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Role of some biotic and abiotic agents in controlling periwinkle root rot disease

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Abstract

This study focuses on identifying and managing root rot disease, a significant threat to annual vinca (*Catharanthus roseus*), caused by *Rhizoctonia solani* and *Fusarium* sp. The most frequently isolated fungi were *Rhizoctonia* spp. (46.15%) and *Fusarium* spp. (38.46%). Various fungal and bacterial bio-agents were evaluated for their effectiveness in inhibiting the growth of these pathogens. *Trichoderma* spp. and mycorrhizal fungi showed the highest inhibition against *F. proliferatum* (80.7%) and *R. solani* 1 (68.8%). Among the bacterial bio-agents, *Bacillus subtilis* exhibited the highest inhibition against *R. solani* 2 (66.6%) and *F. proliferatum* (51.8%). In vivo experiments showed that *Trichoderma* spp. completely inhibited root rot pathogens, except for *Trichoderma album* against *F. solani.* Mycorrhizal fungi reduced disease in-cidence (25%) and severity (40%). *Pseudomonas fluorescens* showed good results, except for *R. solani* 1 (25% disease incidence and 40% severity). The application of safe materials such as silicate, nano silicate, and indole butyric acid prevented infection by root rot pathogens in vivo and improved plant growth parameters, including fresh and dry weight of shoots and roots. These findings contribute insights into identifying and managing root rot disease in *C. roseus*, emphasizing the potential of biological control agents and safe materials as effective strategies.

Keywords: Periwinkle root rot disease, *Catharanthus roseus*, biotic agents, abiotic agents, mycorrhizal fungi; safe materials.

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1. Introduction

Vinca *Catharanthus roseus* (L.) G. Don, commonly known as periwinkle, is a popular ornamental flowering plant from the family Apocynaceae. It is widely cultivated for its attractive blooms and is commonly used in outdoor settings. The plant has gained popularity as an ornamental species in tropical and subtropical regions across the globe [1]. Disease issues pose significant challenges and can lead to reduced yield and quality of plant produce, resulting in financial losses. Vinca is highly vulnerable to *R. solani*, and *Fusarium* spp. are known to be the major soil-borne pathogens causing significant economic damage in bedding plants, including annual vinca [2]. Damping-off diseases, blight, canker, leaf spot, and root rot are common ailments that affect *Catharanthus roseus*. *Rhizoctonia solani* is responsible for stem, crown, and root rot, while *Fusarium* cause root rot disease also causes substantial losses and plant death in periwinkle [3]. Recently, there has been a global shift towards adopting eco-friendly methods to safeguard crops against plant pathogens and pests [4]. Among these methods, biological control and fertility management have proven to be effective approaches for disease control [5]. Not only are they safer and more cost-effective, but also offer environmental benefits. Biological control, therefore, stands as a suitable option that should be pursued. Utilizing soil microorganisms, particularly beneficial rhizosphere fungi that exhibit antagonistic properties against soil-borne pathogens, is a valuable strategy for both biological control and plant fertility management [6]. Biological agents, such as rhizobacteria, contribute to plant health by releasing secondary metabolites and promoting growth through the production of auxin hormone. Rhizobacteria, are known as Plant Growth Promoting Rhizobacteria (PGPR) [7]. Several microbial species, including various *Trichoderma* species, *Pseudomonas fluorescens*, and *Bacillus subtilis*, have demonstrated effective control over plant pathogens [8]. The establishment of a sufficient population on the target site is a crucial factor in utilizing any bio-control agent. Various *Trichoderma* spp. produce microbe-associated molecular patterns (MAMPs) such as xylanases, swollenins, peptaibols, and cerato-platanins, which can elicit plant defense responses [9]. They are capable of controlling pathogens like *Fusarium* sp. through mechanisms in growth chambers and via mycoparasitism. *Trichoderma* spp. also secrete extracellular enzymes that cause the lysis of pathogenic hyphae [10]. *Bacillus* sp. exhibits the ability to inhibit pathogen growth through various compounds such as antibiotics, siderophores, bacteriocins, and extracellular enzymes [11]. It acts as a

growth promoter and shows antagonistic effects against various pathogens both *in vitro* and *in vivo*. *B. subtilis*, possesses multiple mechanisms including promoting plant growth, competition for space and nutrients, pathogen degradation, and stimulating systemic induced resistance (ISR) to enhance disease resistance [12]. *Bacillus subtilis* has been proven to be highly effective in controlling *Fusarium* wilt of chick pea, leading to improved plant growth and disease suppression [13]. Certain strains of *Pseudomonas* spp. such as *P. fluorescens, P. aeruginosa, P. putida, and P. aureofaciens* show promising potential as biocontrol agents by protecting seeds and roots from fungal infections, promoting plant growth, and reducing the severity of fungal diseases due to the production of secondary metabolites including antibiotics, siderophores, protease, and hydrogen cyanide [14]. Arbuscular mycorrhizal fungi (AMF) play a vital role in enhancing plant growth, nutrient uptake, stress tolerance, and disease resistance. They have been consistently shown to control important pathogens like *Fusarium* sp. and *Rhizoctonia* sp. while improving plant antioxidant status [15]. Safe materials like indole butyric acid (IBA) and silicate, specifically nano silicate, have the potential to suppress diseases by promoting plant growth and enhancing defence mechanisms [16]. Exogenous application of auxins, such as IBA, can significantly increase root regeneration in seedlings [17]. Silicate has been shown to control various diseases and improve tolerance against pathogens like *Rhizoctonia solani* [18]. Silicate application also enhances the activity of antioxidative enzymes involved in plant defense [19]. Additionally, the application of nano silicate has demonstrated positive effects on plant growth parameters, increasing shoot and root weights [20]. Nanoparticles (NPs) have unique properties due to their small size, with increased surface area leading to higher solubility and reactivity [21]. Nano-silicon (NSi) is a practical nanomaterial that has shown favorable influences in modern agriculture [22]. The main objectives of this study are to investigate the effectiveness of fungal, bacterial species and mycorrhizal fungi as bio-agents, along with the application of safe abiotic materials like indole butyric acid (IBA), silicate, and nano-silicate, in controlling root rot disease in annual vinca (*C. roseus*) and evaluate their impact on plant growth parameters.

2. Materials and methods

2.1. Sample collection, isolation, and purification of the causal pathogens

Diseased vinca plants with symptoms of damping off, root rot, and crown canker were sampled, stored in paper bags and transported to the laboratory using icebox. Isolation of the causal organisms has been carried out as usual using the methods described by [23] and the obtained isolates were maintained on PDA slants and kept in refrigerator at 5°C for further studies. The frequency percentages of the isolated fungi were calculated using the following equation: % frequency = (number of fungal colonies / total colonies of all fungi) × 100, as described by [24]. The developed colonies were identified based on their morphological characteristics using descriptions provided by [25].

