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Research article

Efficacy of bio-composted agricultural wastes and biocontrol agents in improving soil fertility and controlling dry root rot in King mandarin (*Citrus nobilis*) orchards

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Abstract

Importance of the work: Intensification of King mandarin cultivation has been threatened by dry root rot (DRR) disease, causing unsustainable growth of this citrus fruit.
Objectives: To evaluate the efficiency of bio-fertilizers (BF) in combination with different bio-control agents on improving soil properties and controlling *DRR disease* in King mandarin orchards.
Materials and Methods: Rice straw waste material was composted with isolated fungi from King mandarin orchards and rice fields. Five treatments were conducted in a complete randomized design: 1): common farmer practice (the control); 2): recommended inorganic fertilizers (RIF); 3): RIF plus BF inoculated with *Trichoderma asperellum* (BF1); 4): RIF plus BF inoculated with *Gongronella butleri* (BF2); and 5): RIF plus BF inoculated with *Trichoderma* sp. from a commercial source (BF3).
Results: BF amendment led to significant increases in soil organic matter, soil pH, labile nitrogen, available phosphorus and exchangeable potassium compared to the control with its high dose of inorganic fertilizers. There was a significantly lower density of *Fusarium solani* and DRR suppression (40–82.5%) as result of BF amendment, with BF plus isolated *Trichoderma* and *Gongronella butleri* being the most effective treatment. Consequently, in the second year harvest, fruit yield increased by about 3.5 times that of the control treatments ($p < 0.05$).
Main finding: Using rice straw as waste material to make bio-compost with isolated strains of native fungi could be an optimal practice for improving soil fertility and soil suppression to reduce DRR and enhance fruit yield, promoting an eco-friendly system in King mandarin orchards.

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Introduction

In recent years, King mandarin orchards have been damaged by dry root rot (DRR) disease which causes severe fruit yield losses in the Mekong Delta (Pham, 2004). DDR of citrus is caused by *Fusarium solani* and is one of the most serious diseases attacking mandarin trees, reportedly attacking all citrus varieties (Elgawad et al., 2010). *F. solani* induces toe syndrome of root rot on citrus; initially DRR is confined to the crown and scaffold roots and then to the second, feeder and fibrous roots that are associated with gradual decline of the canopy, leaf curl (witting), defoliation and dieback, with the fibrous roots turning soft and appearing to be water soaked as the their cortex is easily sloughed off by hand (El-Mohamedy et al., 2012; Kurt et al., 2020)

Consequently, farmers have been applying more inorganic fertilizers and chemical fungicides (Nguyen et al., 2018; Chau and Vo, 2020). Old raised beds constructed orchards could be the reason for several adverse effects associated with soil physical, chemical and biological properties. For example, soil degradation was identified in old, raised beds (aged 30 yr) of fruit orchards based on soil compaction, low soil organic matter, and low values for cation exchange capacity and base saturation percentage compared to younger raised beds (Guong et al., 2010). Low soil organic matter, and the imbalanced supply of soil nutrients were found in aging raised beds of orange orchards in the Mekong delta, (Quang and Guong, 2011). The infertile soil conditions can enhance the development of soil pathogenic microorganisms such as serious DRR caused by *Fusarium solani* in King mandarin orchards (Mazin et al., 2016; Ezrari et al., 2022). Amendment using suitable compost can improve soil quality and the microbial community because soil pH and soil organic carbon have been identified the controlling factors of changes in the microbial community, with the application of organic fertilizer resulting in increasing the bacterial community and lessening the extent of the fungal community and suppressing the abundance of *Fusarium* (Chen et al., 2020). In another study, adding organic materials improved soil physico-chemical properties (pH, alkaline nitrogen, available phosphorus, available potassium and organic matter) and significantly reduced the abundance of *Phytophthora nicotianae* and *Fusarium* spp., (Luo et al., 2024). Inoculation of beneficial microorganisms can support nutrient uptake, against soil borne diseases (Ahmad et al., 2018). Biological control of a fungal pathogen is preferred as a form of eco-friendly management for sustainable ecosystem. An important component of the soil microbial population in the soil environment is a rich community of diverse fungal species, such as in the *Trichoderma* genus that have been identified as effective bio-control agents for antagonistic impact on disease-causing fungi (Druzhinina et al., 2011; Adnan et al., 2019; Asad, 2022). The controlling mechanisms of *Trichoderma* against pathogens include antibiosis, mycoparasitism, resistance induction, lytic enzymes and competition for space and nutrients. Therefore, the use *Trichoderma* spp. is a promising strategy due to their interactions with plants and promoting growth and resistance to pathogenic fungus (Gomes et al., 2015; Silva et al., 2019). Information remains limited on amendments of bio-compost with effective microorganisms for improving soil properties and controlling DRR in citrus orchards in the Mekong Delta. Therefore, the objective of the current study was to evaluate the effect of bio-compost from rice straw inoculated with native fungi on improving soil nutrients and the suppression of DRR in King mandarin orchards.

