Effects of Biofertilizers and Organic Amendments on Nutrient Availability in Soil & Plant Growth.

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ACADEMIC ABSTRACT

Applications of fertilizers derived from non-renewable resources, along with improved land management practices have contributed greatly increased crop yields in the past 70 years. Biofertilizers and organic amendments, provide alternative sources of nutrients for increased plant yields and resistance against abiotic stress. The objective of this work was to evaluate the effectiveness of various biofertilizers and an organic amendment on improve plant health and/or crop yield. The first study focused on the organic amendment, glucoheptonate and found that applications of 800-1600 kg/ha can increase available water capacity in fine textured soils by up to 3%. The second study evaluated the effectiveness of dual-inoculating biofertilizers Mung beans (*Vigna radiata* (L.) Wilczek) with two, *bradyrhizobium* spp. and arbuscular mycorrhizal fungi. Dual inoculation significantly increased grain yield (+33%) compared to a synthetic N fertilizer application but did not significantly increase grain yield compared to the control (+22%). Dual inoculation may increase grain yields of mung beans compared to synthetic fertilizer regime but does not show evidence of improving N fixation. The final study was a greenhouse experiment focused on evaluating some mung bean cultivars to determine their susceptibility to salt stress while also evaluating the effect of inoculation in combating saline soils. Germination was significantly decreased at 6 dS/m in all cultivars by about 36% when compared to the control treatment (0 dS/m). Seed yields, pods per plant and seeds per plant, increased as salt concentration increased. No factors recorded where affected by inoculation. Overall, our research suggests that the use of biofertilizers and organic amendments can improve

crop health, but other management and environmental considerations need to be accounted for when reporting effectiveness of such alternative soil amendments

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GENERAL AUDIENCE ABSTRACT

Applications of fertilizers derived from non-renewable resources, along with improved land management practices have contributed greatly to increased crop yields in the past 70 years. Biofertilizers and organic amendments, provide alternative sources of nutrients for increased plant yields and resistance against abiotic stress. The objective of this work was to evaluate the effectiveness of an organic amendment and various biofertilizers to improve plant health and/or crop yield. The first study focused on the organic amendment, glucoheptonate and found that applications of 800-1600 kg/ha can increase available water capacity in fine textured soils by up to 3%. The second study evaluated the effectiveness of dual-inoculating Mung beans (*Vigna radiata* (L.)Wilczek) with two biofertilizers, *bradyrhizobium* spp. and arbuscular mycorrhizal fungi. Dual inoculation significantly increased grain yield (+33%) compared to a synthetic N fertilizer application but did not significantly increase grain yield compared to the control (+22%). Dual inoculation may increase grain yields of mung bean compared to synthetic fertilizer regime but does not show evidence of improving N fixation. The final study was a greenhouse experiment focused on evaluating mung bean cultivars (4) to determine their susceptibility to salt stress while also evaluating the effect of inoculation in combating the effect of saline soils. Germination was significantly decreased by about 36% at a salinity of 6 dS/m across all cultivars compared to the control at 0 dS/m. Seed yields, pods per plant and seeds per plant, increased as salt concentration increased for some cultivars. No factors recorded were observed to be affected by inoculation. Overall, our research suggests that the use of

biofertilizers and organic amendments can improve crop health, but other management and environmental considerations need to be accounted for when reporting effectiveness of such alternative soil amendments.

Dedication

In memory of my grandfathers, Ferdinand and Herbert,

Thank you for teaching me the joy of hard work and conviction.

I dedicate this work to you.

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Chapter 1: Literature Review

Joshua Mott

Organic Soil Amendments

Since the mid 1960's, about 50-75% of global crop yield increases have been attributed to the use of commercial synthetic fertilizers (Roy, 2007; Stewart & Roberts, 2012). While synthetic fertilizers will continue to be a vital part of our agricultural systems, research has continually shown that continued production and mining of these resources is detrimental to our ecosystems. Nitrogen fertilizer manufacturing relies heavily on fossil fuels and contributes to the release of N_2O and CH_4 methane gasses into our atmosphere (Chai et al., 2019; Pachauri et al., 2015; US EPA, 2016). Organic soil amendments have been explored in order improve fertilizer uptake, efficiency or improvement of soil properties to improve yields. Due to various physio-chemical properties, the use of various organic amendments such as, manure or biosolids can reduce greenhouse gas emission potentials by up to 36% when compared to a synthetic fertilizer application when injected into the soil (Kitamura et al., 2021; Savci, 2012).

