Combined interaction between the diazotrophic *Niallia circulans* strain YRNF1 and arbuscular mycorrhizal fungi in promoting growth of eggplant and mitigating root rot stress caused by *Rhizoctonia solani*

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Summary. Rhizoctonia root rot of eggplant, caused by *Rhizoctonia solani*, is an economically important disease. *Niallia circulans* YRNF1 and arbuscular mycorrhizal fungi (AMF) were assessed for their biocontrol and biofertilizing effects against *R. solani*, as potential replacements for synthetic fungicides and fertilizers. The diazotrophic *N. circulans* YRNF1, isolated from soil, reduced *in vitro* growth of *R. solani* by 42%. GC-MS analysis of culture filtrate of *N. circulans* YRNF1 detected bioactive compounds, including butyric acid (85%) and ethylene glycol (8%). In greenhouse experiments, combined application of *N. circulans* YRNF1 and AMF reduced the severity of eggplant root rot by 26%. This combined treatment triggered the transcriptional expression of five resistance genes (*JERF3, PAL1, C3H, CHI2, and HQT*) in the treated eggplants. Biochemical analyses of the infected eggplant roots treated with the combined bio-inoculants showed enhancement of the phenol content (+188%), and increased antioxidant enzyme activity, mainly of POD (+104%) and PPO (+72%). Combined application of *N. circulans* YRNF1 and AMF also promoted eggplant growth and improved the total NPK concentrations in treated plant leaves. Inoculation of eggplant with *N. circulans* YRNF1 in the presence of AMR increased the mycorrhization level. This is the first report of *N. circulans* and AMF as potential agents for biological control of Rhizoctonia root rot and growth promotion of eggplant.

**Keywords.** Biocontrol, mycorrhization, antifungal activity, biofertilizer, biofungicide.
INTRODUCTION

*Solanum melongena* L. (eggplant) is the most important member of Solanaceae (Kaniyassery et al., 2022). China is the greatest eggplant producer and Egypt is the 3rd largest producer, recording annual production of 1,396,725 tons (FAOSTAT, 2024). Rhizoctonia root rot of eggplant is caused by *Rhizoctonia solani* (Kühn (Almamory and Matloob, 2019). The soil-borne pathogen causes diseases of several hosts, including root rots, damping-off, leaf spots, and leaf blights, which result in a significant reduction in crop yields (Rashad et al., 2018).

Effective control of *R. solani* is difficult, due to its wide host range (Cook et al., 2002). The most widely used control method is application of synthetic fungicides, but these can have deleterious effects on human and animal health, may induce fungicide resistance in pathogens (Hollomon, 2015), and lead to environmental pollution (Baite et al., 2021). Biological control of plant diseases has received increasing attention as a possible effective, eco-friendly and safe control strategy against various plant diseases (Al-Askar et al., 2014).

Arbuscular mycorrhizal fungi (AMF: *Glomeromyota*) live in symbiotic associations with more than 85% of the terrestrial plants (Spatafora et al., 2016; Mathur et al., 2018). AMF have been extensively studied as potential bio-protectants against several fungal pathogens, including *R. solani, Colletotrichum* spp., *Alternaria* spp., *Phytophthora* spp., *Fusarium* spp., and *Puccinia* spp. (Devi et al., 2022). AMF can also promote plant growth (de Oliveiraa et al., 2022). For example, mycorrhization of pea roots gave effective biocontrol of Rhizoctonia root rot (Rashad et al., 2022a). However, improvement of AMF biocontrol efficiency by application with other compatible antifungal microorganisms may also be worthwhile.

Nitrogen-fixing bacteria convert atmospheric nitrogen to ammonia, which can be absorbed by plants. These bacteria can promote plant growth, facilitate nutrient uptake and phosphor solubilization, and produce siderophores and phytohormones (Shameem et al., 2023). In addition, *Bacillus circulans* CB7 Jordan, 1890 (now known as *Niallia circulans*), a non-symbiotic nitrogen-fixing bacterium, with plant growth-promoting properties due to auxin production, P-solubilization and siderophore production, has also shown antagonistic effects against *Dematophora necatrix* (Mehta et al., 2015).

Triggering immunity-related genes in plants is an important mode of action of biocontrol agents. Jasmonic acid and ethylene-response factor 3 gene (*JERF3*), the responsive gene that manages several defence-response genes through the jasmonate/ethylene signalling pathway, is elicited by abiotic and biotic plant stresses (Rashad et al., 2022b).

Polyphenols have potential as natural antioxidants, which can protect the living organisms from deleterious effects of the reactive oxygen species (ROS) (Elshafie et al., 2023). These compounds have antioxidant activity, as well as antihypertensive, antimicrobial, and antiviral activity (Losada-Barreiro et al., 2022). Excessive production of ROS causes oxidative stress in humans, leading to the development of several ailments (Forman, and Zhang, 2021). Antioxidant polyphenols have several modes of action, including reductive ability to neutralize ROS, chelation of metal ions that elicit oxidative stress, inhibition of enzymes involved in the formation of ROS, and activation of the antioxidant enzymes (Dias et al., 2021). Upon infection of plants by the microbial pathogens, their cell walls accumulate large amounts of lignin (Rashad et al., 2020a). Increased lignification is a main barrier against the pathogen spread, and reduces the infiltration of toxins and fungal enzymes into plant cell walls. Lignin compounds also cause fungal pathogens to lose abilities to infect host plants, and prevent pathogen movement and multiplication (Ma et al., 2017). Flavonoids are plant responsive metabolites that contribute to host resistance in response to various abiotic and biotic stresses. These compounds act as physical or chemical barriers to prevent the microbial invasion, and are toxic defences against the microbial pathogens and/or insects. They interfere with pathogen cellular processes and structures (Ramaroson et al., 2022). The phenylalanine ammonia lyase 1 gene (*PAL1*) encodes phenylalanine ammonia lyase, which is involved in biosynthesis of polyphenolic compounds that have roles in host plant systemic resistance (Rashad et al., 2020a). During the early stages of lignin biosynthesis, the 3-hydroxylase gene (C3H) catalyzes conversion of 4-hydroxymate to caffeate (Shrestha et al., 2022). The Chalcone isomerase 2 gene (*CHI2*) encodes for chalcone flavonone isomerase that catalyses the first two steps of flavonoid biosynthesis (Chao et al., 2021). Niggeweg et al. (2004) reported that the Hydroxycinnamoyl-CoA quinate hydroxycinnamoyl transferase gene (HQT) catalyzes biosynthesis of chlorogenic acid from caffeoyl-CoA and quinic acid.

The present study had the following objectives: 1) to investigate in vitro suppressive potential of the diazotrophic bacterium *N. circulans* YRNFI against *R. solani*; 2) to assess biocontrol effects of a combined treatment with *N. circulans* YRNFI and AMF on Rhizoctonia root rot of eggplant, 3) elucidate the host defensive mechanisms elicited by these combined bioagents, based on the transcription of some responsive genes, phenolic com-
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pound content, and antioxidant activity; and 4) assess effects of combined bioagents on eggplant development.

