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Original Article

Protection of Sorghum Seedlings by Inoculums and Metabolites of Growth Promoting against Plant Pathogens

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Abstract

Introduction: Plant diseases result in significant agricultural losses. Traditional methods to control these diseases, such as chemical pesticides and pathogen-resistant cultivars, have significant drawbacks, including deleterious environmental consequences. This study aimed to assess the protective potentials of inoculums and metabolites from growth-promoting rhizobacterial strains against sorghum seedlings infected by plant pathogens.

Materials and Methods: Fifteen rhizobacterial inoculums and metabolites were tested against five plant pathogens (*Alternaria* sp., *Aspergillus niger*, *Corynespora* sp., *Fusarium oxysporum*, and *Xanthomonas campestris*) in this study. Four treatment groups were used for the study; infected-only, metabolite- or inoculum-treated-only, infected-treated, and control-group seeds. Following planting, the final germination percentage and the vigor index of seeds in the respective groups were calculated.

Results: In general, all the pathogens showed infectivity on the sorghum seeds. In all cases, significantly higher germination percentages and vigor index values were recorded for the treated-only and the infected-treated seeds when compared with the infected-only and the control setups. This was a constant observation irrespective of the pathogens used to simulate infection or the metabolites or inoculums used for treatment.

Conclusions: The study highlights the potential of using rhizosphere bacterial strains, particularly PGPR, as biocontrol agents against important plant pathogens. Treating infected seeds with the inoculum or metabolite of these rhizobacterial strains improved seed germination and seedling vigor index compared to the infected, untreated seeds.

Keywords: Biopesticide, Phytopathogens, Rhizosphere, Plant Diseases, Plant Pathogens, Seed Infection, Seed Treatment

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Introduction

Plant diseases result in huge losses in agricultural production,[1] and about 20-40% of yield losses have been ascribed to plant pathogens.[2] Conventional or traditional methods for disease management include the application of chemical pesticides, the use of pathogen-resistant cultivars, and crop rotation systems.[3] They usually require field application, which involves a lot of time and labor to implement, and this is typically the case in resource-poor countries. Perhaps, more importantly, the intensification of chemical pesticides can result in negative impacts on health and the environment, contributing heavily to ecological imbalance[4] that can vitiate environmental sustainability.

Although chemical pesticide use can promote plant productivity and yield, it can also cause damage to soil, animal, and human health.[5] The non-target drift of chemical pesticides can decrease photosynthetic rates and seed production capacity.[6] Consumption of these toxic pesticides, when they move up the food chain, can cause human diseases.[7,8] Their application can make the soil fragile and inelastic, decreasing soil respiration and activities of soil macrofauna.[9,10] They can also inhibit the growth-promotion potential of microbial species.[11]

Biopesticides are natural products of plant, animal, or microbial origin with antagonistic or suppressive activity against phytopathogens.[12,13] Microbial pesticides are emerging as an attractive alternative to chemical pesticides. They tend to be low-cost, eco-friendly, target-specific, and do not produce greenhouse gases,[14] and they carry reduced risks.[15] Hence, they can be incorporated into agricultural production in an integrated crop management system (ICMS).[16] Biological control, as an essential component of ICMS, involves using microorganisms for plant growth promotion and protection against pathogens.[17]

Around 57 of 356 bio-pesticidal products have a microbial origin in the USA.[13] There is a growing interest in the exploitation of the microbial world to create biopesticides. The rhizosphere, with a bacterial population 10-1000 times that of bulk soil, holds a rich trove of plant-beneficial

microorganisms.[18] Species of *Pseudomonas* and *Bacillus* have been used as biocontrol agents for plant pathogens.[15,19]

Sorghum [*Sorghum bicolor* (L.)], a member of the economically-important clade Panicoideae, serves various food and industrial needs.[20,21] It is a popular food in many areas of the world, and it is well-adapted to nutrient-deficient soil and dry climate,[22-24] and it is the fifth most-important crop based on production tonnage and cultivated area.[25]

Seed-plant transmission can have significant implications for agriculture, as infected seeds can serve as a primary infection source for crops. It can result in the dissemination of pathogens over large areas and contribute to the persistence and recurrence of diseases from one growing season to another. Seed treatment involves the application of the inoculum or product of the beneficial strain to the seeds. Alabouvette *et al*.[20] stated that using beneficial microorganisms as biocontrol agents involves screening them for pathogen control activity before investigating their mechanisms of action and determining an effective delivery method that ensures optimal efficiency. Since many PGPRs also possess biocontrol potential,[21] this study aimed to assess the protective potentials of inoculums and metabolites from growth-promoting rhizobacterial strains against sorghum seedlings infected by plant pathogens.

Materials and Methods

Source and Viability Testing of Sorghum Seeds

The sorghum seeds used for the study were obtained from a local market in Ado-Ekiti, Ekiti State, Nigeria. The seeds were identified and authenticated at the Herbarium of the University of Ilorin, Kwara State, Nigeria, and a voucher number (UILH/002/1489/2022) was obtained.

Before use, the sorghum seeds from a seed lot were tested for viability. Initially, a large quantity of surface-sterilized seeds was placed in a 1-L beaker filled with water. The seeds that floated to the surface were discarded and deemed to have failed the preliminary viability test. The remaining seeds were tested further for germination potential by planting seven seeds (four replicates) in transparent plastic containers that contained absorbent cotton wool to serve as blotters and incubated under fluorescent light for 5 d at ambient temperature to observe for germination. Seeds from a lot were regarded as viable if at least 70% germination was obtained. Seed lots with less than 70% germination were discarded and not used.

