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Combination on endophytic fungal as the Plant Growth-Promoting Fungi (PGPF) on cucumber (*Cucumis sativus*)

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Abstract. *Syamsia S, Idhan A, Firmansyah AP, Noerfitryani N, Rahim I, Kesaulya H, Armus R. 2021. Combination on endophytic fungal as the Plant Growth-Promoting Fungi (PGPF) on Cucumber (*Cucumis sativus*). Biodiversitas 22: 1194-1202.* Endophytic fungi are known to stimulate plant growth by producing secondary metabolites, including phytohormones (IAA and Gibberellins), siderophore, phosphate-solubilizing metabolites. In this study, a total of six endophytic fungi were successfully isolated from local rice plants and showed different abilities in producing secondary metabolites, during single isolates testing. These six isolates were then combined to obtain 15 combinations for analysis, to determine the best combination for application as a plant growth promoter. Subsequently, each combination was tested for phytohormones (IAA, gibberellins) and siderophore (quantitatively)-producing activity, phosphate-solubilizing ability, and the effect on cucumber (*Cucumis sativus* L) plant growth. F13 showed activity in producing IAA and produced the highest gibberellin levels, while F1 exhibited the highest phosphate-solubilizing activity. In addition, F11 (Na-salicylate) and F1 (catechol) showed the highest siderophore activity, while a combination of F6, F8, F9, and F12 successfully increased plant height growth. Also, F4 increased the root growth, while the fresh weight of cucumber was increased by F8 treatment, under controlled conditions. Molecular analysis showed the tested isolates have close similarity to *Daldinia eschscholtzii*, *Sarocladium oryzae*, *Rhizoctonia oryzae*, *Penicillium allahabadense*, and *Aspergillus foetidus*. The combination of endophyte fungal isolates showed potential as plant growth promoters, however, further testing on several plant types is required before the combination is to be widely applied.

Keywords: Gibberellin, IAA, phosphate-solubilizing, and siderophore

INTRODUCTION

Microorganisms, including a group of plant growth-promoting bacteria and fungi, called plant growth-promoting rhizobacteria (PGPR) and plant growth-promoting fungi (PGPF) respectively, have an important role in plant growth (Pieterse et al. 2012; Zhang et al. 2019). Furthermore, PGPF positively impacts environmental ecology (Zhou et al. 2018), directly as well as indirectly, and stimulates plant growth by secreting growth-promoting hormones (El-Maraghy et al. 2020), thus, supporting plants to obtain the nutrients available in soil (Murali et al. 2012; Abdel-Motaal et al. 2020), and offering protection from pathogenic infections (Jogaiah et al. 2018; Abdel-Motaal et al. 2020).

An endophyte is microorganism of fungi or bacteria origin, with the ability to colonize plant tissues during the life cycle, without causing any adverse symptoms (Tan and Zou 2001; Gunatilaka 2006; Duhan et al. 2020). These microorganisms also promote nutrient absorption and protect host plants from both abiotic and biotic stresses (Fadiji and Bababola 2020). Thus, endophytic fungi are fungi able to live in plant tissue without causing symptoms to the host plants (Hilarino et al. 2011; Afandhi et al. 2018).

These fungi play an important role in the ecosystem as well as plant growth (Zheng et al. 2017; Afandhi et al. 2018), produce bioactive molecules (Spellberg et al. 2008; Vasundhara et al. 2016; Manganyi et al. 2019; Techaoei et al. 2020), phytohormones (IAA, gibberellins, and cytokinins) (Lu et al. 2000; Barka et al. 2002) (Wulandari and Suryantini 2019), as well as siderophore (Neilands 1952; Ghosh et al. 2017), and also act as phosphate solvent (Malinowski and Belesky 1999; Hamayun et al. 2011).

IAA is a highly important phytohormone for the formation and elongation of plant roots, root hairs, as well as lateral root, in order to increase both nutrients and air absorption as well as plant development (Mishra et al. 2009; Ahmed and Hasnain 2010; Hussain et al. 2013; Hagaggi and Mohamed 2020). Meanwhile, auxin also plays an important role in root formation (Casimiro et al. 2001; Sapareng et al. 2017), and the IAA produced by endophytic

fungi has the ability to increase plant growth and productivity (Yuan et al. 2010; Widowati et al. 2016).

According to Khan et al. (2013, 2017), Gibberellin plays a role in plant growth and helps to deal with abiotic stress. This phytohormone is produced by fungi, including *Fusarium, Aspergillus,* and *Penicillium* (Tudzynski 2005; Leitão and Enguita 2016).