2.2. Pathogenicity tests of the isolated fungi

The pathogenicity of nine fungal isolates collected from diseased plants was investigated. Seven isolates of *Fusarium* spp. and three isolates of *Rhizoctonia* spp. were tested for pathogenic potentiality on *C. roseus*. The experiment took place in pots under controlled glasshouse conditions. For soil infestation, pathogenic fungal cultures were separately inoculated at a ratio of 5% (inoculum: soil) and thoroughly mixed to ensure even distribution of the inocula. Plastic pots with a diameter of 20 cm were filled with 2 kg of the infested soil. Each fungal isolate had four pot replications, arranged in a randomized complete block design, and kept at 25 ± 2°C in the glasshouse for 2 weeks. Bioassays were conducted on healthy 6-month-old vinca plants with one plant serving as the control. Each fungal isolate had four replications, and a total of five replications were used for each pathogen. The plants were transplanted into 20 cm pots filled with sandy clay soil (50% sand, 50% clay) after being artificially infested with the tested fungal isolates.

2.3. Disease assessment

Disease incidence was calculated one month after transplanting using the formula described by [26] Disease severity was evaluated one month after transplanting based on the method described by [27].

2.4. Molecular identification of the causal organisms

The fungal isolates that had the greatest impact on disease severity were chosen for further identification based on their DNA sequences. Initially, these isolates, labeled as *Fusarium* spp. Isolates F1, F3, Fx3, and Ft, were identified at the genus level using morphological and microscopic characteristics [28]. Molecular identification was conducted for each isolate by analyzing the sequence of their internal transcribed spacer (ITS) regions 1 and 2 [29]. The Cetyl trimethylammonium bromide (CTAB) buffer method [30] was employed for fungal genomic DNA extraction according to the manufacturer's protocol. The quality and quantity of the extracted DNA were assessed using a 1% agarose gel and a nanodrop spectrophotometer. The extracted DNA was stored at -20°C until further use. The PCR amplification of the internal transcribed spacer (ITS) regions 1 and 2 from *Fusarium* spp. and *Rhizoctonia* spp. isolates was conducted using the universal primers ITS1 (5'-TCCGTTGGTGAACCAGCGG-3') and ITS4 (5'-TCCTCCGCTTATGATATGC-3') [29]. A high-fidelity DNA polymerase (Vent DNA polymerase, NEB Biolabs, England) was used in a 50 µl PCR reaction mixture containing 10× PCR Thermo Pol. buffer, 10 pmol of each primer, 2 µl of dNTPs, 3 µl of template DNA, and 1 unit of Vent DNA polymerase, topped up with nuclease-free water. PCR amplification was carried out using methods of [29]. The PCR products were separated and visualized using a Bio-Rad gel doc system under UV light. The PCR products were purified using the Qiagen PCR purification kit and sent for sequencing at AuGCT, China. Sequencing was performed in both directions using the respective forward and reverse ITS1 and ITS4 primers. The obtained nucleotide sequences were aligned and compared with the sequences in the NCBI database using the Basic Local Alignment Search Tool (BLAST) [31]. Multiple sequence alignment and construction of phylogenetic trees were conducted using the neighbor-joining method [32] with MEGA X software [33].

2.5. In vitro evaluation of antifungal activity of the isolated rhizospheres' fungal isolates against pathogenic fungi

Four isolates of *Trichoderma* spp., including *Trichoderma* sp. 1, *Trichoderma* sp. 2, *T. viride* and *T. album*, were tested for their antifungal activity against the pathogenic fungal isolates. The dual culture technique was applied, following the method described by [34]. Five mm mycelial disc from a 7-day-old culture of each *Trichoderma* species and the soil-borne pathogens (*F. solani*, *F. proliferatum*, *R. solani* 1, and *R. solani* 2) were placed on opposite sides of a plate, equidistant from the periphery. The experiment was set up in a completely randomized design. Control plates contained only a disc of the soil-borne pathogen in the center. At the end of the experiment, the radial growth of the pathogen isolates was measured, and the percentage of inhibition of average radial growth was calculated using the formula of [35].

2.6. In vivo evaluation of antifungal activity of Trichoderma isolates and mycorrhizal fungi against pathogenic fungi

The effectiveness of *Trichoderma* isolates against the aforementioned isolated pathogens was assessed in a greenhouse environment using a modified version of the method described by [36]. Pots with a diameter of 15 cm were filled with sandy-clay soil (50% sand, 50% clay). Each pot was then transplanted with a single 6-month-old vinca (*Catharanthus roseus*) seedling in sterilized soil after soil infestation. The pathogenic fungi, cultivated on barley grains, were mixed with the potting soil at a concentration of 3% (w:w). After 10 days, a spore suspension of each *Trichoderma* isolate (3% v/v) was separately mixed with the soil infested with the pathogens. Each treatment was replicated four times. The experiment consisted of 16 treatments, including non-infested soil as a positive control, and 16 treatments with soil treated with the pathogenic fungi as a negative control. The pots were arranged in a randomized block design, kept under greenhouse conditions, and irrigated as needed. After one month, the plants were uprooted to assess disease incidence and severity, and to measure the dry and fresh weights of both roots and shoots. The mycorrhizal inoculum, consisting of *glomus* isolate, was obtained from the Biological Control Research Institute at the Agricultural Research Center (ARC). The inoculum was supplied as propagules in expanded clay. The vinca plants were grown in pots with a diameter of 15 cm filled with 1 kg of soil mixed with 10% (v/v) mycorrhizal fungus inoculum. The inoculum was prepared by combining and thoroughly mixing the surrounding soil with the root systems of *Glomus* sp. Autoclaved non-mycorrhizal inoculum was also provided as a control. One seedling was transplanted into each pot, with the mycorrhizal inoculum placed 3 cm below the soil surface to promote mycorrhizal plant growth [37]. The soil was infested with the tested pathogenic fungi (*F. solani*, *F. proliferatum*, *R. solani* 1, *R. solani* 2) by cultivating them in potato dextrose broth (PDB) and thoroughly mixed them with the potting soil at a concentration of 3% (w:w). Pots were kept in a greenhouse and irrigated as necessary. After one month, the plants were uprooted to assess disease incidence and severity, as well as measure the dry and fresh weights of roots and shoots.

2.7. In vitro and in vivo evaluation of bacterial activity against pathogenic fungi

The *in vitro* antagonistic effects of three bacterial isolates *P. fluorescens, P. aeruginosa, and B. subtilis* against pathogenic fungi (*F. solani, F. proliferatum, R. solani* 1, *R. solani* 2), were evaluated. The reduction percentage of fungal mycelial growth was calculated according to [38]. The radial growth of *R. solani* and *F. solani* was measured, and the inhibition percentage of growth was calculated. Each antagonistic bacterial strain was utilized in the form of a spore suspension. Six-month-old vinca seedlings were soaked in the respective suspension for a duration of two hours [39]. A pot experiment was conducted. The potting soil was mixed with pathogenic fungi cultivated on barley grains at a concentration of 3% (w:w). Each pot was transplanted with a single six-month-old vinca seedling, and the experiment was replicated four times in a completely randomized design (CRD). The experiment consisted of 16 treatments, including non-infested soil as a positive control, and 16 treatments with soil treated with the pathogenic fungi as a negative control. The pots were kept under greenhouse conditions and irrigated as needed. After one month, plants were uprooted to determine disease incidence and severity as mentioned before, the dry and fresh weights of both roots and shoots, were determined.

