Chemical and Biological Technologies in Agriculture

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Biological control of fusarium root rot of Indian mulberry (*Morinda officinalis* How.) with consortia of agriculturally important microorganisms in Viet Nam

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Abstract

Background: Fusarium root rot disease in Indian mulberry (*Morinda officinalis* How.) (FRRBK), caused by *Fusarium proliferatum* (FP), is widespread and responsible for serious economic losses in Viet Nam. The efficacy of a new bio-product named MICROTECH-1(NL) is compared with other commercial products for suppression of FP under in vitro, pot, nursery as well as in the field conditions.

Results: In in vitro antagonistic assay, MICROTECH-1(NL) significantly inhibited the mycelial growth of FP (72.38%). Under pot conditions, the efficacy of all the bio-products was significantly higher when applied prior to pathogen inoculation. The disease severity of treatments with double application of MICROTECH-1(NL) (applied both in the nursery and in the pot soil) was only 15.56%, significantly lower than control (80%). Thus, the application of MICROTECH-1(NL) significantly reduced the incidence of FP and markedly increased the number of plant beneficial bacteria and actinobacteria in rhizoplane of *M. officinalis* compared to untreated control. In the field conditions, double application of MICROTECH-1(NL) (both in the nursery and in the field soils) significantly decreased disease severity in comparison to single application in nursery or field.

Conclusion: The most effective treatment was double application of MICROTECH-1(NL), which significantly reduced the disease severity and FP population in roots of *M. officinalis* and increased the population of plant beneficial microbes.

Keywords: Ba kich, Biological control, *Fusarium proliferatum*, *Morinda officinalis*

Background

In Viet Nam, Indian mulberry (*Morinda officinalis* How.) is locally known as Ba kich and is widely grown in many mountainous provinces in the north of Viet Nam including Thai Nguyen, Quang Ninh, Ha Tay, Bac Giang, Bac Ninh, Lang Son, Tuyen Quang, Cao Bang, Bac Kan, Lao Cai, Yen Bai, Phu Tho, Lai Chau, Son La, Hoa Binh, Thanh Hoa, and Nghe An. Roots of Ba kich are used for medicinal purposes in traditional medicine as it is rich in various medicinal compounds [1].

Fusarium root rot of Ba kich (FRRBK) is a widespread soil-borne disease, which can cause serious damage and huge economic losses to Ba kich production in Viet Nam. *Fusarium proliferatum* (FP) has been identified as the causal pathogen of FRRBK [1]. It was shown that long periods of continuous cropping of Ba kich with high planting density and intensified soil acidification from chemical N fertilization and less use of organic manures may change in the soil microbiome, subsequently leading to disease outbreak [1]. Since Ba kich is directly used for human consumption, overuse of chemical fertilizers and

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chemical pesticides may be a potential threat for both human and environmental health. Thus, application of antagonistic microorganisms is a promising eco-friendly alternative for the efficient management of FRRBK.

Antagonistic microorganisms play an important role in management of different plant diseases [2, 3] and enhance plant growth [4] via different mechanisms. It was showed that soaking banana suckers in the suspension of *Pseudomonas fluorescens* before planting significantly decreased the wilt incidence of fusarium wilt of banana caused by *F. oxysporum* f. sp. *cubense* [5]. *Bacillus* spp. were used for suppressing tomato fusarium crown and root rot disease [6], significantly inhibited growth of *F. verticillioides* and fumonisin B1 accumulation, reduced rhizoplane and endorhizosphere colonization of the pathogen [7], suppressed growth of *F. oxysporum* in the rhizosphere of cucumber, and protected the host plant from the pathogen [8]. The fluorescent pseudomonads are potential beneficial agents of various soil-borne diseases [9–11] via different mechanisms [12], which promote plant growth [2]. They also produce antimicrobial metabolites [13], trigger induced systemic resistance (ISR) in plants leading to protection against a broad spectrum of diseases [13, 14].

Streptomycetes, are antibiotic producers [15], were reported to significantly inhibit the growth of *Fusarium* spp. [16, 17] and reduce disease incidence and disease severity of different *Fusarium* diseases [16, 18, 19]. Lactic acid bacteria also produce various compounds such as organic acids, diacetyl, hydrogen peroxides, bacteriocins, and bactericidal proteins [20], reduce the soil pH, and create unfavorable environmental condition to soil-borne pathogens [21], and were used to control *Fusarium* diseases caused by *F. nygamai* and *E. amylovora* [22–24].

It was reported that photosynthetic bacteria were widely used to promote plant growth and improve plant quality [25]. Various compounds produced by *Rhodopseudomonas palustris* induced systemic resistance in plants and possessed ISR-inducing properties [26, 27]. In the field conditions, foliar spray of *R. palustris* GJ-22 suspension protected tobacco plants against tobacco mosaic virus (TMV) by colonizing the phyllosphere and inducing resistance against TMV [27]. *Saccharomyces cerevisiae* acts as a natural stimulator [28], plays an essential role in cell division and cell enlargement due to its cytokinin content [29] and acts as a biocontrol agent of soil-borne fungal pathogen *F. oxysporum* in sugar beet seedlings [30]. *Azotobacter* sp., a free living N_2 fixing bacterium, synthesizes and secretes considerable amounts of biologically active substances promoting root growth and improving nutrient uptake by the plants [31]. *Azotobacter* isolate AZT-R7 acted as a potential biocontrol agent against fusarium wilt of chilli in India [32].

The present study was undertaken to investigate our newly developed bio-product named MICROTECH-1(NL) that composed from *P. fluorescens*, *B. subtilis*, *Streptomyces* spp., *L. casei, R. palustris, S. cerevisiae, Azotobacter* sp. against FP and FRRBK in in vitro, nursery, pot, and field conditions, and compared to other commercial bio-products available in Viet Nam.

Materials and methods

Inoculum preparation

Three isolates of FP designated as BKVN, BKDT, and BKPL were used in this study. They were grown on PDA for 3 days in the dark at 25 °C. Mycelial disks (5 mm diameter) were transferred to 100 mL potato dextrose broth in a 500-mL flask and incubated for 7 days at 25 °C with shaking at 120 rpm. The fungal suspensions was harvested and diluted with sterile distilled water. It was adjusted to 5×10^6 cfu mL^{-1} and used as inocula for further experiments [1].

