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Bio-Control of Net-Blotch and Scald Pathogens of Barley Using *Paenibacillus Polymyxa* KAI245 Isolated from Sorghum Rhizosphere in Western Kenya

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Abstract Net-blotch and scald are important foliar diseases of barley. In the present study, the biocontrol activity of *Paenibacillus polymyxa* KaI245 was evaluated against *Drechsclera teres* f. sp *teres* and *Rhynchosporium commune* causing net-form-net-blotch and scald respectively. *In-vitro* efficacy of the bacterial isolate entailed dual culture technique, use of cell-free supernatant and test for volatile-compounds-mediated inhibition. Greenhouse studies were further conducted to evaluate the efficacy of crude bacterial extracts against net-blotch pathogen in barley plants. In dual culture technique, the mycelial growth of *D. teres* f. sp *teres* was impeded by approximately 47.3% while there was no any observable effect in *R. commune* colonies. Food-poison technique was u sed to test the antifungal activity of cell-free supernatant. The cell free supernatant inhibited the growth of *D. teres* by approximately 24.1%. *R. commune* colonies were impeded by 52.9% via volatile organic compounds while *D. teres* f. sp *teres* remained unaffected. Greenhouse studies showed decreased disease incidence (50%) in the crude-extract-treated barley leaves inoculated with *D. teres*. *In-vitro* studies revealed that greater inhibition is imparted by live bacterial cells. The bacterium has the potential to be used as a biocontrol agent against the tested pathogens of barley. Use of bio-control agents to manage crop diseases is one of the alternatives set to replace chemical fungicides that are saddled with lots of negativity due to their hazardous environmental impact.

Keywords: *D. teres f. sp teres, R. commune, P. polymyxa KaI245, net-form-net-blotch, scald, bio-control*

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1. Introduction

Net blotch of barley (*Hordeum vulgare*) is more prevalent in high rainfall areas and temperate regions [1] with stubble retention and cultivation of susceptible varieties aggravating the problem [2]. The causal agent is *Drechsclera teres* which exists in two forms; *D. teres* f. sp *teres* and *D. teres* f. sp *maculata* [3]. *D. teres* f. sp. *teres* infects and feeds as a necrotroph by growing intercellularly and affecting host cells further from the mycelium while *D. teres* f. sp. *maculata* first forms haustorial-like intracellular structures and feeds just like a biotroph but necrotrophic growth ensues almost immediately [4]. *D. teres* f.sp *teres* induces net type net blotch (NTNB) characterized by horizontal and vertical crisscrossed dark brown venation on the barley leaves that may be chlorotic, while *D. teres* f.sp *maculata* induces spot form net blotch (SFNB) characterized by dark brown circular or elliptical spots surrounded by chlorosis [5]. These symptoms have been shown to be caused by phytotoxic low molecular weight compounds (LMWCs) and proteinaceous metabolites produced by the fungi [6]. Both types can cause huge losses due to their negative impacts on the quality of the grains [7]. Losses ranging from 10-40% [8] up to 100% [9] have been reported.

Scald of barley is caused by Rhynchosporium commune (formerly known as Rhynchosporium secalis [10]). The disease is characterized with lesions having dark brown borders. It is particularly common in areas with cool and temperate climates with semi humid conditions [11]. It is classified as a hemi-biotroph because of its long symptomless phase even when there is sporulation and complete life cycle [12]. It has however been recently shown that the pathogen produces three necrosis inducing effectors namely, NIP1, NIP2 and NIP3 [13]. Although generally considered an imperfect fungi, there have been suggestions for the presence of teliomorphic stages [13].

Common control measures include crop rotation, stubble destruction, use of chemical fungicides and resistant barley varieties [14]. Some resistant barley

varieties have been found to be vulnerable with time. Nguzo barley variety was previously resistant to net-blotch has been reported to be susceptible in recent times [15]. With shrinking land-size coupled with growing demand of barley, rotation will become counterproductive in times ahead. Synthetic fungicides have been implicated with negative environmental impacts due to their toxic, non-biodegradable, and indiscriminate nature. This awareness has resulted in the demand of 'chemical-free' agricultural products [16]. Governments continue to enact laws prohibiting the use of certain agrochemicals resulting in fewer options to the farmers. In countries like Kenya where the agrochemicals are imported, exorbitant cost renders agricultural activities unsustainable [17]. To mitigate this, modern day scientist's main duty is to seek ways of managing plant diseases with minimal impact to the environment. The path to finding alternatives has been advanced in several fronts; biological control is one such alternative.

