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Research article

Indigenous fungi from corn as a potential plant growth promoter and its role in *Fusarium verticillioides* suppression on corn

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ABSTRACT

Indigenous fungi can suppress infection by pathogens and produce secondary metabolites that directly or indirectly affect plant growth. This study aimed to test indigenous fungi collected from corn plants as biological control agents and their effects on the viability and vigor of corn seeds. Purposive sampling method was used for sampling where soil samples taken from the rhizosphere zone, corn stem and leaf tissue from three locations namely Maros-South Sulawesi, Bone-South Sulawesi, Sigi-Central Sulawesi, Indonesia. Rhizospheric fungi were isolated from soil collected at the rhizosphere and rhizoplane using a serial dilution technique, while the endophytic fungi isolated from the leaves and stem tissues using surface sterilization method. The isolated fungi were cultured on a potato dextrose agar (PDA) medium. An antagonism test was performed using the dual culture method on PDA media with *F. verticillioides* as target pathogen. Pathogenicity test and the effect of fungi on corn seed germination was carried out using the blotter test method. Parameters observed were; necrotic symptoms on seedlings, growth potential, germination, growth rate, growth simultaneity, vigor index, germination rate, and time needed for 50% of the total germination. The effect of the isolated indigenous fungi on corn growth was carried out *in-planta* using seedling trays. The results of the blotter test and *in-planta* test were further confirmed by a physiological characteristic test. And assessing the fungi's ability to dissolve potassium, phosphate, and produce protease enzymes. A total of 89 fungal isolates were isolated and collected from various parts of the corn plant. Nineteen of the 89 fungal isolates showed inhibitory activity *against F. verticillioides* by $\geq$ 50% inhibition. The fungal isolates JRP 5 MRS, JRP 9 MRS, JRP 10 MRS, JRP 7 MRS, and JEDF 1B BN were selected based on the tests and showed a consistently positive effect on seed viability and vigor with a value of $\geq$90%. The isolates did not cause necrosis in corn, and had the ability to suppress the growth of pathogenic *F. verticillioides* by $\geq$ 50%.

1. Introduction

Corn is the second most important cereal crop in the world after wheat, rice and other food sources, accounting for 94% of all cereal consumption (FAO 2012; Awata et al., 2019). The demand for corn for consumption in developing countries is expected to increase by 1.3% annually until 2020 (Ortiz et al., 2010). Also, it is estimated that by 2050, the demand for corn will increase to reach 3.3 billion tons, and globally in developing countries, corn will become the highest production crop by 2025 (FAO 2016). The Ministry of Agriculture of Indonesia (2020)

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reported an average increase in the national corn production of 18,840, 000 and 19,650,001 tons in 2018 and 2019, respectively. And in 2022, it is estimated to increase to 22,317,194 tons.

Around 47 known diseases have been reported to attack corn. Seven major diseases, include downy mildew (*Peronosclerospora sp.*), leaf blight (*Bipolaris maydis*), leaf rust (*Puccinia polysora*), gray leaf spot (*Cercospora zeae-maydis*), bacterial stalk rot (*Dickeya zeae*), Fusarium stalk rot (*Fusarium verticillioides*), and banded leaf and sheath blight (*Rhizoctonia solani*) have been recorded in recent years as the main diseases limiting the production of corn in Indonesia (CIMMYT Maize Program 2004; Muis et al., 2013; Soenartiningsih et al., 2015; Suriani and Muis 2016). One limiting factor for corn cultivation globally is plant pathogens, which can cause yield losses of about 11% of total production (Suleiman and Omafe 2013). Tsedaley and Adugna (2016) reported that fungal pathogens could cause yield loss of about 50–80% during the storage period if conditions are favorable for pathogen development. The high incidence of fungal contamination in the samples suggest the adaptation of fungal spores in dry or humid conditions during storage (Chandra et al., 2018).

Over the last few years, stem and cob rot caused by *Fusarium verticillioides* have been problematic to farmers in Indonesia (Pakki 2016; Suriani et al., 2018). This pathogen causes obstruction of the transport of nutrients into plant tissues such that the plant growth is inhibited and also affects filling and rotting of cobs (Suriani et al., 2018; Zainuddin et al., 2017). Severe cases cause the plant to die completely. Symptoms of fungal transmission on corn seeds are observed by the presence of white mycelia threads, followed by a change in the color of the seeds from pink to brown. But, usually it does not affect the entire seeds on the cob (Duncan and Howard 2010; Parsons and Munkvold 2012). Appropriate pathogen control technology needs to be implemented to reduce the decrease in corn production caused by *F. verticillioides* infection.

Control of the *F. verticilloides* pathogen is difficult because this fungus can survive for a long time in the soil by forming spores (chlamydospores), even when environmental conditions are unfavorable especially the absence of host plants (Sudantha 2010). Utilization of several alternative disease control can suppress the development of pathogens. One of the alternatives control method is the use of biopesticides in the form of antagonist microbe against the plant pathogens. A study on the use of microbial antagonists has provided recommendations for potential organisms as a substitute for synthetic pesticides. The use of microbial antagonists generally does not have a negative impact on the environment compared to synthetic fungicides (Wartono et al., 2012). This microbe acting as a biocontrol is not only more economical than synthetic fungicides; it is also eco-friendly (Chandra et al., 2020).

In addition to controlling pathogens, increasing crop production needs to be considered. One factor in increasing corn production is the availability of high-quality seeds. High quality seeds are not only determined by the seeds' health, but also by their vigor and viability. High quality seeds can be obtained by preparing and treating them with various nutrients, growth regulators, cultural control, or functional microbes that can act as plant growth inducers (Sutariati 2012; Agustiansyah et al., 2013; Arinasa 2016; Marpaung and Hutabarat 2016). Functional microbes can be native fungi that live permanently and associated with plants both in the root area and in plant tissues. Indigenous fungi are one of innate microbial that inhabits the soil and the surfaces of all living things inside and outside which have the potentiality in biodegradation, bioleaching, biocomposting, nitrogen fixation, improving soil fertility and as well as in the production of plant growth hormones (Kumar and Gopal 2015).

Some fungi associated with plants either live inside plant tissues (i.e endophyte fungal) or in the rhizosphere of plants with the abilities to control plant pathogens and increase plant growth, such fungi are called plant growth-promoting fungi (PGPF) (Murali et al., 2012). Endophytic and rhizospheric fungi are beneficial microbes capable of producing secondary metabolites, which either directly or indirectly affect the growth of their host plants (Agusta 2009; Mirsam et al., 2015).

In recent years, there is increasing trend of research particularly the use of microbes as biological control agents and plant growth inducers. The use of rhizospheric and endophytic microbes in controlling several types of plant pathogens and their effects on plant resistance levels have been widely studied, both from bacteria and fungi (Mirsam et al. 2015, 2016; Damayanti 2013; Singh et al., 2013). However, information regarding the use of antagonistic indigenous fungi in inducing corn seed germination is limited particular in Sulawesi regions. Therefore, this study aimed to test indigenous fungi collected from corn plant as biological control agents and their effects on the viability and vigor of corn seeds.

