Research Article

EXPLORATION OF INDIGENOUS PLANT GROWTH PROMOTING FUNGI (PGPF) AS BIOLOGICAL CONTROL AGENTS AND BIOFERTILIZER

Asniwita^{1,*} ⁽¹⁾, Novalina², M. Syarif¹, Andini Vermita Bestari³, Bruce Ochieng Obura⁴

¹ Faculty of Agriculture, Universitas Jambi, Jambi, Indonesia

² Faculty of Agriculture, Universitas Sumatera Utara, Sumatera Utara, Indonesia

Abstract

³Biomanagement, Institut Teknologi Bandung, Jawa Barat, Indonesia

⁴ Plant Pathology, International Potato Center Sub-Saharan Africa Regional Office, Ilri Campus, Nairobi, Kenya Corresponding author email: <u>wita1812000@gmail.com</u>

Article Info

Recieved: Feb 16, 2024 Revised: Mar 28, 2024 Accepted: May 02, 2024 OnlineVersion: May 14, 2024 This groundbreaking study ventures into uncharted territory to explore the vast potential of Plant Growth Promoting Fungi (PGPF) as multifaceted allies in agricultural sustainability. Departing from traditional paradigms, the research sets out to identify and characterize non-pathogenic fungal isolates with the capacity to serve as potent PGPF agents. Employing a pioneering approach, fungal isolates are meticulously collected from the rhizosphere of plants, heralding a new era of ecological exploration at the microorganism level. Rigorous testing for pathogenicity on soybean seeds unveils a rich reservoir of fungi diversity, with 18 isolates demonstrating remarkable efficacy in enhancing germination rates and promoting vigorous seedling growth. These findings not only underscore the pivotal role of PGPF in bolstering plant health and resilience but also herald a paradigm shift in sustainable agriculture. With the potential to serve as biopesticides for plant protection and biofertilizers for enhancing growth, these PGPF isolates offer a promising avenue for reducing reliance on synthetic inputs and mitigating environmental impacts. Moreover, their integration into integrated disease management strategies holds the promise of synergistic efficacy, paving the way for holistic approaches to agricultural sustainability. This research not only expands the frontiers of knowledge surrounding PGPF but also lays the groundwork for transformative innovations in agroecological practices, ushering in a greener, more resilient future for global agriculture.

Keywords: Biofertilizer, Biopesticide, Germination, Non-Pathogenic, PGPF



© 2024 by the author(s) This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

INTRODUCTION

Plant Growth Promoting Fungi (PGPF) are non-pathogenic fungi that can support plant growth (biofertilizer) and can act as biological control agents (biopesticide) (El-Maraghy et al., 2021; Zhang et al., 2019). According to Murali et al. (2013), PGPF do not cause diseases in plants and have a mutualistic symbiosis with plants. PGPF mostly live in the rhizosphere around the roots and are

influenced by plant roots, which serve as a gathering place for various microorganisms, including fungi (Mendes et al., 2013; Silva et al., 2021). There are also those PGPF that live in plants known as endophytic fungi. Plants and fungi interact with each other, fostering positive interactions that are beneficial to plants. Fungi in the rhizosphere, directly and indirectly, influence the composition and productivity (biomass) of plants (Mohamed et al., 2022; Schnitzer et al., 2011).

Fungi contribute significantly to both the economy and ecology (Hyde et al., 2019), as they serve as valuable resources for biopesticides and biofertilizers. Fungi as a biological control agent have been used to control diseases caused by Phytophthora parasitica (Asniwita, 1989). Some indigenous isolates of endophytic fungi can induce resistance in chili plants against mosaic disease caused by the Tobacco mosaic virus (TMV) (Asniwita, 2016). PGPF can suppress pathogen populations in plants, providing important ecological benefits to agricultural environments (Heimpel & Mills, 2017). PGPF help overcome production issues and protect plants from pathogens (Adedayo & Babalola, 2023) such as viruses, fungi, and bacteria. PGPF are safe, effective, and environmentally friendly in controlling plant diseases, and do not pose any danger to human health (El-Saadony et al., 2022). Endophytic fungi can increase the percentage of chili seed germination and increase the percentage of normal germination (Asniwita & Hayati, 2017). PGPF can increase seed germination of barley (Hordoum vulgare) and rapeseed (Brassica napus) (Brazhnikova et al., 2021). Rhizosphere fungi can promote plant growth and increase crop yields (El-Saadony et al., 2022). Plant growth promoting microorganisms have the potential to replace synthetic chemicals, leading to the transition of conventional agriculture towards integrated, organic, and sustainable practices that are environmentally friendly (Malgioglio et al., 2022). The diversity and population of fungi in the soil can be used to predict plant diversity and productivity (Jahagirdar et al., 2019; Lau & Lennon, 2011).

Research on PGPF is important to study the interaction of PGPF with plants. As a sustainable and renewable agricultural production (including biofuel and bioenergy), PGPF plays a crucial role and offers an environmentally friendly disease control method, increasing productivity while reducing the control costs and the purchase of inorganic fertilizers, supporting sustainable agriculture. Indigenous PGPF isolates are more adaptable to the environment than introduced PGPF isolates. However, research on indigenous PGPF for disease management in plants is still limited.

While previous studies have acknowledged the importance of PGPF in agriculture, there remains a significant gap in understanding the diverse range of non-pathogenic fungal isolates and their specific mechanisms of action. This study addresses this gap by systematically characterizing fungal isolates from the rhizosphere and evaluating their efficacy in enhancing plant growth. By filling this gap, the research contributes to a deeper understanding of the role of PGPF in sustainable agriculture and opens new avenues for research and application in this field. This groundbreaking study ventures into uncharted territory to explore the vast potential of Plant Growth Promoting Fungi (PGPF) as multifaceted allies in agricultural sustainability. Departing from traditional paradigms, the research sets out to identify and characterize non-pathogenic fungal isolates are meticulously collected from the rhizosphere of plants, heralding a new era of ecological exploration at the microorganism level.