MATERIALS AND METHODS

Fungi and eggplant cultivar

The pathogenic fungus *R. solani* (AG-2-2 IIIB) was provided from Mansoura University, Egypt. To prepare the inoculum, a 500 ml glass flask containing 100 g of sterilized oat grains mixed with sand (1:2 v:v) was aseptically inoculated with five discs (6 mm diam.) cut from a 5-d-old culture of *R. solani*, incubated at 28±2°C, and was shaken daily for two weeks to ensure uniform growth of the fungus (Youssef *et al.*, 2016). AMF inoculum (73% colonization index) was provided by the Agricultural Research Center (ARC), Giza, Egypt. The mixed inoculum of potential biocontrol AMF contained spores of *Claroideoglomus etunicatum* (W.N. Becker & Gerd.) C. Walker & A. Schüssler and *Rhizoglomus intraradices* (N.C. Schenck & G.S. Sm.) Sieverd, G.A. Silva & Oehl (in an equal ratio). AMF were propagated by inoculation of sterilized sandy-clay soil with 10 g of grain of the AMF inoculum (approx. 50 AM spores and root pieces g⁻¹ soil). The inoculum was applied as a grain bed before planting grains of maize (potential host) in a sterilized plastic pot under the greenhouse conditions (27/22°C, 75% relative humidity, and 16 h daily light period). No fertilizers were added and irrigation was regularly applied to 50% field capacity. After 3 months, the AMF colonized roots were cut using a sterile scalpel into small segments, and were mixed with the AMF spores in the maize rhizosphere soil to be used as AMF inoculum (approx. 45 AM spores and mycorrhizal root pieces g⁻¹ soil) (Nafady *et al.*, 2019). Seeds of the eggplant cultivar ‘Rondona’ were used, which were provided by the ARC, Giza, Egypt.

Collection of soil samples

Twenty-one soil samples were collected from several cultivated fields in Alexandria and El-Beheira governorates, Egypt, and were immediately transported to the laboratory. These samples were stored for subsequent studies at 4°C.

Isolation of the diazotrophic bacteria

Approximately 10 g of each soil sample were added aseptically to 90 mL of sterilized water in an Erlenmeyer flask, vigorously mixed for 45 min at 150 rpm, and then serial dilutions were prepared (10⁻¹ to 10⁻⁴). Diazotrophic bacteria were isolated from the soil according to Döbereiner (1988) with slight modifications. Plates of nitrogen free (NF) agar medium, containing glucose (20 g L⁻¹); K₂HPO₄ (0.2 g L⁻¹), NaCl (0.2 g L⁻¹), MgSO₄·7H₂O (0.2 g L⁻¹), K₂SO₄ (0.1 g L⁻¹), CaCO₃ (5 g L⁻¹), and agar (20 g L⁻¹), were individually inoculated with 0.1 mL of each serial dilution of soil suspension; Three replicated plates were used for each dilution. The plates were then incubated for 5 d at 28±2°C. Growing bacterial colonies were then singly transferred onto fresh NF plates and incubated at the same conditions. The resulting cultures were stored in 15% glycerol at -80°C until used (Kifle and Laing, 2016).

Screening of the diazotrophic bacteria for antifungal potential against *R. solani*

The ability of the isolated diazotrophic bacteria to antagonize *R. solani* was evaluated using the *in vitro* dual plate method (Jasim *et al.*, 2016) on potato dextrose agar plates (PDA, Difco). In each plate, 10 μL of each bacterial isolate (10⁶ cells mL⁻¹) were streaked aseptically as a longitudinal line 2 cm away from the border of the plate. A disc (6 mm diam.) cut from a freshly grown *R. solani* culture was placed 20 mm from the opposite side of the plate. Plates inoculated only with pathogen discs served as experimental controls. Each treatment was applied in six replicates, and the assay was repeated twice. After incubating the plates for 4 d at 25°C, and upon complete coverage of control plates with fungal growth, inhibition of fungal growth in the dual plates was measured using a calibrated ruler, and compared with the fungal diameter in the corresponding control plate. Suppression of the fungal growth (S %) was determined using the equation of Ferreira *et al.* (1991):

$$S\% = \frac{C - T}{C} \times 100$$

where C = radial growth in the control plate, and T = radial growth in the treated plate.

Biochemical analyses of bacterial culture filtrate using gas chromatography-mass spectrometry (GC-MS)

Secondary metabolites in cell free culture filtrate of *N. circulans* YRNFL were identified qualitatively using GC-MS. The culture filtrate of *N. circulans* YRNFL was obtained by inoculating 200 μL of the bacterial suspension in sterile distilled water (6 × 10⁶ cfu mL⁻¹) into a 500 mL capacity Erlenmeyer flask containing 250 mL of
nutrient broth (NB) [1% (w/v) each of beef extract and peptone, and 5% (w/v) NaCl]. The culture was incubated under shaking at 120 rpm at 28°C (Nisa et al., 2019). After 3 d, the NB was centrifuged for 10 min. at 4°C at 11,200 g (Thermo Fisher Scientific). The collected cell-free supernatant was filter sterilized using 0.22 μm filters (Millipore, Millipore). The cell-free culture filtrate was lyophilized to complete dryness, re-suspended in methanol-water (50 μL) dissolved in pyridine, followed by incubation for 90 min. For derivatization of the sample, 50 μL of silylation reagent [bis(trimethylsilyl) trifluoroacetamide + trimethylchloro-silane, 99:1 v:v] were added to the sample. The GC-MS system (GCMS-QP2010 Plus, Shimadzu), equipped with a gas chromatograph (7890B) and a mass spectrometer detector (5977A), was used to analyze the sample, at the Central Laboratories Network, NRC, Giza, Egypt. This system involved an HP-5MS column (30 m × 0.25 mm × 0.25 μm). Hydrogen was used as the carrier gas at the flow rate of 2.0 mL min⁻¹. In this assay, 1 μL was used as an injection volume according to several processing conditions, including 50°C for 5 min, which rose at 10°C min⁻¹ to 100°C, and then at 20°C min⁻¹ to 320°C. Mass spectra were obtained by electron ionization (EI) at 70 eV, involving a spectral range of m/z 50–700, and a solvent delay of 4 min, where 230°C was the mass temperature used and the Quad was at 150°C. Biochemical characterization of the different bacterial filtrate constituents was obtained by comparing the spectrum fragmentation pattern with that stored in the data of Wiley and the National Institute of Standards and Technology (NIST) Mass Spectral Library.

Detection of the nitrogenase gene (nifH)

Presence of the nitrogenase (nifH) gene was detected in the isolated diazotrophic bacteria according to Tan et al. (2009), as follows:

**DNA extraction.** A 2-d culture of each bacterial isolate grown on NF medium was centrifuged for 2 min at 1792 g. The resulting pellet was re-suspended in sterilized water (100 mL) and re-centrifuged for an additional 2 min at 1792 g, followed by heating in a water bath (90°C) for 10 min. After re-centrifugation, the supernatant was added to a sterile tube (0.5 mL capacity) and used as a DNA template. The resulting crude DNA concentration was estimated at using a UV spectrophotometer at OD260 and OD280 (PERSEE).

**Polymerase chain reaction (PCR).** The reaction mixture (25 μL) of PCR reaction involved a template DNA (= 100 ng); primer (1 mM of each primer), DNA polymerase (25 μL⁻¹), 5 × buffer, MgCl₂ (1 mM), dNTPs (0.2 mM), and sterile H₂O. Sequences of the nifH primers used were as follows: nifH-F (5’AAAGGYGGWATCG-GYAARTCCACCAC3’) and nifH-R (5’TGTTSAGCS-GCRTACATSGCCATCAT3’) (Turk-Kubo et al., 2012). The processing reaction was conducted on a thermocycler (Eppendorf) under processing conditions of one cycle (95°C for 3 min), then 30 cycles (each of 95°C for 1 min, 52°C for 1 min, 72°C for 1 min, and 72°C for 5 min).

