

ประสิทธิภาพของเชื้อราเอนโดไฟต์ *Neosartorya fischeri* UB-SD-B4 ในการยับยั้งการเจริญของ
เชื้อรา *Fusarium oxysporum* f. sp. *lycopersici* ที่เป็นสาเหตุของโรคเหี่ยวในมะเขือเทศ

Efficacy of Endophytic Fungus, *Neosartorya fischeri* UB-SD-B4 against

Wilt-causing Fungus in Tomato, *Fusarium oxysporum* f. sp. *lycopersici*

ทศพร ศิริษะภูมิ^{1*} นาถอนงค์ ยอดสิงห์² และ จิตตรี เชื้อนสันเทียะ¹

Totsaporn Srisapoomi^{1*} Natanong Yodsing² and Jittri Khueansanthia¹

¹คณะนวัตกรรมและเทคโนโลยีการเกษตร มหาวิทยาลัยเทคโนโลยีราชมงคลอีสาน นครราชสีมา

²คณะวิทยาศาสตร์และศิลปศาสตร์ มหาวิทยาลัยเทคโนโลยีราชมงคลอีสาน นครราชสีมา

¹Faculty of Agricultural Innovation and Technology, Rajamangala University of Technology Isan, Nakhon Ratchasima

²Faculty of Sciences and Liberal Arts, Rajamangala University of Technology Isan, Nakhon Ratchasima

*E-mail: totsaporn.sr@rmuti.ac.th

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บทคัดย่อ

งานวิจัยนี้มีวัตถุประสงค์เพื่อศึกษาประสิทธิภาพของเชื้อราเอนโดไฟต์ *Neosartorya fischeri* UB-SD-B4 ในการยับยั้งการเจริญของเชื้อรา *Fusarium oxysporum* f. sp. *lycopersici* ที่เป็นสาเหตุของโรคเหี่ยวในมะเขือเทศ โดยเชื้อราเอนโดไฟต์ที่ใช้ในการศึกษานี้แยกได้จากกิ่งของต้นทรายเด่น (*Polyalthia cerasoides*) และถูกระบุชนิดโดยเทคนิคทางอนุชีววิทยา การทดสอบความสามารถของเชื้อราเอนโดไฟต์ในการยับยั้งเชื้อรา *F. oxysporum* f. sp. *lycopersici* ในระดับห้องปฏิบัติการใช้วิธี dual culture คุณสมบัติส่งเสริมการเจริญเติบโตของพืชของเชื้อราเอนโดไฟต์ตรวจสอบจากความสามารถในการผลิตกรดอินทรีย์และความสามารถในการละลายฟอสเฟต ผลการทดลองแสดงให้เห็นว่าเชื้อราเอนโดไฟต์ *N. fischeri* UB-SD-B4 สามารถยับยั้งการเจริญทางเส้นใยของเชื้อรา *F. oxysporum* f. sp. *lycopersici* ได้ 65.65 เปอร์เซ็นต์ และยังสามารถผลิตกรดอินทรีย์และละลายฟอสเฟต ได้เท่ากับ 8.102 และ 3,607 พีพีเอ็ม ตามลำดับ ความสามารถของเชื้อราเอนโดไฟต์ *N. fischeri* UB-SD-B4 ในการยับยั้งเชื้อราก่อโรคพืช *F. oxysporum* f. sp. *lycopersici* ได้ทำการทดลองกับมะเขือเทศด้วย โดยแบ่งการทดลองออกเป็น 4 ชุดการทดลอง ได้แก่ ชุดการทดลองที่ 1: เมล็ดมะเขือเทศที่ไม่ได้รับเชื้อราใด ๆ ชุดการทดลองที่ 2: เมล็ดมะเขือเทศที่ได้รับเชื้อราเอนโดไฟต์เพียงอย่างเดียว ชุดการทดลองที่ 3: เมล็ดมะเขือเทศที่ได้รับเชื้อราเอนโดไฟต์ร่วมกับเชื้อราก่อโรคพืช และชุดการทดลองที่ 4: เมล็ดมะเขือเทศที่ได้รับเชื้อราก่อโรคพืชเพียงอย่างเดียว หลังจากนั้นนำเมล็ดมะเขือเทศในทุกชุดการทดลองไปปลูก แล้ววัดความสูงของต้น น้ำหนักสดของลำต้นและราก และน้ำหนักแห้งของลำต้นและราก ผลการทดลองพบว่าเชื้อราเอนโดไฟต์ *N. fischeri* UB-SD-B4 ทำให้ต้นพืชที่ติดเชื้อราก่อโรค (ชุดการทดลองที่ 3) มีความสูงของต้น น้ำหนักสดของราก และน้ำหนักแห้งของลำต้นและรากเพิ่มขึ้นอย่างมีนัยสำคัญเมื่อเปรียบเทียบกับต้นพืชที่ติดเชื้อราก่อโรค (ชุดการทดลองที่ 4) งานวิจัยนี้แสดงให้เห็นว่าเชื้อราเอนโดไฟต์ *N. fischeri* UB-SD-B4 มีศักยภาพที่จะใช้เป็นตัวควบคุมทางชีวภาพของโรคเหี่ยวในมะเขือเทศ และอาจมีบทบาทสำคัญสำหรับการเกษตรแบบยั่งยืน