Organic soil amendments are defined as the use of animal or plant by-products that provide benefits to crop growth and or soil health (Goss et al., 2013). Some examples of organic amendments include but are not limited to, biosolids, manures, biochar, compost, and plant based chelating agents. The earliest evidence of the use of organic amendments in agriculture can be traced back to 3000 B.C.E. (Wilkinson, 1982). Proper application and timing of organic amendments is important to ensuring excess nutrients do not inherently become pollutants to our environment. For example, losses of N to the environment (via mechanisms of run-off and volatilization), by application of manures, can be greatly reduced by injecting the manure into the soil rather than a broadcast application (Bierer et al., 2017).

Chelating Agents and Glucoheptonate

Micronutrients, while needed by plants in smaller amounts relative to macronutrients, are essential to plant health and growth. Common micronutrients include iron, manganese, copper, zinc and boron (Clemens et al., 1990). Micronutrient cation availability in soil is dependent on mainly 4 different factors; soil pH, soil structure and texture, organic matter content, as well as mycorrhizal activity. In conditions where the previous factors are not optimal, micronutrients can be aided in plant uptake by chelates, which can be created and released by plant roots, formed in soil humus, or can be produced synthetically and added to soil solution (Weil & Brady, 2017). Chelated metals (micronutrients) are soluble complexes that can supply a micronutrient to a plant the chelating agent, natural or synthetic, will form soluble complexes with other micronutrients given the proper pH of the soil and this reaction essentially protect the micronutrient from reacting with soil particles (Clemens et al., 1990; Weil & Brady, 2017).

This process allows plants to utilize nutrients that would otherwise not be available in soil solution. It has been reported that chelates that are effective for one micronutrient in a particular soil and pH will not necessarily be as effective in a different environment (Clemens et al., 1990). Studies have also shown some chelating agents and nano-chelates to be effective in increasing plant nutrient efficiency in water shortage situations (Ali et al., 2020). Adding the chelating agents was shown to be particularly effective when applied during a water shortage that occurred during the reproductive phase of the plant in question (Janmohammadi et al., 2016).

The study of chelating agents dates back to 1893 with theories of a ring like structure for complexes containing ethylenediamine (EN - $C_2H_4(NH_2)_2$)(Clemens et al., 1990). Ethylenediamine (EN) is an organic compound that used in polymers, solvents, pharmaceuticals, and as a precursor to chelating agents and agrochemicals. It is the precursor specifically to

ethylenediamine tetra acetic (EDTA) (PubChem, 2020). For example, Iron (III) ion will react first with ammonia to form a complex $Fe^{3+} + 6NH_3 \rightarrow [Fe(NH_3)_6^{3+}]$ and when ethylenediamine substitutes for ammonia the complex forms a ring system that is often called a chelated complex (Clemens, 1990). Chelating agents often have higher stability constants because of their ring like structures and the overall structure of chelates is variable. It has been considered that "natural" or organic chelating agents, produced by plants roots or from soil humus are 'easier to absorb' for plants (JH Biotech Inc., 2019).

The two general categories of chelating agents that have been heavily studied are aminopolycarboxylates and hydroxycarboxylates. Some of the aminopolycarboxylates are ethylenediamine tetraacetic acid (EDTA), ethylenediamine di(o-hydroxyphenylacetic acid)(EDDHA), hydroxyethyl ethylenediamine triacetic acid (HEEDTA), and diethylenetriamine pentaacetic acid (DTPA.). There are many hydroxycarboxylates including citric acid, mandelic acid, gluconic acid, and glucoheptonate. In some studies, it has been shown that the hydroxycarboxylates are superior to aminopolycarboxylates because of price and applications in certain soils. Glucoheptonate has been noted to be a better chelating agent for heavy metals in most soil applications (Clemens, 1990).

Glucoheptonate, in particular is relatively affordable in the common market. There are various methods used to produce the compound starting from glucose in corn syrups or other plant derived sources of glucose such as corn syrup or by-products like sugar beet (*Beta vulgaris* L.) molasses (Goos, 2006). Some plants have developed homeostatic mechanisms "in order to maintain the concentration of essential metals within physiological limits and to minimize the detrimental effects of non-essential metals" (Wenger, 2005). Most of the research on

phytosiderophores, or natural chelating agents, is done primarily on Fe(III), zinc, and magnesium.

