

Effects of a Microbial Biostimulant, Mammoth P™, on *Cannabis sativa* Bud Yield

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Abstract

High-value medicinal uses of non-psychoactive non-tetrahydrocannabinol (THC) hemp extracts like cannabidiol (CBD) are expanding to help reduce pain, severity of epileptic seizures and anxiety. This has stimulated the expansion of large indoor Cannabis grow operations in several markets. One key problem facing indoor cannabis producers is finding solutions to increase yield without significantly increasing operating costs. The purpose of this work was to evaluate how inoculation with a new sustainable microbial biostimulant, Mammoth PTM, developed at Colorado State University, affects plant growth rates and characteristics in *Cannabis sativa*. Treatment with Mammoth P led to significant increases in bud yields in *Cannabis sativa* by 16.5%. This increase in yield was accompanied by increases in plant height, and basal stem area. Increasing yield using this sustainable technology will help offset these capital expenses, reducing risk and likely increasing net income for indoor propagation facilities in this newly emerging market.

Keywords: *Cannabis*; Microbial biostimulant; Mammoth P; Cannabidiol

Introduction

The diversity of potential fiber, structural, dietary, and medicinal uses has expanded interest in *Cannabis sativa* cultivation [1]. Though currently limited worldwide production is limited to 65 thousand hectares, cultivation of *Cannabis sativa* for fiber plus hempseed purposes combined grew by 38% between 2010 and 2012 [2]. There is growing interest in medicinal uses of non-psychoactive hemp extracts like cannabidiol (CBD) to reduce pain [3], severity of epileptic seizures [4,5], and anxiety [6]. These medicinal prescriptive uses in conjunction the expansion of indoor Cannabis propagation for psychoactive THC properties have stimulated the expansion of large indoor grow operations in several markets. Hemp is thought of as a relatively low input crop with limited demands for nutrients [7], but ensuring adequate supply of N, P, and K is critical to increase yields [8,9]. Indoor hydroponic and soil-less grow systems enable tight control over nutrient supply to plants, but several studies suggest that soil bacteria can facilitate nutrient uptake by plants, P in particular [10]. Although soil bacteria are ubiquitous in soil systems, they are often lacking or absent in these indoor soil-less or hydroponic agriculture management systems. The purpose of this work was to evaluate how inoculation with a new microbial biostimulant developed by several research scientists at Colorado State University, called Mammoth PTM, affects plant growth rates and characteristics in *Cannabis sativa*. We assessed the capacity of this microbial biostimulant to increase plant growth during the seedling and bloom phases. This is one of the first growth trials for *Cannabis sativa* and among the first tests of microbial biostimulants conducted in hydroponic and soil-less systems.

Materials and Methods

In order to test the effectiveness of Mammoth PTM in boosting *Cannabis sativa* bloom and yields, we conducted a set of two experiments. The first experiment (E1) evaluated response to inoculation with Mammoth PTM when applied to established plants. The second (E2) was focused on assessing impacts on growth during the clone stage. Experiment E1 was conducted in a mixture of coco and perlite buffered with calcium nitrate (Cocotex PX[®] from General Hydroponics). For this experiment, cuttings were selected from a large population of mother

plants to ensure crop uniformity, then randomly allocated to treatment (treated with Mammoth P) or control (without Mammoth P) groups. Plants were transplanted into Cocotex

PX[®] and grown under one of six replicate lights at a density of 16 plants per light. During the first two weeks after transplant, plants were monitored daily in order to ensure uniformity during the vegetative growth phase; about 12% of plants underperformed initially and were replaced. Lights (400 w induction with vegetative spectrum manufactured by iGrow for vegetative growth and 600 w double ended high pressure sodium manufactured by Gavita for late vegetative and flowering phases), irrigation equipment (1/4 hp submersible pump by Everbilt connected to a 30' rain wand by Dramm), and nutrients (CNS Grow 3-1-2 manufactured by Botanicare, CNS Bloom 2-2-3 manufactured by Botanicare, Hygrozyme horticultural enzymatic formula by Spico Bioengineering Inc., CaMg⁺ an organic calcium magnesium supplement by General Hydroponics, Protek Silica by Dyna Grow, and Double Down pH adjuster from Earth Juice) were chosen to meet industry standards and to represent typical grow operations. Independent sets of irrigation equipment were used for control and treated plant groups. Irrigation reservoirs were mixed thoroughly to ensure uniform pH, EC, and nutrient and Mammoth PTM concentrations. Mammoth P inoculum was applied at rates and frequencies recommended by the manufacturer and listed on the label, ranging from 0.6-4 ml/gal with every watering (detailed schedule on label available at <http://mammothmicrobes.com>). Control plants were treated identically with treated plants without the addition Mammoth PTM. Experiment E1 was conducted under six different planting