Bacterial Inoculums and Metabolites Used

Fifteen bacterial inoculums and metabolites were used in the study. The bacterial strains were isolated from rhizospheres within Afe Babalola University in Ado-Ekiti, Nigeria. The bacterial strains were isolated using the standard pour plating procedure and identified using polymerase chain reaction and 16S rRNA gene sequencing techniques. All sequences were deposited in the National Centre for Biotechnology Information (NCBI) database and accession numbers were obtained.

Preparation of the respective inoculums was carried out by subculturing each of the pure strains in sterile nutrient broth at 25 ^{o}C for 48 h. Metabolite extraction was carried out using the cold-extraction method as reported by Akpor *et al*.[22] For metabolite extraction, the broth culture was centrifuged at 5000 rpm for 15 min to obtain cell-free supernatant, which was acidified to a pH of 2 by adding 1 M HCl. To the acidified supernatant, an equal volume of methanol: ethyl acetate (2:1) was added and incubated for 24 h at 4 ^{o}C ± 2 ^{o}C. Following incubation, the mixture was transferred to a separating funnel to separate the solvent phase and the broth phase.

The crude metabolite was dried by placing the beaker containing the extracting solvents in a water bath at 80 ^{o}C. The dried metabolite was then quantified and stored in clean universal bottles at 4 °C ± 2 ^{o}C until needed. For the metabolites, concentrations of 1000 mg/L were used for the study.

Plant Pathogens

Five plant pathogens (*Alternaria* sp., *Aspergillus niger*, *Corynespora* sp., *Fusarium oxysporum*, and *Xanthomonas campestris*) were used for the study. The pathogens were obtained from the International Institute for Tropical Agriculture (IITA), Ibadan, Nigeria.

To ascertain their purity, the pathogens were first sub-cultured in nutrient agar and potato dextrose agar plates, for the bacteria and fungi, respectively. Only isolates that showed single colonies in plates were used for the study. All isolates were stored in nutrient agar (for the bacterium) and potato dextrose agar (for the fungi) slants.

For the infectivity study, the isolates were cultured in nutrient broth (for bacteria) and potato dextrose broth (for fungi) and incubated for 48 and 96 h, respectively. Infectivity study on the seeds by the isolates was carried out by steeping the seeds for known durations in the broth cultures of the pathogens before planting.

Experimental Setup

Four treatment groups were used for the study, which were I) Seeds steeped in broth cultures of the pathogens without treatment, known as the infected-only seeds; II) Seeds steeped in the respective inoculums or metabolites, known as the treated-only seeds; III) Seeds first steeped in broth cultures of the pathogens before treatment in the respective inoculums or metabolites, known as the infected-treated seeds; IV) Seeds steeped first infected in broth cultures of the pathogens before treatment in water, known as the control group. Generally, all the seeds were steeped for a duration of 2 h. The infected-only seeds and treated-only

seeds were steeped for a 2 h duration in the respective broth cultures of the pathogens and the inoculums/metabolites, respectively. For all treatments, seven seeds were withdrawn and planted in transparent plastic cups containing blotters and incubated for eight (8) days. In all the setups, at the expiration of the incubation period, final germination percentage and seedling vigor index were estimated. All experimental setups were carried out in quadruplicate.

Statistical Analysis

Data were presented as means ± standard deviation. Comparison of means was determined using the Student's Independent T-test at 95% confidence level. Statistical analysis was carried out using the Statistical Package for Social Scientists (SPSS) version 23.0.

Results

In general, all the pathogens were observed to show infectivity on the sorghum seeds. In all cases, significantly higher final germination percentage and vigor index values were recorded for the treated-only and the infected-treated seeds when compared with the infected-only and the control setups. This observation was constant irrespective of the pathogen used or the metabolite or inoculum used for treatment.

Germinability of the alternaria-infected Seeds

For the *Alternaria* sp.-infected seeds, significantly higher final germination values were recorded in the treated-only seeds in the presence of some of the inoculums. However, final germination percentages higher than 70% were recorded for the treated-only and the infected then-treated seeds in the presence of the respective inoculums, except for the infected then-treated seeds in the presence of *B. cereus* (OP830502) that showed a value of 64.29% value. In the case of vigor index values, although higher values were recorded in the treated-only seeds than the infected then treated ones, these differences were observed to be significant in the presence of inoculums of *B. cereus* (OP830493), *B. cereus* (OP830500), *B. cereus* (OP830493), *B. cereus* (OP830495), *P. rettgeri* (OP830497), *S. liquefaciens* (OP830504), and *S. liquefaciens* (OP830503) (Table 1).

In the presence of the respective metabolites, final germination values higher than 70% were recorded in both the treated-only and the infected-treated seeds, except for seeds treated with metabolites from *B. cereus* (OP830493), *B. cereus* (OP830495), *P. vermicola* (OP830490), *B.* cereus (OP830499), *S. liquefaciens* (OP830504), and *B. cereus* (OP830501) that showed values of 64.29, 57.14, 64.29, 64.29, 57,17, and 64.29%, respectively, for the infected-treated setup. In the case of vigor index, significantly higher values were observed for the treated-only seeds than the infected-treated ones, except for seeds treated with metabolites

Table 1. Germinability of the Sorghum Seeds Infected with the *Alternaria* sp. in the Presence of the Inoculums and Metabolites

