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RESEARCH

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Potential of endophytic fungi as a pathogenic biocontrol agent and growth promoters in corn seedlings

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Abstract

Background Use of endophytic fungi, as pathogen control of *Bipolaris maydis* and *Curvularia* sp., is an alternative method of control without the use of synthetic pesticides that are more environmentally friendly. This study aimed to determine the potential of endophytic fungi in controlling the growth of pathogens *B. maydis* and *Curvularia* sp. in vitro and in spurring the growth of corn plants. It was consisted of three types of testing three endophytic fungal species (*Aspergillus*_1, *Fusarium*_2, and *Trichoderma*_11), namely (1) testing the antagonistic activity of endophytic fungal against pathogens by double culture method, (2) physiological characterization of endophytic fungal as phosphate solvents and chitinase producers, and (3) testing of corn seed vigor with the blotter test method.

Results The results of testing endophytic fungal isolates against *B. maydis* pathogens showed that the three isolates were able to suppress the development of *B. maydis*, whereas the *Trichoderma*_11 isolate showed higher suppression results than others. The isolate that showed the best ability to dissolve phosphates is *Fusarium_2* with a dissolving index of 1.9 and their effectiveness up to 91.5%. Meanwhile, *Trichoderma*_11 was able to produce the highest chitinase activity index of 1.9 with an effectiveness of 90.6%. The best corn root lengths and plant height were shown on *Fusarium*_2 treatment. Similar outcomes were observed when *Curvularia* sp. was tested. The whole isolates were able to suppress the growth of the pathogen by 16.43–40.44% on the 4th day after incubation. *Trichoderma* sp. isolate was 72.50% more effective at suppressing than the other two isolates. On day 11, the isolate of *Aspergillus* sp. was suppressed by 62.50%, while *Fusarium* sp. showed the lowest suppression of 59.17%.

Conclusions *Trichoderma_11* isolate was potentially the best biocontrol agent against maydis leaf blight and *Curvularia* leaf spot in vitro. Meanwhile, the *Fusarium*_2 isolate had promoted the growth of the corn seedlings.

Keywords Blotter test, Dual culture, Endophytes, *Fusarium* sp., *Trichoderma* sp., *Bipolaris maydis*, *Curvularia* sp.

Background

Corn is one of the Indonesia's priority commodities today. It is one of the foodstuffs that are widely consumed or used as feed in Indonesia. The problem of disease incidences on corn is still a problem that is often encountered in the field. The pathogen *Bipolaris maydis* is one that causes leaf blight in corn. Corn leaf blight caused by *Bipolaris* species has often occurred with complex symptoms, where the typical symptoms caused are very clearly seen in corn; namely, there are longitudinal strip lesions or fusiform (Dai et al. 2020). Pathogenic infections that cause leaf blight in corn are one of the factors

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that decrease the productivity of corn crops in South Sulawesi. Based on data from the Central Statistics Agency of South Sulawesi Province, there was a decrease in corn production by 2.13% from 2014 to 2018. The period was 1 year, from 2017 with an average production value of 56.83 tons/ha, while in 2018 the average productivity was 55.62 tons/ha (BPS 2020).

The average value of leaf blight severity in South Sulawesi reaches the economic threshold value. The level of damage to plants that is commonly used as a basis for control with diseased leaf areas was 30.1–50% (Hooda et al. 2018). As reported in the research of Djaenuddin et al. (2021a, b), the pathogen that causes leaf blight infects corn plants at the age of 70 days after planting (DAP) with an infection rate of up to 72.5%. In addition, the pathogen *Curvularia* sp. is also disease-causing in corn crops. One of the diseases in corn plants in the Palu region, Central Sulawesi, is the *Curvularia* leaf spot, where the intensity of infection ranged from 40 to 90% (Soenartiningsih et al. 2013).

The recent challenge faced by advanced farming is to achieve high yields in an environmentally friendly way. Thus, there is an urgent need to find environmentally friendly solutions, such as the widest application of biocontrol agents (Rajesh et al. 2016). The term biological control (or biocontrol) applies to the use of living organisms to suppress population density or its influence on a particular organism, making it less abundant or less destructive than it should be (Poveda et al. 2020). Control of this pathogen has been widely carried out, one of which is the use of antagonistic microbes. The use of antagonistic microbes such as endophytic fungi is an alternative in the control of this pathogen. Endophytic fungi derived from plant tissues are often used as a biocontrol agent against pathogens, plant growth promoters, biodegradation, biodecomposer, and so on. In addition, the use of botanical pesticides clover leaf extract combined with *B. subtilis* BNt8 can suppress the development of *B. maydis* by up to 13% and increase crop yields by up to 26% (Djaenuddin et al. 2018).