In addition, Microbial phosphate solvent affects plant growth as well as development (Tallapragada and Gudimi 2011; Alori et al. 2017), and is able to improve phosphorus uptake in plants, thus, reducing the use of chemical fertilizers with a negative impact on the environment (Alori et al. 2017). Microorganisms produce siderophores under iron-deficient conditions (Schwyn and Neilands, 1987; Ahmed and Holmström 2014). These siderophores are classified into large groups of catechol, hydroxamate, and carboxylate, depending on the chemical nature of the coordination sites with iron (Ahmed and Holmström 2014). In addition, Calvente et al. (1999) and Halo et al. (2018) reported the siderophore produced by *Rodhotorula glutinis* is able to suppress blue rot disease on apple plants.

The application of PGPF isolates is able to induce plant growth, and the endophytic application on cucumber (*Cucumis sativus* L) plants showed increased biomass and plant growth. However, the inoculation of two or more microbial species produces increased growth and production, compared to a single application. The combination of *Aspergillus terreus* and *Acremonium strictum* inhibits root-knot disease in tomato plants (Elsharkawy and El-Khateeb 2019; Waqas et al. 2012; Navale et al. 1995; Saxena et al. 2015; Singh and Mathur 2010; Halo et al. 2018).

Therefore, the aims of this study were to select a combination of endophytic fungal isolates with the best ability to produce phytohormones, siderophores as well as phosphate-solubilizing metabolites, determine the effect of a combination of endophytic fungal isolates on cucumber germination and growth, and to identify the fungal isolates used, by pairs, followed by sequencing.

MATERIALS AND METHODS

Preparation of endophytic fungi isolates and the combination of endophytic fungal isolates

The six isolates of endophytic fungi were derived from South Sulawesi local rice and cultivated on PDA media at 28ºC, for 7 days.

Combination design of endophytic fungi isolates

The six endophytic fungal isolates were combined to obtain 15 combinations. These are F1-F5 (2 isolates combination), F6-F9 (3 isolates combination), F10 and F11 (4 isolates combination), F12 and F14 (5 isolates combination), as well as F15 (6 isolates combination) (Table 1).

The IAA production analysis

For this analysis, the 15 combinations of endophytic fungi were inoculated on Potato Dextrose Broth (PDB) media, L-tryptophan (0.1 $g.L^{-1}$) was added, and the combinations were left to stand in the dark for five days, at 28°C. Subsequently, the combination cultures were harvested and centrifuged at 6000 rpm for 15 minutes, and 1 mL supernatant was obtained from each consortium and transferred into a test tube containing Salkowski reagent (Glickman and Dessaux 1995). This solution was then placed in the darkroom for 24 hours, at 28°C, and absorbance was measured at a wavelength of 535.

The gibberellins production analysis

The gibberellins production test was performed using the standard method, as described by Borrow et al. (1955). For this analysis, 15 combinations were inoculated on PDB medium for 7 days. Subsequently, the harvested cultures were centrifuged at 8000 rpm for 10 minutes and 15 mL of supernatant was collected and transferred into a test tube. This was followed by adding 2 mL of zinc acetate and leaving the mixture to stand for 2 minutes, before adding 2 mL of potassium ferrocyanide solution. The mixture was then centrifuged at 800 rpm for 10 minutes and 5 mL of supernatant was transferred into a test tube, 30% hydrochloric acid was added, and the mixture was incubated at 27°C, for 75 minutes. Subsequently, absorbance was measured at a wavelength of 254 nm, with a UV-VIS spectrophotometer.

Phosphate solvent test

The combination of endophytic fungi isolates was analyzed to determine the phosphate solubilizing ability quantitatively, using Pikovskaya, and $Ca_3(PO_4)_2$ as a source of phosphate. Pikovskaya media was prepared using 0.5g of $(NH_4)\ 2SO_4$, 0.1g of $MgSO_4 \cdot 7H_2O$, 0.02g of NaCl, 0.02 g KCl, 0.003 g of $FeSO_4 \cdot 7H_2O$, 0.003g of $MnSO_4 \cdot H_2O$, 5g of $Ca_3\ (PO_4)_2$, 10.0g of glucose, 0.5g of yeast extract, 15.0g of agar, and 1000 mL of distilled water (Elias et al. 2016).

Each combination was grown on liquid Pikovskaya media and incubated for 7 days. The cultures of each endophytic fungi isolate consortium were then harvested and filtered using Whatman paper no 42.