Treatment	Inoculum				Metabolite			
	% germination		Vigor index		% germination		Vigor index	
	Treated only	Infected & treated	Treated only	Infected & treated	Treated only	Infected & treated	Treated only	Infected & treated
B. cereus (OP830500)	92.86^{a} ±8.25	100.00^{a} ±0.00	609.29^{a} ±139.39	495.71^{a} ±13.20	92.86^{a} ±8.25	85.71^{a} ±16.50	452.04^{a} ±15.79	457.60^{a} ±128.96
B. cereus (OP830493)	92.86^{a} ±8.25	92.86^{a} ±8.25	648.78^{a} ±118.53	499.69^{a} ±147.17	92.86^{a} ±8.25	64.29^{b} ±8.25	490.00^{a} ±21.44	236.89^{b} ±88.78
B. cereus (OP830502)	100.00^{a} ±0.00	64.29^{b} ±8.25	617.86^{a} ±42.06	292.76^{b} ±63.74	100.00^{a} ±0.00	78.57^{b} ±8.25	565.00^{a} ±76.71	286.53^{b} ±19.79
B. thuringiensis (OP830494)	100.00^{a} ±0.00	92.86^{a} ±8.25	686.43^{a} ±20.62	485.31^{b} ±158.83	92.86^{a} ±8.25	71.43^{b} ±0.00	452.86^{a} ±315.07	331.38^{a} ±15.02
B. cereus (OP830495)	92.86^{a} ±8.25	71.43^{a} ±16.50	601.94^{a} ±108.28	395.31^{a} ±164.25	100.00^{a} ±0.00	57.14^{b} ±16.50	514.29^{a} ±16.50	158.93^{b} ±66.87
P. vermicola (OP830490)	92.86^{a} ±8.25	85.71^{a} ±16.50	667.76^{a} ±126.31	386.94^{b} ±103.33	92.86^{a} ±8.25	64.29^{b} ±8.25	532.45^{a} ±84.60	220.20^{b} ±49.72
P. rettgeri (OP830497)	92.86^{a} ±8.25	71.43^{b} ±0.00	385.51^{a} ±125.60	329.08^{a} ±13.55	78.57^{a} ±8.25	78.57^{a} ±24.74	520.61^{a} ±66.22	396.89^{a} ±225.46
P. rettgeri (OP830496)	92.86^{a} ±8.25	71.43^{a} ±16.50	545.20^{a} ±18.73	345.92^{b} ±125.13	85.71^{a} ±0.00	71.43^{a} ±16.50	546.73^{a} ±33.23	272.04^{b} ±91.67
P. vermicola (OP830492)	92.86^{a} ±8.25	85.71^{a} ±0.00	580.71^{a} ±56.91	335.51^{b} ±35.35	100.00^{a} ±0.00	71.43^{b} ±0.00	584.29^{a} ±34.64	375.51^{b} ±51.84
B. cereus (OP830499)	100.00^{a} ±0.00	78.57^{b} ±8.25	547.14^{a} ±74.23	376.28^{b} ±70.99	100.00^{a} ±0.00	64.29^{b} ±8.25	567.14^{a} ±79.18	254.74^{b} ±83.48
S. liquefaciens (OP830504)	92.86^{a} ±8.25	85.71^{a} ±0.00	669.39^{a} ±81.54	458.57^{b} ±10.60	92.86^{a} ±8.25	57.14^{b} ±16.50	420.20^{a} ±161.42	207.35^{a} ±109.34
S. liquefaciens (OP830503)	92.86^{a} ±8.25	85.71^{a} ±16.50	558.88^{a} ±13.55	424.90^{a} ±123.01	100.00^{a} ±0.00	71.43^{b} ±0.00	547.86^{a} ±33.82	205.10^{b} ±34.17
P. rettgeri (OP830491)	100.00^{a} ±0.00	85.71^{b} ±0.00	620.00^{a} ±11.55	581.63^{a} ±63.63	100.00^{a} ±0.00	71.43^{b} ±16.50	675.00^{a} ±94.85	310.31^{b} ±121.71
P. rettgeri (OP830498)	100.00^{a} ±0.00	85.71^{b} ±0.00	613.57^{a} ±70.11	448.78^{b} ±50.19	85.71^{a} ±0.00	78.57^{a} ±8.25	488.57^{a} ±125.84	337.76^{a} ±40.06
B. cereus (OP830501)	92.86^{a} ±8.25	71.43^{b} ±0.00	647.04^{a} ±52.90	325.26^{b} ±55.08	92.86^{a} ±8.25	64.29^{b} ±8.25	533.37^{a} ±9.31	230.31^{b} ±43.95

The final germination percentage and the seedling vigor index of the infected-only seeds were 21.00 ± 10.10 and 3.47 ± 4.32, respectively. Values are mean ± standard deviation. Values with similar and different superscripts represent "not significantly" and "significantly" different, respectively.

Table 2. Germinability of the Sorghum Seeds Infected with the *Aspergillus niger* in the Presence of the Inoculums and Metabolites

