Evaluation of *Pseudomonas fulva* PS9.1 and *Bacillus velezensis* NWUMFkBS10.5 as Candidate Plant Growth Promoters during Maize-*Fusarium* Interaction

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Abstract: Based on in vitro assessments, molecular and chemical analysis, *Pseudomonas fulva* PS9.1 and *Bacillus velezensis* NWUMFkBS10.5 are candidate biocontrol agents for plant disease management including maize fusariosis, a disease caused by members of the *Fusarium* species. This in vivo study evaluated the bio-protective potential of the aforementioned rhizobacteria strains on maize against the proliferation of the pathogenic fungus *Fusarium graminearum* (*Fg*). The study results show that the bacterized plants were not susceptible to *Fg* aggression and the antagonists displayed the capability to proliferate in the presence of other likely competing microflora. The screen-house data also suggest that the presence of resident soil microbiota impacted the activity of antagonists (PS9.1 and NWUMFkBS10.5). This variation was recorded in the soil treatments (sterilized and unsterilized soil). In all the experimental periods, bacterized maize plants with or without *Fg* inoculation significantly (% = 0.05) grew better in unsterilized soil. Besides, during the experimental periods, all the consortia treatments with or without *Fg* infection regardless of the soil used demonstrated appreciable performance. The result of this study suggests that the microbial agents can actively colonize the surface of their maize plant host, improve plant growth, and suppress the growth of phytopathogens. Considering their overall performance in this screen-house evaluation, *P. fulva* PS9.1 and *B. velezensis* NWUMFkBS10.5 have potential for field applications. All safety issues regarding their use under field conditions and risks associated with their extended-release into the environmental will, however, be assessed prior to further bioformulation, field investigation, and scale-up.

Keywords: *Bacillus*; beneficial microbes; bio-inoculants; in planta; maize-*fusarium* interaction; plant growth; *Pseudomonas*

1. Introduction

Microflora that inhabit or colonize the rhizosphere can be classified based on the effects they have on plants, and plant roots can serve as a portal of entry for both beneficial and pathogenic micro-organisms that influence plant growth and development. This zone where root activity influences the biological interaction taking place between plant, soil, and resident flora significantly is referred to as the rhizosphere [1]. The influence of the beneficial micro-organisms present in the root zone on plant growth has been investigated for decades and these beneficial influences are exerted through direct and indirect mechanisms. The activities of these rhizobacteria could either result in the stimulation of plant growth or protection of the plant against pathogen attack [2–4]. Plant growth promotion involves the direct secretion of plant growth-regulators such as auxin, while biocontrol involves the production of metabolites such as siderophores, antibiotics, and hydrogen cyanide, respectively [5,6].
The large scale production of maize (*Zea mays* L.), which is one of the most important cereal crops cultivated globally, is adversely affected by members of the *Fusarium graminearum* species complex (FSGC) of which *F. graminearum* (*Fg*) has been frequently implicated in several worldwide outbreaks [7]. Infection of maize by this phytopathogen brings about devastating effects on plant parts, grain quality, and yield, resulting in significant economic losses in South Africa where maize is a major staple crop, used by humans for various industrial and agricultural purposes [8–10]. The occurrence of *Fg* in maize fields can be detected at both pre-harvest and post-harvest, and its infection occurs through several routes such as systemic infection through the seeds and the movement from the roots to the stalk, sometimes leading to severe rot of the whole plant [11]. Maize infection by *Fg* can also be via the silk channel, or through kernel and tassel injuries inflicted by insects or birds [12,13]. Furthermore, the mycotoxins (zearalenone, deoxynivalenol (DON)) produced by *Fg* when present in maize and maize-based products pose a health threat to man and their animals [10,14,15].

The biological control of this notorious plant pathogen gained worldwide attention due to the outbreaks of the FGSC in different geographic regions of the world [16,17]. The continued global awareness for less dependence on inorganic crops, chemical fertilizers, and agricultural activities that adversely affect the ecosystem, has also encouraged the introduction of biological disease management practices [1]. To date, few reports on effective strategies for biocontrolling *Fg* exist and the majority have not shown the effectiveness of indigenously developed biocontrol candidates in the control of localized maize fusariosis. The effectiveness of BCAs during in-planta applications is dependent on several environmental factors. Depending on the inherent conditions during pre-harvest or post-harvest of crops, seedling storage conditions, crop diseases, or phytopathogens intended to suppress (e.g., endophytes), bioprotectants can either be applied by drenching and coating seeds, or spraying of plant parts [18–20].

In previous reports, both the cells and crude extracts of *Bacillus velezensis* NWUM-FkBS10.5 (Gram-positive) [21], and *Pseudomonas fulva* HARBPS9.1 (Gram-negative) [22], exerted biosuppressive activity against *Fg*. Here we assess the influence of these candidate biocontrollers on maize plants grown in the presence of the *Fg* pathogen during the maize pot experiment. The use of biological control consortia has been reported to be more effective during controlled bioprotection bioassays than the application of a single biological control agent [23]. In addition, biocontrol consortia strains have demonstrated their compatibility and synergy when applied in planta [24]. As such, this study will further evaluate the compatibility of test antagonists and consortia viability of the antagonists during the maize plant-*Fg* interaction in comparison to singularly applied antagonists. The study did not seek to evaluate disease incidence and severity.

2. Results
2.1. Compatibility of Test Antagonists, Seed Germination Test, and Seed Treatment Preparations

In the compatibility tests, there was no area of inhibition between the two test antagonist-bacteria (PS9.1 and NWUMFkBS10.5). Consequently, the test antagonist-bacteria were considered compatible with one another. During the seed germination test, all the seedlings tested sprouted healthily. Two hundred seeds were grown and they all sprouted. Growth parameters were, however, not recorded for the seed germination test (Figure S1). All the screen-house treatment combinations and soils used (sterile and unsterile) are shown in Table 1.
In Table 1, the pot experiment treatment combinations for soils used (sterile and unsterile) are provided. The table shows the effects of different treatments on plant growth parameters such as shoot length and dry weights. Key: M = Maize; A = PS9.1 inoculant; B = NWUMFkBS10.5 inoculant; P = pathogen inoculant (Fusarium graminearum); Mus = maize plant in unsterilized soil; Ms = maize plant in sterilized soil.

