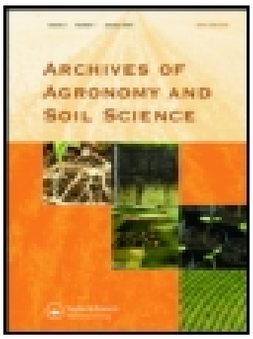

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RESEARCH ARTICLE

Enhanced plant performance in tomato (*Lycopersicon esculentum*) through seed encapsulation with controlled-release fertilizers

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ABSTRACT

Tomatoes are among the most widely grown vegetable crop, with more than 5-million hectares of land dedicated to its cultivation. To enhance production, many growers use conventional fertilizers which also contribute to non-point source pollution. While there are a variety of methods used to administer fertilizers to crops, some require expensive equipment, are labor intensive, or apply fertilizers not efficiently used by plants. This study considered an alternative approach that delivered controlled-released fertilizers to tomatoes using gelatin capsules; wherein both seed and fertilizer were planted together as a single unit. The objectives were to determine if seed encapsulation altered seedling performance, while also considering the possible use of encapsulation to deliver controlled-release fertilizers. Although seed vigor tests suggest gelatin can diminish seedling performance, seed encapsulation had minimal impact on seedling emergence when planted in soils. Capsule treatments (without fertilizers) were taller than controls, and the addition of fertilizers improve plant performance, with higher fertilizer content fostering greater growth. The results suggest that seed encapsulation may be an effective way to deliver fertilizers to crop plants, and that the combination of capsules and controlled-release fertilizer could possibly lead to a reduction in the quantity of fertilizers necessary for tomato cultivation.

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KEYWORDS
Biostimulant; capsule; fertilizer; gelatin; seed encapsulation

Introduction

With production rates in excess of 185 million tonnes in 2020, tomatoes (*Lycopersicon esculentum* Mill.) are among the most widely consumed vegetable crop in the world (FAOSTAT 2022). To achieve this level of fruit production, more than 5 million hectares of agricultural land has been allocated towards its cultivation (FAOSTAT 2022). As with other members of the genus *Lycopersicon*, tomatoes are tolerant to a wide range of environmental and nutritional conditions and, through careful crossings, have been modified to either produce a single-harvest crop or a succession of fruit that could supply markets over extended periods (Hobson and Grierson 1993). Tomatoes also serve an important role in human nutrition through the provision of essential amino acids, vitamins, and minerals, as well as a rich source of antioxidants such as vitamin C and E, and as a primary source of lycopene (Agarwal and Rao 2000; Martí et al. 2016). Indeed, the bioactivities of a vast array of phytochemicals found in tomatoes have been linked to several health benefits in humans including anti-genotoxic, anti-inflammatory, anti-mutagenic, anti-proliferative and chemopreventive properties (Chaudhary et al. 2018).

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In some areas, cultivation of tomatoes does not involve fertilizer amendments, due to the presence of residual nutrients in the soil and/or the comparatively high costs of synthetic fertilizers (Taiwo et al. 2007). Nevertheless, in many areas, driven by increased yields and its concomitant economic benefits, use of chemical fertilizers has become widely accepted. This proliferation of fertilizers to enhance crop production, however, has led to declines in environmental health especially when application rates greatly exceed the amount necessary to sustain crop growth. Excessive nutrients on agricultural lands often contribute to non-point source pollution that degrades land, surface waters, and groundwater supplies (Criss and Davisson 2004). Recently, controlled-release fertilizers have become more attractive to farmers cultivating tomatoes (and other crops) as these fertilizers have demonstrated both positive plant growth with increased fruit yield (Carson et al. 2014; Li et al. 2017). These fertilizers can provide appropriate nutrient release necessary to sustain both growth and physiological demands throughout the plant cycle (Vejan et al. 2021). Moreover, in comparison to conventional chemicals, controlled-release fertilizers can distribute nutrients more evenly, thereby reducing nutrient loss that often follows initial applications, and potentially minimizing environmental perturbations associated with improper nutrient management (Vejan et al. 2021).

There are a variety of methods used to administer fertilizers to plants cultivated in either greenhouses or agricultural fields including banding, broadcasting, drip irrigation, fertigation, liquid injection, and side dressing (Badr et al. 2010; Carson et al. 2014). Some techniques, however, require expensive equipment with varying amounts of maintenance, others are labor intensive, or apply fertilizers in a manner that is not efficiently used by the crop (Sharmasarkar et al. 2001; Hebbar et al. 2004). In this study we explored an alternative approach by delivering controlled-released fertilizers to tomatoes using pharmaceutical capsules. This could reduce labor costs and lessen the amount of fertilizer needed by sowing both the seed and fertilizer together. Capsules are typically made from gelatin (collagen-based material from animal bone or hide) or plant-derived hydroxypropyl methyl cellulose (HPMC). Even without added fertilizers, gelatin and other protein hydrolysates, have been shown to act as plant biostimulants, with enhanced plant growth observed in a variety of crops (Morales-Payan and Stall 2003; Parrado et al. 2008; Ertani et al. 2009; Koukounararas et al. 2013; Amirkhani et al. 2016; Wilson et al. 2018).

Seed encapsulation, using pharmaceutical capsules, can be viewed as an extension of seed coatings (Touchette and Cox 2022). In general, seed coat technologies include encrusting, film coating, or pelleting. Film coating consists of uniformly depositing polymers, plasticisers, and colorants (typically less than 10% of the total seed mass) forming a protective-physical barrier around the seed (Taylor et al. 1998; Pedrini et al. 2017). Pelleting involves depositing material in layers, thus modifying the shape and size of the original seed. This change in conformation can improve plantability for small or irregularly shaped seeds (Barut 2008; Sidhu et al. 2019). Moreover, pelleted materials can include agrichemicals such as fungicides and insecticides that may provide additional benefits to seeds and young developing plants (Heijbroek and Huijbregts 1995; Taylor et al. 2001).

Due to the potential advantages of delivering controlled-released fertilizers through seed encapsulation, we sought to evaluate the efficacy of this technology and to explore its potential as a possible delivery system for polymer-coated controlled-release fertilizers that may enhance tomato performance. More specifically, the objectives of this study were to (*i*.) determine if and to what degree seedling emergence was altered when placed within capsules, (*ii*.) characterize plant growth following emergence of encapsulated seeds, and (*iii*.) evaluate the use of seed encapsulation as a possible vehicle for the delivery of controlled-release fertilizers. These studies were conducted in both sandy and organically rich soils, to determine if the prior soil-nutrient status would influence encapsulated treatment response and performance.