Plants and bio-products

Ba kich seedlings were grown in small plastic pots ($5 \times 15 \times 5$ cm) in the nursery for about 1 year before planting in the field. Seven commercially available bio-products were applied to evaluate their effects on the development of FP and FRRBK. They were (1) Actino Vate (*S. lydicus* WYEC 108 1×10^7 CFU/g, Golden Rice Hau Giang Co. Ltd.), (2) Bionite (*B. subtilis* 10^9 CFU/g, Bio-AgriTech Co. Ltd.), (3) Ketomium (*C. cupreum* 1.5×10^6 CFU/g, Nam Bac Co. Ltd.), (4) BIMA (*Trichoderma* sp. 5×10^6 CFU/g, SaiGon Pesticide Co. Ltd.), (5) TRICHO (consortia of *Trichoderma* spp., *B. subtilis*, *Pseudomonas* sp., and *Streptomyces* spp., Dien Trang Co. Ltd.), (6) SH-BV1 (consortia of *T. viride*, *B. subtilis*, *B. oisengihumi*, *A. beijerinckii*, *S. owasiensis,* and *M. anisopliae*, Plant Protection Research Institute (PPRI), Viet Nam), and (7) MICROTECH-1(NL) (consortia of *P. fluorescens*, *B. subtilis*, *Streptomyces* spp., *L. casei, R. palustris, S. cerevisiae, Azotobacter* sp., PPRI, Viet Nam). Among them, SH-BV1 and MICROTECH-1(NL) are new bio-products developed by PPRI. According to the recommendation from the manufacturers, the bio-products were diluted at the following rates: Actino Vate (0.25 g/L), Bionite (0.3 g/L), Ketomium (1 g/L), BIMA (1 g/L), TRICHO (2.5 g/L), SH-BV1 (3 g/L), and MICROTECH-1(NL) (0.1% v/v). For the pot and soil experiments, all the diluted bio-products were applied as drenches at the rate of 50 mL per plant.

Effect of MICROTECH-1(NL) on mycelial growth of *F. proliferatum*

Petri plate experiments were carried out to investigate the antagonistic effect of MICROTECH-1(NL) on the

mycelial growth of three isolates of FP (BKVN, BKDT, and BKPL). Each experiment had total of eight treatments arranged in completely randomized design with five petri plates per treatment as five replications. The eight treatments were untreated- control and the seven bio-products applied to 100 mL sterilized PDA medium at 60 °C before being divided equally into five petri plates (as five replications) at the following rates: Actino Vate at 0.25 g/L, Bionite at 0.3 g/L, Ketomium at 1 g/L, BIMA at 1 g/L, TRICHO at 2.5 g/L, SH-BV1 at 3 g/L, and MICROTECH-1(NL) at 1% (v/v). The control plates were prepared by replacing the bio-products with the same amount of sterile distilled water. Colony growth in diameter of BKVN, BKDT, and BKP isolates was measured and recorded on the 7th day after treatment. The inhibition percentage (%) of each bio-product on mycelial growth of each FP isolate was determined at the 7th day of incubation following formula $(1-\mathrm{Cn/Co})\times 100$, where Cn is the average diameter of FP colony in treatment plates, and Co is the average diameter of FP colony in control plates.

Effect of MICROTECH-1(NL) on disease severity of FRRBK in pot condition

Three pot experiments were carried out to investigate the effects of MICROTECH-1(NL) and other bio-products on disease severity of FRRBK using three application approaches: (1) In the first experiment, all the bio-products were drenched in the soil 1 week before pathogen inoculation, (2) In the second experiment, all the bio-products and the pathogen spores were applied simultaneously, and (3) In the third experiment, all the bio-products were drenched to the pots 1 week after pathogen inoculation. Each experiment had three replications, eight treatments (untreated control and the seven bio-products applied at the rates mentioned above), arranged in completely randomized design with thirty (30) pots per treatment. One eight-leaf stage (about 1 year old) seedling was transplanted to each pot ($20\times 40\times 20$ cm) containing FP-infested soils.

The disease severity was recorded on 0–3 visual scales, in which 0 was no symptoms; 1 was light yellowing of leaves, light or moderate rot on taproot, secondary roots, and crown rot; 2 was moderate or severe yellowing of leaves with or without wilting, stunting, severe rot on taproot, secondary roots, crown rot with or without hypocotyls rot, and vascular discoloration in the stem; and 3 was dead plants. The disease severity (%) was determined using the following formula:

$$\text{Disease severity}(\%) = \frac{\left(\sum \text{scale} \times \text{number of plants infected}\right)}{\left(\text{Highest scale} \times \text{total number of plants}\right)} * 100$$

Effect of MICROTECH-1(NL) on microorganism in the soil

FP-infested soil was obtained from the field in Vo Nhai district of Thai Nguyen province in which Ba kich had been previously planted and seriously infested with fusarium root rot disease. The spore concentration of FP in this soil was assessed by PDA plate method and resulted of 2.3×10^5 cfu g^{-1} soil.

Treatments were applied to both nursery and pot soils. In the nursery, soils were treated with either SH-BV1 (SHn), or MICROTECH-1(NL) (MIn), or none of SH-BV1 and MICROTECH-1(NL) (Cn) as untreated controls. In the pot, soils were treated with 1% (v/v) MICROTECH-1(NL) (MIp), 1% (w/w) SH-BV1, or none of SH-BV1 and MICROTECH-1(NL) (Cp). Combination of two phases treatments resulted in eight treatments that were: (1) SHn + Cp: SH-BV1-treated nursery soil + untreated pot soils; (2) Cn + SHp: untreated nursery soils + SH-BV1-treated pot soils; (3) SHn + SHp: SH-BV1-treated nursery and pot soils; (4) MIn + Cp: MICROTECH-1(NL)-treated nursery soils, untreated pot soils; (5) Cn + MIp: untreated nursery soils, MICROTECH-1(NL)-treated pot soils; (6) MIn + MIp: MICROTECH-1(NL)-treated nursery and pot soils; (7) SHnp + MInp: SH-BV1-treated nursery and pot soils, MICROTECH-1(NL)-treated nursery and pot soils; and (8) Cn + Cp: untreated nursery and pot soils. All amendments were mixed well with the soil before putting into nursery or pots. To ensure sufficiently high density of FP population, all pot soils were inoculated with the spores of FP isolate at 10^5 cfu g^{-1} soil before putting into pots. Experiment had three replications with randomize design and 50 pots per treatment.