2.2. Harvest of Screen-House Pot Experiments Conducted over Three Experimental Periods

For the first experiment harvested at the V4-V5 stage, although plant growth was significantly ($p = 0.05$) retarded in the maize pots treated with only $F_g$ spores and roots lacked vigor (Ms + P; Mus + P), no rot or wilting was observed (Figure 1; Table 2). In all the pots with non-bacterized seeds (Ms + P; Mus + P; Ms; Mus), the primary and lateral roots were not fibrous despite watering and the mesocotyl were unhealthy. Overall, better plant growth and pathogen suppression were recorded in the unsterilized soil. Although maize pots having treatments with NWUMFkBS10.5 (B + Ms; B + Mus) had better shoot length both in sterile and unsterilized soil, the fresh plant weight of the untreated plants in the pots with non-bacterized seeds (Ms + P; Mus + P; Ms; Mus) lacked vigor (Ms + P; Mus + P), no rot or wilting was observed (Figure 1; Table 2). In all treatments, plants treated with PS9.1 had more fibrous roots and root hairs (Figure 1).

![Figure 1](https://example.com/figure1.jpg)

**Figure 1.** Harvest of plantings at V4-V5 stage (2.5 weeks) after seeding.

The harvest of the second experiment showed that overall, the bacterized seed performed better in unsterilized soil; treatments in unsterilized soil without $F_g$ showed a higher increase ($p = 0.05$) in plant vigor than plants in sterilized soil (Figure 3a(ii),b). The controls (maize pots treated with only $F_g$ spores) had retarded growth as expected. Growth was also retarded in the non-bacterized maize seedlings in sterilized soil.
Table 2. Influence of antagonistic isolates (*Pseudomonas fulva* PS9.1 and *Bacillus velezensis* NWUM-FkBS10.5) applied independently or in consortium against *Fg* and their effect on maize seedling growth parameters in a screen-house pot experiment (harvest of plantings at V4-V5 stage (2.5 weeks) after seeding).

| Treatments  | Shoot Length (cm) | Root Length (cm) | Wet Plant Weight (g) | Dry Plant Weight (g) |
|-------------|-------------------|------------------|----------------------|---------------------|
| B + P + Ms  | 30.5 ± 0.5 d      | 10.9 ± 1.1 eFG   | 2.7 ± 0.3 ef         | 0.4 ± 0.1 de        |
| B + P + Mus | 32.6 ± 0.5 c      | 12.8 ± 1.0 cd    | 3.1 ± 0.2 e          | 0.5 ± 0.0 c         |
| A + P + Ms  | 27.4 ± 2.3 e      | 9.6 ± 1.5 ghi    | 1.9 ± 0.3 FG         | 0.3 ± 0.0 de        |
| A + P + Mus | 30.6 ± 0.4 d      | 11.7 ± 0.6 def   | 2.1 ± 0.3 FG         | 0.4 ± 0.0 d         |
| AB + P + Ms | 24.3 ± 2.4 e      | 11.6 ± 0.7 def   | 2.0 ± 0.3 FG         | 0.4 ± 0.0 de        |
| AB + P + Mus| 28.9 ± 1.6 d      | 13.7 ± 1.3 bc    | 3.1 ± 0.3 FG         | 0.5 ± 0.0 de        |
| AB + Ms     | 31.9 ± 1.2 h      | 18.5 ± 0.4 ghi   | 3.9 ± 0.2 d          | 0.7 ± 0.0 c         |
| AB + Mus    | 35.4 ± 1.7 f      | 19.8 ± 0.5 def   | 4.3 ± 0.2 d          | 0.8 ± 0.0 b         |
| Ms          | 32.4 ± 0.9 c      | 11.7 ± 0.3 def   | 6.3 ± 0.1 ab         | 1.3 ± 0.0 f         |
| Mus         | 36.3 ± 1.2 d      | 17.9 ± 0.3 FG h  | 6.8 ± 0.1 a          | 1.5 ± 0.0 c         |
| B + Ms      | 36.1 ± 1.4 ab     | 14.7 ± 1.6 b     | 5.9 ± 0.2 b          | 0.9 ± 0.0 bc        |
| B + Mus     | 39.6 ± 0.6 a      | 18.6 ± 0.9 a     | 6.3 ± 0.2 b          | 1.1 ± 0.1 a         |
| A + Ms      | 30.0 ± 0.6 d      | 17.9 ± 0.6 FG h  | 4.4 ± 0.1 cd         | 0.7 ± 0.0 d         |
| A + Mus     | 36.0 ± 0.9 b      | 18.7 ± 1.4 cde   | 5.8 ± 0.1 c          | 0.9 ± 0.0 bc        |
| M + Ps      | 17.2 ± 0.9 i      | 8.1 ± 1.2 i      | 0.9 ± 0.1 h          | 0.2 ± 0.0 g         |
| M + Mus     | 23.6 ± 0.6 i g    | 9.8 ± 0.8 hi     | 1.7 ± 0.1 gh         | 0.3 ± 0.0 FG        |

Data represent means of three replicated pots from two repeats—presented as the mean ± standard error of mean (SEM). Columns with the same letter are not significantly different according to Duncan’s Multiple Range Test (*p* = 0.05) at each time of evaluation. Values with different uppercase letters are significantly different.

Figure 2. Cont.
Figure 3. (a) (i,ii): Harvest of the second experiment at V6-V7 germination stage. All values are the means of three replicated pots from two repeats—presented as the mean ± SEM. Treatments are significantly different at p = 0.05 according to Duncan’s Multiple Range Test. (b): Harvest of the second experiment at V6-V7 germination stage.

Although antagonist NWUMFkBS10.5 treatments (B + Mus; B + Ms; B + Mus + P; B + Ms + P) performed significantly (p = 0.05) better than all other treatments in both soils, the consortia treatments (AB + Mus; AB + Ms; AB + Mus + P; AB + Ms + P) showed higher plant weights when compared with the PS9.1 treatments (A + Mus; A + Ms; A + Mus + P; A + Ms + P) (Figure 3a(i,ii),b).

During the harvest of the third experimental period (Table 3), plant growth and fungal suppression after 90 days of infecting maize with Fg spores were recorded. Overall, all the treatments in unsterilized soil showed the best performance.

