

# Applying beneficial microbes as transplanting dipping and post-transplanting foliar spray led to improved rice productivity

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## Abstract

Rice is a popular food in Africa, but current yield achieved is far lower than the yield potential due to abiotic/biotic stresses. We recently demonstrated that commercially formulated products of *Bacillus subtilis* and *Trichoderma asperellum* strains increased rice yield when applied as transplant dipping or post-transplanting foliar sprays in Tanzania (Africa). Further experiments were conducted to investigate: (1) synergies in alternate use of *B. subtilis* and *T. asperellum* products as dipping or foliar spray in Tanzania, and (2) effects of coating seeds with *Serratia nematodiphila* in Kenya (Africa). In Tanzania, using formulated *B. subtilis* and *T. asperellum* products led to > 100% increase in yield. Furthermore, alternative use of *B. subtilis* and *T. asperellum* as transplant dipping or post-transplanting foliar spray led to further yield increase (ca. 32%) over the use of single-organism products at both times. Microbial treatments led to significant reductions in rice blast. In comparison with the fungicide treatment, increased yield with using microbial products appeared to have resulted mostly from improved plant development rather than reduced rice blast *per se*. At the Kenyan site where current yield is relatively high, the overall yield increase associated with microbial products was limited although statistically significant. Coating seeds with one specific *S. nematodiphila* strain led to grain yield comparable to applying microbial products as transplant dipping and post-transplanting foliar spray. The present study suggests that the formulated microbial products can significantly improve rice productivity in subsistence farming and should be applied in alternation over time to exploit their synergies.

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## Introduction

Rice (*Oryza sativa*) is grown in many African countries with substantial increased productivity recently<sup>[1]</sup>, but the overall rice demand in Africa has outstripped local production, requiring 40% imported rice. Tanzania is the most important rice production zone in East Africa; rice is the second most cultivated food and commercial crop in Tanzania after maize. However, rice productivity in Tanzania is still low, with the average yield in the range of 1.5–2.8 t·ha<sup>-1</sup>, due in large part to the fact that small-scale and low-technology farmers account for ca. 70% of the rice growing area. On these farms, fertilisers are often not used because of the cost, limiting yields. Rice blast caused by *Magnaporthe oryzae* is another challenge facing rice farmers<sup>[2–4]</sup>. Good blast control may be achieved with intensive fungicide programmes, but this is both expensive, particularly for smallholder farms, and undesirable<sup>[5]</sup>.

The use of beneficial microbes is being promoted for their potential in sustainable agriculture, by exploiting their biocontrol effects against specific diseases and/or plant growth promoting effects. In addition to conventional applications of biopesticides to soils (e.g., drenching) and plants (e.g., dipping, spraying), coating seeds with low amounts of beneficial microbes can be an efficient way to delivering beneficial microbes for improving seed germination, seedling establishment and suppression of root diseases<sup>[6,7]</sup>. Treating *Brassica*

*napus* seeds with biocontrol organisms and fungicides were, however, less effective than soil drench in reducing clubroot caused by *Plasmodiophora brassicae*<sup>[8]</sup>. Bio-priming rape seed with *Serratia plymuthica* led to reduced development of *Verticillium dahliae*<sup>[9]</sup>. Coating eggplant seeds with *Paenibacillus alvei* or a nonpathogenic *Fusarium oxysporum* strain led to significant suppression of *Verticillium* wilt<sup>[10]</sup>.

To control rice blast, biopesticides may be applied as a seed priming treatment, dipping treatment at transplanting, and foliar sprays post-transplanting<sup>[11–14]</sup>. Promising organisms against rice blast include strains from several common sources for biocontrol agents: *Bacillus*, *Trichoderma*, *Streptomyces* and *Pseudomonas*<sup>[12,13,15,16]</sup>. Several rhizobacteria showed a good efficacy against rice leaf blast when applied as soil drench or foliar spray but the efficacy varying with application methods<sup>[17]</sup>. A recent pilot study in Kenya showed that dipping roots of rice seedlings in commercial formulated *B. subtilis* or *T. asperellum* products led to reduced blast development and increased yield. Following this pilot study, we conducted further field studies and showed that applying formulated *B. subtilis* or *T. asperellum* commercial products as transplanting dipping or as post-transplanting foliar sprays led to large increases in rice yield only at subsistence farming low-yield sites (Tanzania) and the benefit of using the same organism product as dipping or foliar spray varied greatly with seasons<sup>[18]</sup>. Reduction in blast development due to biocontrol

was achieved but did not necessarily lead to corresponding increases in yield<sup>[14]</sup>. It is often recommended to combine biopesticides to achieve better performance, exploiting potential synergies among different microbial organisms. However, such a laudable objective of exploiting synergies has rarely been achieved<sup>[19]</sup>. Mixed use of formulated *B. subtilis* or *T. asperellum* products did not lead to additional benefits in terms of blast control or rice yield<sup>[18]</sup>.

The present study aims to develop strategies of applying commercial biopesticides for rice growers in Africa, extending our previous research<sup>[18]</sup> in the following three specific aspects on the use of formulated microbial products in rice production. Firstly, alternate use of formulated *B. subtilis* and *T. asperellum* products as transplanting dipping or post-transplanting foliar spray were assessed for potential synergistic effects in reducing blast and increasing yield. In the previous research<sup>[18]</sup>, the same biopesticide product was used in dipping and post-transplanting foliar spray. Secondly, we reduced the number of post-transplanting foliar sprays from the previous five/six sprays to two sprays. Finally, the effect of coating rice seeds with one *S. nematodiphila* strain on blast development and rice yield was compared with using microbial products as transplanting dipping and post-transplanting foliar spray. In our previous study, newly formulated product of this *S. nematodiphila* strain performed similarly to formulated *B. subtilis* and *T. asperellum* products when applied as dipping or post-transplanting foliar spraying.

## Materials and methods

### Overall approach

Experiments were conducted at one site in Kenya for two cropping seasons and three sites in Tanzania for one crop season in 2022, at the same sites as the experiments reported previously<sup>[18]</sup>. A randomised block design was used for all sites. Within each block, there was one plot (5 m × 5 m) randomly assigned to one of the treatments; planting space was 20 cm × 15 cm. There was a gap of 75 cm between neighbouring plots.

In Kenya, the study objective was to compare a seed-coating treatment with the formulated product of one specific Real IPM [The Real IPM Co. (Kenya) Ltd., Thika, Kenya] *S. nematodiphila* strain (code SN01) with three other Real IPM formulated products of specific *B. subtilis* strain Bs01 (trade name: REGAIN®), *T. asperellum* strain T-900 (trade name: Sustain®), and the same *S. nematodiphila* strain SN01 when applied as dipping at transplanting and/or post-transplanting foliar spray. In Tanzania, the main objective was to assess whether there was additional benefit in the alternate use of formulated *B. subtilis* and *T. asperellum* products as transplanting dipping or post-transplanting foliar spray. All formulated *B. subtilis*, *T. asperellum* and *S. nematodiphila* products contained  $1.0 \times 10^{10}$  CFUs ml<sup>-1</sup> of specific microbes. The formulated *S. nematodiphila* product and seed coating product with the *S. nematodiphila* strain were not included in Tanzania as they were not approved for use there.