คำสำคัญ: เชื้อราเอนโดไฟต์ โรคเหี่ยวในมะเขือเทศ *Neosartorya fischeri* *Fusarium oxysporum* f. sp. *lycopersici*

Abstract

This research aimed to study the efficacy of endophytic fungus, *Neosartorya fischeri* UB-SD-B4 against wilt-causing fungus in tomato, *Fusarium oxysporum* f. sp. *lycopersici*. The endophytic fungus used in this study was isolated from branches of *Polyalthia cerasoides* and identified by molecular identification techniques. The dual culture technique was used for the *in vitro* investigation of antagonistic activity of the endophytic fungus against *F. oxysporum* f. sp. *lycopersici*. Plant growth-promoting properties of the endophytic fungus was determined by its ability to produce indole acetic acid (IAA) and to solubilize phosphate. The results revealed that the endophytic fungus, *N. fischeri* UB-SD-B4 was able to inhibit the mycelial growth of *F. oxysporum* f. sp. *lycopersici* by 65.65%. Its production of IAA and phosphate solubility were found to be 8.102 and 3,607 ppm, respectively. *In planta* study was also performed to assess the antagonistic activity of endophytic fungus, *N. fischeri* UB-SD-B4 against plant pathogenic fungus, *F. oxysporum* f. sp. *lycopersici* in tomato. Four treatments were set up including Treatment 1: uninoculated tomato seedlings, Treatment 2: tomato seedlings treated with the endophytic fungus only, Treatment 3: tomato seedlings treated with the endophytic fungus together with the plant pathogenic fungus and Treatment 4: tomato seedlings treated with the plant pathogenic fungus only. After tomato seedlings in all treatments were grown, plant height, fresh weights of shoot and root, and dry weight of shoot and root were measured. It was found that the endophytic fungus, *N. fischeri* UB-SD-B4 led the plant infected with pathogenic fungus (Treatment 4) to have significantly higher plant height, fresh root weight, dry root weight and dry shoot weight compared to the plants infected with pathogenic fungus (Treatment 3). This research demonstrates that the endophytic fungus, *N. fischeri* UB-SD-B4 has potential to be a biocontrol agent against wilt of tomato and may play an important role in sustainable agriculture.

Keywords: Endophytic fungus, Wilt of tomato, *Neosartorya fischeri*, *Fusarium oxysporum* f. sp. *lycopersici*

1. Introduction

Tomatoes (*Solanum lycopersicum* L.) are a widely grown vegetable throughout the world. Fusarium wilt, caused by *Fusarium oxysporum* f. sp. *lycopersici*, is one of several soilborne fungal diseases that affect tomatoes. This fungus is one of the most damaging tomato crop diseases in the world [1]. The symptoms first appear on the bottom leaves. Then, the plant wilts, turns brown, and drops its leaves as the disease spreads upward. Vascular tissue discoloration and stunting occur. Roots that appear stable at first, eventually die. In the most severe cases, the entire plant wilts. The most efficient method for controlling Fusarium wilt disease is to use resistant species [2]. Chemical management is ineffective. Biological control is an alternative disease management strategy [3].

Endophytic microorganisms have emerged as appealing, promising and environmentally friendly biological control agents due to their ability to inhibit vascular progression of the target pathogen. This more effectively limits disease incidence and severity. Endophytic fungi, which inhabit millions of unique biological niches, are a rich source of natural bioactive materials. Furthermore, they can also thrive in a variety of environments [4]. Endophytic fungi produce compounds that stimulate growth hormones in plants, causing them to grow faster and become more resistant to pathogens. These compounds include secondary metabolites and antibiotics [5]. Endophyte metabolites have been shown to suppress a wide range of microorganisms, lending credence to this theory.