Paenibacillus polymyxa KaI245, a bio-control agent used in this study, is a plant growth promoting rhizobacterium (PGPR) isolated from the sorghum rhizosphere in western Kenya. The bacterium, formerly known as *Bacillus polymyxa* [18], is a Gram positive, endospore forming, facultative anaerobic, non-pathoenic bacillus [19]. The bacterium has a wide distribution ranging from soil, rhizosphere and marine sediments [18]. *P. polymyxa* has been shown to have great applications in agriculture, industry and medicine [20]. In agriculture, it has properties including nitrogen fixation, plant growth promotion, and soil phosphorous solubilisation [18]. On the downside, *P. polymyxa* KaI245 is the causal agent for potato tuber rot and tomato seedling blight [21]. In a previous study, the bacterium showed promising antimicrobial activity towards *Colletotrichum sublenolium* the causative agent of sorghum anthracnose [22]. In the present study, *in vitro* and *in vivo* bio-assays were conducted to evaluate the possibility of the bio-control agents from the bacterium to suppress net-blotch disease in barley.

2. Materials and Methods

2.1. Isolation and Identification of the Fungal Pathogens

Drechslera teres (net-blotch pathogen) and *Rhynchosporium commune* (scald pathogen) were used in this experiment. Diseased barley leaves were collected from KARI-Njoro (0.3˚N, 35.9˚E), and Chepkoilel in Uasin Gishu county (0.6˚N, 35.3˚E). The leaves with scald symptoms were first left to air dry in paper envelopes for 24hrs. They were then cut into 1 cm fragments and surface sterilised by wetting in 70% ethanol for 10s and 0.5% sodium hypochlorite solution for 1 min, then soaked three times (1 min) in sterile distilled water. Afterwards they were dried between sterilised filter papers. Leave fragments were then transferred onto petri dishes containing Potato Dextrose Agar (PDA) amended with streptomycin and incubated for ten days at 28±1°C (*D. teres*) and 15±1°C (*R. commune*) [23] in continuous darkness to allow mycelial growth. The emerging mycelia were transferred onto petri-dishes containing plain PDA to obtain pure colonies. Riddel slides were prepared for microscopy studies. Identification was done using cultural characteristics of pure cultures and morphological characteristics by microscopy. The fungal cultures were maintained on Potato Dextrose Agar at 4°C.

2.2. *In vitro* Antifungal Study on Pathogenic Fungal Growth

In vitro inhibition of mycelial growth of the isolated fungal phytopathogens by *Paenibacillus polymyxa* KaI245 was tested using the dual culture technique [24]. *P. polymyxa* KaI245 was streaked onto a PDA plate 2cm from the 2 equidistant edges of the petri-dish. Fresh mycelial plugs (6mm diameter) of *D. teres* f.sp *teres* and *R. commune* were placed at the center of the petri dish. Because of its slow growth, *R. commune* was left to grow for 9 days before the bacterium was streaked as described above. *D. teres-P. polymyxa* plates were incubated at 28°C for seven days while *R. commune-P. polymyxa* plates were incubated at 20°C. Readings (7 days for *D. teres* and 18 days for *R. commune*) were taken by measuring the radius of the mycelium towards the bacterium and labelled as 'r' and that of the mycelium not growing towards the bacterium and labeled 'R'. Each experiment was replicated six times. Percent inhibition was calculated using the formula;

$$\frac{R-r}{R}\times 100. \quad (1)$$

Where R: radius of the mycelium away from the bacterium.
r: radius of the mycelium towards the bacterium.

Antagonism test was also performed using food poison technique with the cell free supernatant. A single colony of *Paenibacillus polymyxa* KaI245 was cultured in Luria Bertani (LB) on a mechanical shaker at 28°C and 150 r.p.m for 24 h as seed liquid. Seed liquid (1.5 mL) was then inoculated into 200 mL of Potato Dextrose Broth (PDB) medium and cultured at 30˚C, 120 r.p.m for 7 days [25]. The supernatant was recovered by centrifugation at 4°C, 13000 rpm for 30 min and poured out into a clean container. The supernatant was then used for antifungal assay of *Drechslera teres* f. sp *teres*. The fungal growth medium (PDA) was supplemented with 10% of the supernatant [24].

Fungal mycelium (6mm) was then inoculated on to the centre of the medium. The controls had the fungi inoculated on standard (plain) PDA. The plates were incubated at 28°C. Colony diameters of the cultured fungi were recorded daily for five days. Change in diameters between days were also recorded and subject to analysis. The experiment was done in five replicates.