Materials and methods

Seed germination and vigor

Germination and vigor tests were performed on tomato seeds (*Lycopersicon esculentum* Mill. cv. Cherokee Purple) under laboratory conditions according to Demir et al. (2011). That is, 25 seeds were placed between moisten paper towels within a 90 mm diameter petri dish. Four mL of distilled water (control; n = 3) or treatment solutions (n = 3) were added to each dish. After 24 hrs, to minimize the increased viscosity of the highest gelatin treatment and to maintain suitable moisture conditions for all treatments, an additional 1 mL of distilled water was added to each dish to achieve final treatment concentrations for type-A gelatin (0.08, 0.4, 0.8, or 4.0%) or hydroxypropyl methyl cellulose (HPMC; 0.08, 0.4, 0.8, 4.0%). Seeds were incubated at 20°C and monitored daily for germination as indicated by radicle emergence. At 14 days, total seedling length (combined root and shoot lengths; mm) were recorded in 10 randomly selected seedling from each dish. Total germination (G) was expressed as a percent germination of all seeds within each dish after 14 days. Time to 50-percent germination (T_{50}) were determined according to [AOSA] Association of Official Seed Analysts (2009) and calculated according to equation (1):

$$T_{50} = t_i + \frac{\left(\frac{N}{2} - n_i\right)\left(t_j - t_i\right)}{\left(n_j - n_i\right)} \tag{1}$$

where N is the number of seeds that germinated, and n_j and n_i represent the cumulative number of seeds germinated during adjacent counts at times t_i and t_j ($n_i < N/2 < n_j$). Similarly, mean germination time (MGT) was calculated according to equation (2):

$$MGT = \frac{\sum Dn}{\sum n} \tag{2}$$

where n is the number of seeds germinated on day D, and D is the number of days counted after initial germination. Germination index (GI) was calculated according to Gupta (1993) using the following formula (equation 3):

$$GI = \frac{\text{Number of germinated seeds}}{\text{Day of first count}} + \; - \; - \; - \; - \; + \frac{\text{Number of germinated seeds}}{\text{Day of last count}} \tag{3}$$

Finally, seed vigor index (VI) was calculated according to Gupta (1993) using equation (4):

$$VI = G \times L \tag{4}$$

where G is the percent of germinated seeds and L is the total seedling length (mm).

Experiment-1: controlled-release fertilizers and sandy soils

Seeds of tomato plants (*L. esculentum* cv. Cherokee Purple) were encapsulated in pharmaceutical capsules where one side of the capsule was made from a type-A (involving an acid pretreatment of collagen) bovine-hide gelatin extract and the other side from hydroxypropyl methyl cellulose (HPMC; size 00; Capsuline Inc., Dania Beach, FL) with or without control-released fertilizers. Results from the seed germination and vigor tests indicated significant delays in both germination and vigor in *L. esculentum* when seeds were exposed to elevated levels of gelatin (Table 1), therefore this experiment employed a mixed-material capsule that reduced gelatin content by one-half. The hard-gelatin capsules used in this study were immediate-release dosage forms composed of biopolymers (85–92% protein; Duconseille et al. 2015). External water, consistent with the amount needed to initiate seed germination, is generally the main component necessary for the dissolution of gelatin, with other environmental factors such as temperature, pH, and salinity having additional influences (Chiwele et al. 2000; Duconseille et al. 2015). Unlike soft gelatin, hard gelatin remains intact at

Table 1. Tomato (Lycopersicon esculentum Mill. cv. Cherokee Purple) germination parameters for control and treated seeds exposed to 4-levels of HPMC (0.08, 0.4, 0.8, and 4.0%) or gelatin (0.08, 0.4, 0.8, and 4.0%). Values include percent germination (Germ.) at 14 days, time to 50% germination (T_{50}), mean germination time (MGT), germination index (GI), seeding length at 14 day, and vigor index. Significant differences among treatments are designated by different letters (α = 0.5). Data are presented as means ± 1 SE (standard error; n = 3).

Treatment	Germ. (%)	T50 (d)	MGT (d)	GI	Length (mm)	Vigor
Control	97.4 ± 2.6^{A}	4.8 ± 0.7^{AB}	4.7 ± 0.1^{A}	5.7 ± 0.1^{A}	92 ± 1.4^{A}	8962 ± 287^{AB}
HPMC						
0.08%	96.1 ± 2.3^{AB}	5.0 ± 0.6^{AB}	4.9 ± 0.2^{A}	5.4 ± 0.3^{AB}	101 ± 4.1^{A}	9690 ± 623^{A}
0.4%	97.3 ± 1.4^{A}	4.7 ± 0.7^{A}	4.7 ± 0.02^{A}	5.4 ± 0.2^{AB}	100 ± 5.0^{A}	9721 ± 621^{A}
0.8%	96.1 ± 2.2^{AB}	5.7 ± 1.0^{ABC}	5.0 ± 0.2^{A}	5.3 ± 0.2^{AB}	95 ± 0.9^{A}	9154 ± 257^{A}
4.0%	95.8 ± 2.5^{AB}	5.8 ± 0.4^{ABC}	5.1 ± 0.2^{A}	5.0 ± 0.5^{B}	98 ± 3.2^{A}	9370 ± 286^{A}
Gelatin						
0.08%	98.7 ± 1.3^{A}	6.2 ± 0.7^{BCD}	4.9 ± 0.1^{A}	5.5 ± 0.1^{AB}	81 ± 8.5^{A}	8023 ± 893^{B}
0.4%	98.7 ± 1.3^{A}	6.7 ± 0.4^{CD}	7.0 ± 0.3^{B}	3.7 ± 0.1^{C}	21 ± 0.8^{B}	2051 ± 106^{C}
0.8%	91.9 ± 2.3^{B}	7.0 ± 0.3^{CD}	8.4 ± 0.2^{C}	2.9 ± 0.1^{D}	5 ± 0.7^{C}	475 ± 88^{D}
4.0%	84.6 ± 2.3^{C}	7.3 ± 0.2^{D}	8.8 ± 0.2^{C}	2.8 ± 0.2^{D}	2 ± 0.3^{C}	166 ± 21^{D}

relatively high temperatures. For example, at 60°C hard gelatin will only lose approximately 12% of its strength after 3 hours ([GMIA] Gelatin Manufacturers Institute of America 2019). In properly watered soils with temperatures above 20°C, we have observed gelatin dissolution within minutes to hours, albeit full dissolution may take days in comparatively dryer soils and/or lower temperatures. The HPMC capsule component was also an immediate-release dosage form that undergoes dissolution in the presence of water. As with gelatin, HPMC dissolution will occur in minutes to hours in well-watered soils; although studies suggest it may require additional time to fully breakdown compared to gelatin (especially in non-acidic environments; Al-Tabakha 2010; El-Malah et al. 2007).

Polymer-coated fertilizer treatments included Florikan with nutricote (18:6:8, N:P:K; Florikan, Sarasota, FL) and Osmocote (14:14:14; ICL Specialty Fertilizers, Dublin, OH). Florican control release fertilizer is coated with a polyolefin-type resin, that provides slow release of macro- and micro-nutrients (see Table 2 for elemental composition) over a release time of 360 days at 25°C soil temperature. Osmocote control-release fertilizer is coated with a copolymer resin (dicyclopentadiene and glycerol ester) and has a 3- to 4- month release time at 21°C (Jacobs 2005). For Osmocote, 4-, 6-, or 8- prills (OC-4, OC-6, and OC-8, respectively) were added to each capsule, accounting for approximately 110, 165, and 220 mg fertilizer per capsule, respectively. For Florikan, 2-, 4-, 6-, and

Table 2. Physiochemical properties of materials used in this study including pH, electrical conductivity (EC), total macro- and micro-nutrients for controlled release fertilizers (Florikan and Osmocote), gelatin capsule (Gelatin), and soils (sandy and nutrient-rich organic). Soil data are presented as means ± 1 SE, and significant differences between sandy- and organic-soils are indicated by asterisks (n = 5). Note, data are in percentages for soil carbon and fertilizers when values are available. Gelatin elemental composition is based on data from [GMIA] Gelatin Manufacturers Institute of America (2019).