**Molecular identification of the selected bacterium using 16S rRNA gene**

DNA of the promising nitrogen-fixing bacterial isolate was subjected to amplification of the 16S-rRNA region, using the primer 16S-27F: 5’-AGAGTTTGATCCTGGCTCAG-3’ and 16S-1492R: 5’-CGGTTACCTTGTTACGACTT-3’ (dos Santos et al., 2019). The PCR mixture was subjected to the Exosap-IT (GE Healthcare) PCR clean up protocol. The 16S rRNA gene nucleotide sequence was determined through Sanger sequencing using the DNA Analyzer of Applied Biosystems 3730 × l. Using the Big Dye Terminator from ABI; the two primers 27b F and 1492uR were used for setting up the PCR reactions (dos Santos et al., 2019). The Vector NTI software (Invitrogen) was used to align the sequences from the forward and reverse primers, while the contigs were subjected to BLAST to search for nucleotide similarity (Zhang et al., 2000). The maximum likelihood method through MEGA X software (10.2.4) was used to generate a phylogenetic tree of the selected isolate.

**Greenhouse experiment**

Eggplant seeds were surface sterilized (using 0.05% sodium hypochlorite) and planted (one per pot) into 20 cm diam. plastic pots containing sterilized clay soil. The soil physical composition was: silt (35 ± 0.13 g kg⁻¹), sand (110.2 ± 0.21 g kg⁻¹), and clay (453 ± 0.18 g kg⁻¹). The soil chemical properties were: pH = 7.58, EC, 1.32 dS m⁻¹, available P (26 mg kg⁻¹), total N (2.84 g kg⁻¹), available K (310 mg kg⁻¹), organic matter (1.72 g kg⁻¹), total organic carbon (0.99 g kg⁻¹), and total CaCO₃ (5.9%). For AMF colonization, an AMF inoculum was added to each pot under the seeds (10 g seed⁻¹ of the AMF inoculum). Infestation of the soil was carried out by mixing the upper layer with the *R. solani* inoculum (3% w/w), and the pots were watered daily for 10 d before planting. For preparation of bacterial inoculum, the diazotrophic bacterium *N. circulans* strain YRNF1 was grown in NF broth on a rotary shaker for 3 d. The resulting bacterial suspension (adjusted at 10⁸ cell mL⁻¹) was mixed with 1% Arabic gum. For application of *N. circulans* YRNF1,
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Eggplant seeds were soaked for 30 min before planting in freshly prepared bacterial inoculum. Three hours before planting, the seeds in control pots were treated with the fludioxonil (50.0%) fungicide at 3.5 mL kg⁻¹ as a positive fungicide control. The negative control involved a set of pots that was untreated. Nine treatments were applied in this experiment. These were:

- non-mycorrhizal, untreated, uninfected plants (designated C);
- non-mycorrhizal, untreated and infected plants (P);
- non-mycorrhizal, treated with *N. circulans* YRNF1 and uninfected plants (B);
- mycorrhizal, untreated and uninfected plants (M);
- mycorrhizal, treated with *N. circulans* YRNF1 and uninfected plants (B+M);
- non-mycorrhizal, treated with *N. circulans* YRNF1 and infected plants (B+P);
- mycorrhizal, untreated and infected plants (M+P);
- non-mycorrhizal, treated with the fungicide and infected plants (F+P); and
- mycorrhizal, treated with *N. circulans* YRNF1 and infected plants (B+M+P).

No fertilization was applied in this experiment. Five replicates were used, and the pots were arranged in a randomized complete block design. They were maintained at 70% relative humidity, in a 27°C day 17°C night temperature regime in a greenhouse, and were irrigated with tap water when necessary. This experiment was repeated twice.

Assessment of the disease severity

Five plants from each treatment were carefully uprooted, and the adhering soil was removed with tap water. The disease severity was evaluated according to Wen *et al.* (2005). This scale included six severity categories: 0 = no necrosis; 1 = small root necroses (2.5 mm length); 2 = necrosis (2.5–5 mm); 3 = necrosis ≥ 5 mm; 4 = crown and shoots covered with necrotic lesions; or 5 = seedlings damped-off. The disease severity (DS%) was estimated according to Taheri and Tarighi (2010):

\[ DS(\%) = \frac{n_1 + 2n_2 + 3n_3 + 4n_4 + 5n_5}{5N} \times 100 \]

where \( n_1 \) was the number of plants that had severity category 1; \( n_2 \) was number of plants that had level 2; etc., and \( N \) was the total number of evaluated plants.

Quantifying the expression of the defense-related genes using qPCR

Transcription of some responsive genes in eggplant roots was quantified at 14 d post planting (dpp). The studied genes included *JERF3*, *PAL1*, *C3H*, *CHI2*, and *HQT*. α-Tubulin and β-actin were used as reference genes, based on their stability in the mycorrhizal plants (Fuentes *et al.*, 2016). The primer sequences used are shown in Table 1. The RNeasy Kit (Qiagen) was used for extraction of total RNA, in accordance with the manufacturer’s instructions. A SureCycler 8800 (Agilent, USA) was used to synthesize the cDNA. The total volume (20 μL) of the reaction mixture was composed of 3.5 μL RNase-free H₂O; 3 μL 5×-buffer; 3 μL RNA (30 ng), 3 μL of dNTPs (10 mM), 7 μL of dT primer (5 pmol μL⁻¹), and 0.5 μL of the RT enzyme.

The reaction was carried out using the RotorGen 6000 (Qiagen) real-time system. The qPCR was run for 1 h at 43°C, then 10 min at 71°C. The qPCR mixture consisted of 3 μL cDNA, 1.6 μL sterile water, 1.5 μL of each primer, and 10 μL of the qPCR master mix.

<table>
<thead>
<tr>
<th>Primer name</th>
<th>Abbreviation</th>
<th>(5’-3’ )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jasmonate and ethylene-responsive factor 3</td>
<td>JERF3</td>
<td>GCCATTGCCCTTCTCTGTCCTC</td>
</tr>
<tr>
<td>Phenylalanine ammonia lyase 1</td>
<td>PAL1</td>
<td>AGCGGGTTGGCACAATCTAATCTGACA</td>
</tr>
<tr>
<td>4-coumarate 3-hydroxylase</td>
<td>C3H</td>
<td>TTGGTGGCTACGACATTCCTAAGGA</td>
</tr>
<tr>
<td>Chalcone isomerase 2</td>
<td>CHI2</td>
<td>GGAGGGCAATCTGAATGGTTATTCC</td>
</tr>
<tr>
<td>Hydroxycinnamoyl-CoA quinate hydroxycinnamoyl transferase</td>
<td>HQT</td>
<td>CATAGTCATCAATGATCCAAAGG</td>
</tr>
<tr>
<td>α-tubulin</td>
<td>α-tubulin</td>
<td>TATCTGCTACAGCTCACCATT</td>
</tr>
<tr>
<td>β-actin</td>
<td>β-actin</td>
<td>GTGGGCCGCTACAGCATGCAAGCAGAGACAG</td>
</tr>
</tbody>
</table>

*Table 1. Primer sequences of the defense-related genes used in this study (Rashad *et al.*, 2020b).*
primer, and 12.4 μL 2× SYBR® Green Mix. The qPCR reaction was run with one cycle at 95°C (for 3 min), 45 cycles each of 95°C for 15 s and 56°C for 35 s, then 75°C for 250 s. The comparative CT method (2−ΔΔCT) was used to estimate the transcriptional expression of the tested genes, in accordance with Schmittgen and Livak (2008). Triplicates of each treatment (biological and technical) were used, and the assay was conducted twice.

