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ORIGINAL ARTICLE

2,4-Diacetylphloroglucinol producing *Pseudomonas fluorescens* JM-1 for management of ear rot disease caused by *Fusarium moniliforme* in *Zea mays* L.

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Abstract
Maize (*Zea mays* L.) is a major cereal crop grown in a large number of countries. Loss in maize yield due to biotic stresses including fungal phytopathogens is a matter of immense concern. Control measures applied for eradication of fungal phytopathogens in maize are not up to the mark and more often involve harsh chemical(s)/pesticide(s) that cause deleterious effects both in humans and soil biota. Greener alternatives, such as the use of rhizosphere microbes in the form of bioinoculants, have proven to be very successful in terms of enhancing crop yield and suppressing fungal phytopathogens. In the present study, fluorescent pseudomonads were isolated from the maize rhizosphere and monitored for their plant growth-promoting (PGP) and biocontrol activities against *Fusarium moniliforme*. Based on various PGP traits and biocontrol potential, isolate JM-1 was found to be most effective and as per 16S rRNA gene sequencing analysis was identified as *Pseudomonas fluorescens*. Further experiments showed that the biocontrol potential of JM-1 against ear rot fungus involved the production of antifungal compound 2,4-diacetylphloroglucinol (DAPG). When examined for antagonistic interaction under scanning electron microscopy (SEM), structural abnormality, hyphal lysis, and deformity in fungal mycelium were observed. In the pot experiment, application of talc-based JM-1 containing bioformulation (in pot trials) showed significant enhancement in maize growth parameters (including the seed number and weight) in comparison to control even in presence of the phytopathogen. Ear fresh weight, dry weight, number of seeds per plant, and 100-grain weight were found to increase significantly by 34, 34, 52, and 18% respectively, in comparison to control. *P. fluorescens* JM-1 can therefore be used as a bioinoculant for ear rot disease control and sustainably enhancing maize yield.

Keywords 2,4-DAPG · Pseudomonas · Fluorescent pseudomonads · Biocontrol · Metabolite · Maize · Ear rot

Introduction

Maize (*Zea mays* L.) is grown throughout the world in various agro-climatic zones. The crop is not only being consumed on daily basis by millions of people globally but also used as a key constituent of animal feed and raw material for the manufacture of several industrial products (Scott and Emery 2016; Sheng et al. 2018). A wide variety of naturally occurring phytopathogens are known that affect the yield and quality of maize crop. Moreover, yield loss is also a big threat to food security in the regions where maize is a major staple food (Shiferaw et al. 2011; Pechanova and Pechan 2015). Among all of the reported phytopathogens of maize, fungal infection and consequent contamination with mycotoxins have raised serious health hazards to both humans and animals (Oldenburg et al. 2017). Maize is highly susceptible to fungal diseases caused by *Fusarium* species including *F. moniliforme* (synonym *Fusarium verticillioides*). The pathogen commonly causes infection in maize and grows in all regions of the world except in cooler areas. It is responsible for causing Fusarium Ear Rot (FER) disease in maize (Teich 1989). Ear rot causes a significant yield loss in maize, while production of mycotoxins, such as fusaric acid, fusarins,

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fumonisins, fusaproliferin and moniliformin, affects the quality of grains and pose severe health risks (Munkvold 2017). The most common observable symptom of ear rot disease is the development of tan or brown color at the tip of the ear due to fungal growth (Munkvold 2003). The fungus can survive on maize residues in form of mycelium or other structures, such as microconidia, macroconidia, and perithecia, and transmitted vertically and horizontally to the next generation of plants via clonal infection of seeds and plant debris (Smith and White 1988; Shin et al. 2014).

Every year tons of systemic fungicides are used by farmers for the management of fungal diseases in cereals. Apart from reducing crop loss, these synthetic fungicides also impact the health of the soil and are considered harmful to beneficial microbiomes leading to their deterioration (Kour et al. 2021). Additionally, indiscriminate use of chemical fungicides is polluting land and water bodies and has proven to be ineffective due to resistance development (Arora et al. 2018; Hu and Chen 2021).

Biocontrol strategies for the management of fungal diseases in cereals have been evaluated by many workers (Tsitsigiannis et al. 2012; Köhl et al. 2019). Notable successes have been achieved by the application of bacterial antagonists to control fungal diseases in maize (Figueroa-López et al 2014; Samsudin et al. 2017). Fluorescent pseudomonads (FLPs) are the most promising groups of plant growth-promoting rhizobacteria (PGPR) and are front runners in this regard. They are considered as potential biocontrol agents against plant pathogens due to their excellent root colonization ability, metabolic versatility, and production of antagonistic metabolites (antibiotics, siderophores, and hydrogen cyanide HCN) (Mishra and Arora 2018; Zhang et al. 2021). Antibiotics production by FLPs mainly include phenazines, 2,4-diacetylphloroglucinol (2,4-DAPG), pyrrolnitrin, pyoluteorin, and cyclic lipo-peptides that are effective against a wide range of phytopathogenic fungi and bacteria (Haas and Keel 2003; Jain and Das 2016). Among these, the phenolic antimicrobial compound 2,4-DAPG is known for its broad-spectrum antibacterial, antifungal, antiviral, and nematicidal activity (Bottiglieri and Keel 2006). Mechanisms of action of 2,4-DAPG include membrane damage, inhibition of mitochondrial electron transport chain and V-ATPase activity, and antiglycation activity (Gleeson et al. 2010; Kwak and Weller 2013; Gutiérrez-García et al. 2017). Studies have shown that the application of 2,4-DAPG producing FLPs can lower the incidences of fungal pathogens and also increase the growth and yield of crops (Validov et al. 2005; Andreolli et al. 2019). Hence, FLPs possessing antagonistic activity can play a promising role as sustainable biocontrol agents and serve as a redeemer in achieving food security. They can be exploited in form of bioinoculants with dual support, i.e., plant growth promotion and disease suppression at the same time. The present study demonstrates the potential of 2,4-DAPG producing *P. fluorescens* JM-1-based bioformulations in the management of ear rot disease caused by *F. moniliforme* and simultaneous growth promotion of maize plants.

Materials and methods

Isolation and screening of fluorescent pseudomonads antagonistic to *F. moniliforme*

Forty-two fluorescent pseudomonads were isolated on King's B medium from the rhizosphere of maize plants growing in different regions of Lucknow, 26.45° N 81.00° E in Uttar Pradesh, India. Isolates were morphologically and biochemically identified according to Bergey's Manual of Systemic Bacteriology (Garrity et al. 2005). To select antagonistic fluorescent pseudomonad, a 5 mm mycelial disk of *F. moniliforme* ITCC No. 2193 (procured from the division of Plant Pathology, Indian Type Culture Collection, IARI, New Delhi, India) growing on potato dextrose agar (PDA) was excised and placed at the center of fresh PDA plate. Thereafter, bacterial isolates were streaked on either side of the fungal disk equidistant from the periphery and incubated at 27 °C for 5 days. After 5 days of incubation, radial growth of the fungus was calculated in relation to the control plate (growing fungus alone) by the following equation:

$$L = \frac{C - T}{C} \times 100,$$

where L is the percentage inhibition of radial mycelial growth, C is radial growth of fungus in the control; T is radial growth of the fungus in the presence of bacterial isolate.

