# EFFECTIVENESS OF MYCORRHIZAE AND VERMICULTURE SEED INOCULATION FOR GERMINATION, VEGETATIVE GROWTH, CANNABINOID CONTENT, AND CURED FLOWER WEIGHT OF CBD-RICH HEMP (*Cannabis sativa* L.)

by

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## DEDICATION

This thesis is dedicated to my family and my dearest friends who have seen me through the shining moments and the pits of despair. I particularly want to mention my beloved cat, Dex, for his comfort and companionship throughout my graduate school experience. This thesis is also dedicated to all the wrongfully incarcerated individuals due to the war on drugs and the illegality of all forms of cannabis for so many decades here in the United States. I am so incredibly privileged to be able to study cannabis in its low-THC form and I hope my work will further the science that may one day convince the federal and remaining state governments to correct these grave injustices.

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## ABSTRACT

Effective germination and vigorous growth of hemp varietals is paramount to cultivators' ability to produce high-quality hemp outputs. Mycorrhizae and the beneficial organisms found in vermiculture are known symbionts to plants and are used in regenerative agriculture to increase plant health and crop yield. This study investigated the effect of a single inoculation of beneficial microbes on hemp seed germination rate, plant height, cured flower weight, and cannabinoid content. While the results demonstrate no statistically significant differences between treatments in any of the parameters measured, the study results demonstrate potential for further investigation into seed inoculants for enhanced cured flower weight and cannabidiol (CBD) content of CBD-rich hemp.

# I. INTRODUCTION

Hemp, also known as *Cannabis sativa* L., has a long, widespread history as an oilseed and fiber crop hypothesized to have moved alongside humanity during the pre-agriculture, nomadic stages of society (Cherney & Small, 2016). Hemp cultivation came under official attack in the United States through the Marihuana Tax Act of 1938 whereby lawmakers placed a tax on cultivation and sale of all forms of cannabis, including hemp. This measure also placed enforcement of the tax act and its provisions under the control of the Drug Enforcement Agency (DEA) (Cherney & Small, 2016). After a brief resurgence of hemp cultivation during the Second World War, hemp and marijuana were coupled and made illegal under the 1970 Comprehensive Drug Abuse Prevention and Control Act, regardless of the legislation itself making a distinction between the non-psychoactive, industrial use of hemp and the high-psychoactivity, recreational use of marijuana. The 2014 Farm Bill allowed pilot programs of hemp cultivation in the United States for the first time in forty-four years, with only four states reporting hemp cultivation during the initial year (Brannon, 2019; Mark et al., 2020; Quarles, 2019). The subsequent 2018 Farm Bill re-legalized hemp cultivation in the United States with finalized provisions released as recently as early 2021. Due to this nearly 50-year hiatus, there are a lack of scientific data on the cultivation practices of hemp including best practices for optimal seed germination and effectiveness of microbe-based soil treatments.

As hemp cultivation is being explored more scientifically in North America, interest in sustainable and regenerative methods of crop cultivation for food and fiber crops is also increasing (Kroma, 2006). Regenerative agriculture is a farming practice

that aims to improve soil health, promote ecological sustainability, and ultimately reduce input costs for producers. It is characterized by reduced tillage, diverse crop rotations, cover cropping, livestock integration, and an overall focus on soil microbial health (Figure 1; Khangura et al., 2023).

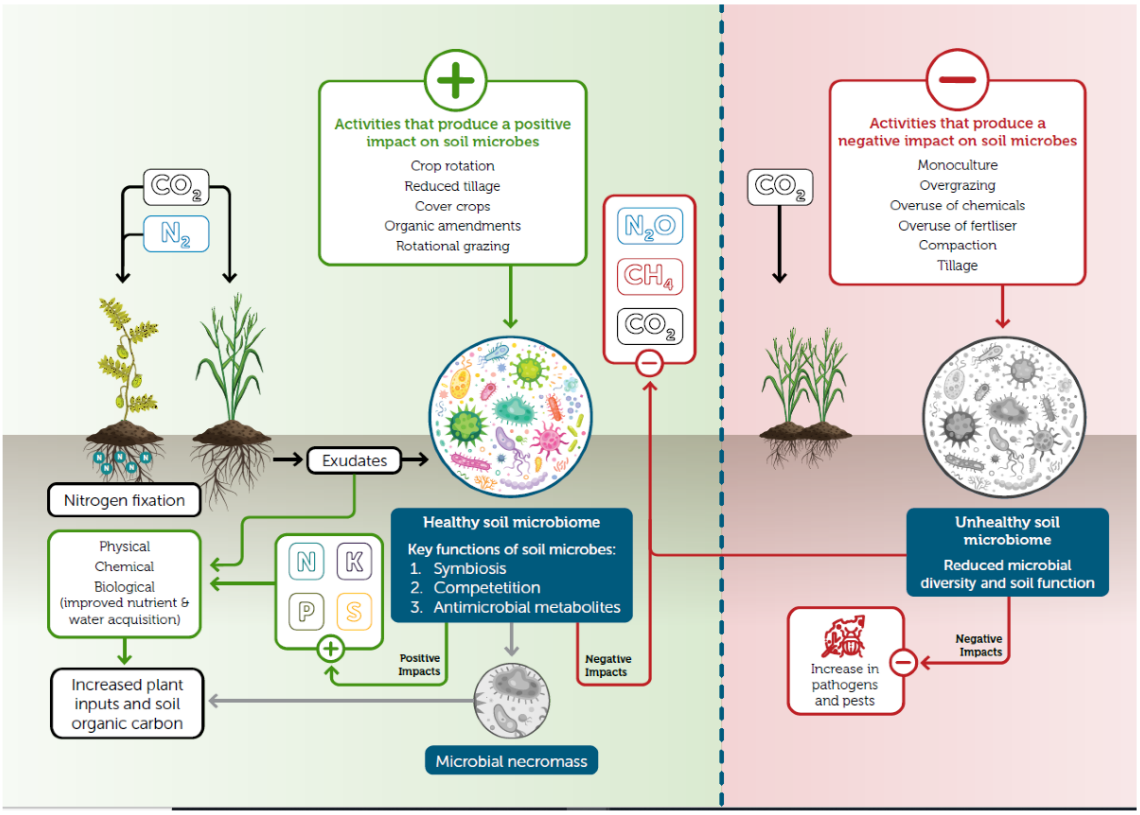


Figure 1. Plant, environment, microbe, and management interactions impacting soil health. Reprinted from "Regenerative Agriculture--A Literature Review on the Practices and Mechanisms Used to Improve Soil Health" by Khangura et al., 2023, *Sustainability*, *15*, p. 24. Copyright by R. Kangura, D. Ferris, C. Wagg, and J. Bowyer.

Soil microorganisms are responsible for important ecosystem functions such as nutrient cycling, carbon sequestration, and disease suppression although the specifics of this complex biological soil fertility are still being explored and are currently under-studied (Johns, 2017). Of the types of soil microbes, mycorrhizae are known to colonize roots and bring soil nutrients to the plant in exchange for plant carbon. Soil bacteria are

decomposers of organic waste that release nutrients into usable forms for plants. The addition of microbes to growth media has been demonstrated to improve germination and growth factors for vegetables (Ahirwar & Hussain, 2015; Atiyeh et al., 2000; Dhanalakshmi et al., 2014; Edwards et al., 2006; Karmegam & Daniel, 2008; Lazcano et al., 2010; Paul & Metzger, 2005; Sallaku et al., 2009) and for cotton (Paul et al., 2011) but the same has not yet been determined for *Cannabis sativa*.

With the limited cultivation-based, peer-reviewed literature available to hemp growers, research on best practices in seed germination is likely to benefit this young industry. By investigating the effects of regenerative-minded microbial seed inoculants on germination rate and growth parameters of hemp, we can better understand early cultivation methods which may affect hemp growth and production.

**Purpose of the Study**

The purpose of this study was to determine if inoculation with beneficial microorganisms, including mycorrhizae and/or vermiculture, affects hemp seed germination, vegetative plant growth, cured flower weight, and cannabinoid content.

**Research Objectives**

*Phase I*

1. Determine if the addition of a seed treatment of beneficial microorganisms, including mycorrhizae and/or vermiculture affects hemp germination rate or time to germination.
2. Determine if the addition of beneficial microorganisms, including mycorrhizae and/or vermiculture to hemp seeds affects early vegetative growth.

*Phase II*

3. Determine if seed inoculation with beneficial microorganisms, including mycorrhizae and/or vermiculture, affects cannabinoid content of cannabis flower, post-harvest.
4. Determine if seed inoculation with beneficial microorganisms, including mycorrhizae and/or vermiculture, affects weight of harvested and cured CBD flower.

**Significance**

By conducting research on cultivation practices for hemp production, we can contribute to the limited repository of research available on hemp cultivation, which would provide foundational knowledge benefitting hemp producers.

**Research Limitations**

Since hemp cultivation was federally legalized in the 2018 Farm Bill, foundational research was limited. Due to the lack of evidenced-based literature, cultivation practices were largely informed by individuals who grew cannabis in its high-THC form in other states where it is legal or in places where it is still illegal and part of the illegal market. Given the two-year timeframe of this study, a greater number of cultivars, site locations, and experiment treatments was not practical. Infrastructure limitations were also inherent due to ongoing and delayed construction in the building where Phase I of the study took place.

This research was conducted in a dry laboratory space that was adapted to use for growing hemp plants and was not ideal for this study as certain environmental factors (i.e., temperature, relative humidity) could not be fully controlled. Further, the transition to the second phase of this study was delayed due to infrastructure construction (i.e., new

bed preparation and irrigation systems), translating to a longer than necessary vegetative period. This delay in transition to Phase II could have impacted plant height data as the plants continued to grow vegetatively indoors under the 18 h artificial light cycle.

**Keywords**

For the purpose of the study, the following terms are defined:

*Cannabinoid*: Any chemical substance that joins with the receptors of the endocannabinoid system, referring, in this study, to the phytocannabinoids naturally present in *Cannabis sativa* plants.

*THC*: Delta-9-tetrahydrocannabinol, the psychoactive cannabinoid found in *Cannabis sativa* plants which remains a Schedule I drug at the federal level when greater than 0.3% by dry weight.

*CBD*: Cannabidiol, the non-psychoactive cannabinoid found in *Cannabis sativa* which has been legalized and is the main sellable component of hemp flower and hemp products.

