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ISOLATION AND CHARACTERIZATION OF RHIZOSPHERE BACTERIA ANTAGONISTIC TO *FUSARIUM OXYSPORUM* F.SP. SESAME, A CAUSE OF WILT DISEASE ON SESAME PLANT

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Abstract

The study aimed to isolate bacteria from the sesame root rhizosphere that can antagonize the fungus *Fusarium oxysporum* f.sp. sesami. The fungus that caused wilt disease in the sesame plants was isolated from naturally infected samples and was then artificially introduced into the healthy sesame seedling to confirm the disease symptom. In parallel, rhizosphere soils of the sesame root collected in Bac Kan, Vietnam were used to isolate bacteria by serial dilution method. The isolated bacteria were then used to screen for their ability in inhibiting the *Fusarium oxysporum* f.sp. sesami at either *in vitro* or field. Among 30 isolated bacteria, only 7 of them could antagonize *Fusarium oxysporum* f.sp. sesami with antibacterial efficiency ranged from 6.15 to 72.17%. Among 7 antifungal isolates, three isolates (NR11, NR22, and NR26 identified as *Bacillus cereus* KN4, *Bacillus amyloliquefaciens* B7, and *Bacillus pumilus* B19) could produce siderophore and degraded cellulose, protein, and chitin. These isolates reduced wilt-disease symptoms and promoted seedling development in sesame under greenhouse and field conditions. These results suggest that NR11, NR22, and NR26 strains could be applied as biocontrol agents in sustainable agriculture.

Key words: Antagonistic bacteria, Biocontrol, *Fusarium oxysporum* f.sp. sesame, Isolation, Sesame.

Introduction

The sesame plant (*Sesamum indicum* L.) is an oil crop, a food plant with high economic value (Bedawy & Moharam, 2018). Sesame seeds are not only used as food for humans (such as roasted, oiled, used as lighting oil) but also used as medicinal herbs.

In Vietnam, sesame is grown all year round due to suitable conditions, the annual sesame growing area varies from 30.000 to 40.000 ha (Lan, 2012). However, sesame cultivation faces many problems due to phytopathogens, mainly fungal pathogens. Among them, *Fusarium oxysporum* f.sp. sesami is one of the most destructive fungal diseases that cause the sudden death of plants and reduces yield by 25-40% (Kolte, 1985, Ngamba *et al.*, 2020).

The *Fusarium* spp. persist for several years in the soil and can still penetrate the host plant (Hammami *et al.*, 2013). There are many methods have been used to mitigate Fusarium wilt damage such as cultivating resistant crops, rotating the cultivars, and fumigating the soil (Assefa *et al.*, 2020; Zhang *et al.*, 2020). It is a fact that control of *F. oxysporum* by chemical methods is often very difficult because they are both parasitic and saprophytic, so they stay in the soil for a long time (Mokhles *et al.*, 2021). In addition, the remaining pesticides stored in agricultural products, soil, and groundwater will affect humans and other living species, pollute the environment, and unbalance the ecology. Moreover, the overuse of pesticides would make pathogens easier to develop resistance and generate new strains (Mokhles *et al.*, 2021). Therefore, it is necessary to find a safer solution to replace pesticides. Currently, biological methods have been applied and have shown positive effects. This solution is based on the interaction among microorganisms in the ecosystem to promote the role of beneficial microorganisms based on their natural ability to antagonize pathogens (Agrios, 2005). The treatment of sesame seeds or sesame roots with antagonistic bacteria such as *Bacillus subtilis*, *Gliocladium virens*, *Pseudomonas fluorescens* MC07 (Jyothi *et al.*, 2011, Amin *et al.*, 2017), or with antagonistic fungal such as *Trichoderma* sp. (Mahmoud & Abdalla, 2018; Mokhles *et al.*, 2021) has been demonstrated the effective protection of sesame plants from wilt disease.

Hence, this study was carried out to select promising bacterial strains antagonizing the *Fusarium oxysporum* f.sp. sesami, a casual of sesame wilt diseases at laboratory and nursery conditions, creating a premise for the production of probiotics to prevent wilt disease in sesame, contribute to reducing environmental pollution, and develop sustainable agriculture.

Material and Methods

Isolation and identification of *F. oxysporum* f.sp. sesami causes wilt disease in sesame: Sesame plants with typical symptoms of wilt were collected from fields grown sesame at Na Ri, Bac Kan, Vietnam during the growing season of 2019. Samples of diseased plants were put in paper bags clearly stating the location and date of sample collection. All samples of soils and plants were transferred to the lab in a day.