2. Materials and methods

2.1. Description of the sampling areas and samples collection

The survey and samples collected were from several corn farm in Maros District-South Sulawesi, Bone District-South Sulawesi, Sigi District-Central Sulawesi, Indonesia (Figure 1). Maros District-South Sulawesi is located in the western part of South Sulawesi between 40°45′-50°07′ S and 109°205′-129°12′ E. Soil types in Maros are dominated by alluvial soils usually gray, brown or black (CBS Maros, 2018). The second location, Bone District-South Sulawesi is located at 4°13' – 5°6′ S and between 119°42′-120°30′ E. Throughout the year, the humidity ranges from 77–86% and the temperature between 24.4°C-27.6 °C. Soil type is dominated by Mediterranean soil (67.6%), Renzina (9.59%), and Litosol (9%) (CBS Bone, 2018). The third location, Sigi District-Central Sulawesi is located between 0°.36″-0°.56″ S and 119°.45" – 121°.1″ E, positioned at the equator line with an altitude of 0–700 m above sea level (ASL). The temperature in Sigi ranges from 23 °C–36.5 °C, with the lowest temperature occurring in January while the highest temperature in October. The average air temperature recorded at the Mutiara Palu Meteorological Station reached 34.3 °C with relative humidity ranging from 64.7 to 78.8%. The soil type is dominated by alluvial soil (CBS Palu, 2018).

Sampling was carried out twice at each study location in February–May 2020 (specify the different sample period, for example from February–March and March–May 2020). Purposive sampling was done by selecting samples based on specific criteria for healthy plants. Purposive sampling was used to select healthy/healthier corn compared to other corn around. That indicated that the corn was healthy because of the influence of biotic factors associated with the corn. The soil in the rhizosphere zone of the plant, stem and leaf tissue of the corn were taken as samples. Samples collected were stored in separate plastic bags and immediately transported to the laboratory using a cool box (Mirsam et al., 2021).

2.2. Fungi isolation

Soil from the rhizosphere (i.e root zone) and rhizoplane (i.e soil attached to the root surface) of corn was taken and weighed 10 g each. The soil was put into Erlenmeyer flasks and 90 ml of distilled water (DW) was added and then homogenized for 30 min using a vortex. One millilitre of the homogenized suspension was diluted to 10^{-4}, 0.1 ml of the suspension was cultured on potato dextrose agar (PDA) medium.

Endophytic fungi isolation was carried out by surface sterilization method reported by Mirsam et al. (2016) with modified NaOCl concentrations. The leaves and stems of the plant were cut into smaller pieces approximately 1–2 cm size and washed under flowing water to remove dirt. Later, the surface sterilization was done t by soaking the samples in 2% NaOCl solutions for 2 min, 70% alcohol for 2 min, then rinsed with DW 3 times. Then the samples were cultured in PDA and observed after 3–5 days. Next the fungi grown on PDA were isolated and cultured on new PDA based on the characteristic of the shape and colour of the colony.

The fungal isolates obtained previously were then re-cultured on inclined PDA media using test tubes as stock, and incubated at 28 °C for 7

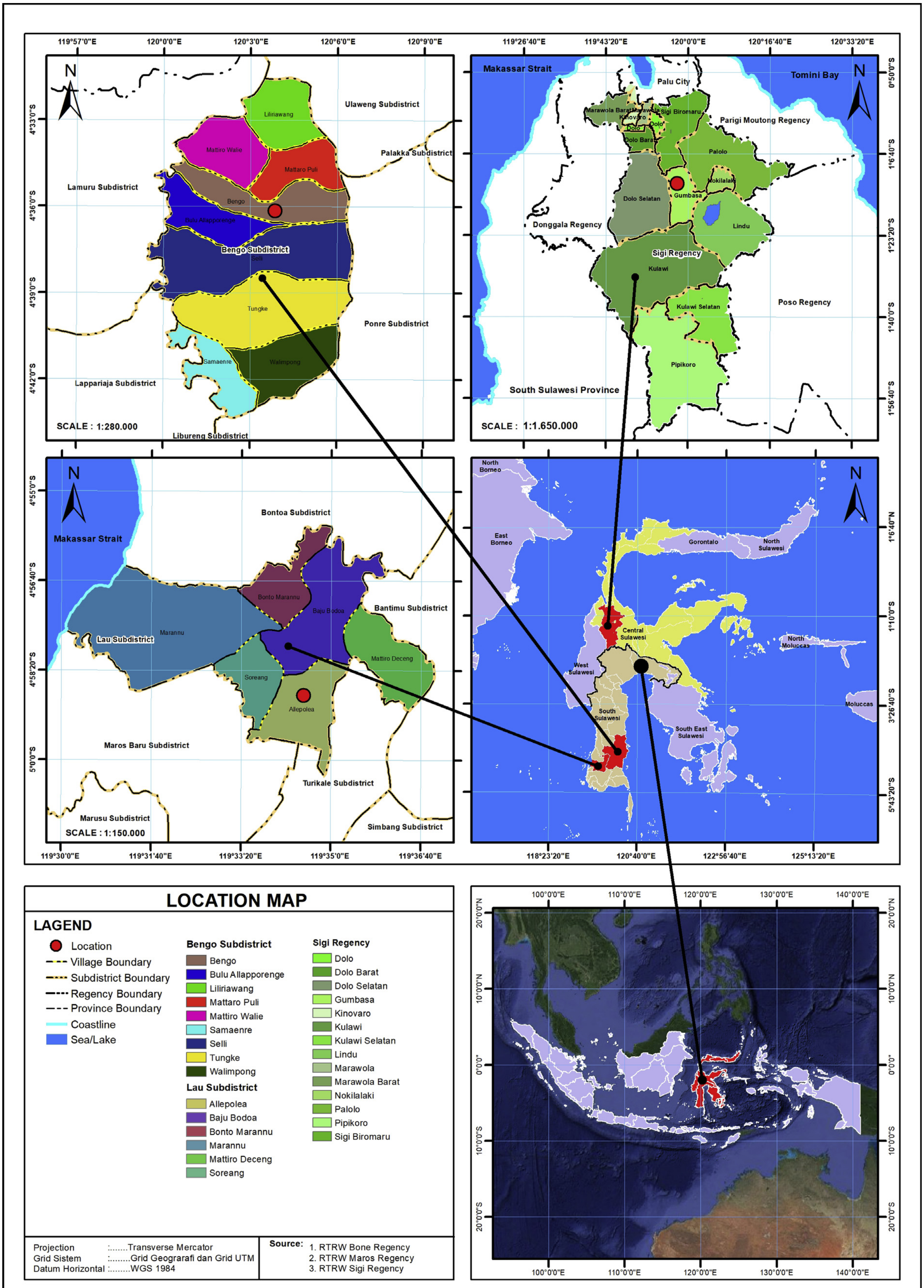

Figure 1. Location of collected samples from corn plantations in three locations: Maros District-South Sulawesi, Bone District-South Sulawesi, Sigi District-Central Sulawesi, Indonesia.

days. After the fungus filled the inclined PDA media, it was stored in a refrigerator at 4 °C and later used as stock for the next test.