Materials and Methods

Site description and field experiment

The experiment was carried out in Vinh Long province, Vietnam, where large areas of King mandarin orchards have been infected by RRD. The study investigated King mandarin plants aged 3 yr at the commencement of the fruiting stage. The raised beds of these orchard had been constructed 22 yr ago on Endo-Protho-Thionic Gleysols (IUSS Working Group WRB, 2022)). The initial soil properties were characterized by: soil bulk density of 1.25; soil pH of 5.43; organic matter (%C) of 1.78; total nitrogen of 0.09%; available nitrogen of 38.76 mg/kg; available phosphorus of 9.45 mg/kg; exchangeable potassium of 0.05 cmol/kg; cation exchange capacity (CEC) of 15.58 cmol/kg; and base saturation percentage of 37.16 %.

Waste material from rice straw was composted using three different biocontrol agents (BF1–BF3): BF1 was rice straw compost inoculated with *Trichoderma asperellum,* a native fungus isolated from the rhizosphere of King mandarin orchards; BF2 was rice straw compost inoculated with *Gongronella butleri,* a fungus isolated from paddy soils; and BF3 was rice straw compost inoculated with

Trichoderma sp. from a commercial product. Before composting, the rice straw was adjusted to a relative humidity of 70%, then covered by a sealed plastic bag The duration of composting was 75 d; at days 15 , 30, 45 and 60, the compost was mixed well and the relative humidity was checked and adjusted to 70%. Then, three different biocontrol agents were inoculated with a final density of 1×10^6 colony-forming units (CFU)/g. The fungal density was determined using the spread-plate method. For each incubation experiment, 1 g of sample was placed in 20 mL of sterile distilled water and shaken on a horizontal shaker at 130 revolutions per minute for 1 hr. The samples were used for serial dilution from 10^{-3} to 10^{-5} and spread-plated on *Trichoderma*-selective medium with the antibacterials streptomycin and chloramphenicol.

The composted rice straw contained 46.67% organic carbon, 1.74% total nitrogen, 0.22% total phosphorus (P_2O_5) and 2.16% total potassium.

The experiment was carried out in a completely randomized design using five treatments and four replicates. BF1, BF2 and BF3 were amended with 20 t/ha and applied one time at the beginning of the crop. Each replicate consisted of two plants. The treatments were: T1, common farmer practice (control 1); T2, recommended inorganic fertilizer (control 2); T3, BF1 plus T2; T4, BF2 plus T2; and T5, BF3 plus T2. The inorganic fertilizer according to common farmer practice (control 1) was applied per plant as 360 g N, 195 g P_2O_5 and 55 g K_2O. The recommended inorganic fertilizer (T2) was added per plant as 250 g N, 50 g P_2O_5 and 250 g K_2O. The fertilization schedule was divided into four times per crop. Initially, a basal application of 2.0 t/ha of $CaCO_3$ was applied for all treatments. Then, at 1 moth of the harvest stage, 25% of the total nitrogen, 50% of total potassium and 100% of total phosphate were applied. Next, when flowering, 25% of total nitrogen was applied. Finally, at 1 month after fruit set, 25% of total nitrogen and 50% of total potassium were applied.