*Cannabis*: Plants of the *Cannabis sativa* species, either hemp or marijuana varietals.

*Cured flower*: After harvest, cannabis flower is partially dried, trimmed of excess plant material like fan leaves and stems, and then aged in a closed container to maintain a desired moisture level and to allow for the optimal level of cannabinoid.

## II. LITERATURE REVIEW

The focus of this study explores whether a mycorrhizal and/or vermiculture inoculation of hemp seed improves germination rate and impacts young plant growth. The focus on soil health through the fostering of soil microbes (e.g., mycorrhizae and rhizobacteria), and use of biofertilizers (e.g., vermiculture) is a critical aspect of regenerative agriculture, as the microbes involved facilitate important ecosystem functions like nutrient cycling and disease suppression (Johns, 2017). The addition of microbes has been demonstrated to improve growth factors for vegetables, wheat, and cotton (Ahirwar & Hussain, 2015; Atiyeh et al., 2000; Choi et al., 2016; Colla et al., 2015; Dal Cortivo et al., 2018; Dhanalakshmi et al., 2014; Edwards et al., 2006; Gholami et al., 2009; Karmegam & Daniel, 2008; Lazcano et al., 2010; Paul & Metzger, 2005; Paul et al., 2011; Sallaku et al., 2009), as summarized in Table 1. The effect of microbial seed inoculations on the germination and growth of *Cannabis sativa* has yet to be determined, highlighting the need for more research in this area.

Soil microbes have been used extensively in crop production since their discovery in the late 18$^{th}$ century. Rhizosphere microbial activity is known to be a major factor in determining nutrient availability for plants, and has consequential influence on plant productivity and health (Jeffries et al., 2003). Beneficial soil microbes, like plant growth-promoting rhizobacteria, provide a direct effect on plant growth by competing with root pathogens for nutrients and space in the rhizosphere, and through production of phytohormones that promote root hair growth (Podile & Kishore, 2006). Enhanced plant growth through the actions of these microbes is quantified as “an increase in seedling emergence, vigor, biomass, proliferation of root system and yield in various plant

species" (Podile & Kishore, 2006, p. 195). Seed inoculation of beneficial microorganisms is the most common method of inoculation compared to foliar and soil inoculation (Souza et al., 2015; Arora et al., 2020). The benefit to seed inoculation is that the introduced organisms colonize seedling roots as soon as they emerge, thus improving plant nutritional availability.

Table 1. Summary of crops with improved germination or growth parameters due to the addition of microbes.

| Plant Species | Microbial Application | Improvements Observed | Reference |
|---|---|---|---|
| Cucumber | Vermicompost soil amendment | Growth parameters*; Relative growth rate of seedlings | Sallaku et al., 2009 |
| Cotton | Mycorrhizae, bacteria | Germination rate<br>Seedling development | Paul et al., 2011 |
| Hyacinth bean | Vermicompost soil amendment | Growth & yield parameters** | Karmegam & Daniel, 2008 |
| Lettuce, Tomato | Vermicompost tea | Germination rate | Arancon et al., 2012 |
| Maize | Rhizobacteria | Germination rate<br>Seedling vigor | Gholami et al., 2009 |
| Maritime pine | Vermicompost soil amendment & vermicompost tea | Germination rate<br>N content | Lazcano et al., 2010 |
| Okra, brinjal, chilli | Vermicompost soil amendment | Germination rate<br>Root & shoot length | Dhanalakshmi et al., 2014 |
| Orchids | Mycorrhizal fungi | Protocorm formation<br>Seedling growth | Shao et al., 2020 |
| Rice | Rhizobacteria | Germination rate, growth parameters, nutrient content*** | Choi et al., 2016 |
| Sunflower | Rhizobacteria | Germination rate<br>Biochemical profile | Den et al., 2021 |
| Wheat | Endophytic fungi | Seedling growth<br>Yield & protein content | Colla et al., 2015 |

* Dry matter per plant, relative leaf expansion rate
** Dry matter production, length of fruits, fruit yield (fresh weight)
*** Fresh and dry weight, grain yield, P, K, and N content of grain

**Hemp Seed Germination**

Seed germination initiates with seed uptake of water, terminates with the emergence of the radicle through the seed's exterior layers, and is fundamental to quality and yield of a crop (Tuan et al., 2019). Early seedling growth is also crucial to ascertain the vigor of young hemp growth based on size, growth rate, and overall health.

To date, there are no published studies of hemp seeds inoculated with beneficial microorganisms. Thus, the studies that included pre-treatments of hemp seeds, either with compounds, phytohormones, or environmental factors, are detailed below. A 2021 study in Australia demonstrated that hemp seed germination rates are negatively impacted by seed pre-treatments of gibberellic acid, chlorine dioxide, and cold temperature with some positive effect on early growth parameters of surviving seedlings (Islam et al., 2021). In this study, hemp seed germination rate varied greatly in both Petri dish assay and glasshouse environments from 17% to 70% and 13% to 92%, respectively (Islam et al., 2021). Pre-treatment of hemp seed with 500 $mg \cdot L^{-1}$ of gibberellic acid demonstrated a nonsignificant decrease in germination rate but a significant increase in seedling vigor index for surviving seedlings (Islam et al., 2021), indicating that pre-treatment of seeds likely affects both germination rate and early seedling growth and vigor.

Seed germination rate for cannabis is affected by seed size, water and ethanol infiltration, inclusion of sand with seed at a 3:1 ratio (Moon et al., 2020), and by "scuffing" seeds with sandpaper prior to planting (Green et al., 2001). Germination rate can be improved by mixing sand with seed at a 3:1 ratio prior to germination in a low-density sprouting situation; specifically, germination rate was 19.9% without sand and

58.7% with sand (Moon et al., 2020). A recent study by Hesami et al. (2021) demonstrated that a moderate inclusion of salt in the growth medium, combined with a moderate supplementation of carbohydrates in the form of sucrose, could bolster the seed germination of hemp in an *in vitro* setting. Germinating hemp seed in an *in vitro* lab setting is appropriate for scientific research, but is unlikely to be utilized by hemp producers as most farmers do not have access to such technologies. Most sources do not state a general germination rate for *Cannabis sativa* seeds, likely because this varies by strain and growing condition.

**Mycorrhizae**

Mycorrhizae are phylogenetically ancient fungi that form symbiotic relationships with nearly all terrestrial species of plants. The use of mycorrhizal fungi in terrestrial applications is explored at length in the book *Mycorrhizal Fungi: Use in Sustainable Agriculture and Land Restoration* (Solaiman & Mickan, 2014) with rhetoric on alleviation of plant water stress, soil remediation, plant health improvement, and contributions to soil carbon sequestration (Solaiman & Mickan, 2014). Alori et al. (2017) notes that mycorrhizal fungi "can be used as biofertilizer to improve soil nitrogen, phosphorus and potassium availability and uptake" (p. 281).

Literature available on mycorrhizal interaction with cannabis seed germination is minimal. A recent study by Kakabouki et al. (2021) determined that inclusion of a type of arbuscular mycorrhiza fungus, *Rhizophagus irregularis*, in high doses increased length, dry biomass, and survival of float system-produced cannabis seedling root. While the method of interaction for each species of mycorrhiza may not be fully elucidated, it is clear these fungi synergistically interact with the root zones of terrestrial plants to provide

structures and products plants utilize naturally and readily (Koide & Mosse, 2004).

Rhizobacteria have been demonstrated to be an effective clone inoculant in a recent study which explored the application of three types of plant growth-promoting rhizobacteria (i.e., *Bacillus* sp., *Pseudomonas* sp., and *Mucilaginibacter* sp.) and their effect on flower yield, physiology, and morphological development of *Cannabis sativa* L. (Lyu et al., 2022). Inoculation of rhizobacteria on hemp clones increased fresh flower weight at harvest by 5.13%-11.45% but did not affect plant height, leaf area, branch number, or node number (Lyu et al., 2022). The link between mycorrhizae and cannabinoid content and improved hemp growth parameters beyond flower weight at harvest is yet to be explored.

Although studies exploring mycorrhizae in hemp production are scant, research investigating other crops shows positive results. Recent literature demonstrates potential for mycorrhizae to play an important role in improving seed germination rates, including for wheat and fava beans (Raklami et al., 2019), as well as orchids (Sebastián et al., 2014; Herrera et al., 2017; Alghamdi, 2019; Shao et al., 2020; Tsulsiyah et al., 2021). Raklami et al. (2019) demonstrated improved growth for fava bean and wheat plants inoculated with both rhizobacteria and mycorrhizae, with an increase of 130-293% in shoot dry weight and an increase of 200-258% in root dry weight. They also determined microbe-inoculated plants had improved shoot content of sugar, proteins, N, P, Ca, K, and Na compared to the control (Raklami et al., 2019).

**Vermiculture**

Vermiculture, the cultivation of annelid worms, relies on earthworms as the primary macrophage in the composting process (Teršič & Gosar, 2012), and

microorganisms to digest organic materials and transform them into valuable soil amendments and plant-available nutrients. Vermicompost refers to soil-like worm castings, and vermicompost tea is the liquid infusion of vermicompost in water. A review on vermicompost highlighted how the earthworm enzymes continue to digest organic matter after expulsion from the worms (Olle, 2019).

While the effects of vermiculture inoculation on hemp seed germination has not yet been investigated, there was a recent study that demonstrated benefits for chlorophyll concentration in plant leaves, as well as hemp stem and seed yield, in accordance with increasing doses of vermicompost application (Stramkale et al., 2021). These researchers applied organic vermicompost or an equivalent amount of mineral nutrients twice to field-grown, oilseed variety hemp plants and compared results to a non-fertilized control group. While the plants in this study were started from seed, the vermicompost was not added as a seed inoculant but rather as a soil amendment (Stramkale et al., 2021). These findings are encouraging for hemp producers interested in replacing synthetic fertilizers with environmentally friendly and organic alternatives.

Studies exploring vermicompost use in hemp production do not exist to date; thus, the rest of this section includes results of vermicompost use in a variety of crops which could provide general insight into hemp production. Arancon et al. (2012) soaked tomato and lettuce seeds in a pure water control and increasing concentrations of vermicompost tea up to 10%; they observed a linear increase in seed germination rate as tea concentration increased. Further, the length of time tomato seeds were soaked in the water and vermicompost tea mix positively impacted germination rate of tomato seeds (Arancon et al., 2012). These findings suggest that the presence of bioactive, water-

extractable compounds like humic acids and other vermicompost-inherent substances could be responsible for improved germination of the tomato seeds (Arancon et al., 2012).