Molecular characterization of the isolate

Molecular identification of selected isolate JM-1 (selected on basis of its excellent biocontrol potential against *F. moniliforme*) was carried out. For polymerase chain reaction (PCR), universal primers (27F 5′-AGAGTTTGATC(A/C)TGGCTCAG-3′ and (1492R 5′-TACGG(C/T)TACCTTGTTACGACTT-3′) (Heuer et al. 1997) were used. Sequence homology was identified using nucleotide Basic Local Alignment Search Tool (BLASTn; https://blast.ncbi.nlm.nih.gov/). The 16S rRNA gene sequence was submitted in the GenBank nucleotide database under the accession number KT734728. For the construction of phylogenetic tree, the 16S rRNA gene sequences of other strains closely related to these isolates were obtained from the NCBI database. Multiple sequence alignments were performed by the Clustal W program. Phylogenetic tree was constructed by the

maximum-likelihood method based on Maximum Composite likelihood model using Molecular Evolutionary Genetics Analysis Version 10.0 (MEGA X).

Plant growth-promoting properties (PGP) of the isolate JM-1

To evaluate PGP characters of the isolate, the following tests were done: phosphate solubilization according to Pikovskaya (1948), zinc (Zn) solubilization according to Saravanan et al. (2007), production of indole acetic acid (IAA) according to Bric et al. (1991), gibberellic acid by Holbrook et al. (1961) and siderophore production as per Schwyn and Neilands (1987).

Screening for hydrogen cyanide (HCN)

Assessment of HCN produced by isolate JM-1 was also carried out. For this, plate of King's B medium amended with 4.4 g/l glycine was prepared and 0.1 ml fresh culture of JM-1 was spread on it. A Whatman No. 1 filter paper dipped in picric acid solution (0.5% picric acid in 2% sodium carbonate) was placed inside the lid of Petri plates. The plates were sealed with parafilm and incubated at 28 °C for 4 days. After incubation, a color change (yellow to brown) in filter paper was visually assessed against the control. Non-inoculated plates were taken as control.

Production, extraction, and purification of non-volatile crude antifungal metabolite produced

Production of the crude metabolite was done according to the methods of Raaijmakers and Weller (2001). For this, an inoculum of JM-1 was prepared in 5 ml KB broth and allowed to grow at 30 °C for 12 h. For batch fermentation, 1% of inoculum (10^8 CFU/ml; optical density (OD_{600}) = 0.5) was added in a conical flask containing 250 ml of pigment-producing medium (peptone 20 g/l, glycerol 20 ml/l, sodium chloride 5 g/l, potassium nitrate 1 g/l, pH 7 ± 0.2) (Kumar et al. 2005) and incubated at 28 ± 2 °C for 5 d on a rotary shaker at 240 rpm. After fermentation, culture was centrifuged at 12,000*g* for 20 min at 4 °C to recover the cell-free supernatant. For extraction, cell-free supernatant was acidified to pH 2 and extracted by an equal volume of chloroform in a separating funnel. The separated organic layers were then combined, dried over anhydrous Na_2SO_4, and subjected to evaporation to 1 ml with a rotary evaporator (40 °C, 250 rpm). After that, the metabolite was re-suspended in 0.5 ml methanol and stored at 20 °C for further analysis. Finally, purification of the crude extract was carried out by thin-layer chromatography (TLC) using silica Gel GRM7480 (Himedia, India) with a solvent system of methanol:chloroform (1:9) according to de Souza and Raaijmakers (2003). TLC spots were visualized by ultraviolet (UV) irradiation at 254 nm and sprayed with diazotized sulfanilic acid (DSA). The R_f value of spots was calculated and compared with previous reports (Rosales et al. 1995). Spots were scratched separately from the TLC plates and transferred into micro centrifuge tubes followed by extraction with methanol. Further, silica residue was removed by centrifugation and the supernatant was used for assessment of in vitro antifungal activity. Crude extract was also fractioned by column chromatography using a glass column (50 cm) packed with slurry of silica gel (60–120 mesh) and eluted with hexane–benzene (1:3) to collect 5 ml fractions with a flow rate of 1 ml/min.

In vitro antifungal activity

To further assess the effect of scratched metabolite against *F. moniliforme*, the agar well diffusion method was used. For this, plates containing PDA were taken and 200 µl of TLC purified metabolite suspended in methanol was placed in a well at a distance of 1.0 cm from the edge of the plate. A mycelial disk of 5 mm in diameter was excised from the edge of an actively growing *F. moniliforme* (on PDA plate), placed at the center of the same plate and incubated at 28 °C for 5 days. In control, only methanol was suspended in wells.

Characterization of purified metabolite

In vitro antifungal activity showing metabolite was further characterized by UV–visible spectrophotometry and Fourier-transmission infrared (FT-IR) spectrophotometry. The absorbance maximum of the fractioned metabolite was determined by scanning its dilute solution in diethyl ether against pure diethyl ether using UV–Visible Spectrophotometer (Evolution 201, Thermo Scientific) in the region of 200–1000 nm. To characterize the functional group of the metabolite, its FT-IR spectrum was measured from 4000 to 500 cm^{-1} by an FT-IR Spectrophotometer (Nicolet TM 6700, Thermo Scientific, USA).

Antagonistic interaction of metabolite with *F. moniliforme* through scanning electron microscope (SEM)

Structural abnormality, hyphal lysis, and deformity in conidia formation induced by fractioned metabolite produced by bacterial isolate were examined under SEM. For this, a method devised by Torres et al. (2016) was applied. In brief, fungal mycelium from near the zone of inhibition (produced by fractioned metabolite) was taken. For control, fungal mycelium grown without the presence of metabolite was taken. The samples were fixed with