Two months after transplanting, numbers of diseased and healthy plants were recorded, and disease severity was calculated. Five plants (with maximum disease symptoms in the aerial parts) in each replication were sampled. Roots were taken from the pots and carefully shaken by hand to remove soil; these soil samples represented rhizosphere soils; soil that still adhered to roots, and the root themselves, were considered as rhizoplane samples. The microorganisms were enumerated by the standard tenfold dilution method. FP was isolated from roots of Ba kich, rhizoplane, and rhizosphere soils using Komada selective medium (KSM) (1 g K_2HPO_4, 0.5 g KCl, 0.5 g $MgSO_4{\cdot}7H_2O$, 0.01 g Fe–Na-EDTA, 2 g L-asparagin, 20 g D-galactose, and distilled water up to 1 L, pH 7.0). Total bacteria and actinobacteria were isolated from rhizoplane and rhizosphere soils using Gauze's medium (20 g soluble starch, 1 g KNO_3, 0.5 g NaCl, 0.5 g K_2HPO_4, 0.5 g $MgSO_4$, 0.01 g $FeSO_4$, 20 g agar, and distilled water up to 1 L, pH 7.2). Total fungi were isolated from rhizoplane and rhizosphere soils using Martin medium (10 g

glucose, 5 g peptone, 1 g KH_2PO_4, 0.5 g $MgSO_4$, 30 g Rose Bengal, 0.03 g streptomycin, and distilled water up to 1 L). The FP populations, bacterial and fungal isolation, and enumeration from soil and root samples were carried out as described previously [33].

Effect of MICROTECH-1(NL) on disease severity in the field condition

Field experiments for effective management of FRRBK were carried out independently in Vo Nhai, Dai Tu, and Phu Luong districts, Thai Nguyen province by applying two bio-products SH-BV1 and MICROTECH-1(NL). Treatments were applied to both nursery and field soil. In the nursery, soils were treated with either SH-BV1 (SHn), or MICROTECH-1(NL) (MIn), or none of SH-BV1 and MICROTECH-1(NL) (Cn) as untreated controls. In the field, soils were treated with 1% (v/v) MICROTECH-1(NL) (MIf), 1% (w/w) SH-BV1 (SHf), or none of SH-BV1 and MICROTECH-1(NL) (Cf). Combination of two phases treatments resulted in ten treatments that were: (1) SHn + Cf: SH-BV1-treated nursery soil, untreated field soil; (2) Cn + SHf: untreated nursery soil, SH-BV1-treated field soil; (3) SHn + SHf: SH-BV1-treated nursery and field soil; (4) MIn + Cf: MICROTECH-1(NL)-treated nursery soil, untreated field soil; (5) Cn + MIf: untreated nursery soil, MICROTECH-1(NL)-treated field soil; (6) MIn + MIf: MICROTECH-1(NL)-treated nursery and field soils; (7) SHn + MIf: SH-BV1-treated nursery soil, MICROTECH-1(NL)-treated field soil; (8) MIn + SHf: MICROTECH-1(NL)-treated nursery soil, SH-BV1-treated field soil; (9) SHnf + MInf: SH-BV1 and MICROTECH-1(NL)-treated nursery and field soils; and (10) Cn + Cf: nursery and field soils were not treated as control. The application rate of SH-BV1 and MICROTECH-1(NL) was 1% (w/w) and 1% (v/w), respectively. Experiment had three replications with randomized block design and 24 m × 6 m in plot size, planted with 100 Ba kich plants per treatment.

Statistical analysis

Treatment effects were assessed by analysis of variance (ANOVA) using IRRISTAT for Windows version 5.0 (Biometric Unit, International Rice Research Institute). Mean separation was performed using the least significant difference (LSD) at $P = 0.05$ and $P = 0.01$ whenever a significant ANOVA ($P < 0.05$) result occurred.

Results

Effect of MICROTECH-1(NL) on growth of *F. proliferatum* in vitro condition

In dual-culture assay, the bio-products significantly suppressed the growth of BKVN, BKDT, and BKPL isolates by at least 52%, and the highest values were obtained with MICROTECH-1(NL). The application of MICROTECH-1(NL) inhibited the mycelial growth of BKVN, BKDT, and BKPL isolates by 70.38, 71.80, and 72.04%, respectively. Except for Actino Vate, Bionite, Ketomium, and BIMA that entailed the low growth inhibition (lower than 60%), TRICHO and SH-BV1 had also significantly suppressed mycelial growth of BKVN, BKDT, and BKPL isolates. The inhibition percentage for TRICHO and SH-BV1 ranged from 60.90% with BKPL isolate to 62.09% with BKVN isolate, and from 66.35% with BKVN isolate to 67.30% with BKDT isolate, respectively (Fig. 1).

Effect of MICROTECH-1(NL) on disease severity of FRRBK in pot condition

Treatment of bio-products 1 week before inoculation with FP significantly reduced disease severity compared to inoculated-untreated control (Fig. 2). Among the tested bio-products, SHBV1 and MICROTECH-1(NL) showed highest suppression of disease severity. The results indicated that in treatment of SH-BV1, disease severity ranged from 7.77% with BKDT isolate to 9.25% with BKVN isolate. In treatment of MICROTECH-1(NL), disease severity ranged from 5.92% with BKDT isolate to 6.66% with BKPL isolate. In other treatments, disease severity ranged from 9.62% for Actino Vate with BKDT isolate to 22.96% for Ketomium with BKPL isolate.

The decrease of disease severity when bio-products were applied simultaneously with inoculation of FP spores showed similar trends, although the disease severity was higher compared to that when bio-products were applied 1 week before inoculation with FP. Disease severity in treatments of SH-BV1 and MICROTECH-1(NL) was statistically differentiated compared to other treatments and inoculated-untreated control. With the exception for Ketomium, the disease severity was about 30% in all of three isolates; the disease severity from all bio-products ranged from 10.37 for MICROTECH-1(NL) with BKVN isolate to 27.78% for Bionite with BKVN isolate (Additional file 1). Treatment of bio-products 1 week after the inoculation with FP did not reduce disease severity as compared to inoculated-untreated control. Disease severity was more than 43% and it reached 65.56% for Ketomium with BKVN isolate (Additional file 2).

Effect of MICROTECH-1(NL) on microorganisms in the soil

The results showed that when Ba kich plants were pretreated with both of SH-BV1 and MICROTECH-1(NL), there was significantly reduced disease severity in comparison to other treatments. The disease severity of this treatment was 11.78%, which is significantly lower than untreated control (80%). The disease severity in

treatments with MICROTECH-1(NL) or SH-BV1 for both of Ba kich plants in nursery and pot soils was 15.56 and 19.33%, respectively. On the contrary, treatments were single application of either SH-BV1 or MICROTECH-1(NL) for Ba kich in the nursery or field soil; the disease severity was more than 22.22%. The results showed that Ba kich plants in the nursery were healthier than the control; however, since the pathogen population was high in the field soil and if the field soil was not pretreated before planting, the seedlings were severely prone to pathogen attack (Fig. 3). Therefore, soil treatment was the apex priority for effective management of FRRBK.