For transplanting dipping treatments, seedling roots were dipped in an appropriate product (5 ml formulated product in 1 L water) for 30 min before transplanting. For post-transplanting foliar application, unlike in the previous study<sup>[18]</sup>, microbial products or fungicides were applied only twice with a knapsack sprayer to protect panicles when they were emerging from the boot, about nine weeks after transplanting; two sprays

were applied 7 d apart. Each plot received approximately 7 L each time at the same concentration as dipping treatments.

### Kenya

A single cultivar (cv. Basmati 370) was used at the National Irrigation Board site (longitude  $-37^{\circ}37'2.53''$  E and latitude  $-0^{\circ}49'24.23''$  S). In this region, the main agricultural activity is monocropping of rice grown in paddies that are irrigated for six months. The soil at the site is of the black cotton soil type (vertisol).

There were five blocks and nine treatments. There were three formulated products (*B. subtilis*, *T. asperellum* and *S. nematodiphila*), each applied as (i) transplanting dipping only, and (ii) both as transplanting dipping and post-transplanting foliar spray. There were three additional treatments: (i) seed coating with *S. nematodiphila* only, (ii) water control [dipping in and foliar spray with water], and (iii) fungicide control [post-transplanting spray only – 'Absolute', a mixture of azoxystrobin (200 g·L<sup>-1</sup>), difenoconazole (125 g·L<sup>-1</sup>) and hexaconazole (50 g·L<sup>-1</sup>)]. For the first cropping season, seeds were sown in early February 2022 and transplanted in early March 2022. For the second cropping season, seeds were sown in late August 2022 and transplanted in late September 2022. Fertilisers were applied *via* broadcasting to the trial on three occasions: muriate of potash at 25 kg per acre, three days after transplanting, and sulphate of ammonia at 50 kg per acre, two and seven weeks after transplanting.

Number of tillers and the height of the highest tiller for each plant was assessed weekly immediately from transplanting, giving a total nine assessments. In each plot, the same five plants (randomly selected on the first assessment date) were assessed for tiller development at all nine time points. For each plot, time to the first and median flowering was recorded. Rice blast assessment commenced four weeks after transplanting and thereafter weekly on five leaves, one leaf from each of five plants randomly selected on each assessment occasion for each plot. As blast was not severe, only presence of rice blast was recorded. In total there were five blast assessments. For each plot, 10 panicles (heads) were randomly selected, one from each of 10 randomly selected plants, to estimate number of grains per panicle. The 1000 seed weight and unshelled (raw) gross grain yield were obtained for each plot.

### Tanzania

Experiments were conducted at three sites [Babati ( $-3.8541^{\circ}$  S,  $35.5235^{\circ}$  E), Kikwe ( $3.3711^{\circ}$  S,  $36.8285^{\circ}$  E) and Moshi ( $3.4197^{\circ}$  S,  $37.3676^{\circ}$  E)]; the soil at all three sites is of the black cotton soil type (vertisol). Seeds were sown on 1/12/2021 (Kikwe), 21/12/2021 (Moshi), and 11/01/2022 (Babati). A single cultivar [cv. SARO 5 (TXD 306)] was used at all three sites.

At each site, there were three blocks and nine treatments. There were two formulated products (*B. subtilis* and *T. asperellum*) that were applied individually in two treatments: only transplanting dipping, and both transplanting dipping and post-transplanting foliar spray. There were a further two treatments with alternate use of the two products: *B. subtilis* dipping/*T. asperellum* spray, and *T. asperellum* dipping/*B. subtilis* spray. Finally, there were three control treatments: two water controls and one fungicide control (SC250ECORE 250 g·L<sup>-1</sup> (difenoconazole), applied at 0.5 L·ha<sup>-1</sup>). One of the two water controls was initially reserved for the *S. nematodiphila* seed coating treatment; unfortunately, this product was not

## Use of beneficial microbes to increase rice yield

registered in Tanzania in time for this experiment. Urea was applied through broadcasting as a top dressing six weeks after transplanting; lambda cyhalothrin or deltamethrin was applied to control stalk borers four weeks after transplanting.

Number of tillers and the height of the highest tiller for each of five plants were assessed three times; five plants were randomly selected for tiller assessment at each time point. At Kikwe, they were measured 6, 14 and 19 weeks after transplanting; at Moshi, 6, 10 and 15 weeks after; and at Barbati, 4, 18 and 28 weeks after. For each plot, times to 30% flowering and to maturity were recorded. Number of seeds per head and percentage of grains with rice blast symptoms were recorded at harvest: five panicles were selected randomly (one from a single plant) for each plot to count number of grains per panicle and with blast symptoms. Finally, both unshelled (raw) and shelled (net) grain yields were obtained for each plot.

### Data analysis

For all experiments, preliminary repeated measurement analysis of variance (ANOVA) of tiller number and height showed that there were no significant interactions between assessment dates and treatments. Thus, only the tiller number and height on the final assessment date were statistically analysed and presented. All statistical analyses and graphing were conducted in R<sup>[20]</sup>.

### Kenya

The data were subject to two stages of ANOVA. For yield, number of seeds per panicle, and days to flowering or maturity, log transformation was applied to ensure that residual errors follow (or closely follow) normal distributions. A logit transformation was applied to the incidence of leaves with blast symptoms before ANOVA. No transformation was needed for the 1000 seed weight, tiller number and height. Firstly, ANOVA was applied to all the treatment data pooled over the two crop seasons where cropping season was treated as a blocking factor in addition to the within-experiment block and treatment factors. When ANOVA indicated significant differences among the nine treatments, the Tukey HSD (honestly significant difference) test was then applied to conduct pairwise comparisons. Secondly, ANOVA was applied to the six treatments involving three microbial products applied as dipping or as foliar spray. In this analysis the six microbial treatments were considered as a two factorial design [three products, and two application strategies (dipping only, dipping + foliar spray)] to assess whether three products differed overall in their effects and whether there were interactions between the three products and two application strategies.