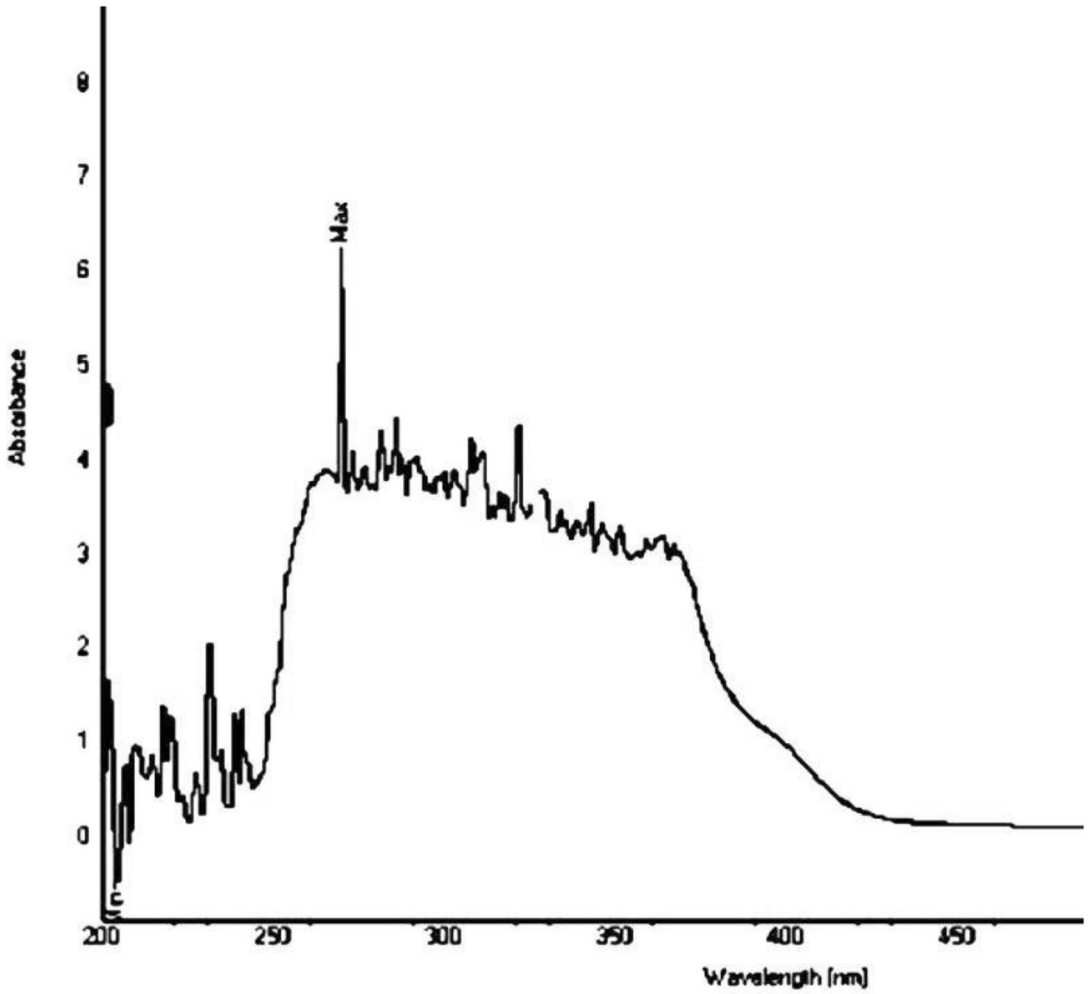


Fig. 1 UV–Vis spectrum of fractioned metabolite (R_f 0.78) showing a tall peak at 270 nm

glutaraldehyde 2.5% v/v in a 0.1 M phosphate buffer (pH 7.2) for 24 h at 4 °C, then dehydrated using 30%, 50%, 70%, 90% and 100% of ethanol. Finally, the metallization of the dehydrated samples was carried out and seen under SEM (Model Jeol Quanta 250).

In vivo pot experiment

Pot experiment was conducted in earthen pots ($50 \times 34 \times 26$ cm) in triplicates during the month of July for two consecutive years (2016–17). For this, 5 kg sandy loam soil having pH 8.78, electrical conductivity (EC) 116 µC cm^{-1}, available phosphorus (P)17.14 kg ha^{-1}, total nitrogen (N) 107.83 kg ha^{-1}, exchangeable potassium (K) 82.80 kg ha^{-1}, and organic carbon (C) 1.72 kg^{-1} was sterilized for 3 h at 15 psi and used. The fungal inoculum was prepared on oat grains (*Avena sativa*) to get 10^4 fungal propagules g^{-1} of soil. Seeds of maize (variety Suvarna NMH589) were surface sterilized using 0.5% NaOCl solution for 1–2 min and rinsed two to three times with sterilized distilled water. For the application of isolate in pot trials, talc-based bioformulations were prepared according to Tewari and Arora (2016) and stored in a sterile polybag. Formulations contained 1.7×10^{10} CFU ml^{-1} at the time of packaging. The seeds coated with talcum powder and 1% CMC slurry served as control. The experiment was conducted in the following set of treatments taking triplicate of each set: T1—non-bacterized seed control; T2—seed coated with JM-1 containing formulation; T3—soil infested with *F. moniliforme*; T4—*F. moniliforme* + JM-1. Total 5 seeds were sown in each pot. Seed germination was tested 10 days after sowing (DAS). Foliar application of talc-based bioformulation was done at 15, 30, 45, and 60 days after showing (DAS). For foliar spray, 20 g of the talc-based formulation was dissolved in 1 l of water (20 g/l) and allowed to settle for 1 h, filtered through muslin cloth, and the filtrate was used for spray. Plants were