Table 3. Influence of antagonistic isolates (Pseudomonas fulva PS9.1 and Bacillus velezensis NWUMFkBS10.5) applied independently or in consortium against Fg and their effect on maize seedling growth parameters (harvest of third screen-house pot experiment).

| Treatment     | Shoot Length (cm) | Root Length (cm) | Fresh Shoot Weight (g) | Dry Shoot Weight (g) | Fresh Root Weight (g) | Dry Root Weight (g) |
|---------------|-------------------|------------------|------------------------|----------------------|-----------------------|--------------------|
| B + P + Ms    | 41.3 ± 0.1         | 17.3 ± 0.1       | 17.3 ± 0.1             | 4.4 ± 0.1            | 12.2 ± 0.2            | 1.8 ± 0.1          |
| B + P + Mus   | 35.7 ± 0.1         | 14.4 ± 0.1       | 16.1 ± 0.1             | 4.4 ± 0.1            | 11.6 ± 0.2            | 1.6 ± 0.1          |
| A + P + Ms    | 45.1 ± 0.1         | 18.3 ± 0.1       | 29.6 ± 0.1             | 8.9 ± 0.1            | 15.1 ± 0.2            | 2.8 ± 0.1          |
| B + P + Mus   | 51.7 ± 0.1         | 21.8 ± 0.1       | 36.4 ± 0.1             | 11.3 ± 0.1           | 18.5 ± 0.2            | 4.1 ± 0.1          |
| A + P + Ms    | 44.4 ± 0.1         | 17.9 ± 0.1       | 24.6 ± 0.1             | 8.6 ± 0.1            | 15.0 ± 0.2            | 2.5 ± 0.1          |
| B + P + Ms    | 50.9 ± 0.1         | 21.7 ± 0.1       | 36.3 ± 0.1             | 10.4 ± 0.1           | 16.3 ± 0.2            | 2.9 ± 0.1          |
| A + P + Ms    | 56.6 ± 0.1         | 24.8 ± 0.1       | 39.5 ± 0.1             | 11.4 ± 0.1           | 19.0 ± 0.2            | 4.9 ± 0.1          |
| B + Ms        | 61.1 ± 0.1         | 24.9 ± 0.1       | 39.6 ± 0.1             | 11.5 ± 0.1           | 19.3 ± 0.2            | 5.9 ± 0.1          |
| B + Mus       | 69.1 ± 0.1         | 26.2 ± 0.1       | 40.5 ± 0.1             | 14.2 ± 0.1           | 20.1 ± 0.2            | 5.1 ± 0.1          |
| B + Mus       | 72.8 ± 0.1         | 26.2 ± 0.1       | 42.2 ± 0.1             | 15.3 ± 0.1           | 20.3 ± 0.2            | 5.3 ± 0.1          |
| B + Ms        | 78.8 ± 0.1         | 32.6 ± 0.1       | 52.7 ± 0.1             | 18.2 ± 0.1           | 21.5 ± 0.2            | 6.4 ± 0.1          |
| A + Mus       | 74.2 ± 0.1         | 29.2 ± 0.1       | 45.2 ± 0.1             | 16.1 ± 0.1           | 21.3 ± 0.2            | 6.3 ± 0.1          |
| A + Ms        | 83.1 ± 0.1         | 38.5 ± 0.1       | 53.8 ± 0.1             | 18.3 ± 0.1           | 22.8 ± 0.2            | 7.4 ± 0.1          |
| Mean          | 58.2 ± 0.1         | 23.8 ± 0.1       | 36.1 ± 0.1             | 11.6 ± 0.1           | 17.8 ± 0.1            | 4.3 ± 0.1          |
| SEM           | 0.6 ± 0.1          | 0.4 ± 0.1        | 0.37 ± 0.1             | 0.1 ± 0.1            | 0.22 ± 0.1            | 0.1 ± 0.1          |

All values are the means of four replicated pots—with overall mean ± SEM. Treatments are significantly different at p = 0.05 according to Duncan’s multiple range test. Values with same letters are not significantly different.

NWUMFkBS10.5 treatments in unsterilized soil (B + Mus) without Fg inoculation, had the highest performance in all the parameters evaluated. The consortia (AB + Mus + P) with Fg inoculation had the best performance when compared with all other consortia treatments (B + Ms + P; B + Mus + P; A + Mus + P; A + Ms + P; AB + Ms + P) without any bacterization dried and died off three weeks after seeding (Figure 4a). The primary and lateral roots were not fibrous despite watering in the pots with non-bacterized seeds (Figure 4b). The bioprotective effect of both antagonists on the root system and tassel development was, however, observed in other treatments (including seeds bacterized alone, seeds bacterized with Fg, and consortia) (Figure 4c).
As observed, the plants with only Fg treatments (Mus + P; Ms + P) without any bacterization dried and died off three weeks after seeding (Figure 3a). The primary and lateral roots were not fibrous despite watering in the pots with non-bacterized seeds (Figure 3b). The bioprotective effect of both antagonists on the root system and tassel development was, however, observed in other treatments (including seeds bacterized alone, seeds bacterized with Fg, and consortia) (Figure 3c).

Figure 3. (a): Fusarium graminearum (Fg) aggression was observed in the non-bacterized maize seedling; (b): Bioprotective and growth-promoting effects of antagonists (Pseudomonas fulva PS9.1 and Bacillus velezensis NWUMFkBS10.5) seen on root system development; (c): Bioprotective and growth-promoting effects of antagonists (Pseudomonas fulva PS9.1 and Bacillus velezensis NWUMFkBS10.5) seen on tassel development.

3. Discussion

Management of grain fusariosis remains unalleviated and it might be necessary to integrate multiple plant disease approaches including efficient cultural practices, use of resistant cultivars for cultivation, or addition of a low concentration of fungicide or a combination along with the biocontrol agents to see how efficient they would be. A combination of several disease management practices has become the popular approach to managing the
continued incidence of cereal grain fusariosis [25–27]. In this study, the influence of two candidate biocontrol rhizobacteria (P. fulva PS9.1 and B. velezensis NWUMFks10.5) on maize development in the presence of a phytopathogen was observed. However, to ascertain that the bacteria antagonist treatments were the only source of nutrients received by the plants during their germination period, no additional external fertilization or fungicide treatment was applied during the planting periods. Our results demonstrate that inoculation of the bacteria strains (PS9.1 and NWUMFks10.5) independently or in consortia did not only improve the maize plant growth under controlled conditions, but also ameliorated the detrimental effects of the Fg pathogen on the maize plant growth. This bioprotection against Fg aggression may be attributed to the antibiotic secreting potential of the bacteria antagonists or possible induction of systemic resistance in maize by the isolates [28,29]. This might be part of a further study to identify the responses elicited by maize as a result of treatments with these antagonists.