Table 1. The combination of six endophytic fungi isolates

Combination code	Combination of endophytic fungi isolates
F1	E1 + E2
F2	E1 + E3
F3	E1 + E4
F4	E1 + E5
F5	E1 + E6
F6	E1 + E2 + E3
F7	E1 + E2 + E4
F8	E1 + E2 + E5
F9	E1 + E2 + E6
F10	E1 + E2 + E3+E4
F11	E1 + E2 + E3 +E5
F12	E1 + E2 + E3 + E6
F13	E1 + E2 + E3 + E4 + E5
F14	E1 + E2 + E3 + E4 + E6
F15	E1 + E2 + E3 + E4 + E5 + E6

Subsequently, the filtrate was centrifuged at 1000 rpm for 15 minutes, and 5 mL of supernatant was transferred into a test tube, with 0.5 mL of concentrated reagent (12 g ammonium molybdate, 0.277 g potassium tinolartate) as well as dye concentrated reagent (0.53 g ascorbic acid), shaken for a few minutes, and left to stand for 30 minutes. The absorbance of the solution was then measured with a spectrophotometer at a wavelength of 693 nm.

The siderophore production analysis

This was carried out by growing the 15 combinations on PDB medium at 28ºC, for 7 days, then harvesting and filtering with a filter paper. The filtrate obtained was centrifuged at 10,000 rpm for 20 minutes, and the supernatant was removed then adjusted to pH 2.0 using HCL solution. Subsequently, 20 mL of this supernatant was collected, macerated with 20 mL of ethyl acetate, and subjected to extraction, twice. This was followed by adding 5 mL of Hathway reagent (1 mL 0.1 M ferric chloride and 1 mL 0.1 N HCl, added to 100 mL distilled water and 1 mL 0.1 M potassium ferricyanide) to 5 mL of the test solution. The quantitative siderophore measurement was then conducted by measuring the absorbance of each endophytic fungal isolate's supernatant with a spectrophotometer at a wavelength of 560 nm, using Sodium Salicylate as standard. Meanwhile, catechol was determined at 700 nm absorbance, using 2.3 DHBA as standard.

The PGPF combination test on cucumber plants

The spore suspension of each endophytic fungal isolate's combination was mixed with distilled water to obtain a spore concentration of 1 x 10^6 CFU (Colony Forming Unit). Subsequently, the surface-sterilized cucumber seeds were planted on rockwool previously soaked with spore suspension and placed in a test tube filled with 50 mL of AM mix nutrient solution. Each treatment of the consortium of endophytic fungi isolates was repeated 3 times, thus, there were 45 treatment tubes and 3 as a control. Meanwhile, the nutrient solution was added after the volume had decreased. After 14 days of planting, the seeds had germinated and grown, and the plants were observed, based on the parameters of plant height, root length, and fresh weight.

The molecular identification of endophytic fungal isolates

This was conducted in the Indonesian Culture Collection (InaCC), Research Center for Biology, Indonesian Institute on Sciences (LIPI), Bogor, Indonesia, using molecular-based partial genetic analysis, at the internal transcribed spacer (ITS) locus of ribosomal DNA of fungi. Meanwhile, DNA isolation was carried out by growing the fungal isolate in (PDB) liquid medium and incubating it for 72 hours. The fungal mycelia biomass was then harvested for DNA extraction, performed using the nucleon reagent PHYTOpure (Amersham LIFE SCIENCE).

In addition, PCR amplification of ITS was carried out using ITS Primer 4: 5`-- TCC TCC GCT TAT TGA TAT GC-3` and ITS Primer 5: 5`--GGA AGT AAA AGT CGT AAC AAG G -3` (White et al. 1990; O`Donnell 1993).

Also, PCR product purification was performed using PEG precipitation method (Hiraishi et al. 1995), and followed by a sequencing cycle. The results of the sequencing cycle were re-purified using the Ethanol purification method, while the nitrogen base sequence-reading was analyzed with an automated DNA sequencer (ABI PRISM 3130 Genetic Analyzer) (Applied Biosystems).

Subsequently, raw data from the sequencing was trimmed and assembled using the BioEdit program (http: //www.mbio.ncsu.edu/BioEdit/bioedit.html). The sequence data were then assembled in a subsequent BLAST, with genomic data registered in DDBJ/DNA Data Bank of Japan (http: //blast.ddbj.nig.ac.jp/) or NCBI/National Center for Biotechnology Information (http: //www.ncbi.nlm.nih.gov/BLAST/) in order to determine the taxa/species with the largest and closest homology/similarity in the molecular site.