2.3. Bacterial Volatile Production-sealed Plate Method

Sealed plate method was used [26]. *Paenibacillus polymyxa* KaI245 was streaked on a PDA in the bottom of a petri dish. A 6mm diameter mycelial plug was cut from the margin of an actively growing fungal cultures and placed at the center of a second petri-plate containing PDA. The plate with mycelial plug was then inverted over the plate inoculated with the bacterium. The plates were

sealed with parafilm and incubated at 28°C (*D. teres* f. sp. *teres*) and 20°C (*R. commune*). The diameter of *D. teres* f. sp *teres* was measured daily (every 24hrs) for 7 days and compared to the control. The diameter of *R. commune* was measured at every 3 days interval for 18 days and also compared to the control. Controls constituted fungal cultures inverted over non-inoculated PDA. Change in diameter of a given fungal colony was calculated by finding the difference between the diameter measured at a specific time from the diameter recorded previously. Five replicates were set up for each treatment. Percent inhibition of the bacterial volatiles were calculated each time the diameters were measured as follows;

$$\text{Percent inhibition} = \frac{D-d}{D} \times 100. \quad (2)$$

Where; D: is average diameter of the controls at the specific time the diameters were measured and,
d: is the average diameter of the fungal colonies exposed to bacterial volatiles at the time of measurement.

2.4. *In vivo* Antifungal Study against *D. teres* in Barley

Nguzo, a barley cultivar susceptible to net-blotch, was used in this experiment. The planting medium was prepared from soil, sand and peat in the ratio of 2:1:1 by volume respectively. The soil mixture was sterilized by subjecting it to 121°C and 1 bar for 2 hours in an autoclave and allowed to stand for 7 days to release toxic substances. The soil was then filled into 20 cm by 10.5 cm plastic pots. Twelve barley seeds were planted in each of the plastic pots in two rows. These pots were kept in the greenhouse and watered and fertilized (a mixture of 2.5 kg fertilization [N-P-K content 14-5-21%, respectively] with 25 litres of water) regularly [27]. After seed germination, thinning was done to leave 6 seedlings per pot.

Fungal conidia suspension was prepared by flooding 7-day-old pure plate cultures of *D. teres* f. sp *teres* with 20 ml of Sterile Distilled Water (SDW). Fungal mycelium was scrapped using the edge of a sterile glass to dislodge the conidia. The conidia suspension was then filtered using sterile cheese cloth to remove mycelia clumps [28,29]. Conidia counts were done by placing a drop of suspension on an improved Neubauer haemocytometer and examined under a microscope. Total conidia counts were adjusted to 400 conidia/ml [30] using SDW.

Inoculation was conducted by spraying 14-day-old plants with a conidial suspension at a concentration of 400 conidia per millilitre using an atomizer, until run off. Treatments for the bio-control experiment were: Plants inoculated with the net blotch pathogen and original concentration of the culture-filtrate, Plants inoculated with the net blotch pathogen and double concentration of the culture-filtrate, Plants inoculated with the net blotch pathogen and tebuconazole (20%w/w), a synthetic fungicide at the rate of 0.2% v/v, Plants inoculated with the pathogen alone (+ve Control), and Non-treated plants (–ve control). The experiments were arranged in the greenhouse in a completely randomized design layout.

The plants were covered using a transparent polythene bag for 72 h to induce high relative humidity conditions of about 100% and 20 ± 5°C for proper germination of conidia and high plant infection. Plants were removed from the polythene chamber after 72 h and placed on greenhouse benches. The percentage necrotic lesions caused by *Drechslera teres* was scored 7 days post-inoculation [30] Severity of net blotch on leaves were evaluated using a scale with 21 levels consisting of 5% intervals; 0 = no symptom, 1 = 0 – 5%, 2 = 5 – 10%, 3 = 10 – 15%, 4 = 15-20%,...,20 = 95 – 100% leaf area affected.

2.5. Statistical Analysis

Data recorded during the course of the study was analyzed independently. Data derived from *in-vitro* experiments were analyzed using student's t-test. Results from *in-vivo* studies were subjected to one-way analysis of variance and the means compared using the Tukey-Kramer Multiple-Comparison test at a 5% probability level.

3. Results

3.1. Isolation and Identification of Barley Fungal Pathogens

After the fungal isolates were cultured (*P. teres*-7 days and 21 days for *R. commune*) they were identified based on their colony morphology as well as microscopically by their conidial shape and hyphal characteristics. Figure 1 shows the net blotch pathogen and net-form-net-blotch symptoms in barley leaf.