2.8. In vivo application of silicate, nano silicate, and Indole Butyric Acid

In vivo experiment was conducted under greenhouse conditions, a rate of 6:8 cm per liter of potassium silicate was applied. Pots containing soil infested with the tested pathogenic fungi (*F. solani, F. proliferatum, Rhizoctonia solani* 1, and *R. solani* 2) were used. The inocula were added to the potting soil at a concentration of 3% (w:w). Two days after soil infestation, the seedlings were sprayed with potassium silicate at the recommended usage rate. The experiment was replicated four times, with one seedling per replicate for each treatment (pathogen). Four pots were left to serve as control without soil infestation and kept in greenhouse conditions and irrigated as necessary. After two weeks, the plants were uprooted to assess disease incidence and severity as mentioned before, as well as the dry and fresh weights of both roots and shoots, were determined. Indole Butyric Acid (IBA) was applied at a rate of 0.25 g per liter by dissolving the required amount in water. After ten days of soil infestation, 6-month-old seedlings were carefully washed and immersed in IBA solutions for 30 seconds. The treated seedlings were then transplanted into pots containing the infested soil. Special attention was given to handling the root systems delicately to avoid any visible root damage during the transplantation process. After one month, the seedlings were removed from the pots, and the soil adhering to the root system was gently washed off. Number and length of roots were measured, and the fresh and dry weights of both roots and shoots were determined following the methods described by [40].

2.9: Statistical analysis

Statistical analysis of the data was done using the Statistix 9 software. Mean separation was determined using Duncan's multiple range test with a significance level set at $p < 0.05$ [41].

3. Results

3.1. Isolation, purification, identification and frequency of the causal pathogens

Various soilborne pathogens belonging to numerous fungal genera were isolated from the suspected diseased vinca plant root samples. These purified and identified microorganisms consist of different isolates of *Fusarium* species and *Rhizoctonia solani*. The data reveal that the most frequently isolated fungal genera from diseased vinca root samples were *Rhizoctonia* species (46.15%), followed by *Fusarium* species (38.46%). However, the lowest frequency percentage was observed in saprophytic bacteria (15.38%) compared to the other pathogens.

3.2. Pathogenicity test

The results of pathogenicity tests were shown in Figure 1 reveal variations among the tested fungi. The highest disease incidence percentage was observed in both isolates F3 (*F. solani*) and Fx3 (*F. proliferatum*), with a value of 50%. And the lowest values of disease incidence was 25%. In terms of disease severity, F3 (*F. solani*) displayed the highest value at 65%. On the other hand, the Fx2 (*Fusarium* sp.) isolate had the least percentage of disease severity at 30%.

3.3 Molecular identification of the causal organisms

The molecular identification of *Fusarium* species was conducted by comparing their internal transcribed spacer (ITS) sequences to those of representative *Fusarium* type-species available in GenBank at the National Center for Biotechnology Information (NCBI). Phylogenetic analysis of all *Fusarium* spp. sequences revealed three major clades and confirmed the presence of three distinct *Fusarium* species: *F. solani*, *F. proliferatum*, and *F. graminearum*. The ITS sequences of isolates F1 and F3 exhibited high similarity to *F. solani* strain ATLOY3 and *F. solani* strain FUS-IS 11, respectively. However, both F1 and F3 isolates were genetically distant, as they fell into two separate subclades with significant branch length differences, supported by bootstrap values indicating the genetic divergence among isolates (see Figure 2). *Fusarium* isolate Fx3 demonstrated considerable sequence similarity with multiple strains of *F. proliferatum* (refer to Figure 2). *Fusarium* isolate Ft showed a close relationship with *F. graminearum* strains displayed in the lower left panel of (Figure 2). All four isolates displayed macroscopic and microscopic characteristics on malt extract agar (MEA) consistent with their respective reference species.

3.4. In vitro assessment of antifungal activity of Trichoderma spp. against vinca root-rot pathogens

The data presented in (Figure 3), demonstrate that the tested fungal bio-agents effectively suppressed the growth of all the examined pathogenic fungi, including *R. solani* 1, *R. solani* 2, *F. solani*, and *F. proliferatum*. The results indicate notable variations among the investigated bio-agents, particularly when targeting *F. solani*. *Trichoderma* sp. (2) exhibited a highly significant impact, resulting in 80.7% reduction in growth of *F. proliferatum* compared to the other bio-agents. In contrast, *T. viride* displayed the lowest efficacy, with a reduction of only 69.9% against the same pathogen. Furthermore, *Trichoderma* sp. (2) showed the highest inhibition percentage being 68.8% against *R. solani* 1. Overall, the mycelial growth of the pathogens was significantly reduced when using *Trichoderma* sp. (2) as a bio-agent, particularly against *F. proliferatum* and *R. solani* 1, which exhibited the highest efficacy.

3.5. Antifungal activity of Trichoderma spp. and mycorrhizal fungi on vinca root rot pathogens in vivo

The presented data in (Table 1) show the impact of various fungal bio-agents on (DI %) and (DS %) of vinca root-rot disease. *Trichoderma* spp. demonstrated complete inhibition of vinca root-rot pathogens, except for *Trichoderma album*, which showed higher disease incidence and severity against *F. solani* isolate (50% and 60%, respectively). On the other hand, treatments with *Trichoderma* sp. (1) and *Trichoderma* sp. (2) resulted in a disease incidence and severity of 25% and 40% for both *F. solani* and *R. solani* 2 pathogens, respectively. Furthermore, the introduction of mycorrhizal fungi to soil inoculated with either *R. solani* 1 or *F. proliferatum* led to a reduction in disease incidence and severity to 25% and 40% for both pathogens. However, mycorrhizal fungi only reduced disease parameters in vinca plants grown in soil inoculated with *R. solani* 2 being 50% and 60% and with *F. solani* to 75% and 80%, respectively. Consequently, the plant growth parameters, including shoot fresh and dry weights (Table 2), root fresh and dry weights (Table 3), were significantly affected. The data in (Table 2 and Figure 5) demonstrate that the highest shoot fresh weight (gm) and shoot dry weight (gm) were observed when *T. album* targeted *Rhizoctonia* solani 2, resulting values of 21.25 gm and 9.4 gm, respectively. Conversely, the least values were recorded when *Trichoderma* sp. (2) was used against *F. proliferatum* (15 gm and 5.8 gm, respectively), the highest root fresh weight (gm) and root dry weight (gm) were observed when *T. album* targeted *R. solani* 2, with values of 4.70 gm and 2.35 gm, respectively. The lowest root fresh and dry weights (gm) were recorded when *Trichoderma* sp. (2) was used against *F. proliferatum* (2.90 gm and 1.45 gm, respectively).