The isolation of *F. oxysporum* f.sp. sesami was done in the lab (after 24 hours of collecting) as the method described by Li *et al*., (2012). Briefly, cut off the stem of the sesame with brown vascularization about 4 cm long, then washed with water to remove dirt and other impurities. The plant samples were removed from the outer skin, wipe the surface with alcohol, and cut off the browned area. The plant surface was disinfected by soaking in 70° alcohol for 30 seconds, then rinsed with sterile distilled water. The

disinfected plant samples were dried by laying on disinfected blotting paper. The stems were cut into small pieces about 2-4 mm and placed on a petri dish containing PDA media. The inoculated plates were kept for 2-3 days at 28°C until mycelia appeared. The fungal isolates were then transferred several times on new PDA plates. After purification, the micromorphological characteristics were observed by the droplet method under the microscope at 400x magnification. Based on the morphology, the color of the fungal colony, and characteristics such as spore form, mycelium, etc to identify the isolated fungus as *F. oxysporum* f.sp. sesami. Pure fungal samples were stored in sterile distilled water for research purposes.

After morphological identification, fungal strains were also identified by ITS-rDNA region sequencing. Extraction of fungal genomes by QIAgen kit, amplified sequence by PCR reaction with the following primer sequences ITS1 (3'-TCCGTAGGTGAACCTGCGG-5') and ITS4 (3'-TCCTCCGCTTATTGATATGC-5'). PCR products (600 bp) were electrophoresed on 1% agarose gel in 1X TBE solution before sending for sequencing at First Base (Singapore). Complete nucleotide sequences were blasted on the NCBI GenBank databank to identify close species (similarity >99%).

The sesame seeds were also artificially infected with isolated fungal samples to confirm the causative agent according to Koch's rule. Briefly, sesame seeds (V6 cultivar) were soaked in warm water with a ratio of 2 boiling: 3 cold for 6 hrs, then sown in the soil (ratio of sand: loam was 1:1, v/v). When the seedlings were 20 days old, artificial infection was carried out by wounding the lower part of the plant with a sterile needle and then attaching a piece of the isolated fungus to the wound site, while also wounding the lower part of the control plant, but not infectious. Used a nylon membrane to cover the infection site. The infected and control plants were observed for the development of wilt (Burgess *et al.*, 2008).

Isolation of bacteria capable of antagonizing fungi: The isolation of antagonistic bacteria was done according to Nguyen *et al*., (2022). Briefly, soil samples were collected from fields growing sesame (about 30 days old), and the soil was collected from 3-5 cm above the topsoil. Each soil sample was collected at 5 locations of sesame fields, 4 locations at 4 corners, and 1 location at the intersection of 2 diagonal lines. The soil samples from each location (0.5 kg) were then mixed together and 0.5 kg from this mixture was used as a representative. The soil was placed in plastic bags labeled with the date and location of sampling and stored at 4-8°C. Two soil samples were used to isolate bacteria by serially diluting up to 10^{-10} by sterilized water. The filtered solution was inoculated on plates containing LB agar medium to observe the bacterial colonies.

The antagonistic ability of bacterial isolates was investigated by using a dual culture method (Trung, 2022). Briefly, the fungus *F. oxysporum* f.sp. sesami was grown on PDA agar plates (Potato Dextrose Agar, Potato: 200g, Dextrose: 20g, agar: 20g). The inoculated plates were incubated for 2 days at 30°C. After that, a plug of the bacteria was placed close (about 1 cm) to the incubated fungal dish. After 5 days, the antifungal zone and the inhibition of fungal growth by bacteria were measured by the formula:

$$I = [(R-r)/R]*100$$

In which: I: Antibacterial efficiency; R: Radius of control mycelium (cm); r: Radius of mycelium on a plate with bacterial strain (cm).

Bacteria with a strong ability in suppressing the mycelial growth of fungi were selected to identify their species by molecular method (Trung, 2022). Briefly, the bacterial strains were cultured in LB broth media overnight, then bacteria cells were collected by centrifuging. After that, the bacterial cells were lysed and extracted DNA by using the specific kit for bacteria (BS8225, Biobasic, Canada) following the instructions of the manufacturer. Then extracted genomic DNA was used to amplify the fragment of the 16S rRNA gene (about 1.5 kb) with specific forward primer (27F, 5′-AGA GTT TGA TCC TGG CTC AG-3′), and reverse primer (1492 R, 5′-TAC GGT TAC CTT GTT ACG ACT T-3′) in PCR methods. The PCR products were cleaned and sequenced by First Base Company (Singapore). The nucleotide sequences were used to search for the close species (similarity was more than 99%) by blast function on the NCBI server. The blasted sequences were cleaned and deposited on the server of GenBank.