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tables with dedicated lights, which were managed independently and served as the experimental replicates for control and Mammoth P treatment groups. Each planting table (16 plants ea.) was fed by independent reservoirs and application devices (i.e., pumps, hoses, and hose end sprayers). Temperature was maintained at 22°C during the light cycle and 18°C when the lights were off. Relative humidity was maintained at a steady 35% throughout the growth cycle. Water use was monitored daily, and fertigation frequency and reservoir loss in the irrigation system were used to assess moisture retention. Mature plants from all experimental units were harvested simultaneously for yield analysis. Plants were harvested uniformly, with cuts made on the main stem one inch above the growth media surface consistently across all plants and among treatment groups. All primary non-resinous fan leaves were removed and each plant was hung upside down to dry for 10 days. Humidity and air movement were kept uniform across the entire drying process to maintain consistency. After total biomass was weighed, flowers were removed from the branches and final dry flower weight was measured for each replicate table. Additionally, plant height and basal stem thickness was measured weekly during the flowering phase using a caliper on three randomly-selected plants from each replicate table. Plant height from floor level to apical meristem was measured weekly. Early crop maturity, increased root proliferation, and flower production were visually assessed daily, and days to visible bud was monitored on days 7-21 of the flowering cycle. Mammoth PTM compatibility with a variety of rooting hormones and growth media was assessed by observing growth of 20 (aeroponic experiment) or 50 replicate (other growth media) clones grown in soil, coconut coir, and rockwool, and aeroponically with addition of several different rooting stimulators (Mykos and Azos from Extreme Gardening, and Clonex Rooting Gel) under 432w T5 florescent 6400 K lights manufactured by Hydrofarm (E2). Clones were evaluated for uniformity, time to callus, and taproot length. Stems visible throughout the cloning process, allowed us to observe days to callus for the aeroponic cuttings. A cloner was deemed callused when 50% of the cuttings showed white callus at the base. Days to visible rooting (roots growing out of pots or 1 cm of root (aeroponic) for 50% of clones) was monitored on all cuttings taken. Success rate of clones was determined at 14 days by identifying whether a rooted cutting was fit for transplant, as indicated by the presence of visible, robust roots at the outer layer of the rooting media or protruding over 1 cm from the stem (in aeroponics).

Statistical Analysis

Six replicate measures of plant height, basal area, and rooting dynamics were compared using two-tailed t-tests. Repeated measures analysis was used to assess differences in stem basal area and plant height over time. For the clone experiment, data on callus formation, root emergence, and rooting were normalized to observed maxima. All statistical analyses were conducted using R [11].

Results

Application of Mammoth P significantly increased bud yields, by an average of 41.8 g per light (2.6 g/plant) over control samples that received fertilizer but no Mammoth P (Figure 1). Plants receiving industry-standard fertilization regimes yielded 253.9 g per light (15.9 g per plant), while average yields for tables treated with Mammoth P were 295.7 g per light (18.5 g per plant). Yield with the use of Mammoth P increased by 16.5% (Figure 1). Stem biomass comprised about 30% of total above ground biomass and did not increase significantly with use of Mammoth P (Figure 2). Plants treated with Mammoth P grew significantly taller (Figure 3). Controls were an average of 46.1 cm tall when harvested while Mammoth P treated plants averaged 50.2 cm,

an increase of 4.1 cm (Figure 3a). Basal stem diameter also increased significantly for the Mammoth P treatment, from 9.2 mm to 11.1 mm, equivalent to +13.5% (Figure 3b). Control and treated plants were not significantly different at the start of the bloom phase, but over the course of the bloom phase the treated plants quickly exhibited significantly greater basal stem area and plant height (Figure 4). Days to visible bud formation (data not shown) was not significantly different between treatments. The responses of callus formation, root emergence, or fully rooting varied as a function of growth medium. Responses tended to be greater for the coco, soil, and soilless systems (Table 1) and faster for the coco and soil systems (Table 2). In most cases, callus formation was faster for Mammoth P treated clones than for untreated clones, though differences were generally not significant. Even when differences between treatments were significant, they tended to be small (1-2 days faster or 1-2 more plants) for all of the clone response metrics (Figure 5).