From the results, treatments in unsterilized soil with or without Fg pathogen showed a higher increase ($p = 0.05$) in plant vigor than plants in sterilized soil. This could be due to the influence or presence of transient microflora. Resident microflora has been implicated in plant growth promotion and biocontrol of phytopathogens in disease suppressive soils [30,31]. Although the antagonists exhibited compatibility in vitro, the consortia treatments (where Fg infection was excluded) had a slightly lower influence on plant growth and Fg suppression when compared to the singular bacteria mix treatments (B + Ms+; B + Mus; A + Ms; A + Mus + P/A + Mus + P). The consortia, however, enhanced plant growth and suppressed Fg activity better where Fg infection was included in the treatments. Whilst antagonist PS9.1 possesses some plant-growth-promoting and biocontrol compounds, rhamnolipids, pyoverdine, and rhizomide [22,32], the antagonist NWUMFks10.5 performed significantly ($p = 0.05$) better than PS9.1 in all the treatments. This could be attributed to the action of its multiple plant-growth-promoting and biocontrol biosynthetic compounds, macrolactin, bacillibactin, mersacidin, bacilysin, surfactin, difficidin, iturin, and fengycin [21,33]. A large number of the Bacillus and Pseudomonas spp. (e.g., Bacillus velezensis and Pseudomonas putida) harbor multiple beneficial genes in their genome [34] that confer on them better proliferative potential. The results of this study agree with previous studies seen under greenhouse and field trials in which species within the genera Pseudomonas and Bacillus suppressed the growth and aggression of Fusarium pathogens in cereal cultivars [35–41]

Despite the study not evaluating disease incidence and severity, the results of this investigation show that the bacterial treatments enhanced maize plant growth compared to the Fg inoculated controls and the untreated and non-bacterized (Mus; Ms) controls. In the non-bacterized Fg inoculated treatments, evidence of pathogen aggression was observed—there were no improvements in plant growth parameters in comparison to bacterized plants. Wilting was observed in the non-bacterized-Fg inoculated treatments—some of the plants died off (Figure 4a). The unhealthiness of the maize pots treated with only Fg spores (without any bacterization) could be attributed to Fg systemic infection [9]. The observable survival of the bacterized maize plants is not unexpected since they are no longer distressed, and they possess a non-diseased root system as shown in Figure 4b. This observation correlates with the report of Pandey, et al. [42]. Treatments with PS9.1 also had better root systems which were seen throughout all the pot experiments. We observed some discolorations in the tassels from the plants harvested from sterilized soils. Tassels from NWUMFks10.5 treated plants were significantly larger than the control and other treatments (Figure 4c). Besides, in the pot treatments with non-bacterized seeds, the primary and lateral roots were not fibrous despite watering and the mesocotyl were unhealthy.

Biocontrol inoculant formulations and their mode of delivery are important for their success under field conditions [1]. From the result we gathered, the root dip treatment of maize seedlings appears to be effective in conferring bioprotective ability on the maize. This correlates with the reports of Pal et al. [43] and Pereira et al. [44], in which pre-sowing of seedlings enhanced the activities of BCAs. The root dip treatment involves an inoculated
release approach—microbial treatments influence plant development as they proliferate on and within plant parts. The root dip approach ensures that the microbial agents do not just actively colonize the surface of the host but may also become endophytic [45], possibly leading to the activation of the plant’s defense system. Although this study neither characterized soil microbiota nor evaluated bacteria persistence, the result from the unsterilized soil treatments suggests the possibility of transient soil microbiota playing a crucial role in the maize plant’s growth. Growth was significantly ($p = 0.05$) retarded in the non-bacterized maize seedlings in sterilized soil. Besides, the bioactivities of the antagonistic bacteria ($P. fulva$ PS9.1 and $B. velezensis$ NWU-MFkB10.5) in unsterilized soil are suggestive of their ability to persist and compete in the environment against resident microflora if they are to be considered as field biocontrol candidates.

In recent years the biosafety status of candidate plant growth-promoting organisms (PGPO) and biocontrol strains have become a topic of major concern [46–48]. Despite strain PS9.1’s beneficial properties [22,32,49,50], some of its genetically related strains have also been recognized as opportunistic pathogens in previous studies [51–56]. Hence, PS9.1 can be considered a strain with overlapping biological impact, which poses a challenge for its use in planta. Because of the safety concerns regarding the release of candidate PGPO with possible overlapping (beneficial and deleterious) attributes [48], we recommend the cautionary use of strain $P. fulva$ PS9.1. According to Kewani, et al. [48], strains of several bacteria genera exhibiting clinical and beneficial overlaps are still utilized in vitro as indicators of plant growth promotion and biocontrol, even though they are not commercially utilized because of the risk of pathogen outbreaks. We consider PS9.1 a prime candidate for the production of microbial synthesized bioinoculants without viable organisms since in silico genome analysis shows the bacterium also harbors biosynthetic genes for synthesis of pyoverdin, lankacidin C, and rhizomides (A, B, and C). Additionally, the candidate biocontroller (PS9.1) will likely be employed strictly for invitro investigative purposes under controlled environments. For example, studies involving the synthesis of agricultural metabolite additives lacking viable organisms will be conducted. We also intend to conduct further bioinformatic analysis of the PS9.1 sequenced genome [32], this should provide additional insights into its beneficial roles and limitations. Information gathered thereafter should be useful in evaluating the biotechnological importance of other beneficial plant-microbes with clinical overlaps.