Research on the diversity of PGPF and the ability to control diseases and stimulate plant growth is crucial to develop integrated disease management and increase soybean production. The research aims to obtain and study the indigenous non-pathogenic fungal isolates, as well as explore fungi that have the potential as indigenous PGPF in increasing soybean seed germination. This study marks the first step in the effort to obtain PGPF to be used as a biological agent and biofertilizer.

RESEARCH METHOD

Collecting and isolating fungi from the rhizosphere. Fungi were collected from various plantations in the lowland and highland regions in Jambi Province, Indonesia. Soil was collected from around the roots of plants. The plants selected were those that had flowered. Soil samples are separated from roots and plant debris, placed in polythene plastic, and then stored in a cooler box before being transported to the laboratory. The isolation of fungi was carried out through: 1 gram of soil from each site was added to 9 ml of distilled water in a test tube and shaken. Then, stratified dilutions of 10⁻⁴, 10⁻⁵, and 10⁻⁶ were made (Doilom et al., 2020). The solutions were used to isolate fungi from the soil sample, and then 1 ml of the suspension was placed in a Petri dish containing Potato Dextrose Agar (PDA) (Merck). It was incubated at room temperature for 2 days, and the growing fungi were isolated again on PDA medium and further incubated at room temperature for 7 days. The grown mycelium was

transferred to the PDA medium to obtain pure colonies, subsequently, the pure fungal isolates were preserved in slant agar and stored at 4°C to be used as a fungi source.

Testing the pathogenicity of fungi on plants. The fungal isolates obtained were pathogenicity tested on soybean seeds to screen isolates that were not pathogenic. Healthy soybean seeds are selected from visual observation: normal-sized, no discoloration, and no defects. Soybean seeds were surface-sterilized with NaOCl, rinsed with distilled water twice, and then sown in Petri dishes containing fungal isolates. Ten seeds were used for each petri dish and a total of 90 soybean seeds were sown on media containing each fungal isolate. If the soybean seeds grow well, it indicates that the fungal isolate was not a pathogen. As a control, soybean seeds were sown on Petri dishes containing only PDA medium.

Data analysis technique. Data on fungal isolates were tabulated in the form of a table, including macroscopic characters of the fungal isolates from the upper side and undersides of the petri dish. Soybean seed germination data were analyzed using descriptive analysis techniques to provide an overview of the average percentage of germination, the average percentage of normal sprouts, the average percentage of non-germinated seeds, and the average percentage of abnormal germinated seeds.

RESULTS AND DISCUSSION

Collecting and isolating fungi around the roots of plants.

Fungi were collected from the root areas in highland and lowland regions. A total of 73 fungal isolates were collected, consisting of 43 isolates from highland regions and 30 isolates from lowland regions. Each isolate was characterized macroscopically. The macroscopic characteristics include colony color on the upper and lower sides of the Petri dish, colony edge, colony center, colony surface texture, and colony pattern. The macroscopic characteristics of each fungal isolate are presented in Table 1.

Table 1. Macroscopic characteristics of rungar isolates obtained from the mizosphere of plants.				
Characters on the upper side of the petri dish	Characters on the underside of the			
	petri dish			
Colonies are white,	Colonies are light yellow.			
The mycelium is thick like velvet.				
Colonies are white, mycelium is thin, granular is yellow.	Colonies are yellowish-white.			
Colonies are greenish white, granules are green,	Colonies are green.			
	Calanias and white			
· · ·	Colonies are white.			
greenish-white.	Colonies are yellowish-white.			
Colonies are white, green granular, thin mycelium.	The colonies are white to yellow.			
5	Colonies are green.			
Colonies are white, yellow granular.	Colonies are yellowish-white.			
	-			
The center of the colony is yellowish-white, the edges are irregular.	The colonies are white.			
6				
Colonies are light brown, the edges of the colonies are smooth the mycelium is thin	Colonies are light brown.			
-	Colonies are white to yellow.			
	Colonies are yellowish-white.			
	Colonies are greenish-white.			
	Colonies are light green.			
	colonies are light green.			
6	Colonies are white to yellow			
÷ •	containes are write to yellow			
e	Colonies are green.			
	colonies de green.			
colony is olderish-green.				
	Characters on the upper side of the petri dish Colonies are white, The mycelium is thick like velvet. Colonies are white, mycelium is thin, granular is yellow. Colonies are greenish white, granules are green, the number of granules is medium. White colonies, thin mycelium. Colonies are white, the edges of the colonies are greenish-white. Colonies are white, green granular, thin mycelium. Colonies are white, green granular. Colonies are white, yellow granular. The center of the colony is yellowish-white, the edges are irregular.			

Table 1. Macroscopic characteristics of fungal isolates obtained from the rhizosphere of plants.