### Tanzania

Similarly a two-stage ANOVA analysis as for the Kenya data were applied. In addition to unshelled (gross) and shelled (net) grain yield, the ratio between the net and gross grain weight was also calculated and analysed. For net gross ratio, log transformation was applied to ensure that residual errors follow (or closely follow) normal distributions. For all other variables, transformation was the same as for the Kenya data. In the first analysis of all nine treatments, the site was treated as a blocking factor in addition to the within-site block and treatment factors. The second ANOVA was applied to the six microbial treatments to determine the effects of multiple treatments and possible synergy from alternate use of *Bacillus* and *Trichoderma* products. In this ANOVA, in addition to site and within-site

block factors, there were two treatment factors: products used as (i) dipping (*Bacillus* or *Trichoderma*), and (ii) as foliar spray (Water, *Bacillus* or *Trichoderma*). The main effect of the foliar spray (two degree-of-freedom, DF) was further decomposed into two single DF orthogonal comparisons: (i) water vs the two microbial products, and (ii) *Bacillus* vs *Trichoderma*. The interaction of the dipping factor with the single DF *Bacillus* vs *Trichoderma* foliar spray comparison indicates whether there were synergies in alternative use of *Bacillus* and *Trichoderma* products over time.

## Results

### Kenya experiments

#### Tiller number and height

Both tiller number and height increased nearly linearly with time and for both variables there were no significant interactions between treatment and time. The overall average final number of tillers per plant across all treatments was 36.7 ( $\pm$  0.50). The final tiller number differed ( $p < 0.001$ ) among the nine treatments: all the six treatments with microbial dipping and/or foliar spray led to more tillers than the water control (Table 1). Overall, there were no additional benefits of applying foliar spray in addition to dipping. The three microbial products did not differ from each other irrespective of the application strategy. In contrast, tiller height did not differ among the nine treatments and among the three products; the overall average final tiller height was 116.0 cm ( $\pm$  1.80).

**Table 1.** Significant pairwise comparisons among the nine treatments applied to rice plants at one site in Kenya across two cropping seasons in 2022, based on the Tukey HSD test. The nine treatments included three biopesticides (*Bacillus subtilis*, *Trichoderma asperellum* and *Serratia nematodiphila*), each applied as dipping at transplanting, and post-transplanting foliar spray as well as dipping, and two controls (water and foliar fungicide spray). The final treatment was seed coating with *S. nematodiphila*.

Treatment 1 (dipping : spraying)	Treatment 2 (dipping : spraying)	Differences	<i>p</i> value
Tiller number on the final assessment date			
Water control	<i>Bacillus</i> : <i>Bacillus</i>	-0.104	0.0247
Water control	<i>Bacillus</i> : Water	-0.111	0.0124
Water control	<i>Serratia</i> : <i>Serratia</i>	-0.099	0.0383
Water control	<i>Serratia</i> : Water	-0.114	0.0094
Water control	<i>Trichoderma</i> : <i>Trichoderma</i>	-0.107	0.0189
Water control	<i>Trichoderma</i> : Water	-0.109	0.0146
Number of seeds per panicle (on the natural logarithm scale)			
<i>Trichoderma</i> : Water	<i>Serratia</i> : Water	0.156	0.0320
Water control	<i>Bacillus</i> : <i>Bacillus</i>	-0.200	0.0015
Water control	<i>Bacillus</i> : Water	-0.181	0.0062
Water control	<i>Serratia</i> : <i>Serratia</i>	-0.184	0.0049
Water control	<i>Serratia</i> : Water	-0.188	0.0036
Water control	<i>Trichoderma</i> : <i>Trichoderma</i>	-0.217	0.0004
Water control	<i>Trichoderma</i> : Water	-0.241	0.0001
Water control	Fungicide	-0.155	0.0331
Incidence of tillers with blast symptoms (on the logit scale)			
Water control	<i>Bacillus</i> : <i>Bacillus</i>	0.685	0.0000
Water control	<i>Bacillus</i> : <i>Trichoderma</i>	0.785	0.0000
Water control	<i>Serratia</i> coating	0.554	0.0000
Water control	<i>Serratia</i> : <i>Serratia</i>	0.618	0.0000
Water control	<i>Serratia</i> : Water	0.608	0.0000
Water control	<i>Trichoderma</i> : <i>Trichoderma</i>	0.639	0.0000
Water control	<i>Trichoderma</i> : Water	0.532	0.0000
Water control	Fungicide	0.597	0.0000

**Days to first flowering**

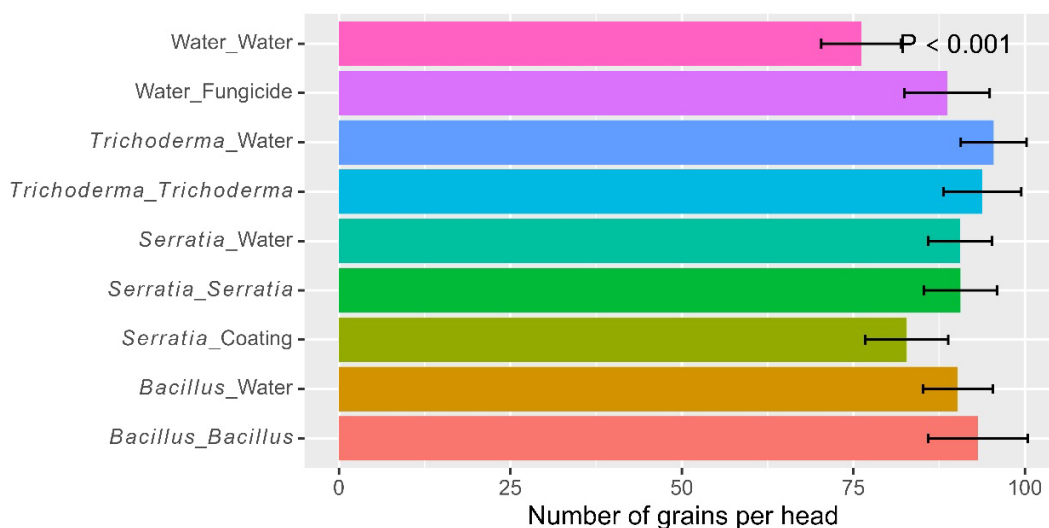
On average, it took 79.0 d ( $\pm 0.53$ ) to reach flowering onset; the length of this time did not differ significantly among the nine treatments as well as among the three products.