Treatment	Inoculum				Metabolite			
	% germination		Vigor index		% germination		Vigor index	
	Treated only	Infected & treated	Treated only	Infected & treated	Treated only	Infected & treated	Treated only	Infected & treated
B. cereus (OP830500)	92.86^{a} ±8.25	78.57^{a} ±24.74	609.29^{a} ±139.39	286.73^{b} ±114.29	92.86^{a} ±8.25	71.43^{a} ±16.50	452.04^{a} ±15.79	180.41^{b} ±82.95
B. cereus (OP830493)	92.86^{a} ±8.25	64.29^{b} ±8.25	648.78^{a} ±118.53	301.84^{b} ±73.29	92.86^{a} ±8.25	57.14^{b} ±16.50	490.00^{a} ±21.44	178.16^{b} ±101.80
B. cereus (OP830502)	100.00^{a} ±0.00	85.71^{a} ±16.50	617.86^{a} ±42.06	439.69^{b} ±130.67	100.00^{a} ±0.00	64.29^{b} ±24.74	565.00^{a} ±76.71	247.65^{b} ±215.98
B. thuringiensis (OP830494)	100.00^{a} ±0.00	85.71^{a} ±16.50	686.43^{a} ±20.62	344.39^{b} ±113.70	92.86^{a} ±8.25	50.00^{b} ±8.25	452.86^{a} ±315.07	125.82^{a} ±52.67
B. cereus (OP830495)	92.86^{a} ±8.25	71.43^{b} ±0.00	601.94^{a} ±108.28	322.96^{b} ±41.83	100.00^{a} ±0.00	57.14^{b} ±0.00	514.29^{a} ±16.50	139.59^{b} ±34.88
P. vermicola (OP830490)	92.86^{a} ±8.25	85.71^{a} ±16.50	667.76^{a} ±126.31	227.65^{b} ±39.00	92.86^{a} ±8.25	71.43^{a} ±16.50	532.45^{a} ±84.60	268.37^{b} ±90.26
P. rettgeri (OP830497)	92.86^{a} ±8.25	78.57^{b} ±8.25	385.51^{a} ±125.60	290.10^{a} ±72.23	78.57^{a} ±8.25	71.43^{a} ±16.50	520.61^{a} ±66.22	235.92^{b} ±112.17
P. rettgeri (OP830496)	92.86^{a} ±8.25	71.43^{b} ±0.00	545.20^{a} ±18.73	285.71^{b} ±10.60	85.71^{a} ±0.00	85.71^{a} ±16.50	546.73^{a} ±33.23	409.08^{a} ±133.03
P. vermicola (OP830492)	92.86^{a} ±8.25	92.86^{a} ±8.25	580.71^{a} ±56.91	399.49^{b} ±117.71	100.00^{a} ±0.00	64.29^{b} ±8.25	584.29^{a} ±34.64	215.20^{b} ±59.03
B. cereus (OP830499)	100.00^{a} ±0.00	92.86^{a} ±8.25	547.14^{a} ±74.23	576.63^{a} ±114.41	100.00^{a} ±0.00	64.29^{b} ±8.25	567.14^{a} ±79.18	213.98^{b} ±51.02
S. liquefaciens (OP830504)	92.86^{a} ±8.25	85.71^{a} ±0.00	669.39^{a} ±81.54	469.59^{b} ±36.05	92.86^{a} ±8.25	78.57^{a} ±24.74	420.20^{a} ±161.42	292.24^{a} ±177.21
S. liquefaciens (OP830503)	92.86^{a} ±8.25	85.71^{a} ±0.00	558.88^{a} ±13.55	382.04^{b} ±12.73	100.00^{a} ±0.00	71.43^{b} ±0.00	547.86^{a} ±33.82	322.45^{b} ±17.67
P. rettgeri (OP830491)	100.00^{a} ±0.00	78.57^{b} ±8.25	620.00^{a} ±11.55	465.51^{b} ±115.71	100.00^{a} ±0.00	78.57^{a} ±24.74	675.00^{a} ±94.85	297.76^{b} ±154.35
P. rettgeri (OP830498)	100.00^{a} ±0.00	78.57^{b} ±8.25	613.57^{a} ±70.11	389.08^{b} ±78.12	85.71^{a} ±0.00	78.57^{a} ±8.25	488.57^{a} ±125.84	116.63^{b} ±53.38
B. cereus (OP830501)	92.86^{a} ±8.25	71.43^{b} ±0.00	647.04^{a} ±52.90	296.94^{b} ±49.49	92.86^{a} ±8.25	64.29^{b} ±8.25	533.37^{a} ±9.31	152.96^{b} ±53.14

The final germination percentage and the seedling vigor index of the infected-only seeds were 14.29.00 ± 0.00 and 4.39 ± 0.43, respectively. Values are mean ± standard deviation. Values with similar and different superscripts represent "not significantly" and "significantly" different, respectively.

from *B.* cereus (OP830500), *B. thuringiensis* (OP830494), *P. rettgeri* (OP830497), *S. liquefaciens* (OP830504), and *P.* rettgeri (OP830498) where no significant difference was observed between the two setups (Table 1).

Germinability of the Aspergillus niger-infected Seeds

When the seeds were infected with *Aspergillus niger*, no significant difference was observed between the treated-only and the infected-treated seeds. This was a constant observation irrespective of the inoculum used for treatment. However, significantly higher vigor index values were recorded in the treated-only seeds than the infected-treated ones in the presence of the respective inoculums, except for seeds treated with inoculums of *P. rettgeri* (OP830497) and *B. cereus* (OP830499), where there was no significant difference between the two groups. (Table 2).

In the presence of the metabolites, significantly higher final germination and vigor index values were recorded for the treated-only seeds than the infected-treated seeds. The vigor index values of the treated-only and infected-treated seeds showed no significant difference in the presence of metabolites from *B. thuringiensis* (OP340494), *P. rettgeri* (OP830496), and *S. liquefaciens* (OP830504) (Table 2).

Germinability of the corynespora-infected Seeds

Generally, for the *Corynespora* sp.-infected seeds, significantly higher final germination and vigor index values were observed for the treated-only seeds than the infected-treated seeds in the presence of most of the inoculums and metabolites. However, there was no significant difference between the treated-only and the infected-treated seeds in the presence of inoculums of *B. cereus* (OP830493), *B. thuringiensis* (OP340494), *P. rettgeri* (OP830496), *P. vermicola* (OP830492), *B. cereus* (OP830499), and *B. cereus* (OP830501), for final germination percentage and inoculums of *B. cereus* (OP830493), *B. cereus* (OP830500), *P. rettgeri* (OP830497), and *B. cereus* (OP830501), for vigor index (Table 3). There was no significant difference in vigor index in the presence of metabolites from *B. thuringiensis* (OP340494), *B. cereus* (OP830495), *P. rettgeri* (OP830496), *S. liquefaciens* (OP830504), and *P. rettgeri* (OP830498) (Table 3).