Fusarium solani density measurement

Soil samples were collected from the King mandarin plant rhizosphere at ratio of 1:10 using sodium pyrophosphate 2% (weight per volume) at room temperature for 30 min. Serial dilutions from the extracted solution were prepared at concentrations from 10^{-1} to 10^{-5} for inoculating living fungi onto agar plates with the desired medium. Counts of living *Fusarium solani* were performed by screening on potato dextrose agar medium with the antibacterials streptomycin and chloramphenicol, in combination with examining the shape and spores of the fungi under a microscope to determine the genus and species (El-Mohamedy et al., 2012).

Dry root rot disease rating after harvesting time on second crop

DRR evaluation followed the method of Jones (1998) using Equation 1:

$$\text{Disease rate (\%)} = (\text{Number of diseased plants} / \text{Total number of plants}) \times 100\% \qquad (1)$$

Diseased plants were identified based on the symptom of yellow leaves on two plants of each replicate.

Soil analysis

Soil samples were collected form 0–20 cm depth in the profile. The pH of air-dried soil samples was measured in a suspension of 1:2.5 (soil-to-water). Soil organic carbon (SOC) was determined based on wet oxidation using the Walkley Black organic carbon method (Nelson and Sommers, 1982). The 2 M KCl method suggested by Curtin and Wen (1999) was chosen to measure labile soil organic nitrogen. Available phosphorus was extracted using the Bray 2 method (Bray and Kurtz, 1945). Exchangeable potassium was measured after extraction of the $BaCl_2$ unbuffered solution, according to the method of Houba et al. (1989). These soil characteristics were collected and analyzed after harvesting the second crop.

Statistical analysis

Data were analyzed using one-way analysis of variance with the Minitab 16.1 software (Minitab Inc.; USA). Differences between mean values of treatments were compared using Duncan's multiple range test, with significance determined at $p < 0.05$.

Results and Discussion

Effect of rice straw bio-fertilizers on soil quality

Amendment of rice straw using the three bio-fertilizers on the King mandarin orchard resulted in significantly improving the soil pH to the near-neutral range (Ph 6.0–6.6), which was beneficial to plants (Fig. 1) compared to control treatment of pH 5.4. This result supported other studies that reported organic fertilization led to improvements in some major soil properties such as pH, soil organic C, nutrient holding and buffering capacity (Laurent et al., 2020; Kumari et al., 2024). In addition, Fagerai (2012) reported that the applications of vermi-compost or bio-composts resulted in the beneficial maintenance of soil health (physical, chemical and microbiological properties) compared to inorganic fertilization alone. Furthermore, increasing the soil pH increases the amounts of dissolved nutrients and soil microbial activity, while at low soil pH, the elements N, P, K and Ca become less available (Fagerai, 2012). Nutrient deficiencies due to changes in the soil pH can lead to a weaker host plant with less ability to fight against soil pathogens (Ortel et al., 2024).

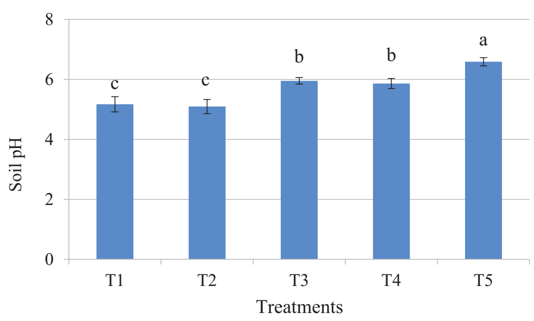

Fig. 1 Effect on soil pH of different soil compost application treatments, where T1 = common farmer practice, T2 = recommended inorganic fertilizer; T3 = T2 + BF1 (compost inoculated with isolated native fungus *Trichoderma asperellum*), T4 = T2 + BF2 (compost inoculated with *Gongronella butleri*), T5 = T2 + BF3 (inoculated with *Trichoderma* sp. from commercial product). Bars represent mean values and error bars represent ±SD. Different lowercase letters above bars indicate significant ($p < 0.05$) differences among treatments.