Fungus isolate	Characters on the upper side of the petri dish	Characters on the underside of the petri dish
FRT 118	Colonies are white to brown, mycelium is thin.	Colonies are white.
FRT 119	Colonies are white to black, granular green.	The colonies are white to black
FRT 121	The center of the colony is yellow, the edge of	The center of the colony is
	the colony is yellowish-white.	yellowish-white, the edge of the
		colony is white.
FRT 122	Colonies are white, granules are green, the	Colonies are yellowish-white.
	number of granules is moderate.	
FRT 123	The colonies are white, the edges of the colonies are yellowish-white.	Colonies are yellowish-white.
FRT 124	The colonies are greenish-yellow, the edges of	Colonies are greenish-yellow.
	the colonies are yellowish-white, the mycelium is	
FDT 125	thick like velvet.	
FRT 125	The center of the colony is white, the edges of the	The center of the colony is white,
EDT 106	colony are hyaline.	the edges of the colony are hyaline.
FRT 126	The center of the colony is green, the edge of the	Colonies are black.
FRT 127	colony is blackish-green. Colonies are greenish-white, green granular,	Colonies are greenish-white.
FKI 127	slightly granular.	Colonies are greenish-white.
FRT 128	Colonies are yellow to white.	Colonies are light brown, the edges
11(1120	colonies die genow to white.	of the colonies are light yellow.
FRT 129	The center of the colony is black, the edge of the	The center of the colony is black,
-	colony is blackish-white, the mycelium is thin.	the edge of the colony is blackish-
		white.
FRT 131	The center of the colony is white, the edge of the	Colonies are white.
	colony is yellowish-white.	
FRT 132	The center of the colony is green, the edge of the	Colonies are greenish-white.
	colony is greenish-white.	
FRT 133	Colonies are green, the mycelium is thick like	The center of the colony is green,
	velvet.	the edge of the colony is yellowish-
FRT 134	Colonico are arean monte arentular	green.
FRT 134	Colonies are green, many granular. Colonies are greenish-white.	Colonies are yellowish-green. Colonies are greenish-white.
FRT 135		Colonies are blackish-white.
111150	colony are blackish-white.	Colonies are blackish-white.
FRT 137	•	Colonies are yellowish-white.
	thickness is medium.	
FRT 138	Colonies are white, granular are green.	Colonies are white.
FRT 139	Colonies are white, mycelium thickness is	Colonies are yellowish-white.
	medium.	
FRT 141	Colonies are white, mycelium is thick.	Colonies are yellowish-white.
FRT 142	Colonies are white, granules are green, granules	Colonies are greenish-white
	are evenly distributed.	
FRT 143	Colonies are blackish-white.	Colonies are greenish-white.
FRT 144	The center of the colony is light green, the edge	Colonies are green.
	of the colony is white.	
FRT 145	Colonies are green.	Colonies are greenish-white.
FRT 146	Colonies are yellowish-white, the mycelium is thick like velvet.	Colonies are yellow.
FRT 147	Colonies are light brown, mycelium thickness is	Colonies are blackish-brown.
111114/	medium.	Colonies are Diackish-DiOwil.
FRR 21	Colonies are white, the mycelium is thick like	Colonies are yellowish-white.
· · · · · · 2 · 1	velvet.	colonies are jono white white.
FRR 22	Mycelium is white, mycelium thickness is	The center of the colony is
	medium.	yellowish-white, the edge of the

Fungus isolate	Characters on the upper side of the petri dish	Characters on the underside of the petri dish	
FRR 23	The center of the colony is greenish-white, the edge of the colony is white.	colony is white. Colonies are light brown.	
FRR 24	Colonies are green, mycelium thickness is medium.	Colonies are whitish-green.	
FRR 25	Colonies are light green, mycelium thickness is medium.	Colonies are greenish-white.	
FRR 26	Colonies are green, the mycelium is thick like velvet.	Colonies are green.	
FRR 27	Colonies are greenish-white, thick mycelium.	Colonies are white.	
FRR 28	The center of the colony is greenish-white, the edge of the colony is white.	Colonies are white.	
FRR 29	Colonies are white, mycelium thickness is medium.	Colonies are yellowish-white.	
FRR 211	Colonies are greenish-white, green granular, slightly granular.	Colonies are white.	
FRR 212	The center of the colony is blackish-brown, the edge of the colony is light brown, the mycelium is thick like velvet.	The center of the colony is black, the edge of the colony is light brown.	
FRR 213	Colonies are white, the mycelium is thick like velvet.	Colonies are light yellow.	
FRR 214	The colonies are brownish-white, the mycelium is thin, the edges of the colonies are smooth.	Colonies are brownish-white.	
FRR 215	Colonies are greenish-white, green granular, slightly granular.	Colonies are greenish-white.	
FRR 216	Colonies are green, the edges of the colonies are hyaline.	Colonies are yellowish-green.	
FRR 217	The center of the colony is blackish-white, the edge of the colony is white, the mycelium is thin.	Colonies are blackish-white.	
FRR 218	The center of the colony is light black, the edge of the colony is whitish-black, the mycelium is thick like velvet.	Colonies are black.	
FRR 219	The center of the colony is yellowish-green, the edge of the colony is yellowish-white.	Colonies are blackish-green.	
FRR 221	The center of the colony is light brown, the edges of the colony are hyaline.	The center of the colony is blackish-brown, the edge of the colony is white.	
FRR 222	The colonies are reddish-white, the edges of the colonies are white.	Colonies are reddish-white.	
FRR 223	The colonies are green, the edges of the colonies are white, the mycelium is thick like velvet.	Mycelium is dark green.	
FRR 224	Colonies are light brown, mycelium is thin.	Colonies are brown.	
FRR 225	The colonies are yellowish-white, the edges of	Colonies are yellow.	
	the colonies are white, the mycelium is thick.	-	
FRR 226	Colonies are greenish-white.	Colonies are brownish-white.	
FRR 227	Colonies are yellowish-white.	Colonies are brownish-white.	
FRR 228	Colonies are white, mycelium thickness is medium.	Colonies are yellowish-white.	
FRR 229	Colonies are blackish-white, thin mycelium.	Colonies are blackish-white.	
FRR 231	The colonies are green, many granular, the mycelium is thick like velvet, there is a	Colonies are green, there is a concentric pattern.	
FRR 232	concentric pattern. The center of the colony is black, the middle of the colony is light black, the edge of the colony is	Colonies are black, edges of colonies are light black.	

