INFLUENCE OF BIOLOGICAL SEED TREATMENT ON SOYBEAN GRAIN YIELD IN THE U.S.

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ABSTRACT

Biological seed treatment in soybean (*Glycine max* (L.) Merr.) is a growing market in the U.S., with multiple microbially active ingredients and several proposed benefits. Some of the claimed benefits include improving nitrogen fixation, stimulation of root growth, increasing phosphorus, sulfur, and other nutrient absorption, and control of diseases, with the aim to increase soybean grain yield. Farmers are often bombarded with marketing claims about biological seed treatments. In many cases, there is little or no third-party evidence of quantitative assessment regarding these biological seed treatments' ability to improve soybean yield. Therefore, this project's objective was to evaluate if biological seed treatments improved soybean yield across the U.S. Field experiments were established using a common protocol during the 2022 growing season at 49 locations across 17 U.S. states, examining the effectiveness of nine commercial biological seed treatments to increase soybean yield. The experimental design was a randomized complete block with six replications. Treatments included microbes from the genera *Bradyrhizobium, Bacillus, Azospirillum, Pseudomonas, Pantoea, Delftia, Trichoderma*, and *Glomus*. Some of the products had multiple active ingredients (microbes). Results showed that the effects of treatments were not significant (P=0.4229) nor varied among the examined locations (P=0.0985). Also, Bayesian analysis indicated that a high probability (>80%) of the yield difference (each treatment minus untreated control) being higher than zero was mainly found in the treatment products that contained *Trichoderma* only, *Bradyrhizobium* only, and the arbuscular mycorrhizal fungi *Glomus* mostly in Southern U.S. states. In these locations, the yield difference ranged between 1.2 to 2.3 bu/acre; however, none was significant (95% credible intervals included zero). Overall results suggest that the biological seed treatments tested in this study in a wide range of environments rarely increased soybean grain yield.

INTRODUCTION

Today's soybean industry faces many challenges, such as high input prices (e.g., fertilizers and pesticides) and an increasing need to produce high-yielding soybeans in an environmentally sustainable manner. Due to these challenges, some products, strategies, or management practices are becoming more available in the market. For example, biological seed treatment for soybean is one of the management practices available; however, the efficacy and use of these products to increase soybean yield need to be better studied.

The benefits of the interaction between microorganisms and plants can be several. For example, the bacteria genus *Azospirillum* has the ability to fix atmospheric nitrogen

(Day and Döbereiner 1976) and can secrete phytohormones (Reynders and Vlassak 1979). Other plant growth-promoting bacteria are from the genera *Bacillus* and *Pseudomonas*. Some *Bacillus* species can improve nutrient supply, secrete phytohormones (Radhakrishnan et al., 2017), and suppress diseases (Hu et al. 2014). Similar to *Bacillus*, the *Pseudomonas* bacteria can promote plant growth by suppressing pathogenic microorganisms and synthesizing phytohormones (Preston, 2004).

Many commercial biological seed treatments contain *Bradyrhizobium* spp., an important bacteria genus known for its ability to fix nitrogen and providing 50 to 60% of soybean N requirement (Salvagiotti et al., 2008). Plants also have a mutualistic relationship with some fungi species such as the fungus genus *Glomus* that promotes phosphorus uptake (Thioub et al., 2019). *Trichoderma*, another fungi genus, showed biocontrol effects against *Macrophomina phaseolina*, fungal causal agent of soybean charcoal rot (Khaledi and Taheri, 2016) and white mold (Macena et al., 2020).

Biological soybean seed treatment is a growing market worldwide. The global market is expecting that the biological market (biopesticides and biostimulants) will grow from $6.9 billion in 2019 to $13.6 billion by 2024 (BCC Research, 2020). Although the soybean seed treatment market is growing, there are limited studies on the efficacy of microorganisms in soybean production in the U.S. Therefore, the objective of this project was to evaluate if biological seed treatments improved soybean yield across the U.S.

MATERIALS AND METHODS

A small plot trial was established at 49 locations across 17 states in the USA (Alabama, Arkansas, Iowa, Indiana, Illinois, Kentucky, Louisiana, Michigan, Minnesota, Mississippi, North Carolina, North Dakota, Ohio, South Carolina, South Dakota, Virginia, and Wisconsin) during the 2022 growing season. The experimental design used was a randomized complete block with six replications. Nine commercially available biological seed treatments were evaluated and compared to the non-treated control (Table 1).

Table 1. List of treatments (products) and active ingredients in each biological product.

Treatment (product)	Active ingredients
1	*Azospirillum brasilense, Bacillus licheniformis, Bacillus amyloliquefaciens, Bacillus subtillis, Pseudomonas fluorescens, Rhizobium*
2	*Trichoderma virens*
3	*Bradyrhizobium spp.*
4	*Bacillus subtillis, Bacillus amyloliquefaciens, Bradyrhizobium japonicum*
5	*Pantoea agglomerans**
6	*Pseudomonas brassicacearum**
7	*Bradyrhizobium elkanii, Delftia acidovorans + Bacillus velezensis*
8	*Bacillus velezensis*
9	*Glomus intraradices, Glomus mosseae, Glomus aggregatum, G. etunicatum*
10	Untreated Control

* Products 5 and 6 were applied only at locations in Illinois, Indiana, Michigan, Minnesota, North Dakota, Ohio, South Dakota, and Wisconsin.

