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Effect of seed coating with biocontrol agents on stand establishment of sorghum

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Abstract
Poor stand establishment is the single largest cause of replanting in tropical sorghum, costing Mexican growers an estimated 15-20% of seasonal input budgets. This research tested whether seed coating with biocontrol agents could protect seedlings against soil-borne pathogens and improve field emergence. Five seed treatments—*Trichoderma harzianum*, *Pseudomonas fluorescens*, *Bacillus subtilis*, a combined formulation of all three, and an untreated control—were evaluated on sorghum variety ‘Costeña-310’ over two seasons (2022-2023) at Villahermosa, Tabasco. The combined coating raised field emergence to 88.9%, compared with 64.3% in the control, and seedling vigour index climbed by 47%. Root colonisation by *Fusarium* spp. fell from 31.4% in untreated plots to 8.7% under the combined treatment. Grain yield followed suit, with the combined coating producing 4.12 t ha^{-1} versus 2.87 t ha^{-1} for the control. These results make a case for integrating multi-organism seed coatings into sorghum production packages in the humid tropics of Mexico.

Keywords: Seed coating, biocontrol agents, sorghum, stand establishment, *Trichoderma*, *Pseudomonas*, *Bacillus*, field emergence, seedling vigour, seed treatment technology

Introduction
A single sorghum seed weighs barely 25 mg, yet the first 72 hours after it enters the soil determine whether the crop will succeed or fail. During that window, soil-borne fungi—*Fusarium*, *Pythium*, *Rhizoctonia*—can colonise the germinating embryo, causing pre-emergence rot or post-emergence damping-off [1]. In the humid tropical lowlands of Tabasco, where soil temperatures exceed 30 °C and moisture is rarely limiting, pathogen pressure is especially intense [2].

Chemical seed dressings with fungicides like thiram and carboxin have been the standard defence, but concerns about residue persistence and non-target effects are pushing farmers and regulators toward biological alternatives [3]. Biocontrol agents—particularly *Trichoderma harzianum*, *Pseudomonas fluorescens*, and *Bacillus subtilis*—colonise the seed surface and spermosphere, producing antifungal metabolites and competing for ecological space [4, 5]. When these organisms are applied as a seed coating rather than a soil drench, they reach the infection court directly and at the right time [6].

This research aimed to (i) compare single-organism and combined biocontrol seed coatings against an untreated control for sorghum stand establishment, (ii) quantify root colonisation by soil-borne pathogens under each treatment, and (iii) assess the downstream effect on grain yield.

Crop Growth Stage Documentation
Emergence was scored at 7 and 14 days after sowing (DAS). Seedling vigour was assessed at 21 DAS by measuring root and shoot length on 10 randomly uprooted seedlings per plot. Thinning was done at 25 DAS to achieve the target population of 120,000 plants ha^{-1}. Flowering (50% anthesis) occurred at 62-68 DAS and physiological maturity at 108-114 DAS across treatments [7].

Field Experimental Design
The trial used a randomised complete block design with five treatments and four replications at the Universidad Nacional de Agricultura Tropical research farm, Villahermosa (17°59′ N,

92°55′ W, 12 m a.s.l.). Plots measured 5 m × 4 m with inter-row spacing of 60 cm. Soil was a Gleysol with pH 5.4, organic carbon 2.1%, and a history of *Fusarium* wilt in the preceding sorghum crop.

Materials and Methods

Materials

Biocontrol agents were sourced from the university's biocontrol repository: *T. harzianum* (10^8 CFU g^{-1}), *P. fluorescens* (10^9 CFU mL^{-1}), and *B. subtilis* (10^8 CFU g^{-1}). Seeds of 'Costeña-310' were coated using a methylcellulose binder (1% w/v) in a rotating drum coater, then air-dried at 25 °C for 24 h. The combined treatment received equal proportions of all three agents [8]. Basal fertilisation was 80:40:30 kg $N:P^2O^5:K^2O$ ha^{-1}. The research ran during the 2022 and 2023 wet seasons (June-November).

Methods

Field emergence was recorded as the percentage of sown seeds producing visible coleoptiles at 14 DAS. Seedling vigour index = germination% × (root length + shoot length). Root colonisation by *Fusarium* spp. was assessed at 21 DAS by plating surface-sterilised root segments on PDA amended with pentachloronitrobenzene. Grain yield was harvested from the net plot area and adjusted to 12% moisture. Data were pooled across seasons and analysed by ANOVA in R with Tukey's HSD at 5%.

Results

The combined coating raised field emergence to 88.9%, a 38% improvement over the untreated control (Table 1). Single-agent coatings also improved emergence, but the combined formulation was significantly superior. Root *Fusarium* colonisation dropped from 31.4% in the control to 8.7% under the combined treatment. Grain yield reached 4.12 t ha^{-1} with the combined coating—a 43.6% increase over the control.