The data presented in Fig. 2A indicate that BF in combination with inorganic fertilizers led to dramatically increased soil organic matter content compared to the control treatments. The application of BF enhanced the pool of soil organic carbon, resulting in more favorable physical, chemical, and biological properties in the soil-plant system. The positive effect on soil quality and soil microbial activity was augmented by releasing plant nutrients upon mineralization, increasing the cation exchange capacity, retaining nutrients that later were available to plants, the positive effect on soil structure and soil aggregate stability and by increasing the evels of total soil microbial and enzyme activity (Diacono and Montemurro, 2011; Sarker et al., 2018; Kumari et al., 2024). Increasing soil organic matter and its labile fraction as particulate organic matter are the important factors affecting nitrogen-dynamics which play a key role in nitrogen mineralization and nitrogen availability to plants (Villarino et al., 2023). Therefore, the BF amendment to the soil in the King mandarin orchard supported optimal management practices for a sustainable soil-plant system.

The application of BF markedly enhanced soil organic labile nitrogen, elevating its concentration from 15 mg/kg in the control to a peak of 36 mg/kg (Fig. 2B). The potential mineralizable organic nitrogen pool for organic amendment soils is important for evaluation of the soil nitrogen supplying capacity (Abagandura et al., 2023). The labile nitrogen fraction can be considered as readily mineralized by soil microorganisms, which contribute to the soil nitrogen supply to plants. The amount of nitrogen requirement of plants is satisfied from the mineralization of soil organic labile fraction, which slowly releases plant nutrients, with lessened leaching to groundwater (Fageria, 2012; Calleja-Cervantes et al., 2015; Thomas et al., 2016).

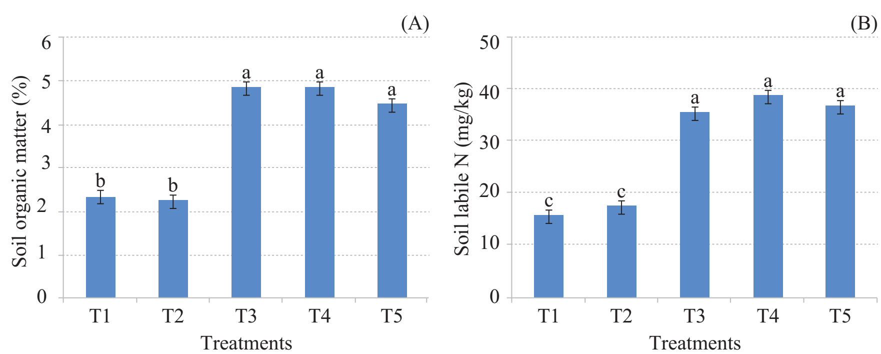

Fig. 2 Effect of different soil compost application treatments on: (A) soil organic matter; (B) soil labile nitrogen, where T1 = common farmer practice, T2 = recommended inorganic fertilizer; T3 = T2 + BF1 (compost inoculated with isolated native fungus *Trichoderma asperellum*), T4 = T2 + BF2 (compost inoculated with *Gongronella butleri*), T5 = T2 + BF3 (inoculated with *Trichoderma* sp. from commercial product). Bars represent mean values and error bars represent ±SD. Different lowercase letters above bars indicate significant ($p < 0.05$) differences among treatments.