Addition of low concentrations of vermicompost to growth media has demonstrated an increase in germination rate and improvement in growth parameters for marigold, lettuce, and tomato (Atiyeh et al., 2000), hyacinth bean (Karmegam & Daniel, 2008), and cucumber (Sallaku et al., 2009), while okra and brinjal seeds were demonstrated to have both improved germination rate and significantly earlier germination (Dhanalakshmi et al., 2014). However, these studies did not explore vermicompost as a seed inoculant.

Vermicompost as an infusion for watering at a 5-20% concentration has been demonstrated to improve bean and pea seedling growth but negative effects on root length and leaf fresh mass were observed beyond 20% vermicompost infusion (Ievinsh, 2011). A similar study demonstrated a 10% improvement in maritime pine seed germination with inclusion of vermicompost in the growth media, and a 16% increase in germination rate when a vermicompost tea was used to water the seeds after planting (Lazcano et al., 2010). A similar study also found germination rate of upland cress, radish, and marigold to be generally improved by vermicompost tea, but generally impaired by inclusion of vermicompost in the growth media (Warman & AngLopez, 2010).

**General Growth Factors for *Cannabis sativa* L.**

When grown in an indoor space, the growth stages of cannabis are adjustable based on photoperiod, among other environmental factors, although the full extent of this

adjustability is still being explored in the literature. The growth stages of cannabis are summarized in Appendix C. Much of the written knowledge on cannabis cultivation reflects industry standards and is captured in books such as *The Cannabis Grow Bible* (Green et al., 2001), *The Cannabis Grow Bible* (Zetta & Paull, 2020), and *Marijuana Horticulture: The Indoor/Outdoor Medical Grower's Bible* (Cervantes, 2006). However, recent literature has begun to detail research on basic cannabis growing practices: i.e., the effectiveness of topping and pruning (Bozzolo & Gonzales-Siemens, 2021), use of LED lights to change cannabinoid profiles (Magagnini et al., 2018), and use of drought stress to increase harvested flower weight and cannabinoid content (Caplan et al., 2019).

Cannabis morphology and cannabinoid profile depend on both genetics and environmental factors. Cannabis, as a species, is nitrophilic (Small, 2015) and generally tolerant of soil pH from 6-7 but, like many plants, is believed to have an optimal pH range of 5.5-6.5 for nutrient uptake (Zetta & Paull, 2020). Similarly, optimal availability of 12 plant nutrients in organic soils has been demonstrated to be in this same range, landing between pH 5.5 and 5.8 (Lucas & Davis, 1961). No recent literature was found to directly demonstrate that hemp optimally benefits from nutrient availability within the proposed pH range of 5.5 and 6.5 by industry standard (Green et al., 2001; Zetta & Paull, 2020).

## III. METHODOLOGY

The purpose of this research was to determine if inoculation with mycorrhizae or vermiculture affected germination rate, early plant growth, flower cannabinoid content, and/or cured flower weight of hemp cultivars of the plant species *Cannabis sativa* L. Most of the current literature does not include research on basic hemp cultivation practices.

The research objectives of this study were to:

*Phase I*

1. Determine if the addition of a mycorrhizae mix or vermiculture to hemp seeds affected germination.
2. Determine if the addition of a mycorrhizae mix or vermiculture to hemp seeds affected early vegetative growth.

*Phase II*

3. Determine if the seed inoculation of mycorrhizae and beneficial microorganisms or vermiculture affected cannabinoid content of cannabis flower, post-harvest.
4. Determine if the seed inoculation of mycorrhizae and beneficial microorganisms or vermiculture affected weight of harvested and cured CBD flower.

Given the limited legality of hemp cultivation in the state of Texas, a hemp research license was acquired through the Texas Hemp Program under the Texas Department of Agriculture (TDA) with TDA License No.: 0874133. TDA compliance

testing was not required due to the plant material remaining on university property through disposal via compost.

**Study Design**

*Phase I*

This study was conducted in a climate-controlled, indoor laboratory at the Department of Agricultural Sciences, Texas State University (San Marcos, Texas). Officially certified hemp seeds were sourced from Ventura Seed Company because they have partnered with the Rodale Institute to perform past and ongoing hemp research (Bozzolo & Gonzales-Siemens, 2021). The varietal chosen for this trial was Cherry Soda because of its early finish time (September) and its vigor and disease resistance (Ventura Seed Company, n.d.). Growth medium for the seed germination trial, Pro-Mix, remained the same for all treatments and the control as it is commercially available and is acceptable media for seed starting. Commercially available Great White Premium Mycorrhizae (GW), which contains three species groups including nine types of endomycorrhiza, seven types of ectomycorrhiza, and 15 types of other beneficial bacteria and fungi (Table 2), and locally sourced vermicompost (V) from Texas Worm Ranch were used as seed inoculants.

A total of 288 seeds were germinated in plug trays, with a sample size of 24 for each of the three treatments and one control, with three replications of all treatments. The control group was planted directly into 100% Pro-Mix growth medium with no seed inoculant and watered with de-chlorinated, municipal water (C). Treatments were: 1) a seed inoculation of Great White (GW), 2) a seed inoculation of vermicompost

(V), and 3) seed inoculation of GW and vermicompost (GW+V) with concentrations of inoculation solutions shown in Table 3.

Table 2. Great White Premium Mycorrhizae ingredient list.

| **Ectomycorrhiza spp.** | **propagules/gram** | **Other fungal/bacterial spp.** | **CFU's/gram** |
|---|---|---|---|
| *Pisolithus tinctorius* | 187,875 | *Azotobacter chroococcum* | 525,000 |
| *Rhizopogon luteolus* | 5,219 | *Bacillus subtilis* | 525,000 |
| *Rhizopogon fulvigleba* | 5,219 | *Bacillus thuringiensis* | 525,000 |
| *Rhizopogon villosullus* | 5,219 | *Bacillus licheniformis* | 525,000 |
| *Rhizopogon amylopogon* | 5,219 | *Bacillus azotoformans* | 525,000 |
| *Scleroderma citrinum* | 5,219 | *Bacillus megaterium* | 525,000 |
| *Scleroderma cepa* | 5,219 | *Bacillus coagulans* | 525,000 |
| **Endomycorrhiza spp.** | **propagules/gram** | *Bacillus pumilus* | 525,000 |
| *Glomus aggregatum* | 83 | *Bacillus amyloliquefaciens* | 525,000 |
| *Glomus intraradices* | 83 | *Paenibacillus polymyxa* | 525,000 |
| *Glomus mosseae* | 83 | *Paenibacillus durum* | 525,000 |
| *Glomus etunicatum* | 83 | *Saccharomyces cerevisiae* | 525,000 |
| *Glomus clarum* | 11 | *Pseudomonas aurofaciens* | 525,000 |
| *Glomus monosporum* | 11 | *Pseudomonas fluorescens* | 525,000 |
| *Glomus deserticola* | 11 | **Biocontrol spp.** | **propagules/gram** |
| *Paraglomus brasilianum* | 11 | *Trichoderma koningii* | 187,875 |
| *Gigaspora margarita* | 11 | *Trichoderma harzianum* | 125,250 |

Table 3. Seed inoculation solution summary by treatment group.

| **Treatment** | **Inoculant Solution** |
|---|---|
| Control (C) | Water |
| Great White (GW) | 1/8 teaspoon per 1 gallon of water |
| Vermicompost (V) | 1 teaspoon per 1 gallon of water |
| Great White & Vermicompost (GW+V) | 1/16 teaspoon GW + 1/2 teaspoon V in 1 gallon of water |

The seeds were watered with de-chlorinated, pH-balanced water as needed during the germination phase and into the seedling phase for all treatments. Water was de-chlorinated in an open-air container with an air stone for a minimum of 24 h and was pH-balanced with pH Up and pH Down products from General Hydroponics. All buckets

and seed trays were disinfected with 91% isopropyl alcohol prior to use. To ensure an 18-hour vegetative photoperiod and prevent premature flowering, full-spectrum LED grow lights from the manufacturer SOEVSI were installed along with an automatic timer. These lights included 5000K, 3000K, 760nm, and 660nm diodes that provided blue, warm white, infrared, and red wavelengths, respectively. Seed germination was expected to be complete with all viable seeds producing seedlings by the end of d 14. Seedlings were then allowed to continue growing in a vegetative state until d 54. Figure 2 depicts

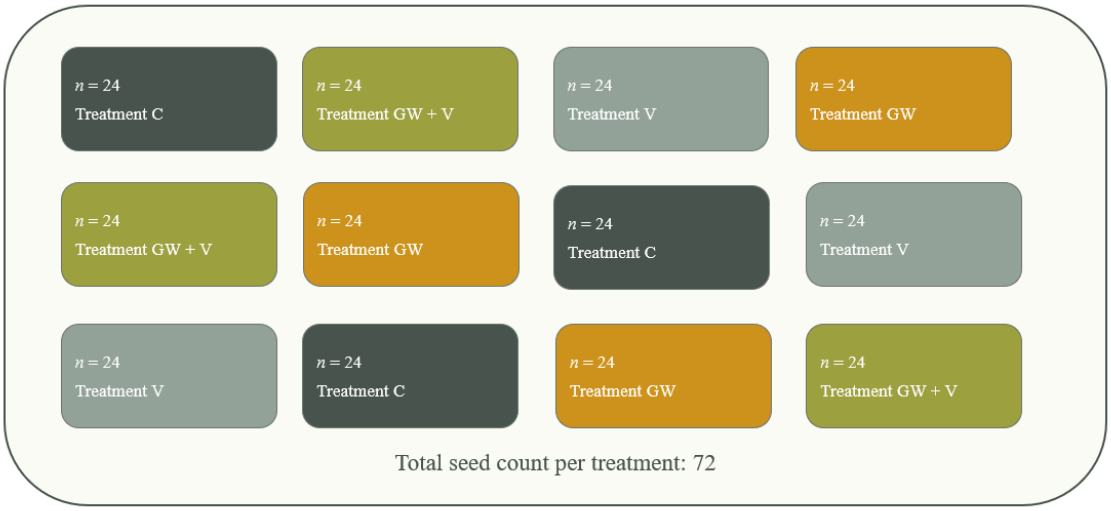


Figure 2. Study design, phase I. C = control, GW = mycorrhizae treatment, V = vermiculture treatment, GW+V = combined treatment of mycorrhizae and vermiculture.

the randomized block design for Phase I seed trays with each replication consisting of 24 seeds per treatment and three replications of the germination trial to ensure proper randomization.