RESULTS AND DISCUSSION

IAA and gibberellin production

According to the IAA and Gibberellin production capability test results on the 15 combinations of endophytic fungal isolates, the F13 combination containing 5 isolates, E1, E2, E3, E4, and E5, produced the best performance (Figure 1). Endophytic fungal are known to have an important role in stimulating the growth of host plants by producing secondary metabolites, including IAA, gibberellins, and siderophore. In this study, six endophytic fungi isolates derived from local rice plants in South Sulawesi, in the previous study, were combined and tested for plant growth-promoting ability. A study by Numponsak et al. (2018) reported only one of twenty-seven endophytic fungi isolates derived from the *Coffea arabica* plant in Northern Thailand, was able to produce IAA. In this study, the F13 consortium produced 3.89 mg.L^{-1} of IAA, and this is almost the same as the 37.034 μg/ml produced by *Lasiodiplodia pseudotheobromae* (Aramsirirujiwet and Kitpreechavanich 2016). This is also similar to the result of gibberellin-producing ability, reported for only 12 fungi (MacMillan 2002; Kawaide 2006; Vandenbussche et al. 2007), including *Fusarium*, *Aspergillus*, and *Penicillium* (Leitão and Enguita 2016; Alori et al. 2017).

Phosphate-solubilizing activity

Based on the results, F1, containing two endophytic fungal isolates, E1 and E2 have the highest phosphate-solubilizing activity (8.64 mg.L^{-1}), compared to other combinations (Figure 2). However, six other combinations, F2, F3, F4, F6, F10, and F15, were also able to dissolve phosphate in the range of 7.27-8.27 mg.L^{-1}. Generally, the endophytic fungal combination's activity in dissolving phosphate is related to the type of endophytic fungal contained. The combination containing the endophytic fungal isolates E1, E2, E3, E4, and E5 had a higher dissolving ability, while the isolate E6 had a lower phosphate dissolving ability (0.68-5.47 mg.L^{-1}). According to the results of molecular identification, E1 and E5 are *Daldinia eschscholtzii*, E2 is *Sarocladium oryzae*, E3 is

Rhizoctonia oryzae, E4 is *Penicillium allahabaden*se, and E6 is *Aspergillus foetidus*. This indicates *Aspergillus foetidus* has low phosphate dissolving ability, even in combination with other endophytic fungi. Reports by Srinivasan et al. (2012), Sharma et al. (2013), and Alori et al. (2017) stated *Achrothcium*, *Alternaria, Arthrobotrys, Aspergillus, Cephalosporium, Cladosporium, Curvularia, Cunninghamella, Chaetomium, Fusarium, Glomus, Helminthosporium, Micromonospora, Mortierella, Myrothecium, Oidiodendron, Paecilomyces, Penicillium, Phoma, Pichia fermentans, Populospora, Pythium, Rhizoctonia, Rhizopus, Saccharomyces, Schizosaccharomyces, Schwanniomyces, Sclerotium, Torula, Trichoderma,* and *Yarrowia*, are examples of fungi with the ability to dissolve phosphates.

Siderophore production

The results showed Na-salicylate and catechol production in the 15 combinations. However, F11 and F1 combinations exhibited the highest concentration of Na-salicylates (7.87 mg.L-1) and catechols (3.90 mg.L^{-1}), respectively (Figure 3).

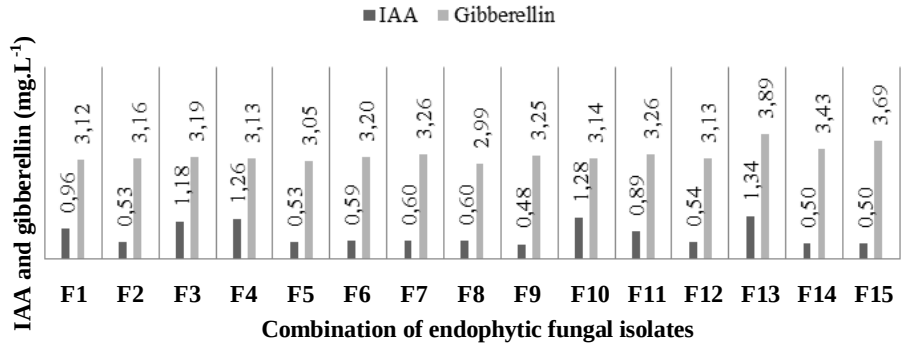

Figure 1. Production of phytohormones (IAA and gibberellin) by the combinations of endophytic fungal isolates