Endophytes appear to sustain their host by defending it against pathogenic fungi, which stimulates biomass production [6]. *Trichoderma* species are antagonistic to a wide range of phytopathogenic fungi, including *F. oxysporum*. They are regarded as promising biological control agents [7]. Among the endophytic fungi, *Nigrospora sphaerica* was screened for bioactivity against various root rot pathogens, *F. solani*, *F. oxysporum*, *Macrophomina phaseolina* and *Phytophthora* sp. [8]. Furthermore, in a dual culture test, *Neosartorya fischeri* KK-KP-P2 produced the most potent extracellular metabolites, inhibiting the growth of *F. oxysporum* f. sp. *lycopersici* with radial growth inhibition of 56.92% [9]. The objectives of the current study are to conduct *in vitro* and *in planta* investigations to assess the efficacy of endophytic fungus, *N. fischeri* UB-SD-B4 against wilt-causing fungus in tomato, *Fusarium oxysporum* f. sp. *lycopersici*.

2. Materials and methods

2.1. Endophytic fungus

The endophytic fungal strain UB-SD-B4 was isolated from the branch tissue of *Polyalthia cerasoides*, collected in Ubon Ratchathani province, Thailand. Phylogenetic taxonomy and internal transcribed spacer (ITS) sequence alignment were used to identify its species. The organism shared over 97% genetic similarity with the *Neosartorya fischeri* strain. This strain was thus identified as *N. fischeri* UB-SD-B4 and was deposited at the Department of Microbiology, Faculty of Science, Khon Kaen University. The isolate was grown on potato dextrose agar (PDA) at 30°C for 5-7 days.

For ITS sequence analysis of *Neosartorya fischeri* UB-SD-B4, the fungal isolate was grown at 30°C on PDA. To extract DNA, a slightly modified protocol and reagents were used [10]. The primers used for ITS amplification were ITS1 (5'-TCC GTA GGT GAA CCT GCG G-3') and ITS4 (5'-TCC TCC GCT TAT

TGA TAT GC-3') [11]. A Gene Q thermal cycler, Model TC-24H/(b) (BIOER Technology, Tokyo, Japan) was employed for polymerase chain reaction (PCR) with the following conditions: an initial denaturation at 94°C for 5 minutes, 30 cycles of 94°C for 1 minute, 54°C for 1 minute, 72°C for 2 minutes, and a final extension at 72°C for 5 minutes. First BASE Laboratories Sdn Bhd provided custom-sequencing service for the DNA sequencing (Selangor, Malaysia). The DNA sequence was subjected to homology analysis using the BLASTN program.

2.2. Plant pathogenic fungus

The plant pathogenic fungus, *Fusarium oxysporum* f. sp. *lycopersici* was obtained from the Department of Microbiology, Faculty of Science Khon Kaen University. This isolate was grown at 30°C for 5-7 days on PDA.

2.3. Study of fungal morphology

Hyphal tips of fungi were transferred onto PDA as a pure culture for slide culture preparation after incubation at 30°C for 5-7 days [12]. Macroscopic characteristics of the fungal isolates were studied, including colony growth patterns, color, and texture, among other features. Characteristics such as spore shape, conidia, ascospore ornamentation, and other characteristics were studied under light microscopy.

2.4. *In vitro* antagonistic assay

The antagonistic activity of the endophytic fungus, *N. fischeri* UB-SD-B4, against *F. oxysporum* f. sp. *lycopersici* was *in vitro* tested using the dual culture technique on PDA plates, as previously described [13]. Briefly, a sterile cork borer was used to carefully remove a 5 mm mycelial disk from the newly developing margins of a culture of this endophyte strain. A similar disk from a culture of *F. oxysporum* f. sp. *lycopersici* was positioned 3 cm

distant from the endophytic strain on the surface of PDA and allowed to develop at 30°C. The pathogen's mycelia colony growth in the presence of the endophytic fungus, as well as alone as a control treatment, were measured from 7 to 10 days after inoculation. Then, the percent growth inhibition of *N. fischeri* UB-SD-B4 versus pathogen (IR) was calculated [14].

$$IR (\%) = [(C2-C1)/C2] \times 100$$

where:

C1 = the diameter of the pathogen's mycelial growth in the presence of the antagonist, and

C2 = the diameter of the pathogen's mycelial growth in the absence of the antagonist.