4. Materials and Methods

4.1. Determination of Compatibility of Test Antagonists

The bacteria antagonists used in this study, $P. fulva$ PS9.1 and $B. velezensis$ NWU-MFkB10.5, were previously isolated from the maize rhizosphere, identified based on the 16S rDNA gene sequence analysis (accession numbers MF098600 and KX353617.1), and analyzed by genome sequencing [21,22,32,33]. The $Fg$ pathogen was provided as a gift. Bacterial cultures were preserved in 15% glycerol at −70°C, Luria Bertani (LB) broth (Sigma Aldrich L3522) at 4°C, and maintained on LB agar (Sigma Aldrich L3147) at 4°C until needed. $Fg$ was maintained on potato dextrose agar (PDA) (Sigma Aldrich P2182) at 4°C until needed.

Before the pot cultivation (treatment of planting seeds and seeding), the compatibility of the rhizobacteria antagonists (PS9.1 and NWU-MFkB10.5) were determined through a dual-culture interaction test described previously by Dubey et al. [57] and Kumar, et al. [24], with slight modifications. An overnight pure culture of each test antagonist was subcultured on fresh LB agar plates. One hundred microliters of the suspension of each antagonist ($OD_{600 \text{ nm}} = 0.5$) were then prepared and spot inoculated or streaked opposite each other on a LB agar for 48 h at 30°C. Additionally, the PS9.1 antagonist was streaked on an overnight LB spread plate of antagonist NWU-MFkB10.5 and vice versa. The assay was incubated for 48 h at 30°C. After incubation, plates were observed for overlapped growth of the antagonists and possible inhibition zones. The absence of inhibition zones and overlapping
of growth indicated compatibility of the test antagonists while the presence of inhibition zone (if applicable) signified incompatibility.

4.2. Surface Sterilization of Maize Seeds and Seed Germination Test

Maize seeds variety DKC 73–72 (200 g) used in this study were obtained from NWK Limited (Econobuild) Mafikeng industrial area, North West Province, South Africa. To ensure the removal of fungicides from the seeds, they were washed in sterile distilled water. The seeds were further soaked in 0.75% Sodium hypochlorite solution for 5 min, followed by rinsing five times with sterile distilled water, and a final soaking on the fifth wash. The fifth wash was then inoculated on sterile nutrient agar plates to determine the sterilization efficiency. The surface disinfection was recorded as the absence of a colony-forming unit on the Nutrient agar (NA) plate (Millipore 70116).

The disinfected maize seeds were then subjected to a seed germination test based on the paper towel method (ISTA 2003) while the percentage germination (%) was calculated according to Abdul-Baki and Anderson [58]. One hundred maize seeds already lined with a paper towel (moistened with 10 mL of sterile distilled water) were arranged in three 1 L beakers (11.8 cm of side). The seeds were again covered with a paper towel (earlier moistened with 10 mL of sterile distilled H₂O). The beaker was covered and incubated at room temperature for 4 days. Thereafter, the number of germinated seeds per beaker was counted to ascertain the germination percentage using the formula:

\[
GP\% = \frac{NG}{TNS} \times 100
\]

where \( GP \) = germination percentage; \( NG \) = Number of Germinated seed; \( TNS \) = total number of seeds.

4.3. Screen-House Pot Experiment

4.3.1. Collection of Soil for Pot Experiments

Planting soil was collected from the North-West University Animal Science Department agricultural planting area. Sterilization of a portion of the soil was done by dry heat for 1 week at 120 °C. Sterilized soil was plated on NA to confirm sterilization, and sterilization was continued until no growth was observed on the NA plates. Planting pots with dimensions 13 cm (diameter) \( \times \) 10 cm (depth) were filled with 80 kg of both sterilized soil and unsterilized soil up to water-holding capacity. The sterile and unsterile soils were utilized to compare the persistence and competitiveness of the two rhizobacteria antagonists.

4.3.2. Pre-Germination of Maize Grains for Pot Experiments

Two hundred grams of disinfected maize grains presoaked in sterile distilled water were placed in a 1 L beaker previously wallpapered with a sterile paper towel moistened with 10 mL of sterile distilled water. The seeds were covered again with the moistened (10 mL sterile distilled water) sterile paper towel. The flask was covered and incubated at 30 °C for 5 days after which only pre-germinated seeds with 2 cm callus were used for the seed-root dip pot experiment.

4.3.3. Seed Treatments Preparations

A modified seed-root dip pot experiment by Cook, Bruckart, Coulson, Goettel, Humber, Lumsdon, Maddox, McManus, Moore, Meyer, Quimby, Stack and Vaughn [45] was employed for seed bacterization. From an overnight LB broth culture of PS9.1 and NWUM-FkBS10.5, 20 µL of each isolate was transferred into 100 mL of LB broth in a 250 mL Erlenmeyer flask, and cultured for 3 days (28 °C) with continuous shaking at 150 g. Bacteria cells were recovered by centrifugation at 8000 \( \times \) g for 20 min and the supernatant was discarded. The pellet of each isolate was re-suspended in 100 mL sterile LB broth and optical density (OD) was adjusted to 0.5:600 nm. For single bacterization, 120 sterile pre-germinated maize seeds were submerged in the 100 mL bacteria inoculum (OD 0.5:600 nm) of each treatment, and the bacterized mixture consisted of 120 pre-germinated seeds sub-
merged in 100 mL of co-inoculated bacteria broth (50:50 v/v) at OD 0.5:600 nm. This was incubated for 2–4 h with continuous shaking at 100 g for homogenization and adherence of bacteria to seeds. Sixty sterile pre-germinated maize seeds were also infected with 10^7 spores mL^{-1} of the *Fg* pathogen. The overnight pre-germinated (120) bacterized seeds above were also air-dried and 60 grains were aseptically removed and submerged in the spore suspension of the *Fg* pathogen (10^7 spores mL^{-1}). All the treatments (Table 1) were incubated overnight to allow for adherence of inoculum to the seeds.

