MURRAY STATE UNIVERSITY

Journal of Agricultural Hemp Research

Volume 3 | Issue 1 Article 1

December 2023

Effectiveness of mycorrhizae and vermicompost seed inoculation for germination, vegetative growth, cannabinoid content, and cured flower weight of CBD-rich hemp (Cannabis sativa L.)

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Cover Page Footnote

We are grateful for diagnostic support from Ionization Labs in Austin, Texas.

After a 50-year hiatus when research and cultivation of hemp were deemed illegal, interest in hemp cultivation in general – and hemp cultivation using regenerative methods more specifically – has steadily increased in North America (Kroma, 2006). Regenerative agriculture aims to improve yield, promote ecological sustainability, and ultimately reduce input costs for producers through practices that reduce soil disturbance, increase soil diversity, and focus on soil microbial health through fostering beneficial soil microorganisms (e.g., mycorrhizae and rhizobacteria) (Khangura et al., 2023).

While there is minimal literature available on the interactions between mycorrhizal and rhizobacteria with cannabis seed germination, preliminary data with other parameters indicate positive results. Specifically, Kakabouki et al. (2021) determined that inclusion of *Rhizophagus irregularis* arbuscular mycorrhiza fungi in high doses increased length, dry biomass, and survival of cannabis seedling roots. Lyu et al. (2022) found that rhizobacteria inoculation of hemp clones increased fresh flower weight at harvest from 5.13% to 11.45%, but did not affect plant height, leaf area, branch number, or node number. Islam et al. (2023) investigated the influence of microbial inoculants with rock mineral fertilizers on two industrial hemp varieties. They found that microbial inoculation through compost affected hemp biomass, yield, and nutrient composition depending on variety and sampling time. In a study of hemp inoculation with *Metarhizium* species, an endophytic, insect-pathogenic fungus, and *Pochonia chlamydosporia*, an endophytic nematode-pathogenic fungus, both endophytic fungi positively influenced shoot length, stem weight, and root weight of hemp (Hu et al., 2023).

Because research on mycorrhizal and rhizobacteria interaction with hemp is limited, studies that explore microbial inoculation with other crops may provide insight. As summarized in table 1, the addition of beneficial microbes through compost applications or microbial

products has improved growth of vegetable, grain, and fiber crops (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).

Research investigating crops other than hemp also demonstrate that mycorrhizae and rhizobacteria play a role in improving seed germination rates (Alghamdi, 2019; Herrera et al., 2017; Raklami et al., 2019, Sebastián et al., 2014; Shao et al., 2020; Tsulsiyah et al., 2021), as well as increasing shoot and root dry weight, and improving shoot content of sugar, proteins, N, P, Ca, K, and Na (Raklami et al., 2019). Researchers who have used vermicompost to inoculate plants with beneficial organisms have reported increased seed germination and improvements in growth in tomato and lettuce (Arancon et al. 2012; Atiyeh et al., 2000); marigold (Atiyeh et al., 2000); hyacinth bean (Karmegam & Daniel, 2008), cucumber (Sallaku et al., 2009); and okra and brinjal (Dhanalakshmi et al., 2014).

While there is limited literature regarding the microbial impacts on cannabis seed germination, plant growth, and plant weight, the above cited studies that confirm positive results for other crops may inform hemp research. By investigating the effects of microbial seed inoculants on germination rate and growth parameters of hemp, we can better understand early cultivation methods which may affect hemp production and farmer net returns. Thus, the purpose of this study was to determine if the addition of a seed treatment of beneficial microorganisms affected 1) hemp germination rate and days to germination, 2) plant height, 3) cannabinoid content of harvested cannabis flower, and 4) weight of harvested, cured cannabidiol flower.

Materials And Methods

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.

Certified hemp seeds were sourced from Ventura Seed Company (Camarillo, California), which has also supported ongoing hemp research at the Rodale Institute (Bozzolo & Gonzales-Siemens, 2021). The cultivar chosen for this trial was Cherry Soda because of its vigor, disease resistance, and short time to harvest. Microbial seed inoculants used in this study were Great White® Premium Mycorrhizae (GW), which contains nine species of endomycorrhiza, seven species of ectomycorrhiza, and 15 species of other beneficial bacteria and fungi (table 2), and locally sourced vermicompost (V) from Texas Worm Ranch (Dallas, Texas).