3.6. In vitro, and in vivo assessment of bacterial bioagents against vinca root-rot pathogens

The data presented in (Figure 4), illustrates the impact of the tested alternative bacterial bio-agents on the growth of pathogenic fungi, including *R. solani* 1, *R. solani* 2, *F. solani*, and *F. proliferatum*. Among the bio-agents, *B. subtilis* exhibited the highest percentage of inhibition of mycelial growth, with reductions of 66.6% for *R. solani* 2 and 51.8% for *F. proliferatum*. However, *F. solani* and *R. solani* 1 showed the least mycelial reduction percentages being 48.1% and 42.4%, respectively. Furthermore, *P. fluorescens* followed *B. subtilis* in suppressing the mycelial growth of *R. solani* 2, resulting in a reduction of 51.8%, while the least value of 24.1% was obtained when tested against *R. solani* 1. The data presented in (Table 1) exhibited that *P. fluorescens* was the most favorable outcomes against vinca root-rot pathogens, except for the R. solani 1 isolate, where disease incidence and severity were 25% and 40%, respectively. Similarly, *P. aeruginosa* showed significant results against most vinca root-rot pathogens, except for the *R. solani* 2 isolate, which displayed higher disease incidence and severity at 50% and 45%, respectively. *B. subtilis* demonstrated consistent percentages of disease incidence (25%) and disease severity (40%) for the tested pathogens, including *R. solani* 1, *F. solani*, and *F. proliferatum*. However, *R. solani* 2

exhibited higher values for both disease incidence and severity, even in the presence of the antibacterial bio-agent *B. subtilis*, with values of 50% and 60%, respectively. Consequently, the plant growth parameters, Tables 2 and 3, significantly differed compared to the control group depending on the specific bacterial bio-agent used. The results presented in (Table 2), along with (Figure 6), indicate the highest shoot fresh weight (SFW) and shoot dry weight (SDW) when vinca plants were treated with Pseudomonas aeruginosa against *R. solani isolate* 2, recording values of 19 gm and 7.7 gm, respectively. Conversely, the lowest SFW and SDW were observed when vinca plants were infected with *R. solani* 1 and treated with *B. subtilis*, recording values of 11.75 gm and 5.17 gm, respectively. In terms of root fresh weight (RFW) and root dry weight (RDW), data of (Table 3) reveal the highest values when vinca plants infected with F. proliferatum were treated with *P. fluorescens*, resulting RFW and RDW of 3.91 gm and 1.96 gm, respectively. The least RFW and RDW were observed when vinca plants infected with *R. solani* isolate 1 were treated with *B. subtilis*, resulting RFW and RDW of 2.59 gm and 1.29 gm, respectively.

3.7. Control of vinca root rot pathogens using safe materials

The data presented in (Table 1) indicates that the application of silicate, nano silicate, and (IBA) effectively prevents infection with root rot pathogens in vinca plants. Consequently, the results related to plant growth parameters, including SFW and SDW (Table 2), as well as RFW and RDW (Table 3), were determined. In Table 2, the highest SFW and SDW were observed in the treatment of *R. solani* 2 with nano silicate yielded significant outcomes, with SFW and SDW of 25. 29gm and 12.87 gm, respectively. Conversely, the least SFW and SDW were recorded in plants grown in soil inoculated with *F. solani* and *F. proliferatum* when treated with silicate, with values of 12.5 gm and 5.27 gm, and 12.5 gm and 5.17 gm, respectively. In Table 3, indicate the highest RFW and RDW were achieved in plants grown in soil inoculated with *F. solani* and treated with IBA, recording values of 6.58 gm and 3.29 gm, respectively. Following *F. solani* treatment, soil inoculated with *R. solani* 2 demonstrated comparable plant parameters, of RFW and RDW being 6.44 gm and 3.22 gm, respectively, when treated with nano silicate.

4. Discussion

The study revealed that *Rhizoctonia* spp. and *Fusarium* spp. are the primary causes of root rot of vinca plants taken from diseased plants, these results might be due to suitable environmental conditions that affect the disease incidence by such fungal genera rather than other neglected genera which recorded least results. These findings align with previous research on root rot pathogens in various ornamental plant species [42]. Studies by [43] identified *Fusarium* species and *R. solani* as fungal pathogens in vinca plant roots. A previous study [44] Reported that *F. proliferatum* and *F. solani* are major pathogens causing root rot in Carnation (*Dianthus caryophyllus* L.) plants. Understanding the prevalence and identity of these pathogens is crucial for developing effective management strategies to protect vinca plants from root rot [45]. In spite of promising results obtained by some chemical treatments in controlling root rot disease, phytotoxicity and chemical residues are major problems leading to environmental pollution and human health hazards. Pathogenicity tests showed variations in disease occurrence and severity among different fungal isolates, especifically those of *F. solani*, *F. proliferatum*, and *R. solani*. Similar studies have confirmed the pathogenic nature of these fungi on various plant species.The pathogenic fungi were readily re-isolated separately from the artificial inoculated vinca plants and were found to be identical with the original cultures used for inoculation. The observations obtained could be clarified on the basis of the presence of different pathotypes specifically those of *Fusarium* species within the same genera differing in their virulent. For instance, an earlier study [46] demonstrated the ability of *F. solani* and *F. proliferatum* to cause similar disease occurrence and severity in different crops. Similarly, Abdelghany *et al*., [47] investigated the pathogenic potential of *R. solani* on different plants, supporting the observation that this pathogen can induce similar disease occurrence and severity as observed in the present study. The molecular identification and phylogenetic analysis confirmed the presence of *F. solani, F. proliferatum,* and *F. grameniarum* in the tested isolates. The genetic distance observed within *F. solani* isolates and the similarity between Fx3 and *F. proliferatum* strains were supported by the phylogenetic tree. These findings align with previous research that also utilized molecular methods to identify these *Fusarium* species. The studies by[48] reported similar results, highlighting the genetic diversity within each species and the consistent clustering patterns. The genetic distance observed in the present study, as well as the subclades within *F. solani*, were also noted in the study by [49], reinforcing the reliability of the molecular approach for species identification in the *Fusarium* genus. The results of this study indicate the potential of fungal bio-agents, specifically *Trichoderma* species, in suppressing the growth of pathogenic fungi in Vinca plants. The inhibitory mechanisms of *Trichoderma* against fungal pathogens might involve competition for nutrients, production of volatile and non-volatile secondary metabolites, and induction of systemic resistance in plants [50]. These findings align with the observed inhibitory effects of *Trichoderma* on *R. solani* and *Fusarium* sp. in the present study. Fortunately, the antagonistic fungi and bacteria were generally useful treatments in significantly minimizing disease incidence and severity. In fact, the action by which the antagonistic microorganisms could suppress the activity of a plant pathogen was quite varied. The hyphae of the bioagent might be in contact with the host either by producing appressorium like strengthen or coiling host hyphae then digest it enzymatically and or producing lytic, toxic, and antibiotic metabolites that might affect growth of the pathogen fungi. Similar research conducted by [51] investigated the biocontrol potential of *Trichoderma* against *Fusarium* wilt in tomato plants. The study demonstrated significant inhibition of *F. solani* growth by *Trichoderma* species, which aligns with the findings of the current study. Another study by [52] explored the efficacy of *Trichoderma* against *R. solani*, a common pathogen causing root rot in various crops. Thus, the mechanisms underlying the biocontrol activity of *Trichoderma* involve mycoparasitism, production of antifungal metabolites, and induction of plant defense responses [53]. The development of biocontrol strategies utilizing *Trichoderma*-based products can contribute to sustainable and environmentally friendly approaches for disease management in horticultural crops.