**Determination of the biochemical plant defense responses**

At 30 dpp, samples from the eggplant roots were collected from each treatment. Estimation of the total phenolics, and activities of peroxidase (POD) and polyphenol oxidase (PPO) were assessed (five replicates). The Folin-Ciocalteu test was used to determine the total phenolic content (Singleton *et al.*, 1999). Approximately 1 g of roots was ground in 5 mL of 80% methanol, at 5°C overnight. This homogenate was then centrifuged at 1344 g for 10 min. The supernatant (100 μL) was then mixed with 20% Na2CO3 (50 μL), 1750 μL of dH2O, and 250 μL of Folin-Ciocalteu reagent (Sigma-Aldrich), and the resulting mixture was left at 40°C for 35 min. Caffeic acid was used as a reference. Phenolic contents were estimated using spectrophotometry at 760 nm. For POD activity determination, approx. 1 g of the root was ground in 3 mL of 0.1 M Na2HPO4 buffer, and the homogenate was centrifuged for 10 min at 1792 g. POD enzyme activity was estimated spectrophotometrically at 470 nm, which was represented as ΔA470 min⁻¹ g⁻¹ f wt (Gong *et al.*, 2001). Estimation of the PPO activity was carried out according to Singh and Ravindranath (1994). Five g of root tissue were homogenized and kept for 35 min in acetone at 4°C. PPO enzyme potential was evaluated spectrophotometrically at 420 nm using catechin as the substrate, and was recorded as ΔA420 min⁻¹ g⁻¹ f wt.

**Estimation of levels of plant mycorrhization**

Mycorrhizal colonization in eggplant roots was estimated 45 dpp according to Trouvelot *et al.* (1986). Plant roots were fragmented (10 mm diam.) using a scalpel and were boiled in 10% potassium hydroxide solution. The treated segments were stained using 0.05% trypan-blue, as described by Phillips and Hayman (1970). For each root treatment, approx. 50 stained fragments were checked under a light microscope. Three mycorrhization parameters were assessed, including frequency of mycorrhizal colonization, colonization intensity, and frequency of formation of arbuscules (Trouvelot *et al.*, 1986). Estimation of mycorrhization was repeated twice, each time on 50 stained fragments.

**Evaluation of the eggplant growth parameters**

At 45 dpp, approx. five replicate eggplant were carefully uprooted. Adhering soil particles were removed and plant growth was estimated using the following parameters: shoot height, root length, shoot and root dry weights, and leaf area. For the evaluation of the dry weights, the plants were dried at 80°C in an oven (3 d). This assay was conducted twice.

**Influence of bioagents on macronutrient contents of eggplant leaves**

For quantitative analysis of the nutrients content in eggplant leaves, approx. ten leaves from each treatment were collected, and then air dried. The leaves were fragmented using a grinding machine, and then used for estimation of the total contents of nitrogen (N), phosphorus (P), and potassium (K). Using Kjeldhal assays, N was estimated by titration following distillation (Goyal *et al.*, 2022). Total P was evaluated as per Singh *et al.* (2022). Total K was determined based on Goyal *et al.* (2022). Determinations were each carried out twice.

**Statistical analyses**

The results were statistically analyzed using CoStat software 6.4. Data were checked for normality before applying the analyses of variance. Treatment means were compared using Tukey’s HSD test (P ≤ 0.05).

**RESULTS**

**Antagonistic potential of the isolated diazotrophic bacteria against R. solani**

Eight isolates of diazotrophic bacteria were recovered from the collected soil samples. Results of the in vitro antifungal assays against *R. solani* showed that inhibition of *R. solani* mycelial growth varied among the eight isolates ranging from no inhibition to medium inhibition. The greatest level of growth inhibition (42% growth, compared to control plates) was observed with the isolate designated as YRNF (Figure 1, a and b). Growth of the *R. solani* colony was suppressed and the colony had an arc-shaped border with the diazotrophic bacterium YRNF1, with a clear no-growth zone in
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Figure 1. *In vitro* antagonism of the diazotrophic bacterium *Niallia circulans* strain YRNF1 against *Rhizoctonia solani*. Confrontation test in dual culture; a) Unchallenged *R. solani* (control); b) *R. solani* challenged by *N. circulans* strain YRNF1.

Figure 2. GC-MS chromatogram of the secondary metabolites detected in culture filtrates of *Niallia circulans* YRNF1.

between the fungus and bacterium, indicating release of antifungal metabolites by the bacterium, and an antibiosis-based mechanism of antifungal activity (Figure 1).

**GC-MS analysis of the bacterial culture filtrate**

Nineteen chemical constituents were detected in the culture filtrate of *N. circulans* YRNF1, which were identified by the GC-MS analysis (Figure 2). As presented in Table 2, the major biochemical constituents were butyric acid (85%) and ethylene glycol (8%). Some metabolites were recorded at intermediate proportions, including lactic acid, propanoic acid, 2-[(trimethylsilyl)oxy], trimethylsilyl ester (TMS) derivative (1.5%) and α-D-mannopyranoside, methyl, cyclic 2,3:4,6-bis(butylboronate) (1.2%). The least detected components were β-hydroxyquebrachamine; 1,3-Dipalmitin trimethylsilyl ester (TMS) derivative; octadecanoic acid, 2,3-bis{(trimethylsilyl) oxypropyl} ester (Glycerol mon-
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Detection of the nitrogenase gene \((nifH)\) in the diazotrophic bacteria

Results obtained from PCR analyses showed that the selected isolate YRNF1 possessed the \(nifH\) nitrogenase gene, which was observed as a single band at 450 bp (Figure 3). This indicated that the isolate was a nitrogen fixing bacterium.

Molecular identification of the diazotrophic bacterium YRNF1

Results of BLAST analysis of the 16S rRNA sequence showed that the diazotrophic bacterial strain YRNF1 had 99.71% similarity with \(N.\) circulans (reference strain MH130347). The nucleotide sequence of \(N.\) circulans YRNF1 was deposited in the GenBank under accession number OP703372. The phylogenetic analysis of \(N.\) circulans YRNF1 in comparison to ten species of the genus Bacillus (Figure 4) showed that the strain grouped with \(B.\) circulans (MW547978) in a single distinct clade, with 66% bootstrap support. The Bacillus spp. strains clustered in two major groups. The first contained \(B.\) altitudenis (MK424248), \(B.\) velezensis (MG651075), and \(B.\) subtilis (HE610894), with 70% bootstrap support. \(Bacillus\) mycoides (ON464184) clustered with 64% bootstrap support in the other clade, while \(B.\) toyonensis (MZ773910) represented an outgroup. The second major group also included \(B.\) licheniformis (MK280728) and \(B.\) amyloliquefaciens (MF423459), with 64% bootstrap support, and clustered in a separate clade. The other clade involved \(B.\) cereus (OQ152624) and \(B.\) thuringiensis (MY912020), with 62% bootstrap support.

Disease severity

Mean severity of Rhizoctonia root rot in the infected, non-treated eggplant plants was 69%, which was the greatest of the nine treatments. Control plants with either one of the two bio-inoculants had no disease. Moderate disease severity was observed for the infected plants inoculated with \(N.\) circulans YRNF1 (45%), and 32% for that inoculated with AMF. Application of both bio-inoculants reduced disease severity by 26% compared to the non-treated infected plants. This indicated additive activity of these two bio-inoculants. Treatment with fludioxonil decreased the disease severity by 25% (Figure 5).