*Phase II*

Thirty plants from each treatment were transplanted into 15.25 m hügelkultur beds (Figure 3) that were homogenously prepared with Suståne 8-2-4 organic slow-release fertilizer incorporated into the top 10 cm of soil at 3.4 kg per 9.29 $m^2$. Plants

were hand-watered after transplanting to ensure sufficient water penetration into the garden beds and then placed on a timed drip hose that released 4 h of water every day for the first three weeks. Drip watering was then reduced to 4 h of water every third day for the remainder of the growth cycle.

Harvest began on d 127 (September 13, 2022), with selection and harvesting of the 12 most mature plants from each treatment group. Plants have reached peak maturity when flower pistils have turned from white to brownish-red and when resin glands on the surface of the flowers have transitioned from clear to milky-white (Cervantes, 2006). These plants were then processed by hanging indoors in a climate-controlled facility to dry for a week (mean temperature of 28.2°C, mean relative humidity of 40.6%). After drying, flower buds were removed from stems and placed in plastic bins to finish curing, following industry standard (Green et al., 2001; Zetta & Paull, 2020).

At the end of the curing stage, weights were taken for the 12 plants from each treatment group and 7-gram composite samples of flower from each treatment were placed in individual plastic bags for cannabinoid testing at Ionization Labs in Austin, Texas.

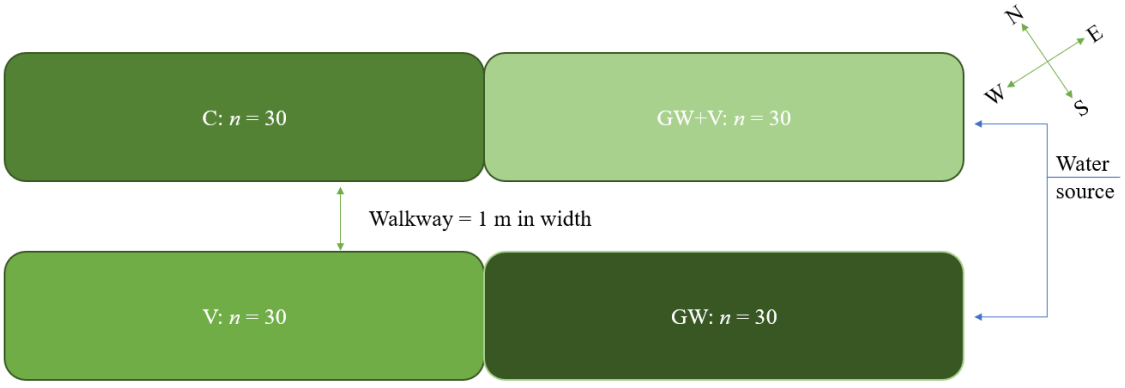


Figure 3. Field design, phase II. C = control, GW = mycorrhizae treatment, V = vermiculture treatment, GW+V = combined treatment of mycorrhizae and vermiculture.

**Data Collection**

Data collection in the 14-d after planting included number of days from seeding to germination, overall germination percentage, environmental temperature, and humidity. Seed germination indices used for evaluating hemp seed germination were adopted directly from Hesami et al. (2021).

1. Germination rate (GR) was computed using the following equation.

   GR = $\frac{G}{n} \times 100$ where G is the number of germinated seeds by d 14 and *n* is the total number of cultivated seeds.

2. A germination index (GI) was calculated based on the following equation.

   GI = $\left(\frac{Number\ of\ germinated\ seeds}{days\ of\ first\ count}\right) + \cdots + \left(\frac{Number\ of\ germinated\ seeds}{days\ of\ last\ count}\right)$

   Seed germination index is a measure of the speed and uniformity of seed germination under controlled conditions. It is a statistical method that is commonly used to quantify the percentage of seeds that germinate within a certain period of time.

*3.* Mean germination time (MGT) was computed based on the following equation.

   MGT = $\sum fd/\mathrm{f}$ where *f* is the number of germinated seeds on day *d*.

   Mean germination time is a measure of the average time required for seeds to germinate under specific conditions.

Plants were transplanted from their germination cells into 10 cm nursery pots after 28 d to continue vegetative growth. Data collection for vegetative growth was completed on d 54, and plants were transported to the outdoor field location for Phase II.

## Data Analysis Overview

Excel for Windows was used to analyze data. Measures of central tendency were calculated, and a single-factor ANOVA test was utilized to determine if the difference in results between treatment groups was significant ($\alpha = 0.05$).

## IV. FINDINGS AND DISCUSSION

Results are organized based on the study's objectives to: 1) determine if the addition of mycorrhizae or vermiculture to hemp seeds affected germination, 2) determine if the addition of mycorrhizae or vermiculture to hemp seeds affected early plant growth, 3) determine if the addition of mycorrhizae or vermiculture to hemp seeds affected flower cannabinoid content, post-harvest, and 4) determine if the seed inoculation of mycorrhizae or vermiculture affected weight of harvested and cured CBD flower.

### Findings Related to Objective 1: Effect on Germination

Hemp seeds were considered germinated upon emergence of the cotyledons and germination was recorded every morning for 14 d. No germinations were observed past d 7 for any treatments. The germination indices are summarized in Table 4. The germination rates are summarized in Figure 4.

Table 4. Summary of germination indices based on $\alpha$=0.05.

| Treatment Group | GR | GI | MGT |
|---|---|---|---|
| Control (C) | 90.28% | 3.51 | 3.89 |
| Great White (GW) | 87.50% | 3.38 | 3.86 |
| Vermiculture (V) | 87.50% | 3.33 | 3.81 |
| GW + V | 86.11% | 3.31 | 3.84 |

C = control, GW = mycorrhizae treatment, V = vermiculture treatment, GW+V = combined treatment of mycorrhizae and vermiculture.

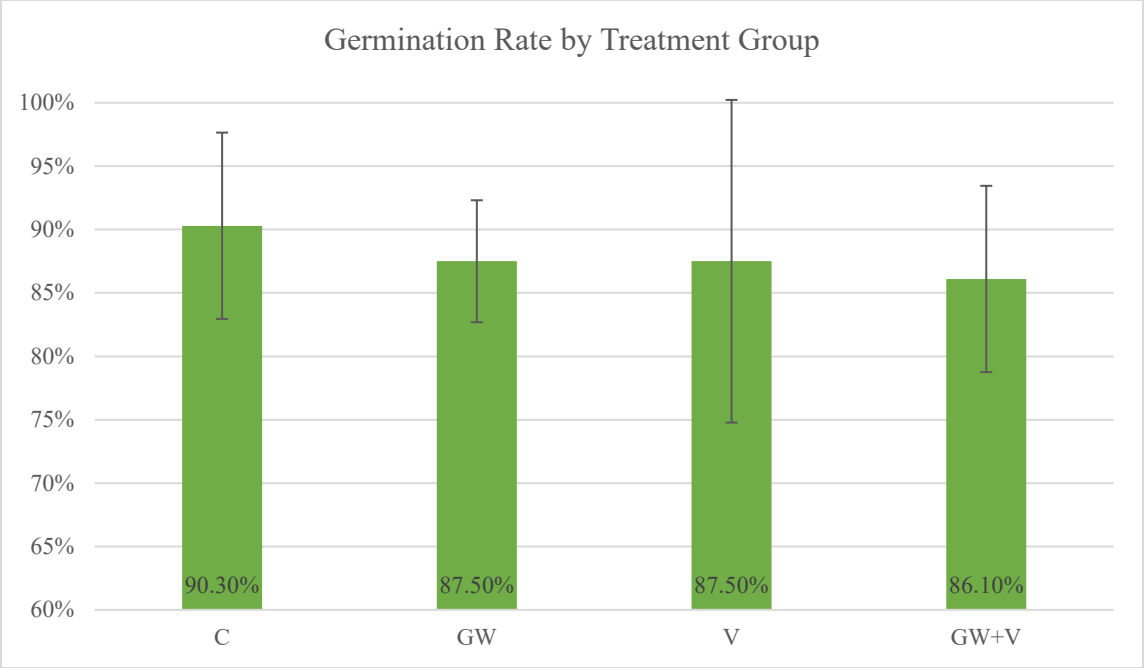


Figure 4. Germination rate by treatment group. C = control, GW = mycorrhizae treatment, V = vermiculture treatment, GW+V = combined treatment of mycorrhizae and vermiculture. ($\alpha = 0.05$; $P = 0.92$)

The germination rates across treatments were found to have a P-value of 0.92 when analyzed by a single-factor ANOVA statistical test such that the null hypothesis was not rejected. The germination rates were found to be independent of seed inoculation treatments and were an average of 87.85% across groups. Germination index results demonstrated the control group to be slightly better performing with respect to rate and uniformity of seed germination, although the difference was not statistically significant compared to the three treatments ($M = 3.38$; $SD = 0.09$). Mean time to germination was also similar among treatments with the vermiculture group taking slightly less average time to germinate, and the control group taking the longest average time to germinate. However, the differences were minute, and all treatments had a mean germination slightly below 4 days ($M = 3.85$; $SD = 0.03$). The full array of time to germination data is summarized in Figure 5. No seeds germinated on the first or second

day of the trial nor after the seventh day of the trial. Of 288 seeds, 35 seeds across all treatments never germinated, ranging from seven seeds in the control group to 10 seeds in the GW+V group ($M = 8.75$; $SD = 1.26$).