**Yield data**

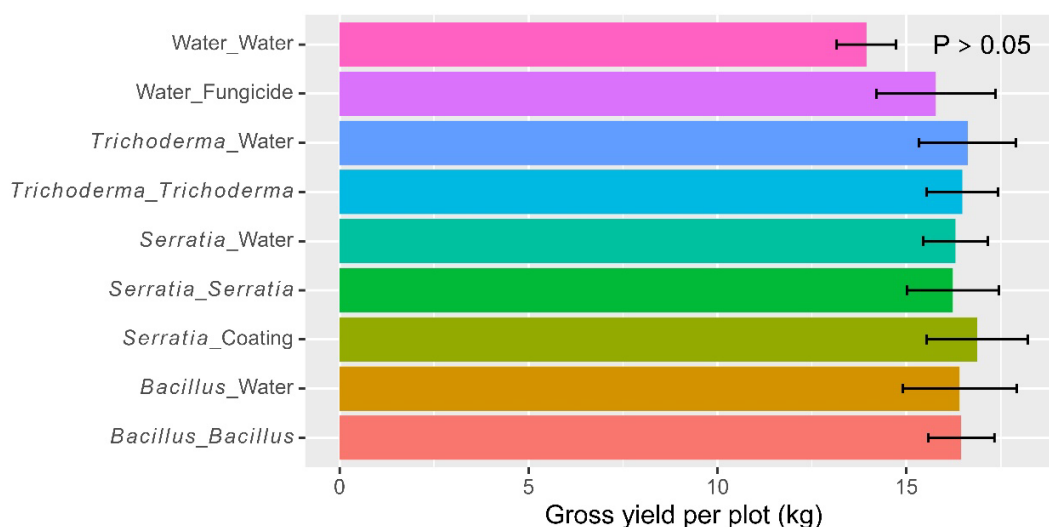
Overall, there were 89.1 seeds ( $\pm 1.91$ ) per panicle. There were significant ( $p < 0.001$ ) differences among the nine treatments in the number of seeds per panicle, due primarily to the fact that the water control had fewer seeds than all the other treatments, except the seed-coating (Fig. 1; Table 1). There were no additional benefits of applying foliar spray in addition to dipping. There were no significant differences among the three products irrespective of the application strategy. The grand average 1000 seed weight was 20.1 g ( $\pm 0.21$ ) and there were no significant differences among the three products or

effects of additional foliar applications.

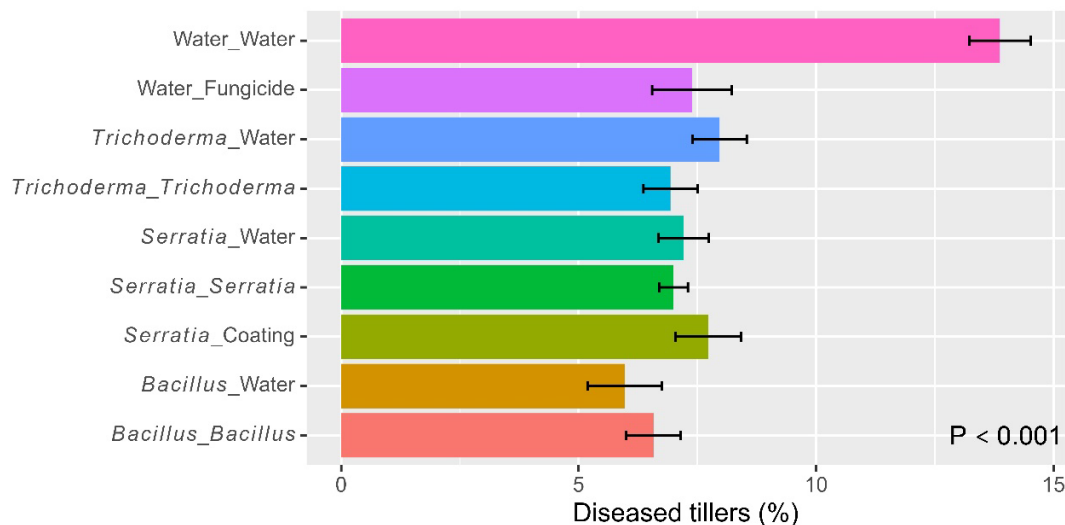
The average gross grain yield was 16.1 kg ( $\pm 0.39$ ) per plot; there were no significant differences among the nine individual treatments (Fig. 2). However, ANOVA of combined data over all three microbes (including seed-coating) showed that applying beneficial microbes once (seeding coating or dipping) or twice (dipping and foliar spray) led to a significant increase ( $p < 0.05$ ) in the gross yield over the water control. The average gross yield per plot was 13.9 kg ( $\pm 0.78$ ), 16.6 kg ( $\pm 0.61$ ) and 16.4 kg ( $\pm 0.57$ ) for the water control, dipping or seed-coating, and both dipping and spray treatments, respectively. No additional benefit was obtained when foliar spray was applied in addition to transplanting dipping. There were no significant differences among the three products when applied as dipping or foliar spray.



**Fig. 1** Number of grains per head of rice plants, treated by one of the four biopesticides at different times (seed coating, dipping at transplanting, post-transplanting foliar spray, or both dipping and spray) at one site in Kenya over two cropping seasons in 2022. Two controls (water as negative, and fungicide foliar spray as positive) were included for comparison. The bar represents one standard error, and P value is associated with the F-test in ANOVA, indicating the overall differences among the nine treatments.



**Fig. 2** Gross yield (kg per plot) of rice plants, receiving one of the four biopesticides applied at different times (seed coating, dipping at transplanting, post-transplanting foliar spray, or both dipping and spray) at one site in Kenya over two cropping seasons in 2022. Two controls (water as negative, and fungicide as positive) were included for comparison. The bar represents one standard error and P value is associated with the F-test in ANOVA, indicating the overall differences among the nine treatments.



**Fig. 3** Percentage of tillers with blast symptoms for those rice plants receiving one of the four biopesticides applied at different times (seed coating, dipping at transplanting, post-transplanting foliar spray, or both dipping and spray) at one site in Kenya over two cropping seasons in 2022. Two controls (water as negative, and fungicide as positive) were included for comparison. The bar represents one standard error, and P value is associated with the F-test in ANOVA, indicating the overall differences among the nine treatments.

### Blast development

Across all treatments, about 7.9% of leaves had blast lesions. There were significant ( $p < 0.001$ ) differences among the nine treatments in the blast incidence – the water control had a higher blast incidence than all the other treatments (Fig. 3, Table 1). Seed-coating or dipping alone managed rice blast as satisfactorily as the fungicide treatment; there were no additional reductions in blast with additional foliar application. The three microbial products did not differ in their effects on blast development when applied as dipping or foliar spray.

### Tanzania experiments

#### Tiller number and height

For both tiller number and height there were no significant interactions between treatment and time. The average final number of tillers per plant was 18.8 ( $\pm 0.62$ ). The final tiller number differed ( $P < 0.001$ ) among the eight treatments: all four treatments with both transplant dipping and foliar applications of *Bacillus* or *Trichoderma* led to more tillers than the water control (Table 2). Foliar spray in addition to dipping led to further increases ( $p < 0.05$ ) in the number of tillers; but there were no synergies in alternate use of *Bacillus* and *Trichoderma*.

