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EFFICACY OF TRICHODERMA HARZIANUM AND BACILLUS SUBTILIS IN THE MANAGEMENT OF BROWN SPOT DISEASE IN RICE (ORYZA SATIVA)

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Abstract

Alternaria brown spot, brought on by Alternaria alternata, poses a significant threat to the adequate production of rice, a staple crop vital for global food security, particularly in Nigeria. Two biocontrol agents, Trichoderma harzianum (TH) and Bacillus subtillis (BS) (alone and in combination), Standard check (SC), Positive control (PC), Negative control (NC) were investigated using a completely randomized design (CRD) with three replications in a laboratory experiment on the germination, growth, and disease severity in rice. Rice was cultivated under controlled conditions in plastic pots. Selected seeds were artificially inoculated with Alternaria alternata spore (10^6) suspension. The biocontrol agents were evaluated prophylactically through seed priming. Primed seeds were inoculated with Alternaria alternata. The standard check was seed dressing with seed plus fungicide. The treated seeds were placed in a medium infested with A. alternata and allowed to germinate and thrive for three weeks. Brown spot disease early symptoms were monitored in growing seedlings. Germination percentage, root length (cm), shoot length (cm), number of leaves, root volume (cm^3), fresh root weight (g), disease incidence (%), and disease severity (%) were all recorded. Minitab Statistical software was used for the data analysis while the collected data were subjected to two-way ANOVA to assess for significance among treatments. The findings revealed that TH only had the highest germination percentage, plant height and number of leaves while TH + BS recorded the highest root length, root volume and fresh root weight. Both sole and combined TH and BS improved plant health and growth relative to other treatments including NC while NC had the highest disease incidence and severity. TH + BS is recommended as a sustainable substitute for chemical fungicide in the control of rice brown spot disease caused by Alternaria alternata.

Keywords: Rice, Alternaria alternata, Trichoderma harzianum, Bacillus subtilis, biological control

Introduction

Sufficient production of rice (*Oryza sativa*), one of the most significant cereal crops and staple food dependent upon by over half of the world's population is threatened by diseases. A number of pathogens, such as bacteria, viruses, and fungus, make it difficult to grow rice particularly in Nigeria (Nalley *et al.,* 2016; Mizubuti and Valent, 2006; Liu, 2021). Of these, *Alternaria alternata*, the causative agent of brown spot disease, is a major hazard to rice production, it lowers grain quality and reduces yield. Tsuge *et al.*, (2013) in his research revealed that *Alternaria alternata* generates toxins that contribute to its pathogenicity and capacity to cause plant disease.

In Nigeria, a significant amount of rice is imported since domestic output has not kept up with the demand due to the problem of disease. The use of chemical fungicides to control plant diseases purposely to improve crop quality and reduce yield loss has raised serious concerns regarding the sustainability of the environment and the associated potential health hazards (Law *et al*., 2017; Agbowuro *et al*., 2020).

Appropriate disease management techniques including the use of biological control technologies are garnering greater attention due to their sustainability and ecologically friendly properties. The use of biological agents like *Trichoderma harzianum* and *Bacillus subtilis* have been discovered as viable options due to their capacity to effectively controls plant disease and increase plant health (Firduet *et al*., 2022). Trichoderma colonizes treated seeds' rhizosphere and root surface, preventing fungal infections and promoting plant development and yield (Brotman *et al*., 2012). Elkomy, (2015) in his report revealed that plant infections can be eradicated by the use of *Trichoderma harzianum*. It was further revealed that *Trichoderma harzianum* improves germination, vigor, seedling development, vegetative growth, photosynthetic rates, stomatal conductance, internal CO_2 concentration, and water use efficiency in addition to its ability to manage disease (Doni *et al*., 2014).