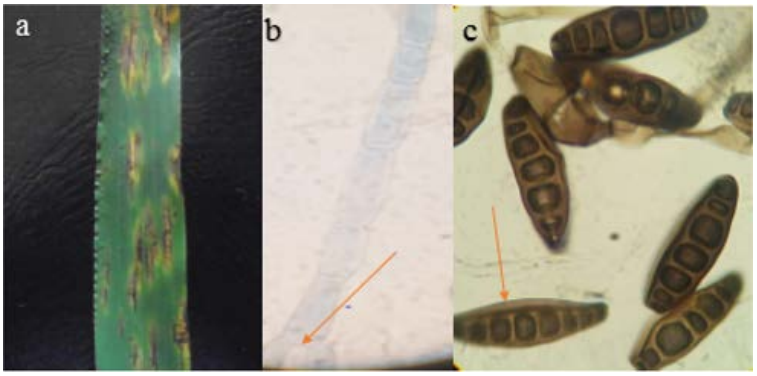

Figure 1. ***Pyrenophora teres.*** **a**; net form net blotch symptoms on a barley leaf. **b**; septate and branched (pointed by the arrow) mycelium. **c**; conidia with five and six (pointed) septa. Photo **a**, was photographed using Sony camera while micrographs **b** and **c**, were photographed using infinix Note 4 smartphone camera. The micrographs were at 1000X magnification

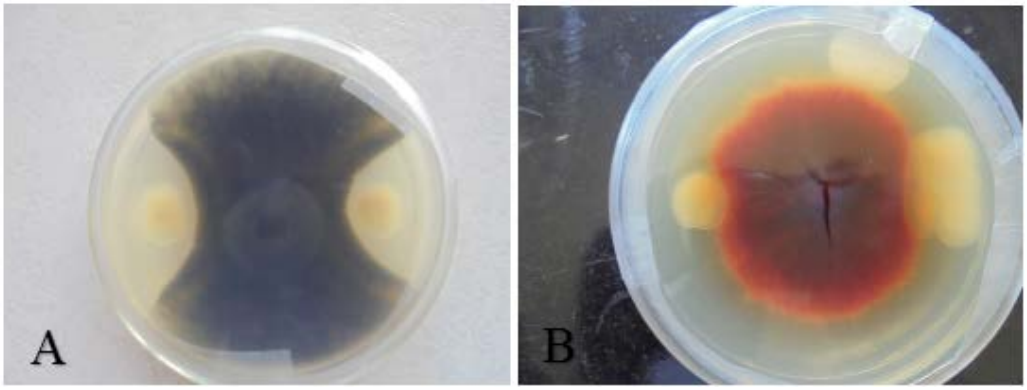

Figure 2. Petri plates showing the rear views of fungal pathogens isolates co-cultured with the antagonistic bacteria. In '**A**', (*D. teres*) inhibition of mycelial growth towards the bacterium is clearly evident. **B**; The inhibition of *R commune* mycelia by the bacteria is not effective

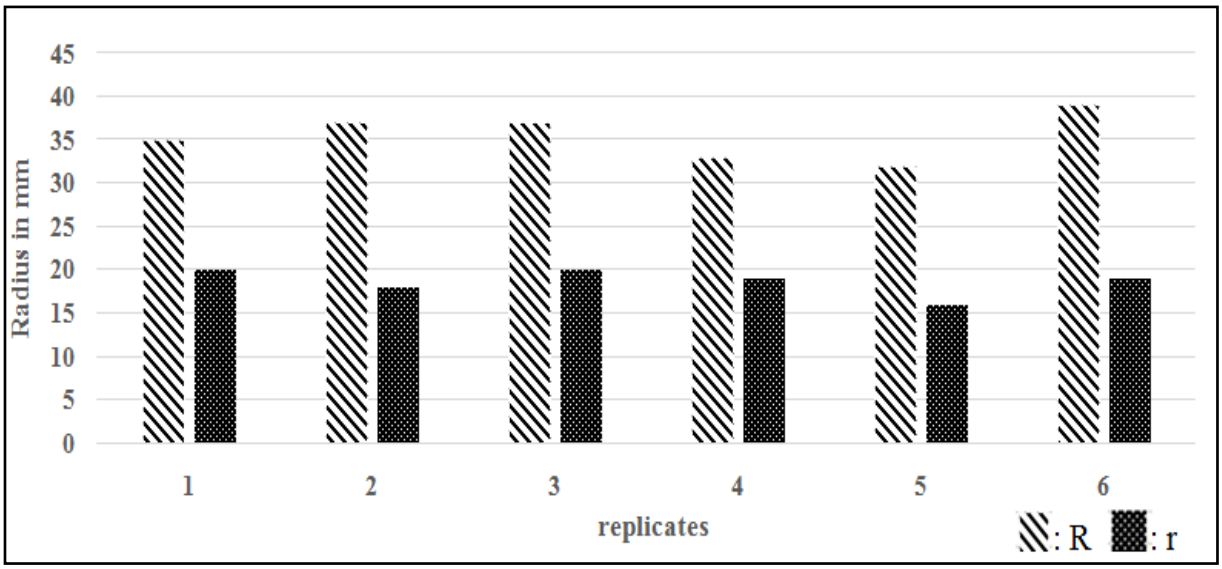

Figure 3. Effects of *P. polymyxa* on the growth of *D. teres* (dual assay)