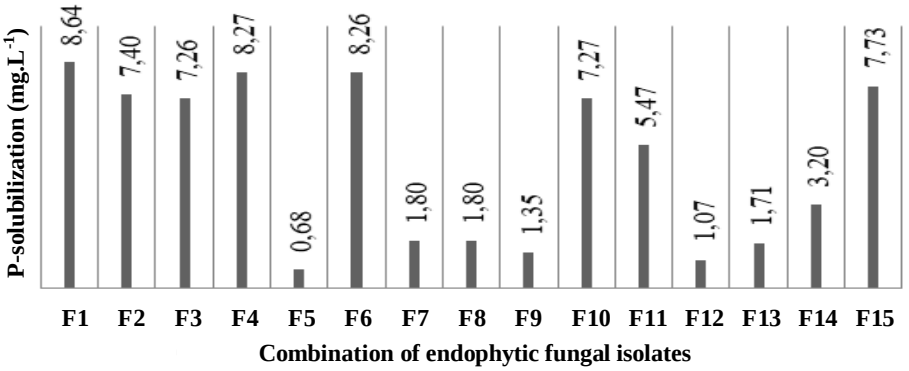

Figure 2. Phosphate solubilization activity of the consortium of endophytic fungal isolates

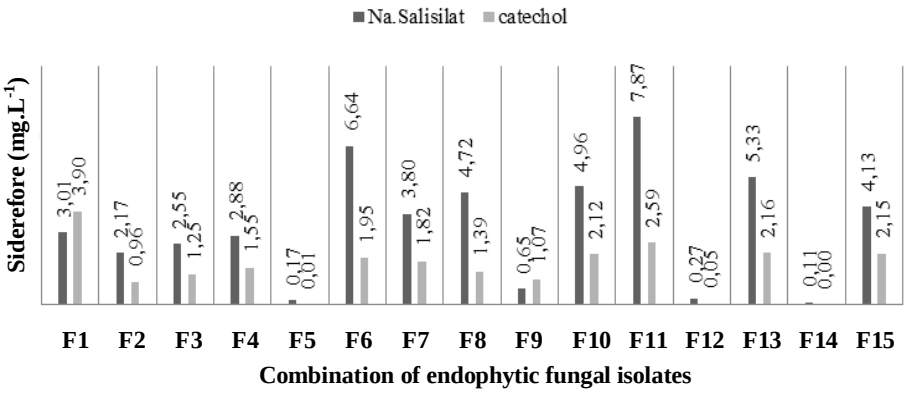

Figure 3. The production of Siderophore (Na-salicylate and catechol) combination by endophytic fungal isolates

The F11 consortium contained E1, E2, E3, and E5 isolates, while F1 contained E1 and E2. In terms of siderophores, F1 consortium shows the highest catechol activity, while F11 exhibits the highest salicylate concentration of 3.90 mg.L^{-1} and 7.87 mg.L^{-1}, respectively. Kesaulya et al. (2018) reported *Bacillus subtilis* HPC21 strain was able to produce 4.21 mg.L^{-1} of siderophore (Verma et al. 2011; Ahmed and Holmström 2014). The effect of endophytic fungal isolates inoculation is able to cause significant increase in the parameters of plant height, root length, and fresh weight, in cucumber plants. In this study, the F6, F8, F9, and F12 combinations showed the best result for the plant height. Meanwhile, F4 combination, containing isolates E1 and E5, resulted in the highest root length of 38.67.

Application of endophytic fungal isolate combination in plants

An endophytic fungi's ability to produce phytohormones, including IAA and gibberellin, is highly significant and necessary for increasing plant growth. According to Strobel et al. (2004) and Syamsia et al. (2015), indole acetic acid is an essential compound for growth, root, and shoot development, produced by microbes. Salazar-Cerezo et al. (2018) also described gibberellin's role, and the phytohormone has a vital function in plant growth processes, including seed germination, stem elongation, flowering, and fruit development. The activity of the fifteen combinations of endophytic fungal isolates in this study varied in the IAA and gibberellin-producing activity. However, isolate F13 was discovered to have the highest phytohormone-producing ability.

After nitrogen, phosphate is the second main nutrient required for plant growth, however, about 95-99% of existing phosphate is insoluble, and therefore not utilizable by plants (Vassileva et al. 1998; Patil et al. 2011). A study by Alori et al. (2017) showed Phosphate Solubilizing Microorganisms (PSM) increase the bioavailability of soil insoluble phosphorus for plant use. The fifteen endophytic fungi combinations showed varied phosphate dissolving activity, with F1 exhibiting the highest activity.

Meanwhile, siderophore is an iron-chelating agent with a small molecular size, produced by microorganisms and plants in iron deficiency conditions (Schwyn and Neiland, 1987; Ahmed and Holmstrom 2014; Syamsia et al. 2020). The siderophore's primary function is to chelate iron and form complex compounds with Cd, Cu, Zn and other heavy metals (Jonstone and Nolan 2015; Syamsia et al. 2020). In this study, the ability of endophytic fungi combinations to produce two types of siderophores varied, with F1 and F11 producing the highest catechol and Na-salicylate levels, respectively.