The average final height was 34.1 cm ( $\pm 1.09$ ). The final plant height differed ( $p < 0.001$ ) among the eight treatments: all six microbial treatments increased plant height over the water control (Table 2). Foliar spraying with *Bacillus* led to higher plants than *Trichoderma* ( $p < 0.05$ ); there were no synergies in alternate use of *Bacillus* and *Trichoderma*.

#### Days to 30% flowering and harvest

On average, it took 66.9 d ( $\pm 1.20$ ) to reach 30% flowering. The time to 30% flowering differed ( $p < 0.001$ ) among the eight treatments, due primarily to the fact that microbial treatments reduced the time to 30% flowering (Fig. 4; Table 2). For instance, the time to 30% flowering for the water control was 73.9 d ( $\pm 1.63$ ), compared to 67.7 d ( $\pm 2.45$ ) for dipping only treatments. Applying microbial products as foliar spray at the booting stage also reduced ( $p < 0.01$ ) the time to 30% flowering, which was due primarily to the synergies in alternate use of

*Bacillus* and *Trichoderma* (Fig. 5): time to 30% flowering was 60.6 d ( $\pm 1.95$ ) for the two treatments of using the two products in alternative, compared to 65.5 d ( $\pm 2.51$ ) for the two treatments using the single products as dipping and foliar spray. The average time to grain maturity was 95.5 d ( $\pm 1.42$ ). The treatment effects on the time to maturity were similar to, but less pronounced than, those on the time to 30% flowering.

#### Yield

Overall, there were 107 seeds ( $\pm 1.91$ ) per panicle. The eight treatments differed ( $p < 0.001$ ) in the number of seeds per panicle, due primarily to the fact that the water/fungicide control had fewer seeds than microbial treatments (Table 2). Foliar spraying led to further increases ( $p < 0.01$ ) in the number of seeds per panicle, due primarily to the synergies in alternate use of *Bacillus* and *Trichoderma*: 88.8 seeds ( $\pm 1.95$ ) (water control), 108 seeds ( $\pm 2.62$ ) (dipping only), 111 seeds ( $\pm 2.76$ ) (dipping + spray with the same product), and 122 seeds ( $\pm 1.56$ ) (alternate use of the two products). This synergy was highly significant ( $p < 0.001$ ).

On average, the net grain yield was 3.13 kg ( $\pm 0.167$ ) per plot. There were significant ( $p < 0.001$ ) differences among the eight treatments (Fig. 5), accounting for 72.9% of the total observed variability. The water control had a lower yield than all the other treatments, and the fungicide treatment had a lower yield than the four treatments with microbial products applied both at dipping and post-transplanting (Table 2). Foliar spraying led to further increases ( $p < 0.001$ ) in the net grain yield, and there was a significant ( $p < 0.01$ ) synergy from alternate use of the *Bacillus* and *Trichoderma* products. The average net grain yields achieved were 1.51 kg ( $\pm 0.123$ ) (water control), 2.77 kg ( $\pm 0.210$ ) (dipping only), 3.56 kg ( $\pm 0.195$ ) (dipping + spray with the same product), and 4.70 kg ( $\pm 0.227$ ) (alternate use of the two products). Overall, the two *Bacillus* and *Trichoderma* products did not differ in grain yield when applied as a dipping or foliar spray.

#### Blast development

On average, the incidence of grain with blast symptoms was 16.1% ( $\pm 1.20\%$ ). There were significant ( $p < 0.001$ ) differences

**Table 2.** Significant pairwise comparisons for all eight treatments applied to rice plants at three trial sites in Tanzania in 2022, based on the Tukey HSD test. The eight treatments included six microbial treatments and two controls (water and fungicide foliar spray).

Treatment 1 (dipping : spraying)	Treatment 2 (dipping : spraying)	Differences	p value
Number of tillers (on a log scale)			
<i>Bacillus</i> : <i>Bacillus</i>	Water control	0.307	0.0108
<i>Bacillus</i> : <i>Trichoderma</i>	Water control	0.328	0.0050
<i>Trichoderma</i> : <i>Bacillus</i>	Water control	0.403	0.0002
<i>Trichoderma</i> : <i>Trichoderma</i>	Water control	0.284	0.0239
Tiller height (cm – on a log scale)			
<i>Bacillus</i> : <i>Bacillus</i>	Water control	0.222	0.0002
<i>Bacillus</i> : <i>Trichoderma</i>	Water control	0.193	0.0016
<i>Bacillus</i> : Water	Water control	0.181	0.0038
<i>Trichoderma</i> : <i>Bacillus</i>	Water control	0.269	0.0000
<i>Trichoderma</i> : <i>Trichoderma</i>	Water control	0.183	0.0032
<i>Trichoderma</i> : Water	Water control	0.179	0.0045
Days to 30% flowering (on a log scale)			
<i>Bacillus</i> : Water	<i>Trichoderma</i> : <i>Bacillus</i>	0.144	0.0098
<i>Trichoderma</i> : <i>Trichoderma</i>	<i>Trichoderma</i> : <i>Bacillus</i>	0.131	0.0256
<i>Trichoderma</i> : Water	<i>Trichoderma</i> : <i>Bacillus</i>	0.155	0.0039
Fungicide	<i>Bacillus</i> : <i>Bacillus</i>	0.179	0.0005
Fungicide	<i>Bacillus</i> : <i>Trichoderma</i>	0.193	0.0001
Fungicide	<i>Bacillus</i> : Water	0.130	0.0285
Fungicide	<i>Trichoderma</i> : <i>Bacillus</i>	0.274	0.0000
Fungicide	<i>Trichoderma</i> : <i>Trichoderma</i>	0.142	0.0110
Fungicide	<i>Trichoderma</i> : Water	0.119	0.0614
Water control	<i>Bacillus</i> : <i>Bacillus</i>	0.148	0.0010
Water control	<i>Bacillus</i> : <i>Trichoderma</i>	0.163	0.0002
Water control	<i>Trichoderma</i> : <i>Bacillus</i>	0.243	0.0000
Water control	<i>Trichoderma</i> : <i>Trichoderma</i>	0.112	0.0296
Number of seeds per pedicle (on a log scale)			
<i>Bacillus</i> : <i>Trichoderma</i>	Fungicide	0.200	0.0022
<i>Trichoderma</i> : <i>Bacillus</i>	Fungicide	0.209	0.0011
<i>Bacillus</i> : <i>Bacillus</i>	Water control	0.248	0.0000
<i>Bacillus</i> : <i>Trichoderma</i>	Water control	0.324	0.0000
<i>Bacillus</i> : Water	Water control	0.200	0.0002
<i>Trichoderma</i> : <i>Bacillus</i>	Water control	0.333	0.0000
<i>Trichoderma</i> : <i>Trichoderma</i>	Water control	0.203	0.0002
<i>Trichoderma</i> : Water	Water control	0.206	0.0001
Shelled yield (kg per plot on a log scale)			
<i>Bacillus</i> : <i>Trichoderma</i>	<i>Bacillus</i> : Water	0.460	0.0086
<i>Trichoderma</i> : <i>Bacillus</i>	<i>Bacillus</i> : Water	0.583	0.0003
<i>Bacillus</i> : <i>Trichoderma</i>	<i>Trichoderma</i> : Water	0.545	0.0009
<i>Trichoderma</i> : <i>Bacillus</i>	<i>Trichoderma</i> : Water	0.669	0.0000
<i>Bacillus</i> : <i>Bacillus</i>	Fungicide	0.514	0.0021
<i>Bacillus</i> : <i>Trichoderma</i>	Fungicide	0.737	0.0000
<i>Trichoderma</i> : <i>Bacillus</i>	Fungicide	0.861	0.0000
<i>Trichoderma</i> : <i>Trichoderma</i>	Fungicide	0.518	0.0019
<i>Bacillus</i> : <i>Bacillus</i>	Water control	0.882	0.0000
<i>Bacillus</i> : <i>Trichoderma</i>	Water control	1.105	0.0000
<i>Bacillus</i> : Water	Water control	0.646	0.0000
<i>Trichoderma</i> : <i>Bacillus</i>	Water control	1.229	0.0000
<i>Trichoderma</i> : <i>Trichoderma</i>	Water control	0.886	0.0000
<i>Trichoderma</i> : Water	Water control	0.560	0.0000
Fungicide	Water control	0.368	0.0202
Incidence of grains with blast symptoms (on the logit scale)			
<i>Bacillus</i> : Water	<i>Bacillus</i> : <i>Trichoderma</i>	0.854	0.0030
<i>Bacillus</i> : Water	<i>Trichoderma</i> : <i>Bacillus</i>	0.683	0.0350
<i>Trichoderma</i> : Water	<i>Bacillus</i> : <i>Trichoderma</i>	0.703	0.0266
Water control	<i>Bacillus</i> : <i>Bacillus</i>	1.250	0.0000
Water control	<i>Bacillus</i> : <i>Trichoderma</i>	1.580	0.0000
Water control	<i>Bacillus</i> : Water	0.726	0.0037
Water control	<i>Trichoderma</i> : <i>Bacillus</i>	1.409	0.0000
Water control	<i>Trichoderma</i> : <i>Trichoderma</i>	1.061	0.0000
Water control	<i>Trichoderma</i> : Water	0.877	0.0002
Water control	Fungicide	1.012	0.0000