Germinability of the Fusarium oxysporum-infected Seeds

In the presence of the inoculum of the respective isolates, seeds infected with the *Fusarium oxysporum* showed significantly higher final germination and vigor index values for the treated-only seeds than the infected-treated ones. However, there was no significant difference between the treated-only and infected-treated seeds in the presence of inoculums of *S. liquefaciens* (OP830504) and *P. rettgeri* (OP830491), for final germination and *P. rettgeri* (OP830497), *S. liquefaciens* (OP830504), and *P. rettgeri* (OP830491), for vigor index (Table 4).

For metabolite treatment of infected seeds, significantly

Table 3. Germinability of the Sorghum Seeds Infected with the *Corynespora* sp. in the Presence of the Inoculums and Metabolites

Treatment	Inoculum				Metabolite			
	% germination		Vigor index		% germination		Vigor index	
	Treated only	Infected & treated	Treated only	Infected & treated	Treated only	Infected & treated	Treated only	Infected & treated
B. cereus (OP830500)	92.86^{a} ±8.25	92.86^{a} ±8.25	609.29^{a} ±139.39	502.55^{a} ±81.18	92.86^{a} ±8.25	50.00^{b} ±24.74	452.04^{a} ±15.79	122.86^{b} ±96.15
B. cereus (OP830493)	92.86^{a} ±8.25	78.57^{b} ±8.25	648.78^{a} ±118.53	381.53^{b} ±74.11	92.86^{a} ±8.25	64.29^{a} ±24.74	490.00^{a} ±21.44	217.04^{b} ±121.24
B. cereus (OP830502)	100.00^{a} ±0.00	100.00^{a} ±0.00	617.86^{a} ±42.06	448.57^{b} ±80.83	100.00^{a} ±0.00	78.57^{b} ±8.25	565.00^{a} ±76.71	97.24^{b} ±40.41
B. thuringiensis (OP830494)	100.00^{a} ±0.00	78.57^{b} ±8.25	686.43^{a} ±20.62	401.02^{b} ±74.23	92.86^{a} ±8.25	64.29^{b} ±8.25	452.86^{a} ±315.07	177.14^{a} ±26.39
B. cereus (OP830495)	92.86^{a} ±8.25	71.43^{a} ±16.50	601.94^{a} ±108.28	373.06^{b} ±109.34	100.00^{a} ±0.00	85.71^{a} ±16.50	514.29^{a} ±16.50	486.22^{a} ±156.12
P. vermicola (OP830490)	92.86^{a} ±8.25	85.71^{a} ±0.00	667.76^{a} ±126.31	381.43^{b} ±28.99	92.86^{a} ±8.25	78.57^{b} ±8.25	532.45^{a} ±84.60	315.92^{b} ±32.52
P. rettgeri (OP830497)	92.86^{a} ±8.25	92.86^{a} ±8.25	385.51^{a} ±125.60	493.27^{a} ±110.05	78.57^{a} ±8.25	64.29^{b} ±8.25	520.61^{a} ±66.22	333.27^{b} ±38.41
P. rettgeri (OP830496)	92.86^{a} ±8.25	64.29^{b} ±8.25	545.20^{a} ±18.73	258.44^{b} ±109.26	85.71^{a} ±0.00	92.86^{a} ±8.25	546.73^{a} ±33.23	563.88^{a} ±12.02
P. vermicola (OP830492)	92.86^{a} ±8.25	71.43^{b} ±0.00	580.71^{a} ±56.91	436.73^{b} ±17.67	100.00^{a} ±0.00	64.29^{b} ±8.25	584.29^{a} ±34.64	290.41^{b} ±62.92
B. cereus (OP830499)	100.00^{a} ±0.00	64.29^{b} ±8.25	547.14^{a} ±74.23	287.96^{b} ±51.61	100.00^{a} ±0.00	71.43^{b} ±0.00	567.14^{a} ±79.18	343.37^{b} ±28.87
S. liquefaciens (OP830504)	92.86^{a} ±8.25	92.86^{a} ±8.25	669.39^{a} ±81.54	372.24^{b} ±14.14	92.86^{a} ±8.25	71.43^{a} ±16.50	420.20^{a} ±161.42	200.61^{a} ±97.80
S. liquefaciens (OP830503)	92.86^{a} ±8.25	85.71^{a} ±0.00	558.88^{a} ±13.55	297.55^{b} ±63.63	100.00^{a} ±0.00	35.71^{b} ±8.25	547.86^{a} ±33.82	50.92^{b} ±30.99
P. rettgeri (OP830491)	100.00^{a} ±0.00	85.71^{a} ±16.50	620.00^{a} ±11.55	307.76^{b} ±86.72	100.00^{a} ±0.00	100.00^{a} ±0.00	675.00^{a} ±94.85	467.14^{b} ±56.09
P. rettgeri (OP830498)	100.00^{a} ±0.00	92.86^{a} ±8.25	613.57^{a} ±70.11	521.73^{a} ±161.30	85.71^{a} ±0.00	85.71^{a} ±16.50	488.57^{a} ±125.84	308.57^{a} ±224.34
B. cereus (OP830501)	92.86^{a} ±8.25	64.29^{b} ±8.25	647.04^{a} ±52.90	288.16^{b} ±44.30	92.86^{a} ±8.25	92.86^{a} ±8.25	533.37^{a} ±9.31	191.43^{b} ±13.20

The final germination percentage and the seedling vigor index of the infected-only seeds were 0.00 ± 0.00 and 0.00 ± 0.00, respectively. Values are mean ± standard deviation. Values with similar and different superscripts represent "not significantly" and "significantly" different, respectively.