The recommended application of glucoheptonate is typically as a foliar spray containing the micronutrient. The use of foliar sprays is not feasible for all crops and regions due to a lack of resources needed to apply the agent and also a possibility of causing damage to the crop (Clemens, 1990). Glucoheptonate is a highly biodegradable organic chelating agent "with as much as 98% degradation occurring within 2 days" and this is cause for concern when looking at the agent for long term soil 'solutions' (Shaddox, 2016; Zak, 1972; Maxwell & SpringerLink, 2004).

Biofertilizers

Another alternative to synthetic fertilization, is the use of biofertilizers. Biofertilizers are a specific set of living organisms that, when applied to seed or soil, exhibit properties that enhance plant health via increased nutrient availability, production of phytohormones, and or protection against environmental stresses (Fasusi et al., 2021; Kumar et al., 2022). There are subsets of biofertilizers based on their exhibited actions when interacting with plants. Nitrogen fixing bacteria are amongst the most commonly applied in agricultural settings and these organisms include but are not limited to *Rhizobium*, *Bradyrhizobium*, and *Azobacter* (Thomas & Singh, 2019). Another subset of biofertilizers are phosphate solubilizing microorganisms, such as Arbuscular Mycorrhizal Fungi and *Pseudomonas striata* (Mehnaz, 2016). The use of biofertilizers can reduce the necessity of synthetic fertilizer applications and enhance nutrient use efficiency in agricultural systems.

Nitrogen fixing bacteria

In agricultural settings, inoculation with nitrogen fixing bacteria is crucial in legume, specifically soybean, production systems. Rhizobacteria can fix atmospheric nitrogen by creating nodules on the plant roots, utilizing materials from the plant, in order to provide the plant with plant available nitrogen. Nodule formation for nitrogen fixation involves many complex interactions and transactions, between the plants and the bacteria and varies across plants. Plants release compounds such as simple sugars, polysaccharides, flavonoids and betaines in the rhizosphere where rhizobacteria interact with infected and non-infected cells of the plants which induce the genes of nodulation (Gage, 2009; Powell & Doyle, 2015). The introduction of the rhizobacteria in order to begin nodulation expression is referred to as 'inoculation'. Once signaled by the plant, the root hairs are infected by the bacteria which in-turn begin to express the proper genes for nodulation and nitrogen fixation. There are two types of nodules, indeterminant and determinant (Gage, 2009). Legumes with persistent meristems are said to be indeterminant and the cells of meristems are typically small and spherical which gives way to the shape of nodules on plants. (Gage, 2009; "Meristem | Definition, Function, Types, Examples, & Facts," 2020).

Field crop legumes that make up most of the global production are inoculated by the genera *Rhizobium, Sinorhizobium, Mesorhizobium, Allorhizobium, or Bradyrhizobium* and are referred to as symbionts. Nitrogen fixation has been measured by extent of root nodulation and nodule color, but these practices only give an indication of nitrogen fixation totals (Peoples et al., 2009). Current methods used to measure N_2 fixation are nitrogen balance, nitrogen difference, acetylene reduction, hydrogen evolution, ureides, and N-15 isotope consumption. The previous methods have potential limitations depending on plant species, cultivar, plant age, drought stress and location of sample (Peoples et al., 2009).

In order to optimize legume production, producers are encouraged to use plant growth-promoting rhizobacteria (PGPR) or proper 'inoculant'. Residual indigenous rhizobium, is largely considered to be the first method of legume inoculation and early experiments of exogenous soil inoculation saw yields 10 times that of plants that were not inoculated (R. H. Miller & May, 1991).

Arbuscular Mycorrhizal Fungi

There are several types of mychorrhizal fungi. The two most commonly observed in nature are ectomycorrhizal fungi (EM) and arbuscular mycorrhizal fungi (AMF) (Toju & Sato, 2018). EM colonize the root surface and form a scaley-sheath around the root (Anderson & Cairney, 2007) while AMF colonize the inner portions of the root cells and extends hyphae from 'infected' plant cells into the surrounding soil (Birhane et al., 2012). Unlike nitrogen fixing bacteria, the majority of EM and AMF colonies are not host specific, lending themselves to more ubiquitous utilization in plant communities (Smith & Read, 2010; Zhu et al., 2010).

Arbuscular mycorrhizal fungi (AMF) form symbiotic relationships with legumes and 80% of other plant families (Begum et al., 2019). AMF colonies solubilize P and other nutrients for the plants, while plants provide various nutrients to support growth for the AMF (Gage, 2009; Suzaki et al., 2019). The phosphate solubilization process begins with arbuscule formation in the root hairs followed by the expression of phosphate transporter genes (Kobae, 2019).