Therefore, it was necessary to conduct research to determine the ability of endophytic fungal to suppress the development of *B. maydis* and *Curvularia* sp. pathogens in vitro and spur the growth of corn seedlings.

Methods

Time and place

This research was carried out at the Plant Pathology Laboratory of the Indonesian Cereals Research Institute from February to May 2021. The research materials used were three isolates of endophytic fungal, namely *Aspergillus* sp. (Aspergillus_1), *Trichoderma* sp. (Trichoderma_11), and *Fusarium* sp. (Fusarium_2), and corn seeds of the Anoman variety (which has a level of resistance against late blight and leaf spot) obtained from UPBS BPSI Cereal Plants. The tools used include tools for the multiplication of fungi on PDA (Oxoid®) medium consisting of Petri dishes, Bunsen lamps, prepared needles, and tweezers. The tool for sterilizing media was an autoclave. In addition, specific media were used for testing, namely *Pikovskaya* media, colloidal chitin, and agar.

Pathogen isolates source

The pathogenic isolates used are isolates of *B. maydis* and *Curvularia* sp. Both isolates are from the collection of the Plant Pathology Laboratory of the Indonesian Cereals Research Institute (ICERI).

Testing of the antagonistic activity of endophytic fungal against pathogens

Three isolates of endophytic fungal, namely *Aspergillus*_1, *Trichoderma*_11, and *Fusarium*_2, were further tested for antagonistic activity against both pathogens (*B. maydis* and *Curvularia* sp.) using a double culture technique. Pure cultures of both pathogenic and endophytic were cut using a cork borer measuring 0.5 cm, and then, each was placed next to the other on the PDA media using a preparation needle. Control was an isolate of a pathogen that was grown without isolates of endophytic fungi. Each treatment and control were repeated four times. It was further incubated at a temperature of 28 ± 2 °C (Vinayarani and Prakash 2018).

Physiological characterization of endophytic fungal as phosphate solvents and chitinase producers

Phosphate solubility

The dissolution potential of inorganic phosphates from isolates of endophytic fungal was evaluated in vitro based on (Jasim et al. 2013). Briefly, Pikovskaya medium (contains, glucose 10 g l^{-1}; Ca3(PO4)2 5 g l^{-1}; (NH4)2SO4 0.5 g l^{-1}; NaCl 0.2 g l^{-1}; MgSO4.7H2O 0.1 g l^{-1}; KCl 0.2 g l^{-1}; FeSO4.7H2O 0.002 g l^{-1}; yeast extract 0.5 g l^{-1}; MnSO4.2H2O 0.002 gl^{-1}; agar 15 g l^{-1}; 1 l d.H2O; pH 6.8) was prepared. The fungal colony was inoculated on Petri containing Pikovskaya media and incubated at 28 °C for 72 h. The diameter (mm) of the clear zone around the fungal colony was measured for the qualitative determination of the phosphate dissolving capacity.

Chitinase generator

Manufacture of Colloidal Chitin. Shrimp chitin colloids were made with a concentration of 20 g of shrimp chitin dissolved in 300 ml of concentrated and homogenized HCl. The solution was then incubated in the refrigerator for 24 h. The solution was then diluted with 200 ml of distilled water which had been cooled to a temperature

of 4 °C for one night, and then filtered with gauze. The filtrate was then neutralized with NaOH 12 N to set pH 7. Then the solution was centrifuged at a rate of 4000 rpm for 10 min. The obtained precipitate was rinsed with sterile distilled water and centrifuged again at 4000 rpm for 10 min. Isolates of endophytic fungal were inoculated on agar chitin media (composition 0.05% MgSO4.7H2O; 0.07% K2HPO4; 0.1% yeast extract; 0.5% colloidal chitin; and 1.5% agar), incubated at room temperature for 48–120 h and then observed clear zone diameter.