Component	Florikan	Osmocote	Gelatin	Sandy Soil	Organic Soil
pH	5.0 (10% Aq.)		5.72 ± 0.06	6.64 ± 0.16	6.02 ± 0.12*
EC (dS m^{-1})			0.15 ± 0.01	0.19 ± 0.01	2.20 ± 0.10*
C (%)			98.5 ± 0.5	2.09 ± 0.59	45.7 ± 0.37*
N (g Kg^{-1})	18%	14%	162 ± 3.0	0.44 ± 0.12	9.14 ± 0.09*
P (g Kg^{-1})	6%	14%		0.06 ± 0.01	1.31 ± 0.04*
K (g Kg^{-1})	8%	14%	0.33 ± 0.05	0.18 ± 0.02	4.54 ± 0.11*
Ca (g Kg^{-1})			0.9 ± 0.1	1.60 ± 0.27	9.77 ± 0.47*
Mg (g Kg^{-1})	1.2%			0.40 ± 0.04	4.06 ± 0.25*
S (g Kg^{-1})	4.0%			0.06 ± 0.01	1.52 ± 0.05*
Fe (g Kg^{-1})	0.2%		0.02 ± 0.01	2.49 ± 0.11	1.98 ± 0.02*
Mn (mg Kg^{-1})	0.06%			14.7 ± 1.79	98.5 ± 1.52*
Zn (mg Kg^{-1})			1.5 ± 0.5	4.21 ± 0.62	16.4 ± 0.22*
Cu (mg Kg^{-1})	0.05%			4.75 ± 0.15	5.27 ± 0.11*
B (mg Kg^{-1})	0.2%			0.90 ± 0.09	4.57 ± 0.04*
Al (g Kg^{-1})				1.11 ± 0.21	1.94 ± 0.03*
Na (g Kg^{-1})			3.6 ± 1.4	0.062 ± 0.003	0.39 ± 0.01*

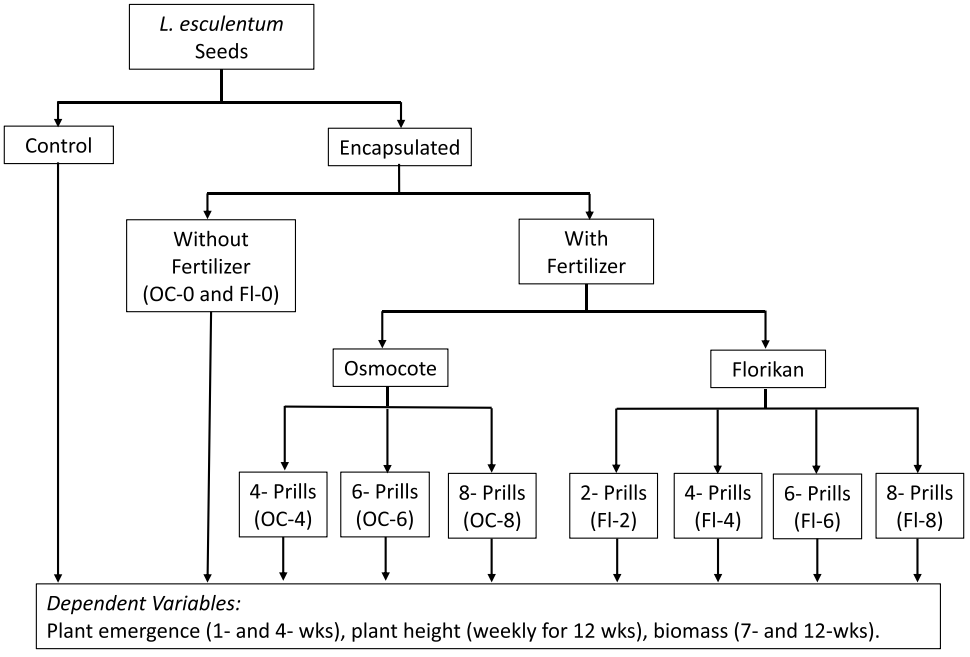

Figure 1. Block diagram for Experiment-1. Controls consisted of untreated seeds planted directly in pots with sandy soil. Encapsulated treatments included no fertilizer (OC-0 and Fl-0) or controlled release fertilizers (Osmocote or Florikan) at different levels (4-, 6-, and 8-prills for Osmocote, labeled OC-4, OC-6, and OC-8, respectively; 2-, 4-, 6-, and 8-prills for Florikan, labeled Fl-2, Fl-4, Fl-6, Fl-8, respectively). The dependent variables included plant emergence, plant height, and biomass.

8- prills (Fl-2, Fl-4, Fl-6, and Fl-8, respectively) were added to each capsule, accounting for 100, 200, 300, and 400 mg fertilizer per capsule. For both controlled-release fertilizer brands, an additional capsule-control was used, consisting of a capsule with seed only (i.e. OC-0 and Fl-0; Figure 1). In this experiment, we did not include any controls that considered both seed and fertilizer without capsules as this would require an additional 50 pots (including both experiments, leading to glasshouse space constraints), we also wanted to focus on the practicality of having a single planting unit containing both seed and fertilizer, and a fertilizer control was not necessary to support the stated research objectives. For all encapsulated treatments (with and without fertilizer), the remaining capsule space was loosely filled with a dried mixture consisting of plant compost (60%) and peat (40%).

To evaluate seedling emergence, three seeds (control) or three encapsulated seeds (treatments) were planted equidistantly in 1.8 L polypropylene pots with low-nutrient sandy soils (see Table 2 for elemental composition) in mid-July. A total of 55 pots were employed in this study (25 pots for Osmocote, and 30 pots for Florikan; n = 5; Figure 1) and were placed in a randomized complete block design that accounted for the north-south orientation of the glasshouse benches (Hartung et al. 2019). The climate-controlled glasshouse maintained temperatures between 25 and 30°C, with relative humidity fluctuating between 34 and 89% throughout the study. Pots were watered daily with approximately 400 mL of water.

Plant emergence was evaluated 1- and 4-weeks after sowing. Successful seedling emergence was characterized by the presence of aerial cotyledons, and reported as percent emergence from each experimental unit (i.e. plant pot). One plant from each pot was selected and evaluated weekly (over 12 weeks) for changes in plant height (growth). The additional plants were harvested for biomass after 7-, and 12-weeks. For biomass, plants were carefully removed from the pots, sorted between aboveground and belowground structures, dried in a laboratory oven at 60°C until constant weight, and massed.