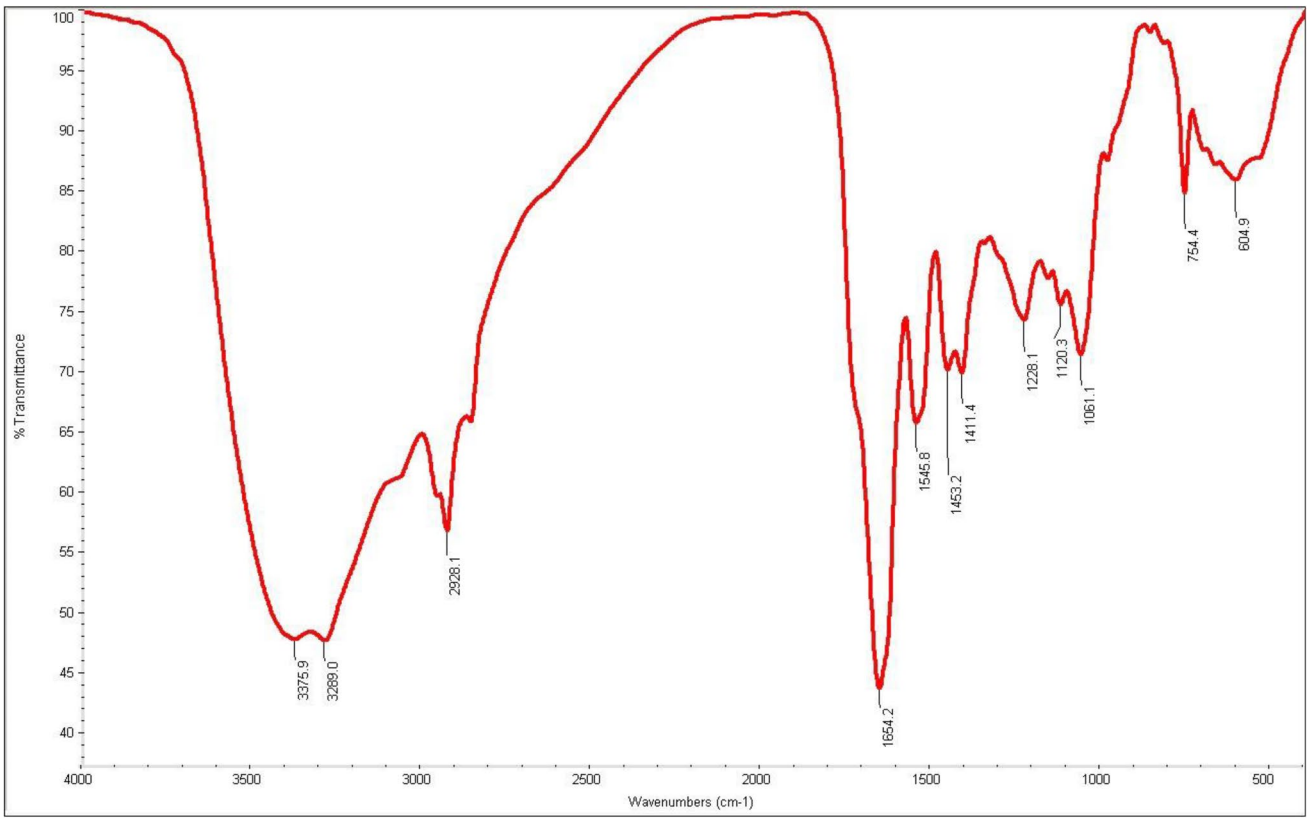


Fig. 2 FT-IR spectrum of TLC fractioned metabolite produced by *P. fluorescens* JM-1

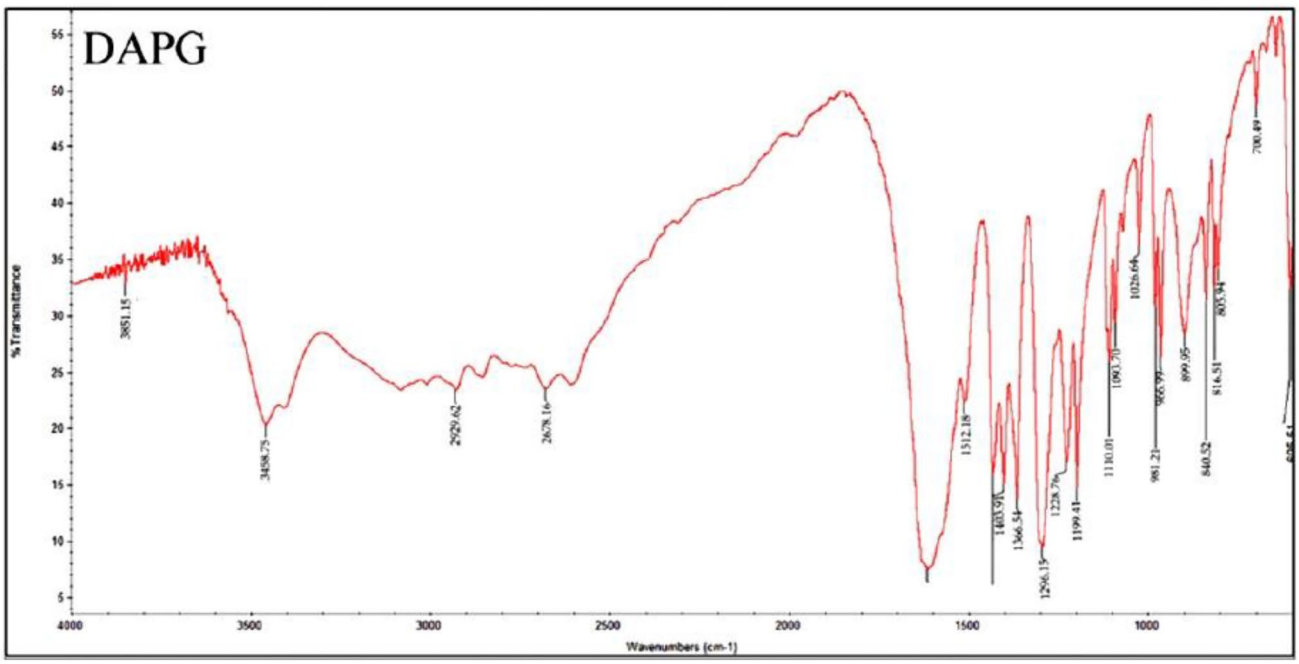


Fig. 3 FT-IR spectrum of synthetic DAPG Source: https://spectrabase.com/spectrum/BuOXfva7g77