2.3. Fungi antagonism test with the dual culture method

The fungal antagonism test was carried out using a dual culture technique on PDA media against *F. verticillioides*. The pathogenic fungi isolates were obtained from the Plant Pathology Laboratory of the Indonesian Cereals Research Institute (ICERI). The isolated potential fungi were placed at 2.25 cm at the edge on the PDA in the Petri dish using Each pathogen isolate was placed on the opposite side. Observations were made on the inhibition zone on the seventh day and the inhibition zone was calculated using Eq. (1) (Mirsam et al., 2015) (see Figure 2).

$$\text{Inhibition Percentage } (\%) = \frac{R1 - R2}{R1} \tag{1}$$

2.4. Fungi pathogenicity test and its effect on corn seed germination

Pathogenicity test was carried out using the blotter test method according to the standard method determined by the International Seed Testing Association (ISTA) (ISTA 2018) was modified using PDA media. This method was a practical test method, simple and capable of detecting isolates of pathogenic or non-pathogenic fungi, although they did not produce spores or conidia. Fungi isolates were tested for their pathogenicity using corn seed (Anoman variety) sprouts as the indicator. The surface sterilization of corn seeds was done based on the method described by Matic et al. (2014) with modified temperature and time. The corn seeds were surface sterilized with 2% NaOCL solution for 5 min, 70% alcohol for 2 min, and rinsed with DW 3 times. Furthermore, the seeds were given a hot water treatment by immersing them in DW at 55 °C for 20 min. Later the seeds were dried on sterile tissue paper and ten seeds were arranged on top of 7-day-old indigenous fungi in a Petri dish covered with sterile heat-resistant plastic. The seeds were incubated for one week at room temperature (specify the room temperature). Control seeds were grown on sterile PDA media. Observations were made by calculating the percentage of normal seed sprouts. Fungal isolates that cause abnormal sprouts and necrotic symptoms were isolates that were pathogenic and/or potentially pathogenic. Furthermore, the effect of isolates on seed viability and vigor was established using the following parameters:

2.4.1. Growth potential (GP)

GP is the percentage calculated based on the number of seeds grown on the 7th day of observation of the tested seeds using Eq. (2).

$$GP = \frac{\sum \text{germinated seeds}}{\sum \text{seeds grown}} \times 100\% \tag{2}$$

2.4.2. Seed germination (G)

Normal germination percentage indicates the potential viability of seeds, it was calculated as the percentage of normal seedling at the first observation (5^{th} day) and the second observation (7^{th} day) after the seeds were grown (Equation 3).

$$G = \frac{\sum \text{1st observation} + \sum \text{2nd observation}}{\sum \text{seeds grown}} \times 100\% \tag{3}$$

2.4.3. Growth rate (GtR) (%/et mal)

The observation of growth rate (GR) for normal seedlings was carried out every day and is calculated using Eq. (4).

$$GtR = \frac{n1}{D1} + \frac{n2}{D2} + \ldots + \frac{n7}{D7} \tag{4}$$

n = percentage of normal seedlings per observation (%)
D = observation time/24 h (etmal)

2.4.4. Growth simultaneity (GS)

The Growth Simultaneity (GS) was carried out on the 6th day after seeds germinated (Equation 5).

$$GS = \frac{\sum \text{normal seedlings during 1st and 2nd observation}}{\sum \text{seeds grown}} \times 100\% \tag{5}$$

2.4.5. Vigor index (%)

The Vigor Index (VI) assessment was carried out by calculating the percentage of normal seedlings that appeared on the first observation (5^{th} day) using Eq. (6).

$$VI = \frac{\sum \text{normal seedlings}}{\sum \text{seeds grown}} \times 100\% \tag{6}$$

2.4.6. Germination rate (GR)

The germination rate was measured by observing the number of days the seeds was needed to germinate (Equation 7).

$$\mathbf{GR} = \frac{N1T1 + N2T2 + \ldots + NxTx}{\sum \text{germinated seeds}} \tag{7}$$

N is the number of seeds germinated each day; T is the amount of time between the start of the test and the end of the specified interval of observation.

2.4.7. Median germination time (T50)

T50 is the time needed to reach 50% of total germination, measured by counting the number of seeds germinated each day. T50 was calculated using Eq. (8).

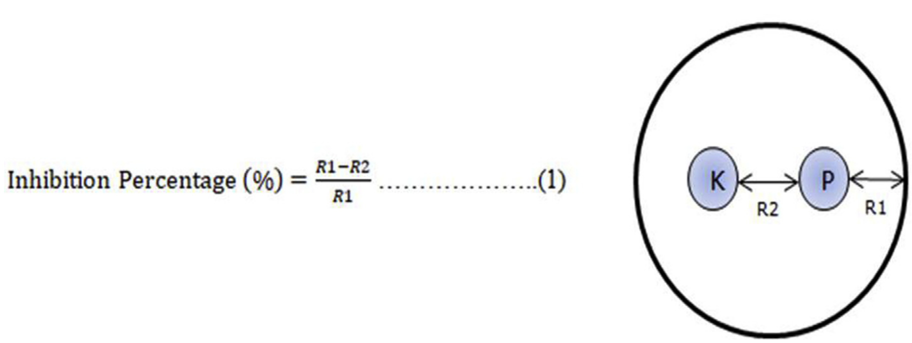

Figure 2. Illustration of the antagonism test of fungal isolates against *F. verticillioides* using a dual culture method. K, the biological agent candidate fungi; P, pathogenic fungus. R1, the colony radius in the opposite direction to the candidate fungi; and R2, the colony radius to the candidate fungi colony.

$$T50 = ti + \left(\frac{n50\% - ni}{nj - ni}\right)(tj - ti) \quad (8)$$

Where n is the total number of germinated seeds, and ni and nj are the total numbers of seeds germinated in adjacent counts at time t_i and t_j, respectively.

2.5. The effect of indigenous fungi on corn growth in-planta

Test of indigenous fungi isolates on corn growth was demonstrated in a greenhouse (*in-planta*) using seedling trays. The seeds of Anoman variety were treated with hot water treatment at 55 °C for 20 min, then air-dried for 20 min on sterile tissue paper. Seven-day-old fungi culture was suspended to 10^8 cfu/ml (calculated using a haemocytometer) by adding DW on the agar culture. Then it was scraped using a spatula and used as a suspension to soak the corn seeds. Seeds were soaked in the fungal suspensions at 160 rpm for 24 h. The soaked seeds were planted in a seedling tray with sterile soil and combined media (1:1). Four seeds were planted for each treatment and replicated three times. The parameters observed at 7 days after planting (DAP) were seedling height, root length, root wet weight, root dry weight, shoot wet weight, and shoot dry weight. The dry weights were calculated after the plant samples were dried in an oven at 60 °C for 72 h.

2.6. Experiment design and data analysis

The test of indigenous fungi on corn growth in-planta was arranged using a randomized block design. The observed data were analyzed statistically followed by the least significant difference test (LSD) at the 5% level (α 0.05).

2.7. Physiological characterization of indigenous fungi

2.7.1. Potassium solubilization test

Potassium solubilization test was done using Alexandrov agar media reported by Prajapati and Modi (2012). The 1 L Alexandrov media composed of 10 g glucose, 0.5 g MgSO4.7H2O, 0.005 g FeCl3, 0.1 g CaCO3, 2 g CaPO4, 5 g potassium aluminium silicate, and 20 g agar (pH 6.5). The test method was the same as the phosphate solvent fungi test. Fungal isolates that can solubilize potassium are identified by the formation of clear zones around the fungal colonies.

2.7.2. Phosphate solubilizing test

The test was done based on the method described by Nautiyal (1999) using Pikovskaya solid media with the composition of 10 g glucose, 2 5 g Ca3 (PO4), 0.5 g (NH4) 2SO4, 0.2 g NaCl, 0.1 g MgSO4.7H2O, 0.2 g KCl, 0.002 g MnSO4.H_2O, 0.002 g FeSO4.7H2O, 0.5 g yeast extract, 20 g agar and 1000 ml distilled water at pH 7.0. Single colonies of indigenous fungi aged 24 h on PDA media were taken and cultured on plates from 2-4 days on pikovskava solid media using sterile corkborer. The activity of phosphate solubilizing fungi is characterized by the formation of a clear halo zone around the colony.

