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BIO-PRIMING TO IMPROVE THE SEED GERMINATION, EMERGENCE AND SEEDLING GROWTH OF KALE, CARROT AND ONIONS

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ABSTRACT: *There is increasing interest in the use of bio-priming as a means of growth promotion through the efficient use of Plant Growth Promoting Rhizobacteria as an economic and efficient means of treating crops. This study was carried out to examine the effect of bio - priming using Bacillus subtilis and Serratia nematodiphila on the seed germination, seed emergence and plant growth of the seedlings of vegetable seeds (kale var. Collard, carrots var. Nantes, and onion var. Detroit red). Bio-priming involved soaking 2 g o f seeds in 5ml/l for both Bacillus subtilis (2.4×10^7 cfu/ml) and Serratia nematodiphila solution (2.4×10^9 cfu/ml) and distilled water (control) for 20 minutes and then left to air dry for 30 minutes. Germination was assessed after 8 days. Seedling emergence and growth was assessed by sowing treated seeds in a seedling media in a controlled environment. The results show that combined germination percentage of all three species for the three treatment was: Water control 59.00%, Bacillus 65.33% and Serratia nematodiphila 69.66%, water being significantly lower (P= 0.05) than the two microbial treatments. The combined mean height of all three species for the three treatments after three weeks was: Water control 4.33 cm, Bacillus subtilis 8.68cm and Serratia nematodiphila 9.31cm, water being significantly lower (P= 0.05) than the two microbial treatments.*

KEYWORDS: Bio-Priming, Bacillus Subtilis, Serratia Nematodiphila, PGPR

INTRODUCTION

Horticultural production is primarily involved in the intensive use of resources, such as land, water, labour and inputs such as fertilizers and pesticides. The use of such resources in a concentrated space and time has the potential to negatively impact on the local environment and worker welfare (Wainwright *et al*.,2014). The use of pesticides and fertilizers is of major concern and to minimize their impact on the environment, safer alternatives have been sort. The use of microbes has been reported over many years to promote growth commonly termed "bio-fertilizers". A group of soil based bacteria that promote growth have been termed Plant Growth-Promoting Rhizobacteria (PGPR). As a consequence, PGPR have the potential to enhance plant health and promote plant growth rate without environmental contamination (Vejan *et al*.,2016). A range of PGPR have been studied, including the species *Pseudomonas, Bacillus, Enterobacter, Klebsiella, Azobacter, Variovorax, Azosprillum* and *Serratia* (Glick, 2012). However, the commercial utilization of PGPR in the agriculture industry is disappointing. The successful use of PGPRs is dependent on many factors, including survival of the organism on the seed and in the soil, the interaction with the microflora in the soils and the crop, and providing consistent results across a range of environments. However, PGPRs work in different ways and there is a need to understand these to ensure the successful adoption. An important component is to apply the PGPR in an efficient and in a manner compatible with

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current agricultural practice. Treating seeds with PGPR offers an economical and efficient application method (O'Callagham, 2016)

Priming seed using osmotic solutions has been around for many decades (Heydecker *et al*., 1975) This is now common commercial practice in selected high value horticultural seeds. This concept was extended to hydro priming in cereal and legume crops and the technique of "on farm" priming has been revived (Harris *et al*., 2001). More recently the term Bio-priming has been adopted where the seed is immersed in a microbial suspension for a pre-determined period, and this can then be followed by drying of the seed to prevent onset of germination. Given the effort involved in this process, it is most appropriate for low-medium volume, high value crops, such as vegetable seed (O'Callagham, 2016). Numerous species of PGPR have been evaluated and these include *Bacillus subtilis* isolates and less frequently, bacteria in the *Serratia g*enus. When *Serratia marcescens* was used in wheat benefits included positive for ACC deaminase activity, phosphate solubilization, production of siderophores, indole acetic acid production, nitrogen fixation, and ammonia production. The inoculation of *S. marcescens* enhanced the growth of wheat plant under salinity stress (Rajnish *et al*., 2016) whilst *Bacillus* species have been extensively reviewed as PGPR by (Kumar *et al*.,1994) (Sivasakthi *et al*., 2014). In this paper, we report the effect of Bio-priming of three vegetable seeds (carrots (*Daucus carota* subsp. sativa (var. Nantes) onion (Allium cepal., (var. Detroit dark red) and kales (*Brassica oleracea* var. Collard) using *Bacillus subtilis*, a plant-beneficial Gram-positive bacterium widely reported as a bio-fertilizer and *Serratia nematodiphila* on their germination, emergence rate and seedling growth.