Fungus isolate	Characters on the upper side of the petri dish	Characters on the underside of the petri dish
FRR 233	white, the mycelium is thin. The colonies are white, the edges of the colonies are yellowish-brown.	Colonies are yellowish-white.

The purified fungal isolates from the rhizosphere were preserved in slant agar and stored at a temperature of 4°C, to be used in subsequent tests and as a source of PGPF.

Pathogenicity testing of fungal isolates on soybean seeds.

The pathogenicity testing of fungi on soybean seeds was conducted by sowing 10 soybean seeds on Petri dishes containing each fungal isolate, a total of 90 soybean seeds were tested for each fungal isolate. As a control, soybean seeds were sown in Petri dishes containing PDA media (Table 2).

	Table 2. Response of soybean seeds to each fungal isolate.			
Isolate Code	The seeds	Seeds germinate	Seeds germinate	The seeds do not
Isolate Code	germinate	normally	abnormally	germinate
Control	82 (91.11%)	82 (91.11%)	0 (0.00%)	8 (8.89%)
FRT 11	85 (94.44%)	85 (94.44%)	0 (0.00%)	5 (5.56%)
FRT 12	84 (93.33%)	84 (93.33%)	0 (0.00%)	6 (6.67%)
FRT 13	81 (90.00%)	80 (88.89%)	1 (1.11%)	9 (10.00%)
FRT 14	44 (48.89%)	32 (35.56%)	12 (13.33%)	46 (51.11%)
FRT 15	82 (91.11%)	82 (91.11%)	0 (0.00%)	8 (8.89%)
FRT 16	82 (91.11%)	81 (90.00%)	1 (1.11%)	8 (8.89%)
FRT 17	82 (91.11%)	82 (91.11%)	0 (0.00%)	8 (8.89%)
FRT 18	55 (61.11%)	23 (25.56%)	32 (35.56%)	35 (38.89%)
FRT 19	81 (90.00%)	79 (87.78%)	2 (2.22%)	9 (10%)
FRT 111	81 (90.00%)	80 (88.89%)	1 (1.11%)	9 (10%)
FRT 112	35 (38.89%)	23 (25.56%)	12 (13.33%)	55 (61.11%)
FRT 113	84 (93.33%)	84 (93.33%)	0 (0.00%)	6 (6.67%)
FRT 114	85 (94.44%)	85 (94.44%)	0 (0.00%)	5 (5.56%)
FRT 115	82 (91.11%)	81 (90%)	1 (1.11%)	8 (8.89%)
FRT 116	86 (95.56%)	86 (95.56%)	0 (0.00%)	4 (4.44%)
FRT 117	80 (88.89%)	78 (86.67%)	2 (2.22%)	10 (11.11%)
FRT 118	80 (88.89%)	77 (85.56%)	3 (3.33%)	10 (11.11%)
FRT 119	82 (91.11%)	82 (91.11%)	0 (0.00%)	8 (8.89%)
FRT 121	81 (90.00%)	79 (87.78%)	2 (2.22%)	9 (10.00%)
FRT 122	84 (93.33%)	84 (93.33%)	0 (0.00%)	6 (6.67%)
FRT 123	60 (66.67%)	39 (43.33%)	21 (23.33%)	30 (33.33%)
FRT 124	80 (88.89%)	78 (86.67%)	2 (2.22%)	10 (11.11%)
FRT 125	80 (88.89%)	79 (87.78%)	1 (1.11%)	10 (11.11%)
FRT 126	82 (91.11%)	81 (90.00%)	1 (1.11%)	8 (8.89%)
FRT 127	82 (91.11%)	82 (91.11%)	0 (0.00%)	8 (8.89%)
FRT 128	79 (87.78%)	78 (86.67%)	1 (1.11%)	11 (12.22%)
FRT 129	48 (53.33%)	37 (41.11%)	11 (12.22%)	42 (46.67%)
FRT 131	79 (87.78%)	79 (87.78%)	0 (0.00%)	11 (12.22%)
FRT 132	80 (88.89%)	79 (87.78%)	1 (1.11%)	10 (11.11%)
FRT 133	84 (93.33%)	84 (93.33%)	0 (0.00%)	6 (6.67%)
FRT 134	85 (94.44%)	85 (94.44%)	0 (0.00%)	5 (5.56%)
FRT 135	80 (88.89%)	79 (87.78%)	1 (1.11%)	10 (11.11%)
FRT 136	40 (44.44%)	29 (32.22%)	11 (12.22%)	50 (55.56%)
FRT 137	82 (91.11%)	82 (91.11%)	0 (0.00%)	8 (8.89%)
FRT 138	81 (90.00%)	80 (88.89%)	1 (1.11%)	9 (10.00%)
FRT 139	84 (93.33%)	84 (93.33%)	0 (0.00%)	6 (6.67%)
FRT 141	86 (95.56%)	86 (95.56%)	0 (0.00%)	4 (4.44%)

Jurnal Ilmiah Ilmu Terapan U	Universitas Jambi
------------------------------	-------------------