Corn seed vigor testing

Three isolates of endophytic fungal, namely *Aspergillus* sp., *Trichoderma* sp., and *Fusarium* sp., were then tested for seed vigor by the blotter test method. Seeds were sterilized on their surface by soaking them in 1% sodium hypochlorite for 3 min. Next, the seeds were dried on sterile filter paper and placed in a Petri dish. Furthermore, as many as 10 seeds were planted in PDA media, whose entire surface had been covered with each isolate of endophytic fungi. Furthermore, the PDA media that was filled with seeds was wrapped using a sterile clear plastic and incubated at room temperature for 7 days. Control was a PDA medium that did not have endophytic isolates in it (Camargo et al. 2017).

Observation variables

Testing of the antagonistic activity of endophytic fungi against pathogens

Growth from pathogens presented in treatments and control was measured at 4, 6, 8, and 11 days after incubation (DAI), using the crossbar. Furthermore, the percentage of growth inhibition was calculated using the following formula (Hamzah et al. 2018):

$$I = (C - T/C) \times 100 \quad (1)$$

where I was the amount of inhibition in percent (%), C was the diameter of the pathogen colony at the control in mm, and T was the diameter of the pathogen colony in mm.

Physiological characterization of endophytic fungi as phosphate solvents and chitinase producers

After measuring the diameter of the clear zone in the treatment, the dissolution/activity index (phosphate and chitinolytic) and its effectiveness were then calculated. The formula for the dissolving index/activity and the effectiveness of the dissolution/activity was as follows:

$$\text{Dissolving index} = \frac{\text{Diameter colony} + \text{Diameter clear zone}}{\text{Diameter colony}} \quad (2)$$

$$\text{Dissolution effectiveness} = \frac{\text{Diameter clear zone}}{\text{Diameter colony}} \times 100\%. \quad (3)$$

Vigor seed testing

Observations were made by calculating the percentage of the number of seeds germinated (%), plant height (cm), and root length (cm) on the treatment compared to the control after 7 DAI.

Data analysis

The data obtained in the analysis of diversity and the different data were continued with the honest real difference test (Tukey's HSD) at 5%. Data processing was done using the computer program STAR 2.0.1 for Windows (IRRI 2013).

Results

Testing of the antagonistic activity of endophytic fungal against pathogens

The results of testing endophytic fungal isolates against *B. maydis* pathogens showed that the three isolates were able to suppress the development of *B. maydis* since day 4, which was 21.90–33.33%. Furthermore, it was seen that the *Trichoderma* sp. isolate showed the highest suppression results than the others, namely 64.76% on day 11, while the *Fusarium* sp. isolate showed the lowest suppression of 29.52%, and the *Aspergillus* sp. isolate showed an emphasis on the pathogen *B. maydis* by 42.86% (Fig. 1).

Of the three isolates, only *Trichoderma* sp. could suppress the pathogen *B. maydis* by more than 50% on the 11th day. This suggests that this isolate had the potential to be used as a controlling agent for corn leaf blight caused by the pathogen *B. maydis.* Testing of the pathogen *Curvularia* sp. also showed similar results. On the 4th day after incubation, the entire isolates were able

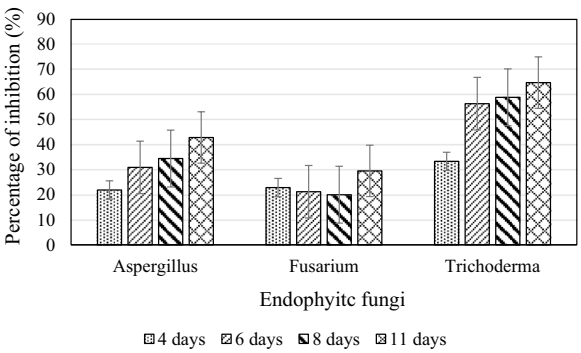

Fig. 1 Percentage of inhibition of *Bipolaris maydis* pathogen growth against three isolates of endophytic fungi on each day of observation

to suppress the development of the pathogen by 16.43–40.44%. *Trichoderma* sp. isolate was capable of suppressing better than the other two isolates, namely 72.50%. The same result was obtained where *Fusarium* sp. showed the lowest suppression of 59.17%, and the *Aspergillus* sp. isolate was suppressed by 62.50% on day 11 (Fig. 2).