MATERIAL AND METHODS

Germination studies

By definition, according to (Berley, 1995) germination incorporates those events that commence with the uptake of water by the quiescent dry seed and terminate with the elongation of the embryonic axis. The visible sign that germination is complete is usually the penetration of the structures surrounding the embryo by the radicle. While according to **(**Perry, 1984**)** Seedling emergence is as the result of a complex interaction between seed quality and seedbed environment.

Two bacterial strains *Bacillus subtilis* (BS01) and *Serratia nematodiphila* (Sn) were supplied by the Real IPM Company (K) Ltd and were used in this trial having been previously isolated from Kenyan soils. These strains were identified by CABI microbial identification service, Egham, UK. The vegetables seeds (carrots (Daucus carota subsp. sativa (var. Nantes) onion (*Allium cepa*., (var. Detroit dark red) and kales (*Brassica oleracea* var. Collard) were sourced from Royal Seeds company in Kenya. Two grams of each vegetable seeds varieties were weighed and soaked in 5ml/l *Bacillus subtilis* or *Serratia nematodiphila* solution containing 2.5×10^{7}cfu/ml and 2.4×10^{9} cfu/ml respectively or distilled water (control) for 20 minutes and then removed and allowed to air dry for 30 minutes. Germination was assessed by placing seeds on moisture filter paper in covered petri dishes. The petri dishes were covered with the lid cut on the top for ventilation purpose and they were placed in a germination room and incubated in the dark for 3 days for 25℃ and subsequently subjected to light (12h light/12 h dark) for another 5 days at 25℃ for germination. There were 20 seeds per replicate, three replicates per treatment and these were arranged in a complete random design. This experiment

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was repeated to validate the results. Daily count was performed for eight consecutive days then germination percentage was calculated.

$$\textbf{Germination}\ (\%) = \frac{\textbf{Number of seeds germinated}}{\textbf{Total number of seeds kept for germination}}\ \textbf{x 100}$$

To assess Seed emergence, treated seeds were sown individually into modular seed trays, each modular being 15 x 15 x 15 mm. Seeds were sown into sieved coco peat that had been previously washed three times prior to sowing and kept at 25℃ ± 2in the day time and 18℃ ± 2at night, no fertilizer was applied to any treatment during the trial. Each treatment had 11 replicates and were replicated three times. The trial was repeated a second time to validate the results. The emergence seeds were recorded on daily basis. To assess the growth rate, 5 plants were randomly sampled from each replicate, labelled and plant height was taken weekly until the third week.

Statistical Analysis

Data was subjected to Analysis of Variance with General Linear Model (GLM) procedures using Genstat software and means separated by LSD Tukey at $p \leq 0.05$.

RESULTS

Germination and emergence

Bio-priming of vegetables seeds (kales, carrots and onion) with *Bacillus subtilis* and *Serratia nematodiphila* resulted in a significant increase in seed germination after 8 days when compared with water soaking. The combined germination percentage of all three species for the three treatment was: Water control 59.0%, *Bacillus* 65.3% and *Serratia nematodiphila* 69.7%, water being significantly lower (P= 0.05) than the two microbial treatments. Kale had the highest percentage of seed germination after 8 days of all vegetables and the two bio-priming treatments were significantly higher than the water control with values of 92.0% (*Bacillus subtilis*) 95.0% (*Serratia nematodiphila*) 90.0% for the water priming (Fig 1A). For onion seeds, seeds bio-primed with *Serratia nematodiphila* gave a higher germination percentage (88.0%) than *Bacillus subtilis* (75.0%) whilst both were significantly higher than water treated (65.0%) (Fig 1B). Carrot germination was the slowest of the three vegetables tested and after 8 days the two bio-priming treatments were significantly higher than the water control with values of 28.0% (*Bacillus subtilis*) 26.0% (*Serratia nematodiphila)* 22.0% for the water priming (Fig 1C).