Each trial was replicated five times and the entire experiment was repeated twice.

2.5. Study of plant growth-promoting properties of *N. fischeri* UB-SD-B4

2.5.1. Indole acetic acid production

A modified version of the methods was used to determine the production of indole acetic acid (IAA) by the endophyte isolate [15]-[17]. The endophytic isolate was grown in 100 mL of potato dextrose broth (PDB) supplemented with 0.2% L-tryptophan and incubated in the dark with shaking for 7 days under a constant temperature of 28°C. Then, the culture was centrifuged at 4,000 rpm for 10 minutes. One mL of the supernatant was combined with two mL of Salkowski reagent and 35% perchloric acid to make the color developing reagent. A pink color was observed after 30 minutes of incubation in the dark at 28°C, indicating IAA production. The optical density (OD) at 530 nm was spectrophotometrically measured. The degree of IAA production was estimated by comparing these OD readings at 530 nm to an IAA standard.

2.5.2 Phosphate solubilization

Pikovskaya (PVK) broth medium was used as a liquid medium to culture the endophytic fungus and assess its phosphate solubilization capability. Agar disks (5 mm diameter) were cut from the apical margin of a fungal colony grown on PDA and used as inocula. One hundred mL of PVK broth (pH 7.2) was placed in a conical flask. For each flask containing test media, ten fungal agar disks (5 mm diameter) were used as an inoculum. The test flask was treated in the same way as the control, but the control flask was not inoculated with fungal agar disks. The flasks were incubated for 21 days at 30°C. After 7 days, the solubilized phosphate concentration was measured three times [18]. The culture broths were centrifuged at 4,000 rpm for 10 minutes after being passed through Whatman No. 1 filter paper. Culture pH and solubilized phosphate were measured in the culture broth supernatants. The absorbance of solubilized phosphate was spectrophotometrically measured at 820 nm. Murphy and Riley's method was used to determine the amount of soluble phosphate [19].

2.5.3. Siderophore production

The siderophore production of the endophytic fungus, *N. fischeri* UB-SD-B4, was determined by a modified method [17], [20], [21]. The endophyte isolate grown on PDA plates at 28°C for 5-7 days was cut and inoculated on chrome azurol S (CAS) agar and incubated in the dark at 28°C for 3-7 days. The color shift advance in chrome azurol S (CAS) agar was determined from the CAS reaction rate. A colony with orange zone was described as a siderophore-producing isolate. An uninoculated CAS-agar control plate was incubated under the same condition as the inoculated plate.

2.6. *In planta* antagonistic assay

In planta antagonistic assay was carried out using *F. oxysporum* f. sp. *lycopersici* as a pathogen and endophytic *N. fischeri* UB-SD-B4 as a test antagonist. *N. fischeri* UB-SD-B4 and the pathogenic fungus, *F. oxysporum* f. sp. *lycopersici*, were cultured on PDA at 30°C for 7 days for preparation of the spores of the endophytic fungus. Samples, 5 mm in diameter, were taken from the edge of each fungal colony and placed in 50 mL conical flasks. Agar surfaces were washed with distilled water mixed with Tween 20 to collect the spores. The spore counts were determined using a hemacytometer. *N. fischeri* UB-SD-B4 spore suspension was diluted to 2×10^6 spores/mL, while *F. oxysporum* f. sp. *lycopersici* spore suspension was diluted to 2×10^7 spores/mL [22].

Tomato seedlings were first surface sterilized for 5 min with 1.5% sodium hypochlorite, rinsed twice with sterile distilled water and dried under laminar airflow on sterile paper. For single treatment, tomato seedlings were treated with 1 mL of spore suspension (*N. fischeri* UB-SD-B4 spore suspension or *F. oxysporum* f. sp. *lycopersici* spore suspension) at 30°C for 7 days. For double treatment, tomato seedlings already treated with *N. fischeri* UB-SD-B4 were treated again with 1 mL of *F. oxysporum* f. sp. *lycopersici* spore suspension at 30°C for 7 days.