Table 4. Germinability of the Sorghum Seeds Infected with the *Fusarium oxysporum* in the Presence of the Inoculums and Metabolites

Treatment	Inoculum				Metabolite			
	% germination		Vigor index		% germination		Vigor index	
	Treated only	Infected & treated	Treated only	Infected & treated	Treated only	Infected & treated	Treated only	Infected & treated
B. cereus (OP830500)	92.86^{a} ±8.25	71.43^{b} ±0.00	609.29^{a} ±139.39	291.84^{b} ±31.81	92.86^{a} ±8.25	64.29^{b} ±8.25	452.04^{a} ±15.79	198.78^{b} ±20.27
B. cereus (OP830493)	92.86^{a} ±8.25	64.29^{b} ±8.25	648.78^{a} ±118.53	276.22^{b} ±66.34	92.86^{a} ±8.25	50.00^{b} ±8.25	490.00^{a} ±21.44	132.35^{b} ±40.41
B. cereus (OP830502)	100.00^{a} ±0.00	71.43^{b} ±16.50	617.86^{a} ±42.06	313.27^{b} ±117.59	100.00^{a} ±0.00	78.57^{b} ±8.25	565.00^{a} ±76.71	335.92^{b} ±39.12
B. thuringiensis (OP830494)	100.00^{a} ±0.00	50.00^{b} ±8.25	686.43^{a} ±20.62	158.37^{b} ±27.34	92.86^{a} ±8.25	78.57^{b} ±8.25	452.86^{a} ±315.07	315.31^{a} ±38.88
B. cereus (OP830495)	92.86^{a} ±8.25	50.00^{b} ±8.25	601.94^{a} ±108.28	164.80^{b} ±53.85	100.00^{a} ±0.00	85.71^{b} ±0.00	514.29^{a} ±16.50	339.18^{b} ±8.48
P. vermicola (OP830490)	92.86^{a} ±8.25	78.57^{b} ±8.25	667.76^{a} ±126.31	393.47^{b} ±56.09	92.86^{a} ±8.25	78.57^{b} ±8.25	532.45^{a} ±84.60	267.96^{b} ±52.55
P. rettgeri (OP830497)	92.86^{a} ±8.25	78.57^{b} ±8.25	385.51^{a} ±125.60	397.14^{a} ±60.33	78.57^{a} ±8.25	78.57^{a} ±8.25	520.61^{a} ±66.22	305.20^{b} ±59.03
P. rettgeri (OP830496)	92.86^{a} ±8.25	64.29^{b} ±8.25	545.20^{a} ±18.73	250.10^{b} ±54.08	85.71^{a} ±0.00	71.43^{b} ±0.00	546.73^{a} ±33.23	295.41^{b} ±14.73
P. vermicola (OP830492)	92.86^{a} ±8.25	78.57^{b} ±8.25	580.71^{a} ±56.91	424.39^{b} ±45.83	100.00^{a} ±0.00	64.29^{b} ±8.25	584.29^{a} ±34.64	219.69^{b} ±44.42
B. cereus (OP830499)	100.00^{a} ±0.00	64.29^{b} ±8.25	547.14^{a} ±74.23	199.80^{b} ±16.26	100.00^{a} ±0.00	50.00^{b} ±8.25	567.14^{a} ±79.18	138.67^{b} ±43.48
S. liquefaciens (OP830504)	92.86^{a} ±8.25	92.86^{a} ±8.25	669.39^{a} ±81.54	686.73^{a} ±124.19	92.86^{a} ±8.25	85.71^{a} ±0.00	420.20^{a} ±161.42	322.04^{a} ±7.07
S. liquefaciens (OP830503)	92.86^{a} ±8.25	78.57^{b} ±8.25	558.88^{a} ±13.55	458.88^{b} ±73.88	100.00^{a} ±0.00	71.43^{b} ±0.00	547.86^{a} ±33.82	301.02^{b} ±17.67
P. rettgeri (OP830491)	100.00^{a} ±0.00	85.71^{a} ±0.00	620.00^{a} ±11.55	532.04^{a} ±113.82	100.00^{a} ±0.00	92.86^{a} ±8.25	675.00^{a} ±94.85	425.71^{b} ±36.29
P. rettgeri (OP830498)	100.00^{a} ±0.00	78.57^{b} ±8.25	613.57^{a} ±70.11	476.73^{b} ±67.40	85.71^{a} ±0.00	71.43^{b} ±0.00	488.57^{a} ±125.84	343.37^{a} ±30.05
B. cereus (OP830501)	92.86^{a} ±8.25	57.14^{b} ±16.50	647.04^{a} ±52.90	246.12^{b} ±118.77	92.86^{a} ±8.25	64.29^{b} ±8.25	533.37^{a} ±9.31	167.96^{b} ±20.50

The final germination percentage and the seedling vigor index of the infected-only seeds were 0.00 ± 0.00 and 0.00 ± 0.00, respectively. Values are mean ± standard deviation. Values with similar and different superscripts represent "not significantly" and "significantly" different, respectively.