The combined emergence percentage of all three vegetables species for the three treatment was: Water control 28.5%, *Bacillus subtilis (*39.4%) and *Serratia nematodiphila* (37.6%), water being significantly lower (P= 0.05) than the two microbial treatments. Kale had the highest seed emergence after 21 days of all vegetables and the two bio-priming treatments were significantly higher than the water control with values of 49.1% (*Bacillus* subtilis) 47.7% (*Serratia nematodiphila*) 33.6% for the water priming (Fig 1A). For onion seeds bio-primed with *Bacillus* gave a higher emergence percentage (27.0%) than *Serratia nematodiphila (*25.0%) whilst both were significantly higher than water treated (24.0%) (*Fig 1B).* Carrot emergence for the two bio-priming treatments were significantly higher than the water control

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with values of 42.0% (*Bacillus* subtilis) 40.0% (*Serratia nematodiphila*) 28.0 % for the water priming (Fig 1C). Highest emergence rate of seed was observed in kale seed.

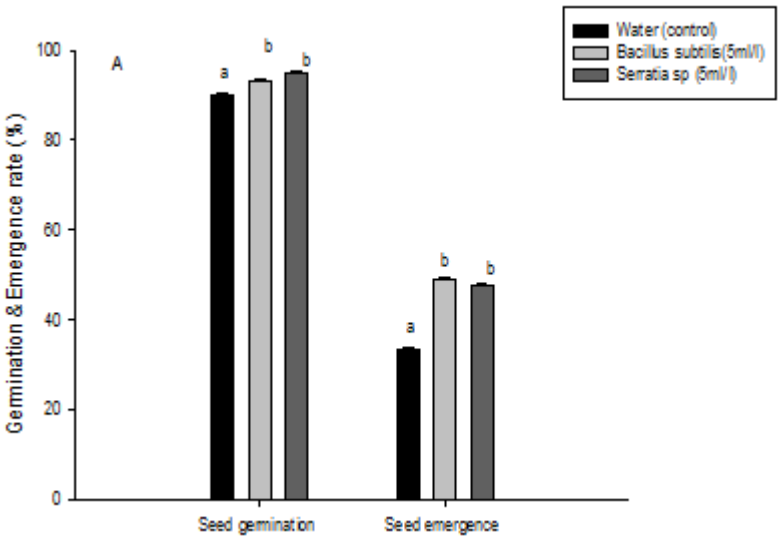

Figure 1A. Kale var. collard

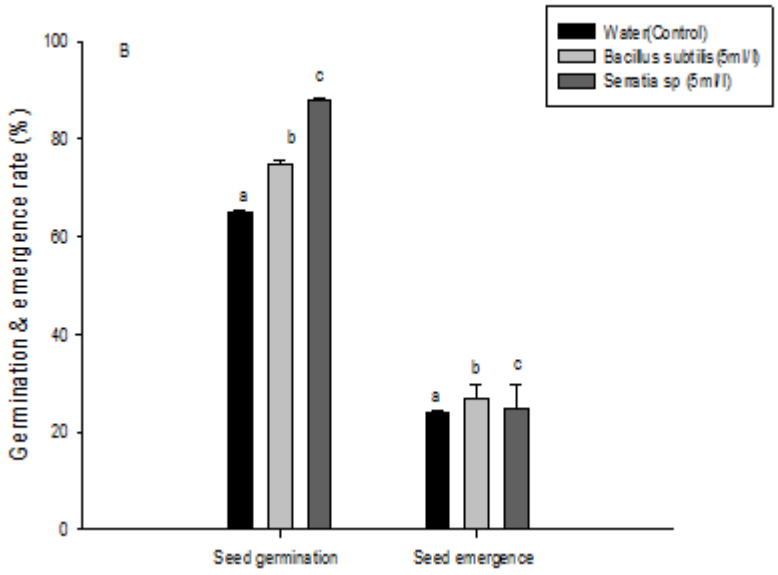

Figure 1B. Carrot var. Nantes

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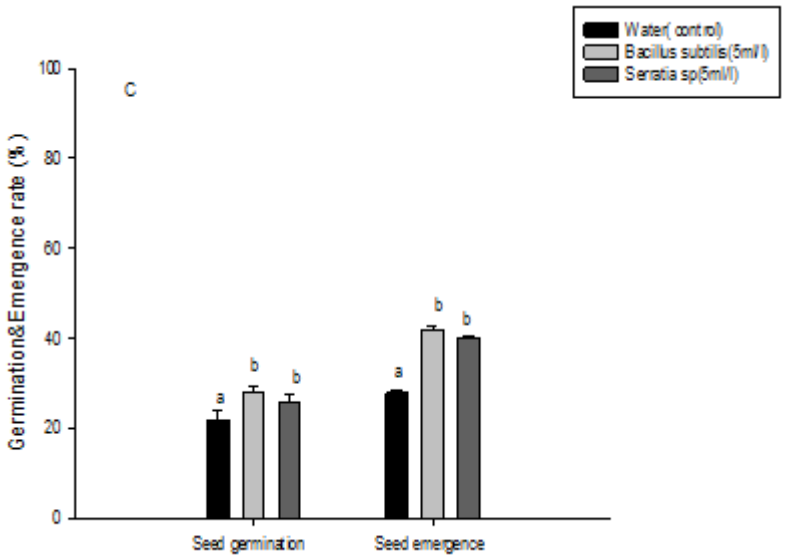

Figure 1C. Onion var. Detroit

Figure 1A, 1B and 1C: Comparison of mean percentage (±SE) of seed germination after 8 days and emergence after 21 days after bio- priming three vegetables seed: kale var. collard (Fig 1A), carrot var. Nantes (Fig 1B) and onion var. Detroit (Fig 1C) using *Bacillus subtilis* and *Serratia nematodiphila*. Different letters for germination or emergence indicate statistical differences (P ≤ 0.05). Error bars represent standard error value