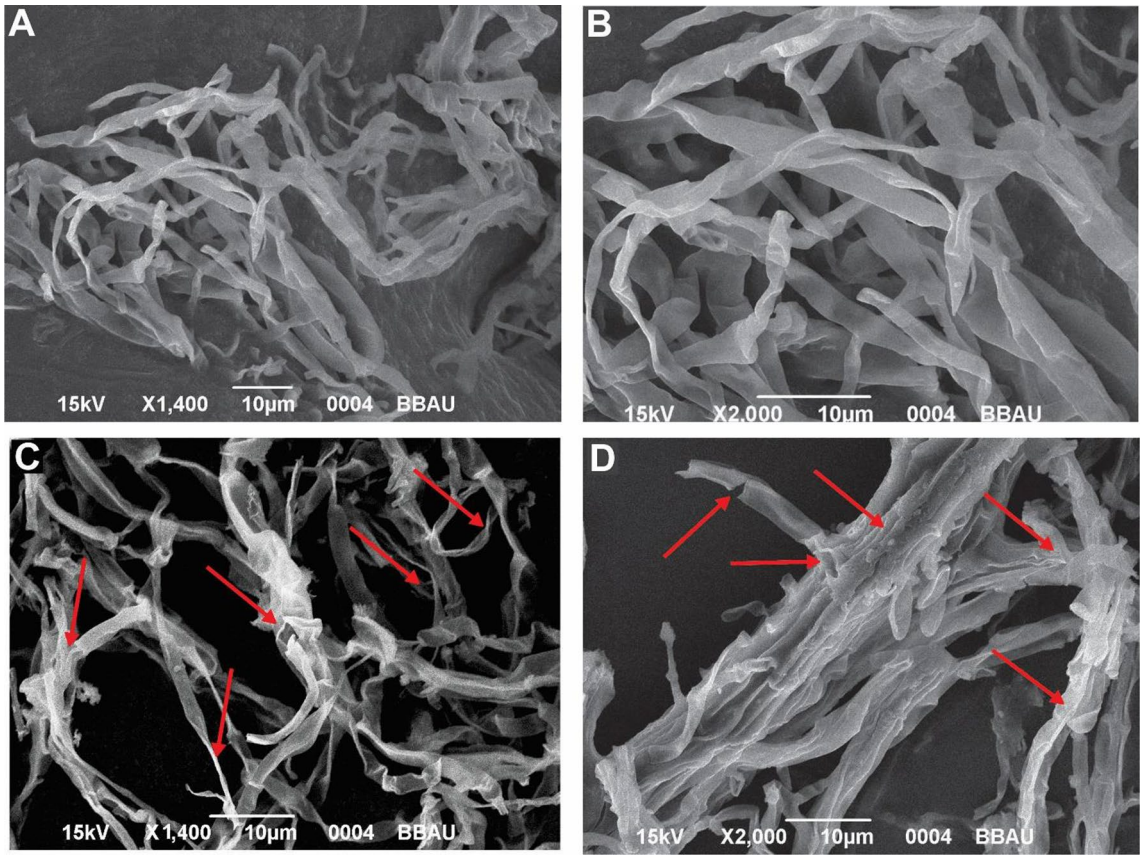


Fig. 4 SEM microphotographs (**A** and **B**) of *F. moniliforme* growth without the presence of antifungal metabolite (control) and (**C** and **D**) growth of *F. moniliforme* in presence of metabolite. Arrows indicate site of deformity

also visually observed for lateral root formation after 45 DAS. Plant growth parameters including shoot and root length, lateral root formation, fresh and dry weight of the plant, ear fresh and dry weight, number of seeds per plant, and 100-grain weight were measured 90 DAS.

Statistical analysis

One-way analysis of variance (ANOVA) was used to determine the effect of the treatments on growth parameters of maize. Duncan's multiple range test ($P < 0.05$) was used for comparing the means, using IBM SPSS statistics version 25.

Results and discussion

Isolation and screening of fluorescent pseudomonads antagonistic to *F. moniliforme*

Fluorescent pseudomonads were isolated from the rhizosphere of the maize plant. Isolates were Gram-negative, motile, curved rod having yellowish green slimy mucoid colonies showing positive results for oxidase, catalase, citrate, urease, arginine dehydrolase, gelatinase and negative for indole, methyl red, Voges–Proskauer, lipase, starch, and casein hydrolysis that are characteristic of genus *Pseudomonas* (Garrity et al. 2005). Out of 42 isolates, 26 showed antagonistic activity against *F. moniliforme*. Isolate JM-1 showed the highest antagonistic activity and hence was selected for further characterization. Molecular identification through Nucleotide-BLAST and 16S rRNA sequencing showed more than 99% homology with *P. fluorescens* strains confirming it to be *Pseudomonas fluorescens* (accession number of KT734728). The constructed phylogenetic tree based on the 16S rRNA gene sequences showed that isolate JM-1 shared a cluster with *P. fluorescens* (Supplementary Fig. a). Fluorescent pseudomonads are one of the dominant rhizospheric microbes and have also been reported from many cereal crops, such as rice, wheat, and maize (Yasmin et al. 2017; Sun et al. 2021). *F. moniliforme* is reported as the major soil-borne fungi causing severe crop loss. In the present study, *P. fluorescens* JM-1 effectively antagonized the ear rot pathogen *F. moniliforme* and showed 69% inhibition of radial growth on dual culture plates.

PGP properties of the isolate JM-1

Analysis of PGP traits confirmed that *P. fluorescens* JM-1 can solubilize phosphate and Zn, produce phytohormones IAA and gibberellic acid. Availability of nutrients, such as P and Zn, is essential for grain yield and in enhancing biomass in maize (Hussain et al. 2017). Phosphorous (P) is necessary for several vital functions of plants, such as photosynthesis, root development, and overall crop maturity (Suleman et al. 2018), but due to its insoluble form in soils, the availability of P is a limiting factor in agricultural systems. Results showed that the phosphate solubilization index (PSI) of isolate JM-1 was 4.372. Earlier in a study, Hameeda et al. (2008) found that application of phosphate-solubilizing *Pseudomonas* sp. CDB 35 significantly improved maize growth parameters and increased maize yield by 64% as compared to the un-inoculated control. Recently Blanco-Vargas et al. (2020) found that phosphate-solubilizing activity of *Pseudomonas* sp., enhanced growth of *Allium cepa* L. in greenhouse conditions. Zn is another imperative micronutrient that is required for optimum plant growth. Zn plays important role in carbohydrate metabolism, auxin metabolism and acts as a potent antioxidant (Kamran et al. 2017). In maize, deficiency of Zn causes severe chlorosis coupled with slight whitish blotches near the base of top early leaves, reduces transpiration rate and stomatal conductance (Mallikarjuna et al. 2020). Zn-solubilizing rhizobacteria are the potential candidates for enhancing the bioavailability of Zn in many crops. In the present study, isolate JM-1 was able to solubilize Zn by 3.213 ZSI (Zn solubilization index). Studies showed that the application of Zn-solubilizing *P. fluorescens* not only enhances the bioavailability of Zn but also facilitates the uptake of other nutrients in plants (Fahsi et al. 2021; Lanna et al. 2021). In a study by Sirohi et al (2015), application of Zn-solubilizing *P. fluorescens* strain Psd was found to improve the growth and productivity of wheat. Abbaszadeh-Dahaji et al (2020), reported that the application of a Zn-solubilizing PGPR strain, *P. fluorescens* T17-24 with exogenous iron source (Fe–EDTA) also increased uptake of other micronutrients like copper (Cu) and manganese (Mn) in sorghum grown in a low-fertile calcareous soil. Production of IAA, an integral plant hormone, is another important PGP trait exhibited by FLPs. IAA is responsible for the proliferation of cells and stimulates the uptake of water, minerals, and nutrients in the host (Patten and Glick 2002; da Silva et al 2020). In the present study isolate JM-1 produced 23.19 mg/l of IAA. Similar findings were shown by Pavlova et al (2017), where the application of IAA producing *P. fluorescens* promoted germination of *Dendrobium nobile*. Similarly, *P. fluorescens* Ms-01 isolated from grapevine rhizosphere produced 32 μgml^{-1} IAA and its inoculation promoted the growth of wheat plants (Kadmiri et al 2018). Like IAA, gibberellins are necessary plant hormone required for important growth processes in plants including stem elongation, seed germination, flowering, and photosynthesis (Olanrewaju et al 2017). Isolate JM-1 was positive for GA production (4.353 mg/l). In same context, a PGPR isolate, *P. fluorescens* PF-8 isolated from paddy rhizosphere showed production of GA (5.96 µg/ml) (Sivasakthi et al 2013). Saber et al (2015) showed inoculation of GA and IAA producing FLP strain SE8 increased nitrogen (N), P, and potassium (K) content in tomato. Iron (Fe) has an essential role in the completion of reproductive stages and maintaining yield in maize (Xue et al. 2014). Application of siderophores producing rhizobacteria is found to enhance germination percentage, shoot and root length, and dry weight in maize (Sharma and Johri 2003; Shirley et al. 2011). Siderophore production by JM-1 was checked and it was found to produce 4.58 psu (percent siderophore unit) by CAS liquid assay. In a study, Sah et al. (2017) reported that inoculation of siderophore-producing *Pseudomonas* increased shoot length, root length, cob length, grain number, iron content of stem and leaf in maize. There are other examples where the application of siderophore-producing FLPs have improved growth parameters in plants (Lurthy et al. 2020; Oliveira et al. 2021).