Bacillus subtilis is a spore-forming, Gram-positive bacteria that has been shown to be an effective biocontrol agent in agriculture because of its capacity to promote plant growth, inhibit plant diseases, and enhance soil health. The role of conserved genes that mediate biofilm formation in giving plant pathogen resistance was emphasized by Chen *et al*. (2012) as they used *Bacillus subtilis* isolates to study the biocontrol of tomato wilt disease. Understanding how *Bacillus subtilis* shields plants against bacterial diseases is crucial. Hardy endospores and antibiotics produced by Bacillus species, especially *Bacillus subtilis*, are well-known for controlling a broad range of plant diseases. The versatility of Bacillus species as biocontrol agents was investigated by Shafi *et al.,* (2017) who emphasized how they can control plant diseases by producing antimicrobial compounds. The capacity of *Bacillus subtilis* to decrease diseases and encourage plant growth has also been investigated. It has been discovered that the bacterial strains enhance plant health, suppress soil-borne diseases, and encourage tomato plant development. The beneficial benefits of *Bacillus subtilis* on tomato plants were demonstrated by Qiao *et al*., (2017) highlighting the bacteria's potential as a biocontrol agent for soil-borne diseases. According to Rais *et al*., (2017) the bacterium is acknowledged as a good biocontrol agent against a variety of phytopathogens by establishing systemic resistance and enhancing efficacy through consistency in field settings. Numerous metabolites produced by *Bacillus subtilis*, such as hydrolytic enzymes (HE), lipopolysaccharides (LPS), salicylic acid (SA), siderophores, and antibiotics, either directly or indirectly reduce pathogens by strengthening plant defenses. The goal of this study is to determine whether *T. harzianum* and *B. subtilis* are effective in managing rice brown spot disease in order to create a safe and sustainable management plan.

Materials and Methods

Experimental Site Description

The study was carried out at the Federal University of Technology's Department of Crop, Soil, and Pest Management Laboratory in Akure, Ondo State, Nigeria (7°16'N and 5°12'E). Akure is located in Nigeria's rain forest region, which experiences an average 1300–1600 mm of rainfall annually and 27°C temperatures. The relative humidity is less than 60 % during the dry season and between 85 and 100 % during the wet season. The elevation of Akure is roughly 351 meters above sea level. The rainfall pattern is bimodal, peaking in June and July and September, with a brief dry spell in August in between.

Experimental Procedure and Treatments Application

The FARO 44 rice cultivar's certified seeds were acquired from the International Institute of Tropical Agriculture (IITA). The floating method was used to evaluate the seeds for germination. Department of

Crop, Soil, and Pest Management at Federal University of Technology, Akure provided the *Bacillus subtilis* and *Trichoderma harzianum*. After being isolated from contaminated rice seeds, the pathogen *Alternaria alternata* was cultivated on potato dextrose agar (PDA). Rice seeds were inoculated with *Alternaria alternata* spore suspensions and subjected to the treatments involving sole and combination of *T. harzianum* and *B. subtilis* to produce the following six treatments: Priming with *T. harzianum* (10^5/s/ml), priming with *B. subtilis* (10^4/cfu/ml), priming with *B. subtilis* + *T. harzianum,* standard check (seed dressing with Seed Plus), negative control (priming with water), *and* positive control (priming with water only). The experiment was arranged in a completely randomized design (CRD) with three replications

Determination of Disease incidence (DI, %) **and severity** (DS, %)

Disease incidence was determined using the formular:

$$\text{Disease incidence} = \frac{\text{No of infected leaves}}{\text{Total number of leaves}} \times 100$$

The disease severity was assessed by using 0 to 5 rating scale (Shahzad and Bhat, 2005). Five disease categories were made on the basis of percent leaf area infected.

Disease severity (DS) was calculated by using formula:

$$\text{Disease Severity} = \frac{\Sigma\,(n \times v)}{N \times G} \times 100$$

Where, Σ=Summation; n = Number of leaves in each category; v = Numerical value of each category; N = Total Number of leaves examined; G = Maximum numerical value.

