Bacillus Induced to Biosynthesize VOCs & Nitriles May Benefit Agriculture

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ABSTRACT:

The scope of the project was to identify the possible agricultural applications for bacteria induced to synthesize nitriles and VOCs. The study was randomized. Cucurbit seeds and *Bacillus licheniformis* were selected as the plant and microbial models for two trial studies. In trial 1, 90 cucumber seeds were cultured with B.licheniformis induced to synthesize VOCs (including ethanol, 3-methyl-1-butanol, pentanol), esters (ethyl acetate), and acetonitriles. After 2 weeks the induced bacteria increased seed germination by 68% compared to control samples. Several seedlings were transferred to a small garden, infested with soil nematodes. Roots of control and induced samples appeared affected. Control samples appeared stunted in growth with decreased productivity, but cucumber plants initially planted with induced bacteria were noticeably larger in size with good productivity. Induced Bacillus increased the number of blossoms and cucumber per plant by 125% compared to control samples. Induced Bacillus did not increase solubility of nitrogen, phosphorous, or potassium in the soil, but appeared to increase plant health and defenses against pathogenic infections. Though the study findings are preliminary, soil microbes induced to synthesize VOCs and nitriles may improve plant health and productivity in cucurbit plants.

Keywords: Organic, Plant Growth Promoting Rhizobacteria, Bacillus, Crops, Flowers, Garden

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INTRODUCTION:

The US farming industry is heavily dependent on chemical fungicides and fertilizers to maintain crop health and productivity (Al-Busaidi, 2013; Johnson, 2014). These chemicals have harsh side effects, cause microbial resistance, and negatively impact of climate and ecosystems (Ribaudo et *al.,* 2011; Banayo et *al.,* 2012; Ishfani, 2012; Aggani, 2013). Farmers need ecofirnedly and efficient alternative to treat seeds and protect crops, (Trostle and Seeley, 2013). Plant growth promoting rhizobacteria (PGPR) such as Azobacter, Bacillus, Azosprillum, Acetobacter, Pseudomonas, and Rhodococcus improve plant development by increasing solubility of nitrogen, phosphorous, and potassium in the soil, (DeBont and Albers, 1976; Miller et al., 1987; Kloepper et. *al* 1991; Vandeputte et al., 2005; Arshad et al., 2007; Ahemad and Kibret, 2014; Vacheron et *al*., 2013). Soil microbes can also convert hydrocarbons like ethylene into VOCs (including ethanol, 3-methyl-1-butanol, pentanol), esters (ethyl acetate), and acetonitriles compounds that can increase seed germination and enhance plant development (DeBont *et al.,* 1974; Germon and Knowles, 1988; Perry, 2011; Nelson et *al.,* 2012; Downes et *al.,* 2013; Perry and Perry, 2019).

In this study Bacillus licheniformis will be cultured to induce VOC, ester, and nitrile compounds as described in Perry and Perry, 2014. Bacillus were inoculates for cucumber seeds to determine if the benefits of the microbes in the soil improved seed germination and/or productivity.

MATERIALS AND METHODS:

Microbial Induction

Bacillus licheniformis (ATCC 12759) purchased from Diagnostic were cultured on nutrient agar for 3 d, scrapped from agar, suspended in 15 ml of (1X) PBS buffer (0.8% NaCl, 0.02% KCl, 0.02M PO4, and pH 7.2), then transferred to a to a IL flask that contained $CoCl_2$ 0.201 (g/L), Urea

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7.5 (g/L), Glucose 5 (g/L), Ethylene 15% (v/v), and 300 ml Minimal Media for 3 d at 30˚C with shaking at 120 rpm (Perry, 2016; Perry, 2011; Shadowen and Sciortino, 1989). Cells were harvested & re-suspended to 1.37 10^5 CFU/ml.

Germination Study

Uncoated cucumber seeds were purchased from Ferry Morse Co. and stored at 23 °C (40% RH) until potted. Germination period of 14 d and required soil pH 5.5- 7.0 (Elzebroek and Wind, 2008). Seeds were planted in peat pots and induced Bacillus was mixed with potting soil; 90 control; 90 experimental seeds (two seeds were planted in each peat soil pot 1.3 in. deep). Seedlings were counted, imaged, and harvested on 12th day. Seeds were watered every 3rd-d. No seeds were planted on designated negative control rows (pink flag). After 3 wks several seedlings were transferred to a untilled garden, with a soil nematode problem. At 8 wks plants were imaged & harvested.