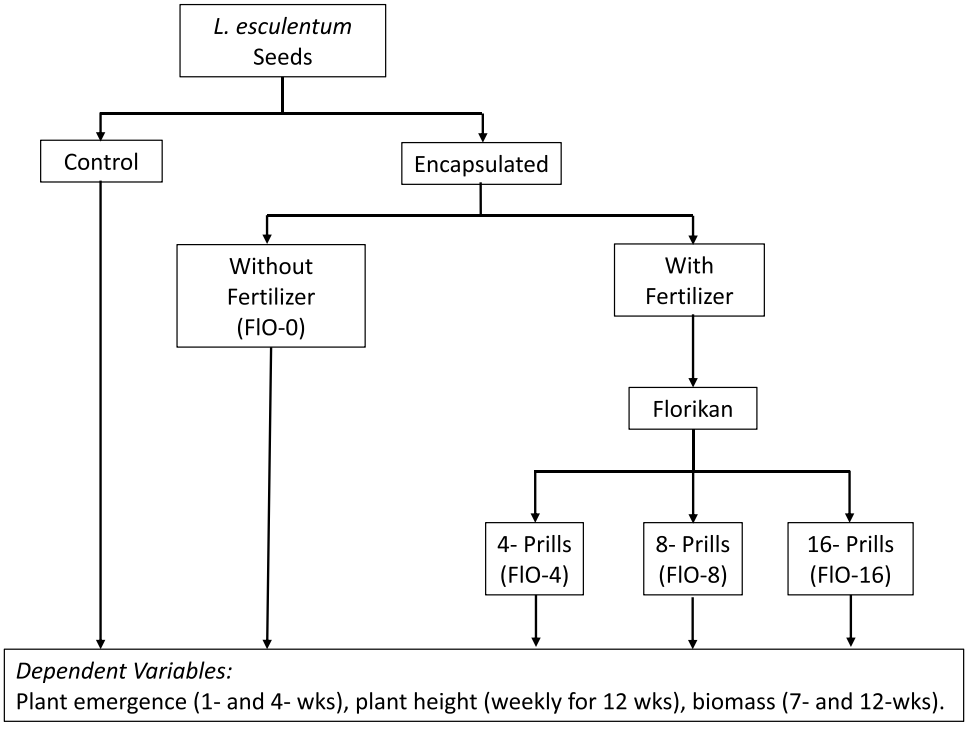

Figure 2. Block diagram for Experiment-2. Controls consisted of untreated seeds planted directly in pots with nutrient-rich organic potting soil. Encapsulated treatments included no fertilizer (FlO-0) or the controlled release fertilizer, Florikan, at different levels (4-, 8-, and 16-prills, labeled FlO-4, FlO-8, and FlO-16, respectively). The dependent variables included plant emergence, plant height, and biomass.

Experiment-2: Florikan controlled-release fertilizer and organic soils

As before, seeds of tomato (*Lycopersicon esculentum* Mill. Cv. Cherokee Purple) were encapsulated with or without the controlled-release fertilizer, Florikan. In this experiment, 4-, 8- and 16- prills (FlO-4, FlO-8, and FlO-16, respectively) were added to each capsule, accounting for 200, 400, and 800 mg fertilizer per capsule, respectively. Seeds were also placed in capsules that lacked controlled-released fertilizer (FlO-0; Figure 2). The remaining void space within the capsule was loosely filled with a dried mixture of compost (60%) and peat (40%).

Three seeds (control) or three encapsulated seeds (treatments) were planted equidistantly in 1.8 L polypropylene pots with organically-rich commercial potting soil (Miracle-Gro All Purpose Potting Mix, Scotts Miracle-Gro Co., Marysville, OH; see Table 2 for elemental composition) in mid-August. A total of 25 pots were placed in a similar randomized complete block design as described above and watered daily with approximately 400 mL of water. Plant emergence was evaluated 1- and 4-weeks after sowing. One plant from each pot was selected and evaluated weekly (over 12 weeks) for changes in plant height, and the remaining plants were again harvested for biomass after 7-, and 12-weeks (as described above).

Material and soil analyses

Sandy soils (Experiment-1) and organically-rich commercial potting soils (Experiment-2) were evaluated for pH and electrical conductivity according to Mylavarapu et al. (2020; n = 5, for each soil type). Hard gelatin capsules were evaluated for pH and electrical conductivity according to [GMIA] Gelatin Manufacturers Institute of America (2019), wherein 1 g of gelatin was dissolved in 100 mL at

45°C and cooled to room temperature before measuring. Additionally, soil samples were dried at 80°C, ground using a Wiley mill (Thomas Scientific, Swedesboro, NJ) and passed through a 1-mm mesh screen. Total soil N concentrations were determined using oxygen combustion gas chromatography with an elemental analyzer (NA1500; CE Elantech Instruments; Lakewood, NJ) according to [AOAC] Association of Official Analytical Chemists (2006). Samples used to measure the remaining total soil macronutrients (P, K, Ca, Mg, and S) and micronutrients (Fe, Mn, Zn, Cu, B, Al, and Na) underwent closed-vessel HNO_3 digestion in a microwave digestion system (MARS 6 Microwaves; CEM Corp; Matthews, NC) as described in Campbell and Plank (1992). The digested samples were diluted to 50 mL with DI water and passed through acid washed filter paper (Laboratory Filtration Group, Houston, TX). Total nutrient concentrations were subsequently determined using inductively coupled plasma-optical emission spectrometry (ICP-OES; Spectro Arcos EOP, Ametek, Mahwah, NJ; Donohue and Aho 1992).

Data analyses

Seed germination and vigor parameters were statistically evaluated using generalized linear models (GLM) that compared control and treated (HPMC or gelatin) seeds. Post hoc least significant difference (LSD) tests were performed when comparisons were identified as significant by GLMs. Similarly, physiochemical properties of the two soil types (from Experiments-1 and −2) were statically compared using GLMs followed by post hoc LSD analyses. Due to the longitudinal nature of the data collected in Experiments-1 and −2, emergence (recorded on 2 dates), biomass (measured on 2 dates), and weekly changes in plant height over 3 months, we employed generalized estimating equations (GEE), which is an extension of GLMs used for repeated-measures designs (Zeger and Liang 1986; Ballinger 2004). GEEs were selected because of the model's ability to evaluate non-normal longitudinal data that is sometimes characteristic of continuous and count data. Wald chi square tests were performed on parameters identified by GEE to have significant treatment responses. All statistical analyses were conducted using SPSS software version 26 (IBM Corp 2019), where comparisons were considered significant at $\alpha = 0.05$.

Results

There were notable differences in germination and vigor metrics for both HPMC and gelatin treated seeds (Table 1). In comparison to controls, seed treatments had little effect on percent germination during the first 14 days, with the exception gelatin at concentrations at or above 0.8%. In those conditions there were significant decreases in seed germination when exposed to higher gelatin levels ($p \leq 0.022$). Moreover, both time to 50% germination (T_{50}) and mean germination time (MGT) were similar between controls and HPMC treated seeds (Table 1). In contrast, gelatin levels at or above 0.4% resulted in a 1- to 3- day delay in seed germination; with higher levels of gelatin resulting in greater time delays ($p \leq 0.009$). Seedling length at 14 days was also influenced by gelatin treatments. Gelatin treated seedlings were significantly smaller than control and HPMC treated seeds when exposed to 0.4% or more gelatin ($p < 0.001$). Indeed, seeds treated with 0.8% or more gelatin had minimal growth beyond the initial radicle emergence over the 14-day period. Consistent with germination and length results, seed vigor was significantly reduced in gelatin treatments when concentrations were 0.4% or greater (Table 1; $p < 0.001$).