From these results, it is known that the three isolates of endophytic fungi were able to suppress the development of the pathogen *Curvularia* sp. above 50% after 11 days of incubation. This was of course the potential of the three isolates, especially *Trichoderma* sp. as control of leaf spot disease caused by the pathogen *Curvularia* sp.

From the appearance of all treatments on PDA media, it can be seen that *Trichoderma* sp. was a mycoparasite against both pathogens, namely *B. maydis* and *Curvularia* sp. *Aspergillus* sp. isolate was a mycoparasite in *Curvularia* sp. pathogens but space and nutrient competition against *B. maydis* pathogens, while *Fusarium* sp. isolate was the opposite, namely space competition and nutrition against *Curvularia* sp. pathogens and mycoparasites against *B. maydis* pathogens (Fig. 3).

Physiological characterization of endophytic fungal as phosphate solvents and chitinase producers

Solubility of phosphates and chitinolytic activity was characterized by the formation of clear zones around the colony of fungi on media containing Phikovskaya tricalcium phosphate (Ca3PO4) for phosphate solvents and media containing chitin colloids for chitin-producing (Fig. 4).

The dissolving indices of phosphates, as well as chitinolytic activity and their effectiveness by endophytic fungi, show varying values (Table 1). The isolates that showed the best ability to dissolve phosphates were *Fusarium*_2 isolates with a dissolving index of 1.9 and their effectiveness up to 91.5%. Meanwhile, isolate *Trichoderma*_11 was able to produce the highest chitinase activity index of 1.9 with an effectiveness of 90.6%.

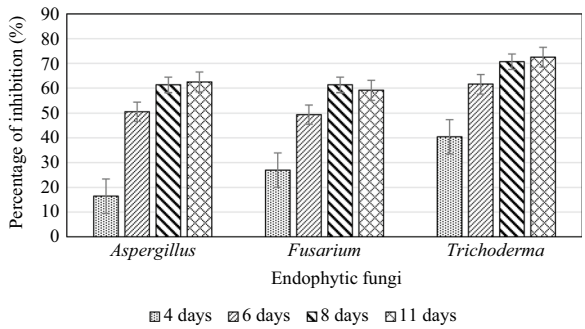

Fig. 2 Percentage of inhibition of the growth of the pathogen *Curvularia* sp. against all three isolates of endophytic fungi on each day of observation

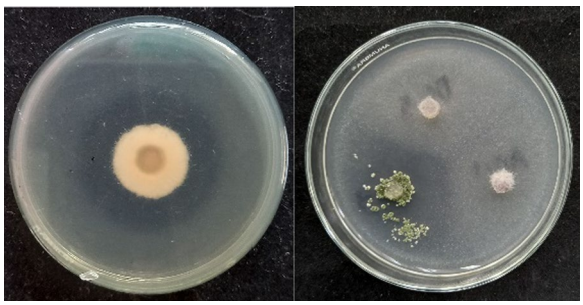

Fig. 4 Activity of phosphate dissolution by *Fusarium*_2 (left) and the activity of chitinase by *Trichoderma*_11 (right) are characterized by the appearance of a clear zone around the colony of fungi