The germination and vegetative phase of this study was conducted in a climate-controlled, indoor laboratory at the Department of Agricultural Sciences, Texas State University in San Marcos, Texas. Seeds were germinated in seedling trays with Pro-Mix growth medium, with a sample size of 24 for each of the three treatments and one control, with three replications. Treatments were: 1) seed inoculation of Great White® Premium Mycorrhizae (GW), 2) seed inoculation of vermicompost (V), and 3) seed inoculation of GW and vermicompost (GW+V) with concentrations of inoculation solutions shown in table 3 applied as a soil drench to seedling trays. The control group was directly sown into Pro-Mix medium with no additional seed inoculant. Seed trays were arranged in a randomized block design with three replications consisting of 24 seeds per treatment. All seeds were consistently watered with de-chlorinated, municipal pH-balanced water during the germination and vegetative phase. To ensure an 18-hour

vegetative photoperiod and prevent premature flowering, full-spectrum LED grow lights from the manufacturer SOEVSI were installed on an automatic timer. These lights included 5000K, 3000K, 760nm, and 660nm diodes that provided blue, warm white, infrared, and red wavelengths, respectively.

For 14 days after sowing, data collected included the number of days from seeding to germination and overall germination percentage. After 28 days, seedlings were transplanted from their germination cells into 10-cm nursery containers to continue vegetative growth in a climate-controlled laboratory. On day 54, plant height was measured before plants were transplanted in outdoor hügelkultur beds at Texas State University Freeman Center, located in USDA Zone 8b. Growing beds were homogenously prepared with Suståne 8-2-4 organic slow-release fertilizer, which was incorporated into the top 10 cm of soil at 3.4 kg per 9.29 m^2. Plants were spaced 50 cm apart in blocks. Plants were hand-watered with well water after transplanting to ensure sufficient water penetration and then all beds received the same amount of irrigation water via a timed drip hose for the remainder of the growth cycle.

On September 13, 2022, 127 days after seeds were sown, the 12 most mature plants from each treatment group were harvested. Peak maturity was identified when flower pistils turned from white to brownish-red, and resin glands on the surface of the flowers transitioned from clear to milky-white (Cervantes, 2006). Plants were then processed by hanging upside down indoors in a climate-controlled facility to dry for one week at a mean temperature of 28.2°C and mean relative humidity of 40.6%. After drying, flower buds were removed from stems and placed in plastic bins to finish curing, following the 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 composites of flowers from each treatment were tested for cannabinoids at Ionization Labs in Austin, Texas.

Data analysis. Indices to evaluate hemp seed germination were adopted directly from Hesami et al. (2021), which included germination rate (GR), germination index (GI), and mean germination time (MGT). Germination rate was computed using the following equation:

$$\text{GR} = \frac{G}{n} \times 100$$

where *G* is the number of germinated seeds by day 14 and *n* is the total number of cultivated seeds.

A GI was calculated based on the following equation:

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

Seed GI 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. MGT, a measure of the average time required for seeds to germinate under specific conditions, was computed based on the following equation:

$$MGT = \sum (n \times d)/N$$

where n is the number of germinated seeds on each day, d is number of days from the beginning of the test period, and N is the total number of seeds germinated at the termination of the test period (Ellis and Roberts, 1981).

For GR, days to germination, and plant height, a single-factor ANOVA test was utilized to determine if the difference in results between treatment groups were significant ($\alpha = 0.05$) using MS Excel. Measures of central tendency were calculated for cannabinoid content and weight of harvested and cured flowers.

Results and Discussion

Germination rate and days to germination. Hemp seeds were considered germinated upon emergence of the cotyledons and germination was recorded for 14 days after seeds were inoculated and placed in growing media. All seedlings that germinated did so before seven days. Germination rates are summarized in figure 1 and GI are summarized in table 4.