In Experiment-1, seedling emergence in control tomatoes grown in sandy soils were between 66.5 ± 0.3 and 80 ± 13% (mean ± 1 S.E.) after week-1, and 80 ± 13% by week-4 (Figures 3a and 4a). While there were no significant differences in plant emergence between control and encapsulated treatments using Florikan ($p = 0.76$; Figure 4a), there were significant declines in capsule treatments (OC-0) using Osmocote ($p = 0.039$). In that case, seedling emergence was 30% lower in OC-0 compared to the control (Figure 3a). Interestingly, there were no significant declines in emergence when seeds were encapsulated with different quantities of Osmocote ($p \geq 0.38$). Indeed, there was

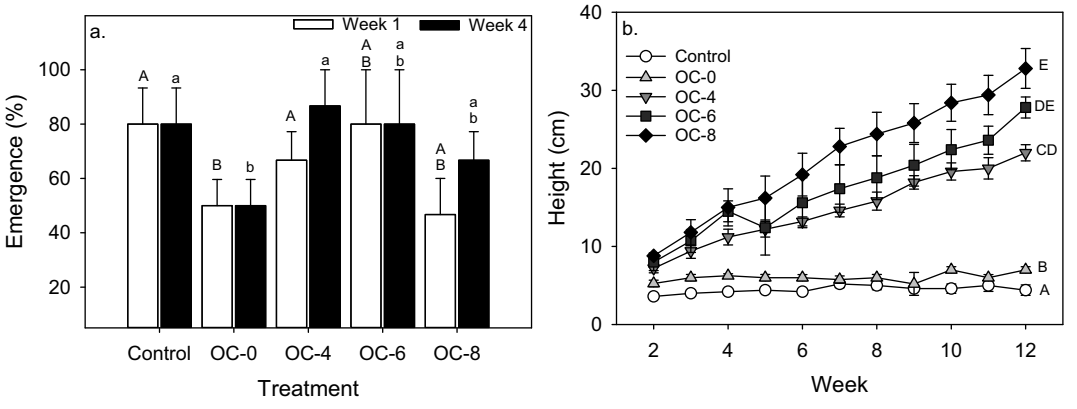

Figure 3. Seedling emergence and plant height over time- Osmocote. Percent seedling emergence in sandy soils on week-1 and −4 (panel-A), and plant height over time (panel-B) for control and encapsulated treatments including capsule only (OC-0), and capsules with controlled-released Osmocote, with four- (OC-4), six- (OC-6), and eight-fertilizer prills (OC-8). Significant differences among treatments, based on GEEs following a repeated-measures design, are identified by letters above the bars (panel-A), or to the right of the trend lines (panel-B), wherein different letters identify significant differences among treatments (n = 5).

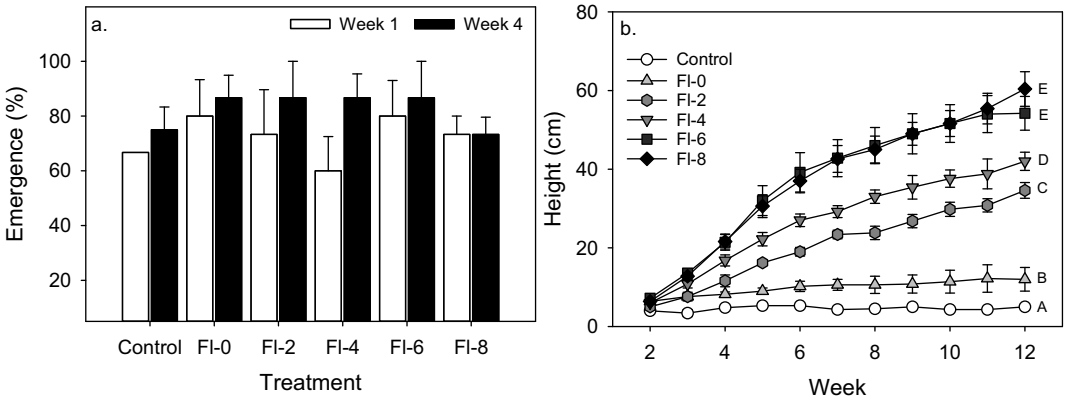

Figure 4. Seedling emergence and plant height over time- Florikan. Percent seedling emergence in sandy soils on week-1 and −4 (panel-A), and plant height over time (panel-B) for control and encapsulated treatments including capsule only (Fl-0), and capsules with controlled-released Florikan, with two- (Fl-2), four- (Fl-4), six- (Fl-6) and eight-fertilizer prills (Fl-8). Significant differences among treatments are identified by letters above the bars (panel-A), or to the right of the trend lines (panel-B), wherein different letters identify significant differences among treatments (n = 5).

a significant increase in emergence (by 36.7%; after 4-weeks) for OC-4 when compared to OC-0 treatment alone ($p = 0.012$). Interestingly, the emergence or germination rates observed in the glasshouse studies were notably lower than rates recorded in the seed germination/vigor testing from the laboratory (including controls).

Plant growth, as indicated by changes in plant height over time, was significantly higher for encapsulated treatments when compared to controls in both Osmocote and Florikan fertilizers grown in sandy soils ($p < 0.001$). For Osmocote, there was a stepwise increase in plant height, wherein plants from the OC-0 treatment were taller than the controls ($p < 0.001$), and OC-8 was taller than OC-4 ($p = 0.001$; Figure 3b). At 12 weeks, control plants were 4.4 ± 0.7 cm and the fertilizer treatments were 22.0 ± 1.0, 27.8 ± 1.4, and 32.8 ± 2.6 cm for OC-4, OC-6, and OC-8, respectively. Similar patterns were observed with Florikan, wherein Fl-0 treatments were taller than controls ($p < 0.001$), Fl-4 was taller than Fl-2 ($p < 0.001$) and both Fl-8 and Fl-6 were taller than Fl-4 ($p \leq 0.002$;

Figure 4b). By the end of the study, the height of the control was 5.0 ± 0.03, compared to 34.6 ± 2.0, 42.0 ± 2.3, 54.2 ± 4.3, and 60.4 ± 4.4 cm for Fl-2, Fl-4, Fl-6, Fl-8, respectively.

Similar to changes in plant height, there were marked differences in plant mass when comparing controls against encapsulated treatments grown in sandy soils. In Osmocote treatments, there was a significant increase in belowground mass for OC-0 treatments compared to controls by week-12 ($p < 0.001$; Table 3). This was also reflected in elevated root/shoot ratios for OC-0 when compared to controls ($p = 0.014$). There were no differences between OC-0 and controls, however, for both aboveground- and total- biomass by week-12 ($p = 0.16$ and 0.33, respectively). For capsules with Osmocote, there was a stepwise increase in both aboveground mass and total plant mass over 12-weeks. Total mass, for example, increase by 3,220, 6,080, and 8,560% (when compared to controls) for OC-4, OC-6, and OC-8, respectively (Table 3). Unlike Osmocote fertilizer, the Fl-0 treatments using Florikan had significantly larger aboveground-, belowground-, and total-mass after 12 weeks compared to controls ($p < 0.001$, for all three parameters; Table 4). Fl-0 treatments, as before, had greater root/shoot ratios compared to the controls at 12 weeks ($p = 0.001$); a response that was not observed when Florikan was added to the capsule (i.e. Fl-2, Fl-4, FL-6, and Fl-8), wherein root/shoot ratios were often lower than the controls during that same period ($p = 0.055$ for Fl-2, and $p \leq 0.015$ for other Florikan treatments). The addition of Florikan, nevertheless, resulted in significant increases in plant mass, with an overall trend of increasing aboveground-, belowground-, and total-mass with

Table 3. Tomato biomass- Osmocote. Dry mass measured on plants including aboveground-, belowground-, and total- dry mass, and root/shoot mass ratios for control, capsule (OC-0), and capsule plus Osmocote controlled-release fertilizer treatments (OC-4, OC-6, and OC-8) grown in sandy low nutrient soils. Plants were harvested on 7-, and 12- weeks after planting. Significant differences are indicated by letters; wherein different letters identify differences among control and treatments within each sample date (α = 0.05). Data are presented as means ± 1 SE.