The disease severity (DS, %) rating is based on these five scales of no infection (1); poorly infected (2); slighthly infected (3); strongly infected (4) and severely infected (5).

Data Collection and Analysis

Data were collected on germination percentage, root length (cm), shoot length (cm), number of leaves, root volume (cm^3), fresh root weight (g), disease incidence (%) and severity (%). All data were analyzed using Minitab Statistical Software. The collected data were subjected to two-way ANOVA to assess for significance among treatments.

Results and Discussions

***Trichoderma harzianum* (TH) and *Bacillus subtilis* (BS) seed priming's effects on germination, plant height and root length of rice**

The findings on the impact of *Trichoderma harzianum* (TH) and *Bacillus subtilis* (BS) on the laboratory-grown rice germination rate, plant height (cm), and root length (cm) are shown in Table 1. In comparison to the negative control (NC), all of the tested treatments markedly increased the rice's germination rates, plant height, and root length. When compared to other treatments, *Trichoderma harzianum* (TH only) notably had the highest germination rate and plant height (92.83 % and 24.11 cm respectively), while BS + TH recorded the maximum root length (13.75 cm). At a significance level of 5 %, the data for plant height and germination percentage acquired for PC did not differ substantially from those recorded for BS only and BS + TH. Both the negative control (NC) and standard check (SC) exhibited significantly lower germination rates (78.33 and 75.33, respectively) compared to other treatments.

Table 1: *Trichoderma harzianum* (TH) and *Bacillus subtilis* (BS) seed priming's effects on germination, plant height and root length of rice.

Treatments	Germination Rate (%)	Plant Height (cm)	Root Length (cm)
TH Only	92.83a	24.11a	12.67ab
BS Only	88.17b	17.60b	11.80bc
BS + TH	86.67c	15.28bc	13.75a
SC	78.33d	15.03bc	10.35c
PC	87.33bc	17.95b	11.93b
NC	75.33e	14.00c	5.15d

Values followed by similar letters under the same column are not significantly different at p = 0.05. TH = *Trichoderma harzianum,* BS = *Bacillus subtillis*, SC = Standard check, PC = Positive control, NC = Negative control

***Trichoderma harzianum* (TH) and *Bacillus subtilis* (BS) seed priming's effects on number of leaves, root volume (cm³), and fresh root weight (g) of rice**

Table 2 displays the findings on the impact of *Trichoderma harzianum* (TH) and *Bacillus subtilis* (BS) on fresh root weight, root volume, and leaf count. The greatest result for the number of leaves (6.17) was recorded for TH only was followed by SC. There was no discernible difference among the results recorded for BS only, BS + TH, and PC on the number of leaves while NC recorded with the least number of leaves. BS in combination with TH obtained the highest values of root volume and fresh root weight and they were significantly higher than the results obtained for other treatments. The results on root volume and fresh root weight recorded for TH only and PC were not significantly different from each other but significantly higher than other treatments while NC had the least value of number of leaves, root volume and fresh root weight.

Table 2: *Trichoderma harzianum* (TH) and *Bacillus subtilis* (BS) seed priming's effects on number of leaves, root volume (cm³), and fresh root weight (g) of rice

Treatments	No. of Leaves	Root Volume (cm³)	Fresh Root Weight (g)
TH Only	6.17a	47.21b	63.50b
BS Only	2.67bc	41.33c	52.83c
BS + TH	2.67bc	66.61a	118.83a
SC	3.00b	35.67d	32.50d
PC	2.67bc	46.00b	59.25b
NC	1.67c	19.75e	13.67e

Values followed by similar letters under the same column are not significantly different at p = 0.05. TH = *Trichoderma harzianum,* BS = *Bacillus subtillis*, SC = Standard check, PC = Positive control, NC = Negative control, NL= Number of leaves, RV = Root volume, FRW = Fresh root weight.