The impact of *Trichoderma* spp. on vinca root-rot pathogens in greenhouse settings. *Trichoderma* spp. exhibited significant antifungal activity against these pathogens, leading to complete inhibition in most cases. These results align with previous research, highlighting the potential of *Trichoderma* spp. as biocontrol agents for effectively managing root-rot diseases in vinca plants. For instance, [54] conducted similar research and found that *Trichoderma* spp. exhibited strong inhibitory effects on fungal diseases, corroborating the current study's findings. Their findings also support the current study, emphasizing the ability of *Trichoderma* spp. to suppress disease incidence and severity attributing to mechanisms including mycoparasitism, competition for nutrients, and the production of antifungal metabolites [55]. These mechanisms likely contribute to the observed inhibition of vinca root-rot pathogens in this study. The influence of different *Trichoderma* strains on plant growth parameters, namely shoot fresh weight (SFW), shoot dry weight (SDW), root fresh weight (RFW), and root dry weight (RDW), was conducted. The application of various *Trichoderma* strains yielded diverse effects on plant growth parameters. *Trichoderma album* and *Trichoderma viride* exhibited promising results in enhancing shoot and root weights, while *Trichoderma* sp. (2) had a less pronounced impact. It is obviously noticed that the biocontrol agent exhibits best results in controlling the disease incidence in consequence parameters of shoot and root weights were increased. This might be due to the rapid growth and establishment of the bioagent and colonizing the sphere surrounding the plant root system thus resulting a reduction of disease incidence and improve plant vigor. These results align with previous studies [56] and [57] that emphasize the growth-promoting potential of *Trichoderma* spp. of different plant cultivars. The growth-promoting mechanisms of *Trichoderma* spp. also involve various factors such as the production of plant growth regulators, nutrient solubilization, induction of defense responses, and enhancement of root system development [58]. These mechanisms likely contributed to the observed improvements in shoot and root parameters in the current study.

The effectiveness of mycorrhizal fungi in biocontrolling vinca root rot pathogens was examined, consistent with previous research highlighting their potential. The ability of mycorrhizal fungi to form mutualistic associations with plant roots, enhancing nutrient uptake and providing a physical barrier against pathogen invasion, contributes to their effectiveness in controlling root rot pathogens [59]. Mycorrhizal fungi combat soil-borne plant pathogens through various mechanisms. They establish mutualistic associations with plant roots, triggering defense responses and enhancing resistance against pathogens. Additionally, mycorrhizal fungi compete with pathogens for soil resources and release antimicrobial compounds that hinder their growth. Furthermore, they stimulate the production of defense-related compounds in plants, supporting systemic resistance. These multifaceted mechanisms contribute to the effective control of soil-borne plant diseases by mycorrhizal fungi [15,60]. *B. subtilis* showed the highest inhibition percentage against *R. solani* 2 and *F. proliferatum* mycelial growth, while *P. fluorescens* demonstrated potential suppressing growth of *R. solani* 2. As for, the ability of bacterial strains to inhibit growth of the pathogens *in vitro* and to produce certain secondary metabolites which cause inhibition and lyses of the pathogenic fungi has been claimed to be of importance for biological control. These results support previous research indicating the antifungal activity of these bacterial bio-agents. In a study by [61], the antifungal potential of *Bacillus subtilis* against various plant pathogens was investigated, confirming its ability to inhibit fungal growth. Similarly, an earlier study [62] examined the antagonistic effects of *Pseudomonas fluorescens* against *Fusarium solani* in different crops, demonstrating its inhibitory activity. Several mechanisms have been suggested for disease control by *P. fluorescens* including siderophores production, volatile compounds, and hydrolytic enzymes as well as stimulating phytoalexins or by competing with pathogens for nutrients or colonization zone. The activity of these bacterial bio-agents may be due to various factors such as the production of antimicrobial compounds, nutrient competition, and induction of systemic resistance in plants [63]. On the other hand, the application of bacterial bio-agents had a significant influence on the growth parameters of vinca plants affected by root-rot pathogens. *P. aeruginosa* and *P. fluorescens* displayed promising results in enhancing plant growth, while the efficacy of *Bacillus subtilis* was varied. These findings align with previous research highlighting the role of bio-agents in promoting plant growth and managing root diseases [64]. A previous study [65] and [66] described the role of *Pseudomonas aeruginosa* in enhancing the growth of mustard plants infected with root pathogens. Significant improvements in plant growth parameters, such as shoot length, root length, and dry weight, were observed in the treatments with *P. aeruginosa*, indicating its potential as a growth-promoting bio-agent. These findings, along with the previous studies, provide additional evidence of the positive influence of bacterial bio-agents like *P. aeruginosa*, *P. fluorescens*, and *B. subtilis* on the growth parameters of plants affected by root-rot pathogens. The use of these bio-agents resulted in enhancements of plant height, root length, and biomass, indicating their potential for promoting plant growth. Generally, biological control of soil borne pathogens is often attributed to improved nutrition that boosts host defenses or to direct inhibition of pathogen growth and activity. Table 1 demonstrate that the application of silicate, nano silicate, and indole butyric acid (IBA) effectively prevent infection by root rot pathogens in vinca plants.

Table 1: In vivo effect of various bioagents against vinca root-rot pathogens on disease incidence (DI %) and disease severity (DS %)

Fungal bioagents	DI%					DS%				
	***R. solani* 1**	***R. solani* 2**	***F. solani***	***F. proliferatum***	**Cont (+)**	***R. solani* 1**	***R. solani* 2**	***F. solani***	***F. proliferatum***	**Cont (+)**
T. album	0	0	50	0	0	0	0	60	0	0
T. virdi	0	0	0	0	0	0	0	0	0	0
Trichoderma sp. (1)	0	0	25	0	0	0	0	40	0	0
Trichoderma sp. (2)	0	25	0	0	0	0	40	0	0	0
Mycorrhizae	25	50	75	25	0	40	60	80	40	0
Cont. (-)	100	100	100	100	0	60	65	70	30	0
L.S.D.										
Fungi			14.96					12.85		
Bioagents			13.85					11.90		
Fungi* Bioagents			11.24					9.66		
Bacterial bioagents										
B. subtilis	25	50	25	25	0	40	60	40	40	0
P. fluorescens	25	0	0	0	0	40	0	0	0	0
P. aeruginosa	0	50	0	0	0	0	45	0	0	0
Cont. (-)	100	100	100	100	0	60	65	70	30	0
L.S.D.										
Fungi			23.08					18.26		
Bioagents			20.14					15.93		
Fungi* Bioagents			15.80					12.50		
Abiotic agents										
Silicate	0	0	0	0	0	0	0	0	0	0
Nano silicate	0	0	0	0	0	0	0	0	0	0
IBA	0	0	0	0	0	0	0	0	0	0
Cont (-)	100	100	100	100	0	60	65	70	30	0
L.S.D.										
Fungi			22.50					14.88		
Materials			19.63					12.99		
Fungi*Materials			15.41					10.19		