Evaluating the antagonistic properties: The siderophore production of antagonistic bacteria was investigated as a method described by Schwyn & Neilands (1987). Bacterial strains would be cultured on CAS-blue agar. After 3 days, the diameter of the yellow circle around the colony was measured to evaluate the ability of bacteria to produce a siderophore. The formula for siderophore production: (Yellow circle diameter – Colony diameter).

The ability to decompose chitin of isolates was performed on a YEG medium (Yeast extract: 4g, Glucose: 20g, Agar: 20g) supplemented with 1% chitin suspension (Mokhles *et al.*, 2021). The plate incubation was done at 30 °C for 3 days. The ability of bacteria to degrade chitin was determined by staining with Lugol's solution. Chitin-degrading bacteria will form a colorless circle around the colony, measuring the diameter of the decomposition ring to determine the ability to decompose chitin in the environment. The formula for chitin degradation is calculated: (Decomposition diameter – Colony diameter).

The cellulose decomposition experiment was carried out on CMC agar (10g CMC; 1g $(NH_4)_2SO_4$; 1g K_2HPO_4; 0.5g $MgSO_4.H_2O$; 0.001g NaCl; 15g Agar) (Mokhles *et al.*, 2021). The inoculated plates were then stained with 0.1% Congo Red solution and rinsed with 1M NaCl to detect CMC-degrading bacteria, which formed a colorless circle around the colony (Teather and Wood, 1982). The formula for calculating cellulose degradation ability: (Disintegration ring diameter – Colony diameter).

The protease activity of the bacterial strains was evaluated by their ability to form proteolytic rings in a milk medium (Priest, 1993). Experiments were carried out on SMA (Skim Milk Agar) medium supplemented with 2%

agar. The proteolytic capacity of the bacteria was determined by using a proteolytic ring diameter measure. The formula for calculating protein degradation: (Disintegration ring diameter – Colony diameter).

Disease suppression of the antagonistic bacteria in the greenhouse condition: The antagonistic bacteria were further investigated in their abilities to inhibit the *F. oxysporum* f.sp. sesami under laboratory conditions.

The bacteria with the antagonistic ability against the fungus *F. oxysporum* f.sp. sesami (NR11, NR22, and NR26) were cultured in Luria-Bertani (LB) at 28°C for 48 hrs. After that, the bacterial cultures were centrifugated and prepared in a bacterial suspension (at 10^8 cfu mL^{-1}).

Fungus *F. oxysporum* f.sp. sesame was grown on PDA media at 28°C for seven days. Conidia of the fungus were collected by pouring sterilized water on the plate, then using a sterile brush to suspend the conidia into the water. The solution was filtered through sterilized cheesecloth to get the spore. The concentration of spore solution was adjusted to around 1×10^6 spores mL^{-1}).

Sesame seeds were surface sterilized by the following procedure: the seeds were soaked for 3 min with 1% sodium hypochlorite (SH) solution, then rinsed four times (1m for each time) with sterile distilled water (SDW). The sterilized seeds were dried on the sterilized filter paper. In parallel, a mixture that included equal parts of peat, sand, and field soil was prepared and autoclaved twice for 20 min at 120°C with 24 h between autoclavings.

The pots (35 cm in diameter) were sterilized and filled with autoclaved soil. After that, 35 ml of a suspension of *F. oxysporum* (10^6 spore/ml) was added and let grow for 7 days. After that, 35 ml of the antagonistic bacteria suspension (10^8 cfu mL^{-1}) was also added as an inoculant. Each pot was sown with 10 sterilized sesame seeds V6 cultivars. Each experiment was repeated three times and the control was the pot containing inoculum of *F. oxysporum* only. The pots were kept in the nursery (with a temperature of 30°C and constant humidity of 85-95%). After two and four weeks from sowing, the percentage of seedlings presenting pre and/or post-emergence damping-off was recorded. The survival seedlings were weekly observed for the symptoms of wilt disease that appeared. During the experiments, the number of wilted plants was recorded and was used to determine the percentage of wilt disease.