2.7.3. Protease enzyme activity

The protease enzyme activity was tested using skim milk agar media. The proteolytic activity was indicated by the presence of a clear halo zone around the fungal colonies, were observed 48 h after inoculation.

3. Results

3.1. Exploration and isolation of indigenous fungi

A total of 89 fungus isolates were isolated from the rhizosphere, rhizoplane, and endophytic region of the plant consisting of 44 isolates from Maros, 29 isolates from Bone, and 16 isolates from Palu. The results of the isolation showed that the highest number of fungal colonies were obtained from the rhizosphere. While based on the location of origin, the highest number of fungal colonies were obtained from Maros, followed by Palu and Bone, 6.04×10^4, 3.20×10^4, and 1.14×10^4 cfu/g, respectively (Table 1). The total number of fungi (cfu/g) obtained from the samples from Maros were 2.85×10^4 (Rhizoplane), 2.91×10^4 (Rhizosphere), 2.83×10^3 (Endophyte), and from Palu were 1.45×10^4 (Rhizoplane), 1.64×10^4 (Rhizosphere), 1.11×10^3 (Endophyte). While the total number of fungi (cfu/g) from Bone was 3.18×10^3 (Rhizoplane), 6.82×10^3 (Rhizosphere), and 1.43×10^3 (Endophyte) (Table 1). In this study, the results of fungal isolation showed differences in each location. Further, the difference in the number of colonies obtained was due to the different rhizosphere areas from one location to another. One of the most important factors thought to be responsible for the Rhizosphere effect is the large variation in organic compounds available in the root area by the root exudates (sap) released by the roots, both directly and indirectly affecting the quality and quantity of microorganisms in the root zone.

3.2. The antagonistic activity of indigenous fungal isolates against *Fusarium verticillioides*

The antagonistic test of 89 fungal isolates against *F. verticillioides* on PDA media showed that there were 19 fungal isolates capable of inhibiting the growth of *F. verticillioides* with an inhibition of ≥50% ranging from 51.04% - 75.00% (Table 2). The fungal isolates exhibiting the antagonistic ability were grouped into three groups based on the type of interaction between the antagonistic fungi and pathogenic fungi, including antibiosis, competition, and parasitism (Figure 3). The competition reaction was shown by the interaction between JEDF 1B BN and *F. verticillioides*, where the growth of fungal hyphae of JEDF 1B BN isolates quickly overgrown the *F. verticillioides* thus the pathogen was unable to grow properly. The antibiosis reaction showed by the formation of a clear zone between the JRP 10 MRS fungal isolate and *F. verticillioides*. While the parasitism reaction was shown by the interaction between the SRF 1 MRS fungal isolate and *F. verticillioides*, where the hyphae of SRF 1 MRS grow over the *F. verticillioides.* Nineteen isolates that showed an inhibitory index of ≥50% were selected for further tests.

3.3. Effect of indigenous fungi on corn seed viability and vigor in vitro

The test results showed that there were 10 fungi isolates that showed necrotic symptoms in corn seedlings, namely JRP 6 MRS, JRF 2 MRS, SRP 2 MRS, SRP 5 MRS, JRP 4 PL, JEDF 4A PL, JEDF 2B PL, JRP 1 BN, JRF 9 BN, and JEDF 1A BN isolates. Aside from causing necrosis, these isolates also affected seed vigor with normal seedling percentage ≤80% so it was classified as a pathogenic fungus. Meanwhile, 9 other fungal isolates had the potential to promote germination, which showed a higher percentage of normal seedlings compared to the control (≥70%) and did not cause necrotic symptoms (Table 3).

A total of 7 out of 19 fungal isolates tested showed a positive effect on growth potential, germination, and vigor index of corn seeds with a value ≥ 90%, namely JRP 5 MRS, JRP 7 MRS, JRP 9 MRS, JRP 10 MRS, SEDF 6A MRS, SEDF 7A MRS, and JEDF 1B BN isolates (Table 4 and Figure 4). Not all of the isolates tested have a positive effect on the viability of corn seeds, some of them were causing abnormal seed germination even some resulting the seed inability germinate, especially those isolates that initially caused the seeds to germinate well but later experiencing

Table 1. Total Plate Count of indigenous fungi obtained from the different study regions.

Locations	Number of colony per gram samples (cfu/g)			Total
	Rhizoplane	Rhizosphere	Endophyte	
Maros, South Sulawesi	2.85×10^4	2.91×10^4	2.83×10^3	6.04×10^4
Bone, South Sulawesi	3.18×10^3	6.82×10^3	1.43×10^3	1.14×10^4
Palu, Central Sulawesi	1.45×10^4	1.64×10^4	1.11×10^4	3.20×10^4

Table 2. Inhibition activity of indigenous fungi isolates against *F. verticillioides.*