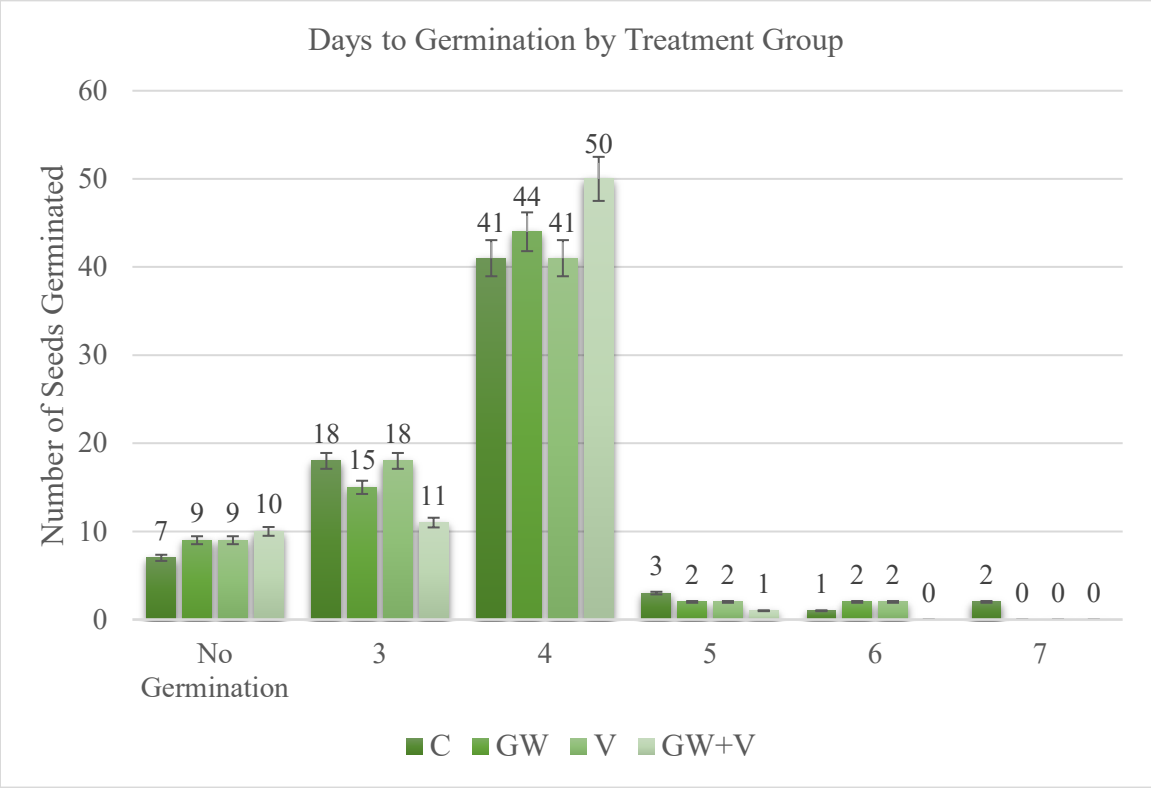


Figure 5. Days to germination by treatment group. C = control, GW = mycorrhizae treatment, V = vermiculture treatment, GW+V = combined treatment of mycorrhizae and vermiculture.

**Findings Related to Objective 2: Effect on Early Growth**

The second objective of this research was to identify any significant effect on early vegetative growth due to seed inoculation. A single-factor ANOVA test demonstrated no statistically significant difference in plant height by treatment group ($P = 0.55$). The range, mean, and standard deviation of plant heights by treatment group are summarized in Table 5. The height of all plants at the transition to Phase II are shown in

Figure 6, from shortest to tallest. The normal distribution of plant height by treatment is represented in Figure 7.

Table 5. Summary of height data by treatment group.

| | C | GW | V | GW+V |
|---|---|---|---|---|
| Range | 30.0 - 95.5 | 34.5 - 87.5 | 34.5 - 89.5 | 27.0 - 90.5 |
| Mean | 68.10 | 67.83 | 65.56 | 62.81 |
| SD | 17.50 | 14.96 | 18.93 | 18.73 |

Units = cm. $P = 0.55$. C = control, GW = mycorrhizae treatment, V = vermiculture treatment, GW+V = combined treatment of mycorrhizae and vermiculture.

Thirty-five plants from each of the four groups were measured for vegetative plant heights prior to a final cull of plants to $n$=30 for each treatment before transplanting. The control and GW groups had a section of heights which were taller than the corresponding plants in the vermiculture and GW+V groups, but the difference was not found to be significant ($P = 0.55$).

Mean plant height across treatments was 66.07 cm with the C group having the tallest mean plant height (68.10 cm) and the GW+V group having the shortest mean plant height (62.81 cm). The GW treatment group had the lowest standard deviation of all groups ($SD = 14.96$), meaning the heights in the GW group were clustered more closely around the mean height, while the other three groups had heights spread further from the mean height ($SD = 17.50 – 18.93$).

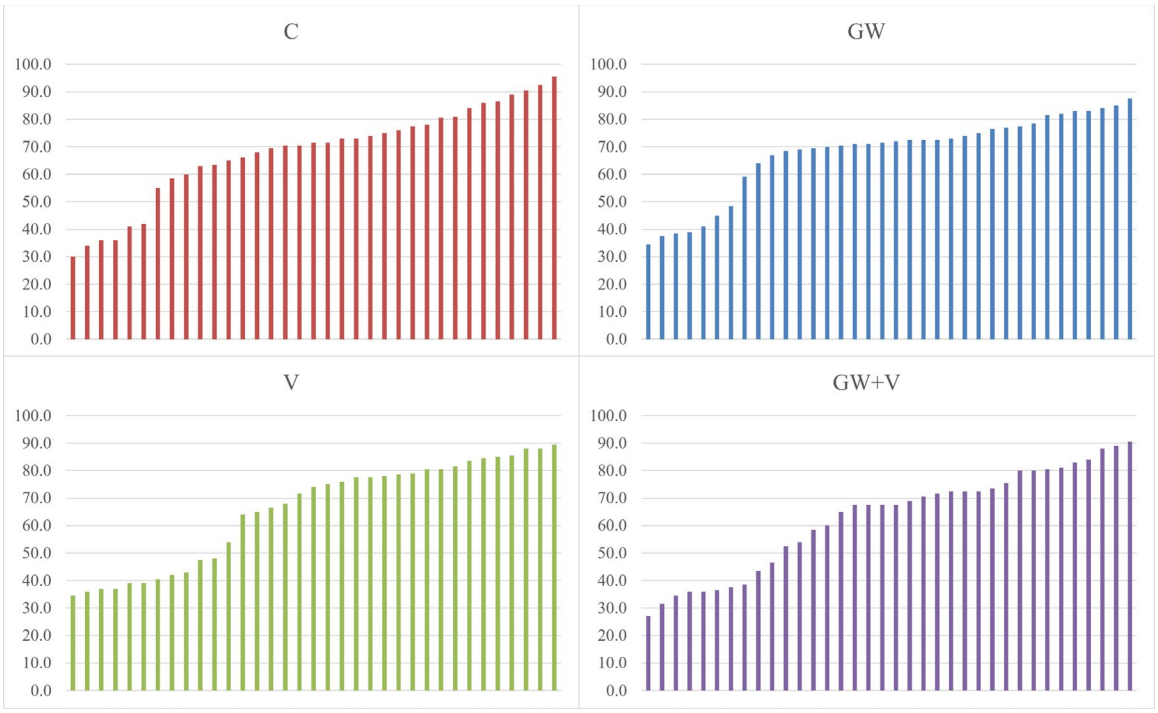


Figure 6. Vegetative plant height by treatment group. Measured in cm. C = control, GW = mycorrhizae treatment, V = vermiculture treatment, GW+V = combined treatment of mycorrhizae and vermiculture.

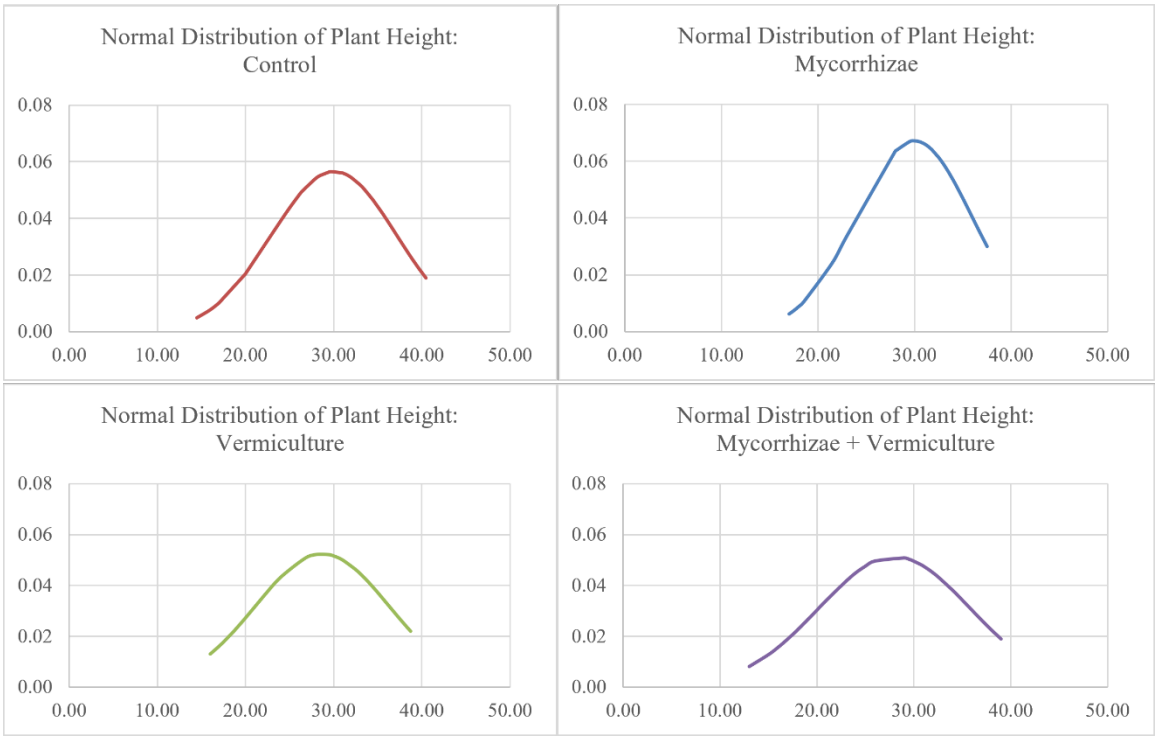


Figure 7. Normal distributions of plant heights by treatment group.

When examining the normal distribution of plant heights by treatment, it was noted that both groups containing vermiculture appeared to have a more balanced distribution compared to the C and GW treatments, which both favored the left side of the graph.