Discussion

Treatment with Mammoth P led to significant increases in bud yields in *Cannabis sativa*. Increases in yield were linked to increases

| Growth media | Callus | Root emergence | Full rooting |
|--------------|--------|----------------|--------------|
| Aeroponic* | -2 | -2 | -2 |
| Rockwool | 0 | -1 | -2 |
| Coco | 1 | 1 | 0 |
| Soil | 2 | 1 | 2 |
| Soilless | 1 | 2 | 2 |

*The aeroponic treatment had 20 replicate clones whereas the other treatments had 50.

Table 1: Difference in number of plants ultimately achieving different stages of growth for clones. Positive numbers indicate more clones realized a given growth phase when treated with Mammoth P.

| Growth media | Callus | Root emergence | Full rooting |
|--------------|--------|----------------|--------------|
| Aeroponic* | 3 | -16 | -3 |
| Rockwool | 16 | -9 | -32 |
| Coco | 8 | 6 | -6 |
| Soil | 12 | 10 | 16 |
| Soilless | -7 | -4 | -2 |

*The aeroponic treatment had 20 replicate clones whereas the other treatments had 50.

Table 2: The number days earlier or later those groups of clones achieved different stages of growth. Positive numbers indicate that clones achieved a given growth phase faster with Mammoth P than without.

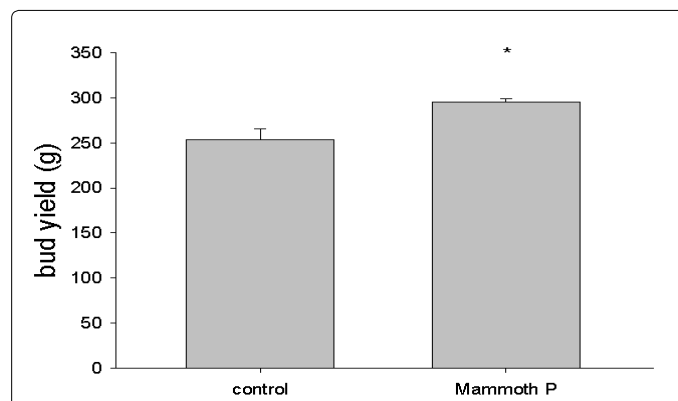


Figure 1: *Cannabis sativa* bud yield (g per light ± 1 SE) for plants that received conventional fertility management in comparison with samples that were inoculated with Mammoth P. Asterisks indicate significant (P<0.05) differences at a given observation date.

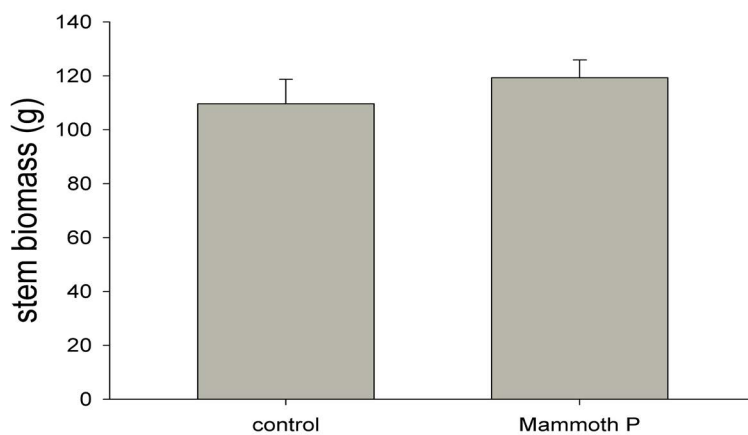


Figure 2: *Cannabis sativa* stem biomass (g per light \pm 1 SE) for plants that received conventional fertility management in comparison with samples that were inoculated with Mammoth P.