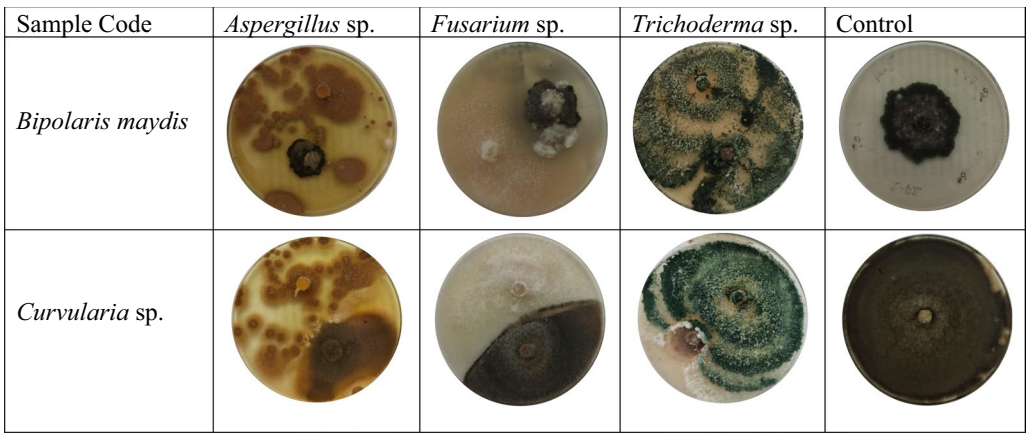

Fig. 3 Appearance of antagonistic activity of isolates endophytic fungal *Aspergillus* sp., *Trichoderma* sp., and *Fusarium* sp. against pathogens of *Bipolaris maydis* and *Curvularia* sp. compared pathogen control on the 11th day after incubation

Table 1 Value of the dissolution/activity index and the effectiveness of the dissolution (%) by endophytic fungal

Sample	Dissolution/activity index		The effectiveness of the dissolution (%)	
	Phosphate	Chitinase	Phosphate	Chitinase
*Aspergillus*_1	1.0 b	1.5 a	2.1 b	52.5 a
*Fusarium*_2	1.9 a	1.1 a	91.5 a	11.8 a
*Trichoderma*_11	1.0 b	1.9 a	0.0 b	90.6 a

Numbers followed by different letters in the same column show marked differences based on the Tukey's test of 0.05

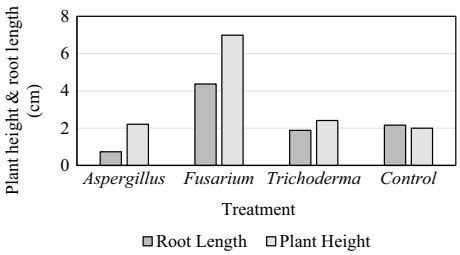

Fig. 5 Comparison of corn's root length (cm) and plant height (cm) of each isolate treatment of endophytic fungal with controls at 7 days after inoculation

Fig. 6 Comparison of root length and plant height of maize in each endophytic fungal tested (above) and the appearance of corn seed growth on PDA media applied by endophytic fungal (bottom) at 7 days after inoculation

Corn seed vigor testing

In testing corn seed vigor on PDA media that was grown endophytic fungal isolates, it was known that all corn seeds were able to germinate 100%. From the length of the roots, the treatment of *Fusarium* sp. showed better results than the other isolates and control with a length of 4.37 cm. The isolate *of Aspergillus* sp. showed smaller root length value compared to the control, which was 0.73 cm. Similar to the length of the roots, the height of the corn plant was better than other isolates, and the control was seen in the *Fusarium* sp. treatment, which was 6.99 cm. But from the height of the plant, it can be seen that the entire isolate was able to grow taller than the control (Fig. 5).

From the results obtained above, it is known that *Fusarium* sp. isolate could spur the growth of corn plants and had the potential to be growth-promoting agents, while the other two isolates, namely *Aspergillus* sp. and *Trichoderma* sp., were considered to have no potential to be used as a growth-promoting agent for corn plants. This is more clearly seen in (Fig. 6) which showed the ratio between root growth and plant height between each isolate.

Discussion

Isolate of *Trichoderma* sp. was potentially a biocontrol agent with an emphasis on developing the pathogen *B. maydis* and *Curvularia* sp. In line with the results of the study (Khan et al. 2017), testing *T. harzianum* against *B. maydis* showed that the inhibition of growth of this pathogen was 81.9–83.2% at 14 DAI. *T. viridae* can suppress corn leaf blight infection in the range of 89.36–93.48% and *T. aureoviridae* suppresses up to 73.94% (Kumar et al. 2021). Testing for inhibition of the development of the pathogen *Curvularia spicifera*, which causes *Curvularia* leaf spot on tomatoes using several consortia *Trichoderma* species, was able to suppress disease infections by about 50–55% (Rao et al. 2020).