Effect of seed priming treatments on plant height (cm) of three vegetables (kale var. collard, carrot var. Nantes and onion var. Detroit) seeds after week 1,2 and 3 after planting. The combined mean height of all three species for the three treatments after three weeks was: (Water control) 4.33 cm, (*Bacillus subtilis*) 8.68cm and (*Serratia nematodiphila*) 9.31cm, water being significantly lower (P= 0.05) than the two microbial treatments. For kales at week one, the plant height significantly higher for *Bacillus subtilis* treated seeds than *Serratia nematodiphila* treated seeds and both were significantly higher than the water treated seeds. For week two and three there was no significance difference between the *Bacillus subtilis* and *Serratia nematodiphila* treated seeds but they were significantly higher (P<0.01) than the water control (Fig 2A).

In onion seedlings, at week one *Serratia nematodiphila* treated seeds were significantly higher than *Bacillus subtilis*, and *Bacillus subtilis* was significantly higher in height than water treated seeds. However, by week two there was no significant difference between *Bacillus subtilis* and *Serratia nematodiphila* treated seeds but both treatments were high than the water control (Fig 2B). In carrot at week one Bacillus subtilis treated seeds were significantly higher than *Serratia nematodiphila* treated seeds, and *Serratia nematodiphila* was significantly higher in height than water treated seeds. However, by week two there was no significant difference between *Bacillus subtilis* and *Serratia nematodiphila* treated seeds but both treatments were high than the water control (Fig 2C).

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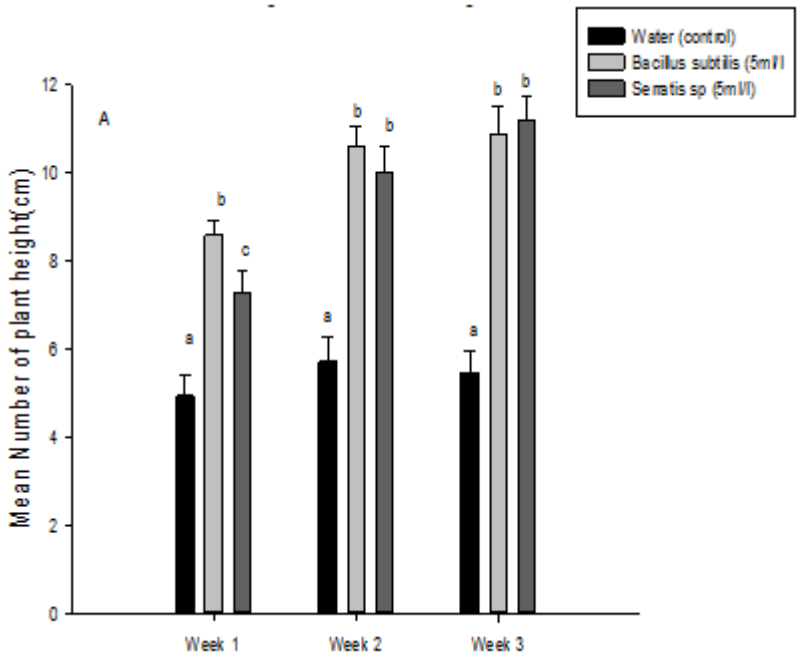