BF amendment led to a substantial increase in available phosphorus concentration, reaching 52–80 mg P/kg, compared to 18 mg P/kg in the inorganic fertilizer treatment (Fig. 3A). According to Obreza et al. (2008), about 65 mg P/kg available phosphorus is considered sufficient for citrus growth, while Srivastava and Singh (2006) reported the optimum value for available phosphorus in a citrus soil orchard was 21.2–45.6 mg P/kg available phosphorus. Based on the results in the current study from using BF, the available phosphorus in the soil was in the optimum range for plant growth. Organic amendment supports the build-up of available phosphorus of the soil through the decomposition of organic phosphorus compounds and the death of microbial cells (Fageria, 2012).

The application of the BF played a significant role in almost doubling the exchangeable potassium in the soil compared to the control treatment with solely inorganic fertilization (Fig. 3B). Potassium is one of the most important nutrients for improving fruit yield and fruit quality as sufficient potassium enhances the resistance capacity to soil-borne disease in fruit orchards (Bailey and Lazarovits, 2003). The impaired physiological processes due to a potassium deficiency in plants can be easily infected by soil pathogens; thus, an adequate supply of potassium may lead to reductions in fungal and bacterial diseases (Ortel et al., 2024). Therefore, in the current study, BF amendment resulting in improved potassium availability in the soil could be one of the factors controlling DRR in King mandarin orchards.

In general, the results in the current study supported those from other studies on the effect of biofertilizer containing biocontrol agents to promote soil qualities in different crop productions. For example, Ye et al. (2020) confirmed that bio-fertilizer containing the *Trichoderma harzianum* strain T7 significantly enriched soil fertility, as the soil organic matter increased by 55–75%, total N by 25–36%, total P by 116–123% and total K by 99%–100% after the fertilization over four seasons. Similarly, *G. butleri* was reported to have the highest cellulase and protease activities among the microorganism studied for biocomposting ability and significantly accelerated the degradation of the organic wastes (Valsalan et al., 2022). According to Wang et al. (2023), amendment of *G. butleri* in biofertilizer helped to improve soil aggregate stability and soil organic matter by 20.72% compared to the control. Fu et al. (2020) reported that the potential mechanisms allowing *T. asperellum* to maintain a better soil microbiome for plant growth in saline-alkali soils on a cyclic basis could be explained by plant growth perhaps being improved initially by enhanced plant root growth, followed by the better root growth influencing soil microbes through the supply of more root exudates or rhizodeposition, with the soil microbes, in turn, altering plant performance through higher diverse microflora and more frequent interactions, resulting in more available nutrients. More bioavailable nutrients cause better root growth, facilitating more microbial colonization in the rhizosphere, finally promoting greater nutrient uptake by the inoculated plants (Fu et al., 2020).

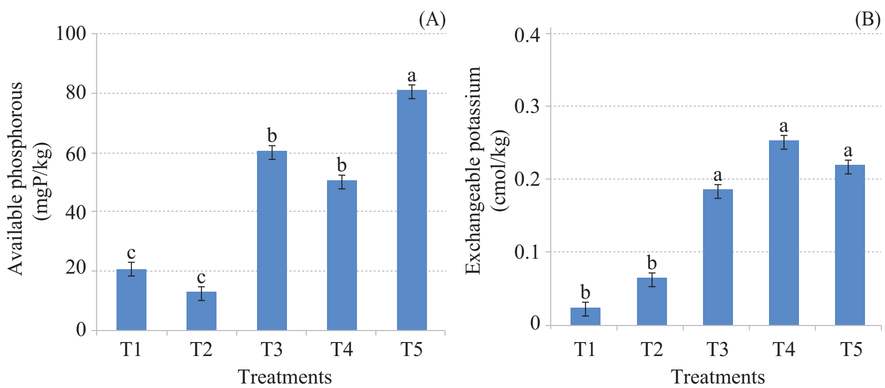

Fig. 3 Effect of different soil compost application treatments on: (A) available soil phosphorus; (B) exchangeable potassium, where T1 = common farmer practice, T2 = recommended inorganic fertilizer; T3 = T2 + BF1 (compost inoculated with isolated native fungus *Trichoderma asperellum*), T4 = T2 + BF2 (compost inoculated with *Gongronella butleri*), T5 = T2 + BF3 (inoculated with *Trichoderma* sp. from commercial product) Bars represent mean values and error bars represent ±SD. Different lowercase letters above bars indicate significant ($p < 0.05$) differences among treatments.