**Findings Related to Objective 3: Effect on Cannabinoid Content**

Cannabinoid profiles of cured flower by treatment group are summarized in Table 6. The difference in cannabinoid content between groups was not statistically significant ($P = 0.19$). THCD9, the compound illegal at the federal level, was under the 0.3% limit in all groups with the highest level in the GW+V group at 0.15%.

Table 6. Cannabinoid content of post-harvest flower by treatment group. C = control, GW = mycorrhizae treatment, V = vermiculture treatment, GW+V = combined treatment of mycorrhizae and vermiculture.

| | C | GW | V | GW+V |
|---|---|---|---|---|
| CBDV | 0% | 0% | 0% | 0% |
| CBDVA | 0.03% | 0.04% | <LOQ | 0.06% |
| THCV | 0% | 0% | 0% | 0% |
| CBD | 0.92% | 1.01% | 1.01% | 1.34% |
| CBG | 0.09% | 0.11% | 0.10% | 0.13% |
| CBDA | 11.09% | 10.78% | 12.46% | 12.91% |
| CBGA | 0.19% | 0.17% | 0.24% | 0.29% |
| CBN | 0% | 0% | 0% | 0% |
| THCD9 | 0.12% | 0.12% | 0.12% | 0.15% |
| THCD8 | 0% | 0% | 0% | 0% |
| CBC | 0.07% | 0.07% | 0.07% | 0.09% |
| CBNA | 0.04% | <LOQ | <LOQ | <LOQ |
| THCA | 0.44% | 0.46% | 0.54% | 0.51% |
| CBCA | 0.45% | 0.47% | 0.56% | 0.50% |
| Total | 13.44% | 13.23% | 15.11% | 15.97% |
| Total THC | 0.51% | 0.52% | 0.60% | 0.60% |
| Total CBD | 10.65% | 10.47% | 11.94% | 12.67% |

<LOQ = less than a measurable amount was detected. C = control, GW = mycorrhizae treatment, V = vermiculture treatment, GW+V = combined treatment of mycorrhizae and vermiculture.

Total cannabinoid content in hemp leaves was significantly lower than the levels

found in cured flower ($P<0.01$) as well as total THC content ($P<0.01$) and total CBD content ($P<0.01$). Between treatment groups, the leaves had less variation than cured flower in total cannabinoid content ($SD = 0.0055$ versus 0.0132, leaves and flower respectively) and total CBD content ($SD = 0.0042$ versus 0.0105, leaves and flower respectively). The level of THCD9 in all leaf samples was a tenth of the legal allowable limit or less.

Table 7. Cannabinoid content of post-harvest leaves by treatment group. C = control, GW = mycorrhizae treatment, V = vermiculture treatment, GW+V = combined treatment of mycorrhizae and vermiculture.

| | C | GW | V | GW+V |
|---|---|---|---|---|
| CBDV | 0% | 0% | 0% | 0% |
| CBDVA | <LOQ | 0% | 0% | 0% |
| THCV | 0% | 0% | 0% | 0% |
| CBD | 0.22% | 0.36% | 0.33% | 0.25% |
| CBG | 0.07% | 0.09% | 0.10% | 0.07% |
| CBDA | 3.72% | 3.77% | 4.03% | 2.99% |
| CBGA | 0.06% | 0.05% | 0.06% | 0.05% |
| CBN | 0% | 0% | 0% | 0% |
| THCD9 | <LOQ | 0.04% | 0.03% | <LOQ |
| THCD8 | 0% | 0% | 0% | 0% |
| CBC | <LOQ | <LOQ | <LOQ | <LOQ |
| CBNA | 0% | 0% | 0% | 0% |
| THCA | 0.15% | 0.14% | 0.16% | 0.11% |
| CBCA | 0.12% | 0.13% | 0.14% | 0.10% |
| Total | 4.34% | 4.57% | 4.84% | 3.57% |
| Total THC | 0.13% | 0.16% | 0.17% | 0.10% |
| Total CBD | 3.49% | 3.67% | 3.86% | 2.88% |

<LOQ = less than a measurable amount was detected. C = control, GW = mycorrhizae treatment, V = vermiculture treatment, GW+V = combined treatment of mycorrhizae and vermiculture.

**Findings Related to Objective 4: Effect on Cured Flower Weight**

A single-factor ANOVA test of the cured flower weight between treatments lead the researcher to not be able to reject the null hypothesis and demonstrated no significant

difference in cured flower weight between treatment groups ($P = 0.71$).

The average total plant weight of the 12 harvested plants per treatment group was 811.40 grams while the average weight per plant was found to be 67.60 grams ($SD$ = 139.05 and 11.59, respectively). The control group was demonstrated to have the least amount of cured flower post-harvest at 681.20 total grams and 56.77 average grams per plant. The treatment group with the greatest amount of cured flower by weight was the GW+V group at a total of 974.30 grams of flower and an average of 81.19 grams of flower per plant. Even though this difference was not statistically significant, a 217.2-gram average difference in final product weight between adding an inoculation treatment and no inoculation *could* be of economic interest to growers depending on market price of CBD flower and size of harvest.

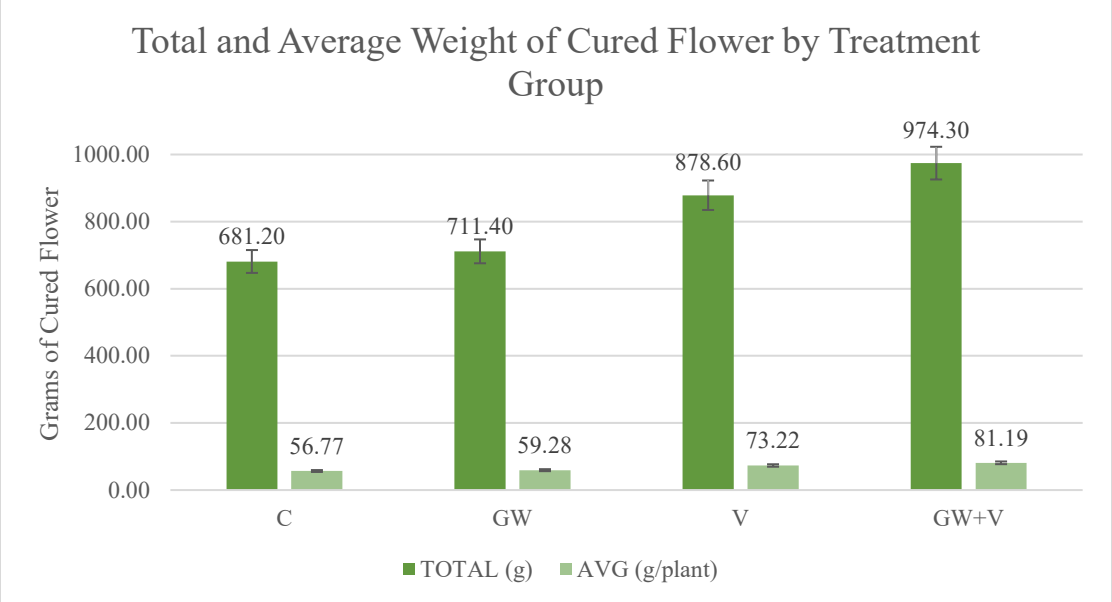


Figure 8. Summary of cured flower weight data by treatment group. AVG = average, C = control, GW = mycorrhizae treatment, V = vermiculture treatment, GW+V = combined treatment of mycorrhizae and vermiculture.

The treatment with the least difference in weight compared to the control group was the mycorrhizae group with a difference in total weight of just 30.20 grams and a

2.50-gram difference in average weight per plant. Both treatment groups containing vermiculture demonstrated to have between 197.40 and 293.10 grams more cured flower than the control group and between 167.20 and 262.90 grams more cured flower than the mycorrhizae-only group. Similarly, both treatment groups containing vermiculture measured between 16.45 and 24.43 grams per plant more than the control group and between 13.93 and 21.91 grams per plant more than the mycorrhizae-only group.

Using the control treatment as the baseline with the lowest overall and average cured flower weight, the mycorrhizae-only treatment had 4.43% more weight while the two vermiculture groups, vermiculture-only and mycorrhizae plus vermiculture, had 28.98% and 43.03% more weight, respectively. These results suggest that more research is needed to support whether both mycorrhizae and vermiculture inoculation can improve overall flower yield of hemp.

# V. SUMMARY, CONCLUSIONS, AND RECOMMENDATIONS

## Summary

The purpose of this research was to determine if inoculation with a mycorrhizae mix or vermiculture affects germination rate, early plant growth, flower cannabinoid content, or cured flower weight of hemp cultivars of the plant species *Cannabis sativa*. Most of the current literature does not include basic cultivation practices of hemp; accordingly, this study aimed to elucidate the effect of a single inoculation step in the cultivation process on several important growth parameters.

The addition of mycorrhizae or vermiculture seed inoculant did not demonstrate any statistically significant differences in any of the parameters measured in this study. Given previous studies that have shown significant differences of growth parameters due to microbial inoculation, as well as the minor (albeit non-significant) differences found in this study's results, future research is needed on this topic of growing interest in regenerative agriculture.

## Conclusions

### *Seed germination*

The average germination rate across treatments was 87.85% and there was no effect of treatment ($P = 0.92$). The expected germination rate for this seed variety (Cherry Soda) was 80% (Ellis, 2023) and a common germination rate for outdoor-sewn oilseed and fiber hemp seed is 70% (Conley et al., 2018). This study's seeds were germinated in an indoor space with consistent ventilation and air flow, 18 h of LED light daily, and an average ambient temperature of 28.2°C, resulting in 7.85% higher average germination rate than expected by the seed producer and 17.85% higher average

germination rate than outdoor-sewn hemp seed. The cost of hemp seeds used in this study was $1.00 per seed under 500 seeds ordered (Ventura Seed Company, n.d.) which is much higher than a similar smokeable plant, tobacco, at $0.04 to $0.0002 per seed (Urban Farmer, 2023) or than a similar fiber plant, cotton, at $0.07 to $0.008 per seed for an heirloom, non-GMO varietal (True Leaf Market, n.d.). Therefore, even a marginal increase in germination rate may be beneficial to hemp producers.