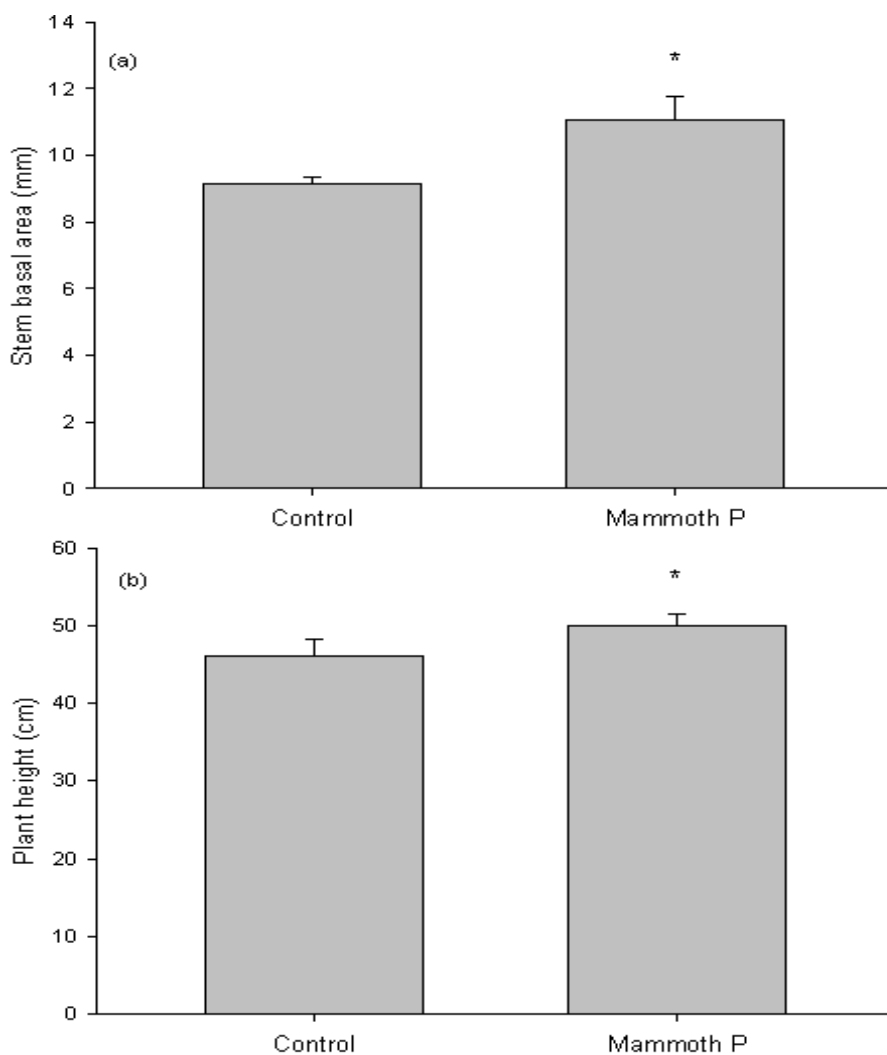


Figure 3: Plant height (a) and basal stem diameter (b) of *Cannabis sativa* for plants grown with and without Mammoth P. Error bars represent standard errors across 3 replicates and asterisks indicate significant ($P < 0.05$) differences at a given observation date.