AMF colonies in soil have been shown to decrease effects of water stress in plants, by promoting regulated gas exchange in the roots of plants allowing for leaves to maintain stomatal control and subsequent water balance (Augé, 2004). Mycorrhizal fungi are also largely responsible for maintaining or improving soil structure (R. Miller & Jastrow, 2000), which can

allow for enhanced plant growth. The AMF hyphae, physically entangle smaller soil particles and microaggregates eventually becoming stable macroaggregates (Rillig & Mummey, 2006).

Inoculation of plants in agricultural settings by non-native AMF colonies has become popular over the past several, with evidence of improved plant health greater than that of plants without the additional inoculation (Begum et al., 2019). There is also a great deal of evidence stating that there is no effect of AMF inoculation due to native colonies and with some studies reporting negative affects to biodiversity and ecosystem functionality due to non-native AMF inoculation (Hart et al., 2017).

Soil Physical Properties of Concern

Water Holding Capacity

Soil water holding capacity is a crucial component of soil quality. Without the soils ability to hold water plants it would have to be constantly supplied water. The capacity of a soil to hold water is not just about how much water is in the soil at one time but it is more so about the amount of water retained in capillary spaces of soil after gravitational water loss into deeper layers of the soil. This information also allows producers to know how long to wait between irrigations and give a solid understanding of when their soils may be oversaturated (Olorunfemi, 2016). A soil's water holding capacity is heavily dependent upon the porosity and bulk density of the soil. Both of these factors are dependent upon the climate, the percentage of sand, silt, and clay in the soil, and the effects of disturbances. For example, after a field has been tilled and wetted, a common disturbance in agriculture, the soil porosity and bulk density usually decrease because the soil matrix will usually collapse upon itself decreasing porosity and bulk density over time (Zhang, 2018, Eden, 2017). This would effectively increase the water holding capacity

soil but if this disturbance continues then this increase could possibly be a detriment to the soil and plants. It has been shown in certain studies that "...plowing and artificial re-compaction for seedbed preparation also contributes to irreversible changes of soil structure" (Duttman et, al. 2014). This continued land degradation can lead to what is called soil-water drought especially when an area typically has periods of drought. The irregular timing of precipitation events could have major socio-economic impacts on an entire region (Wildemeersch et al., 2015).

The field capacity of soils is also determined by previous water holding history. If a soil has been saturated then dries, it will have a higher field capacity as opposed to a soil that is frequently saturated. This is mainly due to hysteresis (Kirkham, 2005). It is also important to note that the field capacity of a soil is not the is not the maximum available water to the plant for uptake. Field capacity is used as a reliable figure that factors in the fact that water that is over that number will more than likely be loss to gravitational forces. Any water that is above the field capacity is still plant available unless air pore space is less than 10% (Kirkham, 2005).

Studies concerning the field capacity of the soil can easily be recreated in a greenhouse pot. Even though there is no underlying soil to pull water downwards "deep through a soil profile" (Kirkham, 2005) there are similar gravitational forces that are similar to what would be seen in a soil profile. This timing differs and does not directly translate to field capacity so it is often referred to as "pot capacity" (Kirkham, 2005).

To determine the amount of water that would be available to the plant there would need to be a prior understanding of the soil's field capacity and wilting point. Field capacity is defined as "the amount of water held in soil after excess water has drained away and the rate of downward movement has materially decreased" (Sun & Yang, 2013). The wilting point of a soil is defined when the moisture left in the soil is no longer available to that plant which is typically

around −1500 J/kg (or −15 bar) of suction pressure. This is typically measured by using a Pressure Plate Extractor. The difference between these two amounts, field capacity and wilting point, is defined as the available water capacity or plant-available water capacity (Eden et al., 2017; Sun & Yang, 2013). In recent literature it has been noted that the full range between field capacity and wilting point may only apply in areas that are not susceptible to drought conditions, due to a lack of water present in the environment.

It is also important to note that irrigation patterns can also affect the water holding capacity of certain soils. In areas where water resources are not scarce it is recommended to irrigate to 30% field capacity daily in order to avoid wetting and drying cycles in soils that often lead to decreased water infiltration, due to deteriorating soil aggregation and increased bulk densities (Soil Physical, 2019).

Soil Salinity

Saline soil is characterized by having an electrical conductivity of 4.0 mmhos/cm and a pH less than 8.5. Approximately 23% (0.34×10^9 ha) of arable land are considered to have saline soils, with that percentage increasing as climatic changes continue (Yang et al., 2007). Soil salinization is largely due to continued salinization of freshwater irrigation sources due to salt water intrusion and infrequent/irregular rainfall patterns (McFarlane & Williamson, 2002). Soils with a high salt content often express less than ideal physical characteristics such as weak structure and aggregate stability, low porosity, and considerable nutrient toxicities (Imadi et al., 2016).