Isolate Code	The seeds	Seeds germinate	Seeds germinate	The seeds do not
Isolate Code	germinate	normally	abnormally	germinate
FRT 142	85 (94.44%)	85 (94.44%)	0 (0.00%)	5 (5.56%)
FRT 143	45 (50.00%)	38 (42.22%)	7 (7.78%)	45 (50.00%)
FRT 144	81 (90.00%)	81 (90.00%)	0 (0.00%)	9 (10.00%)
FRT 145	82 (91.11%)	82 (91.11%)	0 (0.00%)	8 (8.89%)
FRT 146	81 (90.00%)	80 (88.89%)	1 (1.11%)	9 (10.00%)
FRT 147	80 (88.89%)	80 (88.89%)	0 (0.00%)	10 (11.11%)
FRR 21	84 (93.33%)	84 (93.33%)	0 (0.00%)	6 (6.67%)
FRR 22	82 (91.11%)	81 (90%)	1 (1.11%)	8 (8.89%)
FRR 23	85 (94.44%)	85 (94.44%)	0 (0.00%)	5 (5.56%)
FRR 24	80 (88.89%)	80 (88.89%)	0 (0.00%)	10 (11.11%)
FRR 25	81 (90.00%)	80 (88.89%)	1 (1.11%)	9 (10.00%)
FRR 26	82 (91.11%)	81 (90.00%)	1 (1.11%)	8 (8.89%)
FRR 27	40 (44.44%)	21 (23.33%)	19 (21.11%)	50 (55.56%)
FRR 28	79 (87.78%)	79 (87.78%)	0 (0.00%)	11 (12.22%)
FRR 29	79 (87.78%)	78 (86.67%)	1 (1.11%)	11 (12.22%)
FRR 211	20 (22.22%)	12 (13.33%)	8 (8.89%)	70 (77.78%)
FRR 212	79 (87.78%)	79 (87.78%)	0 (0.00%)	11 (12.22%)
FRR 213	80 (88.89%)	79 (87.78%)	1 (1.11%)	10 (11.11%)
FRR 214	86 (95.56%)	86 (95.56%)	0 (0.00%)	4 (4.44%)
FRR 215	85 (94.44%)	85 (94.44%)	0 (0.00%)	5 (5.56%)
FRR 216	81 (90.00%)	80 (88.89%)	1 (1.11%)	9 (10.00%)
FRR 217	81 (90.00%)	81 (90.00%)	0 (0.00%)	9 (10.00%)
FRR 218	45 (50.00%)	40 (44.44%)	5 (5.56%)	45 (50.00%)
FRR 219	81 (90.00%)	81 (90.00%)	0 (0.00%)	9 (10.00%)
FRR 221	56 (62.22%)	46 (51.11%)	10 (11.11%)	34 (37.78%)
FRR 222	80 (88.89%)	79 (87.78%)	1 (1.11%)	10 (11.11%)
FRR 223	44 (48.89%)	20 (22.22%)	24 (26.67%)	46 (51.11%)
FRR 224	82 (91.11%)	82 (91.11%)	0 (0.00%)	8 (8.89%)
FRR 225	82 (91.11%)	81 (90.00%)	1 (1.11%)	8 (8.89%)
FRR 226	82 (91.11%)	82 (91.11%)	0 (0.00%)	8 (8.89%)
FRR 227	86 (95.56%)	86 (95.56%)	0 (0.00%)	4 (4.44%)
FRR 228	82 (91.11%)	80 (88.89%)	2 (2.22%)	8 (8.89%)
FRR 229	14 (15.56%)	4 (4.44%)	10 (11.11%)	76 (84.44%)
FRR 231	84 (93.33%)	84 (93.33%)	0 (0.00%)	6 (6.67%)
FRR 232	85 (94.44%)	85 (94.44%)	0 (0.00%)	5 (5.56%)
FRR 233	82 (91.11%)	81 (90.00%)	1 (1.11%)	8 (8.89%)

Various colors of fungal isolates are observed, including white, greenish-white, yellowishwhite, brownish-white, blackish-white, reddish-white, green, light green, yellowish-green, light yellow, light brown, brown, black, and light black. The dominant colony color is white, followed by green, yellow brown, and the least color is black. Some colonies form granules (16 isolates); these granules are either evenly distributed on the medium or grouped. Typically, the granules are green, and their quantity varies from few, to moderate, to numerous. The thickness of the mycelium also varied, ranging from thick, velvety mycelium to those with thin mycelium.

Based on the pathogenicity tests conducted on the 73 fungal isolates obtained from the rhizosphere of plants, it is known that there are fungal isolates that can inhibit seed germination or cause abnormal seed germination. Some fungal isolates do not affect seed germination, in this case, seed germination is the same as the control (without fungal isolates). Additionally, there are fungal isolates that can increase seed germination and increase normal seed germination. It can be said that the interaction between fungi and soybean seeds can either reduce, enhance, or have no effect on soybean seed germination. Fungal isolates that can increase seed germination and increase seed germination and increase normal seed germination have the potential as PGPF. The results of this study align with Mohamed et al., (2022),

that fungi in the rhizosphere can increase the percentage of seed germination, these isolates demonstrate potential as PGPF.

The research obtained 18 fungal isolates (FRT 11, FRT 12, FRT113, FRT 114, FRT 116, FRT 122, FRT 133, FRT 134, FRT 139, FRT 141, FRT 142, FRR 21, FRR 23, FRR 214, FRR 215, FRR 227, FRR 231, and FRR 232) that could increase the germination percentage and the percentage of normal seed germination. The percentage of seed germination and the percentage of normal seed germinated to 93.33% - 95.56%, while in the control it was 91.11%. Abnormal germinated seeds were 0%. Non-germinated seeds decreased to 4.44%-6.67%, while in the control it was 8.89%. These 18 fungal isolates have the potential as PGPF candidates. In previous research, Asniwita & Hayati (2017) obtained 65 endophytic fungal isolates from the roots, stems, and leaves of chili plants. Among of the 65 isolates there are 13 isolates that could increase the percentage of chili seed germination and normal seed germination compared to the control. Mirta (2023) obtained 40 isolates of cocoa and mahogany rhizosphere fungi in agroforestry land, 28 isolates of fungi were pathogenic, while 12 isolates were non-pathogenic.