Figure 2A. Kale var. collard

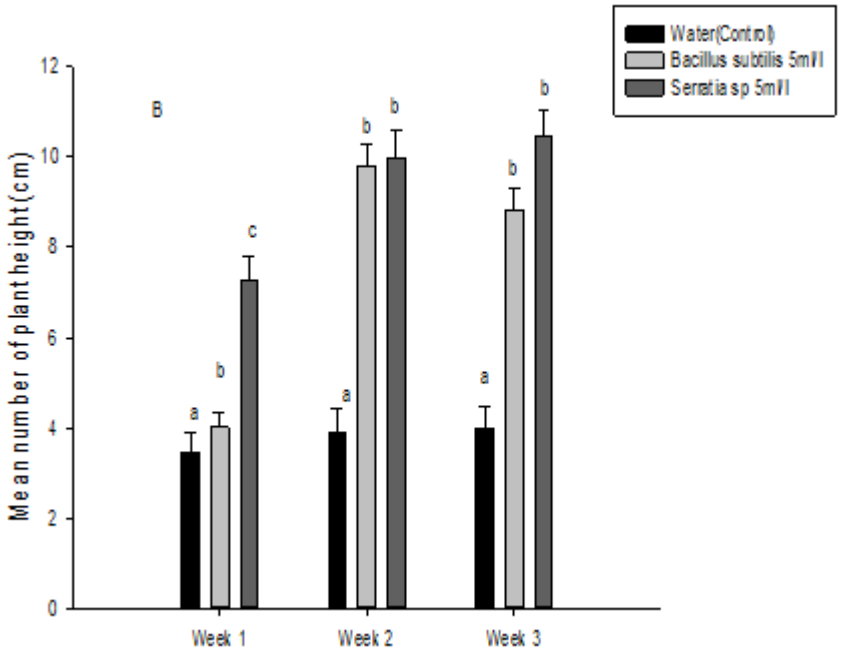

Figure 2B. Carrot var. Nantes

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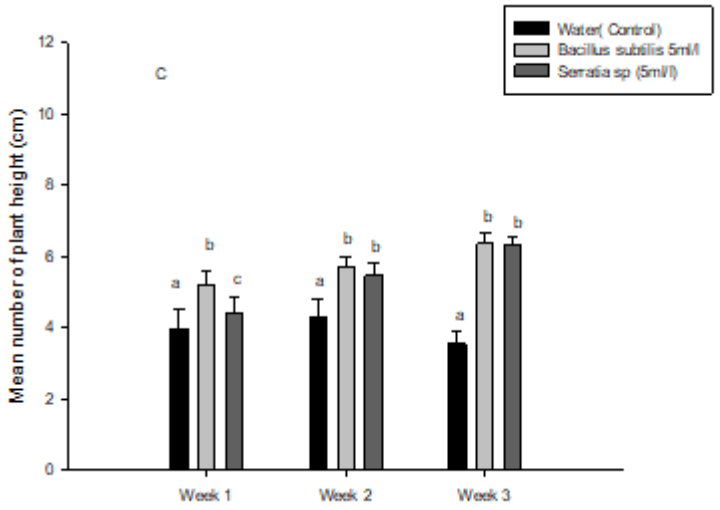

Figure 2C. Onion var. Detroit

Fig 2A, 2B and 2C. Measurement of plant height(cm) of Vegetable seed (kale var. collard (Fig 2A), carrot var. Nantes (Fig 2B) and onion var. Detroit (Fig 2C) with bio-priming treatments (*Bacillus subtilis and Serratia nematodiphila)* after 1, 2 and 3 weeks from sowing. Different letters in any single week indicate statistical error ($p \leq 0.05$). Error bars represent standard error values.