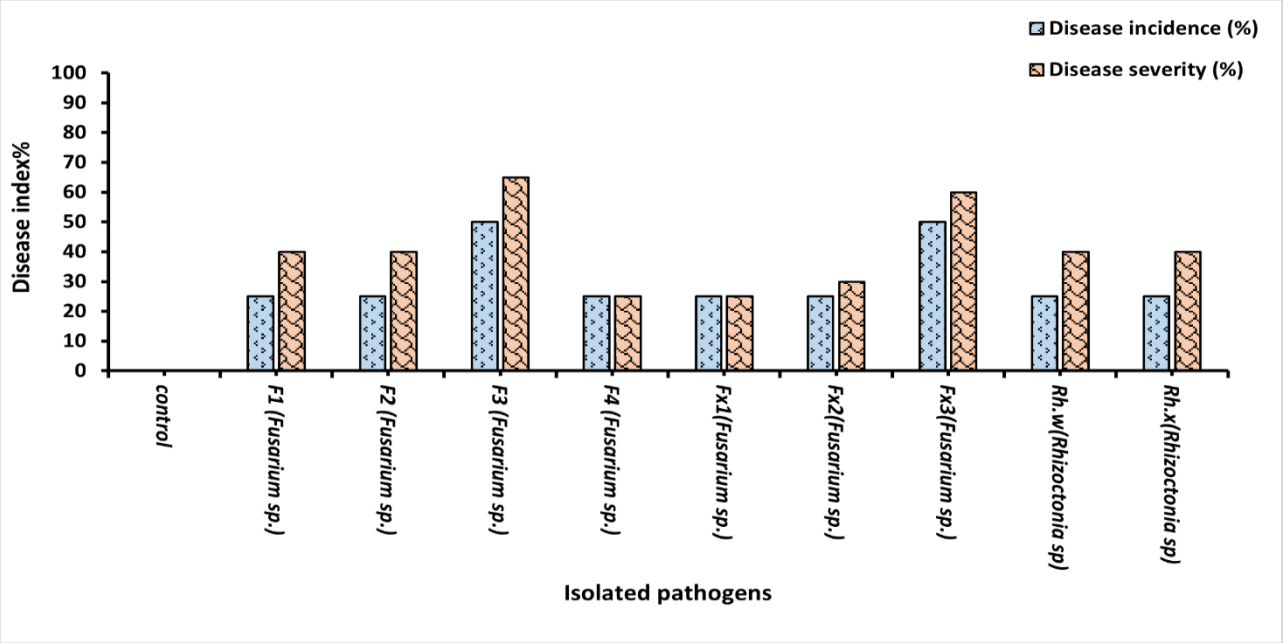

Figure 1: Pathogenicity assessment of nine isolated pathogenic genera from Vinca rosa roots under controlled greenhouse conditions.

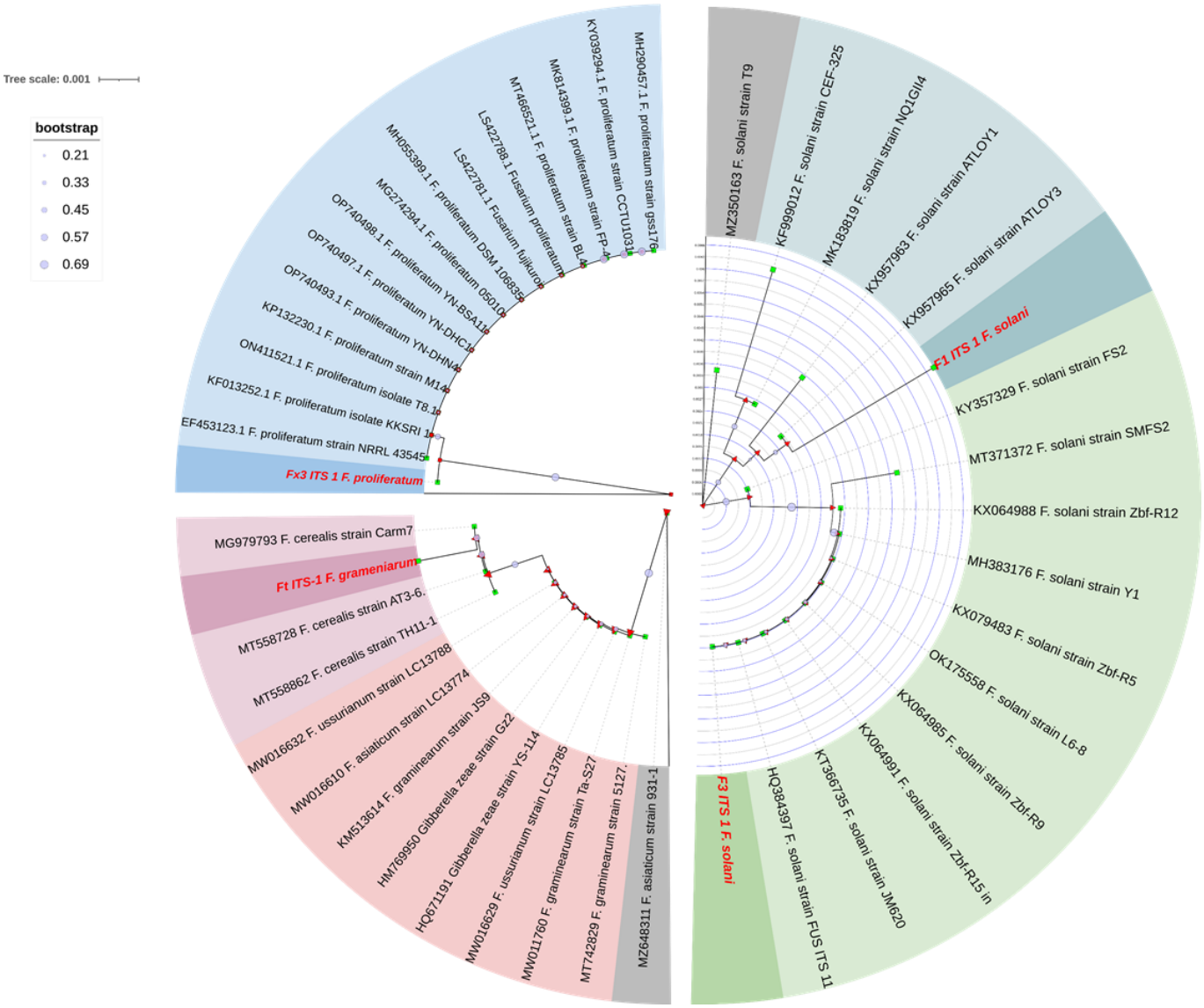

Figure 2: Phylogenetic tree illustrating the relationships of isolates F1, Ft, F3, and Fx3 to closely related species within the genus *Fusarium* using the neighbor-joining method.