Table 1. % mycelia inhibition of *D teres* f sp. *teres* by *Paenibacillus polymyxa* KaI245

Replicate.	R	r	%inhibition.
1	35	20	42.9
2	37	18	51.4
3	37	20	46.0
4	33	19	42.6
5	32	16	50.0
6	39	19	51.3
Mean	35.5	18.7	47.3
SD.	2.7	1.5	4.1
P	0.000		

R: radius away from the antagonist. **r**: radius towards the antagonist. **SD**: Standard Deviation. The data was recorded on the 7th day of the fungal growth.

3.2. Dual Culture

Live bacterial cells were tested against both *D. teres* f sp. *teres* and *R. commune in vitro* (Figure 2). Mycelial growth inhibition was strongest on *D. teres*. The strongest percentage inhibition was achieved at 51% and the lowest at 42% (Table 1). Average percent inhibition was found to be 47.3%. Further, the difference between 'R' and 'r' was found to be significant $t(10) = 13.47$ at $P = 0.05$. Dual plating of *Paenibacillus polymyxa* KaI245 with *R. commune* showed less than full effect in the control of the fungi. The apparent less 'r' compared to 'R' can only be attributed to the physical barrier to growth by the bacterial cells rather than to any antagonistic activity.

3.3. Fungal Inhibition by Bacterial Volatile Organic Compounds

Volatile organic compounds produced by *Paenibacillus polymyxa* KaI245 suppressed the growth of *R. commune* by an average of 52.9%. The inhibition rate did not significantly change with time; the lowest was 50.8% (between days 9-12) the highest was 54.1% (between days 3-6) (Table 2). After 18 days, *R. commune* colonies grown with the bacteria *P. polymyxa* on the lower plate recorded an average diameter of 25.8mm compared to an average of 56mm on the controls. Growth rate was also significantly inhibited. The colonies cultured with bacteria underneath had an average growth rate of 0.8mm/day while the controls had an average of 1.8mm/day. This is shown more clearly by the pictures depicting smaller colony diameters in Figure 4A compared to colonies with relatively larger diameters in Figure 4B. The line graph in Figure 5 relays information on growth rates. There was no

significant difference on the inhibition of growth rate between the days. Despite the live cell cultures having a strong inhibition on *Drechslera teres* as described earlier, the bacterial volatiles did not show any observable effect on the growth rate of the fungal mycelium (Figure 6).

Table 2. Inhibition effect of volatile metabolites of *P. polymyxa* KaI245 on *R. commune*

Day.	Colony diameters±SD (mm)		Percent (%) inhibition.
	Control.	Antagonized.	
3	29.2±1.5	13.6±1.1	53.4
6	36.6±1.1	16.8±1.3	54.1
9	42.4±2.4	20.2±2.3	52.4
12	48.4±4.3	23.8±4.0	50.8
15	53.2±6.1	25.2±3.1	52.6
18	56±9.6	25.8±3.9	53.9
Average.			**52.9**
SD.			**1.2**
***P* value.**	**0.009**		

The diameters represent averages from five replicates. The diameters were recorded at 3 days interval for 18 days. SD: Standard Deviation. Percentage inhibition was calculated from the average diameters of the antagonized compared to that of control on the same day ((C-A/C)x100). C: Control diameter, A: Antagonized diameter.