Evaluating the disease suppression of antagonistic bacteria on the sesame seedlings in the field: The field experiments were randomly designed and done at Na Ri, Bac Kan in 2021. The sesame seeds (V6 cv.) were disinfected and inoculated with antagonistic bacteria. The bacterized seeds were sown in the plots. Each experiment was designed randomly with three plots (1.8×2.4m), three rows per pot, six hills (20 cm) per row, and five seeds per hill. About 70g of pathogenic fungus were applied to each hill at the same time as seed sowing. For control, the sterilized sesame seeds were sown in plots infected with fungal pathogens only. The care for sesame cultivation was a commonly known practice. The following parameters were estimated: the percentage of seedlings that presented pre-and post-emergence damping-off and the appearance of wilt disease and its severity.

Effect of bacterial isolates on the development of sesame plant: At the time of harvesting, in each pot, ten healthy plants were collected randomly to determine some parameters including the plant length (cm), number of pods per plant, number of bearing branches per plant, seed yield per plant (g), and percentage of oil content (Ziedan *et al*., 2011).

Statistical Analysis

Data were analyzed by using one-way ANOVA with significance ($p<0.05$) for treatment effects, followed by *posthoc* comparisons (Tukey's HSD).

Result and Discussion

Isolation of pathogenic fungi: From a sample of browned sesame stems collected in Na Ri, Bac Kan, Vietnam, a pure strain of fungi has been isolated. On PDA medium, the mycelium of *Fusarium oxysporum* was dry and smooth, initially white, turning to pale pink after 3 days of inoculation (Fig. 1). After 6 days of inoculation, the fungal colony diameter reached 7.5 cm, and after 9 days of inoculation, the fungi grew over the plate surface (Fig. 1).

In addition, the result also showed that the fungal mycelium was branched, and septate; the spores have several types of shapes such as oval to elliptical, straight or slightly curved. This result was consistent with the description of Burgess *et al*., (2008) when isolating and evaluating the harmful ability of *Fusarium oxysporum* f.sp. sesami causes wilt disease in sesame plants. Furthermore, the molecular method indicated that the isolated fungus was close to *Fusarium oxysporum* KAML01. The ITS sequence of the isolate was deposited on the Genbank with accession number OP315278.

After 20 days of sowing sesame seeds, the pathogenicity test was carried out. After 7 days of inoculation, the plants began to show symptoms of wilt disease, the lower leaves turned yellow, but the upper leaves were still green. The tree withered when it was hot, green again in the cool of the afternoon, the next 7 days the cycads were not green again, wilted, and died. Collecting samples of this diseased plant and performing the same fungal isolation procedure as the initial strain yielded the same fungal strains as before the artificial inoculation. That confirmed the isolated fungus was causal of the wilt disease in sesame.

Isolation of bacteria with the antagonistic ability: From 2 soil samples of the sesame root zone collected in Bac Kan, 30 bacterial colonies with different characteristics were selected. The results showed that most of the bacterial strains were fast-growing and formed colonies after 24 hours, most of them had round, milky white colonies, buoyancy, and undulating cover, and most of the bacterial cells had rods and single bonds.

In addition, the results for screening their antagonistic ability against *F. oxysporum* f.sp. sesame showed that 7 isolates produced a clear zone around the bacterial colony by inhibiting the growth of mycelium. The results were represented in Fig. 2 and presented in Table 1.