In this study, *Trichoderma*_11 showed its ability to suppress the growth of *B. maydis* and *Curvularia* sp. pathogens in vitro better than the isolates *Aspergillus*_1 and *Fusarium*_2. It is presumed that the volatile compound produced by *Trichoderma* sp. is capable of mediating its antifungal activity against both pathogens. The presence of such inhibition can influence the growth of pathogens (Manzar et al. 2022). Among the volatile antifungal compounds produced by *Trichoderma* sp., the

most important and well documented, was 6-pentyl-α-pyron (6-PAP), eight isolates of *Trichoderma* sp., among *T. atrovirid, T. citrinoviridae, T. hamatum, T. harzianum, T. koningii*, and *T. viridae* disqualified were able to produce 6-*n*-pentyl-2H-pyran-one (6-PAP) (Jeleń et al. 2014). The volatile compound Alkyl pyrone produced by *Trichoderma* was an antifungal compound that can inhibit the development of mycelia *Colletotrichum capsici* (Howell 2003). In line with that according to (Chattapadhyay and Dureja 2006), as antifungal alkyl pyrone compounds denature proteins, disrupting the lipid layer, which leads to cell wall damage. However, not all *Trichoderma* interactions with plants are beneficial, as some strains of fungal may be an opportunistic endophyte. For example, during interaction with corn, *T. virens* modified and degraded plant cell walls for internal colonization (TariqJaveed et al. 2021).

In this study, the activity of dissolving phosphates for all strains of fungal was qualitatively assessed on Pikovskaya media supplemented with tricalcium phosphate as a source of inorganic phosphates. Of the endophytic fungal strains tested, all of them were able to form clear zones demonstrating the ability to dissolve phosphates. However, endophyte Fusarium_2 had the highest phosphate dissolving effectiveness and was confirmed at the best plant vigor testing. Djaenuddin et al. (2021a, b) concluded that TM4 bacterial isolates can produce chitinase, while BNt8 isolates have better ability to dissolve phosphates, and both bacterial isolates can increase the growth of corn plants in vivo. Phosphorus is one of the macronutrients needed in high amounts to support plant growth (Khalil et al. 2021). *Fusarium* sp. isolates have the potential to be a growth booster for corn plants when viewed from their ability to spur root growth and plant height twice as much as the controls. The use of endophytic fungus isolates as growth promoters is well known.

All endophytes can produce chitinase, one of the important enzymes responsible for lysing cell walls. As per the results of the research of Putri et al. (2022), an isolate of the endophytic fungi *T. asperellum* can inhibit *Pyricularia oryzae* and can produce hydrolytic enzymes, such as chitinase and cellulase, which function to degrade the cell wall of pathogenic fungi. In this study, the *Trichoderma*_11 isolate was known to have a high potential in inhibiting the development of pathogens of the fungal *B. maydis* and *Curvularia* sp. in vitro and it turns out to have the highest chitinase effectiveness among other endophytes. This is in line with the statement of (Sunny and Kumar 2018); chitinase plays an important role in the biocontrol of many plant pathogenic fungi by lysing the cell walls of fungi.

Aspergillus sp. isolates are known to be able to inhibit infection from the pathogen *Curvularia* sp. range 16.43–62.50% and pathogen *B. maydis* range 21.90–42.86%. The ability to inhibit the endophytic fungus *Aspergillus* sp. was quite high than the controls; this is due to antioxidant compounds actively inhibiting the development of pathogens. This is reinforced by the statement of Singh et al. (2014) that in general the antimicrobial compounds produced by *Aspergillus* are neutral and polar, and have a phenol group. The phenol content in the endophytic fungus *Aspergillus* sp. actively inhibited the movement of the pathogenic fungus *Curvularia* sp. and *B. maydis,* and this suggests that the content of antifungal compounds from the isolates of *Aspergillus* sp. was more specific than that of the isolates of *Curvularia* sp. and *B. maydis*.

Conclusion

Trichoderma_11 isolate was the best biocontrol agent against Maydis Leaf Blight and Curvularia Leaf Spot in vitro with 64.76 and 72.50% inhibition values, respectively, followed by the highest chitinase activity effectiveness of up to 90.6%. Meanwhile, the *Fusarium*_2 isolate had the best promoted the growth of the corn seedlings with the highest phosphate dissolution effectiveness of 91.5%.

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Author contributions
RF, ED, ND, AM, and NN conceived the study; RF, ND, AM, and NN did the morphological examination; ED did statistical analysis; RF wrote the manuscript with major contributions from AM, ND, NN, and ED; and all authors discussed the findings and contributed to the final draft.

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Availability of data and materials
The data and materials of this study are presented in the manuscript.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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