Title: Test the combined effect of high calcium and biocontrol on reducing aflatoxin contamination

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Brief & concise statement of objectives

- 1. Assay the impact of CDB on peanut growth and pod development.
- 2. Determine the interaction between CDB and *A. flavus* on plates.
- 3. Determine the effect of CDB to reduce pre-harvest *A. flavus* infection.

Summary of results from Year 1

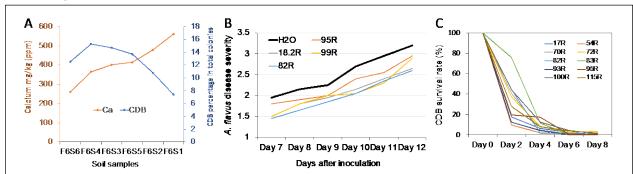


Figure A: A negative correlation in soil calcium level and CDB percentage was observed in samples collected from a peanut field in Mitchel county, GA. **Figure B**: co-inoculation of A. flavus and CDB reduced the disease severity in a root radicle assay. 1: no fungal growth; 4: *A. flavus* cover a seedling. **Figure C**: Most CDB strains do not persist after being applied in soil.

- 1. CDB strains have no negative impact on peanut growth. We assayed the impact of 10 CDB strains on seedling growth by dipping seeds in the bacterial suspension. Seeds coated with CDB showed the same or slightly high germination compared to control-treated seeds, indicating that CDB are not pathogenic.
- 2. Negative correlation between soil calcium level and CDB population in peanut field (**Figure A**). We previously observed a negative correlation between soil calcium level and CDB population in lab conditions and in an experimental field. In the summer of 2021, we surveyed 90 samples from 15 peanut-growing fields in three counties (Mitchel, Tifton and Sumter). In four fields, we observed a negative correlation (correlation coefficient < -0.5), indicating that soil calcium influences CDB population in peanut fields.
- 3. Three CDB strains reduced propagation of A. flavus in a root radicle assay (**Figure B**). When co-inoculated with one of these CDB strains on peanut seeds, A. flavus growth was reduced compared to control treatment. Interestingly, no inhibition was observed when CDB strains and A. flavus were included in a plate competition assay on Potato Dextrose Agar (PDA) medium.
- 4. Applied CDB do not survive long in soil (**Figure C**). We generated mutated CDB strains resistant to Rifampicin and used these strains to monitor the survival rate of CDB after being applied to soil. In 10 CDB strains tested, we found that added CDB did not survive more than 4 days at 28°C. This observation indicates a major challenge in applying CDB in soil.