Effect of rice straw bio-fertilizers on reducing Fusarium spp. density in soil and supressing dry root rot disease

Results presented in Fig. 4 reveal a marked suppression of *Fusarium* spp. density by BF amendments, reducing populations by approximately 50% (1.3–3.8 × 10^4 CFU/g) compared to the notably higher levels observed in inorganic fertilizer application (7.0–7.5 × 10^4 CFU/g; $p < 0.05$). Ezrari et al. (2022) noted that DRR is caused by a complex of *Fusarium* spp., of which *Fusarium solani* was the most predominant. In the current study, the considerable reduction in *Fusarium* spp. density was a reasonable explanation for the effective control of DRR in King mandarin plants (Fig. 5). The level of DRR disease in the BF-amended treatments was significantly reduced by about 40–82% compared to the common farmer practice treatment, with BF1 inoculation with isolated native fungi (*Trichoderma* and *Gongronella butleri*) was the most effective, compared to BF with commercial product (BF3 treatment). Furthermore, the isolated native *Trichoderma asperellum* (BF1) was significantly better at suppressing *Fusarium* spp. than the commercial *Trichoderma* sp. (BF3). This could be explained by BF1 being a native fungus isolated from the rhizosphere in King mandarin orchards. In addition, *Gongronella butleri* (BF2) had a positive effect on *Fusarium* spp. suppression but this was not significantly different from BF1 and BF3. The current results were in agreement with another study that bio-organic amendments containing microbial antagonists improve soil properties—such as organic matter content, nutrient supply, and buffering capacity—thereby enhancing microbial activity, maintaining soil health, and suppressing disease, and have been recognized as a promising alternative to chemical control (De Sousa et al., 2023). Furthermore, organic fertilization has been reported to result in setting up microbial resistance in the rhizosphere to suppress fungal pathogen development (Garbeva et al. 2004; Noble and Conventry, 2005; Chen et al., 2020).

Role of rice straw bio-fertilizers on improving fruit yield

In the first harvest, fruit yield significantly increased from 5 t/ha in the inorganic fertilizer treatments to 15 t/ha in the BF-amended treatments (data not shown). In the second year of BF application, the fruit yield was approximately 35 t/ha or 3.5 time higher than the control treatments (Fig. 6). All fungi (*Trichoderma asperellum, Gongronella butleri* and *Trichoderma sp.*) from the commercial product compost inoculation produced the same effect of fruit yield augmentation.

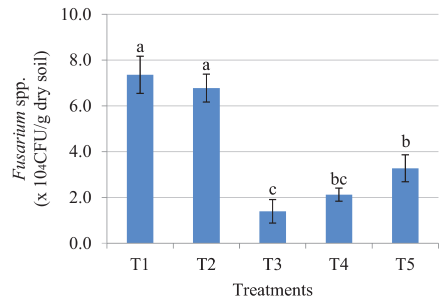

Fig. 4 Effect of different soil compost amendment treatments on suppressing *Fusarium* spp. in King mandarin orchard, where T1 = common farmer practice, T2 = recommended inorganic fertilizer; T3 = T2 + BF1 (compost inoculated with isolated native fungus *Trichoderma asperellum*), T4 = T2 + BF2 (compost inoculated with *Gongronella butleri*), T5 = T2 + BF3 (inoculated with *Trichoderma* sp. from commercial product). Bars represent mean values and error bars represent ±SD. Different lowercase letters above bars indicate significant ($p < 0.05$) differences among treatments.