Parameter (wk)	Control	OC-0	OC-4	OC-6	OC-8
Aboveground (g)					
Wk-7	0.006 ± 0.001^{A}	0.018 ± 0.001^{B}	0.08 ± 0.04^{B}	0.25 ± 0.06^{C}	0.21 ± 0.07^{C}
Wk-12	0.014 ± 0.010^{A}	0.020 ± 0.004^{A}	0.44 ± 0.06^{B}	0.92 ± 0.06^{C}	1.33 ± 0.22^{D}
Belowground (g)					
Wk-7	0.003 ± 0.001^{A}	0.010 ± 0.000^{B}	0.05 ± 0.02^{BC}	0.12 ± 0.04^{CD}	0.12 ± 0.03^{D}
Wk-12	0.006 ± 0.003^{A}	0.015 ± 0.003^{B}	0.26 ± 0.03^{C}	0.38 ± 0.05^{D}	0.49 ± 0.06^{D}
Total mass (g)					
Wk-7	0.009 ± 0.001^{A}	0.029 ± 0.007^{B}	0.13 ± 0.06^{B}	0.37 ± 0.10^{C}	0.33 ± 0.10^{C}
Wk-12	0.021 ± 0.013^{A}	0.035 ± 0.008^{A}	0.70 ± 0.08^{B}	1.30 ± 0.10^{C}	1.82 ± 0.28^{D}
Root/Shoot					
Wk-7	0.46 ± 0.10	0.65 ± 0.26	0.65 ± 0.10	0.46 ± 0.06	0.59 ± 0.12
Wk-12	0.54 ± 0.08^{A}	0.75 ± 0.05^{B}	0.61 ± 0.07^{C}	0.41 ± 0.04^{AD}	0.38 ± 0.03^{D}

Table 4. Tomato biomass- Florikan. Dry mass measured on plants including aboveground-, belowground-, and total- dry mass, and root/shoot mass ratios for control, capsule (Fl-0), and capsule plus Florikan controlled-release fertilizer treatments (Fl-2, Fl-4, Fl-6 and Fl-8) grown in sandy low-nutrient soils. Plants were harvested on 7-, and 12- weeks after planting. Significant differences are indicated by letters; wherein different letters identify differences among control and treatments within each sample date (α = 0.05). Data are presented as means ± 1 SE.

Parameter (wk)	Control	Fl-0	Fl-2	Fl-4	Fl-6	Fl-8
Aboveground (g)						
Wk-7	0.006 ± 0.001^{A}	0.039 ± 0.007^{B}	0.30 ± 0.05^{C}	1.04 ± 0.12^{D}	1.35 ± 0.24^{D}	1.95 ± 0.08^{E}
Wk-12	0.011 ± 0.002^{A}	0.027 ± 0.003^{B}	1.68 ± 0.26^{C}	2.62 ± 0.41^{D}	4.48 ± 0.78^{E}	5.64 ± 0.55^{E}
Belowground (g)						
Wk-7	0.003 ± 0.001^{A}	0.014 ± 0.002^{B}	0.12 ± 0.01^{C}	0.29 ± 0.05^{D}	0.45 ± 0.15^{DE}	0.46 ± 0.06^{E}
Wk-12	0.006 ± 0.002^{A}	0.026 ± 0.004^{B}	0.59 ± 0.08^{C}	0.77 ± 0.08^{C}	1.07 ± 0.35^{CD}	1.67 ± 0.32^{D}
Total mass (g)						
Wk-7	0.009 ± 0.001^{A}	0.054 ± 0.007^{B}	0.42 ± 0.06^{C}	1.34 ± 0.16^{D}	1.81 ± 0.24^{D}	2.41 ± 0.13^{E}
Wk-12	0.017 ± 0.005^{A}	0.053 ± 0.007^{B}	2.27 ± 0.33^{C}	3.40 ± 0.48^{D}	5.55 ± 1.08^{E}	7.31 ± 0.84^{E}
Root/Shoot						
Wk-7	0.61 ± 0.20^{A}	0.43 ± 0.13^{AB}	0.39 ± 0.04^{A}	0.29 ± 0.04^{B}	0.43 ± 0.22^{AB}	0.24 ± 0.02^{B}
Wk-12	0.56 ± 0.11^{A}	0.95 ± 0.07^{B}	0.36 ± 0.04^{C}	0.31 ± 0.03^{CD}	0.23 ± 0.05^{D}	0.29 ± 0.04^{CD}

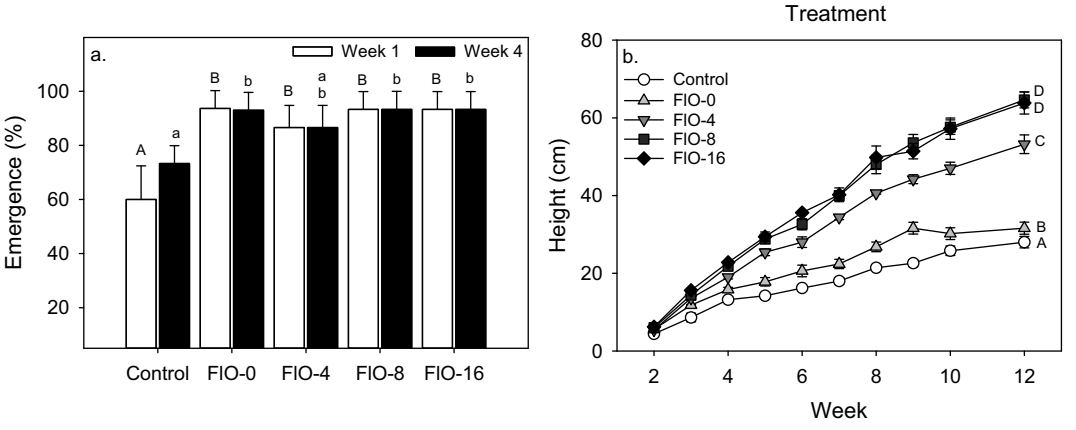

Figure 5. Seedling emergence and plant height over time- Florikan in organic soils. Percent seedling emergence in organic nutrient-rich soils on week-1 and −4 (panel-A), and plant height over time (panel-B) for control and encapsulated treatments including capsule only (FIO-0), and capsules with controlled-released Florikan, with four- (FIO-4), eight- (FIO-8) and sixteen-fertilizer prills (FIO-16). Significant differences among treatments are identified by letters above the bars (panel-A), or to the right of the trend lines (panel-B), wherein different letters identify significant differences among treatments (n = 5).

increasing fertilizer content ($p < 0.001$; Table 4). After 12 weeks, there was an increase of 13,250, 19,840, 32,550, 42,940% (compared to controls) in total mass for FI-2, FI-4, FI-6, and FI-8, respectively.

Experiment-2 considered if the addition of a controlled-release fertilizer, Florikan, encapsulated with tomato seeds would provide growth benefits to plants in nutrient-rich soils (see comparisons between sandy and nutrient-rich soils; Table 2). For the most part, encapsulated seeds had significantly higher rates of emergence in comparison to the controls (Figure 5a). After four weeks, seedling emergence was 73.3 ± 6.7% for the controls, compared to 93.3 ± 6.6% in the FIO-0 treatment ($p = 0.018$). Furthermore, the inclusion of Florikan in the capsules did not appear to adversely impact seedling emergence as both FIO-8 and FIO-16 had significantly higher germination compared to controls ($p = 0.018$, for both treatments). Only FIO-4 was found to have comparable emergence (86.7 ± 8.2%) rates as controls ($p = 0.157$).