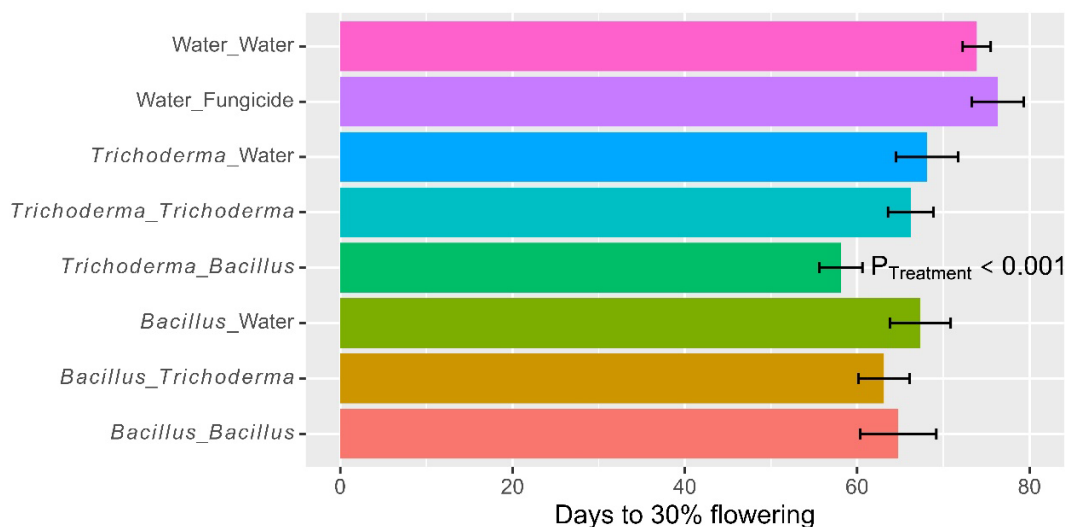
among the eight treatments (Fig. 6), accounting for 57.9% of the total observed variability. The water control had a higher blast incidence than the other treatments and the six microbial treatments did not differ in the blast incidence from the fungicide control (Fig. 6 & Table 2). Foliar spraying led to further reductions ( $p < 0.001$ ) in the blast incidence, and there was a significant ( $p < 0.05$ ) synergy in blast control from alternate use of the two *Bacillus* and *Trichoderma* products. The incidences of grains with blast were 28.9% ( $\pm 1.94\%$ ) (water control), 15.5% ( $\pm 1.62\%$ ) (dipping only), 11.4% ( $\pm 1.36\%$ ) (dipping + spray with the same product), and 8.49% ( $\pm 1.24\%$ ) (alternate use of the two products). Overall, the two *Bacillus* and *Trichoderma* products did not differ in the blast incidence when applied as dipping or foliar spray.

Yield increase achieved by the two microbial products was unlikely due entirely to the reduced blast development. All the six microbial treatments did not differ in the blast incidence from the fungicide control, but four microbial treatments where microbial products were applied both as dipping and foliar spray resulted in higher yields than the fungicide control (Table 2). Figure 7 shows grain blast incidence plotted as against grain yield for individual plots. Many microbial-treated plots had much higher yields than the fungicide-treated plots although the blast incidence was similar.