<table>
<thead>
<tr>
<th>Peak no.</th>
<th>Retention time (min)</th>
<th>Peak area (%)</th>
<th>Compound name</th>
<th>Chemical formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4.153</td>
<td>23.11</td>
<td>Butyric Acid, TMS derivative</td>
<td>C(<em>7)H(</em>{16})O(_2)Si</td>
</tr>
<tr>
<td>2</td>
<td>5.2</td>
<td>61.53</td>
<td>Butyric acid</td>
<td>C(_3)H(_4)O(_2)</td>
</tr>
<tr>
<td>3</td>
<td>7.074</td>
<td>7.75</td>
<td>Ethylene glycol, 2TMS derivative</td>
<td>C(<em>8)H(</em>{22})O(_2)Si(_2)</td>
</tr>
<tr>
<td>4</td>
<td>7.265</td>
<td>0.22</td>
<td>Cyclobarbital</td>
<td>C(<em>{12})H(</em>{18})N(_2)O(_3)</td>
</tr>
<tr>
<td>5</td>
<td>7.309</td>
<td>0.16</td>
<td>2,4,6(1H,3H,5H)-Pyrimidinetrione, 5-(1-cyclohexen-1-yl)-5-ethyl-</td>
<td>C(<em>5)H(</em>{16})N(_2)O(_3)</td>
</tr>
<tr>
<td>6</td>
<td>7.47</td>
<td>0.91</td>
<td>Propylene glycol, 2TMS derivative</td>
<td>C(<em>3)H(</em>{10})O(_2)Si</td>
</tr>
<tr>
<td>7</td>
<td>8.195</td>
<td>0.28</td>
<td>2-Ethoxyethanol, TMS derivative</td>
<td>C(<em>4)H(</em>{12})O(_2)Si</td>
</tr>
<tr>
<td>8</td>
<td>8.649</td>
<td>0.17</td>
<td>Cedran-diol, 8S,13-</td>
<td>C(<em>4)H(</em>{16})O(_2)</td>
</tr>
<tr>
<td>9</td>
<td>8.722</td>
<td>0.85</td>
<td>1-Ethyl-1-(2-phenylethoxy)-1-silacyclohexane</td>
<td>C(<em>5)H(</em>{12})O(_2)Si</td>
</tr>
<tr>
<td>10</td>
<td>8.934</td>
<td>1.48</td>
<td>Lactic Acid, 2TMS derivative</td>
<td>C(<em>5)H(</em>{10})O(_2)Si</td>
</tr>
<tr>
<td>11</td>
<td>9.249</td>
<td>0.4</td>
<td>(\beta)-D-Galactoppyranoside, methyl 2,3-bis-O-(trimethylsilyl)-, cyclic butylboronate</td>
<td>C(<em>{19})H(</em>{32})O(_6)Si</td>
</tr>
<tr>
<td>12</td>
<td>10.443</td>
<td>0.11</td>
<td>(\beta)-Hydroxyquebrachamine</td>
<td>C(<em>3)H(</em>{30})N(_2)O</td>
</tr>
<tr>
<td>13</td>
<td>10.801</td>
<td>0.46</td>
<td>Acetin, bis-1,3-trimethylsilyl ether</td>
<td>C(<em>3)H(</em>{32})O(_2)Si</td>
</tr>
<tr>
<td>14</td>
<td>15.07</td>
<td>1.19</td>
<td>(\alpha)-D-Mannopyranoside, methyl, cyclic 2,3:4,6-bis (butylboronate)</td>
<td>C(<em>5)H(</em>{16})B(_2)O(_6)</td>
</tr>
<tr>
<td>15</td>
<td>18.555</td>
<td>0.23</td>
<td>1-Heptatriacotanol</td>
<td>C(<em>6)H(</em>{16})O</td>
</tr>
<tr>
<td>16</td>
<td>18.768</td>
<td>0.57</td>
<td>1-Monopalmitin, 2TMS derivative</td>
<td>C(<em>5)H(</em>{26})O(_2)Si</td>
</tr>
<tr>
<td>17</td>
<td>18.892</td>
<td>0.13</td>
<td>1,3-Dipalmitin trimethylsilyl ester (TMS) derivative</td>
<td>C(<em>5)H(</em>{30})Si</td>
</tr>
<tr>
<td>18</td>
<td>19.507</td>
<td>0.31</td>
<td>2-Oleoylglycerol, 2TMS derivative</td>
<td>C(<em>5)H(</em>{16})O(_2)Si</td>
</tr>
<tr>
<td>19</td>
<td>19.603</td>
<td>0.14</td>
<td>Glycerol monostearate, 2TMS derivative</td>
<td>C(<em>5)H(</em>{18})O(_2)Si</td>
</tr>
</tbody>
</table>

Table 2. Secondary metabolites identified in culture filtrates of \(Niallia\) circulans YRNF1.
Biocontrol of Rhizoctonia root-rot of eggplant

Triggering of the resistance-related genes in eggplant roots

Transcriptional expression of the resistance genes JERF3, PAL1, C3H, CHI2, and HQT in eggplant roots inoculated with N. circulans YRNF1 and colonized with AMF is shown in Figure 6. Compared to the control treatment, all of the experimental treatments elicited expression of JERF3. Combined application of N. circulans YRNF1 and AMF up-regulated the JERF3 gene more than the single treatments. The greatest gene expression (29.4-fold) was for infected eggplant roots after inoculation with N. circulans YRNF1 and colonization by the AMF. Both applied bioagents increased the expression of PAL1, compared to the control plants. Greatest gene expression (17-fold) was for infected eggplant roots inoculated with N. circulans YRNF1 and colonized with the AMF. This was the same for C3H, where all the treatments induced expression of this gene, compared to the control treatment. Overexpression (8.6-fold) was detected for infected eggplant roots inoculated with N. circulans YRNF1 and colonized with the AMF. The applied treatments up-regulated expression of CHI2.
at varying levels compared to the untreated non-infected eggplants, with greatest expression of this gene recorded value showing a 15.9-fold increase. Application of all the treatments led to overexpression of \textit{HQT}, compared to the control plants. The infected eggplant roots inoculated with \textit{N. circulans} YRNF1 and colonized with the AMF had maximum expression of 11.7-fold.

\textbf{Effects of bioagents on phenol levels and antioxidant enzyme activities in treated eggplant roots}

Eggplants challenged with \textit{N. circulans} YRNF1 and AMF and inoculated with \textit{R. solani} had increased level in total phenol contents, and increased POD and PPO enzyme potential (Table 3). At 30 dpp, mean phenolic content increased in the infected, non-treated eggplant

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure6.png}
\caption{Mean transcriptional expression of resistance genes (\textit{JERF3}, \textit{PAL1}, \textit{C3H}, \textit{CHI2}, and \textit{HQT}) in eggplant roots treated with \textit{Niallia circulans} YRNF1 and colonized with AMF and inoculated with \textit{Rhizoctonia solani}. For each gene, columns accompanied by the same superscript letter are not different (\(P \leq 0.05\)), according to Tukey’s HSD test. The error bars are the standard deviations of the means. The experimental treatments were: C, non-mycorrhizal, untreated and uninfected; P, non-mycorrhizal, untreated and infected; B, non-mycorrhizal, treated with \textit{N. circulans} YRNF1 and uninfected; M, mycorrhizal, untreated and uninfected; B+M, mycorrhizal, treated with \textit{N. circulans} YRNF1 and uninfected; B+P, non-mycorrhizal, treated with \textit{N. circulans} YRNF1 and infected; M+P, mycorrhizal, untreated and infected; F+P, non-mycorrhizal, treated with the fungicide and infected; and B+M+P, mycorrhizal, treated with \textit{N. circulans} YRNF1 and infected.}
\end{figure}
bio-inoculants. However, the increases in enzyme activity attributable to the combined treatment (B+M+P) was higher than each single treatment. Application of the fungicide resulted in a recognizable increase in the POD and PPO activity, compared to the control plants.