There was a total of 12 seedling trays: three trays for each treatment and three control seedling trays. The seedlings from one of the vermiculture-treated trays had 100% germination, while the two lowest germination rates (tied at 79%) were from the vermiculture group (V) and the mycorrhizae plus vermiculture group (GW+V). Germination rate differences between treatments was not significant. However, the conditions under which these seeds were germinated were demonstrated to provide a level of germination higher than the expected rate of 80% (Ellis, 2023, p. 8). More replications of this trial should be conducted to demonstrate this result consistently. Although not statistically significant, the numerical difference between the expected and achieved germination rate likely has practical and economic value for the hemp producer.

This study may be one of the first investigations of the use of full-spectrum LED lights on the effectiveness of hemp seed germination, as no publications to date have been identified to use this light parameter. Further investigation is warranted to explore whether LED lights affect hemp seed germination rate. Because LED lighting lasts up to 50,000 h while T5 fluorescent tube bulbs last between 10,000 and 15,000 h, the use of LED lights for germination could be beneficial to producers (Karmakar et al., 2016). LED lighting has a longer lifespan than fluorescent alternatives, but the higher cost of

LED lights currently offsets this lifespan advantage. However, it is worth noting that fluorescent bulbs contain mercury, which is harmful to the environment, while LED bulbs and fixtures do not (Karmarkar et al., 2016). This is an important consideration for conscientious producers.

*Early plant growth*

Early vegetative growth for hemp producers can be crucial to plan for field canopy heights, staking and trellis requirements, and for harvest planning. The effect of seed inoculation did not demonstrate a significant difference in early plant growth. The control group had the tallest mean plant height at 68.10 cm (*SD* = 17.50) followed closely by the GW treatment group with a mean height of 67.83 cm (*SD* = 14.96). The V and GW+V treatment groups both had shorter average plant heights (65.56 and 62.81 cm, respectively) but larger standard deviations (18.92 and 18.73, respectively).

For a hemp producer, the tendency to have more plants at a similar height is beneficial to canopy planning when transplanting to the field. The canopy refers to the uppermost layer of leaves and branches and canopy planning typically involves determining ideal plant spacing and training techniques to manage the growth and development of the plants (Matzneller et al., 2022). The goal is to create a uniform and productive canopy that maximizes the yield and quality of the cannabis crop. Canopy management is important for cannabis growers because of its effects on light interception, air circulation, transpiration, yield, and product quality (Matzneller et al., 2022). Measuring the morphological characteristics of a canopy can be difficult, as the morphology is constantly changing throughout the life of the crop, and plant height at the transition from vegetative to flowering growth stages is just one parameter to consider

(Matzneller et al., 2022). Further investigation is needed to determine if plant height is affected by seed inoculation, or if the differences in plant height across treatments and within treatments were due to other factors such as natural genetic variation between seeds and environmental factors, such as incidental differences in light access during early plant growth.

*Cannabinoid content*

The reason cannabinoid content is important is that "the major market for *C. sativa* is as a source of cannabinoids, the two most abundant of which are THC and cannabidiol (CBD)" (Toth et. al., 2020, p. 213). Finding production techniques that increase the amount of these cannabinoids is crucial to hemp producers who hope for their crop to be economically viable. The differences in flower cannabinoid content were not statistically significant between treatments. Total CBD content in the vermiculture treatment groups increased between 1.29% and 2.02% from the control group; while this was not statistically significant, any increase in CBD content could make a hemp product more competitive in the market. The growing market for CBD-based products has prompted many companies to explore different avenues for increasing CBD content in their products. Currently, Greenwich Biosciences formulates an approved prescription medication named Epidiolex® which contains CBD (Toth et al., 2020), and many companies in the United States have various tinctures, salves, and ointments on the market which contain CBD as their primary active ingredient. These products market their potency based on milligrams of CBD, charging more for increased amounts of CBD present (Medterra, n.d.).

The cannabinoid content in hemp cultivated with vermiculture or a mix of

mycorrhizae and vermiculture measuring higher than the control and mycorrhizae-only groups could mean that the inoculation with the vermiculture introduced beneficial microbes to emerging roots and gave those plants marginally improved nutrient uptake ability. Studies have demonstrated that cannabinoid concentrations can be affected by external drivers such as sunlight hours (De Prato et al., 2022), temperature, precipitation (Sikora et al., 2011), soil type and nutrients (Bócsa et al., 1997). Additionally, there is evidence of external drivers affecting THC and CBD concentrations differently (Sikora et al., 2011), but there is yet to be a study directly linking soil microbial activity to a change in cannabinoid production. Given the lack of hemp studies on this topic, future research is warranted.

*Cured CBD flower weight*

A study published after the initiation of this research concluded that three rhizobacteria, (i.e., *Bacillus* sp., *Mucilaginibacter* sp. and *Pseudomonas* sp.) increased the fresh flower weight of a hemp varietal of *C. sativa* by 5.13%, 6.94%, and 11.45%, respectively (Lyu et al., 2022), compared to a control. These researchers noted that the plants inoculated with these plant growth-promoting microbes did not have changes in other factors such as leaf area, branch number, node number, or plant height in concert with the increase in flower weight (Lyu et al., 2022). While our study did not measure these parameters, our results demonstrate that flower weight after harvest did appear to increase in the presence of soil microbes from one inoculation, although the increases were not found to be significant. Compared to the control, our results demonstrate an increase in flower weight of 4.43% with mycorrhizae inoculation and a flower weight increase of 28.98% and 43.03% with vermiculture and vermiculture plus mycorrhizae

inoculation, respectively. These results demonstrate a larger increase than the Lyu et al. (2022) study, however our study started with hemp seeds while the Lyu et al. (2022) study started with hemp plant cuttings. Additionally, both studies used different microbe inoculations. Additional iterations of similar studies are warranted to confirm the optimal application timing and composition of beneficial soil microbes.

**Recommendations for Future Study**

Based on findings from this study, there are several potential avenues for future research. Further investigation may examine the effect of hemp seed inoculants on other aspects of plant growth, such as seedling vigor, branching, and overall biomass accumulation. Additionally, this study only evaluated single-step inoculation whereas multiple inoculations at crucial growth transitions (e.g., at transplanting) warrant further investigation (White, 2020). While this study found no statistical differences in mean time to germination and plant height between treatments, further optimization of inoculation methods to maximize plant growth and yield could elucidate relationships. Further research could explore different inoculation methods or variations in inoculant composition to determine the most effective treatment for promoting hemp plant growth and cannabinoid production. Additional opportunities for investigation include the effects of hemp seed inoculants on other varieties of cannabis, other seed sources, and other batches of the same seed type, as natural genetic variation between seeds could have contributed to the observed differences in plant height.

As a follow-up to the use of LED lights for the germination stage of growth, a larger-scale trial with a greater number of replications could further investigate the effectiveness of LED lights for hemp seed germination, especially compared to

fluorescent bulbs, and their impact on seed germination rates and plant growth. A cost-benefit analysis of the use of LED lights versus fluorescent lights for the seed germination stage would also be informative for hemp producers, especially when considering lifespan and environmental cost of each light source. LED technology is still evolving and information on the efficiency and effectiveness of these lights in the seed germination process is still scant.

Finally, this study did not find statistically significant differences in cannabinoid content or cured flower weight between treatments. Future research with larger sample sizes and more robust statistical analyses may be able to detect more subtle effects of inoculation on these important outcomes. Future research should focus on examining the effect of inoculation on the quality and quantity of cannabinoids produced over the entire lifecycle of the plant. There is also room for investigation into different harvesting and processing techniques on cannabinoid content of hemp flowers, as well as the potential impact of other pre- and post-harvest environmental factors such as photoperiod, soil pH, and nutrient availability. Overall, further research is needed to fully understand the benefits and optimize these treatments.

# APPENDIX SECTION

## APPENDIX A

## Texas Worm Ranch Biological Soil Amendment Report

earthfort

# Biological Analysis
# Soil Amendment

**Report prepared for:**
Texas Worm Ranch
Heather Rinaldi
2636 National Circle
, TX 75238 USA
txwormranch@gmail.com

Report Sent: 1/21/2016
Sample#: 01-122313 | Submission:01-025104
Unique ID: TexasWormRanch
Plant:
Invoice Number: 13139
Sample Received: 1/13/2016

For interpretation of this report please contact:
Earthfort Labs
info@earthfort.com
(541) 257-2612

*Consulting fees may apply*

| Organism Biomass Data | Dry Weight | Active Bacteria (µg/g) | Total Bacteria (µg/g) | Active Fungi (µg/g) | Total Fungi (µg/g) | Hyphal Diameter (µm) |
|---|---|---|---|---|---|---|
| **Results** | 0.440 | 175 | 1955 | 16.0 | 3733 | 2.9 |
| Comments | In Good Range | Above range | In range | In range | Above range | |
| Expected Range Low | 0.2 | 3 | 300 | 3 | 300 | |
| Expected Range High | 0.8 | 30 | 3000 | 30 | 3000 | |

| | Protozoa (Numbers/g) | | | Total | Mycorrhizal Colonization (%) | |
|---|---|---|---|---|---|---|
| | Flagellates | Amoebae | Ciliates | Nematodes #/g | ENDO | ECTO |
| **Results** | 130763 | 6301340 | 13077 | 43.8 | Not Ordered | Not Ordered |
| Comments | High | High | High | Good | | |
| Expected Range Low | 10000 | 10000 | 0 | 10 | | |
| Expected Range High | 100000 | 100000 | 2000 | 100 | | |

| Organism Biomass Ratios | Total Fungi to Tot.Bacteria | Active to Total Fungi | Active to Total Bacteria | Active Fungi to Act.Bacteria | Nitrogen Cycling Potential (lbs/ac) |
|---|---|---|---|---|---|
| **Results** | 1.91 | **0.004** | 0.09 | 0.09 | 300+ |
| Comments | Good | Low | Good | Good | |
| Expected Range Low | 0.01 | 0.01 | 0.01 | 0.01 | |
| Expected Range High | 10 | 0.1 | 0.1 | 10 | |

**Nematode detail (# per gram or # per mL)**
Classified by type and identified to genus.
(If section is blank, no nematodes identified.)

| | | |
|---|---|---|
| Bacterial Feeders | 29.60 | |
| Achromadora | | 0.64 |
| Butlerius | | 10.29 |
| Cephalobus | | 0.64 |
| Diplogasteritus | | 1.29 |
| Geomonhystera | | 0.64 |
| Plectus | | 1.29 |
| Rhabditidae | | 14.80 |
| Fungal/Root Feeders | 12.22 | |
| Ditylenchus | Stem & Bulb nematode | 1.93 |
| Filenchus | | 10.29 |
| Predatory | 1.93 | |
| Mononchoides | | 1.93 |