Table 5. Germinability of the *Xanthomonas campestris*-Infected Seeds in the Presence of the Inoculums and Metabolites

Treatment	Inoculum				Metabolite			
	% germination		Vigor index		% germination		Vigor index	
	Treated only	Infected & treated	Treated only	Infected & treated	Treated only	Infected & treated	Treated only	Infected & treated
B. cereus (OP830500)	92.86^{a} ±8.25	85.71^{a} ±0.00	609.29^{a} ±139.39	595.71^{a} ±17.67	92.86^{a} ±8.25	64.29^{b} ±8.25	452.04^{a} ±15.79	160.78^{b} ±78.28
B. cereus (OP830493)	92.86^{a} ±8.25	100.00^{a} ±0.00	648.78^{a} ±118.53	477.14^{b} ±39.59	92.86^{a} ±8.25	78.57^{b} ±8.25	490.00^{a} ±21.44	271.22^{b} ±9.19
B. cereus (OP830502)	100.00^{a} ±0.00	78.57^{b} ±8.25	617.86^{a} ±42.06	406.73^{b} ±104.39	100.00^{a} ±0.00	71.43^{b} ±16.50	565.00^{a} ±76.71	261.02^{b} ±93.08
B. thuringiensis (OP830494)	100.00^{a} ±0.00	92.86^{a} ±8.25	686.43^{a} ±20.62	511.53^{b} ±87.31	92.86^{a} ±8.25	64.29^{a} ±24.74	452.86^{a} ±315.07	246.73^{a} ±136.44
B. cereus (OP830495)	92.86^{a} ±8.25	57.14^{b} ±0.00	601.94^{a} ±108.28	165.71^{b} ±16.02	100.00^{a} ±0.00	78.57^{b} ±8.25	514.29^{a} ±16.50	278.78^{b} ±13.20
P. vermicola (OP830490)	92.86^{a} ±8.25	71.43^{b} ±0.00	667.76^{a} ±126.31	309.69^{b} ±15.91	92.86^{a} ±8.25	71.43^{a} ±16.50	532.45^{a} ±84.60	317.14^{b} ±72.11
P. rettgeri (OP830497)	92.86^{a} ±8.25	92.86^{a} ±8.25	385.51^{a} ±125.60	419.90^{a} ±127.13	78.57^{a} ±8.25	78.57^{a} ±8.25	520.61^{a} ±66.22	233.47^{b} ±31.58
P. rettgeri (OP830496)	92.86^{a} ±8.25	71.43^{a} ±16.50	545.20^{a} ±18.73	290.20^{b} ±60.80	85.71^{a} ±0.00	78.57^{a} ±8.25	546.73^{a} ±33.23	275.82^{b} ±95.79
P. vermicola (OP830492)	92.86^{a} ±8.25	92.86^{a} ±8.25	580.71^{a} ±56.91	457.14^{a} ±92.38	100.00^{a} ±0.00	71.43^{a} ±32.99	584.29^{a} ±34.64	202.04^{b} ±88.37
B. cereus (OP830499)	100.00^{a} ±0.00	85.71^{a} ±16.50	547.14^{a} ±74.23	407.55^{b} ±11.08	100.00^{a} ±0.00	85.71^{b} ±0.00	567.14^{a} ±79.18	443.27^{b} ±35.35
S. liquefaciens (OP830504)	92.86^{a} ±8.25	85.71^{a} ±0.00	669.39^{a} ±81.54	389.39^{b} ±35.35	92.86^{a} ±8.25	64.29^{b} ±8.25	420.20^{a} ±161.42	133.47^{b} ±12.73
S. liquefaciens (OP830503)	92.86^{a} ±8.25	78.57^{b} ±8.25	558.88^{a} ±13.55	520.61^{a} ±73.29	100.00^{a} ±0.00	78.57^{b} ±8.25	547.86^{a} ±33.82	224.49^{b} ±25.92
P. rettgeri (OP830491)	100.00^{a} ±0.00	71.43^{b} ±16.50	620.00^{a} ±11.55	339.80^{b} ±67.16	100.00^{a} ±0.00	85.71^{a} ±16.50	675.00^{a} ±94.85	476.73^{a} ±160.48
P. rettgeri (OP830498)	100.00^{a} ±0.00	42.86^{b} ±16.50	613.57^{a} ±70.11	112.04^{b} ±45.01	85.71^{a} ±0.00	85.71^{a} ±0.00	488.57^{a} ±125.84	409.59^{a} ±10.60
B. cereus (OP830501)	92.86^{a} ±8.25	57.14^{b} ±16.50	647.04^{a} ±52.90	251.22^{b} ±78.71	92.86^{a} ±8.25	71.43^{b} ±0.00	533.37^{a} ±9.31	220.92^{b} ±17.08

The final germination percentage and the seedling vigor index of the infected-only seeds were 21.43 ± 10.10 and 11.33 ± 13.42, respectively. Values are mean ± standard deviation. Values with similar and different superscripts represent "not significantly" and "significantly" different, respectively.

higher final germination and vigor index values were observed for the treated-only seeds than the infected-treated ones. However, no significant difference was observed for infected seeds treated with the metabolites from *P. rettgeri* (OP830497), *S. liquefaciens* (OP830504), and *P. rettgeri* (OP830491), for final germination percentage and *B. thuringiensis* (OP340494), *S. liquefaciens* (OP830504), and *P. rettgeri* (OP830498), for vigor index (Table 4).

Germinability of the Xanthomonas campestris-infected Seeds

There was no significant difference in final germination percentage for the treated-only and the infected-treated seeds in the presence of most of the inoculums. In the presence of the inoculums, final germination percentages of over 70% were observed for seeds in the two setups, except seeds treated with the inoculums of *B. cereus* (OP830501) and *P. rettgeri* (OP830498), which showed values of 42.86 and 57.14%, respectively, for the infected-treated setup. A comparison of the vigor index values showed the highest values in the presence of the most of the inoculums for the treated-only seeds. No significant difference was observed for vigor index values between seeds in the two setups in the presence of the inoculums of of *B. cereus* (OP830493), *B. cereus* (OP830500), *P. rettgeri* (OP830497), *P. vermicola* (OP830492), and *S. liquefaciens* (OP830503) (Table 5).

For the metabolite-treated seeds, final germination values higher than 70% were recorded for the seeds in the treated-only and the infected-treated seeds, apart from those infected seeds treated with the metabolites from *B. cereus* (OP830500) and *B. thuringiensis* (OP830494), which showed final germination of 62.29 % for the infected-treated category. In the case of vigor index, significantly higher values were observed for the treated-only seeds than the infected-treated ones, except for seeds treated with the metabolites from *B. thuringiensis* (OP830494), *P. rettgeri* (OP830491), and *P. rettgeri* (OP830491) (Table 5).