No significant differences were determined in GR across treatments **(***P* = 0.92). Of 288 seeds, 35 seeds across all treatments did not germinate (12.2%), ranging from seven seeds in the control group to 10 seeds in the GW+V group (*M* = 8.75; *SD* = 1.26). Mean time to germination was similar among treatments with no statistical differences detected (figure 2). All treatments had a MTG slightly below 4 days (*M* = 3.85; *SD* = 0.03). No seeds germinated on the first or second day of the trial nor after the seventh day of the trial.

While this study's seed germination results are not consistent with non-cannabis crop studies that have reported improved seed germination rates due to mycorrhizae and rhizobacteria as well as vermicompost inoculation, some studies show no effect or decreased germination. For example, Varga (2015) showed that the presence of arbuscular mycorrhizal spores in the soil decreased seed germination of *Geranium sylvaticum* but did increase subsequent plant growth. Furthermore, Ievinsh (2011), who studied vegetable crops, concluded that vermicompost can inhibit seed germination and early seedling development, and that the effect on germination is crop and concentration specific, such that caution should be exercised regarding the use of vermicompost for plant propagation. Overall, further investigation of microbial influence of cannabis seed germination is needed due to the preliminary nature of our study. Additionally, varying inoculant concentration rates and methods of inoculation should be considered in future

cannabis germination studies, for example via seed soaking as opposed to the soil drench method explored in this study.

Plant height. 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 by treatment did not demonstrate a significant difference in vegetative plant growth with respect to height ($P = 0.55$). As shown in table 5, 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 (18.93 and 18.73, respectively). This finding was consistent with results from Lyu et al. (2022) who found that, while inoculation of rhizobacteria on hemp clones increased fresh flower weight at harvest from 5.13% to 11.45%, inoculation did not affect plant height.

Cannabinoid content. Cannabinoid profiles of cured flower by treatment are summarized in table 6. Tetrahydrocannabinol (THC)-delta-9, the compound illegal at the federal level, was under the mandated 0.3% limit for all treatments with the highest level in the GW+V group at 0.15%. Compared with the control, the GW+V treatment had a 17.6% higher total THC content and a 19.0% higher total cannabidiol (CBD) content. The V treatment had a 17.6% higher total THC content and a 12.1% higher total CBD content than the control. The smallest qualitative differences were shown in the GW treatment, which had a 2.0% higher total THC content than the control and a 1.7% lower total CBD content than the control. These results suggest further investigation is needed to quantify THC and CBD differences among treatments. Because replications are needed, statistical significance regarding percentage differences are unknown. However, preliminary observations show interesting trends that must be further investigated.

Cured flower weight. As shown in figure 3, the control group had the lowest total and average cured flower weight. The GW treatment had a 4.4% higher cured flower weight than the control group, while the V and GW+V treatments had an increase of 29.0% and 43.0%, respectively, over the control group. These results are consistent with those of Lyu et al. (2022) who 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 5.1%, 6.9%, and 11.5%, respectively. Our study began with the propagation of hemp seeds, however, while Lyu et al.'s (2022) study began with hemp plant cuttings. Our qualitative results suggest further investigation is needed to quantify whether inoculation through vermicompost and commercial products like Great White is profitable depending on inoculation input cost and market price of CBD flower and size of harvest.

Conclusion

Our study represents some of the early research exploring the impact of seed inoculation on hemp plant productivity, which is critical foundational knowledge of appropriate practices to maximize the economic viability of hemp production. Despite no significant differences between treatments for GR and plant height, there are several potential avenues for future research. Qualitative differences were found between treatments, specifically the GW+V treatment had the highest cured flower weight, as well as total CBD and total THC content over the control. Thus, future investigation is needed to quantify the effect of hemp seed inoculants on cured flower weight and total THC and total CBD content.

Another avenue of future research is the investigation of different inoculant sources (e.g., *Bacillus* sp., *Mucilaginibacter* sp. and *Pseudomonas* sp.). For example, Paul et al. (2011)

identified an opportunity to investigate the impact of a combined seed inoculant of *Azobacter chroococcum* and arbuscular mycorrhizal fungi on the seed germination and seedling development of cotton, leading to potential improvements for this fiber crop. Similarly, a species-specific investigation for hemp seed inoculation may further examine beneficial inoculant combinations.