Four treatments were set up including Treatment 1 (T1): uninoculated tomato seedlings, Treatment 2 (T2): tomato seedlings treated with *N. fischeri* UB-SD-B4 only, Treatment 3 (T3): tomato seedlings treated with *N. fischeri* UB-SD-B4 together with *F. oxysporum* f. sp. *lycopersici* and Treatment 4 (T4): tomato seedlings treated with *F. oxysporum* f. sp. *lycopersici* only. After tomato seedlings in all treatments were grown, plant growth parameters including plant height (cm), fresh weights of shoot and root (g), and dry weight of shoot and root (g) of

infected and non-infected plants were recorded. The experiments were performed in triplicate and the plant growth parameters were measured after 2 weeks of plantation.

2.7. Statistical analysis

The data collected from the aforementioned experiments were statistically analyzed, and the values were represented as their mean \pm SE. SPSS statistical analysis software was used to perform one-way analysis of variance (ANOVA) with Scheffe's post hoc test (Version 26.0; IBM SPSS). Differences with p-values of 0.05 or less were considered statistically significant.

3. Results and discussion

3.1. Morphological characteristics of endophytic fungus and plant pathogenic fungus

N. fischeri UB-SD-B4 endophytic fungal colony on PDA spread broadly, white to yellowish white in color, with a thin mycelial felt and abundant cleistothecia, few conidiogenesis, brown exudates absent, reverse yellowish brown (Figure 1A, 1B). Its conidial head was short to loosely columnar, conidiophores were mostly long, smooth, vesicles were usually flask-shaped, faintly to strongly colored in grayish green shades, and phialides were in a single, crowded cluster, usually in pale to dull greenish shades (Figure 1C). Conidia were globose to subglobose and had a delicate roughening. The cells were irregularly flattened, asci mature quickly, and were globose to subglobose 8-spored (Figure 1D). Based on its nuclear ribosomal RNA sequences, the *Neosartorya* isolate was identified as *N. fischeri* (ITS). Sequence analysis revealed that the strain, UB-SD-B4, had a 98% sequence similarity to the *N. fischeri* strain FJZ2 (JN390830.1) from GenBank (data not shown), and was thus identified as *N. fischeri*. *N. fischeri* was found in the majority of soil samples. Previous

studies discovered this species in rice, cotton, potato, groundnuts, leather, paper, and canned products [23]. *N. fischeri* was found in the rhizosphere soil of the terrestrial orchid, *Goodyera procera*. [24].

For *F. oxysporum* f. sp. *lycopersici* colony on PDA, color varied from white to pink to violet mycelia, reverse pinkish (Figure 2A, 2B), and formed macroconidia and microconidia (Figure 2C). The size, shape and number of septa of macroconidia and microconidia (Figure 2D) were variable.

3.2. *In vitro* evaluation of antagonistic activity of *N. fischeri* UB-SD-B4 against *F. oxysporum* f. sp. *lycopersici*

Under *in vitro* condition, the antagonistic activity of *N. fischeri* UB-SD-B4 was investigated against *F. oxysporum* f. sp. *lycopersici*. The percent of endophyte growth inhibition was determined

against the pathogen in a 7 day old mycelia culture of *N. fischeri* UB-SD-B4 and *F. oxysporum* f. sp. *lycopersici* using the dual culture technique on PDA. The results revealed that the endophytic fungus, *N. fischeri* UB-SD-B4 was able to inhibit the mycelial growth of *F. oxysporum* f. sp. *lycopersici* by 65.65% (Figure 3A, 3B). From previous report, *F. oxysporum*, *Alternaria brassicicola*, *Colletotrichum capsici*, and *Curvularia oryzae* mycelial growth was inhibited by 53.9-58.3% by *N. fischeri* [25]. In a dual culture test, *N. fischeri* KK-KP-P2 produced the most successful extracellular metabolites, inhibiting 56.92% of the mycelial growth of *F. oxysporum* f. sp. *lycopersici* [9]. Alternatively, endophytic fungi were reported to be able to inhibit pathogens by producing antibiotics or directly competing with them [26]. *F. oxysporum* growth was inhibited by *N. fischeri* in the absence of direct interactions between the fungi, implying the formation of antifungal metabolites [27].

