (Table 3).

Identification of selected isolates

Based on morphological identification, the five isolates belong to the genus *Aspergillus* were observed in the present study (Figure 2). The five fungal isolates showed similar characters, as were colony has dark brown to blackish colored colonies with 0.5 to 4 in size. All isolates had a round black conidial head with a dark brown to black layer of conidiophores (Gilman 1971; Gandjar et al. 1999; Gautam et al. 2012). *Aspergillus* is a phosphate-solubilizing fungus that is widely isolated from the rhizosphere of various types of plants (sugar cane, tomato, cabbage, asparagus, maize, piper betel) (Alam et al. 2005; Oliveira et al. 2009; Tallapragada & Seshachala 2012; Ruangsanka 2014; Elias et al. 2016). They are also known for their high ability to dissolve phosphate. The genus *Aspergillus* produces is economically important growth regulators such as indole acetic acid, which are widely used in fermentation process including producing organic acids (Khan et al. 2007; Sharma et al. 2013) and biosurfactants and also in producing enzymes (Reddy et al. 2014). Filamentous fungi particularly black *Aspergillus* have been reported for various properties of biotechnological importance such as biocontrol, biodegradation, phosphate-solubilization, and biofertilizer (Yadav et al. 2011b).

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Based on molecular identification, the five isolates of phosphate-solubilizing fungi have 100% similarity with *Aspergillus niger* (Table 4). *A. niger* is one of the fungi with high phosphate dissolving capability so that it has the potential to be used as a biofertilizer and biocontrol agent (Das et al. 2013; Ruangsanka 2014; Islam et al. 2019). *A. niger* a filamentous fungus that belongs to the class *Ascomycetes*, is a member microbial community in the soil and producer of citric acid (Rashid 2004). *A. niger* has an important role in the global carbon cycle (Gautam et al. 2011). These microbes are saprophytic and involved in the process of decomposition of organic material containing lignocellulose (Baker 2006).

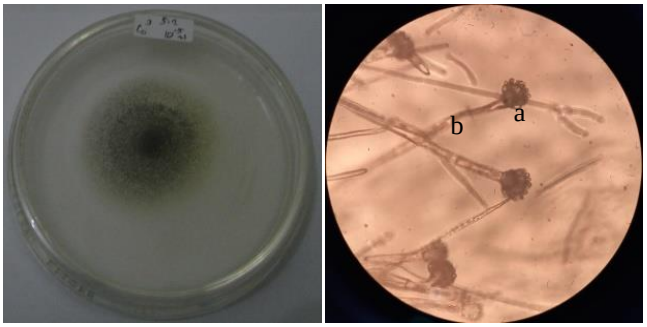

Figure 2. A. Phosphate-solubilizing fungi (*Aspergillus*) on Pikovskaya medium, B. Microscopic structures of *Aspergillus*, a. conidia, b. conidiophores.

Tabel 4. Molecular identification of phosphate-solubilizing fungi

Isolate code	Species identified	Similarity (%)
FG5	*Aspergillus niger*	100
FG8	*Aspergillus niger*	100
FG9	*Aspergillus niger*	100
FG11	*Aspergillus niger*	100
FG12	*Aspergillus niger*	100

Table 3. Types and amounts of organic acids and indole acetic acid production by phosphate-solubilizing fungi isolates

Isolate code	Concentration of organic acids (mg/kg)						Indole acetic acid (mg/kg)
	Citric	Oxalate	Malic	Acetate	Butyric	Lactate	
FG1	-	0.566	0.237	-	0.626	-	1.415
FG2	0.549	0.524	-	-	1.066	1.272	0.624
FG3	0.505	0.084	-	1.374	1.602	0.084	1.798
FG4	-	0.183	0.168	-	1.479	0.244	0.615
FG5	0.477	0.058	0.063	0.190	1.109	-	1.985
FG6	-	-	-	-	1.136	-	1.297
FG7	-	0.490	-	1.087	1.241	-	0.624
FG8	0.230	1.033	-	0.164	1.188	-	1.819
FG9	1.109	0.477	0.063	0.190	0.058	-	2.004
FG10	0.058	-	-	0.446	0.792	-	1.521
FG11	1.574	0.145	-	0.979	1.519	-	2.597
FG12	0.606	0.621	0.045	0.526	1.648	0.247	2.129

Note: -: not detected

Selected phosphate-solubilizing fungi isolated from peat soil have the potential to be used on acid soils, because quantitatively they can dissolve P from $AlPO_4$ and $FePO_4$. Usually, acid soils rich in Al^{3+} and Fe^{3+} (Havlin et al. 1999). In acidic soils, Al and Fe fix P nutrients so that their availability becomes low for plants. Phosphorus fixation causes the use of P fertilizer to be inefficient, so it is often given in high doses (Havlin et al. 1999; Schroder et al. 2011). Utilization of phosphate-solubilizing fungi makes it possible to increase the efficiency of P fertilizer use (Sharma et al. 2013). Therefore, the five isolates of *Aspergillus niger* can be used as biological fertilizers for the management of soil fertility and sustainable crop production.

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