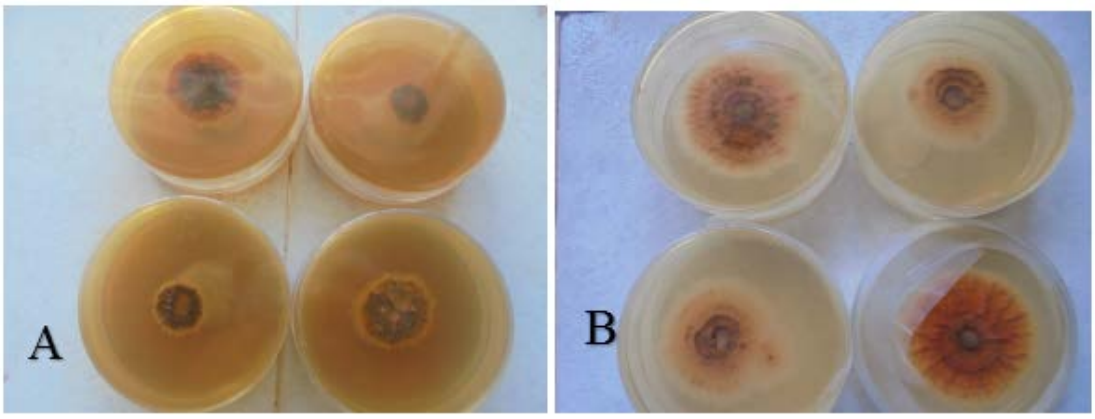

Figure 4. Figures depicting the effect of bacterial volatiles (sealed plate method) on the growth of *R. commune*. Both represent 18 days old colonies. 'A' represent the colonies having the bacteria growing on the lower plate while 'B' represents the plates having non inoculated media (PDA) on the lower plate