***Trichoderma harzianum* (TH) and *Bacillus subtilis* (BS) seed priming's effects on dry root weight, disease incidence, and disease severity**

Combined *Trichoderma harzianum* (TH) and *Bacillus subtilis* (BS) produced the highest dry root weight (60.50g) and it was far higher than all of the studied treatments. Dry root weight recorded for TH alone (37.07 g) was significantly higher than what obtained for BS Only and PC (27.38 g and 24.00g) respectively. Although, BS Only and PC were not significantly different from each other but showed Significant increase compared with the closed dry root weight recorded for SC and NC. Over the course of the laboratory experiment, NC had the lowest values of dry root weight, highest values of disease incidence and disease severity (6.17g, 29.20 and 1.67 %, respectively).

Table 3: *Trichoderma harzianum* (TH) and *Bacillus subtilis* (BS) seed priming's effects on dry root weight, disease incidence and disease severity

Treatments	Dry Root Weight (g)	Disease Incidence (%)	Disease Severity (%)
TH Only	37.07b	7.51b	1.00b
BS Only	27.38c	7.72b	1.00b
BS + TH	60.50a	0.90c	1.00b
SC	12.33d	7.51b	1.00b
PC	24.00c	0.52c	1.00b
NC	6.17e	29.20a	1.67a

Values followed by similar letters under the same column are not significantly different at p = 0.05. TH = *Trichoderma harzianum,* BS = *Bacillus subtillis*, SC = Standard check, PC = Positive control, NC = Negative control, DRW = Dry root weight, DI = Disease incidence, DS = Disease severity.

Discussion

Synthetic chemicals are increasingly being used to cure plant diseases, although this practice is known to have detrimental impacts on human, animal, and environmental health. A safe and efficient method for controlling soil and foliar diseases in a range of crops is the biological control technique. This study focuses on the ability of *Bacillus subtilis* and *Trichoderma harzianum* as biological agents to control rice brown spot disease by triggering a plant defense response. The treated rice plants (i.e., the isolates and the combination of the isolates) were subjected to data collection on germination %, plant height, root length, number of leaves, root volume, fresh root weight, dry root weight, disease incidence, and infection severity. The findings of this investigation demonstrated the efficacy of *Bacillus subtilis* and *Trichoderma harzianum* in lowering the frequency and intensity of rice brown spot disease. The capacity of both biocontrol agents to reduce disease incidence and severity made them beneficial in increasing germination rates and encouraging healthy plant growth when compared to the standard check (SC) and negative control (NC). Alfredo *et al*. (2011) found that the use of biocontrol agents significantly increases rice germination, vigor, seedling growth, vegetative growth, photosynthetic rate, stomatal conductance, internal CO_2 concentration, and water use efficiency. According to Brotman, *et al*., (2012), Trichoderma spp. can colonize the rhizosphere and root surface of treated seeds, shielding them against fungal diseases and promoting plant development and yield. According to Puyam (2016), Trichoderma spp. have been found to be an eco-friendly biological control agent for plant diseases, enabling a decrease in the use of chemical fungicides. The capacity of Trichoderma species to develop cell walls that can break down enzymes when they identify other plant pathogenic organisms—especially fungi—is well recognized. The bacterium genus Bacillus is widely distributed and has been used as a biocontrol agent (Devi *et al*., 2022). The capacity of Bacillus species to produce endospores, which confers resistance to severe environmental conditions, makes them suitable candidates for biocontrol agents. Because of its antagonistic activity, Bacillus may create siderophores and extracellular metabolites (Miljaković *et al*. 2020).

Conclusion

Trichoderma harzianum and *Bacillus subtilis* have the potential to be sustainable substitutes for chemical fungicides in the treatment of rice brown spot disease, according to this study. When used separately and together, these treatments greatly decreased the frequency and intensity of disease while encouraging plant development. Utilizing these biocontrol agents presents a viable approach to environmentally responsible disease control in rice farming systems.

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