### 4.3.4. Seed Cultivation and Planting Experimental Periods

A randomized complete block experimental design (with four replications per pot treatment) based on a modified protocol by Bacon and Hinton [59] was employed in this section. The germinated bacterized seeds, *Fg* inoculated seeds, and consortia were transferred to both soils (sterile and unsterile), with four seeds in one pot (pots in quadruplicate). Uninoculated seeds were used as a control and pots were watered with sterilized water. Furthermore, treatments consisting of 10 mL bacteria antagonist, 10 mL mixtures of both antagonists, and 10 mL of pathogen spore suspensions (10^7 spores mL^{-1}) were applied to the plants using a sterile syringe after one week of seeding in pots according to the treatments listed above. Planting was conducted in three experimental periods. The first experiment to determine the bioprotective capability of PS9.1 and NWUMFkB10.5 during maize germination lasted 2.5 weeks in January 2017 after which the plants were harvested at the V4–V5 stage. The second experimental period occurred after the second inoculation of plants with *Fg*, and was for 40 days during February 2017–March 2017 and the third experimental period was for 3 months July 2017–September 2017. However, during the third experimental period, treatments consisting of 10 mL bacteria antagonist, 10 mL mixtures of both antagonists, and 10 mL of pathogen spore suspensions (10^5 spores mL^{-1}) were applied (following the experimental design and treatments) after 1 week of seeding, using a sterile syringe. During the third experimental period, the plants were harvested at the tasseling stage (VT). At the end of each experimental period, harvested plants were evaluated for growth and survival and growth parameters were recorded. The parameters measured at harvest were wet plant weight, shoot length, root length, and dry plant weight.

### 4.4. Statistical Analysis

Multivariate general linear model, Duncan Multiple Range Test, and Tukey test (highest significant different test (HSD)) were used to analyze and compare observed treatment means, pathogen–antagonist relationship, treatment effects, and interactions in SPSS statistical software (version 22) at the significance level of 5%. Prism 9.1.0 was used for further data analysis and visualization.

### 5. Conclusions

Integrating biocontrol approaches into the current cereal disease management practices will provide novel alternatives (in form of biofungicides) for cereal farmers and the grain industry for the control of inherent *Fusarium* pathogens. The significant findings of the study are the in planta potential of the *P. fulva* PS9.1 and *B. velezensis* NWUMFkB10.5 and their capability to bioprotect maize plants against *Fg* fusariosis and their effectiveness in unsterilized soil. Considering biosafety issues, strain NWUMFkB10.5 is the preferred candidate for the development of a biofertilizer, biostimulant, and biofungicide product. In as much as the maize germination was protected against *Fg* aggression, further work to determine the disease severity, reduction of mycotoxin contamination, and level of nutrient uptake by treated seeds after application of the rhizobacteria duo will be required to ascertain the complete bioprotective potential of these rhizobacteria strains.

**Supplementary Materials:** The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/plants11030324/s1, Figure S1: Germination of maize seedlings
prior to in vitro and in vivo usage: pre-germinated seeds submerged in the 100 mL bacteria inoculum (OD 0.5:600 nm) of each treatment.

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**References**

1. Babalola, O.O. Beneficial bacteria of agricultural importance. *Biotecnol. Lett.* 2010, 32, 1559–1570. [CrossRef]
2. Bloemberg, G.V.; Lugtenberg, B.J.J. Molecular basis of plant growth promotion and biocontrol by rhizobacteria. *Curr. Opin. Plant Biol.* 2001, 4, 343–350. [CrossRef]
3. Souza, R.d.; Ambrosini, A.; Passaglia, L.M.P. Plant growth-promoting bacteria as inoculants in agricultural soils. *Genet. Mol. Biol.* 2015, 38, 401–419. [CrossRef] [PubMed]
4. Shanmugaiah, V.; Nithya, K.; Harikrishnan, H.; Jayaprakashvel, M.; Balasubramanian, N. Biocontrol mechanisms of siderophores against bacterial plant pathogens. In *Sustainable Approaches to Controlling Plant Pathogenic Bacteria*; Bastas, K.K., Ed.; CRC Press: Boca Raton, FL, USA, 2015; pp. 167–186. [CrossRef]
5. Bashan, Y.; Holguin, G. Azospirillum-plant relationships: Environmental and physiological advances (1990–1996). *Can. J. Microbiol.* 1997, 43, 103–121. [CrossRef]
6. Babalola, O.O.; Glick, B.R. Indigenous African agriculture and plant associated microbes: Current practice and future transgenic prospects. *Sci. Res. Essays* 2012, 7, 2431–2439.
7. Summerell, B.A.; Leslie, J.F. Fifty years of *Fusarium*: How could nine species have ever been enough? *Fungal Divers.* 2011, 50, 135–144. [CrossRef]
8. Lamprecht, S.C.; Twedtedemthin, Y.T.; Botha, W.J.; Calitz, F.J. *Fusarium graminearum* Species Complex associated with maize crowns and roots in the KwaZulu-Natal province of South Africa. *Plant Dis.* 2011, 95, 1153–1158. [CrossRef] [PubMed]
9. Janse van Rensburg, B.; McLaren, N.W.; Flett, B.C.; Schoeman, A. Fumonisins producing *Fusarium* spp. and fumonisin contamination in commercial South African maize. *Eur. J. Plant Pathol.* 2015, 141, 491–504. [CrossRef]
10. Mnggawa, P.; Shephard, G.S.; Green, I.R.; Ngobeni, S.H.; de Rijk, T.C.; Katerere, D.R. Mycotoxin contamination of home-grown maize in rural northern South Africa (Limpopo and Mpumalanga Provinces). *Food Addit. Contam. Part B* 2016, 9, 38–45. [CrossRef]
11. Doohan, F.M.; Brennan, J.; Cooke, B.M. Influence of climatic factors on *Fusarium* species pathogenic to cereals. *Eur. J. Plant Pathol.* 2003, 109, 755–768. [CrossRef]
12. Nelson, P.; Plattner, R.; Shackelford, D.; Desjardins, A. Fumonisin B1 production by *Fusarium* species other than *F. moniliforme* in section Liseola and by some related species. *Appl. Environ. Microbiol.* 1992, 58, 984–989. [PubMed]
13. Shephard, G.S.; Thiel, P.G.; Stockenström, S.; Sydenham, E.W. Worldwide survey of fumonisin contamination of corn and corn-based products. *J. AOAC Int.* 1996, 79, 671–687. [CrossRef]
14. Wang, J.-H.; Ndoye, M.; Zhang, J.-B.; Li, H.-P.; Liao, Y.-C. Population Structure and Genetic Diversity of the *Fusarium graminearum* Species Complex. *Toxins* 2011, 3, 1020–1037. [CrossRef] [PubMed]
15. Zhang, H.; Van der Lee, T.; Waalwijk, C.; Chen, W.; Xu, J.; Xu, J.; Zhang, Y.; Feng, J. Population Analysis of the *Fusarium graminearum* species complex from wheat in China show a shift to more aggressive isolates. *PLoS ONE* 2012, 7, e31722. [CrossRef]
16. McMullen, M.; Bergstrom, G.; De Wolf, E.; Dill-Macky, R.; Hershman, D.; Shaner, G.; Van Sanford, D. A unified effort to fight an enemy of wheat and barley: *Fusarium* Head Blight. *Plant Dis.* 2012, 96, 1712–1728. [CrossRef] [PubMed]
17. Varga, E.; Wiesenberger, G.; Hametner, C.; Ward, T.J.; Dong, Y.; Schöfbeck, D.; McCormick, S.; Broz, K.; Stückler, R.; Schuhmacher, R. New tricks of an old enemy: Isolates of *Fusarium graminearum* produce a type A trichothecene mycotoxin. *Environ. Microbiol.* 2015, 17, 2588–2600. [CrossRef] [PubMed]
18. Fravel, D.R. Commercialization and implementation of biocontrol. *Annu. Rev. Phytopathol.* 2005, 43, 337–359. [CrossRef]
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19. Babalola, O.; Berner, D.; Amusa, N. Evaluation of some bacterial isolates as germination stimulants of Striga hermonthica. Afr. J. Agric. Res. 2007, 2, 27–30.