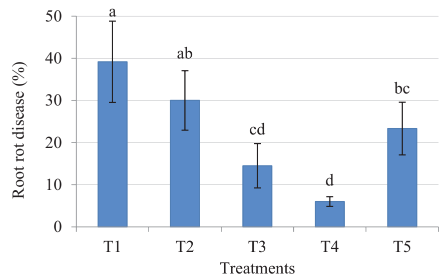

Fig. 5 Effect of different soil compost amendment treatments on suppressing root rot disease in King mandarin orchard, where T1 = common farmer practice, T2 = recommended inorganic fertilizer; T3 = T2 + BF1 (compost inoculated with isolated native fungus *Trichoderma asperellum*), T4 = T2 + BF2 (compost inoculated with *Gongronella butleri*), T5 = T2 + BF3 (inoculated with *Trichoderma* sp. from commercial product). Bars represent mean values and error bars represent ±SD. Different lowercase letters above bars indicate significant ($p < 0.05$) differences among treatments.

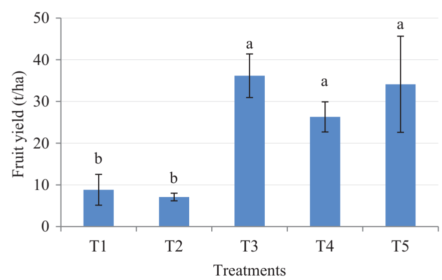

Fig 6. Effect of different soil compost amendment treatments on improving fruit yield after two seasons in King mandarin orchard, where T1 = common farmer practice, T2 = recommended inorganic fertilizer; T3 = T2 + BF1 (compost inoculated with isolated native fungus *Trichoderma asperellum*), T4 = T2 + BF2 (compost inoculated with *Gongronella butleri*), T5 = T2 + BF3 (inoculated with *Trichoderma* sp. from commercial product). Bars represent mean values and error bars represent ±SD. Different lowercase letters above bars indicate significant ($p < 0.05$) differences among treatments.

The addition of 20 tons/ha of BF combined with a reduced dose of recommended inorganic fertilizers in the King mandarin orchard enhanced soil nutrient supply by increasing organic carbon content, boosting labile nitrogen (readily decomposable for nitrogen release), and raising levels of available phosphorus and exchangeable potassium . Guong et al. (2016) reported that amendment with sugarcane filter cake compost inoculated with *Trichoderma* spp. led to significant increases in soil organic matter content, available nitrogen and phosphorus, cation exchange capacity, base saturation percentage, soil respiration and soil aggregate stability and significantly reduced soil compaction on old raised beds in citrus orchards in the Mekong delta . In the current study, in addition to the positive effect of improved soil quality, there was an important benefit to biological control through the reduction of the level of *Fusarium solani* in the soil and significant suppression of DRR that contributed greatly to fruit yield enhancement. These current results supported other studies that reporting that soil amended with compost in combination with native microbial inoculant promoted beneficial microbial communities which induced disease suppression (Giuliano et al., 2016; Adnan el al., 2019; Vida et al., 2020; Ugo, 2020, 2022; Luo et al., 2024).

Conclusions

Fruit yield loss was reduced by amending bio-fertilizers with biological control agents (fungi consisting of *Trichoderma asperellum*, isolated from the citrus rhizosphere and *Gongronella butleri* isolated from a rice field), in combination with low doses of inorganic fertilizers. These effective management practice induced changes in the physicochemical and biological soil quality, which suppressed soil-borne pathogens (*Fusarium* spp.) causing DRR disease. Therefore, bio-fertilizer from rice straw waste material, in combination with biological control agents, should be considered as a sustainable and environment-friendly approach to enhancing nutrient uptake and the production of King mandarin plant under low-input conditions. The application of such bio-systems should further reduce the use of traditional fertilizers and pesticides, thereby increasing the quality of fruit and reducing environmental pollution.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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