PGPF have the potential to increase plant growth. These fungi can colonize roots and solubilize phosphate (Begum et al., 2019; Cycoń et al., 2019). Seed treatment with PGPF can accelerate and increase germination and seedling vigor in sunflowers (Nagaraju et al., 2012), soybeans (Islam et al., 2011), and tomatoes (Jogaiah et al., 2013), chili (Asniwita & Hayati, 2017), cucumber (Halo et al., 2018; Syamsia et al., 2021), sugarcane (Sektiono et al., 2023). PGPF can produce various metabolites, including indole acetic acid (IAA), gibberellins (Khan et al., 2017), cytokinins, abscisic acid, salicylic acid (SA), jasmonic acid (JA), ethylene (Egamberdieva et al., 2021), more solubilizers (Jogaiah et al., 2020), hydrolytic enzymes, cellulolytic (Brazhnikova et al., 2021), phosphate solubilizers (Jogaiah et al., 2013), siderophore (Ghosh et al., 2017), β -1,3-glucanase, and peptaibols, which can stimulate plant growth and control diseases (El-Maraghy et al., 2020).

PGPF represents a diversification effort in utilizing active ingredients for biopesticide raw materials. As a biopesticide and biofertilizer, PGPF is profitable from an economic point of view (El-Saadony et al., 2022). We are optimistic that the PGPF obtained in this research can serve as a source of new biological control agents and potential biofertilizers, ultimately reducing disease control and fertilizer expenses. This is especially applicable in sustainable and environmentally friendly agricultural practices.

This groundbreaking study ventures into uncharted territory to explore the vast potential of Plant Growth Promoting Fungi (PGPF) as multifaceted allies in agricultural sustainability. Departing from traditional paradigms, the research sets out to identify and characterize non-pathogenic fungal isolates with the capacity to serve as potent PGPF agents. Employing a pioneering approach, fungal isolates are meticulously collected from the rhizosphere of plants, heralding a new era of ecological exploration at the microorganism level. Implication: The findings of this study have profound implications for agricultural sustainability and environmental stewardship. By demonstrating the efficacy of PGPF isolates in promoting plant growth and resilience, the research highlights the potential for these microorganisms to reduce reliance on synthetic inputs, such as chemical fertilizers and pesticides. This, in turn, can lead to a more sustainable and environmentally friendly approach to agriculture, with benefits for both farmers and the ecosystem. Building on the insights gained from this study, future research should focus on further elucidating the mechanisms underlying the plant growthpromoting effects of PGPF isolates. Additionally, efforts should be made to scale up production methods for these beneficial microorganisms and explore their integration into existing agricultural practices. Collaboration between researchers, farmers, and policymakers is essential to ensure the successful adoption of PGPF-based strategies for sustainable agriculture. The Limitation of this study represents a significant advancement in our understanding of PGPF, it is not without limitations. The research focused primarily on fungal isolates collected from a specific geographic region and may not capture the full diversity of PGPF present worldwide. Furthermore, the efficacy of PGPF isolates may vary depending on environmental conditions and plant species, necessitating further research to validate their effectiveness across different agricultural contexts.

CONCLUSION

Based on the research conducted, 73 indigenous fungal isolates were obtained from the rhizosphere of plants. Out of these 73 isolates, 18 isolates were identified to increase seed germination and increase normal seedling. These 18 fungal isolates have potential as PGPF and can be further

utilized as candidates for biofertilizers and biological control agents (biopesticide), and can be combined with other compatible control techniques in integrated disease management.

ACKNOWLEDGMENTS

The authors would like to thank fellow authors and parties involved for their contributions and cooperation so that this research can be carried out properly. The authors gratefully acknowledge The Ministry of Education, Culture, Research, and Technology Indonesia for the financial provision of this research through the 'Penelitian Dasar Unggulan Universitas' scheme.

AUTHOR CONTRIBUTIONS

Conceptualization, A.A.; Methodology, A.A., N.N., M.S. and A.V.B; Software, A.A.; Validation, A.A., N.N., M.S., A.V.B. and B.O.O.; Formal Analysis, A.A. and A.V.B; Investigation, A.A., N.N., M.S. and A.V.B; Resources, A.A.; Data Curation, A.A. and M.S; Writing – Original Draft Preparation, A.A.; Writing – Review & Editing, A.A., A.V.B. and B.O.O; Visualization, A.A.; Supervision, A.A.; Project Administration, A.A., A.V.B ; Funding Acquisition, A.A., N.N. and M.S.

CONFLICTS OF INTEREST

The author(s) declare no conflict of interest.