Figure 4 shows the cucumber seeds began to germinate on the third day after planting, as indicated by the presence of plumules and roots, and all the seeds were fully germinated by the seventh day. The plants were maintained up to 14 days by controlling the volume of nutrients in the tube daily (Figure 5). According to the results, consortium F8 produced the highest plant fresh weight of 26.83 g. A study by Syed et al. (2020) showed the PGPR consortium application (*Aspergillus niger*; *A. flavus*; *Fusarium oxysporum*) resulted in higher germination and root induction rates, compared to the control, while a report by Halo et al. (2018) stated the isolates of *Aspergillus terreus* (65P and 9F) were able to increase germination by 10 to 20%, in cucumber plants, under controlled condition. Meanwhile, Hamayun et al. (2011) used the gibberellin-producing *Neosartorya* fungus to stimulate growth in *Brassica rapa* L ssp. pekinensis Kursiv.

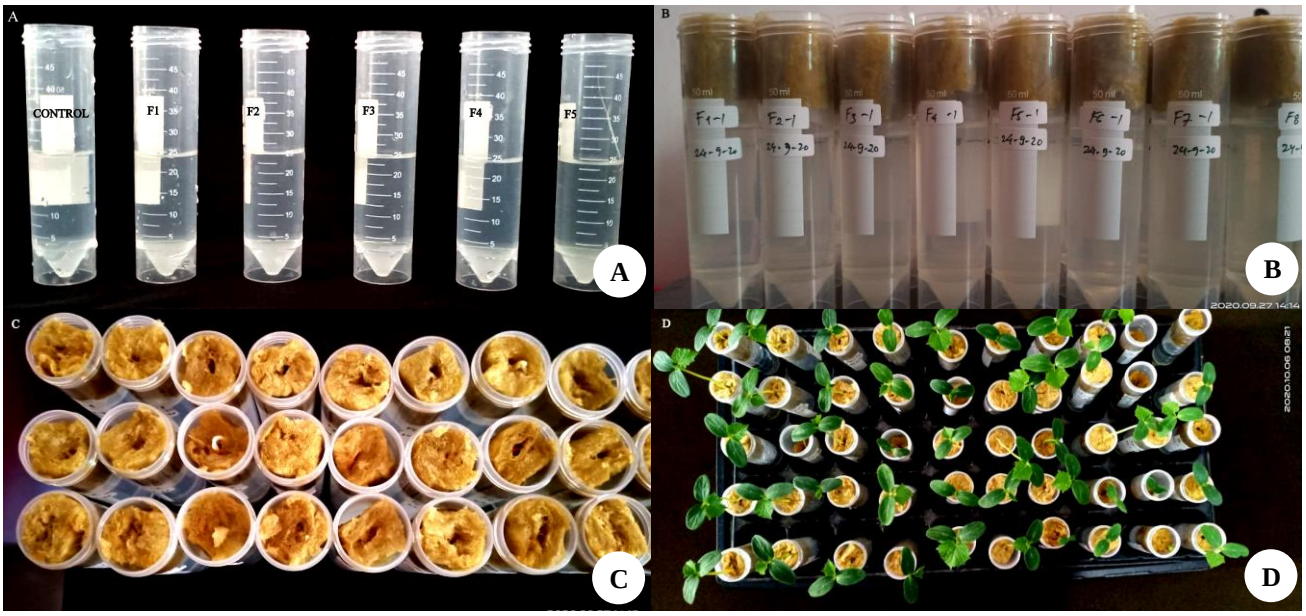

Figure 4. Cucumber seed germination and growth in the inoculation treatment, using the endophytic fungal isolate combinations