Discussion

The final germination percentage and the seedling vigor index values of the infected-only seeds for the five pathogens were significantly lower than those observed for the treated-only and the infected-treated seeds, respectively, for all comparisons. This is an indication that sorghum seed infection was successfully simulated. Even though some of the pathogens used in this study are not established etiological agents of any disease conditions in sorghum, they significantly reduced the final germination percentage and seedling vigor index values of the infected-only seeds when compared with the treated-only and infected-treated seeds, respectively. An indication that saprophytic fungi can cause seed quality issues,[27] affecting germination and seedling growth. Fungal attacks can occur on the field and during storage.[29]

In this study, the observation of statistical parity between the infected-treated and the treated-only seeds that were both treated with the same rhizobacterial strain was taken to be indicative of the ameliorative effect of the rhizobacterial strains on the infected-treated seeds. All the PGPR strains showed inhibition activity against at least one pathogen, going by the absence of a significant difference between the final germination and, more importantly, seedling vigor of the seeds treated with the PGPR only and infected seeds treated with the PGPR. This further lends credence to the suitability of seed treatment as an effective delivery method for bioinoculants, as highlighted by O'Callaghan.[30] It also shows the effectiveness of these rhizobacterial strains in suppressing seed-borne microorganisms that can produce deleterious effects on germination and development. Gram-positive and Gram-negative bacteria have shown potential as potent biocontrol agents against plant pathogens.[31-33] This present study included seven (7) Gram-positive bacteria and eight (8) Gram-negative bacteria.

In this study, statistical results for inoculum treatment comparisons were not always replicated in the metabolite comparisons. Biocontrol of pathogens is not formulaic but depends on a complex interplay of biocontrol strategies[34] and the interactions between the suppressive agent, pathogen, and the plant material.[35] The absence of the bacterium could produce a different result, as this study indicates. Besides, in this study, only shoot length was measured and it was used to calculate the seedling vigor index. It is possible that a live pathogen preferentially attacks either the shoot or root region of a plant.

Some of the *Bacillus* spp used in this study showed promising results against the infectious agents. Bacteria such as *Bacillus amyloliquefaciens*, *Bacillus velezensis*, and *Paenibacillus polymyxa* showed inhibition potential against *Fusarium thapsinum* isolated from leaves of sorghum with leaf spot.[36] Bacteria isolated from sorghum rhizosphere showed significant antagonistic activity *in vitro* and *in vivo* against the fungi *Pythium ultimum*, the causal agent of root rot in sorghum.[37] Seed treatment of bean seeds with *Bacillus* sp. produced a reduced incidence of root rot disease caused by *Fusarium solani* f. sp. *Phaseoli*.[38]

Serratia liquefaciens (OP830504), one of the two strains of *Serratia* in this study, performed well. In some studies, strains of *Serratia* were effective for the control of phytopathogens against the causal agent (*Fusarium oxysporum*) of *Fusarium* wilt in Banana;[39] and against the causal agent of brown root rot in tea, *Fomes lamaoensis*.[40] A strain of *Serratia* obtained from the rhizosphere of sorghum showed activity against *Pythium ultimum* in sorghum.[37] The *Providencia* strains also exhibited ameliorate effects on the infected-treated seeds in this study. *Providencia vermicola* AAU PR1 isolated by Panpatte et al.[41] inhibited fungal pathogens, including different type strains of *Alternaria, Aspergillus, and Fusarium*. The *in vivo* investigation of *Providencia rettgeri* and *Providencia vermicola* revealed the suppressive abilities against *R. solanacearum*, the causal agent of bacterial wilt disease, within the rhizosphere of potato.[42]

This study shows that plant growth promoters can also function as biocontrol agents. Both classical growth-promotion and pathogen-inhibition traits can be present in an organism.[40] The biocontrol potential of any microbial agent depends on several mechanisms. Common biocontrol mechanisms include antibiotic and lytic enzyme production, parasitism, competition, siderophore, activation of systemic resistance, and hydrogen cyanide production.[43-45] Biopesticides, specifically microbial pesticides, present an alternative to chemical pesticides for plant disease management.[12,13] The drawbacks of conventional methods, such as chemical pesticide use, including negative impacts on health, environment, and soil quality,[3] are typically absent in microbial pesticides.

Conclusion

The study highlights the potential of using rhizosphere bacterial strains, particularly PGPR, as biocontrol agents against important plant pathogens. Treatment of seeds with these bacterial strains or their metabolites improves seed germination and seedling vigor index compared to untreated infected seeds. The effectiveness of specific treatments varies depending on the pathogen. Findings suggest that selected rhizosphere bacterial strains can be used as biocontrol agents to enhance seed germination and seedling vigor, providing a potential alternative to conventional disease control measures. Further research and optimization of treatments are necessary to fully understand the mechanisms of action and improve the efficacy of these biocontrol agents. Since microorganisms can attack stored seeds; therefore, seed treatment before planting should be given adequate consideration.

Authors' Contributions

AOA carried out literature search, experimental analysis, analyzed and interpreted the data, and contributed to the manuscript draft. OJI carried out literature review, experimental analysis interpreted, and analyzed the data. TAO carried out experimental analysis and contributed to the manuscript draft. OBA conceptualized the study, approved the methodology, carried out experimental analysis, analyzed and interpreted the data carried literature review and contributed to the manuscript draft. All authors approved the manuscript's final draft.

Conflict of Interest Disclosures

The authors declare that they have no conflicts of interest.

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