REFERENCES

- Adedayo, A. A., & Babalola, O. O. (2023). Fungi That Promote Plant Growth in the Rhizosphere Boost Crop Growth. *Journal of Fungi*, 9(2). <u>https://doi.org/10.3390/jof9020239</u>
- Asniwita. (1989). Pengujian waktu pemberian Trichoderma harzianum Rifai pada tanah yang terinfestasi Phytophthora parasitica penyebab penyakit damping off pada bibit tembakau (Nicotiana tabacum L). Universitas Andalas.
- Asniwita. (2016). Pemanfaatan cendawan endofit isolat lokal untuk meningkatkan produksi dan menginduksi ketahanan tanaman cabai terhadap infeksi virus. Universitas Jambi
- Asniwita, & Hayati, I. (2017). Eksplorasi Cendawan Endofit Isolat Lokal dan Pengaruhnya terhadap Perkecambahan Benih Cabai (*Capsicum annuum*). Jurnal Ilmiah Ilmu Terapan Universitas Jambi, 1(2), 178–184. <u>https://doi.org/10.22437/jiituj.v1i2.4280</u>
- Begum, N., Qin, C., Ahanger, M. A., Raza, S., Khan, M. I., Ashraf, M., Ahmed, N., & Zhang, L. (2019). Role of Arbuscular Mycorrhizal Fungi in Plant Growth Regulation: Implications in Abiotic Stress Tolerance. *Frontiers in Plant Science*, 10(September), 1–15. <u>https://doi.org/10.3389/fpls.2019.01068</u>
- Brazhnikova, Y., Ignatova, L., Omirbekova, A., Mukasheva, T., Kistaubayeva, A., Savitskaya, I., Egamberdieva, D., Usmanova, A., & Batlutskaya, I. (2021). Effect of plant growth promotion fungi on agricultural crops. *BIO Web of Conferences*, 40. https://doi.org/10.1051/bioconf/20214001004
- Cycon, M., Mrozik, A., & Piotrowska-Seget, Z. (2019). Antibiotics in the soil environment degradation and their impact on microbial activity and diversity. *Frontiers in Microbiology*, 10(MAR). <u>https://doi.org/10.3389/fmicb.2019.00338</u>
- Doilom, M., Guo, J. W., Phookamsak, R., Mortimer, P. E., Karunarathna, S. C., Dong, W., Liao, C. F., Yan, K., Pem, D., Suwannarach, N., Promputtha, I., Lumyong, S., & Xu, J. C. (2020). Screening of Phosphate-Solubilizing Fungi From Air and Soil in Yunnan, China: Four Novel Species in Aspergillus, Gongronella, Penicillium, and Talaromyces. *Frontiers in Microbiology*, 11(October), 1–24. https://doi.org/10.3389/fmicb.2020.585215
- Egamberdieva, D., Wirth, S. J., Alqarawi, A. A., Abd-Allah, E. F., & Hashem, A. (2017). Phytohormones and beneficial microbes: Essential components for plants to balance stress and fitness. *Frontiers in Microbiology*, 8(OCT), 1–14. <u>https://doi.org/10.3389/fmicb.2017.02104</u>
- El-Maraghy, S. S., Tohamy, A. T., & Hussein, K. A. (2020). Role of plant-growth promoting fungi (PGPF) in defensive genes expression of Triticum aestivum against wilt disease. *Rhizosphere*, 15. <u>https://doi.org/https://doi.org/10.1016/j.rhisph.2020.100223</u>
- El-Maraghy, S. S., Tohamy, A. T., & Hussein, K. A. (2021). Plant protection properties of the Plant Growth-Promoting Fungi (PGPF): Mechanisms and potentiality. *Current Research in Environmental and Applied Mycology*, 11(July), 391–415. <u>https://doi.org/10.5943/CREAM/11/1/29</u>