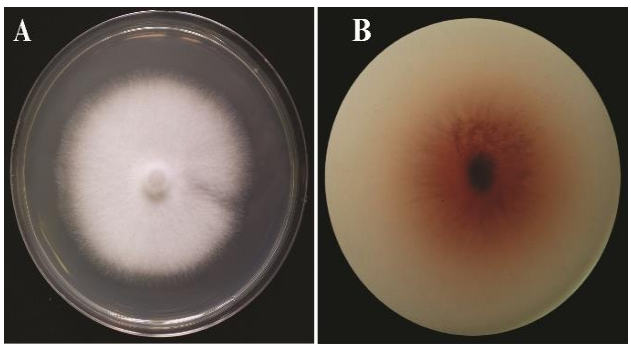

Fig. 1. Fungal colonies on PDA medium. A: Front side, B: Backside

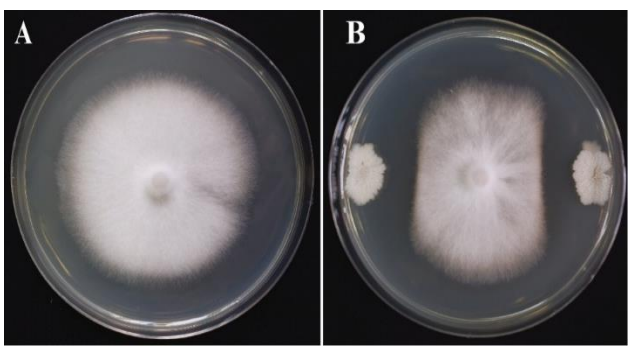

Fig. 2. Bacteria inhibiting fungal growth on an agar plate.
A: *F. oxysporum* f.sp. on PDA media (control), B: the antagonistic ability of NR11 isolate against *F. oxysporum* f.sp.

Table 1. The growth inhibition of *Fusarium oxysporum* f.sp. sesami by bacterial antagonists on PDA medium under *In vitro* condition.

Bacteria strains	Inhibition of fungal growth (%)	Level of antagonism
NR02	10.21 ± 1.31^{e}	+
NR05	15.71 ± 1.25^{de}	+
NR11	72.17 ± 1.57^{a}	+++
NR13	6.15 ± 1.26^{f}	+
NR22	64.21 ± 1.13^{b}	+++
NR26	59.13 ± 1.12^{c}	+++
NR27	17.12 ± 1.51^{d}	+
F. oxysporum f.sp. sesami + sterile-distilled water		-

Values in the same column with the different letter(s) are significantly different as determined by the HSD test ($p<0.05$)
Weak '+' (1-5.99 mm), moderate '++' (6-10.99 mm), and strong '+++' (11-20 mm), inhibition effects on the growth of the pathogen

As can be seen from Table 1, the growth inhibition of isolates varied in a range of 6.15 to 72.17%. The strong inhibition effect (approximately 60% or more) was observed for a group including NR11, NR22, and NR26 (Table 1, Fig. 2). The molecular identification result showed that they were close to *Bacillus cereus* KN4, *Bacillus amyloliquefaciens* B7, and *Bacillus pumilus* B19. The nucleotide sequences of their 16S rDNA fragment were deposited in GenBank with accession numbers ON352659, ON352664, and ON352668, respectively. The results suggest three isolates with high antagonistic ability could be used to develop biocontrol agents for sustainable agriculture.

This result is consistent with the result of Lee *et al*., (1995) when studying the effectiveness of bacteria from the sesame rhizosphere soil in the biological control of sesame wilt disease caused by the fungus *F. oxysporum*. The bacteria capable of inhibiting the mycelial growth was highest at 72.17% and the lowest at 6.15% was recorded after 6 days of inoculation. At the same time, this result is higher than the results of Cazorla *et al*., (2007) and Prashar *et al*., (2013) who investigated the antagonistic ability of bacterial strains isolated from avocado rhizosphere soil and tomato root with *F. oxysporum* caused wilt disease in tomatoes. The authors reported the inhibition rate of fungal mycelium ranging from 40 to 53% and 16.74 to 47.77%, respectively, corresponding to the above two studies after 5 days of inoculation.

Antagonistic properties of bacterial isolates: The results of the investigation of antagonistic properties showed that, out of 7 strains of bacteria capable of antagonism, 5 were capable of producing siderophore (0.13-0.9 cm), 6 were capable of degrading cellulose (0.6-1.73 cm), 5 chitinolytic lines (0.73-2.97 cm) and 5 proteolytic lines (0.3-2.2 cm) (Table 2).

The data in Table 2 indicated that the characteristics of each bacterial strain varied, however, it is noted that 3 bacterial strains are showing at least 2 characteristics, 1 strain showed 3 characteristics, and the remaining strains (NR11, N22, and N26) showed all 4 characteristics. The results also showed that the strains with high antagonistic performance were all strains with all four antagonistic properties. In addition, their antagonistic activities were all average or higher and at least one activity reached the highest level out of a total of 7 investigated bacterial strains. This corresponds to the results that up to 3/7 bacterial strains have high antifungal efficiency of over 60%. In general, the more the combination of antagonistic properties, the more effective the bacterial strains are against fungi.

Table 2. Biochemical properties of antagonistic bacteria on LB media.