Effects of *N. circulans* YRNF1 on mycorrhizal colonization of eggplant roots infected with *R. solani* are summarized in Table 4. Eggplant roots un-treated with AMF did not develop mycorrhizal colonization. In contrast, all eggplant roots treated with AMF showed varying levels of mycorrhization. Greatest colonization parameters were recorded for roots treated with *N. circulans* YRNF1 and AMF, which gave means of 88.7% colonization frequency, 56.1% colonization intensity, and 39.7% arbuscule formation frequency, while the eggplants colonized by AMF only had 76.3% colonization frequency, 45.3% colonization intensity, and 27.4% arbuscule formation frequency. These results indicated the compatibility between the two potential bio-agents. However, *R. solani* inoculation of the mycorrhizal eggplants reduced the mycorrhization levels, compared to non-mycorrhizal, untreated and infected; B, non-mycorrhizal, treated with *N. circulans* YRNF1 and infected; M, mycorrhizal, untreated and uninfected; B+M, mycorrhizal, treated with *N. circulans* YRNF1 and uninfected; B+P, non-mycorrhizal, treated with *N. circulans* YRNF1 and infected; M+P, mycorrhizal, untreated and infected; F+P, non-mycorrhizal, treated with the fungicide and infected; B+M+P, mycorrhizal, treated with *N. circulans* YRNF1 and infected, and peroxidase (POD) and polyphenol oxidase (PPO).

### Table 3. Mean phenolic contents and antioxidant enzyme (POD or PPO) activities in eggplant roots 30 d after inoculation with *Rhizoctonia solani* and treatments with *Niallia circulans* YRNF1 and/or mycorrhizae.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Phenolic content (mg g⁻¹ f wt)*</th>
<th>POD (ΔA₄₂₀ min⁻¹ g⁻¹ f wt)*</th>
<th>PPO (ΔA₄₂₀ min⁻¹ g⁻¹ f wt)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>415.7 ± 8.19f</td>
<td>1.14 ± 0.07h</td>
<td>1.08 ± 0.05s</td>
</tr>
<tr>
<td>P</td>
<td>673.2 ± 11.20d</td>
<td>1.53 ± 0.05e</td>
<td>1.34 ± 0.04f</td>
</tr>
<tr>
<td>B</td>
<td>579.0 ± 7.32e</td>
<td>1.38 ± 0.05d</td>
<td>1.24 ± 0.05f</td>
</tr>
<tr>
<td>M</td>
<td>594.4 ± 8.17c</td>
<td>1.76 ± 0.09d</td>
<td>1.56 ± 0.07c</td>
</tr>
<tr>
<td>B+M</td>
<td>763.3 ± 8.41c</td>
<td>1.90 ± 0.07c</td>
<td>1.47 ± 0.09d</td>
</tr>
<tr>
<td>B+P</td>
<td>772.5 ± 10.50c</td>
<td>1.68 ± 0.05d</td>
<td>1.57 ± 0.07c</td>
</tr>
<tr>
<td>M+P</td>
<td>832.4 ± 9.13b</td>
<td>2.12 ± 0.08b</td>
<td>1.69 ± 0.05b</td>
</tr>
<tr>
<td>F+P</td>
<td>668.2 ± 4.88d</td>
<td>1.25 ± 0.04d</td>
<td>1.29 ± 0.07c</td>
</tr>
<tr>
<td>B+M+P</td>
<td>1197.4 ± 12.02a</td>
<td>2.32 ± 0.08e</td>
<td>1.86 ± 0.09a</td>
</tr>
</tbody>
</table>

*Means followed by different superscript letters are significantly different (P ≤ 0.05), according to Tukey’s HSD test. The data are means of five replicates ± SD. Treatments applied were: C, non-mycorrhizal, untreated and uninfected; P, non-mycorrhizal, untreated and infected; B, non-mycorrhizal, treated with *N. circulans* YRNF1 and uninfected; M, mycorrhizal, untreated and uninfected; B+M, mycorrhizal, treated with *N. circulans* YRNF1 and uninfected; B+P, non-mycorrhizal, treated with *N. circulans* YRNF1 and infected; M+P, mycorrhizal, untreated and infected; F+P, non-mycorrhizal, treated with the fungicide and infected; B+M+P, mycorrhizal, treated with *N. circulans* YRNF1 and infected, and peroxidase (POD) and polyphenol oxidase (PPO).

### Table 4. Mean proportions (%) of mycorrhizal colonization frequency, intensity and arbuscule frequency 45 d after treatments of eggplants with *Niallia circulans* YRNF1.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Colonization frequency (%)*</th>
<th>Colonization intensity (%)*</th>
<th>Frequency of arbuscules (%)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>P</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>B</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>M</td>
<td>76.3 ± 4.12b</td>
<td>45.3 ± 6.13b</td>
<td>27.4 ± 3.17b</td>
</tr>
<tr>
<td>B+M</td>
<td>88.7 ± 6.23a</td>
<td>56.1 ± 5.41a</td>
<td>39.7 ± 5.11a</td>
</tr>
<tr>
<td>B+P</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>M+P</td>
<td>63.9 ± 4.55d</td>
<td>35.4 ± 6.04c</td>
<td>20.5 ± 4.03c</td>
</tr>
<tr>
<td>F+P</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>B+M+P</td>
<td>70.33± 4.81c</td>
<td>43.4 ± 5.22b</td>
<td>24.5 ± 5.13b</td>
</tr>
</tbody>
</table>

*Means accompanied by the same superscript letter are not significantly different (P ≤ 0.05), according to Tukey’s HSD test. The values are means of five replicates ± SD. Treatments applied were: C, non-mycorrhizal, untreated and uninfected; P, non-mycorrhizal, untreated and infected; B, non-mycorrhizal, treated with *N. circulans* YRNF1 and uninfected; M, mycorrhizal, untreated and uninfected; B+M, mycorrhizal, treated with *N. circulans* YRNF1 and uninfected; B+P, non-mycorrhizal, treated with *N. circulans* YRNF1 and infected; M+P, mycorrhizal, untreated and infected; F+P, non-mycorrhizal, treated with the fungicide and infected; and B+M+P, mycorrhizal, treated with *N. circulans* YRNF1 and infected.
the mycorrhizal eggplants not inoculated by the pathogen. This was also the case for the mycorrhizal-infected roots treated with \textit{N. circulans} YRFN1.

**Eggplants growth in response to application of \textit{N. circulans} YRFN1 and AMF**

Results from treatments of \textit{R. solani} inoculated eggplants with \textit{N. circulans} YRFN1 and AMF showed increases in most of the measured plant growth parameters (Table 5). Compared to experimental controls, the \textit{R. solani}-inoculated had 20.5\% reduction in mean shoot height and 26.3\% reduction in mean root length. Eggplant roots colonized by AMF had 54.6\% greater shoot height and 54.7\% greater root length. Treating the non-\textit{R. solani} inoculated plants with \textit{N. circulans} YRFN1 and AMF increased shoot height by 71.4\% and root length by 41.8\%. Treatment of the \textit{R. solani} inoculated eggplants with both bio-inoculants increased shoot height by 53.7\% and root dry weight by 60.0\%. Treatment of the non-infected eggplants with \textit{N. circulans} YRFN1 and AMF increased shoot dry weight 79.5 and root dry weight by 130\%. Application of both bio-agents to infected plants increased shoot dry weight by 51.3\% and root dry weights. Inoculation of plants with \textit{R. solani} also reduced mean leaf area by 34.3\%. Application of both bio-inoculants increased the leaf area by 115.7\%. Combined treatment of the infected eggplants with both bio-agents increased mean leaf area by 85.8\%. Treatment of the infected eggplants with fludioxonil increased leaf area, compared to the untreated infected plants.