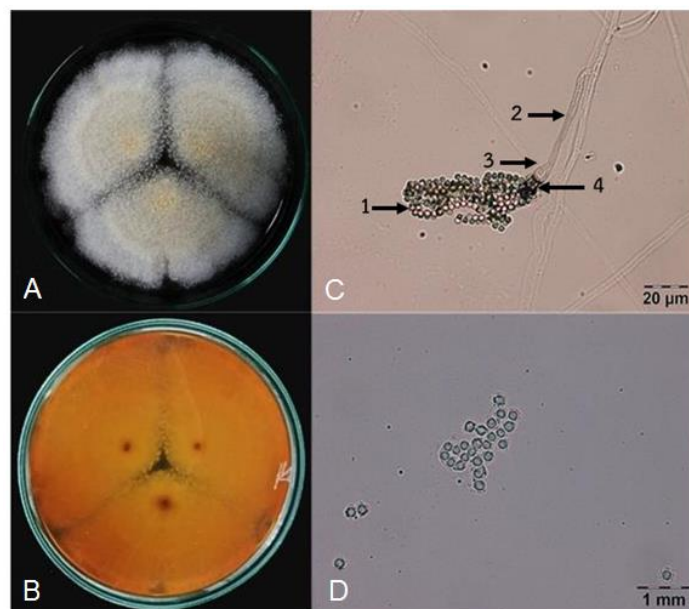


Figure 1 Morphological characteristics of *N. fischeri* UB-SD-B4

(A) Colony morphology after 7 days on PDA; (B) Growth of *N. fischeri* UB-SD-B4, bottom of the plate; (C) Conidial head (1), Conidiophores (2), Vesicles (3) and Phialides (4); (D) Asci and ascospores

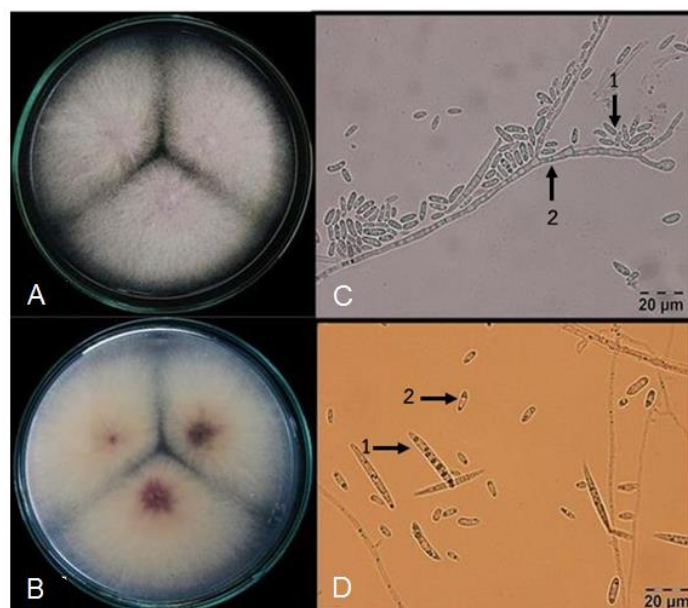


Figure 2 Morphological characteristics of *F. oxysporum* f. sp. *lycopersici*

(A) Colony morphology after 7 days on PDA; (B) Growth of *F. oxysporum* f. sp. *lycopersici*, bottom of the plate; (C) Conidial heads (1) and Conidiophores (2); (D) Macroconidia (1) and Microconidia shape (2)

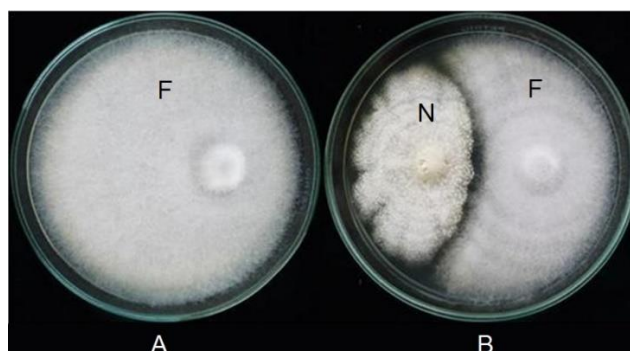


Figure 3 Antagonistic activity of the endophytic fungus, *N. fischeri* UB-SD-B4 (N) against the pathogenic fungus, *F. oxysporum* f. sp. *lycopersici* (F) on PDA after 7 days at 30°C

(A) Colony of *F. oxysporum* f. sp. *lycopersici* (F) on PDA; (B) *N. fischeri* UB-SD-B4 (N) inhibited mycelial growth of *F. oxysporum* f. sp. *lycopersici* (F)

3.3. Study of plant growth-promoting properties of *N. fischeri* UB-SD-B4

The IAA production, phosphate solubilization and siderophore production of *N. fischeri* UB-SD-B4 were determined. This isolate produced 8.102 ppm of IAA and solubilized 3,607 ppm of phosphate, whereas siderophores were not detected around the colony (Table 1). In previous studies, endophytes were shown to exhibit IAA production.