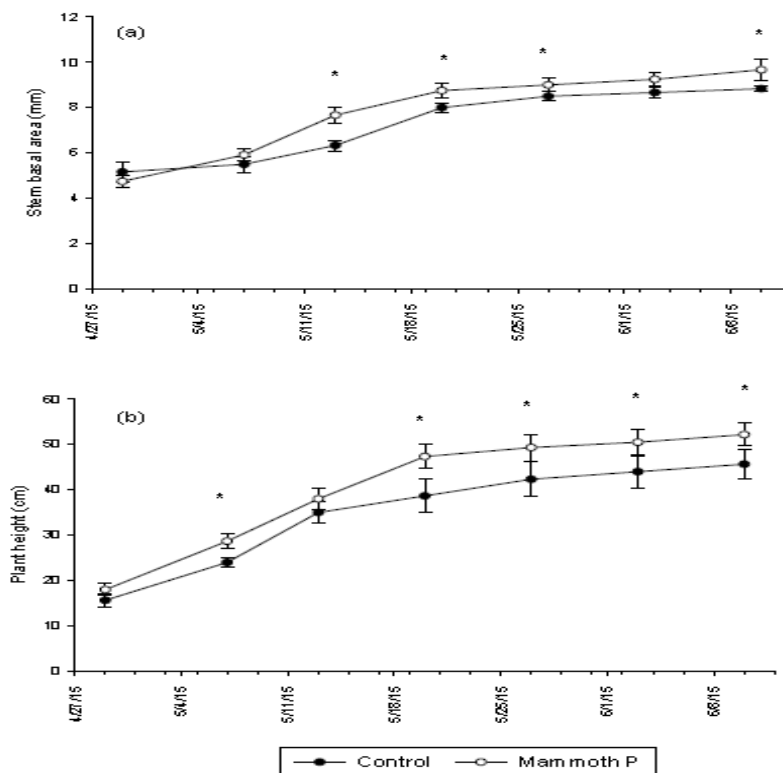


Figure 4: Temporal trends in basal stem area (a) and plant height (b) of *Cannabis sativa* for plants grown with and without Mammoth P. Asterisks indicate significant ($P < 0.05$) differences at a given observation date.

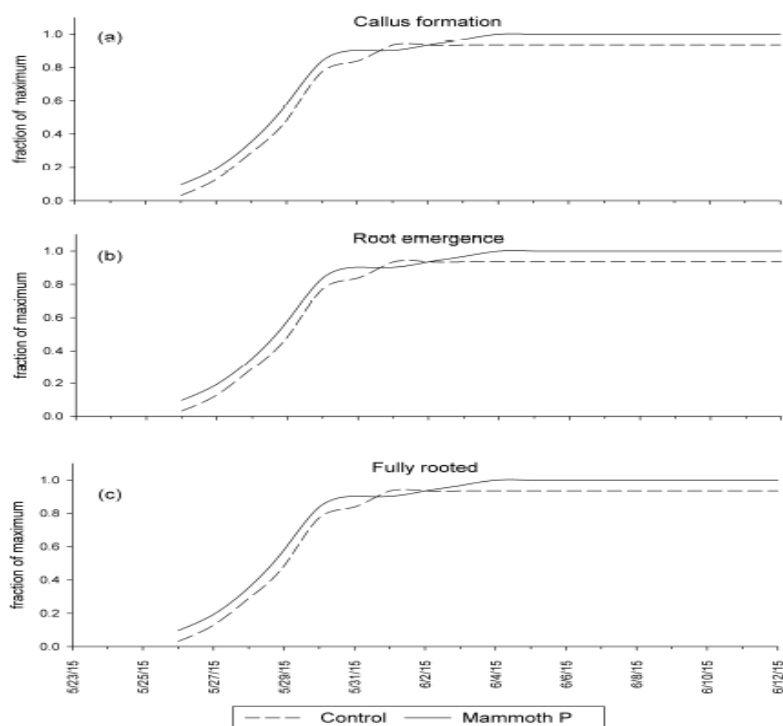


Figure 5: Rates of callus formation (a) root emergence (b), and full root formation (c) treated with Mammoth P and with conventional fertility management. All data were normalized against the maximum number of plants that exhibited each metric (i.e., 1=Max number of plants to callus, exhibit root emergence, or fully root) on a given date. Data are shown are for soil systems. Responses to Mammoth P for other growth media are summarized in Table 1.

in plant height and stem basal area, but no significant changes in stem biomass. Mammoth P led to larger, more robust plants that yielded 16.5% more product. *Cannabis sativa* yields in indoor grow operations are not commonly published, but our yields with and without Mammoth P compare favorably with previous studies using similar density and lighting conditions normalized for wattage [12,13]. In the clone study, Mammoth P did not lead to substantial changes in speed to callus, root emergence or full rooting. However, observations in the experiment on adult plants suggested that Mammoth P-treated plants may have reached full maturity earlier than untreated controls. The rate of height increase for plants treated with Mammoth P were greater than those for control plants through the first 4 weeks of the bloom phase, but the untreated plants caught up some during the final 3 weeks. Similar patterns were observed in basal stem observations. Our experimental design was established a priori, and stipulated harvest after 7 weeks of bloom phase for all treatments. Thus, we were unable to confirm that Mammoth P contributed to faster blooming and bud development, but our height and basal area data indicate some evidence of faster bloom development and growth. Investments in indoor grow operations of *Cannabis sativa* require substantial capital investment [14]. Increasing yield by 16.5% will help offset these capital expenses more quickly, likely increasing net income and reducing risk [15]. Integration of Mammoth P into the grow operation was seamless, requiring no additional revision of standard practices. In sum, while use of Mammoth P did not seem to increase or accelerate plant development during the clone phase, use of Mammoth P led to more robust and more *Cannabis sativa* growth during the bloom phase.

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