RESULTS:

Comparison of Germination Rate

Bacillus licheniformis wascultured and then mixed with induction media. The bacteria were used as a soil inoculum. Seed were planted andimaged after 14 days. Induced bacteria increased seed germination by 68% compared to control samples, Image 1, 2. Several seedlings were transferred to a small garden, infested with soil nematodes. Roots of control and induced samples appeared affected. Control samples appeared stunted in growth with decreased productivity, but cucumber plants initially planted with induced bacteria were noticeably larger in size with good productivity. Induced Bacillus increased the number of blossoms and cucumber per plant by 125% compared to control samples, Image 3, 4, 5.

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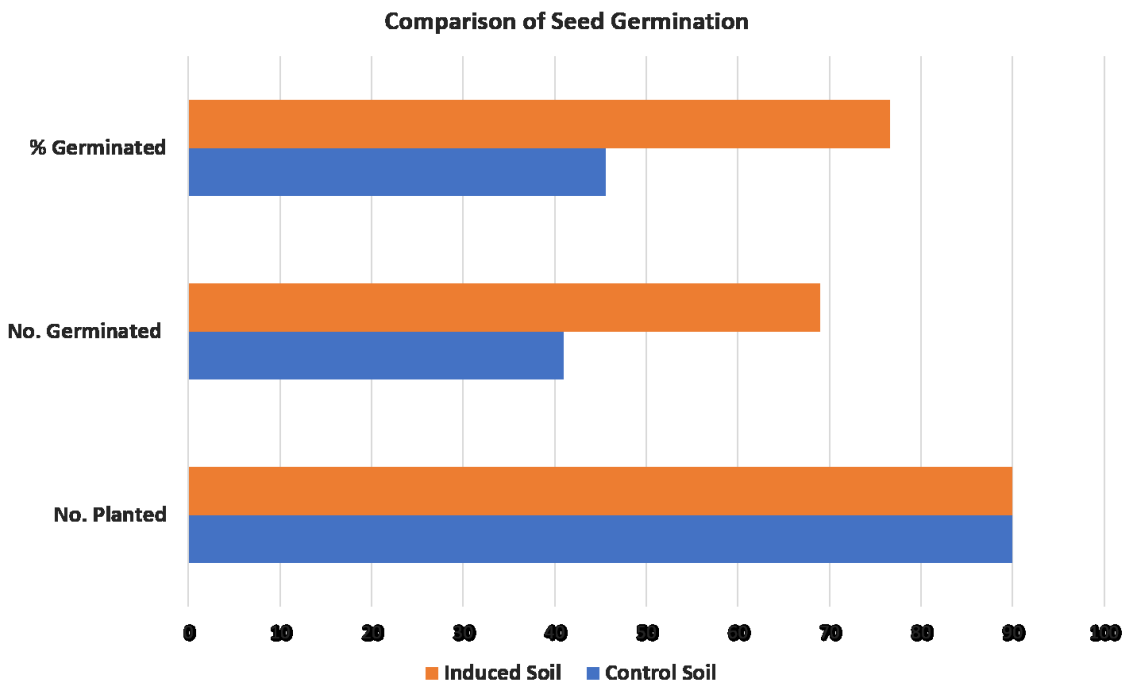

Image (1): Comparison of Germination Rate for Control & Induced Soil: Induced soil contained Bacillus species induced to produce VOCs, esters, and nitriles. Induced Bacillus increased cucumber germination by 68%.

Image (2): Comparison of Germination Rate for Control & Induced Soil: (A) Control Soil (B) Soil Inoculated with Induced Bacillus. Induced Bacillus increased germination by 68%.