FP population in roots in treatments SHn + Cp and MIn + Cp was lower compared to control (Cn + Cp); these two treatments decreased FP population density by 48.00 and 52.89%, respectively. FP population in treatments of Cn + SHp, SHn + SHp, Cn + MIp, MIn + MIp, and SHnp + MInp was significantly lower as compared to control (Cn + Cp). In five treatments, the FP population density decreased by 69.63, 80.67, 81.19, 84.52, and 93.26%, respectively. FP population in roots in treatment of Cn + MIp was highly decreased compared with MIn + Cp (81.19% compared with 52.89%). FP population in roots in treatment of Cn + SHp and SHp + Cp showed a similar trend (69.63% compared with 48.00%). These results indicated that nursery and pot application, double application of SH-BV1, and MICROTECH-1(NL) significantly decreased FP population in rhizoplane of Ba kich (Fig. 4).

A significant decrease of FP population was detected in rhizoplane soil in treatments Cn + SHp, SHn + SHp, Cn + MIp, MIn + MIp, and SHnp + MInp as compared to control (Cn + Cp), resulting in 80.37, 83.26, 80.99, 84.71, and 88.01% decrease, respectively. Nursery-only application with either SH-BV1 or MICROTECH-1(NL) also decreased the population of FP in rhizoplane soil resulting in 53.09 and 55.98% decrease compared to control (Cn + Cp) (Fig. 5).

The FP population in the rhizosphere soil also showed a similar trend. FP population in treatments Cn + SHp, SHn + SHp, Cn + MIp, MIn + MIp, and SHnp + MInp was significant lower as compared to control (Cn + Cp). In these five treatments, the FP population decreased by 83.55, 86.84, 87.34, 92.96, and 95.39%, respectively. Meanwhile, FP population in nursery-only treatment of SH-BV1 (SHn + Cn) or MICROTECH-1(NL) (MIn + Cp) was lower as compared to control, but significantly higher from that of pot-only treatments (Cn + SHp and Cn + MIp) or double application with either SH-BV1 or MICROTECH-1(NL) (SHn + SHp, MIn + MIp, and SHnp + MInp) (Fig. 6).

Total fungal count in rhizoplane soils of treatments Cn + SHp, SHn + SHp, Cn + MIp, MIn + MIp, and SHnp + MInp decreased by 57.91, 67.35, 61.87, 72.08, and 79.19%, respectively, as compared to that of control. Total bacterial count in the rhizoplane soils of the above treatments increased by 80.37, 82.99, 77.7, 83.44, and 88.59%, respectively, as compared to that of control.

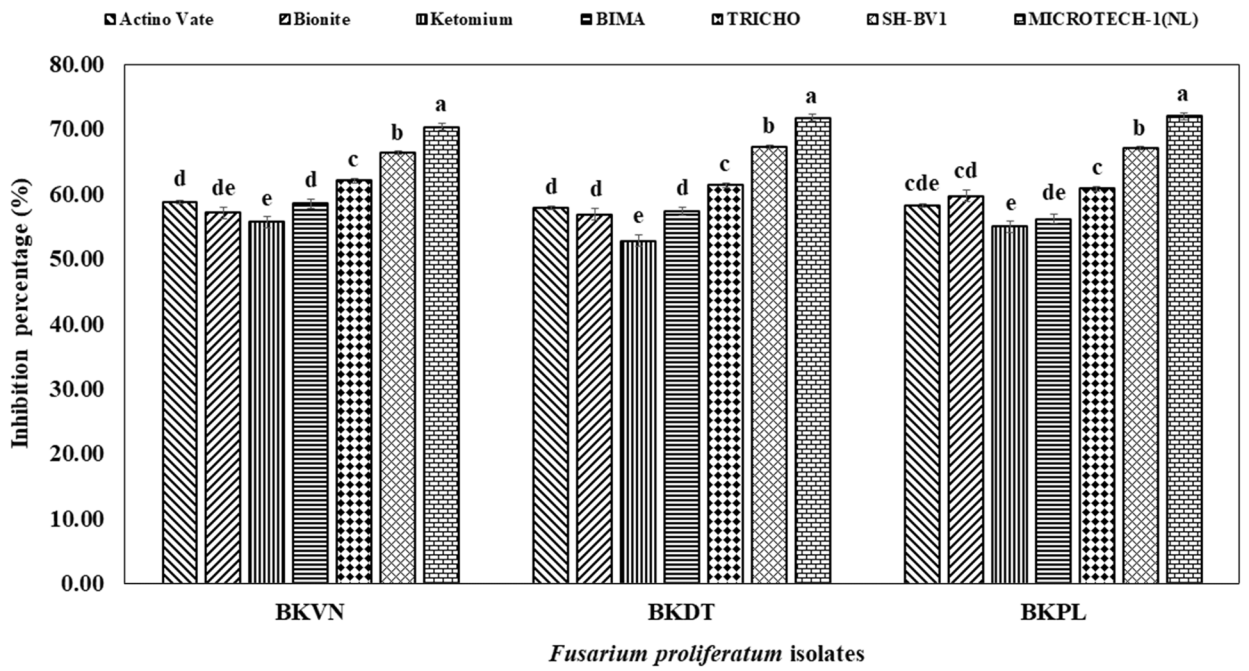

Fig. 1 Inhibition effects of MICROTECH-1(NL) on mycelial growth of *F. proliferatum*. Bars represent ± standard error of the mean (SEM) ($n = 5$). Different letters indicate significant difference by $LSD_{0.05}$