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2636 National Circle
, TX 75238 USA
txwormranch@gmail.com

Report Sent: 1/21/2016
Sample#: 01-122313 | Submission:01-025104
Unique ID: TexasWormRanch
Plant:
Invoice Number: 13139
Sample Received: 1/13/2016

For interpretation of this report please contact:
Earthfort Labs
info@earthfort.com
(541) 257-2612

*Consulting fees may apply*

| | |
|---|---|
| Dry Weight: | Within normal moisture levels. |
| Active Bacteria: | Bacterial activity above expected level. |
| Total Bacteria: | Good bacterial biomass. |
| Active Fungi: | Fungal activity within normal levels. |
| Total Fungi: | Excellent fungal biomass. |
| Hyphal Diameter: | Good balance of fungi. |
| Protozoa: | Should provide a good inoculum of protozoa. High ciliate numbers suggest recent anaerobic conditions. |
| Total Nematodes: | Good numbers and diversity. |
| Mycorrhizal Col.: | |
| TF/TB: | Fungal dominated. |
| AF/TF: | Low fungal activity relative to total biomass |
| AB/TB: | Good bacterial activity. |
| AF/AB: | Fungal dominated, becoming more bacterial. |

Interpretation Comments:

Actinobacteria Biomass = 150 ug/g
Good fungal diversity, hyphal diameter: 1.5 to 6.0um

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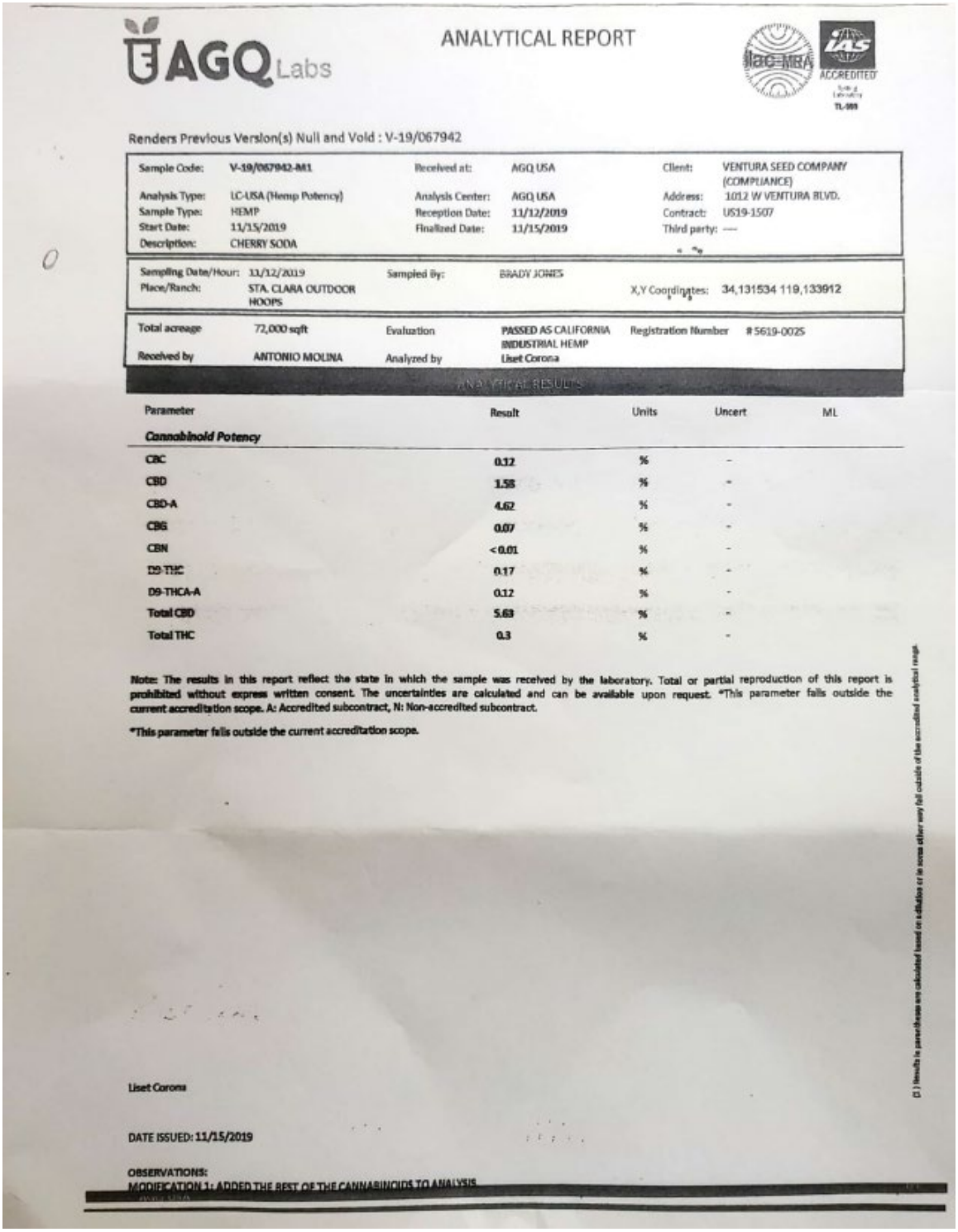
01-122313: Page 2 of 2

APPENDIX B

Cannabinoid report from Ventura Seeds for Cherry Soda strain of *Cannabis sativa*.



AGQ Labs

ANALYTICAL REPORT

Renders Previous Version(s) Null and Void : V-19/067942

| | | | | | |
|---|---|---|---|---|---|
| Sample Code: | V-19/067942-M1 | Received at: | AGQ USA | Client: | VENTURA SEED COMPANY (COMPLIANCE) |
| Analysis Type: | LC-USA (Hemp Potency) | Analysis Center: | AGQ USA | Address: | 1012 W VENTURA BLVD. |
| Sample Type: | HEMP | Reception Date: | 11/12/2019 | Contract: | US19-1507 |
| Start Date: | 11/15/2019 | Finalized Date: | 11/15/2019 | Third party: | --- |
| Description: | CHERRY SODA | | | | |

| | | | | | |
|---|---|---|---|---|---|
| Sampling Date/Hour: | 11/12/2019 | Sampled By: | BRADY JONES | | |
| Place/Ranch: | STA. CLARA OUTDOOR HOOPS | | | X,Y Coordinates: | 34,131534 119,133912 |
| Total acreage | 72,000 sqft | Evaluation | PASSED AS CALIFORNIA INDUSTRIAL HEMP | Registration Number | # 5619-0025 |
| Received by | ANTONIO MOLINA | Analyzed by | Liset Corona | | |

ANALYTICAL RESULTS

| Parameter | Result | Units | Uncert | ML |
|---|---|---|---|---|
| ***Cannabinoid Potency*** | | | | |
| CBC | 0.12 | % | - | |
| CBD | 1.58 | % | - | |
| CBD-A | 4.62 | % | - | |
| CBG | 0.07 | % | - | |
| CBN | < 0.01 | % | - | |
| D9-THC | 0.17 | % | - | |
| D9-THCA-A | 0.12 | % | - | |
| Total CBD | 5.63 | % | - | |
| Total THC | 0.3 | % | - | |

Note: The results in this report reflect the state in which the sample was received by the laboratory. Total or partial reproduction of this report is prohibited without express written consent. The uncertainties are calculated and can be available upon request. *This parameter falls outside the current accreditation scope. A: Accredited subcontract, N: Non-accredited subcontract.

*This parameter falls outside the current accreditation scope.

Liset Corona

DATE ISSUED: 11/15/2019

OBSERVATIONS:
MODIFICATION 1: ADDED THE REST OF THE CANNABINOIDS TO ANALYSIS

# APPENDIX C

The following outline of cannabis growth stages explains the stages of production which are investigated through this study, adapted from *Marijuana Horticulture* (Cervantes, 2006):

1. **Germination**: This process begins when moisture, heat, and oxygen are absorbed by the sturdy outer layer of the seed, activating hormones like cytokinins, gibberellins, and auxins. The embryo inside the seed expands as it feeds on the food reserves stored within. After 3-7 days, the seed coat breaks open, a rootlet emerges and grows downward, while a shoot emerges and grows upwards, seeking light.
2. **Seedling**: During the seedling stage, the primary root continues to grow downwards and branch out, while the stem grows upwards and branches out above the soil level. The roots serve to anchor the young plant to the growing medium and begin absorbing vital nutrients and water. To ensure robust and healthy growth, it is recommended to provide the plant with 16-18 hours of light during this stage.
3. **Vegetative Growth**: To maintain this growth phase, it is recommended to provide the plant with 16-24 hours of light each day. During this period, the plant undergoes further growth and maturation, with the roots developing specialized functions. The root tips continue to elongate into the growth medium, seeking more nutrients and water, while single-celled root hairs become the primary site for water and nutrient absorption. The stem continues to lengthen and strengthen with cellulose in response to air movement, and the apical bud grows directly

upward, while the lateral buds develop into branches and elongate to access light. The leaves of the plant expand and grow from axillary bud points along the main and side branches, performing photosynthesis in the presence of sufficient water and $CO_2$.

4. **Cloning**: This is a propagation technique where cuttings are taken from the tips of branches of vegetative plants and rooted for 10-20 days to produce clones. To maintain the clones in the vegetative stage, it is necessary to provide them with 18-24 hours of light.
5. **Flowering**: During outdoor growth, cannabis plants typically flower in the fall when the days become shorter and the photoperiod reduces to 12 hours of uninterrupted darkness, although some varietals that have adapted to tropical regions may flower with less darkness. The change in light signals to the plants that their life cycle is approaching its end, triggering changes in their functions. Flowers start to form during the final stage of growth, and if left unpollinated, the female flowers develop without seeds, resulting in what is commonly referred to as "sinsemilla." When fertilized with male pollen, seeds start to develop within the flower buds. The unpollinated female flowers continue to enlarge and produce resin that is rich in cannabinoids, while waiting for pollination from a male flower. After several weeks of intense flowering, the cannabinoid production reaches its peak, and the flowers are ready for harvest.

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