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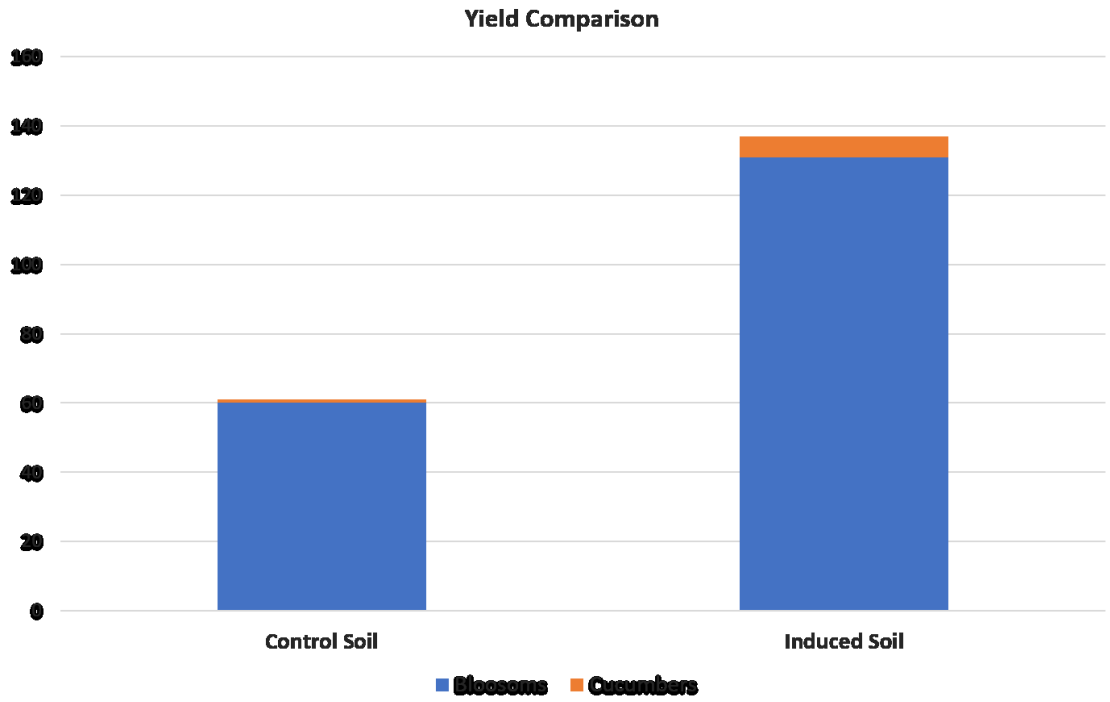

Image (3): Comparison of Germination Rate for Control & Induced Soil: Induced soil contained Bacillus species induced toproduce VOCs, esters, and nitriles. Induced Bacillus increased cucumber plant productivity by 125%, even with a potential soil nematode infection.

Image (4): Comparison of Productivity of Cucumber Plants: (A) Transferred Controls (B) Transferred Induced Samples. Visible nodules on roots, damge to roots, indicated potential soil nematode infection. Pathogenic nematodes burrow into the root to feed and reproduce (Noling, J.W., 1999). Induced Bacillus increased productivity by 125%, even with a potential soil nematode infection.

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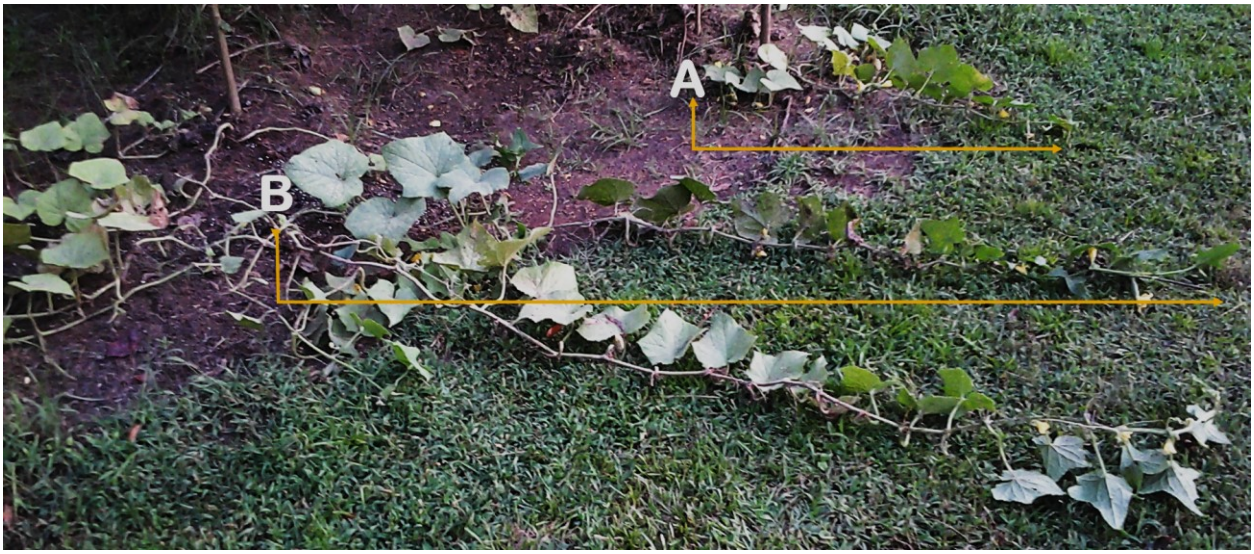