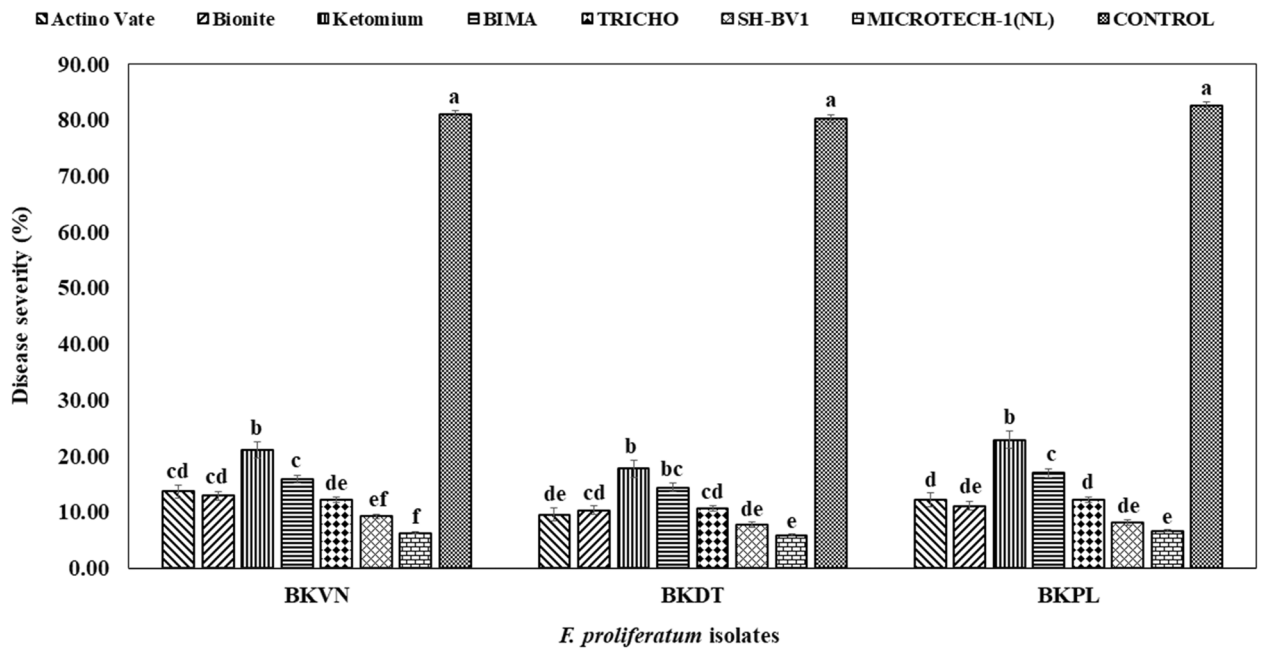

Fig. 2 Effect of the bio-products applied to soil 1 week before *F. proliferatum* inoculation on the disease severity of fusarium root rot of Ba kich. Bars represent ± SEM ($n = 3$). Different letters indicate significant difference by $LSD_{0.05}$

The total actinobacteria count in the rhizoplane soil also increased and resulted in 64.22, 68.85, 65.47, 74.17, and 77.73%, respectively, compared to that of control. In addition, double application of either SH-BV1 or MICROTECH-1(NL) increased total bacteria and actinobacteria count in comparison to nursery-only applications (SHn + Cp and MIn + Cp) (Additional file 3).

The changes in microbial count in the rhizosphere soil showed similar trends, although the values were lower than those in the rhizoplane soils. Total fungal count in the rhizosphere soil of the treatments Cn + SHp, SHn + SHp, Cn + MIp, MIn + MIp, and SHnp + MInp decreased by 58.09, 68.15, 62.79, 73.47, and 80.93%, respectively, compared to that of control. The total bacterial count in the rhizosphere soil of the above treatments increased by 44.61, 73.06, 58.73, 74.40, and 81.42%, respectively, as compared to that of control. The total actinobacteria count in the rhizosphere soil also increased and resulted in 64.22, 61.74, 70.27, 63.05, 74.75, and 87.17%, respectively, as compared to that of control (Additional file 4).

Effect of MICROTECH-1(NL) on disease severity of FRRBK in the field conditions

Double application of SH-BV1 and MICROTECH-1(NL) both in the nursery and in the field significantly decreased disease severity as compared to single nursery application or field application and in comparison to respective controls in Vo Nhai, Dai Tu, and Phu Luong districts. Disease severity in treatments SHnf + MInf, MIn + MIf, SHn + MIf, SHn + SHf, and MIn + SHf were significantly decreased by 90.47, 87.10, 84.16, 79.33, and 78.01%, respectively, as compared to control (Cn + Cp). The disease severity in nursery-only treatments MIn + Cf and SHn + Cf was decreased by 40.32 and 39.15%, respectively, as compared to control. Whereas, those in field-only treatments Cn + MIf and Cn + SHf resulted in 73.02 and 70.09% decrease compared with control, respectively. The decrease in disease severity in Dai Tu and Phu Luong districts showed similar trends. These data indicate that double application of SH-BV1 and MICROTECH-1(NL) in nursery and in the field leads to low disease severity of FRRBK (Fig. 7).

Discussion

Mounting disease pressure in high value crops requires frequent and sometimes injudicious applications of hazardous pesticides, impacting both human and environmental health. Thus, the search for ecologically safer, cost effective, and sustainable alternative for the effective management of fusarium root rot is a top priority in Viet Nam [34, 35].

In the present study, various bio-products were tested for their effectiveness against FP and FRRBK in in vitro, pot, and in the field conditions. Previous reports indicated that different antagonistic microorganism inhibit the growth of *Fusarium* spp. that are the causal pathogens of different diseases [7, 8, 16, 17]. The introduction of MICROTECH-1(NL) into culture medium significantly suppressed the mycelial growth of FP isolates by 70.38–72.04% higher than that from other tested bio-products