The soybean variety and management practices (e.g., row spacing, seeding rate, soybean relative maturity, cropping history, etc.) were representative of each region. Also, seeds were treated with the same commercial fungicide + insecticide seed treatment to be representative of farmer practices. Biological seed treatments in this experiment were compatible with fungicide and insecticide seed treatments according to each company. Also, the application of biological on soybean seeds was followed by using the guidelines and rates provided by each company. Soybean yield was adjusted to 13% moisture concentration prior to data analysis.

Data were analyzed in SAS 9.4 using frequentist (PROC MIXED) and Bayesian (PROC BGLIMM) analysis approaches. In the first approach, location, treatment and their interaction were treated as fixed effects. Replication nested within locations was a random effect, and means were adjusted for multiple comparisons. In the second approach, the Bayesian analysis was modeled within each state.

RESULTS AND DISCUSSION

Grain yield

The main effect of location showed significant results because the trials were conducted in different regions under different environmental conditions, and under low or high yielding areas. The main factor treatment nor the interaction between location and treatment showed significant results ($\alpha = 0.05$) (Table 2). When the grain yield from each treatment was plotted against the untreated control, most of the points were close to the x=y line, showing that there were no substantial differences on grain yield when applying products (Figure 1).

Table 2. Analysis of variance (ANOVA) for location, treatment, and location x treatment.

Source of variation	F Value	Prob > F
Location	109.46	<.0001
Treatment	1.02	0.4229
Location*Treatment	1.10	0.0985

In the Bayesian analysis, high probabilities (>70%) of the yield difference (each treatment minus the untreated control) being higher than zero was mainly found in the treatments that contained *Trichoderma* only, *Bradyrhizobium* only, and the arbuscular mycorrhizal fungi *Glomus* mostly in Southern states (Table 3). Although the yield differences between each treatment minus the untreated control ranged from -6.1 to 4.2 bu/acre, none was significant (95% credible intervals included zero). Similar studies in the USA have been showing inconsistent results. For example, in a study conducted in 13 states in the U.S., Leggett et al. (2017) found a yield difference of 0.9 bu/acre between inoculated soybean seeds with *Bradyrhizobium japonicum* and non-inoculated seeds. Differently, Carciochi et al. (2019) did not find significant yield gain after inoculating seed with *B. japonicum* in any of the environments where the trial was conducted in four USA states. A recently published study in the USA found that the average response to applying *Azospirillum brasilense* in soybean was 1.8 bu/acre, with a probability chance of only 5.3% (de Borja Reis et al. 2022).