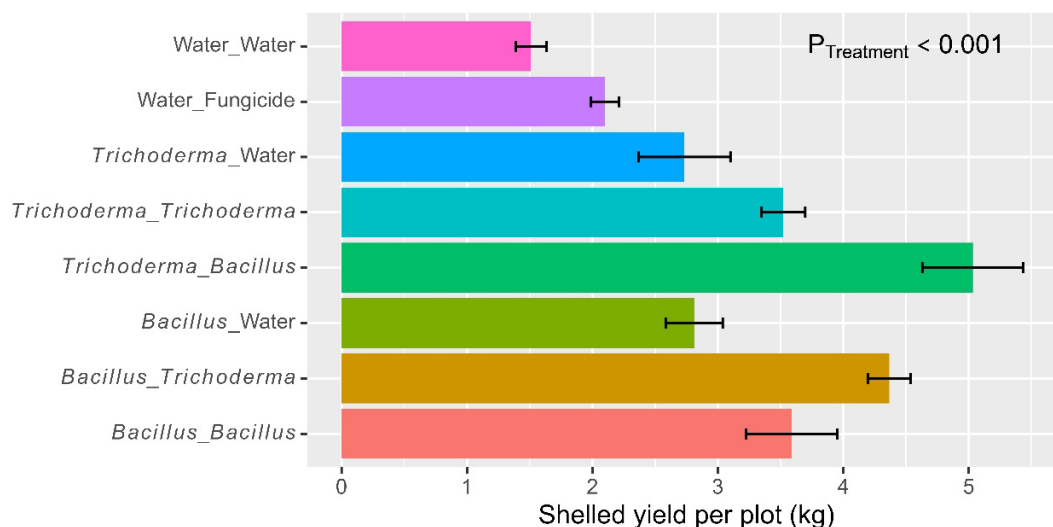
## Discussion

We extended our previous research to assess the effects of using commercial microbial products on rice blast and yield in Tanzania (low yield region - subsistence agriculture) and Kenya (relatively high yield and irrigated paddy rice production region). Low yield obtained in Tanzania, compared to that in Kenya, may have resulted from several factors, including variety genetic potential, blast development (higher in Tanzania), soil fertility, irrigation (used in the Kenya) and climatic conditions. Using microbial products led to significant reductions in blast development with the control efficacy similar to the fungicide treatment. In addition to agreeing with the previous study<sup>[18]</sup> on the increased yield due to microbial products in the subsistence farming region, the present results demonstrated that alternate use of *B. subtilis* and *T. asperellum* at transplanting (as dipping) or post-transplanting (as foliar spray) led to further yield increase (ca. 32.2%, 457 kg per ha) over the use of a single microbial product over time. Alternate use of *B. subtilis* and *T. asperellum* resulted in about 123.8% (1.04 ton per ha) and 211.0% (1.28 ton per ha) increase in grain yield over the fungicide and the water controls, respectively. Comparison with the fungicide control suggested that increased yield resulted mostly from improved plant development rather from reduced blast incidence.

All microbial treatments led to significant reductions in the blast incidence and achieved an efficacy similar to the fungicide control, although the blast incidence was lower than previous studies at the same site<sup>[18]</sup>. This agrees with previous studies demonstrating that other *B. subtilis* and *Trichoderma* strains can reduce rice blast development<sup>[14–16,21]</sup>. The blast fungus can colonise roots and lead to systemic invasion and classical disease symptoms on the above-ground plant parts<sup>[22]</sup>. In addition to direct competition with the blast fungus in the roots, root dipping and seed-coating may affect the pathogen via their effects on rhizosphere and endophyte microbiome.



**Fig. 4** Number of days to 30% flowering of rice plants that had received water/fungicide or one of the two biopesticides as dipping at transplanting only, or both as dipping and as foliar spray at three sites in 2022 in Tanzania. The bar represents one standard error, and P value is associated with the F-test in ANOVA, indicating the overall differences among the nine treatments.



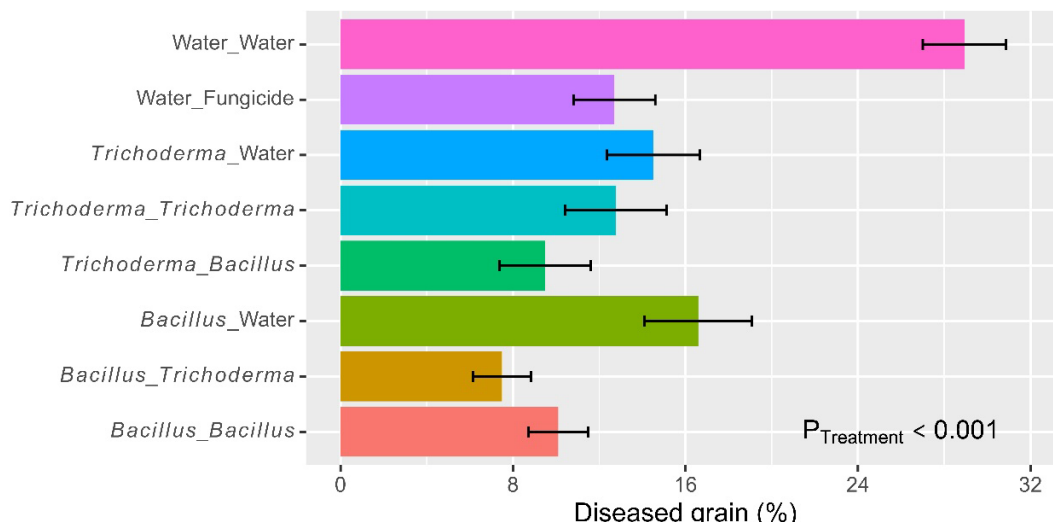
**Fig. 5** Net grain yield (kg) per plot (size 5 m × 5 m) of rice plants that had received water/fungicide or one of the two biopesticides as dipping at transplanting only, or both as dipping and as foliar spray at three sites in Tanzania in 2022. The bar represents one standard error, and P value is associated with the F-test in ANOVA, indicating the overall differences among the nine treatments.

However, we recently demonstrated that rhizoplane and root endophytes of rice seedlings were not much affected by dipping in either *B. subtilis* Bs01 or *T. asperellum* T-900 (Xu, unpublished). Reduced blast development could also have resulted from the indirect effect through plant defence responses induced by the applied beneficial strains. Numerous studies have demonstrated that plant defence responses induced by *Bacillus* or *Trichoderma* spp. led to improved plant tolerance or resistance against specific pathogens and/or improved plant development<sup>[23–28]</sup>.

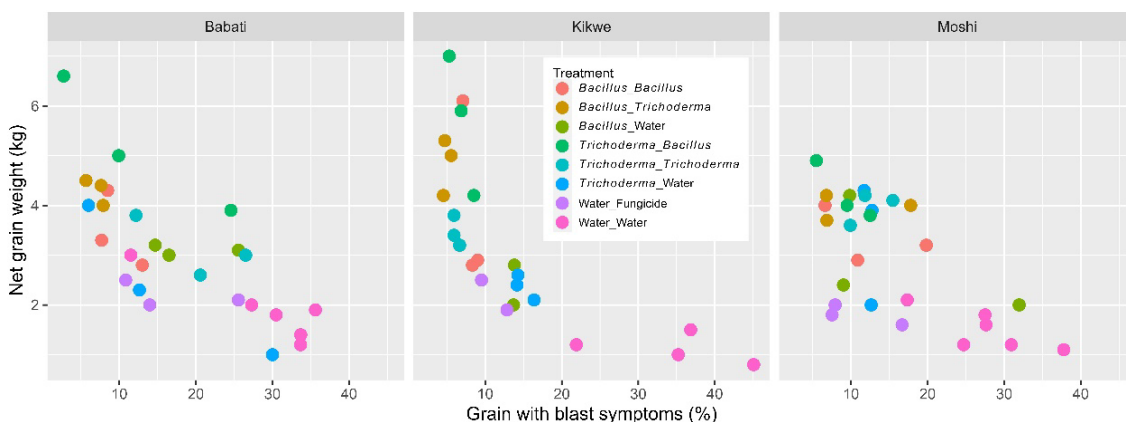
Reduction in blast development is, however, not necessarily equivalent to yield gains, especially for low yielding sites in Tanzania: several microbial treatments led to much greater yield than the fungicide control although the blast incidence was similar. At low yielding sites, applying microbial products led to large increases in grain yield as demonstrated previously<sup>[18]</sup>, due primarily to the increased number of tillers