Bacteria strains	Siderophore production (cm)	Cellulose degradation (cm)	Chitinolytic ability (cm)	Proteolytic ability (cm)
NR02	-	0.61^{d}	1.17^{b}	0.94^{cd}
NR05	0.62^{bc}	0.75^{cd}	-	-
NR11	0.57^{c}	1.67^{b}	0.93^{bc}	1.83^{b}
NR13	-	0.93^{c}	-	0.8^{d}
NR22	0.73^{b}	1.73^{a}	0.73^{c}	2.2^{a}
NR26	0.91^{a}	1.13^{bc}	2.97^{a}	1.2^{c}
NR27	0.71^{b}	-	1.53^{ab}	-

Values in the same column with the different letter(s) are significantly different as determined by the HSD test ($p<0.05$)

Table 3. A different effect of antagonistic bacteria on the incidence of wilt disease in the sesame seedlings.

Bacteria strains	Percentage of damping-off (%)		Percentage of survival plants (%)	Percentage of wilt (%)
	Pre-	Pos-		
NR11	4.43[c]	3.37[c]	92.56[a]	14.13[c]
NR22	7.83[b]	7.69[b]	89.53[b]	17.29[b]
NR26	8.31[b]	7.51[b]	90.36[b]	16.35[b]
Control	11.23[a]	26.57[a]	62.71[c]	75.23[a]

Values in the same column with the different letter(s) are significantly different as determined by the HSD test (p<0.05)

Table 4. Effect of bacterial inoculation on the incidence of wilt disease on the sesame seedlings under field conditions.

Bacteria strains	Percentage of damping-off (%)		Percentage of survival plants (%)	Percentage of wilt (%)
	Pre-	Pos-		
NR11	3.01[c]	2.12[c]	94.87[a]	16.03[c]
NR22	4.72[bc]	3.25[bc]	92.09[b]	17.75[bc]
NR26	4.87[b]	4.24[b]	90.85[c]	24.92[b]
Control	17.15[a]	27.08[a]	56.02[d]	58.51[a]

Values in the same column with the different letter(s) are significantly different as determined by the HSD test (p<0.05)

Table 5. Effect of bacterial inoculation on growth, yield, and seed oil percentage of treated sesame seeds.

Inoculated agent	Plant height (cm)	Number of fruit/ per plant	Number of capsules/ per plant	Seed yield/ per plant (g)	Oil percentage (%)
NR11	192.65[a]	8.34[a]	277.23[a]	39.26[a]	59.37[a]
NR22	181.87[b]	7.45[b]	228.94[b]	33.27[b]	53.81[b]
NR26	179.76[b]	7.61[b]	214.37[c]	32.83[b]	52.89[b]
Control	170.35[c]	6.23[c]	155.82[d]	21.93[c]	47.15[c]

Values in the same column with the different letter(s) are significantly different as determined by the HSD test (p<0.05)

Many studies also reported that bacterial strains could antagonize the fungal growth by producing toxic metabolites such as hydrogen cyanide (HCN), ammonia, lytic enzymes such as protease, cellulase, lipopeptides such as surfactin, fengycin, iturin, or volatile compounds; and/or they could compete for nutrients or live on pathogens (Cazorla *et al.*, 2007; Prashar *et al.*, 2013; Madriz-Ordeñana *et al.*, 2022). A recent study by Madriz-Ordeñana *et al.*, (2022) demonstrated the mechanism of *Bacillus* strains in protecting the plant Kalanchoe from *Fusarium oxysporum* is the production of genes involving the jasmonic and salicylic acid defense pathways. These pieces of evidence suggest the potential of isolates in the biocontrol of the wilt disease on the sesame plant.

Inoculation with antagonistic bacteria inhibited the wilt symptoms on the seedlings under nursery and field conditions: The results of the greenhouse experiment showed that sesame seedlings grown from seeds inoculated with strains NR11, NR22, and NR26 presented a significant decrease in the incidence of damping-off and wilted disease caused by *F. oxysporum* compared with non-treated control (Table 3).

As can be seen from Table 3, seeds inoculated with either NR22 or NR26 strain presented no significant differences in the percent of seedlings that were inoculated with antagonists before or after sowing. The results also indicated that the NR11 strain showed a better reduction of pathogenic incidence and wilt disease to 4.43% and 3.37%, and 14.13%, respectively.

In the field experiments in the 2021 cultivating seasons, a significant decrease in the percentage of disease incidence was observed in the inoculated seedlings compared with the control for all three isolates (NR11, NR22, and NR26) (Table 4).