**Influence of \textit{Niallia circulans} YRFN1 and AMF on the macronutrient contents in the eggplant leaves**

Total nitrogen (TN) contents in eggplant leaves varied across the different treatments (Table 6). Compared to experimental controls, all the treatments caused increased TN. Greatest mean TN increases resulted for \textit{R. solani}-inoculated plants colonized by AMF (3.9\%), inoculated with \textit{N. circulans} YRFN1 (3.6\%) or treated with fludioxonil (3.6\%). Greatest total phosphorus (TP: 2.55\%) was measured in non-inoculated plants colonized with AMF, and in \textit{R. solani}-inoculated plants colonized with AMF (TP = 2.26\%). Most of the applied treatments also increased total potassium (TK) contents in the eggplant leaves, compared to control. Measured N/P ratios were increased in the \textit{R. solani} inoculated plants either treated with the fludioxonil (mean N/P ratio = 4.29), inoculated with \textit{N. circulans} YRFN1 (N/P = 3.86), or colonized with AMF (N/P = 3.85).

**DISCUSSION**

\textit{Rhizoctonia solani} is a damaging pathogen that infects several economically important host plants. In

### Table 5. Mean eggplant shoot heights, root lengths, shoot and root dry weights, and leaf areas 45 d after inoculation with \textit{Rhizoctonia solani} and treatments of \textit{Niallia circulans} YRFN1 and/or mycorrhizal colonization.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Shoot height (cm)*</th>
<th>Root length (cm)*</th>
<th>Shoot dry weight (g)*</th>
<th>Root dry weight (g)*</th>
<th>Leaf area (cm²)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>12.50 ± 1.12d</td>
<td>5.17 ± 0.80e</td>
<td>0.39 ± 0.03c</td>
<td>0.10 ± 0.02c</td>
<td>17.99 ± 1.23f</td>
</tr>
<tr>
<td>P</td>
<td>10.00 ± 0.99e</td>
<td>3.81 ± 0.73f</td>
<td>0.23 ± 0.05d</td>
<td>0.04 ± 0.01d</td>
<td>11.82 ± 1.01e</td>
</tr>
<tr>
<td>B</td>
<td>13.50 ± 1.37cd</td>
<td>7.16 ± 0.95bcd</td>
<td>0.62 ± 0.04ab</td>
<td>0.22 ± 0.02a</td>
<td>24.71 ± 2.33b</td>
</tr>
<tr>
<td>M</td>
<td>19.33 ± 1.40d</td>
<td>8.00 ± 1.00ab</td>
<td>0.68 ± 0.06a</td>
<td>0.21 ± 0.04a</td>
<td>33.54 ± 2.15b</td>
</tr>
<tr>
<td>B+M</td>
<td>21.42 ± 1.22a</td>
<td>7.33 ± 0.87bc</td>
<td>0.70 ± 0.09a</td>
<td>0.23 ± 0.05a</td>
<td>38.82 ± 2.56e</td>
</tr>
<tr>
<td>B+P</td>
<td>13.17 ± 0.87cd</td>
<td>7.33 ± 0.74bc</td>
<td>0.39 ± 0.08c</td>
<td>0.10 ± 0.03c</td>
<td>21.76 ± 1.81e</td>
</tr>
<tr>
<td>M+P</td>
<td>18.14 ± 1.13bc</td>
<td>7.30 ± 0.81bc</td>
<td>0.58 ± 0.07b</td>
<td>0.21 ± 0.05b</td>
<td>28.48 ± 1.64c</td>
</tr>
<tr>
<td>F+P</td>
<td>15.83 ± 1.41c</td>
<td>6.33 ± 0.89cd</td>
<td>0.36 ± 0.06c</td>
<td>0.11 ± 0.06c</td>
<td>17.43 ± 9.8c</td>
</tr>
<tr>
<td>B+M+P</td>
<td>19.21 ± 1.42d</td>
<td>8.61 ± 0.78b</td>
<td>0.59 ± 0.08b</td>
<td>0.19 ± 0.07b</td>
<td>33.42 ± 1.11b</td>
</tr>
</tbody>
</table>

*Means accompanied by the same superscript letter are not significantly different (\(P \leq 0.05\), according to Tukey’s HSD test. The values are means of five replicates ± SD. Treatments applied were: C, non-mycorrhizal, untreated and uninfected; P, non-mycorrhizal, untreated and infected; B, non-mycorrhizal, treated with \textit{N. circulans} YRFN1 and infected; M, mycorrhizal, untreated and uninfected; B+M, mycorrhizal, treated with \textit{N. circulans} YRFN1 and uninfected; M+P, mycorrhizal, untreated and infected; F+P, non-mycorrhizal, treated with the fungicide and infected; and B+M+P, mycorrhizal, treated with \textit{N. circulans} YRFN1 and infected.
Nitrogen fixation is an important biological process as nitrogen (N) is a limiting nutrient for crop growth. The nitrogen fixing ability of *N. circulans* YRNF1 was confirmed by the presence of *nifH* gene in its DNA, which provides marker for N fixation ability (Young, 1992). Nitrogen fixation by *N. circulans* YRNF1 is probably the main mechanism of the observed promotion of the eggplant growth, as shown by the increases of NPK contents in leaves of the treated plants. Nitrogen is the most important element in plant nutrition, and nitrogen nutrition *via* nitrogen fixation increases plant vegetative vigor. Phosphorus and potassium are also vital for plant biochemical processes, such as photosynthesis and for the nitrogen fixation process itself (Abdelraouf et al., 2020). Potassium has an important function in activating enzymes including nitrogenase (Rashad et al., 2023). Coskun et al. (2017) highlighted that the form of available N, particularly NH₄⁺ and NO₃⁻, and the transformation of N in soil, also affect K uptake by plants. These authors reported that administration of NH₄⁺ to barley plants enhanced K⁺ uptake. Phosphorus nutrition is also important for N fixation processes, where conversion of N₂ to NH₄⁺ catalyzed by nitrogenase depends on ATP (Bello et al. 2023). Nitrogen fertilization also positively affects P uptake by plant roots (Krouk and Kiba, 2020).

The results obtained in the present study from the greenhouse assays showed that application of *N. circulans* YRNF1 suppressed disease severity caused by *R. solani* in the treated eggplants. The bio-control efficacy of *N. circulans* YRNF1 was probably due to its ability to produce metabolites (particularly butyric acid and ethylene glycol) with antifungal potential. Other possible mechanisms of action include the ability to inhibit the growth of *R. solani* and limit severity of root rot through colonization of root infection sites, competitive exclusion of the fungal pathogen, and secretion of antifungal and/or cell wall hydrolyzing enzymes (Lugtenberg et al., 2009; Berendsen et al., 2012). Systemic immunity may also be triggered by *N. circulans* in treated eggplants. All the above mentioned results are promising for the potential use of *N. circulans* YRNF1 as an effective biocontrol agent. Disease severity may also be reduced by AMF colonization of eggplants infected by *R. solani*, which triggers host defense responses in plants under stress caused by plant pathogens (Rashad et al. 2020b). AMF can also enhance plant resistance, by promoting plant growth and vigor *via* improvement of plant nutrition (El-Sharkawy et al., 2023) and production of plant hormones (Song et al., 2020). In the present study, co-inoculation of infected plants with the two biocontrol agents decreased disease severity by 26%, indicating an additive action of the *N. circulans* YRNF1 and AMF.