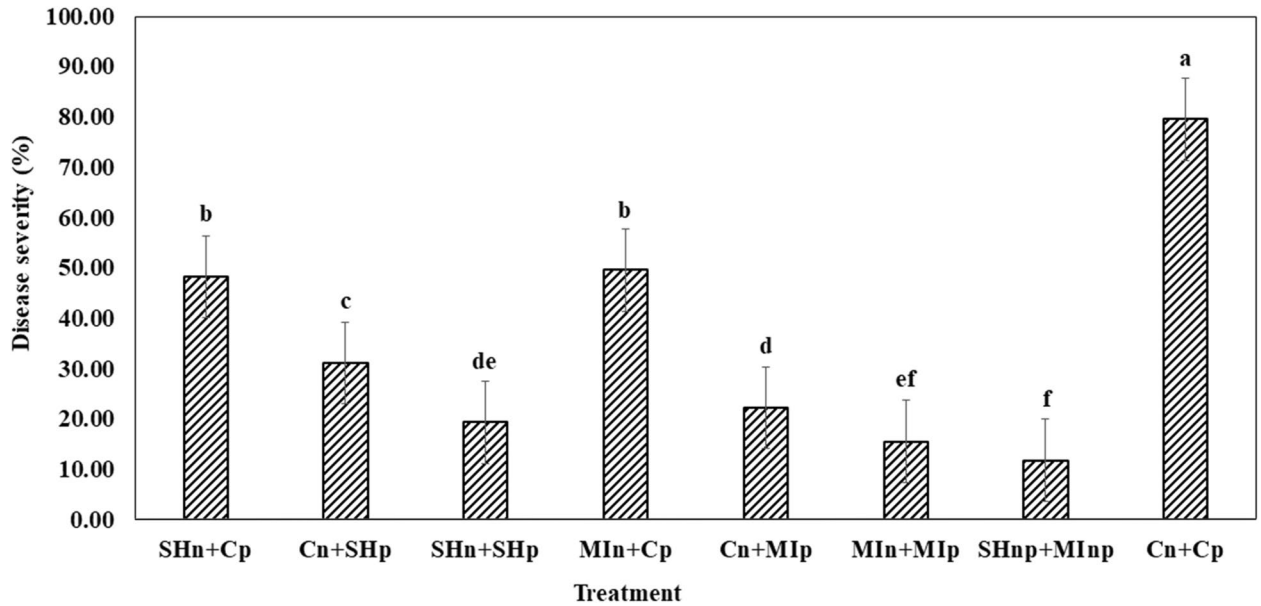

Fig. 3 Effect of MICROTECH-1(NL) on disease severity of FRRBK under nursery and pot conditions. Bars represent ± SEM ($n = 3$). Different letters indicate significant difference by $LSD_{0.05}$

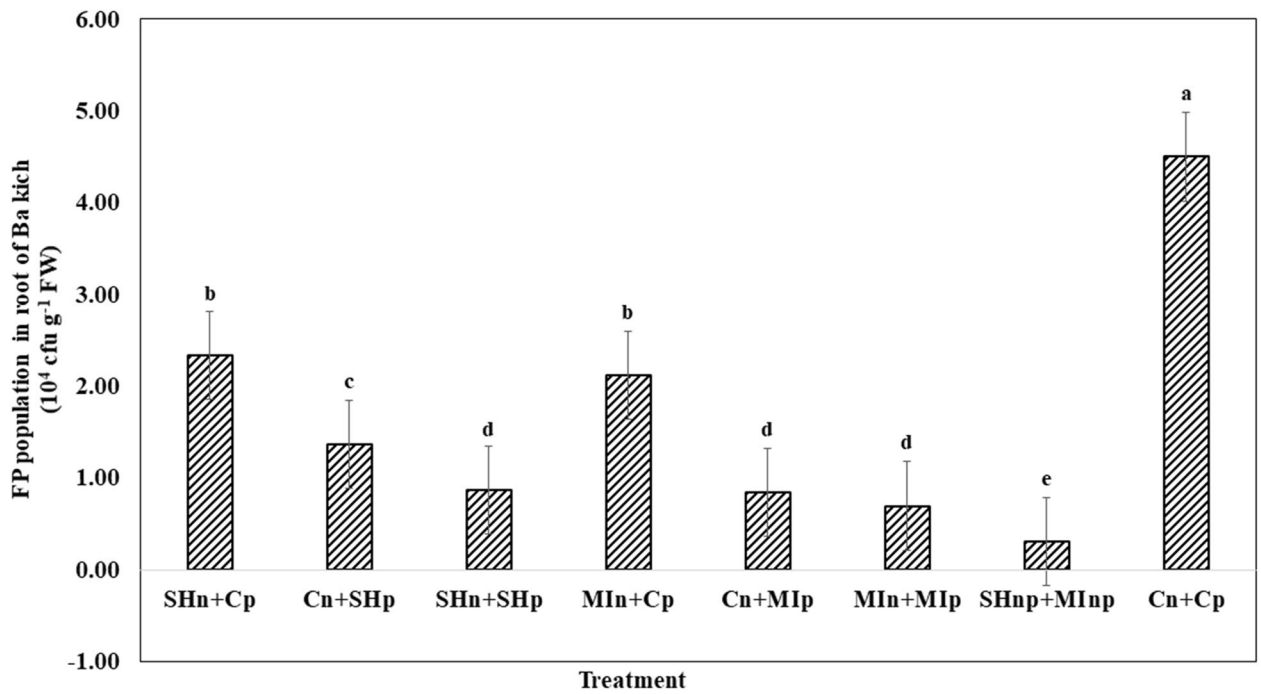

Fig. 4 Effect of different treatments on pathogen population in root of Ba kich. Bars represent ± SEM ($n = 3$). Different letters indicate significant difference by $LSD_{0.05}$

(52.84% for Ketomium to 67.30% for SH-BV1) (Fig. 1). This suggests that the diversity of consortia of antagonistic microorganism species in MICROTECH-1(NL) resulting in higher inhibition percentage compared with that produced by consortia of species in SH-BV1 and other bio-products composed by single species or less diversity of antagonistic microorganisms. One of the biggest challenges of production of bio-products composed from different antagonistic microorganism is the compatibility of all the species. In our case, they were separately