Screening for hydrogen cyanide (HCN)

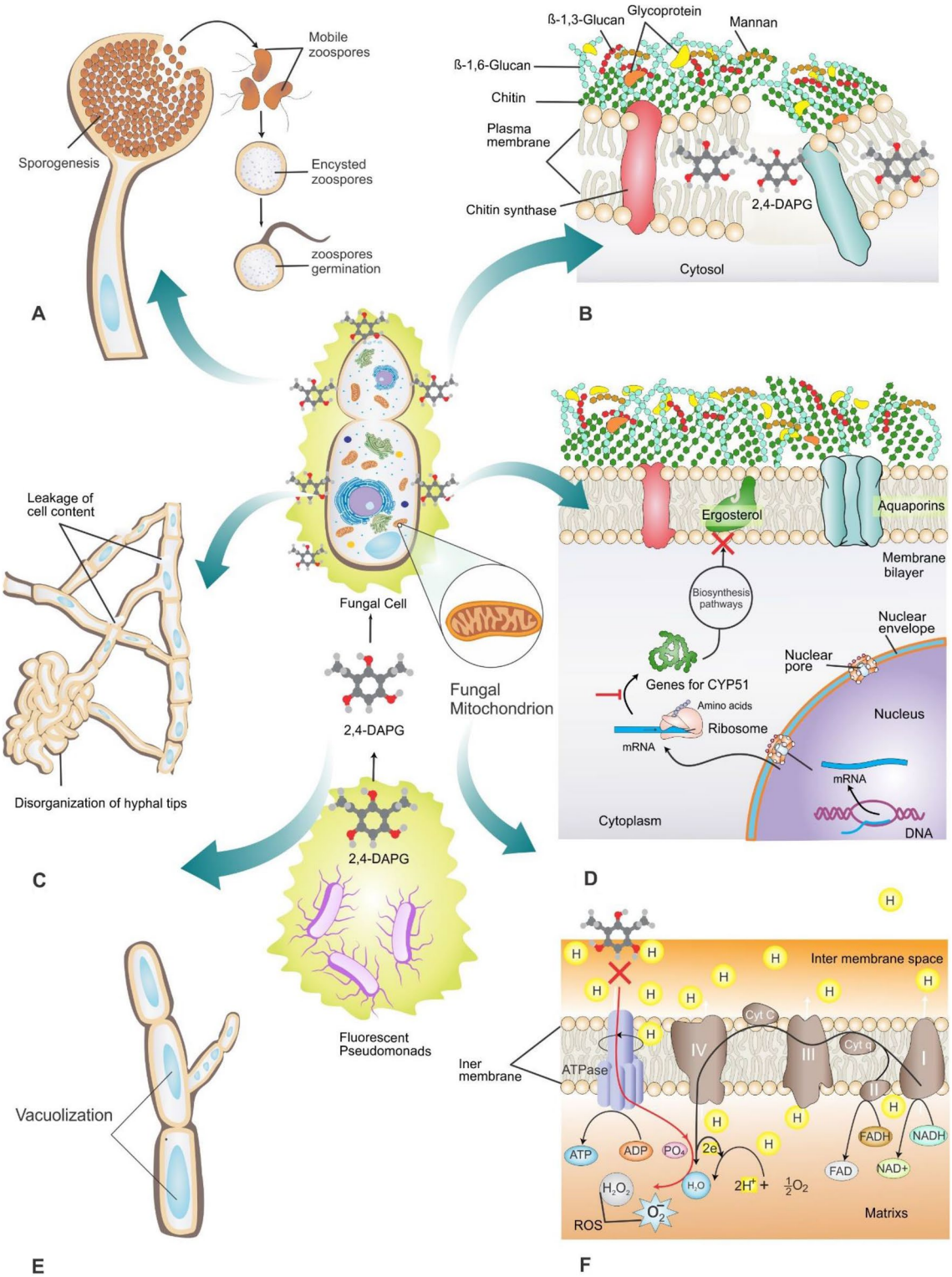
Visual observation of glycine amended King's B plate inoculated with JM-1 did not show color change which indicates that the isolate was unable to produce HCN.

Production, extraction, purification and in vitro antifungal activity characterization of antifungal metabolite

Purification of metabolite by TLC showed the presence of two spots with R_f values 0.78 and 0.74 (Supplementary Fig. b). These were similar as previously reported by Ayyadurai et al. (2006). According to Rosales et al. (1995) under ultraviolet light, pyocyanin metabolite absorbed at retardation factor (R_f) of 0.51–0.53 and appeared as blue, pyrrolnitrin absorbed at R_f 0.23–0.28 while, bright yellow spots at R_f value of 0.35–0.88 belong to 2,4-DAPG (Rosales et al. 1995; Kavitha et al. 2005; Ayyadurai et al. 2006; Showkat et al. 2012). The spot with an R_f value of 0.78 showed inhibition of *F. moniliforme* in the agar well diffusion method.