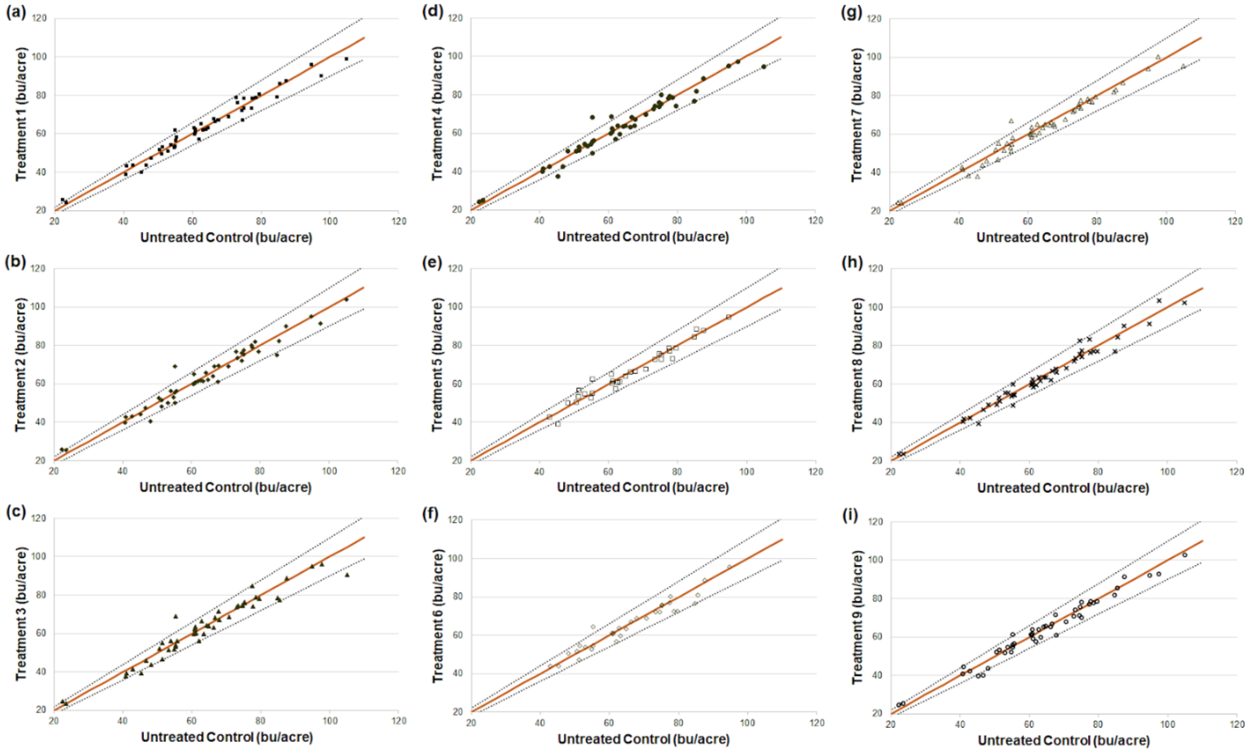

Figure 1. Average grain yield (kg/ha) at each site for each treatment (product) plotted against the average grain yield (kg/ha) of the untreated control (treatment 10) at the same site. Each symbol within a graph represents one site. Solid red lines represent x = y, and the dashed lines represent ±10% of the yield.

IMPLICATIONS

The effects of treatments were not significant (*P*=0.4229), nor were the location x treatment interaction (*P*=0.0985). The Bayesian analysis indicated that a high probability (>70%) of the yield difference (each treatment minus untreated control) being higher than zero was mainly found in the treatment products that contained *Trichoderma* only, *Bradyrhizobium* only, and the arbuscular mycorrhizal fungi *Glomus* mostly in Southern U.S. states. The yield difference ranged from -6.1 to 4.2 bu/acre; however, none was significant. In general, results suggest that the biological seed treatments tested in this study in various environments rarely increased soybean grain yield. These results are preliminary, and the project was repeated in 2023.

Table 3. Summary of the mean yield differences (Yd, in bu/acre) for each treatment minus untreated control, and probabilities for a difference>0 (P, in %) analyzed using Bayesian procedure for each U.S. participant state in 2022.

State	Trt 1		Trt 2		Trt 3		Trt 4		Trt 5		Trt 6		Trt 7		Trt 8		Trt 9	
	Yd	P	Yd	P	Yd	P	Yd	P	Yd	P	Yd	P	Yd	P	Yd	P	Yd	P
	bu/acre	%	bu/acre	%	bu/acre	%	bu/acre	%	bu/acre	%	bu/acre	%	bu/acre	%	bu/acre	%	bu/acre	%
Alabama	-1	33	0.3	55	-1.4	27	0.3	55	-	-	-	-	1.2	71	-0.1	48	0.7	62
Arkansas	1.9	86	0.5	62	-1.3	22	-0.3	43	-	-	-	-	0	50	-0.3	44	2	87
Illinois	0.6	62	-0.9	34	-2.3	15	-2	19	-0.7	38	-2.3	16	-0.3	44	-1.9	20	-0.1	47
Indiana	-0.5	35	-1.2	17	-1.1	19	-0.5	35	-0.5	34	-1	21	-0.1	46	-1.1	18	0.6	69
Iowa	-2	34	-5.3	15	-1.9	36	-5.5	13	-	-	-	-	-4	21	-6.3	11	-0.5	46
Kentucky	0.8	62	-1.2	32	-2.3	18	-2.8	13	-	-	-	-	-3.2	10	-2.5	17	-1	34
Louisiana	0.1	52	2.1	92	2.1	91	1.4	83	-	-	-	-	1	74	0.8	70	2.2	93
Michigan	-0.2	47	-3.6	8	-2.6	16	1.6	73	1	65	-1	35	-2.6	16	0.8	61	-0.8	37
Minnesota	0.7	61	0.4	56	1.6	71	0.4	55	-1.8	26	1.1	66	-3.5	10	-0.6	42	-3.1	13
Mississippi	0.3	59	2.3	94	1.2	79	0.5	64	-	-	-	-	0.5	64	0.3	59	1.4	83
North Carolina	-6.1	3	-3.1	17	-2.6	21	-3.7	13	-	-	-	-	-1.6	31	4.2	91	-0.5	43
North Dakota	-0.7	29	-0.5	34	-1	22	0.4	63	0.5	66	0.2	56	-2.5	3	-2.8	2	-2	7
Ohio	-0.7	33	-1.5	15	-1.4	17	-1.9	9	0.2	56	-1.4	16	-0.4	38	-1	25	-0.1	46
South Carolina	1.2	75	1.4	78	1.4	78	-1	28	-	-	-	-	0.7	65	0.3	57	0.7	65
South Dakota	-1.2	9	-0.8	17	-0.2	41	-0.1	47	-0.4	30	-0.2	42	-0.9	16	-0.7	21	-0.7	20
Virginia	-0.8	38	2.1	81	0	49	-2.5	16	-	-	-	-	-3.2	9	-0.8	37	-5	2
Wisconsin	-1.8	3	1.5	93	0.4	65	0.4	64	-0.8	20	-1.3	9	-0.8	20	-0.9	19	-2.1	2

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