In contrast to sandy soils, organic soils appeared to foster better growth in control plants (Figure 5b). After 12 weeks, the height of control tomatoes in nutrient-rich soils was 28.0 ± 1.5 cm; compared to an overall 4.6 ± 0.4 cm when grown in sandy conditions. However, as observed in the first experiment, seed encapsulation appeared to enhance long-term plant growth ($p < 0.001$). In this case, growth over time was slightly improved in FIO-0 treatments in comparison to controls ($p < 0.001$). Overall plant growth was even more pronounced in Florikan treatments, where there was a 90, 130, and 128% increase (relative to the controls) for FIO-4, FIO-8, and FIO-16 after 12 weeks ($p < 0.001$, for all Florikan treatments).

This increase in growth was reflected in plant mass. In this case, the FIO-0 treatment had significantly higher belowground mass at 4 weeks than controls ($p = 0.001$; Table 5). However, no additional biomass benefits were observed in the FIO-0 treatment at either 4- or 12- weeks. For seeds encapsulated with Florikan, there were also increases in aboveground-, belowground-, and total-biomass compared to controls ($p < 0.001$, for all three treatments at 12 weeks). That is, total plant biomass was 325, 575, and 730% higher (compared to control) for FIO-4, FIO-8, and FIO-16, respectively. In addition, FIO-8 and FIO-16 had significantly greater aboveground-, belowground-, and total- biomass than FIO-4 by week 12 ($p \leq 0.001$, ≤ 0.002, and ≤ 0.004, for above-, below- and total- biomass, respectively), but there were no differences in biomass observed between FIO-8 and FIO-16 ($p = 0.45$, 0.13, and 0.14, for above-, below-, and total- biomass). Unlike the Experiment-1, there were no significant differences in root/shoot ratios in plants from encapsulated treatments ($p = 0.54$).

Table 5. Tomato biomass- Florikan in organic soils. Dry mass measured on plants including aboveground-, belowground-, and total- dry mass, and root/shoot mass ratios for control, capsule (FlO-0), and capsule plus Florikan controlled-release fertilizer (FlO-4, FlO-8, and FlO-16) grown in organic nutrient-rich soils. Plants were harvested on 4-, and 12- weeks after planting. Significant differences are indicated by letters; wherein different letters identify differences among control and treatments within each sample date ($\alpha = 0.05$). Data are presented as means ± 1 SE.

Parameter (wk)	Control	FlO-0	FlO-4	FlO-8	FlO-16
Aboveground (g)					
Wk-4	0.10 ± 0.02^{A}	0.13 ± 0.02^{A}	0.21 ± 0.04^{B}	0.32 ± 0.03^{C}	0.31 ± 0.02^{C}
Wk-12	0.74 ± 0.13^{A}	0.97 ± 0.17^{A}	3.33 ± 0.41^{B}	5.00 ± 0.51^{C}	5.60 ± 0.70^{C}
Belowground (g)					
Wk-4	0.031 ± 0.006^{A}	0.058 ± 0.007^{B}	0.09 ± 0.01^{C}	0.12 ± 0.01^{C}	0.11 ± 0.01^{C}
Wk-12	0.38 ± 0.06^{A}	0.50 ± 0.07^{A}	1.44 ± 0.16^{B}	2.59 ± 0.35^{C}	3.71 ± 0.76^{C}
Total mass (g)					
Wk-4	0.13 ± 0.03^{A}	0.18 ± 0.03^{A}	0.30 ± 0.05^{B}	0.44 ± 0.03^{C}	0.42 ± 0.01^{C}
Wk-12	1.12 ± 0.17^{A}	1.47 ± 0.23^{A}	4.77 ± 0.54^{B}	7.59 ± 0.76^{C}	9.31 ± 1.08^{C}
Root/Shoot					
Wk-4	0.34 ± 0.03	0.49 ± 0.06	0.46 ± 0.04	0.40 ± 0.08	0.37 ± 0.05
Wk-12	0.54 ± 0.06	0.54 ± 0.05	0.44 ± 0.04	0.52 ± 0.06	0.70 ± 0.18

Discussion

This study considered the possible use of seed encapsulation, along with controlled-release fertilizers, to enhance tomato performance in sandy and nutrient rich soils. In contrast to seed vigor results with gelatin, seed encapsulation appeared to have minimal impact on seed emergence; maintaining comparable germination rates in controls at 1- and 4- weeks. The discrepancy between vigor tests and the encapsulation experiments (with and without gelatin) may be attributed, in part, to considerable material differences in substratum used between glasshouse and laboratory studies, dilution and drainage of capsule material within larger glasshouse containers, environmental conditions (e.g. temperature, moisture), and/or the use of different seed lots between the two studies. Nevertheless, for both experiments, gelatin-based capsules alone (i.e. OC-0, Fl-0, and FlO-0 treatments) provided some benefits including small (but significant) increases in plant height over time, and for the most part, increases in aboveground-, belowground-, and total- plant dry mass. While it is possible that the addition of compost in capsule-control treatments may have contributed to some observed plant growth in nutrient-pore sandy soils, the use of compost is less likely to be a factor in promoting growth in organically-rich potting soil. It is conceivable that the small amount of gelatin used in these studies was behaving as a biostimulant, which is consistent with other studies involving protein hydrolysates (Taylor et al. 1998; Calvo et al. 2014; Skwarek et al. 2020). Tomato cuttings, for example, when exposed to plant-based protein hydrolysates developed larger aboveground- and belowground- structures, with roots that were longer, with greater diameter, and more surface area after eight days (Colla et al. 2014). In another study involving pharmaceutical gelatin capsules, there were significant increases in both leaf area and plant mass after 28 days for tomatoes planted adjacent to as little as one-half of a capsule (Wilson et al. 2018). These biostimulant-like properties are not restricted to tomatoes, as other studies have shown similar performance improvements using animal- and plant-derived protein hydrolysates in arugula, broccoli, cucumber, kiwifruit, maize, papaya, passionfruit, pea, pepper, and snapdragon (Quartieri et al. 2002; Morales-Payan and Stall 2003; Ertani et al. 2009; Colla et al. 2014; Cristiano et al. 2018; Wilson et al. 2018).

While gelatin capsules alone may have had modest biostimulatory benefits, the addition of controlled-release fertilizers within capsules greatly improved tomato height and biomass. Tomatoes typically have relatively high N demands (Hebbar et al. 2004; Duan et al. 2019), and when considering the shorter release times for Osmocote (between 3- to 4- months), with presumably greater concentrations of continuously supplied nutrients, it would be reasonable to assume that Osmocote control-released fertilizer would have an advantage over Florikan (for at least 12 weeks). However, in sandy soils, Florikan appeared to provide better growth than Osmocote. When plants were given low quantities of fertilizer (OC-4 and Fl-2), accounting for approximately 100

to 110 mg fertilizer (15 to 18 mg N, for OC- and Fl-2, respectively), mean plant height after 12 weeks was 22.0 ± 1.1 and 34.6 ± 2.0 cm. Similarly, when given approximately 200 to 220 mg controlled-release fertilizer, plant heights were 32.8 ± 2.6 and 42.0 ± 2.3 cm, for OC-8 and Fl-4. It is likely that the improved performance of Florikan was due, in part, to additional macro- and micronutrients released by this fertilizer. This would be particularly important in sandy soils which tend to be nutrient depleted from higher rates of leaching and lower cation exchange capacities (Huang and Hartemink). Owing to the improved plant growth, especially with Florikan treatments, a second experiment was conducted that considered the use of controlled-release fertilizers in nutrient-rich soils. That study also revealed increased growth in encapsulated treated plants as fertilizer content increased. Interestingly, when comparing growth between Fl-8 and FlO-8, there was little difference in plant height after 12 weeks; 60.4 ± 4.4 and 64.6 ± 2.1 cm, respectively. This suggests that 400 mg of Florikan encapsulated with tomato seeds was sufficient to support plant growth in sandy low-nutrient soils for at least 3 months.