DISCUSSION

In all three vegetables (kales, carrot and onion) germination percentage was significantly increased by bio-priming with *Bacillus subtilis* and *Serratia nematodiphila* when compared to a water priming. The assessment of final germination percentage was made at day 8, which for the slower germinating carrot, gave relatively low germination percentages. Further work would require assessing germination over a longer period to fully understand the impact of bio-priming on slow germination species like carrot. In kales and onions emergence rates were lower than germination rates though emergence like germination was improved by Bio-priming with *Bacillus subtilis* and *Serratia nematodiphila*. However, for carrot emergence was greater than germination, though this can be explained by emergence was assessed after 21 days and germination which was assessed after 8 days. Carrot has been shown to be responsive to drum priming by improved emergence and that bio-priming with *Clonostachys rosea* IK726 further improved emergence time *(Amanda et al*., 2009). A commercial bio-fertilizer Supernitroplast, which consisted of *Bacillus subtilis*, *Pseudomonas fluorescens*, *Azospirillum spp*. When used to bio-prime wheat seeds increased several agro-morphological traits including grain number per spike, tiller number and shoot length of wheat plants (Rajnish *et al*., 2016).

In growth rate the two bio-priming treatments significantly increased the growth rate that resulted to have the better plant height (cm) in all the three vegetables seedlings. Kales and onions having a greater growth rate from week 1 to week 3 when bio-primed, however statistically the two treatment did not differ significantly. Although there was an increase in growth height in carrot seeds from week one to week 3 the rate was slow as compared to

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untreated seed in both treatments. Our data shows a slowing down of the growth rate in weeks 2 and 3 after sowing (Fig 2 A, B and C) and a possible cause might be a nutrient shortage as there was no artificial fertilizer applied. Water priming seeds had very slow growth compared to the bio-primed seeds and the contribution of the nutrients used to produce the liquid cultures of *Bacillus subtilis* and *Serratia nematodiphila* cannot be discounted as growth promoters. However, as these bacteria were manufactured and formulated as totally fermented products there was a mixture of bacteria, metabolites and nutrients in the solution used to bio-prime the seeds. Therefore, the bio-priming may be working through many modes of action.

The effect of bio-priming with *Serratia marcecens* CDP-13 showed many plant growth promoting traits increased including ACC deaminase activity, phosphate solubilization, production of siderophores, indole acetic acid production, nitrogen fixation, and ammonia production (Rajnish *et al*., 2016). Previous studies have shown that soil bacteria are able to grow plants more successfully helping them respond to environmental stresses, compared with plants without inoculation (Kloepper *et al*., 1988). Growth promoting bacteria are able to promote the growth and biomass production in different plant species, in this regard, one study in particular points out that some species of *Pseudomonas* spp. promote plant growth by increasing nutrient absorption (e.g., N, P, K) and providing hormones in the rhizosphere,(Díaz *et al*.,2001) (Duda and Orlikowski,2004). Thus, the results reported here are similar to results of other studies, where they report the potential use of rhizobacteria in agricultural production systems to help increase crop yield while reducing the fertilization costs (Vessey, 2003) (Fuentes-Ramírez *et al*., 2006).

CONCLUSION

Based on this study it was found that the use bio- priming with PGPR (*Bacillus subtilis* and *Serratia nematodiphila)* improved germination rate for three vegetables seeds), emergence and seedling growth. The use of bio-priming when compared to film coating, slurry treating or pelleting has considerable advantages including it is effective at getting the organism into the seed and achieves longer term survival of the micro-organisms when compared to other methods (O'Callagham, 2016). What is interesting is that three diverse vegetable families have showed potential to bio-priming with two different micro-organisms. The treatment process has not been optimized and numerous questions remain to be further studied including priming time, concentration of bio-priming solution, and the volume of seed to solution ratio. The addition of fertilizers in the post priming period needs to be assessed along with the long-term effects of both yield and biotic stress. Similarly, the underlying mechanism of how these treatments promote germination and growth are unclear and complex. However, from a practical horticultural stand point bio-priming with *Bacillus subtilis* and *Serratia nematodiphila* remain an exciting prospect

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