Because this study only evaluated single-step inoculation, multiple inoculations at critical points of influence (e.g., at transplanting), as well as the incorporating of rock mineral fertilizers to facilitate mineralization, also warrant further investigation. While this study found no statistical differences in mean time to germination nor plant height between treatments, further optimization of inoculation methods to maximize yield and cannabinoid production could elucidate potential relationships.

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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 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 Seedling vigor	Gholami et al., 2009
Maritime pine	Vermicompost soil amendment & vermicompost tea	Germination rate N content	Lazcano et al., 2010
Okra, brinjal, chili	Vermicompost soil amendment	Germination rate Root & shoot length	Dhanalakshmi et al., 2014
Orchids	Mycorrhizal fungi	Protocorm formation Seedling growth	Shao et al., 2020
Rice	Rhizobacteria	Germination rate, growth parameters, nutrient content***	Choi et al., 2016
Sunflower	Rhizobacteria	Germination rate Biochemical profile	Den et al., 2021
Wheat	Endophytic fungi	Seedling growth 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

Table 2. Great White Premium Mycorrhizae species list

Ectomycorrhiza spp.	**propagules/gram**	**Other fungal/bacterial spp.**	**CFUs/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	Water
Great White (GW)	0.41 g per 3.78 L of water
Vermicompost (V)	3.30 g per 3.78 L of water
GW+V	0.21 g GW + 1.65 g V per 3.78 L of water

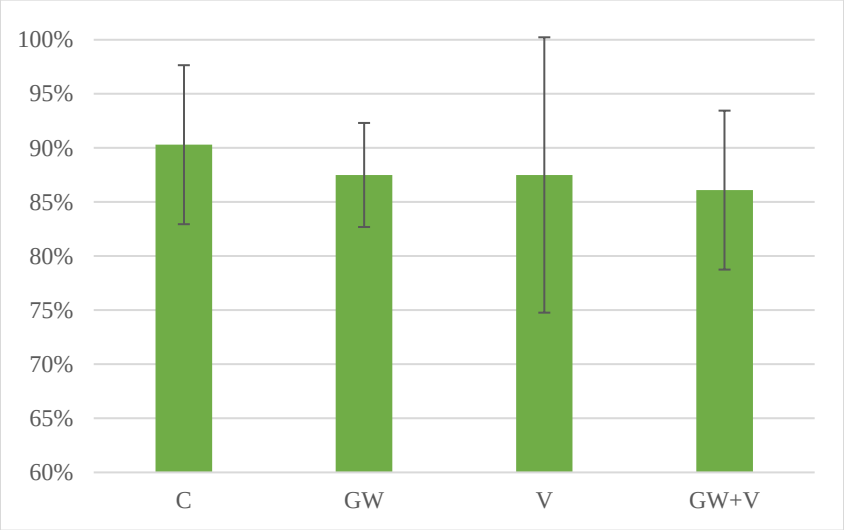

Figure 1. Germination rate by treatment where C = control, GW = mycorrhizae treatment, V = vermicompost treatment, GW+V = combined treatment of mycorrhizae and vermicompost. (α = 0.05; P = 0.92)