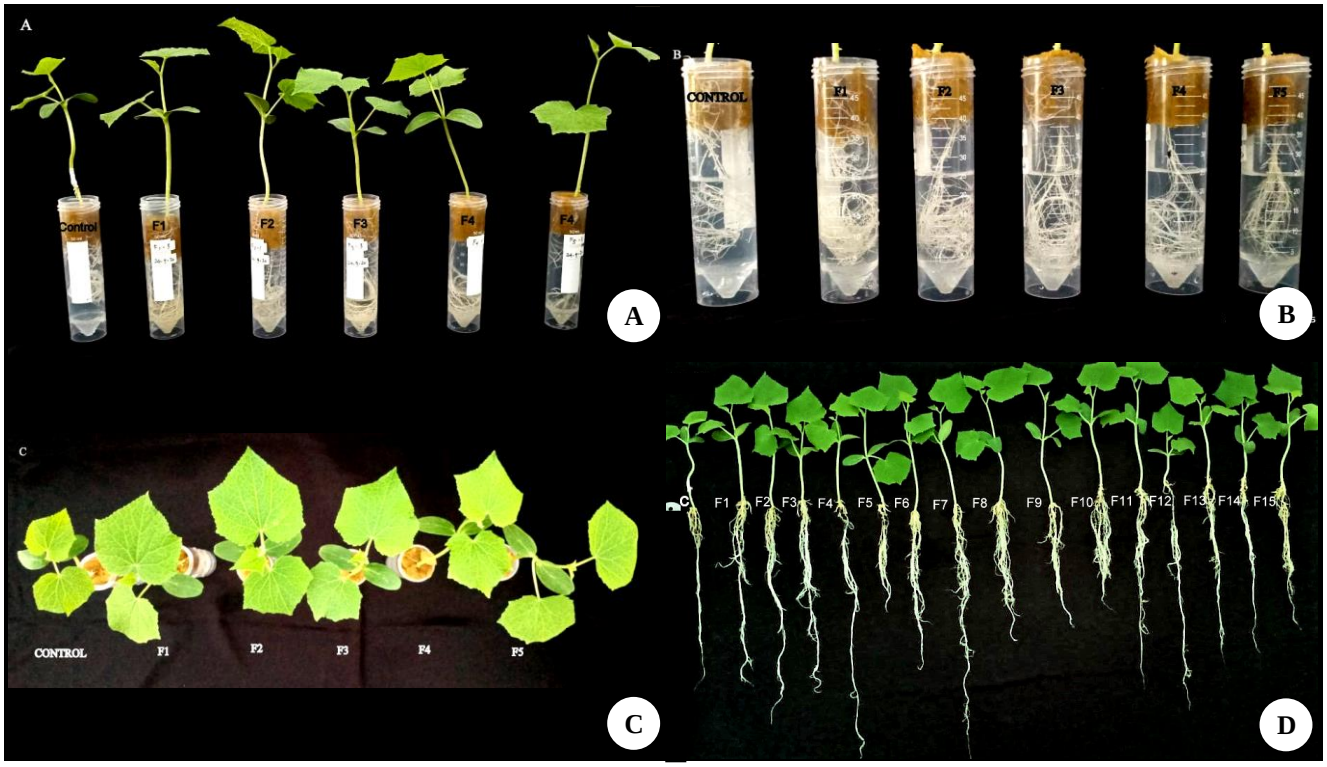

Figure 5. Plant growth of cucumber plant treated with a combination of endophytic fungal isolates, at age 14 days (A), roots (B), leaves (C), and plant (D)

Table 2 shows the inoculation of endophytic fungal isolate combinations was able to significantly increase the plant height, root length, and fresh weight of cucumber. The combinations F6, F8, F9, and F12 showed the best result for plant height, while the F4 and F8 resulted in the best root length, and plant fresh weight, respectively. Based on data analysis, all the results were significantly different.

Table 2. The effect of endophytic fungi combination inoculation on plant height, root length, and fresh weight of cucumber at 14 days after planting.

Treatments	Shoot length (cm)	Root length (cm)	Fresh weight (g)
Control	11.00.^{d}	21,10^{h}	12.10^{g}
F1	11.00^{d}	26.63^{g}	12.25^{g}
F2	11.17^{cd}	30.67^{d}	23.13^{b}
F3	14.00^{b}	28.50^{ef}	21.57^{c}
F4	10.17^{e}	38.67^{a}	15.96^{f}
F5	11.83^{cd}	15.33^{l}	17.97^{e}
F6	15.17^{a}	22.67^{h}	21.97^{bc}
F7	12.93^{c}	36.33^{b}	21.97^{bc}
F8	15.15^{a}	22.67^{h}	26.83^{a}
F9	15.12^{a}	17.67^{k}	22.40^{bc}
F10	15.12^{a}	18.33^{j}	20.36^{d}
F11	14.28^{b}	29.33^{e}	18.23^{e}
F12	15.23^{a}	34.67^{c}	21.53^{c}
F13	7.00^{f}	27.33^{fg}	14.86^{f}
F14	12.17^{c}	27.67^{f}	19.40^{d}
F15	10.67^{e}	20.33^{i}	19.33^{d}