20. Pérez-Garcia, A.; Romero, D.; de Vicente, A. Plant protection and growth stimulation by microorganisms: Biotechnological applications of Bacillus in agriculture. Curr. Opin. Biotechnol. 2011, 22, 187–193. [CrossRef]

21. Adeniji, A.A.; Aremu, O.S.; Babalola, O.O. Selecting lipopeptide-producing, Fusarium-suppressing Bacillus spp.: Metabolic and genomic probing of Bacillus velezensis NWUMFkB10. 5. MicrobiologyOpen 2019, 8, e00743. [CrossRef]

22. Adeniji, A.A.; Aremu, O.S.; Loots, D.T.; Babalola, O.O. Pseudomonas falva HARBP9.1: Candidate anti-Fusarium agent in South Africa. Eur. J. Plant Pathol. 2020, 157, 767–781. [CrossRef]

23. Xie, L.; Lehvävirta, S.; Timonen, S.; Kasurinen, J.; Niemikapee, J.; Valkonen, J.P.T. Species-specific synergistic effects of two plant growth—Promoting microbes on green roof plant biomass and photosynthetic efficiency. PLoS ONE 2019, 13, e0209432. [CrossRef]

24. Adeniji, A.A.; Babalola, O.O. Selecting lipopeptide-producing, Bacillus amyloliquefaciens ameliorates drought stress in chickpea (Cicer arietinum L.). Plant Signal. Behav. 2016, 11, e1071004. [CrossRef] [PubMed]

25. Wegulo, S.N.; Baenziger, P.S.; Hernandez Nopsa, J.; Bockus, W.W.; Hallen-Adams, H. Management of Fusarium head blight of wheat and barley. Crop Prot. 2015, 73, 100–107. [CrossRef]

26. Wegulo, S.N.; Bockus, W.W.; Nopsa, J.H.; De Wolf, E.D.; Eskridge, K.M.; Peiris, K.H.; Dowell, F.E. Effects of integrating cultivar resistance and fungicide application on Fusarium head blight and deoxynivalenol in winter wheat. Plant Dis. 2011, 95, 554–560. [CrossRef] [PubMed]

27. Ooi, Y.; Divakara, S.T.; Nayaka, S.C.; Hariprasad, P.; Niranjana, S.R. Application of beneficial rhizospheric microbes for the management of seed-borne mycotoxigenic fungal infection and mycotoxins in maize. Biocontrol Sci. Technol. 2015, 25, 1105–1119. [CrossRef]

28. Kumar, M.; Mishra, S.; Dixit, V.; Kumar, M.; Agarwal, L.; Chauhan, P.S.; Nautiyal, C.S. Synergistic effect of Pseudomonas putida and Bacillus amyloliquefaciens ameliorates drought stress in chickpea (Cicer arietinum L.). Plant Signal. Behav. 2016, 11, e1071004. [CrossRef] [PubMed]

29. Aliyaz, M.; Divakara, S.T.; Nayaka, S.C.; Hariprasad, P.; Niranjana, S.R. Application of beneficial rhizospheric microbes for the mitigation of seed-borne mycotoxigenic fungal infection and mycotoxins in maize. Biocontrol Sci. Technol. 2015, 25, 1105–1119. [CrossRef]

30. Gómez Exposíto, R.; de Brujin, I.; Postma, J.; Raaijmakers, J.M. Current Insights into the role of rhizosphere bacteria in disease suppressive soils. Front. Microbiol. 2015, 6, 745. [CrossRef]

31. Jayaraman, S.; Naorem, A.K.; Lal, R.; Dalal, R.C.; Sinha, N.K.; Patra, A.K.; Chaudhari, S.K. Disease-suppressive soils—Beyond food production: A Critical Review. J. Soil Sci. Plant Nutr. 2021, 21, 1437–1465. [CrossRef]

32. Pereira, P.A.; Ayangbenro, A.S.; Loots, D.T. Genome sequence resource of Pseudomonas falva HARBP9.1 candidate biocontrol agent. Phytopathology 2020, 111, 896–898. [CrossRef]

33. Perry, T.; Sumarah, M.; Wang, A.; Abbasi, P.A. Characterisation of antagonistic Pseudomonas strains for biological control of plant diseases. Appl. Microbiol. Biotechnol. 2019, 103, 3669–3682. [CrossRef]

34. Schisler, D.; Slininger, P. Formulation of Bacillus. Res. Appl. Microbiol. 2009, 54, 103–111. [CrossRef]

35. Khan, N.; Schisler, D.; Boehm, M.; Slininger, P.; Bothast, R. Selection and evaluation of microorganisms for biocontrol of Fusarium head blight of wheat infected by Gibberella zeae. Plant Dis. 2001, 85, 1253–1258. [CrossRef]

36. Khan, N.I.; Schisler, D.A.; Boehm, M.J.; Lipps, P.E.; Slininger, P.J. Field testing of antagonists of Fusarium head blight infected by Gibberella zeae. Biol. Control 2004, 29, 245–255. [CrossRef]