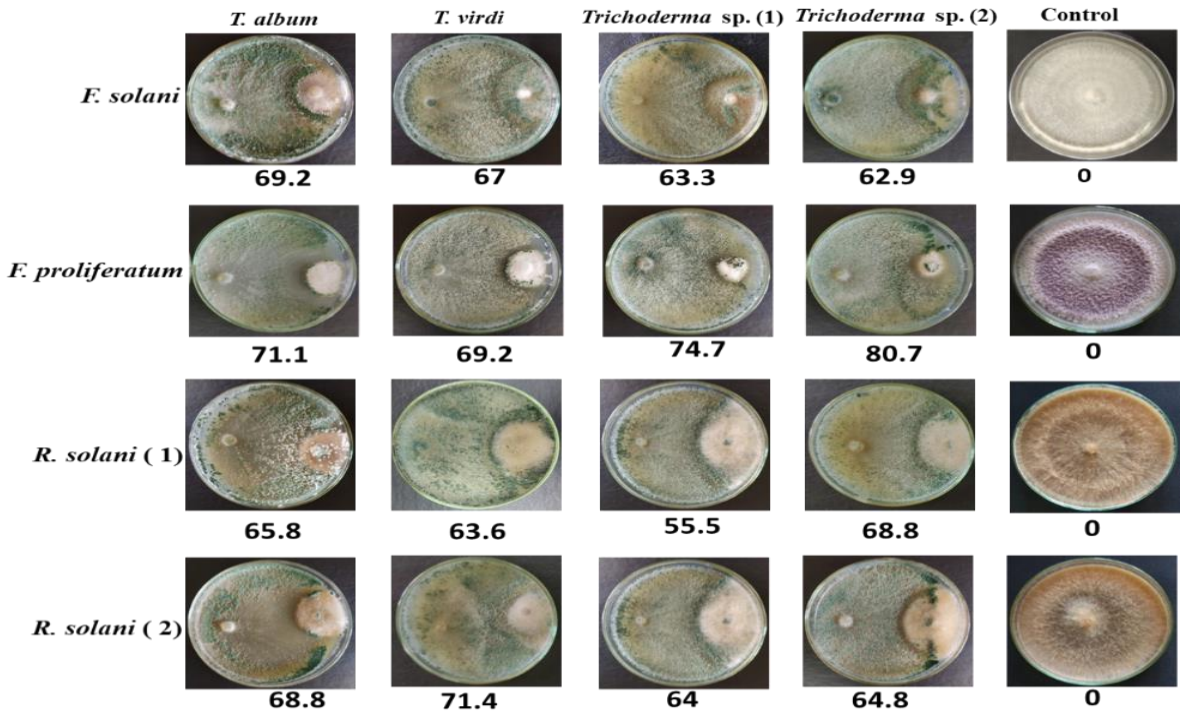

Figure 3: Inhibition percentage of mycelial growth in four isolated pathogens from vinca root by four tested *Trichoderma* species. The numbers below each picture in this figure refer to the inhibition percentage value.

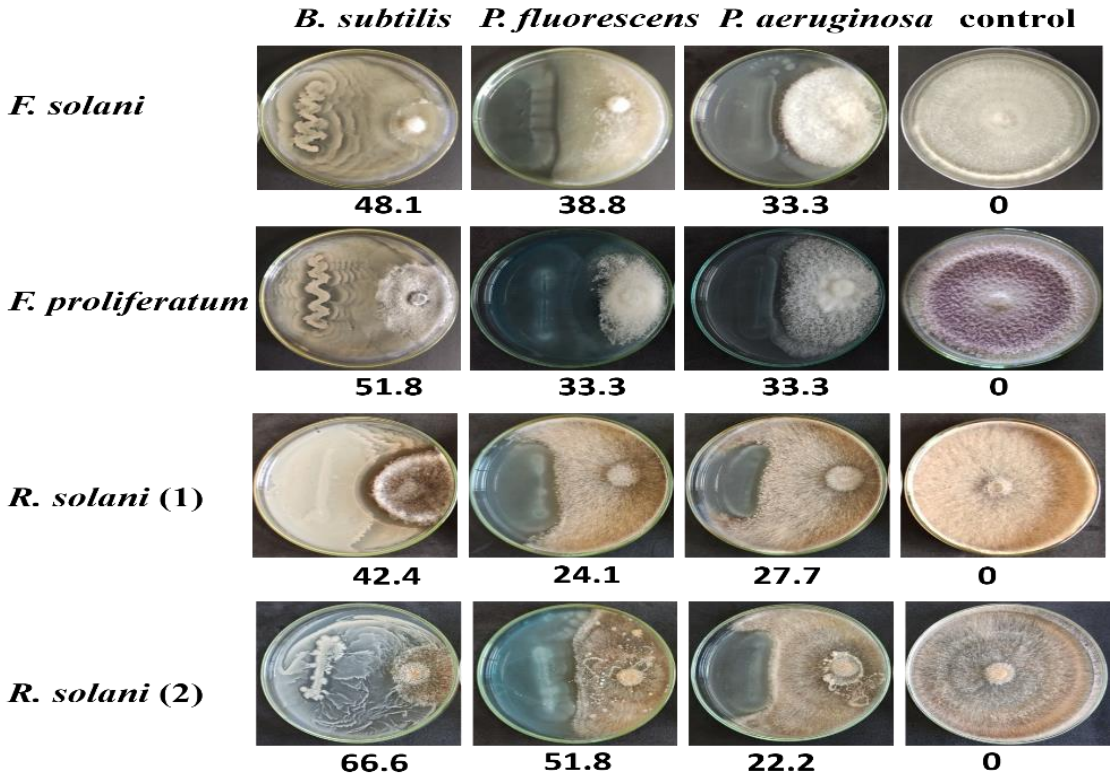

Figure 4: Inhibition percentage of mycelial growth in vinca root-rot pathogens by *P. fluorescens*, *P. aeruginosa*, and *B. subtilis*. The numbers below each picture in this figure correspond to the inhibition percentage values.

Fig. 5: Evaluation of the effectiveness of fungal bioagents against pathogens (*F. solani, F. proliferatum; R. solani 1; and R. solani* 2) in different treatment groups (1: control; 2: infected and treated with bioagent; 3: infected with pathogens) utilizing various combinations: a) *Trichoderma* sp. (2) + *F. solani*; b) *Trichoderma virdi* + *F. proliferatum*, c) *Trichoderma virdi* + *R. solani* 1, and d) *Trichoderma album* + *R. solani* 2.