Characterization of antifungal metabolite

Partial characterization of in vitro antifungal activity showing metabolite by UV–Visible spectrophotometer showed the presence of the highest peak (λ_{max}) at 270 nm (Fig. 1)



which coincides with that of authentic phloroglucinol (Brucker et al. 2008). The FT-IR spectrum of metabolite showed characteristic absorption bands from 3300 cm^{-1} to 600 cm^{-1} (Fig. 2). Functional groups of an average of 40 scans in this frequency range were determined as described by Socrates (2001). The FT-IR spectrum showed (cm^{-1}) 3400–3200 (OHbr) 1600–1400 (aromatic conjugated C=O with intramolecular hydrogen bonds and aromatic C=C),

◂**Fig. 5** Proposed mode of action for antifungal activity of 2,4-DAPG. **A** 1,4-DAPG targets asexual stages of the life cycle. It inhibits the process of sporogenesis and affects the motility and germination of the encysted zoospore. **B** Plasma membranes are more sensitive toward 2,4-DAPG. It causes alteration in structure and permeabilization of the plasma membrane. **C** In many fungi, due to 2,4-DAPG disorganization of cellular content and leakage occurs in hyphal tips. **D** Exposure of 2,4-DAPG influences fungal cell membrane formation by inhibiting the expression of cytochrome P450 14α-demethylase (CYP51) enzyme (see red arrow). CYP51 is responsible for the biosynthesis of ergosterol that helps in maintaining the fluidity of the lipid bilayer. **E** Vacuoles cover many physiological aspects of fungal cells. Exposure of 2,4-DAPG accelerates the rate of vacuolization or hypa space-filling by the vacuole. **F** 2,4-DAPG also acts as an uncoupler of oxidative phosphorylation and ATP synthesis. Acting as a proton ionophore it induces loss of mitochondrial membrane potential and increases oxygen consumption (respiration) but ATP synthesis cannot take place and hence growth is inhibited. Apart from this, due to 2,4-DAPG free radical leakage and the production of reactive oxygen species (ROS) occurs in the mitochondrion

1200–1000 (COH) that are similar to synthetic 2,4-DAPG (Fig. 3) (Pragna Lakshmi et al 2017).

Antagonistic interaction of purified metabolite with *F. moniliforme* through scanning electron microscope (SEM)

There are reports that 2,4-DAPG produced by certain strains of *P. fluorescens* suppresses fungal pathogens causing black root rot of tobacco (*Thielaviopsis basicola*), take-all of wheat (*Gaeumannomyces graminis* var. tritici) (Keel et al. 1992; Mazzola et al. 1995), and damping-off of sugar beet (*Pythium ultimum*) (Fenton et al. 1992; Shanahan et al. 1992). Assessment of the effect of bacterial metabolite on fungal growth and development may provide new insights into the biocontrol trait of FLPs. However, very few studies are available where the effect of bacterial metabolites on fungal deformity is evaluated (Gupta et al. 2001; Minaxi and Saxena 2010; Simionato et al. 2017). In the present study, we noticed that 2,4-DAPG produced by *P. fluorescens* JM-1 caused morphological deformity in *F. moniliforme* (Fig. 4). The SEM images revealed that morphological abnormalities including perforation, fragmentation, swelling, shriveling, and lysis of hyphae in *F. moniliforme* are directly involved in diminishing the growth of the fungal pathogen. Earlier studies have confirmed that membranes of the fungal pathogen are sensitive to 2,4-DAPG (Kwak et al. 2010). According to de Souza et al. (2003), 2,4-DAPG causes retraction and disruption in the plasma membrane, vacuolization, and cell content disintegration in hyphal tips of *Pythium ultimum.* However, there are also evidences suggesting 2,4-DAPG produced by FLPs can arrest cell growth by denucleation, degradation of cytoplasmic content, and impairment of mitochondrial function in many fungal pathogens (Suresh et al. 2021). Using *Saccharomyces cerevisiae* as a model, it was confirmed that 2,4-DAPG inhibits cellular-level respiration and ATP synthesis that led to cell death (Troppens et al. 2013). However, this is for the first time that the impact of metabolite 2,4-DAPG was observed on fungal hyphae. In Fig. 5, antifungal mode of actions of 2,4-DAPG that are involved in the suppression of various fungal pathogens is depicted.

In vivo pot experiment

Seed biopriming with JM-1 showed a significant increase in growth parameters of maize as compared to non-primed seeds (control) (Table 1). Seed treated with JM-1 containing formulation showed an increase in germination percentage by 36% while application of JM-1 in soil infested with fungal pathogen showed a 27% increase. This was probably due to the production of antifungal metabolite and phytohormones at the early stages. Application of talc-based bioformulation showed an increment in shoot and root length, plant fresh and dry weight, by 25%, 51%, 31%, and 34% respectively in T2 (seed coated with JM1) compared to control. JM-1-treated plants also showed a higher number in lateral root formation both in presence and absence of phytopathogen. Ear fresh weight, dry weight, number of seeds per plant, and 100-grain weight increment were found to be 34, 34, 52, and 18% respectively in JM1-treated plants (T2) over control. Even in presence of the pathogen,

Table 1 Effect of different treatments on growth parameters of maize under pot trial conditions (90 DAS)

Treatments	Shoot length (cm)	Root length (cm)	Fresh weight (g/plant)	Dry weight (g/plant)	Ear fresh weight (g/plant)	Ear dry weight (g/plant)	Number of seeds /plants	100 grain weight (g)
T1	139.50 ± 3.11^{a}	19.13 ± 0.87^{b}	277.16 ± 2.49^{b}	31.59 ± 1.30^{ab}	184.09 ± 3.54^{b}	43.21 ± 1.61^{b}	334.00 ± 3.61^{b}	26.69 ± 0.46^{b}
T2	174.50 ± 3.17^{c}	28.97 ± 3.79^{c}	363.99 ± 3.83^{c}	42.42 ± 2.03^{c}	247.24 ± 4.70^{d}	57.81 ± 1.37^{c}	509.00 ± 5.57^{d}	31.58 ± 0.70^{c}
T3	129.13 ± 3.39^{a}	11.60 ± 1.33^{a}	234.04 ± 1.50^{a}	28.09 ± 1.11^{a}	164.56 ± 4.40^{a}	37.14 ± 2.50^{a}	273.67 ± 4.06^{a}	15.38 ± 0.42^{a}
T4	155.97 ± 4.47^{b}	20.50 ± 1.51^{b}	278.51 ± 4.10^{b}	35.65 ± 0.74^{b}	227.33 ± 4.42^{c}	53.62 ± 1.62^{c}	417.33 ± 4.06^{c}	27.60 ± 0.54^{b}

Results are mean ± SD ($n = 3$). Mean in the column followed by the same superscript letters indicates no significant difference ($P = 0.05$) by Duncan's Multiple range test. Three samples were analyzed for each replication and each treatment consisted of three replications

T1 non-bacterized seed control, *T2* JM-1, *T3 F. moniliforme*, *T4 F. moniliforme* + JM-1

JM-1-treated plants (T4) showed significant improvement in growth parameters. JM-1-treated plant showed an increase of 20% in shoot length, 77% root length, 19% plant fresh weight, 27% plant dry weight, 38% ear fresh weight, 44% ear dry weight, 52% in number of seed per plant, and 79% of 100-grain weight in comparison to *F. moniliforme* pathogen (T3) control (Table 1).

The use of beneficial rhizosphere bacteria to seeds (bacterization) has been widely used for growth improvement and for controlling soil-borne plant pathogens in plants (Mahmood et al. 2016; Sundar et al. 2021). In the present study, seed bacterization with JM-1 not only controlled disease incidence of *F. moniliforme* but also improved growth and yield parameters in maize over control.

