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 ≥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 ≥90%. The isolates did not cause necrosis in corn, and had the ability to suppress the growth of pathogenic *F. verticillioides* by ≥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)
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; Soenartiningti, 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 (Saleman and Omame 2013). Tseladze and Adogna (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 storage period if conditions are favorable for pathogen development.

The second location, Bone District-South Sulawesi is located at 4°12’–12°30’ S and between 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 10⁻³, 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 b y 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 days.

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 days.
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. 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).

\[
\text{Inhibition Percentage} = \frac{R_1 - R_2}{R_1}
\]

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).

\[
\text{GP} = \frac{\sum \text{germinated seeds}}{\sum \text{seeds grown}} \times 100\%
\]

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 (5th day) and the second observation (7th day) after the seeds were grown (Equation 3).

\[
G = \frac{\sum \text{1st observation} + \sum \text{2nd observation}}{\sum \text{seeds grown}} \times 100\%
\]

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

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

\[
\text{GrR} = \frac{n_1 \times D_1 + n_2 \times D_2 + \ldots + n_7 \times D_7}{D} \times \frac{n}{D}
\]

\[n = \text{percentage of normal seedlings per observation} \%
\]

\[D = \text{observation time/24 h (etmal)}
\]

2.4.4. Growth simultaneity (GS)

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

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

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 (5th day) using Eq. (6).

\[
\text{VI} = \frac{\sum \text{normal seedlings}}{\sum \text{seeds grown}} \times 100\%
\]

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).

\[
\text{GR} = \frac{N_1T_1 + N_2T_2 + \ldots + N_xT_x}{\sum \text{germinated seeds}}
\]

\[N \text{ is the number of seeds germinated each day; } T \text{ is the amount of time between the start of the test and the end of the specified interval of observation.}
\]

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).

\[
T_{50} = \sum \text{germinated seeds} / \sum \text{seeds grown}
\]

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.
n = the total number of germinated seeds, and ni and nj are the total numbers of seeds germinated in adjacent counts at time ti and tj, 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 obtained from the rhizosphere. While based on the location of origin, the fungi were grouped as 29 isolates from Maros, 29 isolates from Bone, and 16 isolates from Palu. 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 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. 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 Maros, followed by Palu and Bone, 6.04 × 10^4, 3.20 × 10^4, and 1.14 × 10^3 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 × 10^4 (Endophyte). While the total number of fungi (cfu/g) from Bone was 3.18 × 10^4 (Rhizoplane), 6.82 × 10^3 (Rhizosphere), and 1.43 × 10^4 (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 overgrew 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 fungal isolates that showed necrotic symptoms in corn seedlings, namely JRP 6 MRS, JRF 2 MRS, SRF 2 MRS, SRF 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, JRF 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

| Locations | Number of colony per gram samples (cfu/g) | Total |
|-----------|-------------------------------------------|-------|
| Maros, South Sulawesi | 2.85 × 10^4, 2.91 × 10^4, 2.83 × 10^3 | 6.04 × 10^4 |
| Bone, South Sulawesi | 3.18 × 10^4, 6.82 × 10^3, 1.43 × 10^4 | 1.14 × 10^5 |
| Palu, Central Sulawesi | 1.45 × 10^4, 1.64 × 10^4, 1.11 × 10^4 | 3.20 × 10^4 |
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.

### 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     | JRP 1 MRS  | 35.71          | Not Selected    | 60     | JRP 6 BN   | 42.86          | Not Selected    |
| 17     | JRP 2 MRS* | 64.29          | Parasitism      | 61     | JRP 7 BN   | 45.3           | Not Selected    |
| 18     | JRP 3 MRS  | 37.5           | Not Selected    | 62     | JRP 8 BN   | 42.78          | Not Selected    |
| 19     | JRP 4 MRS  | 29.38          | Not Selected    | 63     | JRP 9 BN   | 42.86          | Not Selected    |
| 20     | JRP 5 MRS  | 41.07          | Not Selected    | 64     | JRP 10 BN  | 16.67          | Not Selected    |
| 21     | JRP 6 MRS  | 7.14           | Not Selected    | 65     | JEDF 1A BN*| 54.46          | Antibiosis      |
| 22     | JRP 7 MRS  | 41.07          | Not Selected    | 66     | JEDF 2A BN | 26.42          | Not Selected    |
| 23     | JRP 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     | JRP 1 PL   | 39.79          | Not Selected    |
| 31     | SRP 1 MRS* | 67.54          | Parasitism      | 75     | JRP 1 PL   | 48.21          | Not Selected    |
| 32     | SRP 2 MRS  | 19.23          | Not Selected    | 76     | JRP 2 PL   | 41.07          | Not Selected    |
| 33     | SRP 3 MRS  | 37.07          | Not Selected    | 77     | JRP 3 PL   | 25.79          | Not Selected    |
| 34     | SRP 4 MRS  | 29.06          | Not Selected    | 78     | JRP 4 PL*  | 51.67          | Antibiosis      |
| 35     | SRP 5 MRS  | 26.19          | Not Selected    | 79     | JEDF 1A PL | 41.87          | Not Selected    |
| 36     | SRP 6 MRS  | 9.09           | Not Selected    | 80     | JEDF 2A PL | 37.82          | Not Selected    |
| 37     | SRP 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.

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 g (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...
3.5. Physiological characteristics of indigenous fungal isolates

produce certain metabolites that can stimulate growth. On the 7th day, 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.

| 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    | -      |         |
| JRF 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    | +      |         |
| JRF 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    |        |         |

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

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.

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 ≥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...
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 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 |
| JRP 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 |
| JRP 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; JRP 9 MRS; JRP 10 MRS; SEDF 6A MRS; SEDF 7A MRS; JEDF 1B BN; and Control.
### 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-b | 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 |
| SRF 2 MRS  | 18.74 de | 12.63 b-f | 0.81 b-g | 0.098 d-h | 0.848 g-i | 0.059 d |
| SRF 1 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 A 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.
The ability of indigenous fungi to stimulate growth is also highly related to the production of growth regulators (Saharan and Nehra 2011; Bhataryya and Jha 2012; Glick 2012). The ability of indigenous fungi to stimulate growth is also highly related to the production of growth regulators. This character allows biological agents to be able to protect plants from pathogens by improving plant health so as to avoid disease (Narisawa et al., 2002) and environmental stress (Schulz 2007) such as increase tolerance to mineral stress (Maliowski 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).

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 (Maliowski 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).

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  | -                  | -                | -                | -        |
| JRP 2 MRS  | -                  | -                | -                | -        |
| JRP 5 MRS  | -                  | -                | -                | -        |
| JRP 1 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; Bhat-tacharyya and Jha 2012; Glick 2012).

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 hydrolizations 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 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 Azqil: Muhammad Azrai; Syahirri Pakki; Amran Muis; Nurariasih Djaenuddin: Performed the experiments.  
Abdul Wahid Rauf: Muslimin: Contributed reagents, materials, analysis tools or data.

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

Declaration of interest statement
The authors declare no conflict of interest.

Additional information
No additional information is available for this paper.

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