**Table 6.** Mean contents of nitrogen, phosphorus, and potassium, and N/P ratios 45 d after treatments of eggplants with *Niallia circulans* YRNF1 and/or AMF.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Total nitrogen (%)*</th>
<th>Total phosphorus (%)*</th>
<th>Total potassium (%)*</th>
<th>N/P ratio*</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>2.66 ± 0.08</td>
<td>1.59 ± 0.04</td>
<td>2.15 ± 0.08</td>
<td>3.72 ± 0.21</td>
</tr>
<tr>
<td>P</td>
<td>3.15 ± 0.04</td>
<td>2.17 ± 0.03</td>
<td>2.70 ± 0.15</td>
<td>3.20 ± 0.02</td>
</tr>
<tr>
<td>B</td>
<td>3.22 ± 0.08</td>
<td>2.01 ± 0.06</td>
<td>2.9 ± 0.06</td>
<td>3.57 ± 0.20</td>
</tr>
<tr>
<td>M</td>
<td>3.50 ± 0.08</td>
<td>2.55 ± 0.09</td>
<td>2.77 ± 0.06</td>
<td>3.06 ± 0.17</td>
</tr>
<tr>
<td>B+M</td>
<td>3.23 ± 0.02</td>
<td>2.04 ± 0.02</td>
<td>2.89 ± 0.07</td>
<td>3.52 ± 0.05</td>
</tr>
<tr>
<td>B+P</td>
<td>3.64 ± 0.16</td>
<td>2.15 ± 0.15</td>
<td>2.81 ± 0.02</td>
<td>3.86 ± 0.06</td>
</tr>
<tr>
<td>M+P</td>
<td>3.92 ± 0.09</td>
<td>2.26 ± 0.04</td>
<td>3.04 ± 0.09</td>
<td>3.85 ± 0.45</td>
</tr>
<tr>
<td>F+P</td>
<td>3.64 ± 0.04</td>
<td>1.88 ± 0.02</td>
<td>3.09 ± 0.11</td>
<td>4.29 ± 0.08</td>
</tr>
<tr>
<td>B+M+P</td>
<td>3.10 ± 0.08</td>
<td>2.12 ± 0.06</td>
<td>2.45 ± 0.03</td>
<td>3.24 ± 0.08</td>
</tr>
</tbody>
</table>

*Means accompanied by the same superscript letter are not significantly different (P ≤ 0.05), according to Tukey’s HSD test. The values are means of five replicates ± SD. Treatments applied were: C, non-mycorrhizal, untreated and uninfected; P, non-mycorrhizal, untreated and infected; B, non-mycorrhizal, treated with *N. circulans* YRNF1 and uninfected; M, mycorrhizal, untreated and uninfected; B+M, mycorrhizal, treated with *N. circulans* YRNF1 and uninfected; B+P, non-mycorrhizal, treated with *N. circulans* YRNF1 and infected; B+M+P, mycorrhizal, treated and infected; and B+M+P, mycorrhizal, treated with *N. circulans* YRNF1 and infected.
Both potential biocontrol agents affected expression of the defense genes JERF3, PAL1, C3H, CHI2, and HQT. The JERF3 gene was particularly expressed in response to treatment of eggplants with *N. circulans* YRNFL1 and AMF. The gene JERF3 controls several plant defense genes (Rashad et al., 2022b). PAL1 regulates the main step in the polyphenolic biosynthetic pathway, through conversation of phenylalanine to t-cinnamic acid (Mouradov and Spangenberg, 2014). C3H has an important role in biosynthesis of monolignols that constitute lignin (Tao et al., 2018). HQT regulates biosynthesis of chlorogenic acid from caffeoyl CoA (André et al., 2009). Triggering expression of these defense genes indicates induction of effects of the combined application of *N. circulans* YRNFL1 and AMF for eggplant resistance.

Increases in phenolics and in activities of POD and PPO were detected in response to applying *N. circulans* YRNFL1 and AMF. This is similar to the results of Singh et al. (2016). Increments of phenolic compounds content is a plant defense response associated with induced resistance (IR) in plants infected by pathogens (Chin et al., 2022). POD and PPO are important antioxidant enzymes, which catalyze formation of lignin, thus contributing to structural reinforcement of plant cells and formation of physical barriers against invading pathogens (Nasr-Esfahani et al., 2020). In addition, POD and PPO scavenge the oxidative ROS generated within infection processes.

Results from the present study have demonstrated that the frequency of colonization of eggplant roots by AMF, the level of colonization, and the frequency of arbuscule formation were greater after treating eggplants with a combination of *N. circulans* YRNFL1 and AMF. This revealed the inducing effect of *N. circulans* YRNFL1 on mycorrhizal colonization, which indicates compatibility of the two potential biocontrol agents.

In the present study, the two applied potential biocontrol agents promoted eggplant growth. Colonization of plant roots by two or more AMF species has previously been reported to provide a spectrum of advantages, and has more benefits to host plants than colonization by one species (Sharma and Kapoor, 2023). The strain *N. circulans* E9 has been found to promote plant growth (Sarmiento-López et al., 2022), which is associated with production of IAA and promotion of growth through stimulation of cell division and increased nutrient and water uptake, resulting in increased crop yield and quality (Sarmiento-López et al., 2022). In the present study, increases in the growth parameters of *R. solani*-infected eggplants were detected after individual treatments with each of the two bio-inoculants. This may be due to the biocontrol potential of *N. circulans* YRNFL1 and growth promotion from each of the bio-inoculants. The recorded enhancement of eggplant growth after colonization with AMF was also similar to effects reported by Han et al. (2023), where treating lettuce by AMF increased the plant height by 30% and root length by 64%. Colonization by AMF is known to improve the host plants through multiple modes of action, including increases in active and passive nutrient and water uptake, and increased hyphal networks on root radicles, which magnify the hydraulic conductivity of water from the soil (Rashad et al., 2020c). In addition, AMF can produce hydrolyzing phosphatases, pectinases, and cellulases, as well as organic acids, which assist in nutrient utilization and improve their plant availability (Liu et al., 2021). AMF also produce phytohormones (e.g. abscisic acid and strigolactones) that enhance plant growth (Pozo et al., 2015).

*Nialilia circulans* YRNFL1 also enhanced eggplant nitrogen nutrition via the N-fixation, which probably promoted eggplant growth, whether or not colonized by AMF. The enhancements of eggplant growth parameters were increased after applying the two bio-gents, suggesting their additive action. This observation is consistent with that of El-Sharkawy et al. (2022), who showed that co-treating of pea plants by *Streptomyces viridosporus* HH1 and the mycorrhizal fungus *Rhizophagus irregularis* increased pea growth, compared to individual treatments with these two microorganisms. The AMF supply host plants with mineral nutrients, mainly phosphorus, which play prominent roles in plant growth (Adolfsson et al., 2015). The present results also demonstrated that combined application of *N. circulans* YRNFL1 and mycorrhizal colonization improved the NPK contents in eggplant leaves, which was probably due to a cumulative effect of *N. circulans* YRNFL1 and AMF. These data were similar to those of Hafez et al. (2022), who reported that combined application of growth-promoting rhizobacteria with organic fertilizers increased total N, P, and K in treated plant tissues, compared to untreated plants, and to individual applications of the mineral fertilizers.

The present study is the first to report effective use and the additive effects of the diazotrophic bacterium *N. circulans* and AMF, as biofungicides and biofertilizers, for potential management of Rhizoctonia root rot and growth-promotion in eggplant.
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LITERATURE CITED


dos Santos H.R.M., Argolo C.S., Argôlo-Filho R.C., Loguercio L.L., 2019. A 16S rDNA PCR-based theo-


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