There are studies where the application of both PGP and biocontrol activity showing FLPs not only improved plant growth but also reduced disease incidence in plants, such as sorghum (Funnell-Harris et al. 2008), pearl millet (Hameeda et al. 2006), wheat (Fischer et al. 2010) and sunflower (Tewari and Arora 2016). In the present study, the strain JM1was efficient both in terms of PGP traits (IAA, GA, solubilized phosphate, and Zn) and showing biocontrol activities via production of antifungal metabolite 2.4-DAPG against *F. moniliforme*. There is evidence that indicates IAA and DAPG produced by FLPs may regulate the growth of the plant (Brazelton et al. 2008). Both IAA and DAPG inhibit the growth of primary roots and stimulate the formation of lateral roots (Brazelton et al. 2008; Khare and Arora 2010). As lateral roots form the major portion of the root systems of maize and are mainly involved in the uptake of water and nutrients (Husakova et al. 2013), the ability of JM-1 to produce IAA and DAPG could have helped in improving growth parameters in maize. Studies also indicate that colonization of *F. moniliforme* is primarily found in lateral roots and mesocotyl tissue of maize (Oren et al. 2003). In such conditions, stimulation of lateral root formation by IAA and 2,4 DAPG produced by JM-1 could be acted as a second line of defense to protect maize against *F. moniliforme.* Phytohormone GA also controls various aspects of seed germination, including the loss of dormancy and the mobilization of endosperm reserves in maize (Kaya et al. 2006). Along with IAA and DAPG, the production of GA by JM-1 may also have triggered the activity of specific enzymes that promoted germination of maize and subsequently improving plant health.

For maximizing the yields of maize, phosphate and Zn are required in sufficient concentration (Calderón-Vázquez et al. 2011; Ruffo et al. 2016). Under low phosphate, root elongation, lateral root development, and root architecture of maize are affected (Jia et al. 2018). While low to moderate deficiency of Zn affects root growth parameters including root structure, antioxidant enzyme activity, hydraulic conductivity, aquaporin gene expression, and leaf water potential, chlorophyll content, leaf area, and gas exchange parameters (Zhang et al. 2021). In this context, the ability of JM-1 to solubilize insoluble forms of phosphate and Zn would have helped to enhance maize growth parameters. In a study by Viruel et al. (2013), inoculation of maize with phosphate-solubilizing *Pseudomonas tolaasii* IEXb and *Pseudomonas koreensis* SP28 was found to enhance growth parameters and yield of maize.

Every year soil-borne pathogenic fungi cause severe loss to various crops. Fungal rot diseases are more prevalent in different parts of the globe and are responsible for a huge yield loss (Gai et al. 2018). For the protection of maize against seed-borne fungal diseases, its seeds are often pre-treated with a fungicide, such as captan, etridiazole, or thiram. Application of these and other similar fungicide is hazardous and also target other beneficial microorganisms (Yang et al. 2011). In most of the incidences, the use of these fungicides also develops resistance among phytopathogens (Andriolli et al. 2016). While breeding methods for developing resistance to Fusarium ear rot and fumonisin contamination in maize are also found to be labor-intensive, time-consuming, and expensive (Butoto et al. 2021), in the last few years, eco-friendly greener approaches to disease suppression that involve potential biocontrol agents have gained attention (Wang et al. 2021). Research has shown that inoculation of biocontrol and PGP traits of genetically diverse *Pseudomonas* improved wheat and maize grain yields (Agaras et al. 2020).

Studies have shown that often non-volatile metabolites produced by FLPs are involved in the suppression of fungal pathogens (Frapolli et al., 2007; Shirzad et al. 2012; Asadhi et al. 2013). The 2,4-DAPG is a non-volatile polyketide antibiotic produced by FLPs. Studies indicate that at very low concentrations, the antibiotic may inhibit a vast array of phytopathogens including soil-borne fungi, oomycetes, bacterial pathogens, and nematodes (Biessy et al. 2021). 2,4-DAPG targets the different structures of fungus during its life cycle. For instance, in oomycetes zoospores are most sensitive while in higher fungi exposure of 2,4-DAPG directly affects the growth of vegetative mycelium (Gong et al. 2016; Palma-Guerrero et al. 2021). In the present study, 2,4-DAPG also caused structural changes in the actively growing mycelium of *F. moniliforme*. The ear rot fungus *F. moniliforme* may infect maize either through airborne conidia that first infect the silks and later on its kernel or via the systemic infection of seed. Systemic infection begins with the growth of fungal conidia or mycelia that are either carried inside the seeds or on the seed surface (Oren et al. 2003). Systemic infection is more vulnerable in early stage of maize growth. Hence, it is imperative to control disease severity by suitable means in the very beginning.

Owing to their diversity, multifarious PGP properties, and biocontrol activity, FLPs are among the most recognized

PGPR strains (Novinscak and Filion 2020). They are highly competent in the rhizosphere, produce a wide variety of plant growth-promoting compounds, and mitigate adverse effects of biotic stress in plants (Gómez-Lama Cabanás et al. 2018; Mishra et al. 2020). Unlike their synthetic counterparts, FLPs-based formulations are cheaper, reliable, effective, eco-friendly, and provide sustainable solutions against fungal pathogens. Hence, it can be undoubtedly stated that the application of FLPs-based bioinoculants can provide dual benefits, i.e., growth enhancement and protection from phytopathogens in plants. In the present study, it is clear that both PGP and biocontrol activities of *P. fluorescens* JM-1 not only helped in enhancing maize growth but also conferred protection against ear rot disease. To the best of our knowledge, this is the first study in which through SEM, direct involvement of 2,4-DAPG in causing aberration, deformity, and lysis in the mycelium of *F. moniliforme* is reported. Such destruction of the fungal mycelium had impacted a lot in controlling the ear rot disease of maize. Such a PGPR strain can be used as an effective biostimulant for maize in tropical regions where *F. moniliforme* infestation is common. However, further studies including mechanistic insights and field trials will be required before using *P. fluorescens* JM-1in agro-ecosystems.

Conclusion

The study confirmed that the application of 2,4- DAPG producing *P. fluorescens* JM-1 in the form of bioformulation is effective in controlling ear rot disease caused by *F. monoliforme* in maize. Apart from biocontrol of *F. monoliforme*, PGP properties (IAA production and solubilization phosphate and Zn) of *P. fluorescens* JM-1 also helped in the improvement of growth parameters in maize. Bioformulations based on 2,4-DAPG producing *P. fluorescens* JM-1 can serve as a competitive strategy to control phytopathogens and can help in achieving targets of sustainable agriculture.

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Declarations

Conflict of interest The authors declare that they have no conflict of interest in the publication.

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