Molecular identification

The nearest taxon of BLAST homology results in DDBJ/NCBI ([http: //www.ncbi.nlm.nih.gov/) showed the endophytic fungal isolates E1, E5, and E6 obtained 100% homology and 100% query coverage with *Daldinia eschscholtzii* isolate UZ_108_16 and *Daldinia eschscholtzii* culture-collection JMRC: SF: 11930 for E1, *Talaromyces allahabadensis* (anamorph: *Penicillium allahabadense)* isolate HNMF013 and *Daldinia eschscholtzii* isolate UZ_108_16 for E5, as well as *Aspergillus foetidus* CBS 121.28 *Aspergillus foetidus* isolate BM13, foe E6. Meanwhile, isolate E2 showed 99.83% homology and 99% query coverage with *Sarocladium oryzae* culture collection CBS 399.73, as well as 98.80% homology and 99% query coverage with *Sarocladium oryzae* isolate Saro11. In addition, isolate E3 showed 90.01% homology and 100% query coverage with *Rhizoctonia oryzae* CBS 273.38 strain, as well as 98.20% homology and 99% query coverage with *Rhizoctonia oryzae* CBS 475.82 strain, while isolate E4 showed 100% homology and 56% query coverage with *Talaromyces allahabadensis* (anamorph: *Penicillium allahabadense* isolate Yu3-6 and *Talaromyces allahabadensis* (anamorph: *Penicillium allahabadense*) isolate HNMF013 (Table 3).

Based on the identification, several endophytic fungal isolates, including *Daldinia eschscholtzii*, have also been isolated from the genus *Xestospongia* sponge and are able to produce secondary metabolites (Sibero et al. 2020). According to Sunariasih et al. (2014), the fungus *Sarocladium oryzae* isolated from rice seeds has an inhibitory power against *Pyricularia oryzae*, the cause of blast disease in rice plants.

Table 3. Molecular identification of endophytic fungi isolates

Fungal isolate	GenBank acc. no.	Homology (%)	Max. score	Total score	Query coverage (%)	E-value	Max. identities (%)	Gabs (%)	Endophytic fungi
E1	KY79260	100	1051	1051	100	0.0	100	0	*Daldinia eschscholtzii* isolate UZ_108_16
	KU304335	100	1051	1051	100	0.0	100	0	*Daldinia eschscholtzii* culture-collection JMRC: SF: 11930
E2	HG965027	99.83	1066	1066	99	0.0	99	0	*Sarocladium oryzae* culture collection CBS 399.73
	KT291722	98.80	1033	1033	99	0.0	99	0	*Sarocladium oryzae* isolate Saro11
E3	MH855962	99.01	1092	1092	100	0.0	99	0	*Rhizoctonia oryzae* strain CBS 273.38
	MH861520	98.20	1072	1072	100	0.0	98	0	*Rhizoctonia oryzae* strain CBS 475.82
E4	MG827185	100	992	992	56	0.0	100	0	*Talaromyces allahabadensis* (anamorph: *Penicillium allahabadense* isolate Yu3-6
	HNMF013	100	992	992	56	0.0	100	0	*Talaromyces allahabadensis* (anamorph: *Penicillium allahabadense)* isolate HNMF013
E5	KY792620	100	1051	1051	100	0.0	100	0	*Daldinia eschscholtzii* isolate UZ_108_16
	KU304335	100	1051	1051	100	0.0	100	0	*Daldinia eschscholtzii* culture-collection JMRC: SF: 11930
E6	NR163668	100	1101	1101	100	0.0	100	0	*Aspergillus foetidus* CBS 121.28
	MK910068	100	1101	1101	100	0.0	100	0	*Aspergillus foetidus* isolate BM13

Meanwhile, *Rhizoctonia oryzae* has the ability to cause midrib disease in plants, and *Aspergillus flavus* is a producer of aflatoxin, the most dangerous mycotoxin. However, several studies indicate *Aspergillus flavus* is able to inhibit the growth of *Septobasidium* sp. and also has the potential to act as a biological agent (Suswanto et al. 2018), in addition to absorbing chromium, and is, therefore, suitable for processing waste containing hexavalent chromium (Vinay and Dwivedi 2019) and increasing tomato's resistance to blight (Abdel Motaal et al. 2020). Reports by Berg and Hallmann (2006), as well as Sucipto et al. (2015) show endophytic fungi's mechanism of direct inhibition against pathogens is through antibiosis, competition, as well as lysis, while the indirect mechanism involves inducing plant resistance and increasing plant growth. However, the endophytic fungi tested in this study need to be tested for tobacco plants' pathogenicity. Furthermore, a hemolysis test was conducted to determine the effect on human health before being widely applied to plants as a growth enhancer and biocontrol.

In this study, a combination of endophytic fungi isolates showed IAA, gibberellin and siderophore production, as well as varying phosphate-dissolving activities. In addition, the fungal isolate combination was able to increase the plant height, root length as well as plant fresh weight, and the best combination of endophytic fungi isolates was F1, F4, F8, and F13. However, further studies on several plant types are required before the combination is to be widely applied.

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