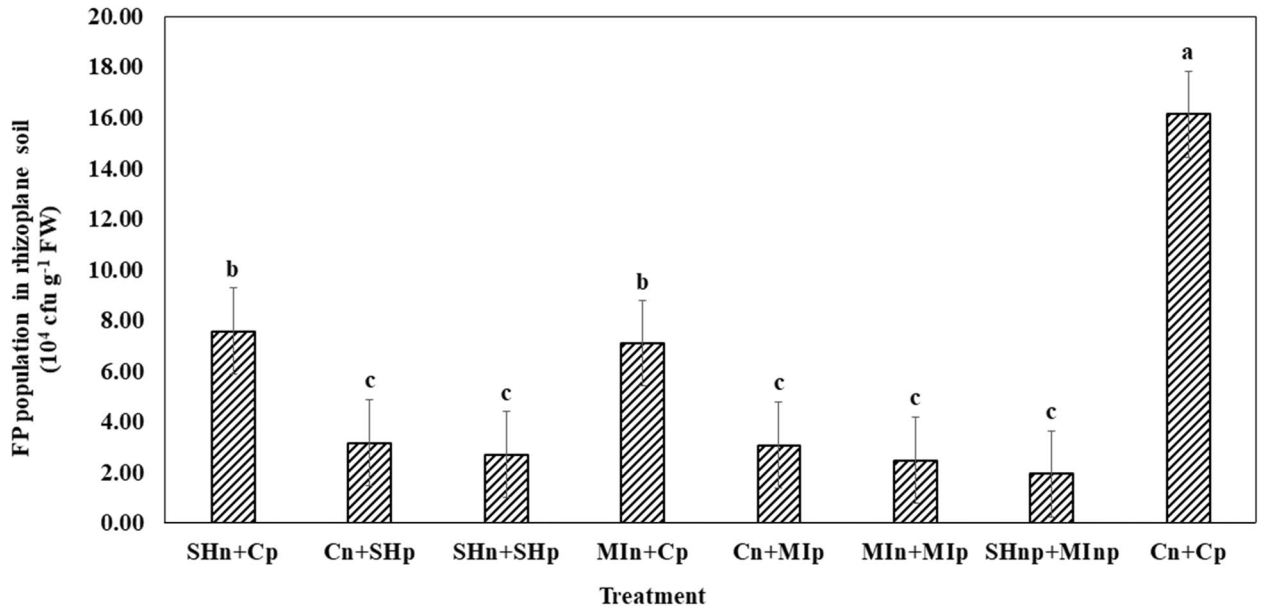

Fig. 5 Effect of MICROTECH-1(NL) on *F. proliferatum* population in rhizoplane soil. Bars represent ± SEM ($n = 3$). Different letters indicate significant difference by $LSD_{0.05}$

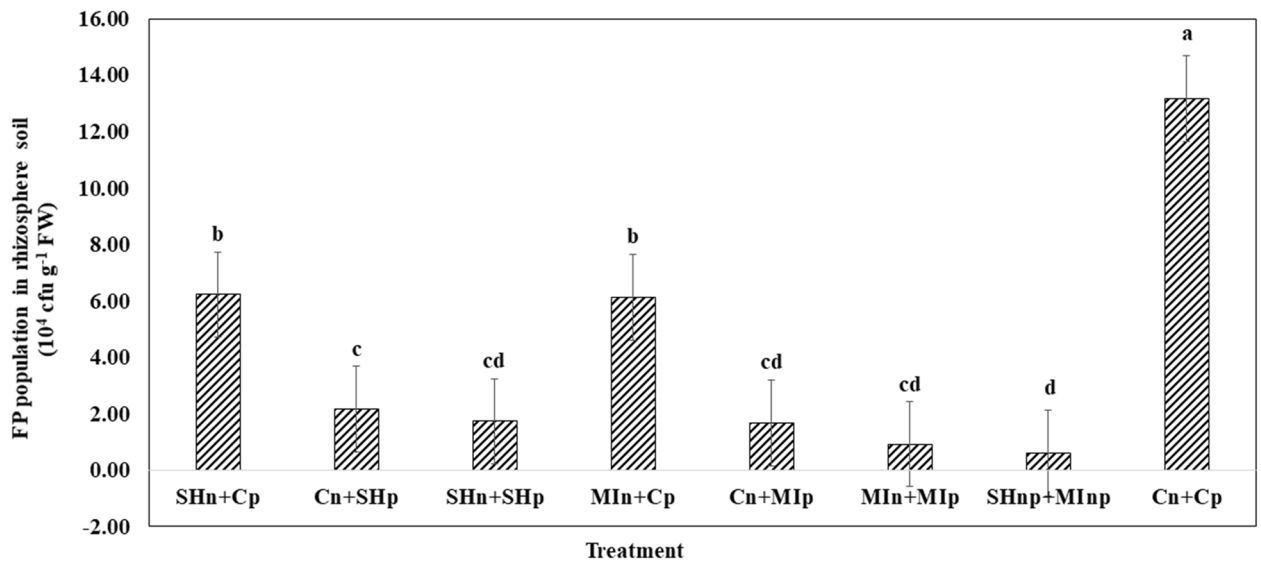

Fig. 6 Effect of MICROTECH-1(NL) on *F. proliferatum* population in rhizosphere soil. Bars represent ± SEM ($n = 3$). Different letters indicate significant difference by $LSD_{0.05}$

cultured in different media before composed into an environment with the same ratio. The efficacy of MICROTECH-1(NL) was tested every 2 months after composing until 12 months. The results indicated that its efficacy was unaltered during storage at room temperature (data not shown) suggesting that the consortia of antagonistic microorganisms in MICROTECH-1(NL) are compatible.

Addition of the tested bio-products before or simultaneous inoculation with pathogen significantly reduced the disease severity of FRRBK. The disease severity reduction was greater when bio-products were applied into the soil 1 week before inoculation with FP. The disease severity ranged from 5.92% (for MICROTECH-1(NL) with BKDT isolate) to 22.96% (for Ketomium with

BKPL isolate) as compared to inoculated-untreated control, in which disease severity ranged 80.37 to 82.59%, respectively. Specially, MICROTECH-1(NL) highly suppressed the development of FRRBK resulting up to 92.6% of disease severity reduction as compared to inoculated-untreated control (Fig. 2). Simultaneous application of bio-products and FP spores also significantly reduced the disease severity resulting in 60.3 to 86.2% as compared to inoculated-untreated control. Among them, MICROTECH-1(NL) also showed highest efficacy in comparison with other bio-products (Additional file 1). However, applying these bio-products 1 week after inoculation with pathogen reduced disease severity, but the reduction was not exceeding 46% (Additional file 2), suggesting that the aforementioned bio-products need to be applied into field soil before transplantation. Previous studies showed that pre-treatment of soil with antagonistic microorganisms such as *T. harzianum*, *Pythium oligandrum*, *B. subtilis*, *B. pumilus*, etc., induces systemic responses in host plants [36–39], triggers various defense mechanisms such as accumulation of pathogenesis related (PR) proteins, deposition of structural barriers [40], and accumulation of antimicrobial phenolics [41], and stimulates plant resistance/tolerance [37, 42, 43] that protects plants against soil-borne diseases [44, 45].