Number	Isolate ID	Inhibition (%)	Interaction type	Number	Isolate ID	Inhibition (%)	Interaction type
1	Control	0.00	Not Selected	45	JRF 2 BN	48.38	Not Selected
2	JRP 1 MRS	28.57	Not Selected	46	JRF 3 BN	44.44	Not Selected
3	JRP 2 MRS	28.57	Not Selected	47	JRF 4 BN	36.38	Not Selected
4	JRP 3 MRS*	67.86	Antibiosis	48	JRF 5 BN	48.00	Not Selected
5	JRP 4 MRS	30.36	Not Selected	49	JRF 6 BN	30.95	Not Selected
6	JRP 5 MRS*	71.43	Competition	50	JRF 7 BN	41.87	Not Selected
7	JRP 6 MRS*	64.29	Antibiosis	51	JRF 8 BN	37.5	Not Selected
8	JRP 7 MRS*	75.00	Competition	52	JRF 9 BN*	51.67	Antibiosis
9	JRP 8 MRS	4.55	Not Selected	53	JRF 10 BN	39.66	Not Selected
10	JRP 9 MRS*	57.14	Antibiosis	54	JRF 11 BN	25.00	Not Selected
11	JRP 10 MRS*	51.79	Antibiosis	55	JRP 1 BN*	51.07	Antibiosis
12	JRP 11 MRS	28.57	Not Selected	56	JRP 2 BN	14.81	Not Selected
13	JRP 12 MRS	28.57	Not Selected	57	JRP 3 BN	30.36	Not Selected
14	JRP 13 MRS	25.55	Not Selected	58	JRP 4 BN	48.21	Not Selected
15	JRP 14 MRS	28.57	Not Selected	59	JRP 5 BN	14.81	Not Selected
16	JRF 1 MRS	35.71	Not Selected	60	JRP 6 BN	42.86	Not Selected
17	JRF 2 MRS*	64.29	Parasitism	61	JRP 7 BN	45.3	Not Selected
18	JRF 3 MRS	37.5	Not Selected	62	JRP 8 BN	43.78	Not Selected
19	JRF 4 MRS	29.38	Not Selected	63	JRP 9 BN	42.86	Not Selected
20	JRF 5 MRS	41.07	Not Selected	64	JRP 10 BN	16.67	Not Selected
21	JRF 6 MRS	7.14	Not Selected	65	JEDF 1A BN*	54.46	Antibiosis
22	JRF 7 MRS	41.07	Not Selected	66	JEDF 2A BN	26.42	Not Selected
23	JRF 8 MRS	21.43	Not Selected	67	JEDF 3A BN	32.31	Not Selected
24	SRP 1 MRS	25.82	Not Selected	68	JEDF 4A BN	49.31	Not Selected
25	SRP 2 MRS	7.85	Not Selected	69	JEDF 5A BN	47.59	Not Selected
26	SRP 3 MRS	18.18	Not Selected	70	JEDF 6A BN	29.8	Not Selected
27	SRP 4 MRS	2.94	Not Selected	71	JEDF 1B BN*	73.33	Competition
28	SRP 5 MRS*	51.04	Antibiosis	72	JEDF 2B BN	46.43	Not Selected
29	SRP 6 MRS	31.25	Not Selected	73	JEDF 3B BN	44.64	Not Selected
30	SRP 7 MRS	12.96	Not Selected	74	JRF 1 PL	39.79	Not Selected
31	SRF 1 MRS*	67.54	Parasitism	75	JRP 1 PL	48.21	Not Selected
32	SRF 2 MRS	19.23	Not Selected	76	JRP 2 PL	41.07	Not Selected
33	SRF 3 MRS	37.07	Not Selected	77	JRP 3 PL	25.79	Not Selected
34	SRF 4 MRS	29.06	Not Selected	78	JRP 4 PL*	51.67	Antibiosis
35	SRF 5 MRS	26.19	Not Selected	79	JEDF 1A PL	41.87	Not Selected
36	SRF 6 MRS	9.09	Not Selected	80	JEDF 2A PL	37.82	Not Selected
37	SRF 7 MRS	27.75	Not Selected	81	JEDF 3A PL	42.86	Not Selected
38	SEDF 1A MRS	32.69	Not Selected	82	JEDF 4A PL*	51.07	Antibiosis
39	SEDF 2A MRS	10.17	Not Selected	83	JEDF 1B PL	26.79	Not Selected
40	SEDF 3A MRS*	70.97	Antibiosis	84	JEDF 2B PL*	56.06	Antibiosis
41	SEDF 5A MRS	10.08	Not Selected	85	JEDF 1D PL	37.75	Not Selected
42	SEDF 6A MRS*	69.64	Competition	86	JEDF 2D PL	39.29	Not Selected
43	SEDF 7A MRS*	54.87	Parasitism	87	JEDF 3D PL	48.15	Not Selected
44	JRF 1 BN	47.51	Not Selected	88	JEDF 4D PL	36.77	Not Selected
				89	JEDF 5D PL	17.96	Not Selected

Remarks: * are fungal isolate able to inhibit the growth of the pathogen *F. verticillioides* on PDA media with ≥50% inhibitory index.

necrotic such as JRP 6 MRS isolates, JRF 2 MRS, SRP 2 MRS, SRP 5 MRS, JRP 4 PL, JEDF 4A PL, JEDF 2B PL, JRP 1 BN, JRF 9 BN, and JEDF 1A BN.

3.4. Effect of indigenous fungi on corn growth in planta

The introduction of fungal isolates had no significant effect according to the LSD 5% test against corn plant height on seedling trays. However, some fungal isolates showed a significant effect on other observed parameters. The treatment of SEDF 3A MRS isolate was significantly able to increase primary root length, root dry weight, shoot wet weight, and shoot dry weight, such as . 20.52 cm, 0.133 g, 1.37 g, and 0.08 g, respectively, which was greater than the control. In addition, the treatments which showed the potential to induce growth were SEDF 6A MRS isolate increased root dry weight by 0.128 g and JEDF 4A PL isolate increased shoot wet weight by 1.325 gr (Table 5).

The variables observed as a benchmark for the selection process of fungal isolates in this study were focused on the level of antagonism test, pathogenicity test, and supported by the potential of fungal isolates in increasing viability and vigor of corn seeds as and several variables of plant growth rate. Therefore, the results showed that isolates JRP 5 MRS, JRP 9 MRS, JRP 10 MRS, JRP 7 MRS, and JEDF 1B BN consistently stimulated growth both in vitro and in planta. The isolates stimulated plant height at the early growth which was higher than the control value (Table 5). The effect of seed soaking with indigenous fungal suspensions

Figure 3. Types of interactions between indigenous fungi and the pathogenic fungi using a dual culture method on PDA media. a, competition (isolate JEDF 1B BN); b, antibiosis (isolate JRP 10 MRS); c, parasitism (isolate SRF 1 MRS); pf, fungi with potential antagonistic activity; fv, *F. verticillioides.*

Table 3. Effect of rhizosphere and endophytic fungi isolates on corn seed vigor on the 7th day.

Isolate ID	Observed Parameters (%)			
	Dead Seeds	Abnormal	Normal	Necrotic
JRP 3 MRS	3.33	10.00	86.67	-
JRP 5 MRS	0.00	6.67	93.34	-
JRP 6 MRS	3.33	63.33	33.33	+
JRP 7 MRS	0.00	16.67	83.33	-
JRP 9 MRS	0.00	0.00	100.00	-
JRP 10 MRS	0.00	3.33	96.66	-
JRF 2 MRS	3.33	30.00	66.66	+
SRP 2 MRS	0.00	83.33	16.66	+
SRP 5 MRS	0.00	30.00	56.67	+
SRF 1 MRS	3.33	13.33	83.33	-
SEDF 3A MRS	6.67	13.33	80.00	-
SEDF 6A MRS	0.00	26.67	73.33	-
SEDF 7A MRS	0.00	3.33	96.66	-
JRP 4 PL	33.33	10.00	56.67	+
JEDF 4A PL	26.67	13.33	60.00	+
JEDF 2B PL	0.00	16.67	83.33	+
JRP 1 BN	10.00	13.33	76.67	+
JRF 9 BN	0.00	23.33	76.67	+
JEDF 1A BN	10.00	50.00	40.00	+
JEDF 1B BN	3.33	10.00	86.67	-
Control	0.00	30.00	70.00	

showed that JRP 5 MRS, JRP 9 MRS, JRP 10 MRS, JRP 7 MRS, and JEDF 1B BN isolates consistently stimulated growth both *in vitro* and *in planta*. These isolates were able to promote plant height at the beginning of growth, higher than the control (Table 5). The results showed a significant increase in plant growth on treated seeds compared to untreated seeds (Figure 5). These results indicated that these isolates were able to produce certain metabolites that can stimulate growth.

3.5. Physiological characteristics of indigenous fungal isolates

Based on the physiological characterizations on *Alexandrov* medium, *pikovskaya* medium, and skim milk agar medium, three fungal isolates had the ability to solubilize potassium (JRP 5 MRS, JRP 9 MRS, JRP 10 MRS), one isolate has the ability to solubilize phosphate (JRP 5 MRS), and two isolates were observed capable of producing protease enzymes (JRP 9 MRS, JRP 10 MRS) (Table 6). The activity of potassium and phosphate solubilization and protease enzymes in solid media can be observed from the formation of clear halo zones around the fungal colonies.

4. Discussions

The number of fungal colonies obtained from rhizosphere tended to be higher and more diverse than the endophytic fungi. It is suspected that the microorganisms that live in the root area are more abundant than the number of microorganisms in plant tissues that are limited to the intercellular space of the plant cells. In addition, the large number of fungi obtained from the root areas is thought to be due to the presence of root exudate compounds produced by plants which are known to attract microorganisms and stimulate microbial development. According to (Rao et al., 2013), the rhizosphere is the area around the roots where interaction and interrelation occur between microorganisms and roots, implying that the activity of microorganisms in the zone will be highly influenced by the secreted root exudate. The rhizosphere is characterized by higher microbiological activity compared to the soil further away from plant roots.