Image (5): Comparison of Productivity of Cucumber Plants: (A) Transferred Controls (B) Transferred Induced Samples. Controls show stunted growth, reduced flowers, and smaller leaves, indicative of nematode infection (Noling, J.W., 1999). While induced samples plants are larger, increased flowers, and produced cucumbers even with potential nematode infection. Visible nodules on roots, damge to roots, indicated potential soil nematode infection. Induced Bacillus increased productivity by 125%.

Image (6): Comparison of Productivity of Cucumber Plants: Induced Samples produced cucumbers even with potential nematode infection. and increased productivity by 125%.

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DISCUSSION/ CONCLUSION:

Soil microbes can be induced to efficiently convert ethylene into cellular metabolites like ethanol, aldehydes, nitriles, esters, or VOCs, Image 6 (Hartmans *et al.,* 1991; Elsgaard 1998; Elsgaard 2000; Perry, 2011; Perry and Perry, 2019). These VOCs include ethanol, acetonitrile, 2-methyl 1-propanol, butanol, 2-pentanone, 2-pentanol, and 3-methyl 1-butanol, may work synergistically to inhibit pathogenic infections and enhance plant development including seed germination (Strobel et. al, 2001; Fialho et. al, 2011; Rezende et.al, 2015; Jantasorn et al., 2016; Liarzi et. al, 2016). The data suggest induced Bacillus provided benefits to cucucmber seeds during early stages of germination and resistance to the effects of pathogens. The mechanism used by the *Bacillus licheniformis* is unknown and the data ispreliminary. Additional experimentation is required to understand this process.

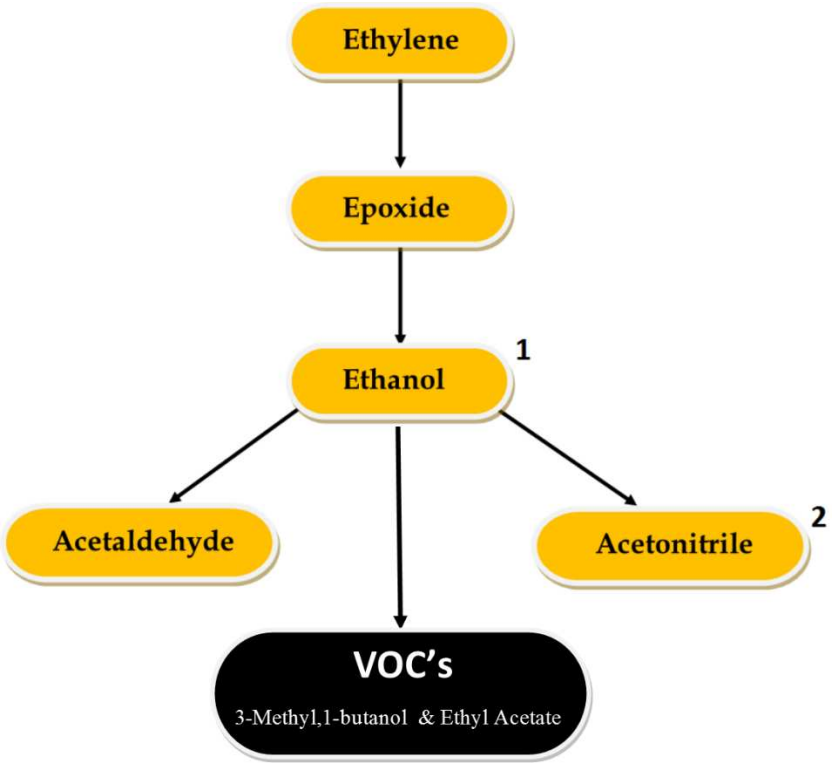

Figure 6: Proposed Pathway, based on GC Mass Spec Data. Ethanol may be converted to VOCs by microbes in low oxygen and high pressure conditions.

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