- El-Saadony, M. T., Saad, A. M., Soliman, S. M., Salem, H. M., Ahmed, A. I., Mahmood, M., El-Tahan, A. M., Ebrahim, A. A. M., Abd El-Mageed, T. A., Negm, S. H., Selim, S., Babalghith, A. O., Elrys, A. S., El-Tarabily, K. A., & AbuQamar, S. F. (2022). Plant growth-promoting microorganisms as biocontrol agents of plant diseases: Mechanisms, challenges and future perspectives. *Frontiers in Plant Science*, 13(October), 1–19. https://doi.org/10.3389/fpls.2022.923880
- Ghosh, S. K., Banerjee, S., & Sengupta, C. (2017). Bioassay, characterization and estimation of siderophores from some important antagonistic fungi. *JBiopest*, *10*(2), 105–112.
- Halo, B. A., Al-Yahyai, R. A., & Al-Sadi, A. M. (2018). Aspergillus terreus inhibits growth and induces morphological abnormalities in *Pythium aphanidermatum* and suppresses pythiuminduced damping-off of cucumber. *Frontiers in Microbiology*, 9(FEB), 1–12. https://doi.org/10.3389/fmicb.2018.00095
- Heimpel, G. E., & Mills, N. (2017). Biological Control: Ecology and Application. Cambridge University Press. <u>https://doi.org/10.1017/9781139029117</u>
- Hyde, K. D., Xu, J., Rapior, S., Jeewon, R., Lumyong, S., Niego, A. G. T., Abeywickrama, P. D., Aluthmuhandiram, J. V. S., Brahamanage, R. S., Brooks, S., Chaiyasen, A., Chethana, K. W. T., Chomnunti, P., Chepkirui, C., Chuankid, B., de Silva, N. I., Doilom, M., Faulds, C., Gentekaki, E., ... Stadler, M. (2019). The amazing potential of fungi: 50 ways we can exploit fungi industrially. *Fungal Diversity*, 97(1), 1–136. https://doi.org/10.1007/s13225-019-00430-9
- Islam, M., Rahman, M., Bulbul, S., & Alam, M. (2011). Effect of Trichoderma on Seed Germination and Seedling Parameters in Chili. Int. J. Expt. Agric, 2(1–2), 21–26. <u>https://doi.org/10.3329/jsf.v8i1-2.14637</u>
- Jahagirdar, S., Kambrekar, D., Navi, S., & Kunta, M. (2019). Plant Growth-Promoting Fungi: Diversity and Classification. In *Bioactive Molecules in Plant Defense: Signaling in Growth and Stress*. Springer Nature. <u>https://doi.org/10.1007/978-3-030-27165-7</u>
- Jogaiah, S., Abdelrahman, M., Tran, L. S. P., & Shin-Ichi, I. (2013). Characterization of rhizosphere fungi that mediate resistance in tomato against bacterial wilt disease. *Journal of Experimental Botany*, 64(12), 3829–3842. <u>https://doi.org/10.1093/jxb/ert212</u>
- Khan, A. L., Gilani, S. A., Waqas, M., Al-Hosni, K., Al-Khiziri, S., Kim, Y. H., Ali, L., Kang, S. M., Asaf, S., Shahzad, R., Hussain, J., Lee, I. J., & Al-Harrasi, A. (2017). Endophytes from medicinal plants and their potential for producing indole acetic acid, improving seed germination and mitigating oxidative stress. *Journal of Zhejiang University: Science B*, 18(2), 125–137. <u>https://doi.org/10.1631/jzus.B1500271</u>
- Lau, J. A., & Lennon, J. T. (2011). Evolutionary ecology of plant-microbe interactions: Soil microbial structure alters selection on plant traits. *New Phytologist*, 192(1), 215–224. <u>https://doi.org/10.1111/j.1469-8137.2011.03790.x</u>
- Malgioglio, G., Rizzo, G. F., Nigro, S., du Prey, V. L., Herforth-Rahmé, J., Catara, V., & Branca, F. (2022). Plant-Microbe Interaction in Sustainable Agriculture: The Factors That May Influence the Efficacy of PGPM Application. *Sustainability (Switzerland)*, 14(4), 1–28. https://doi.org/10.3390/su14042253
- Mendes, R., Garbeva, P., Jos, M., & Raaijmakers. (2013). The rhizosphere microbiome: significance of plant beneficial, plant patogenic, and human patogenic microorganisms. *FEMS Microbiol Rev*, 37, 634–663. <u>https://doi.org/10.1111/1574-6976.12028</u>
- Mirta, B. (2023). Eksplorasi Plant Growth Promoting Fungi (PGPF) pada Tutupan Lahan Agroforestri serta Uji Efektivitasnya terhadap Persemaian Bibit Cabai Merah (Capsicum annum L.). Thesis. Universitas Hasanuddin.
- Mohamed, A. H., Abd El-Megeed, F. H., Hassanein, N. M., Youseif, S. H., Farag, P. F., Saleh, S. A., Abdel-Wahab, B. A., Alsuhaibani, A. M., Helmy, Y. A., & Abdel-Azeem, A. M. (2022). Native Rhizospheric and Endophytic Fungi as Sustainable Sources of Plant Growth Promoting Traits to Improve Wheat Growth under Low Nitrogen Input. *Journal of Fungi*, 8(2). <u>https://doi.org/10.3390/jof8020094</u>
- Murali, M., Sudisha, J., Amruthesh, K., Ito, S., & Shekar Shetty, H. (2013). Rhizosphere fungus *Penicillium chrysogenum* promotes growth and induces defence-related genes and downy mildew disease resistance in pearl millet. *Plant Biol*, 15, 111–118. <u>https://doi.org/10.1111/j.1438-8677.2012.00617.x</u>

- Nagaraju, A., Sudisha, J., Mahadevamurthy, S., & Ito, S. (2012). Seed priming with *Trichoderma harzianum* isolats enhances plant growth and induces resistance against *Plasmopara halstedii*, an incitant of sunflower downy mildew disease. *Australasian J Plant Pathol*, 41, 609–620. https://doi.org/10.1007/s13313-012-0165-z
- Naziya, B., Murali, M., & Amruthesh, K. N. (2020). Plant growth-promoting fungi (PGPF) instigate plant growth and induce disease resistance in *Capsicum annuum* L. upon infection with *Colletotrichum capsici* (syd.) butler & bisby. *Biomolecules*, 10(1), 4–6. <u>https://doi.org/10.3390/biom10010041</u>
- Schnitzer, S., Klironomos, J., & Lambers, J. (2011). Soil microbes drive the classic plant diversity– productivity pattern. *Ecology*, 92(2), 296–303. <u>https://doi.org/10.1890/10-0773.1</u>
- Sektiono, A. W., Habtuti, N., Sandy, Y. A., & Setiawan, Y. (2023). Potential of Endophytic Fungi as Plant Growth-Promoting Fungi (PGPF) Against Growth of Single Bud Set Seedlings on Sugarcane Plants (Saccharum officinarum L). PLANTROPICA: Journal of Agricultural Science, 008(1), 71–79. https://doi.org/10.21776/ub.jpt.2023.008.1.8
- Silva, J. M. da, Montaldo, Y. C., Almeida, A. C. P. S. de, Dalbon, V. A., Acevedo, J. P. M., Santos, T. M. C. dos, & Lima, G. S. de A. (2021). Rhizospheric Fungi to Plant Growth Promotion: A Review. *Journal of Agricultural Studies*, 9(1), 411. <u>https://doi.org/10.5296/jas.v9i1.18321</u>
- Syamsia, S., Idhan, A., Firmansyah, A. P., 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(3), 1194–1202. https://doi.org/10.13057/biodiv/d220315
- Zhang, C., Zhang, Y., Ding, Z., & Bai, Y. (2019). Contribution of Microbial Inter-kingdom Balance to Plant Health. *Molecular Plant*, 12(2), 148–149. <u>https://doi.org/10.1016/j.molp.2019.01.016</u>