The rhizospheric fungi as one of the biotic factors has the ability to induce plant resistance to disease and also act as a biofertilizer (Fety et al., 2015). These results were also in line with the theory of Syahputra et al. (2017) that reported the presence of various fungal species in the rhizosphere of agricultural soils was caused by several factors, such as the availability of nutrients in the form of organic compounds in the form of decaying organic waste. These organic compounds provided fungal species the advantage to live in the soil rhizosphere.

The number of endophytic fungal colonies obtained in this study was lower than that of rhizospheric fungi. However, according to Hamayun et al. (2010), endophytic fungi have the ability as antagonists and plant growth inducers depending on a number of growth-promoting metabolites produced. The mechanism of endophytic microbes in inducing resistance has also been reported to colonize plant tissues in order to stimulate plants to increase the production of metabolites in the form of peroxidase enzymes that play an essential role in plant resistance (Harni and Ibrahim 2011; Mirsam et al., 2021), and growth regulators such as gibberellins, auxins, and cytokinins (Khan et al., 2012).

Based on the observation of the tested seeds, rhizospheric and endophytic fungi can be classified as pathogenic, potential pathogenic, and non-pathogenic fungi. Some of the tested fungi isolates caused necrotic symptoms and reduce the germination potential so that they could be classified as either potential pathogenic or pathogenic fungi. Irawati et al. (2017) explained that fungi can be classified as pathogenic or potential pathogenic based on their effect on seed viability and vigor. Pathogenic fungi can cause the inability of seeds to germinate, while potential pathogenic fungi may not cause the inability seed to germinate but resulting in abnormal growth.

There were nineteen isolates of indigenous fungi capable of inhibiting the growth of *F. verticillioides* with inhibition of $\geq$50% on PDA medium. These fungi isolates inhibit the growth of pathogenic fungi by various types of inhibitory mechanisms. The classifications of the mechanism of interaction that occurs between antagonistic fungi and pathogenic fungi are based on the criteria proposed by Porter (1924), Skidmore and

Table 4. The effect of rhizosphere and endophytic fungi isolates on Growth Potential (GP), Seed Germination (G), Growth Rate (GtR), Growth Simultaneity (GS), Vigor Index (VI), Germination Rate (GR), and T50 of corn seeds.

Isolate ID	Observed Parameters						
	GP (%)	G (%)	GtR (%/etmal)	GS (%)	VI (%)	GR (average days)	T50 (days)
JRP 3 MRS*	100.00	91.67	56.25	91.67	50.00	3.67	2.14
JRP 5 MRS*	100.00	100.00	70.83	100.00	100.00	2.25	1.50
JRP 6 MRS	100.00	91.67	54.72	91.67	91.67	2.67	2.50
JRP 7 MRS*	100.00	100.00	66.67	100.00	100.00	2.33	1.50
JRP 9 MRS*	100.00	100.00	88.89	100.00	91.67	2.42	1.50
JRP 10 MRS*	100.00	100.00	88.89	100.00	91.67	2.42	1.50
JRF 2 MRS	100.00	100.00	52.78	100.00	83.33	3.17	2.50
SRP 2 MRS	91.67	58.33	40.97	58.33	33.33	4.00	3.55
SRP 5 MRS	100.00	100.00	76.39	100.00	91.67	2.67	2.50
SRF 1 MRS	100.00	100.00	44.44	100.00	58.33	3.42	2.50
SEDF 3A MRS	100.00	100.00	66.67	100.00	100.00	2.33	1.50
SEDF 6A MRS*	100.00	100.00	75.00	100.00	100.00	2.42	1.50
SEDF 7A MRS*	100.00	100.00	65.28	100.00	83.33	2.92	2.50
JRP 4 PL	100.00	91.67	56.25	91.67	50.00	3.67	2.50
JEDF 4A PL	100.00	100.00	66.67	100.00	100.00	2.33	1.50
JEDF 2B PL	58.33	41.67	40.97	41.67	33.33	1.08	0.50
JRP 1 BN	100.00	100.00	66.67	100.00	100.00	2.33	1.50
JRF 9 BN	100.00	83.33	57.50	83.33	50.00	3.83	1.50
JEDF 1A BN	100.00	83.33	37.50	83.33	0.00	4.33	3.50
JEDF 1B BN*	100.00	100.00	80.56	100.00	91.67	2.58	1.50
Kontrol	100.00	100.00	90.28	100.00	83.33	2.42	1.50

Remarks: * are fungal isolates that consistently showed a positive effect on growth potential, germination, and vigor index of corn seeds with a value of ≥90% and did not cause necrotic.

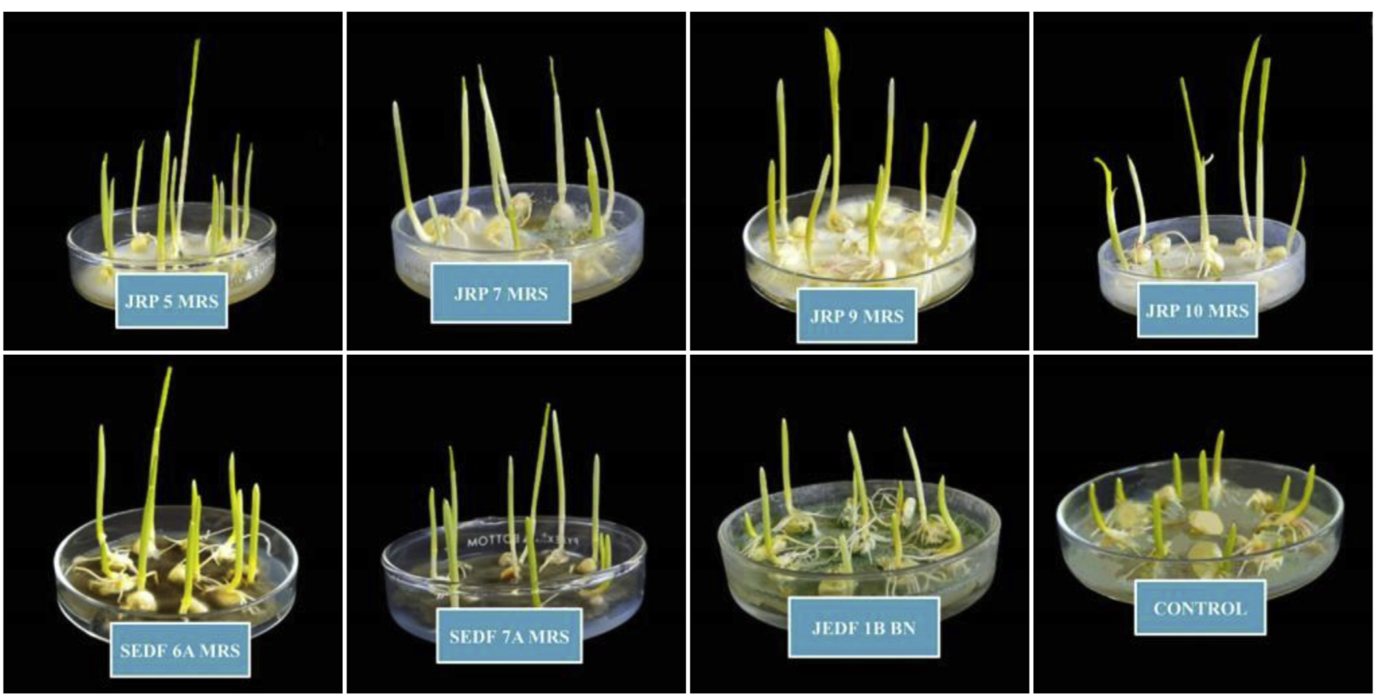

Figure 4. Isolates of indigenous fungi that have consistently positive effects on seed viability and vigor *in-vitro*, i.e. isolates JRP 5 MRS; JRP 7 MRS; JRPS 9 MRS; JRP 10 MRS; SEDF 6A MRS; SEDF 7A MRS; JEDF 1B BN; and Control.