Tomatoes, in general, respond well to higher nutrient levels, and often achieve greater biomass and superior fruit production as soil N, P, and K levels increase (Hebbar et al. 2004; Amr et al. 2007; Shedeed et al. 2009). Timing of fertilizer application is also important as elevated nutrient demands occur as plants mature and initiate fruit set (Miller et al. 1979; Amr et al. 2007). Furthermore, higher yields with extended late-season fruit production, have been observed in tomatoes that received repeated drip-applied nutrients (between 200 to 230 kg N ha^{-1}) in comparison to a single preplant-nutrient application using NPK fertilizers (Locascio et al. 1989; Shedeed et al. 2009). In this study, encapsulation of controlled-release fertilizers (with release times between 3- and 12- months) along with tomato seeds, could allow for sustained nutrient availability throughout the growing season, including periods of higher nutrient demand. Moreover, improved fertilizer-use efficiency in tomatoes can be accomplished through direct application into areas with maximum root activity (Hebbar et al. 2004). In subsurface drip fertigation, for example, applying N and K directly to the root zone has been shown to be more efficient, with better plant performance, in comparison to more conventional fertilizer application techniques (Hernandize et al. 1991; Hebbar et al. 2004). This form of fertigation, however, is often restricted in its ability to apply P as there is a tendency for this element to form insoluble precipitates by reacting with naturally occurring Ca and Mg found in irrigation waters (Hebbar et al. 2004). Seed encapsulation with controlled-release fertilizers, however, may offer multiple advantages including the ability to apply nutrients directly to the root zone throughout a plant's growing season while also integrating P during the same application.

Because this study considered emergence and early growth of plants germinated from encapsulated tomato seeds, flower and fruit production was not considered. Nevertheless, some tomato studies suggest that dry mass fruit production is directly proportional to vegetative dry mass (Heuvelink 1996). Often in fruit plants, competition for assimilates between fruit and vegetative organs has been observed (e.g. apple, citrus, strawberries; Heim et al. 1979; Lenz 1979; Heuvelink 1996). For those plants, allocation to fruit mass is at the expense of vegetative growth, and excessive allocation towards fruit can adversely affect the capacity for future production. Indeed, too much assimilate allocated towards fruit growth may also lead to higher rates of flower and/or fruit abortion (Bertin 1995; Heuvelink 1996). In some instances, such as low light or high pruning, competition between vegetative and fruit mass can also be induced in tomatoes (Heuvelink and Buiskool 1995; Heuvelink 1996). More characteristically, however, is that the ratio between the sink strength of the vegetative component and the sink strength of the fruit in tomatoes approaches 3.0 (Heuvelink 1996). Therefore, larger plants can be expected to produce greater fruit dry mass, and as we have observed increased vegetative dry mass production in tomatoes germinated from capsules with and without fertilizer, it is likely that these larger plants will also maintain higher fruit yields.

A remarkable observation from this study was the amount of plant growth derived from comparatively low amounts of controlled-released fertilizer. While there are a number of variables, including plant size and growth behavior (determinant and indeterminant), planting densities of tomato on agriculture fields appear to be optimal between 16,000 and 26,000 plants per ha (Tuan

and Mao 2015; Maboko and Du Plooy 2018), with fertilizer applications for highest yields around 375 kg N per ha (Duan et al. 2019). It is important to recognize that with three seeds/capsules planted per pot, the remaining plant (after harvesting other plants for biomass) may be benefiting from residual fertilizers. Although this does not directly impact the three primary goals of this study, namely, to determine if encapsulation alters seedling emergence, influences growth, and can serve as a possible vehicle for applying fertilizer, it does limit our ability to precisely determine fertilizer applications. Nevertheless, based on the maximum possible fertilizer usage from this study, with the highest controlled-release dose (FlO-16) of 2,400 mg fertilizer per pot (432 mg N) and a planting density of 26,000 plants per ha, the amount of fertilizer used would be approximately 11.25 kg N per ha. Recognizing that this study was conducted in a glasshouse and did not consider plant productivity beyond three months, including long-term flower and fruit production, the growth that was achieved with just three percent of optimal fertilizer application is noteworthy. Even applying 10-times more controlled-release fertilizer than employed in this study (which could be achieved by using larger capsules), would still reduce fertilizer application rates by 260 kg N per ha. It is unlikely that controlled-release fertilizers provided through seed encapsulation would be sufficient to sustain most crops through the entire growing season, especially in nutrient-poor soils. Under more favorable soil nutrient conditions, however, supplemental control-released fertilizers delivered through encapsulation may be a viable alternative, by providing nutrients more effectively to the roots over extended periods of time and allowing for greater sustained plant productivity. We believe that more research is necessary to determine if seed encapsulation with controlled-release fertilizers could be an effective tool in reducing overall fertilizer applications in both field and greenhouse crops and help mitigate poor-nutrient application practices.

From a more applied perspective, seed encapsulation can be viewed as a form of seed coating, which is comprised of a variety of techniques including pelleting, encrusting, and film coating (Jolayemi 2019; Qiu et al. 2020; Touchette and Cox 2022). Dry powder coatings often do not adhere well to seed surfaces resulting in poor dousing, loss of uniformity, and dust formation, while thick seed coatings may to break or disintegrate before sowing (Halecky et al. 2016; Jolayemi 2019; Qiu et al. 2020). Moreover, externally applied film coatings may not be able to provide enough dosage of plant-beneficial material to be effective (Qiu et al. 2020). This study considered an alternative approach, using pharmaceutical capsules, to enclose seeds along with controlled-release fertilizers. Capsules can provide sufficient void space for greater volumes and/or multiple types of plant-benefitting materials. Gelatin alone has been shown to act as a biostimulant (Calvo et al. 2014; Wilson et al. 2018), and the addition of other components may provide additive benefits without concerns for material loss or human exposure to agrochemicals. Gelatin capsules also promote uniformity in the seeds and can be easily sowed using mechanical planters. Finally, animal-protein hydrolysates have been found to be a safe and sustainable product, with no harmful or toxic effects on soil microbiota or the environment (Corte et al. 2014; Jolayemi 2019). Therefore, seed encapsulation using gelatin capsule may provide unique advantages that are not offered by other forms of seed enhancement (Touchette and Cox 2022). The results of this study support the notion that seed encapsulation can improve tomato performance, and that controlled-release fertilizers can be successfully delivered using gelatin capsules. Further research is necessary to determine best ways to utilize this technology, especially its potential role in reducing fertilizer use in crops that are often attributed to deleterious environmental outcomes.

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