In addition, double application of MICROTECH-1(NL) into both the nursery and the pot soils, significantly reduced disease severity (Fig. 3), suppressed FP population in root of Ba kich (Fig. 4), in rhizoplane soil (Fig. 5), and in rhizosphere soil (Fig. 6); increased total bacteria and actinobacteria count in rhizoplane and rhizosphere soils (Additional files 3, 4) and effectively increased the growth of Ba kich compared to single application and non-treatment control (data not shown). Suggesting that MICROTECH-1(NL) has a broad spectrum of effects on FP and FRRBK, improves the antagonistic microorganisms' population in the soil, improves soils quality, suppresses the growth of FP in the rhizoplane and rhizosphere, and protects the Ba kich plant from the pathogen attack. Similar results were obtained in the field condition, double use of MICROTECH-1(NL) into both nursery and field soils highly reduced disease severity of FRRBK in comparison to single use in nursery or field soil (Fig. 7). These results are relatively consistent with previous studies showing the effect of antagonistic microorganisms in reducing the incidence of different *Fusarium* diseases [5, 16–19, 30, 46–49]. They act against pathogens by synthesizing antimicrobial secondary metabolites [13, 15, 20, 26, 28, 31], induce systemic resistant in plants [7, 8, 16, 17, 26, 27], inhibit the growth of pathogens, and suppress disease incidence and severity [5, 8–11, 13, 14, 16, 18, 19, 21–24, 30, 32].

The growth of Ba kich plants in pot and in the field conditions on application of MICROTECH-1(NL) was higher than that of untreated control (data not shown). This suggests that certain antagonistic strains of MICROTECH-1(NL) colonize the soil, root surfaces of the plants to the same extent as the phytopathogens [50], or secrete

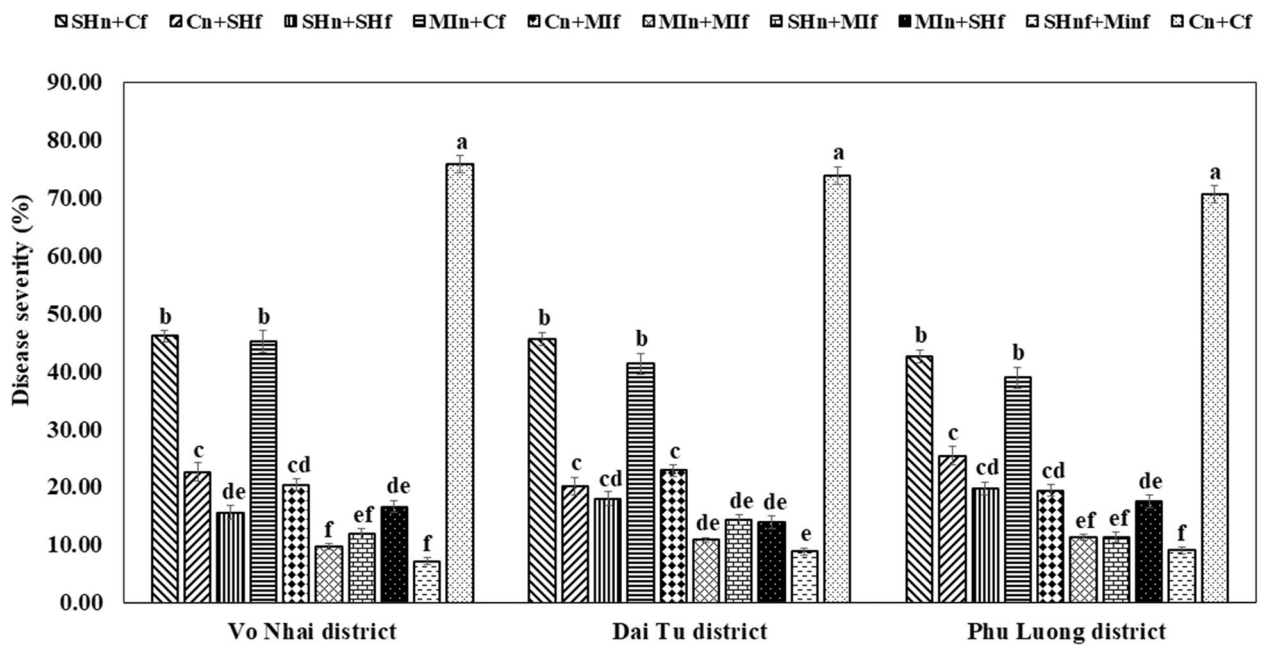

Fig. 7 Effect of MICROTECH-1(NL) on disease severity of FRRBK under the field condition. Bars represent ± SEM ($n = 3$). Different letters indicate significant difference by $LSD_{0.05}$

considerable amounts of biologically active substances that promote root growth, improve nutrient uptake by the plants [31], and increase plant quality [2, 25].

Conclusion

The results in this study demonstrated that MICROTECH-1(NL) containing a consortium of compatible agriculturally important microorganisms with varied biological functions proved as an effective tool for sustainable management of fusarium root rot of Ba kich. For Ba kich plants in the nursery, spraying MICROTECH-1(NL) (1%) 2–3 times per year and in field conditions, pre-treatment of field soils with MICROTECH-1(NL) (1%) 1–2 weeks before planting; and after planting in the field, applying of MICROTECH-1(NL) (1%) 2–3 times per year are highly recommended. Though determination of the precise mode of action of MICROTECH-1(NL) is still required.

Supplementary information

Supplementary information accompanies this paper at https://doi.org/10.1186/s40538-019-0168-x.

Additional file 1. Effect of application of bio-products at the same time with *F. proliferatum* on the disease severity of fusarium root rot of Ba kich.

Additional file 2. Effect of application of bio-products 1 week after inoculation with *F. proliferatum* on the disease severity of fusarium root rot of Ba kich.

Additional file 3. Effects of application of MICROTECH-1$^{(NL)}$ on microorganism density in rhizoplane soil (cfu g^{-1} soil).

Additional file 4. Effects of application of MICTROTECH-1$^{(NL)}$ on microorganism density in rhizosphere soil (cfu g^{-1} soil).

Abbreviations

FRRBK: fusarium root rot of Ba kich:; FP: *Fusarium proliferatum*; LSD: least significant difference.

Acknowledgements

The authors are grateful to the Ministry of Education and Training of Viet Nam for providing financial support through Grant Number: B2018-TNA-59.

Authors' contributions

PVT and TXH conceived the idea, and planned the experiments. DTN, NCH, NVH, and HTBT conducted the experiments, analyzed the data, and prepared the draft of the manuscript. PVT, TXH, and CK revised and edited the manuscript. All authors read and approved the final manuscript.

Funding

This work was conducted as a part of research project funded by the Ministry of Education and Training of Viet Nam [Grant Number: B2018-TNA-59].

Availability of data and materials

Data will be made available on request.

Ethics approval and consent to participate

Not applicable.

Consent for publication

All authors have provided their consent for submission of the manuscript to CBTA.

Competing interests

The authors declare that they have no competing interests.

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Received: 28 May 2019 Accepted: 24 October 2019
Published online: 15 November 2019

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