Dickinson (1976), and Trigiano et al. (2008), namely 1) competition, if the antagonistic fungi colonies overgrow the pathogenic colonies and fill the 9 cm diameter Petri dish. At the contact area, pathogenic hyphae undergo lysis; 2) antibiosis, if a clear zone forms between the pathogenic fungi and the antagonistic fungi, there is an alteration in the shape of the pathogenic hyphae, and a pigment is produced on the lower surface of the antagonistic fungi; and 3) parasitism, if the antagonistic fungal hyphae grow on top of the pathogenic hyphae, in the contact area, the antagonistic fungal hyphae was found wrapped around the pathogenic hyphae and causing lysis.

Isolates JRP 5 MRS, JRP 9 MRS, JRP 10 MRS, JRP 7 MRS, and JEDF 1B BN have the ability to stimulate growth both in-vitro and in-planta.

Table 5. The effect of indigenous fungi suspension on the growth of corn plants at 7 DAP.

Isolate ID	Observed Parameters*											
	Plant height (cm)		Root length (cm)		Root wet weight (g)		Root dry weight (g)		Shoot wet weight (g)		Shoot dry weight (g)	
JRP 3 MRS	20.39	a-d	12.73	b-e	0.80	c-g	0.104	c-g	1.093	c-g	0.070	b-d
JRP 5 MRS	21.68	a-c	15.49	bc	0.99	b-d	0.113	b-f	0.949	e-i	0.069	b-d
JRP 6 MRS	20.14	b-e	8.29	e-g	0.65	f-h	0.122	b-d	0.805	hi	0.067	b-d
JRP 7 MRS	21.69	a-c	12.38	b-g	0.87	b-f	0.103	d-g	1.156	b-e	0.070	b-d
JRP 9 MRS	23.01	a	16.14	ab	1.04	a-c	0.104	c-f	1.177	b-e	0.062	cd
JRP 10 MRS	22.30	a-c	14.75	b-d	0.92	b-e	0.096	e-h	1.116	b-f	0.055	d
JRF 2 MRS	18.98	de	7.78	g	0.46	hi	0.097	e-h	0.706	ij	0.054	d
SRP 2 MRS	18.74	de	12.63	b-f	0.81	b-g	0.098	d-h	0.848	g-i	0.059	d
SRP 5 MRS	19.77	c-e	15.42	bc	0.92	b-e	0.116	b-e	1.115	b-f	0.054	d
SRF 1 MRS	21.15	a-d	7.98	fg	0.76	d-g	0.117	b-e	0.922	e-i	0.061	cd
SEDF 3A MRS	22.13	a-c	20.52	a	1.05	ab	0.133	b	1.366	b	0.080	ab
SEDF 6A MRS	19.82	c-e	16.38	ab	0.83	b-f	0.128	bc	0.858	f-i	0.056	d
SEDF 7A MRS	22.34	a-c	15.03	bc	1.27	a	0.174	a	1.668	a	0.094	a
JRP 4 PL	20.37	a-d	12.44	b-g	0.37	i	0.092	f-h	0.419	k	0.064	b-d
JEDF 4A PL	21.72	a-c	16.63	ab	0.71	e-g	0.108	c-f	1.325	bc	0.077	bc
JEDF 2B PL	22.71	ab	13.22	b-d	0.43	hi	0.080	gh	0.712	ij	0.062	cd
JRP 1 BN	22.49	ab	14.67	b-d	0.82	b-f	0.098	d-h	1.253	b-d	0.063	cd
JRF 9 BN	22.85	e	10.86	c-g	0.57	g-i	0.077	h	0.521	jk	0.059	d
JEDF 1A BN	17.63	e	10.11	d-g	0.39	i	0.078	h	0.698	ij	0.059	d
JEDF 1B BN	21.87	a-c	13.00	b-d	0.85	b-f	0.098	d-h	1.249	b-d	0.067	b-d
Control	20.68	a-d	15.77	b	0.87	b-f	0.098	d-h	1.006	d-h	0.062	cd
LSD 5%	2.65		4.70		0.25		0.024		0.264		0.017	
CV	7.64		21.21		19.28		13.79		16.05		15.75	

Remarks: *Means in the same column followed by same letter are not significantly different according to 5% LSD (α 0,05).

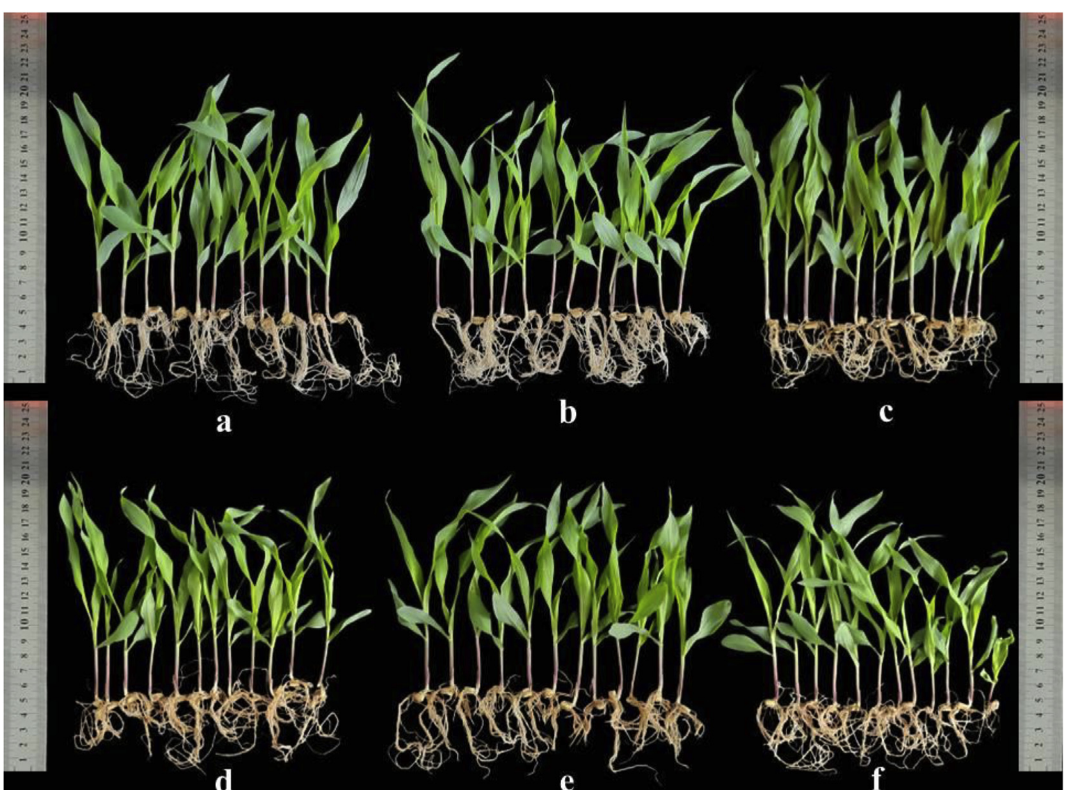

Figure 5. Effect of indigenous fungi isolates on corn growth in seedling trays. a, JRP 5 MRS; b, JRP 9 MRS; c, JRP 10 MRS; d, JRP 7 MRS; e, JEDF 1B BN; f, control.