37. Palazzini, J.M.; Ramírez, M.L.; Torres, A.M.; Chulze, S.N. Potential biocontrol agents for Fusarium head blight and deoxynivalenol production in wheat. Crop Prot. 2007, 26, 1702–1710. [CrossRef]

38. Pereira, P.; Nesci, A.; Castillo, C.; Etcheverry, M. Field studies on the relationship between Fusarium verticillioides and maize (Zea mays L.): Effect of biocontrol agents on fungal infection and toxin content of grains at harvest. Int. J. Agron. 2011, 2011, 7. [CrossRef]

39. Pereira, P.; Nesci, A.; Palmi, L.M.S.; Hebbar, K. Suppression of damping-off in maize seedlings by Pseudomonas corrugata. Microbiol. Res. 2001, 156, 107–108. [CrossRef]

40. Pal, K.K.; Tilak, K.V.B.R.; Saxena, A.K.; Dey, R.; Singh, C.S. Suppression of maize root diseases caused by Macrophomina phaseolina, Fusarium moniliforme and Fusarium graminearum by plant growth promoting rhizobacteria. Microbiol. Res. 2001, 156, 209–223. [CrossRef]

41. Pereira, P.; Nesci, A.; Castillo, C.; Etcheverry, M. Impact of bacterial biological control agents on fumonisin B1 content and Fusarium verticillioides infection of field-grown maize. Biol. Control 2010, 53, 258–266. [CrossRef]
45. Cook, R.J.; Bruckart, W.L.; Coulson, J.R.; Goettel, M.S.; Humber, R.A.; Lumsden, R.D.; Maddox, J.V.; McManus, M.L.; Moore, L.; Meyer, S.F.; et al. Safety of microorganisms intended for pest and plant disease control: A framework for scientific evaluation. *Biol. Control* **1996**, *7*, 333–351. [CrossRef]

46. Gupta, R.; Mathimaran, N.; Wiemken, A.; Boller, T.; Bisaria, V.S.; Sharma, S. Non-target effects of bioinoculants on rhizospheric microbial communities of Cajanus cajan. *Appl. Soil Ecol.* **2014**, *76*, 26–33. [CrossRef]

47. Trabelsi, D.; Mhamdi, R. Microbial inoculants and their impact on soil microbial communities: A Review. *BioMed Res. Int.* **2013**, *2013*, 863240. [CrossRef]

48. Keswani, C.; Prakash, O.; Bharti, N.; Vilchez, J.I.; Sansinenea, E.; Lally, R.D.; Borris, R.; Singh, S.P.; Gupta, V.K.; Fraceto, L.F. Re-addressing the biosafety issues of plant growth promoting rhizobacteria. *Sci. Total Environ.* **2019**, *690*, 841–852. [CrossRef]

49. Pokojska-Burdziej, A.; Strzelczyk, E.; Dahm, H.; Li, C. Effect of endophytic bacterium *Pseudomonas fulva* on growth of pine seedlings (*Pinus sylvestris*), formation of mycorrhizae and protection against pathogens. *Phytopathol. Pol.* **2004**, *32*, 33–47.

50. Thiem, D.; Złoch, M.; Gadzała-Kopciuch, R.; Szymańska, S.; Baum, C.; Hrynkiewicz, K. Cadmium-induced changes in the production of siderophores by a plant growth promoting strain of *Pseudomonas fulva*. *J. Basic Microbiol.* **2019**, *58*, 623–632. [CrossRef] [PubMed]

51. Sheng, Q.; Luo, M.; Guan, J.; Zhang, X.; Zhang, C. Characterization of *Pseudomonas fulva* as a new pathogen of pepper leaf spot isolated from Xinjiang in China. *J. Plant Prot.* **2017**, *44*, 260–268.

52. Liu, Y.; Liu, K.; Yu, X.; Li, B.; Cao, B. Identification and control of a *Pseudomonas* spp (*P. fulva* and *P. putida*) bloodstream infection outbreak in a teaching hospital in Beijing, China. *Int. J. Infect. Dis.* **2014**, *23*, 105–108. [CrossRef]

53. Rebolledo, P.A.; Vu, C.C.L.; Carlson, R.D.; Kraft, C.S.; Anderson, E.J.; Burd, E.M.; Doern, G.V. Polymicrobial ventriculitis involving *Pseudomonas fulva*. *J. Clin. Microbiol.* **2014**, *52*, 2239–2241. [CrossRef]

54. Cobo, F.; Jiménez, G.; Rodríguez-Granger, J.; Sampredo, A. Posttraumatic Skin and Soft-Tissue Infection due to *Pseudomonas fulva*. *Case Rep. Infect. Dis.* **2016**, *2016*, 8716068. [CrossRef] [PubMed]

55. Brovedan, M.A.; Marchiaro, P.M.; Diaz, M.S.; Faccone, D.; Corso, A.; Pasteran, F.; Viale, A.M.; Limansky, A.S. *Pseudomonas putida* group species as reservoirs of mobilizable Tn402-like class 1 integrons carrying blaVIM-2 metallo-β-lactamase genes. *Infect. Genet. Evol.* **2021**, *96*, 105131. [CrossRef] [PubMed]

56. Seok, Y.; Shin, H.; Lee, Y.; Cho, I.; Na, S.; Yong, D.; Jeong, S.H.; Lee, K. First report of bloodstream infection caused by *Pseudomonas fulva*. *J. Clin. Microbiol.* **2010**, *48*, 2656–2657. [CrossRef]

57. Dubey, S.C.; Singh, V.; Priyanka, K.; Upadhyay, B.K.; Singh, B. Combined application of fungal and bacterial bio-agents, together with fungicide and *Mesorhizobium* for integrated management of Fusarium wilt of chickpea. *BioControl* **2015**, *60*, 413–424. [CrossRef]

58. Abdul-Baki, A.A.; Anderson, J.D. Vigor determination in soybean seed by multiple criteria. *Crop Sci.* **1973**, *13*, 630–633. [CrossRef]

59. Bacon, C.W.; Hinton, D.M. In planta reduction of maize seedling stalk lesions by the bacterial endophyte *Bacillus mojavensis*. *Can. J. Microbiol.* **2011**, *57*, 485–492. [CrossRef]