This substance could have a variety of effects on plants including increased cell division and cell elongation, with all of the consequences for plant growth and development [28], [29]. Sati and Prabha [30] discovered that phosphate solubilization by the endophytic aquatic fungus, *Tetraccladium setigerum*, promoted plant growth and stress tolerance. This fungus could be used as a bio-fertilizer in agriculture due to its ability to solubilize phosphate.

3.4. *In planta* evaluation of antagonistic activity of *N. fischeri* UB-SD-B4 against *F. oxysporum* f. sp. *lycopersici*

In this experiment, tomato growth of 4 treatments were evaluated. In T1 (the control without any fungus inoculation), all plant growth parameters (plant height, fresh weights of shoot and root, and dry weight of shoot and root) were found to be highest among all treatments while the opposite results were observed in T4 (the infected plant with *F. oxysporum* f. sp. *lycopersici*) (Table 2) indicating the pathogenic effect of *F. oxysporum* f. sp. *lycopersici* on the plant. In T3 (the plant treated with *N. fischeri* UB-SD-B4 and *F. oxysporum* f. sp. *lycopersici*), all plant growth parameters, except

fresh shoot weight, were found to be significantly higher than those of T4 indicating the antagonistic activity of *N. fischeri* UB-SD-B4 against *F. oxysporum* f. sp. *lycopersici* (Table 2). Previous research used endophytes alone or in combination with other antagonistic organisms to suppress other *Fusarium* spp. diseases [31]. The mechanisms involved in the reduction of *Fusarium* wilt caused by *N. fischeri* in our study require further investigation. However, they were thought to be related to nutrient rivalry, antibiosis by enzymes and secondary metabolites, inhibition of pathogen mycelial growth and spore germination, and activation of plant defense mechanisms. Furthermore, inhibition may be affected by specific abiotic and biotic conditions [13], [32], [33].

Table 1 Analysis of IAA production, phosphate solubilization and siderophore production of *N. fischeri* UB-SD-B4

Endophytic fungus	IAA production (ppm)	Phosphate Solubilization (ppm)	Siderophore production
<i>N. fischeri</i> UB-SD-B4	8.102	3,607	inactive

Table 2 Effect of *N. fischeri* UB-SD-B4 on plant growth parameters of uninoculated and inoculated tomato plants

Treatment	Plant height (cm)	Fresh shoot weight (cm)	Dry shoot weight (cm)	Fresh root weight (cm)	Dry root weight (cm)
T1	10.94±0.42897 ^a	1.24±0.13408 ^a	0.16±0.01298 ^a	0.47±0.04071 ^a	0.04±0.00407 ^a
T2	9.35±0.19774 ^b	0.83±0.04853 ^b	0.09±0.00321 ^b	0.44±0.03002 ^a	0.03±0.00084 ^b
T3	8.43±0.17595 ^b	0.70±0.05161 ^{bc}	0.08±0.0046 ^b	0.36±0.02297 ^a	0.03±0.0019 ^b
T4	5.95±0.11676 ^c	0.42±0.030004 ^c	0.04±0.00198 ^c	0.22±0.02027 ^b	0.01±0.00068 ^c

Data are means ± standard deviations. Data with different letters within the same column are significantly different (p<0.05).

4. Conclusion

The endophytic fungus, *N. fischeri* UB-SD-B4d, had effective *in vitro* antagonistic activity inhibiting radial growth of wilt-causing *F. oxysporum* f. sp. *lycopersici*. It was also shown to be capable of IAA production as well as phosphate solubilization for improved tomato plant growth. *In planta* study was

also demonstrated the antagonistic activity of the endophytic fungus against plant pathogenic fungus, *F. oxysporum* f. sp. *lycopersici* in tomato. However, further investigation is required to understand the mechanisms responsible for the antagonistic activity of *N. fischeri* UB-SD-B4 against *F. oxysporum* f. sp. *lycopersici*.

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