Table 6. Physiological characteristics of indigenous fungal isolates.

Isolate ID	Physiological Test		
	K solubilization	P solubilization	Protease
JRP 3 MRS	-	-	-
JRP 5 MRS	+	+	-
JRP 6 MRS	-	-	-
JRP 7 MRS	-	-	-
JRP 9 MRS	+	-	+
JRP 10 MRS	+	-	+
JRF 2 MRS	-	-	-
SRP 2 MRS	-	-	-
SRP 5 MRS	-	-	-
SRF 1 MRS	-	-	-
SEDF 3A MRS	-	-	-
SEDF 6A MRS	-	-	-
SEDF 7A MRS	-	-	-
JRP 4 PL	-	-	-
JEDF 4A PL	-	-	-
JEDF 2B PL	-	-	-
JRP 1 BN	-	-	-
JRF 9 BN	-	-	-
JEDF 1A BN	-	-	-
JEDF 1B BN	-	-	-
Control	-	-	-

These results indicated that these isolates are able to produce certain metabolites that can stimulate plant growth. The plant growth-promoting effect may be due to the ability of fungal isolates to colonize roots and provide minerals or nutrients needed by plants. This research examined the potential of indigenous fungi either non-pathogenic saprophytes and endophytes as plant growth promoters and as biological control agents. The ability of indigenous fungi to stimulate growth is also highly related to the production of growth regulators (Saharan and Nehra 2011; Bhattacharyya and Jha 2012; Glick 2012).

The effect of rhizospheric fungi in stimulating plant growth and biological control has been widely reported by many researchers. Plant growth promoting fungi (PGPF) are non-pathogenic saprophytic fungi that are reported to be able to suppress diseases caused by plant pathogens either by inducing plant resistance or direct antagonism, the PGPF fungus is also known to have positive effects on plant growth (Hossain et al., 2017; Muslim et al., 2019; Zhang et al., 2018). PGPF's ability to colonize plant roots is considered the most important mechanism involved in preventing pathogenic infection, assisting in absorbing nutrients, and promoting plant growth (Hossain et al., 2017; Zhang et al., 2018; Murali et al., 2013). According to Dewi et al. (2020), another indirect mechanism was biological agents have the potential to support plant growth with the ability to solubilize phosphate in order to produce growth regulators. This character allows biological agents to be able to protect plants from pathogens by improving plant health so as to avoid pathogen attacks.

Endophytic microbes may aid the host plants increasing tolerance to biotic and abiotic stresses (Redman et al., 2001; Rodriguez et al., 2009). The endophytic microbes in plants have the beneficial roles to increase plant resistance to disease (Narisawa et al., 2002) and environmental stress (Schulz 2007) such as increase tolerance to mineral stress (Malinowski and Belesky 2000), drought and high temperatures conditions (Lehtonen et al., 2005). Endophytic fungi are known to be able to produce growth hormones to help plants in countering the abiotic stress and known to produce bioactive secondary metabolites with various activities (Khan et al., 2013; Gupta et al., 2019).

Isolates JRP 5 MRS, JRP 9 MRS, JRP 10 MRS were able to grow on the Aleksandrov medium and form clear halo zones indicating the ability of microbes in solubilizing potassium (Rajawat et al., 2014). According to Ghevariya and Desai (2014), microbes that are able to form clear halo zones are considered as potassium solubilizing microbes. Basak and Biswas (2009), explained that each potassium solubilizing microbe produces different types and amounts of organic acids, and that one type of microbe may produce more than one type of organic acid.

The ability of the fungus to solubilize inorganic insoluble phosphorus can be determined by growing the fungi isolate on medium containing insoluble phosphate. Fungal isolates that have the ability to solubilize phosphate are indicated by the formation of a clear halo zone surrounding the colony (George et al., 2002). George et al. (2002) added that the clear zone formed on pikovskaya medium was caused by the activity of the phosphatase enzyme produced by phosphate solubilizing microorganisms. The mechanism of microorganisms in solubilizing phosphate from an insoluble form can be attributed with the microbial ability to produce enzymes of phosphatase, phytase, and organic acids resulted from metabolisms such as acetic acid, propionic, glycolic, fumarate, oxalate, succinate, tartrate, citrate, lactate, and ketoglutarate. P solubilization activity is also displayed by microorganisms that do not produce organic acids through (1) protons release (H^+ ions) from the respiration process, (2) assimilation of ammonium (NH4 +), and (3) competition between organic anions with orthophosphates on the colloid surface which can also cause orthophosphate mobilization (Illmer and Schinner 1992).

JRP 9 MRS and JRP 10 MRS isolates were able to form clear zones on skim milk media, this indicates that the fungal isolates produced protease enzymes which could hydrolyze the proteins contained in skim milk media into a much simpler form. According to Naiola and Widhyastuti (2002), the results of protein-polymer hydrolyzations are shown by the presence of a clear halo zone which indicates that the protein has been converted into peptides and amino acids which are soluble in the media. The hydrolytic ability of proteolytic fungi isolates was measured by comparing the diameter of the clear halo zone around the colony with the diameter of the fungal colony. Conversely, isolates that do not indicate proteolytic properties, marked by the absence of a clear zone around the colony, may be caused by a mismatch between the type of the casein substrate present in the skim milk agar medium with the protease produced by the isolate or the isolate does not produce the protease enzyme (Yuanita and Wikandari 2014).

5. Conclusions

A total of five fungal isolates namely JRP 5 MRS, JRP 9 MRS, JRP 10 MRS, JRP 7 MRS, and JEDF 1B BN isolates were selected based on the tests and showed a consistently positive effect on seed viability and vigor with a value of ≥90%. Also, the isolates also did not cause necrosis in corn, and have the ability to suppress the growth of pathogenic *F. verticillioides* by ≥ 50%. These results indicated that isolate JRP 5 MRS, JRP 9 MRS, JRP 10 MRS, JRP 7 MRS, and JEDF 1B BN have potential as biological control agents and plant growth inducers.

This research is a basic study carried out on a small scale in the laboratory and greenhouse to obtain candidate biological control agents so that further research on a wider scale in the field is needed to confirm the effectiveness of candidate biological control agents that have been obtained in laboratory and greenhouse testing.

Declarations

Author contribution statement

Hishar Mirsam: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Septian Hary Kalqutny: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Suriani: Performed the experiments; Contributed reagents, materials, analysis tools or data.

Muhammad Aqil; Muhammad Azrai; Syahrir Pakki; Amran Muis; Nurasiah Djaenuddin: Performed the experiments.

Abdul Wahid Rauf; Muslimin: Contributed reagents, materials, analysis tools or data.

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Data availability statement

Data included in article/supplementary material/referenced in article.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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