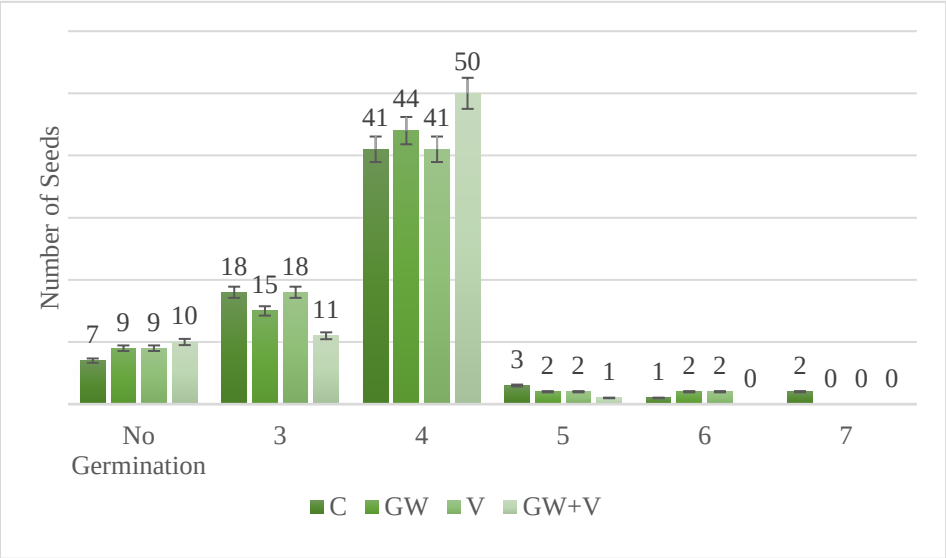

Figure 2. Days to germination by treatment where C = control, GW = mycorrhizae inoculation, V = vermicompost inoculation, GW+V = combined inoculation of mycorrhizae and vermicompost. The first set of bars shows the number of seeds that did not germinate.

Table 4. Summary of germination indices: germination rate (GR), germination index (GI), and mean germination time (MGT) in days.

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

Table 5. Summary of plant height in cm by treatment group ($P = 0.55$)

	Control	Great White (GW)	Vermicompost (V)	GW+V
Range (cm)	30.0 - 95.5	34.5 - 87.5	34.5 - 89.5	27.0 - 90.5
Mean (cm)	68.10	67.83	65.56	62.81
SD	17.50	14.96	18.93	18.73

Table 6. Cannabinoid content of post-harvest flower by treatment group: C = control, GW = mycorrhizae treatment, V = vermicompost treatment, GW+V = combined treatment of mycorrhizae and vermicompost (<LOQ = less than a measurable amount was detected)

	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%

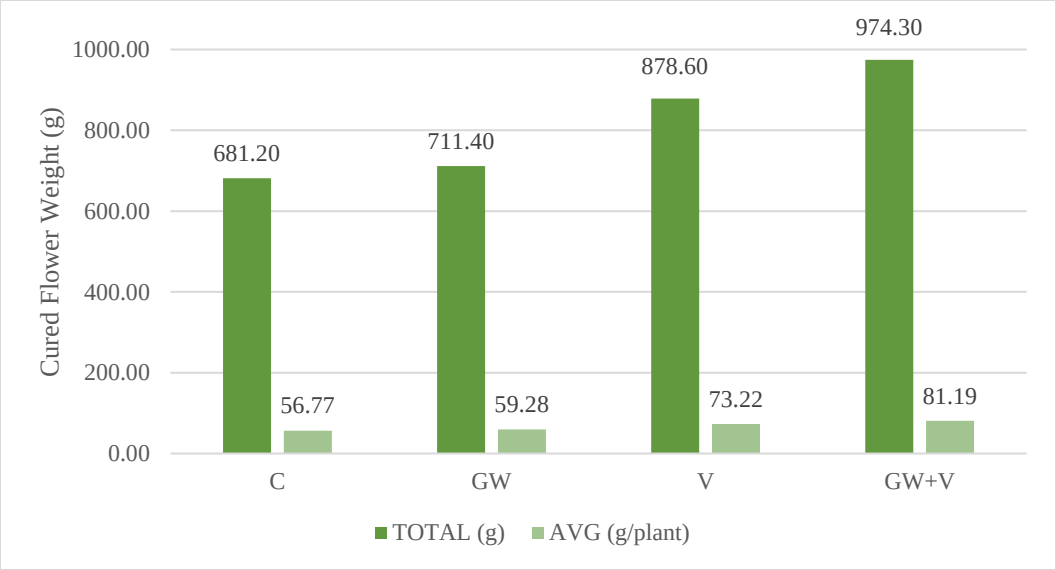

Figure 3. Total and average (AVG) weight of cured flower weight by treatment group where C = control, GW = mycorrhizae treatment, V = vermicompost treatment, GW+V = combined treatment of mycorrhizae and vermicompost