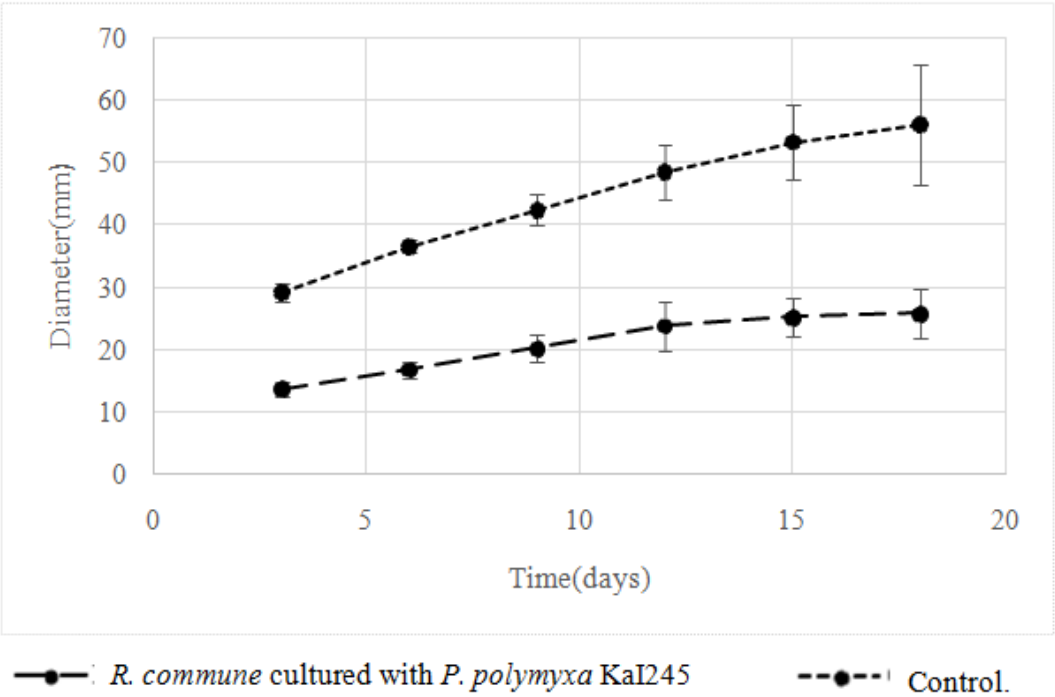

Figure 5. Effects of *P. polymyxa* volatiles on the growth rate of *R. commune*

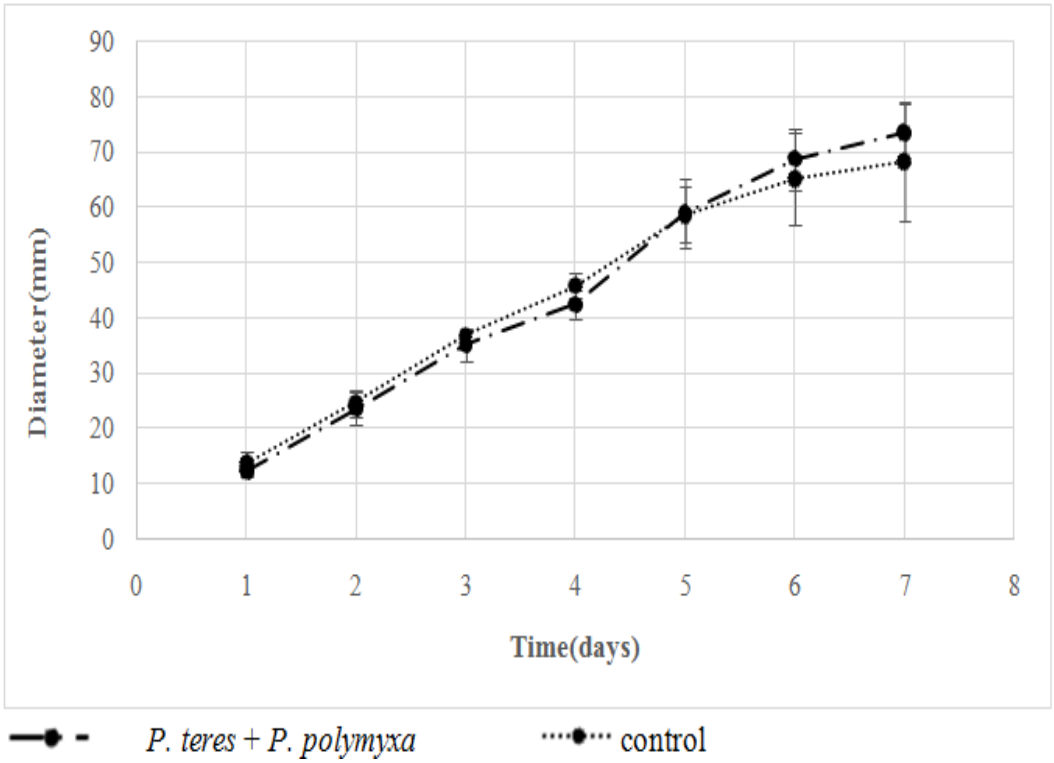

Figure 6. *D. teres* growth rate not affected by *P. polymyxa* volatiles

Table 3. Mean disease score and percent reduction of infection of net blotch on the leaf of nguzo barley variety

Treatment	Mean disease score±sd	% reduction of disease score.
Untreated (+ve control)	3.6±0.7[a]	–
D. teres + orius	1.0±0.8[b]	72.2
D. teres+double conc filtrate	1.8±0.8[c]	50
D. teres+normal conc filtrate	1.8±1.1[c]	50
P value		**0.00428**

Means followed by the same letter in each column are not significantly different at $P = 0.05$. The means were obtained from five replicates.

Figure 7. Different reactions by barley to *D. teres* fsp *teres*. A_1; plants inoculated with *D. teres* f. sp *teres* alone, B_1; plants inoculated with *D. teres* f. sp *teres* and the normal concentration of the crude bacterial extract. C_1; plants inoculated with *D. teres* fsp *teres* and double concentration of the crude bacterial extract. D_1; plant inoculated with *D. teres* f. sp *teres* and Orius (a chemical fungicide). E_1; non-inoculated plants. The pictures in the lower row provides detailed evidence of the lesions (or lack of) of the corresponding plants above them

3.4. Greenhouse Evaluation of *Paenibacillus polymyxa* KaI245 Culture Filtrate in Controlling Net Blotch

The crude bacterial extract was assessed for its efficacy in controlling the pathogen *D. teres* f sp. *teres* in the greenhouse. A susceptible barley cultivar, Nguzo, was used for this experiment. Foliar applications of both the double concentration of the extract and the normal concentration had a mean disease score of 1.8 with no significant difference between them at $P \leq 0.05$ (Table 3). They both represented 50% reduction in disease score as compared to the control. The crude extract was however not equally effective as the chemical fungicide 20%w/w tebuconazole. The foliar spray of the chemical fungicide recorded a mean disease score of 1.0 representing 72.2% disease reduction.

The positive control had a mean disease score of 3.6 and was significantly different from the rest. The maximum disease score of 5 was recorded in the untreated control compared to 3 and 2 for the crude bacterial extracts and chemical fungicide respectively. A minimum disease score of 0 was recorded for the chemical fungicide while a minimum of 1 and 2 was recorded for bacterial extracts and the untreated control respectively. These are depicted in Figure 7. Except for the non-inoculated plants (E_1 and E_2), each pair of leaves in the lower row represent a maximum and a minimum score for each of the treatments.

4. Discussion

In the present investigation, bacterial antagonist *Paenibacillus polymyxa* KaI245 strongly impeded growth of *Drechslera teres* f sp. *teres* as evidenced by prominent zones of antibiosis due to diffusible metabolites (Figure 2A). The live bacterial cells inhibited the growth of the fungus by approximately 47% (Table 1). The bacterium acts through production of substances that may be toxic to the fungi. Microbes acting through such mechanism of action have wide action spectrum and is more effective than any other mode of action [31]. This is also true for this bacterium since it was also shown to inhibit growth of *Colletotrichum sublenolium*, a pathogen of sorghum [22]. Further, it was shown that *Paenibacillus polymyxa* strain E681 produces fusaricidin with capability to control *Phytophthora capsici* the causative agent of phytophthora blight in red-pepper [32]. It has been shown that the bacterium *Pseudomonas fluorescens* BL915 produces pyrrolnitrin, hexyl-5-propylresorcinol and HCN and enzymes such as chitinase and gelatinase [33]. Like the bacteria used in the current research, *P. fluorescens* BL915 produced zones of antibiosis against *Rhizoctonia solani*.