Table 2: *In vivo* effect of various bioagents against root-rot pathogens on vinca shoot fresh (SFW gm) and shoot dry (SDW) weights (gm)

Fungal bioagents	**SFW**					**SDW**				
	***R. solani* 1**	***R. solani* 2**	***F. solani***	***F. proliferatum***	**Cont. (+)**	***R. solani* 1**	***R. solani* 2**	***F. solani***	***F. proliferatum***	**Cont. (+)**
T. album	19.25	21.25	19	16.5	26.75	8.12	9.4	8.4	7.025	13.1
T. virdi	19.75	13.25	19.25	21	26.75	8.97	5.22	8.67	9.75	13.1
Trichoderma sp. (1)	16.75	17.12	14.75	15.5	26.75	7.07	7.05	6.22	6.25	13.1
Trichoderma sp. (2)	18	14.37	19.5	15	26.75	7.12	5.82	8.97	5.8	13.1
Mycorrhizae	16.5	22.5	21.5	14.75	26.75	6.9	8.3	9.7	5.95	13.1
Cont. (-)	10.5	9.5	9.5	8.5	26.75	4.3	3.5	3.37	2.95	13.1
L.S.D.										
Fungi Bioagents Fungi* Bioagents	0.9897 1.0841 2.4241					0.4675 0.5122 1.1452				
Bacterial bioagents										
B. subtilis	11.75	15.87	14	17.5	26.75	5.17	5.9	5.4	7.25	13.1
P. fluorescens	15.25	15.25	17	18	26.75	5.57	6.6	7.2	7.82	13.1
P. aeruginosa	10.75	19	17.75	14.5	26.75	4.7	7.7	7.53	5.35	13.1
Cont. (-)	10.5	9.5	9.5	8.5	26.75	4.3	3.5	3.37	2.95	13.1
L.S.D.										
Fungi Bioagents Fungi* Bioagents	1.2069 1.0795 2.4139					0.7433 0.6648 1.4865				
Abiotic agents										
Silicate	16	16.75	12.5	12.5	26.75	7.02	7	5.27	5.17	13.1
Nano silicate	25.29	29.25	21	26.5	26.75	11.17	12.87	9.37	11.9	13.1
IBA	27	31.5	29.75	26.75	26.75	11.87	13.5	13.15	11.6	13.1
Cont (-)	10.5	9.5	9.5	8.5	26.75	4.3	3.5	3.37	2.95	13.1
L.S.D.										
Fungi Materials Fungi*Materials	1.3236 1.1838 2.6471					0.6188 0.5535 1.2376				

Fig. 6: Efficacy of bacterial bioagents against pathogens (*F. solani*, *F. proliferatum*; *R. solani* 1; and *R. solani* 2) in different treatment groups (1: control; 2: infected and treated with bioagent; 3: infected with pathogens) utilizing various combinations (a: *P. aeruginosa* + *F. solani*, b: *P. fluorescens* + *F. proliferatum*, c: P. fluorescens + *R. solani* 1, and d: *P. aeruginosa* + *R. solani* 2)

Table 3: *In vivo* effect of various bioagents against root-rot pathogens on vinca Root fresh (RFW gm) and Root dry (RDW) weights (gm)

Fungal bioagents	**RFW**					**RDW**				
	***R. solani* 1**	***R. solani* 2**	***F. solani***	***F. proliferatum***	**Cont. (+)**	***R. solani* 1**	***R. solani* 2**	***F. solani***	***F. proliferatum***	**Cont. (+)**
T. album	4.06	4.70	4.21	3.51	6.94	2.03	2.35	2.11	1.76	3.47
T. virdi	4.49	2.61	4.34	4.88	6.94	2.24	1.31	2.17	2.44	3.47
Trichoderma sp. (1)	3.54	3.53	3.11	3.13	6.94	1.77	1.76	1.56	1.56	3.47
Trichoderma sp. (2)	3.56	2.91	4.49	2.90	6.94	1.78	1.46	2.24	1.45	3.47
Mycorrhizae	3.45	4.16	4.85	2.98	6.94	1.73	2.08	2.43	1.49	3.47
Cont. (-)	2.16	1.75	1.69	1.48	6.94	1.08	0.88	0.84	0.74	3.47
L.S.D.										
Fungi Bioagents Fungi* Bioagents	0.2338 0.2561 0.5726					0.1167 0.1279 0.2859				
Bacterial bioagents										
B. subtilis	2.59	2.95	2.71	3.63	6.94	1.29	1.48	1.36	1.81	3.47
P. fluorescens	2.79	3.30	3.60	3.91	6.94	1.39	1.65	1.80	1.96	3.47
P. aeruginosa	2.35	3.86	3.76	2.68	6.94	1.18	1.93	1.88	1.34	3.47
Cont. (-)	2.16	1.75	1.69	1.48	6.94	1.08	0.88	0.84	0.74	3.47
L.S.D.										
Fungi Bioagents Fungi* Bioagents	0.3716 0.3324 0.7433					0.1863 0.1666 0.3725				
Abiotic agents										
Silicate	3.51	3.50	2.64	2.59	6.94	1.76	1.75	1.32	1.29	3.47
Nano silicate	5.59	6.44	4.69	5.95	6.94	2.79	3.22	2.34	2.98	3.47
IBA	5.94	6.76	6.58	5.81	6.94	2.97	3.38	3.29	2.91	3.47
Cont (-)	2.16	1.75	1.69	1.48	6.94	1.08	0.88	0.84	0.74	3.47
L.S.D.										
Fungi Materials Fungi*Materials	0.3094 0.2767 0.6188					0.1547 0.1384 0.3094				

A previous studies [67] investigated the efficacy of silicate and nano silicate in controlling root rot diseases in olive trees and found that they suppressed the growth and activity of root rot pathogens, indicating their potential as safe materials for disease control. The use of safe materials like silicate, nano silicate, and IBA can contribute to disease suppression by influencing plant growth and defense mechanisms [16]. Furthermore, present the effects of IBA and nano silicate on plant growth parameters, including SFW, SDW, RFW, and RDW. The findings indicate that the application of IBA and nano silicate positively influences plant growth parameters, leading to an increase in shoot and root weights. IBA has been reported to stimulate root development and nutrient uptake, promoting overall plant growth [68]. IBA (indole-3-butyric acid) also enhances plant defense mechanisms against pathogens. It activates defense-related genes, leading to the production of antimicrobial proteins and secondary metabolites. It also stimulates the generation of reactive oxygen species (ROS) and triggers the accumulation of phytohormones like salicylic acid (SA) and jasmonic acid (JA), which activate defense pathways. These mechanisms aid in the activation of plant defense against pathogens [69]. Silicate amendments have been shown to improve plant growth and increase biomass production [70]. An earlier studies [20] also found that the application of nano silicate on pea plants resulted an increase of shoot and root weights, further supporting its positive influence on plant growth parameters. Silicate plays a crucial role in plant disease management by enhancing plant defense mechanisms and preventing pathogen infection. It stimulates the synthesis of defense-related compounds, strengthens cell walls to hinder pathogen penetration, and promotes the production of reactive oxygen species (ROS) for signaling defense responses [18]. These mechanisms contribute effectively to plant disease management.

5. Conclusion

Vinca plants are highly susceptible to *Rhizoctonia solani* and *Fusarium* sp., the primary causal agents of root rot disease. *Trichoderma* spp. and mycorrhizal fungi showed effective control against these pathogens. *P. fluorescens*, *P. aeruginosa*, and *B. subtilis* also exhibited inhibition of pathogen growth. The application of silicate, nano silicate, and indole butyric acid prevented pathogen infection and improved plant growth parameters. Overall, the present study provides further evidence that may facilitate simple nontoxic chemicals as silicate, and indole butyric acid and bacterial genera for controlling vinca plants root rot as their low cost, low toxicity to environment and human health make them suitable for disease control under all conditions resulting ideal plant growth parameters. These control strategies effectively reduced disease incidence and severity while promoting plant growth in vinca. By implementing these effective strategies, sustainable production can be achieved while minimizing environmental impact, contributing to a greener and healthier agricultural ecosystem

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