per plant and grains per panicle. In addition, microbial treatment, particularly dipping at the transplanting stage, shortened the time to flowering and maturity. Thus, we speculate that increased yield is due largely to improved plant development induced by applied microbes, rather than due directly to reduced blast development. Even at the high yielding site in Kenya, microbial treatments led to an overall significant increase (ca. 20%) in grain yield over the untreated control. However, as the microbial treatments had similar levels of blast development and grain yield as the fungicide control at the Kenya site, it is not possible to exclude the possibility that the yield increase associated with microbial treatments is due entirely to reduced blast development.

The three formulated products (*B. subtilis*, *T. asperellum* and *S. nematodiphila* strains) did not differ significantly in terms of rice blast development and grain yield at the Kenya site when applied as dipping at transplanting or post-transplanting foliar



**Fig. 6** Percent grain with blast symptom in each plot of rice plants that had received water/fungicide or one of the two biopesticides as dipping at transplanting only, or both as dipping and as foliar spray at three sites in Tanzania in 2022. The bar represents one standard error, and P value is associated with the F-test in ANOVA, indicating the overall differences among the nine treatments.



**Fig. 7** Rice grain yield against the % grain with blast symptom in each plot of rice plants that had received water/fungicide or one of the two biopesticides as dipping at transplanting only, or both as dipping and as foliar spray at three sites in Tanzania in 2022.

spray. Similarly, the *B. subtilis* and *T. asperellum* products did not differ significantly at three sites in Tanzania. Overall, these results are consistent with our previous study<sup>[18]</sup> although the previous study did show some-site specific differences between *B. subtilis* and *T. asperellum* products in Tanzania. Moreover, seed-coating with one specific *S. nematodiphila* strain led to a similar effect as transplanting dipping with or without foliar spray. In addition to induced host defence responses, seed-coating may also influence rhizosphere microbiome (as with dipping) that may affect nutrient uptake. Coating seeds with beneficial microbes is an efficient system to deliver beneficial microbes for improving seed germination and seedling establishment<sup>[6,7]</sup>. Efficacy of seed priming or dipping seedlings with beneficial microbes have been demonstrated in controlled conditions to suppress rice blast development, including *B. subtilis* and *Trichoderma* strains<sup>[14–16,21]</sup>. In the present study, we demonstrated the positive effect of seed-coating with beneficial microbes on rice productivity under commercial production conditions.

Additional foliar spray following a transplant dipping treatment with the same product did not lead to any additional

benefit in Kenya. In contrast, additional spray with the same product as for dipping led to additional yield increases (average 313 kg per ha) in Tanzania, though not as much as increases (average 504 kg per ha) achieved by the dipping only treatments. As we argued above, increased yield is likely due primarily to induced plant responses. Thus, additional foliar applications of the same products as used in dipping (around 6–9 weeks after transplant dipping) may have strengthened plant development pathways previously induced by dipping. Such strengthened plant responses may be more important for plants grown under more stressful conditions. The Kenya experiment was conducted in the irrigation area. In contrast, the three Tanzanian experimental sites relied on rain-fed water, and thus plants may have experienced some drought stress at these sites. Indeed, second season crops were planted in the same sites in order to repeat the trials, as we did in Kenya. However, these repeat trials failed to produce grains because of drought in late 2022 in Tanzania. The differing degree of abiotic stress between Kenya and Tanzania experimental sites may thus explain the differences in the effects associated with additional foliar sprays.



## Use of beneficial microbes to increase rice yield

Variable biocontrol efficacies and consistencies have led to the suggestion of using multiple biopesticides simultaneously to exploit possible synergies among biopesticides and hence improve performance as well as consistencies. However agonistic interactions between component microbes have often been observed, and thus synergistic interactions among biopesticides have rarely been achieved<sup>[19]</sup>. Our previous study<sup>[18]</sup> demonstrated antagonistic interactions between *B. subtilis* and *T. asperellum* when used in a mixture as dipping or foliar spray. Thus, in the present study, we investigated alternate use of *B. subtilis* and *T. asperellum* products over time for their effects on rice productivity. To our surprise, alternate use of the two products consistently outperformed the use of single products, leading to an average 32.2% increase in grain yield (458 kg per ha) over the single products. This additional benefit resulting from the alternate use of the two products may have resulted from the fact that the two (*B. subtilis* and *T. asperellum*) strains do not induce the same host responses either qualitatively (i.e., inducing different pathways) or quantitatively (i.e. same pathways but induced to a different degree). Thus, applying the two products alternately over time may lead to a more complete plant response. Strains from *B. subtilis* and *Trichoderma* spp. can induce systemic defence response on many plant species<sup>[23,26–31]</sup>. When applied in a mixture, direct competition between the two strains may have weakened the induced plant response. In addition, plant responses at a given time might be limited and hence may not be able to fully respond to the simultaneous use of the two strains. To quantify and statistically test the synergy in the alternative use of the two strains is difficult because the exact definition of synergy (hence statistical test) depends on the extent of overlap in induced plant responses by the two microbes and also dose-response relationships<sup>[19]</sup>.

In summary, all microbial treatments led to significant reductions in rice blast and increased rice grain yield, particularly in the low yielding region in Tanzania. Alternate use of formulated *B. subtilis* and *T. asperellum* products at transplanting (as dipping) or post-transplanting (as foliar spray) led to additional yield increase in the low yielding region over the use of a single organism product at both times. Seed coating with one *Serratia* strain led to blast control and grain yield comparable to applying microbial products as transplant dipping and post-transplanting foliar spray as well as the fungicide control. Thus, present research results support the use of microbial products, particularly alternative use of different microbial products over time, to improve rice productivity in subsistence farming. Future research is needed to exploit potential benefits of combining seed coating with transplant dipping and/or post-transplanting foliar spray.

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## Conflict of interest

Xu X and Robinson-Boyer L declare no conflict of interest; Murunde R and Ringo G are employed by Real IPM who produced the three commercial biopesticides evaluated in the present study. The funders had no role in the design of the

study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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