Among isolates, the NR11 strain presented the strongest ability in protecting the sesame seedlings from the effect of the fungal pathogen which was demonstrated by a reduction in the means of pre- (3.01%), post-damping-off (2.12%), and wilt (16.03%), respectively, compared with the non-treated control.

It was reported that isolates of *Bacillus cereus*, *Bacillus amyloliquefaciens*, and *Bacillus pumilus* could have antagonistic activity against *F. oxysporum* (Heidarzadeh & Baghaee-Ravari, 2015; Tian *et al.*, 2021; Madriz-Ordeñana *et al.*, 2022). The obtained results in this study showed that seeds inoculated with strains NR11, NR22, and NR26 produced a significant decrease in the incidence of damping-off and wilt disease in the greenhouse as well as under field conditions. These results agreed with some previous studies which demonstrated the ability of fungal disease suppression of bacteria on many crops such as tomatoes (Heidarzadeh & Baghaee-Ravari, 2015), bananas (Tian *et al.*, 2021), and sesames (Kouighat *et al.*, 2022). These results suggest the potential use of these bacteria to manage various diseases on many crops.

Effect of bacterial inoculation on plant health and plant growth parameter of sesame: Together with the reduction of pathogenic incidence, the result also showed that bacterial inoculation with either NR11 or NR22, or NR26 produced a significant stimulation of plant health and growth promotion of sesame plants compared with the control (Table 5).

For example, the plant height, the number of fruiting branches per plant, the number of capsules per plant, plant seed yield, and the percentage of oil content of treated plants increased from 5.23 to 11.5%, 18.13 to 25.29%, 27.52 to 43.79%, 10.9 to 44.14%, and 10.85 to 12.22%, respectively, in comparison with the control. The results

also indicated that the NR11 and NR26 strains corresponded to the highest and lowest rates of sesame plant health and growth parameters during the field experiment, while the values of growth parameters of the NR22 strain fell into that range.

It is well known that the bacteria antagonizing the pathogenic fungi could play roles in promoting plant growth (Trung, 2022; Kouighat *et al.*, 2022). In this study, the increase of sesame plants was observed in experiments, in which sesame seeds were treated with selected bacteria (NR11, NR22, and NR26). This could be because the inoculated bacteria suppressed the pathogenic fungus reducing the effect of the wilt disease to plant development. This observation was demonstrated by some previous reports, which also worked on sesame plants (Babychan & Simon, 2017; Mahmoud & Abdalla, 2018). For example, Mahmoud & Abdalla (2018) demonstrated that inoculation with *Trichoderma viride* reduced the wilt disease incidence (70-77%) and improved the sesame plant development (20-21%) in comparison with the infected control under *In vitro* and greenhouse conditions. Another example was the report of Heidarzadeh & Baghaee-Ravari (2015), in which tomato plants treated with *Bacillus pumilus* ToIrMA produced an enhancement in the length of root (60%) and shoot (84%) as well as a decrease in disease incidence (73%) under *In vivo* conditions, over control. The plant growth-promoting ability of the bacterial inoculants could be explained by the alteration of phytohormone balance (such as siderophore) in the treated plant compared with the control (Vinothini *et al*., 2020, Ramírez *et al.*, 2021). These results indicated the promise of the isolated bacteria in the development of bacterial consortium to biocontrol phytopathogenic fungi in the future.

Conclusions

The study isolated 7 strains of bacteria capable of antagonizing *Fusarium oxysporum* causing wilt disease on sesame from 2 soil samples of the sesame root area collected in Na Ri, Bac Kan, Vietnam. The rate of *in vitro* inhibition of mycelial growth of bacterial strains ranged from 6.15 to 72.17%, with 3/7 bacterial strains having the ability to antagonize 59% or more. Through investigation of antagonistic properties, there were 5/7 bacterial strains capable of producing siderophore, 6/7 strains capable of degrading cellulose, 5/7 strains capable of proteolytic, and 5/7 strains having the ability to degrade chitin. Moreover, the bacterial strains with high resistance (NR11, NR22, NR26) presented their ability to reduce wilt disease in sesame in greenhouse and field conditions and to promote plant development. In conclusion, this study suggests that strains NR11, NR22, and NR26 identified as *Bacillus cereus* KN4, *Bacillus amyloliquefaciens* B7, and *Bacillus pumilus* B19 may be used as a biocontrol agent and biofertilizer in the field experiments.

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