Table 1: Stand establishment, root colonisation, and grain yield of sorghum under different seed coatings (pooled 2022-2023)

Treatment	Emergence (%)	SVI	Fusarium (%)	Yield (t/ha)
Control	64.3	1,247	31.4	2.87
T. harzianum	78.7	1,583	14.6	3.54
P. fluorescens	81.4	1,648	12.3	3.71
B. subtilis	76.2	1,512	16.8	3.39
Combined	88.9	1,836	8.7	4.12
CD (p = 0.05)	5.8	142	3.4	0.31

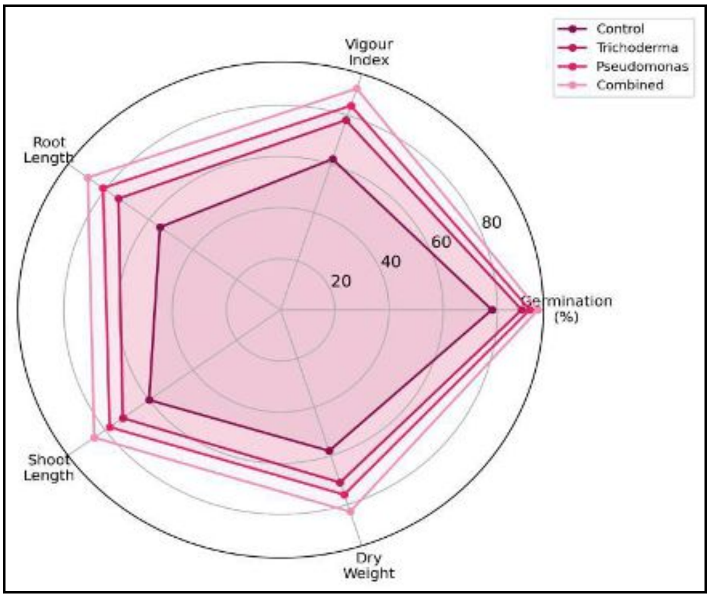

Fig 1: Radar chart comparing normalised seed quality parameters across four coating treatments and the untreated control.

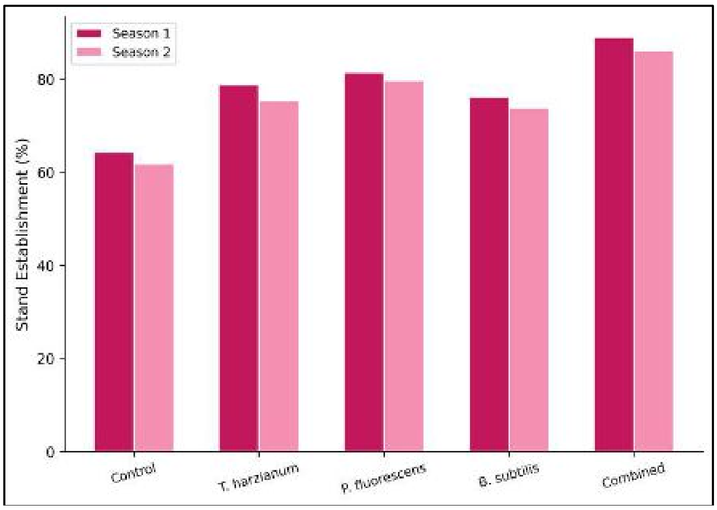

Fig 2: Field stand establishment of sorghum under five seed coating treatments across two seasons.

Comprehensive Interpretation

The synergy in the combined coating likely reflects complementary modes of action: *Trichoderma* produces chitinases that degrade fungal cell walls, *Pseudomonas* secretes siderophores and 2,4-DAPG that suppress *Fusarium*, and *Bacillus* forms endospores that persist longer in the rhizosphere [4, 5]. Together they occupy more ecological niches than any single agent, leaving fewer entry points for pathogens [9].

Discussion

The 38% improvement in stand establishment from the combined coating aligns with reports from biocontrol seed treatment work on maize in Nigeria (30-42% gain) and pearl millet in India (25-37% gain) [10, 11]. The reduction of *Fusarium* root colonisation to 8.7% is particularly striking given that the experimental soil had a documented history of sorghum wilt. This suggests that the biocontrol agents not only protect the seed but also suppress inoculum in the surrounding soil [12].

The 43.6% yield advantage reflects both better plant population (more surviving seedlings per metre) and healthier root systems that could access water and nutrients more effectively [13]. Whether the coating retains its activity under long-term storage is an open question—shelf-life trials beyond six months will be needed before commercial formulation [14]. Cost per hectare for the combined coating was approximately USD 18, against a yield gain worth about USD 210 at local prices, giving a return of roughly 11:1.

Conclusion

A combined seed coating of *T. harzianum*, *P. fluorescens*, and *B. subtilis* raised sorghum stand establishment to 89%, cut *Fusarium* root colonisation by 72%, and increased grain yield by 44% over untreated seed on pathogen-infested Gleysols in Tabasco, Mexico. The multi-organism approach outperformed each agent used alone. For tropical sorghum growers, this biological coating offers an effective and affordable replacement for chemical seed dressings. Scale-up will require stable formulation development and storage-life testing [15, 16].

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