The same results could however not be replicated with *R. commune*. Figure 2B reveals the less than satisfactory zones of inhibition produced by *R. commune*/KaI245 dual assy. The apparent zones of antibiosis can only be attributed to the physical presence of the bacterium that only prevents the growth of the fungal mycelium beyond the point of bacterium inoculation. Such a phenomenon can happen with any bacteria regardless of whether or not it produces antifungal metabolites. This variation in response by different fungi to a bacterial inhibitor has been shown to be common. *Bacillus subtilis* has been shown to inhibit different fungal pathogens to varying degrees even after it was shown to produce different mycolytic enymes [34]. This is an indication that fungi have different tolerance levels to bacterial antifungal systems. It has been reported that antibiotic production by bacteria varies depending on the competing organism [34]. *R. commune* used in this experiment may hence not stimulate the bacteria to release the antifungal metabolites. Because these are biological systems, experiments here reveal more than just the apparent need for competition for more production of antimicrobials. The phytopathogens, depending on their modes of nutrition (whether necrotroph, biotroth or hemibiotroph) produces different metabolites and probably differently alters the production of antimicrobials of a given antagonist. Necrotrophs are known to be robust in the production of phytotoxic metabolites. Net blotch pathogen itself has been shown to produce phytotoxic proteinaceous metabolites in culture [6]. This could be the trigger for the bacterium to produce more antifungal compounds and hence appear more antagonistic towards *D. teres*. Indeed, it has been shown that co-inoculation of the bacterial antagonist with another organism enhances the production of secondary metabolites [35,36]. This could inform on future developments that the range of activity of a given bio-control agent could be biased against a group of phytopathogens based on their mode of nutrition.

Despite the bacterial cells producing strong antifungal diffusible metabolites in dual assay, there was no effect of bacterial volatile organic compounds on the growth of *D. teres*. Figure 6 shows more or less the same colony diameters of the controls and the colonies exposed to bacterial volatiles. The volatiles strongly inhibited *R. commune* colonies though the bacterium did not show any antagonism in dual plate assay. The rate of growth of *R. commune* was inhibited by approximately 53%. It has been shown that susceptibility of bacterial volatile compounds varies greatly between different targets when the fast growing *Rhizoctonia solani* was little affected even by a strongly inhibiting strain of *Pseudomonas* strain SO4 [37]. Growth rate is therefore one of the factors affecting the sensitivity of a fungi to volatiles. This is reasonably so since between the antagonist and the target there is no point of contact. In the current experiment, *D. teres* had a significantly higher growth rate as compared to *R. commune*. The greater susceptibility of *R. commune* to the bacterial volatiles serves to reinforce the line of thought that a fungus with a slower growth is more sensitive to gaseous substances.

Moreover, a striking pattern here is that, the bacterium was inhibitory to *D. teres* via diffusible metabolites but not inhibitory via volatile emission and vice versa for *R. commune*. Given that these fungi belong to the same class, this pattern is surprising. These experiments provide enough evidence that the most effective bacteria in dual assay may not necessarily produce equally effective volatile organic compounds and vice versa. The observation is critical since it informs on future such experiments. Many scientists are tempted to choose only the most effective antagonists in dual assay for volatile production studies [38]. This experiment affirms that

degree of effectiveness of dual plate assay in antagonism should not always be the basis upon which a bacterium (or any other antagonist for that matter) is assessed for volatile mediated control.

5. Conclusion

This study highlights the potential of the sorghum rhizobacterium isolate in bio-control of *D. teres.* It is clear that *P. polymyxa* KaI245 isolated from the sorghum rhizosphere produced extracellular products effective against *D. teres.* The volatile organic compounds from the bacterium however didn't show any inhibition towards *D. teres* but strongly inhibited *R. commune* growth *in-vitro*. Barley leaves infected with *D. teres* and sprayed with *P. polymyxa* culture filtrate showed 50% reduction in net blotch symptoms compared to the controls. This performance however, didn't match that presented by Orius 25EW (20% tebuconazole) which presented 72.2% symptom reduction. Optimization of the antifungal activity of the bacterium filtrate needs can be done to enhance its activity.

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Statement of Competing Interests

The authors have no competing interests.

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