Salt stress in plants effects physiological and morphological traits such as overall plant growth & yield potential, and can lead to plant mortality (HanumanthaRao et al., 2016; Yang et al., 2007). The accumulations of Na^+, Mg^{2+}, and Cl^- in soil can prohibit adequate water uptake

required for proper growth and results in plant injury (Stoytcheva & Zlatev, 2013; Yang et al., 2007). The application of gypsum is a common efficient method of reclaiming salt affected soils by replacing the sodium ions with calcium ions (HanumanthaRao et al., 2016). Nodulation of legumes is significantly affected by soil salinity, with some plants showing no signs of nodulation at 6 dS m^{-1} (HanumanthaRao et al., 2016; Zurayk et al., 1998). At higher concentrations, germination, root growth, and shoot length are all detrimentally affected for many plants (Roychoudhury & Ghosh, 2013).

Legume Growth and Production

The fabacean family or more commonly referred to as "legumes", account for about 27% of the worlds agricultural production and provide at least 33% of the worlds dietary N needs due to symbiotic relationships with various bacteria and fungi to take atmospheric N and convert it to a plant available form of N. This symbiosis is a primary factor of agricultural sustainability, economically and environmentally (Dommergues & Ganry, 1986; Mousavi-Derazmahalleh et al., 2019; Vance et al., 2000). Legumes ranges is from large trees to small herbs, but the most important legumes used for grain and forage are soybean (*Glycine max)*, common bean (*Phaseoulus vulgaris*), alfalfa (*Medicago sativa*), cowpea (*Vigna unguiculata*), lentil (*Lens culinaris*), chickpea (*Cicer arietinum*), peanut (*Arachis hypogaea*), and faba bean (*Vicia faba; hologalegina clade*) (Mousavi-Derazmahalleh et al., 2019). Pulses, typically referring to subset of legumes including, mung bean (Vigna radiata), cowpea, lentils, and chickpea, are the dried edible portion of the plants and they serve as a major source of protein for developing countries (Calles et al., 2019; Eapen, 2008; Snapp et al., 2018). Legume crops used for oil extraction, forages, or vegetable such as soybean, , alfalfa, and green peas, are not considered to be pulses (Calles et al., 2019). The increased consumption of pulses over animal protein sources and

overall inclusion into typical diets, can prevent heart disease, type 2 diabetes and even some cancers (Snapp et al., 2018; WHO (World Health Oragnization), 2008).

The most prominent legume crop produced in the United States is soybean (*Glycine max*). In 2017, the United States harvested approximately 90 million acres of soybeans accounting for about 26% of total cropland in the United States (USDA, 2020; USDA, ERS, 2020). While it is generally true that legumes will make significant N contributions to soil while in crop rotations, if the plant residue is removed from the surface then there can actually be significant net losses of N from that field, therefore post-harvest management is crucial to successful soil fertility improvements (Phoomthaisong et al., 2003).

Mungbean

Mungbean (*Vigna radiata* [L.]) is a warm season legume with a short life cycle, which is approximately 60 days to harvest (Kim et al., 2015). The crop is mainly produced by small holder farmers in developing countries within Asia's southern and eastern regions due to its nutritional content, nitrogen fixation abilities, short life cycle, and multiple harvests, with about 50% of the world annual production being in India (Kang et al., 2014). Mungbean, like other legumes works in symbiosis with *Rhizobium,* or *Bradyrhizobium* fixing up to 110 kg N ha^{-1}, which will not only supply enough nutrients for its own growth but can supplement the nutrient requirements of the following crops thereby increasing soil fertility without further external inputs. (Nair et al., 2013; Peoples & Herridge, 1990; Shah et al., 2003). In addition to being a rich source of protein, mungbean is also high in folate, which is essential nutrient for pregnant women and nursing women (Nair et al., 2013). The pulse is an excellent source calcium and phosphorus containing 118 mg (Ca) 100 g of $seed^{-1}$ and 340 mg (P) per 100 g of seed.

Mungbean is also a very versatile pulse crop that can be turned into flour, noodles, ice-cream or even plant-based animal product alternatives (Kang et al., 2014).

Mungbean is closely related to cowpea (*Vigna unguiculate*) and so it is assumed that many of the nutrient requirements are theorized to be similar for both plants (Kim et al., 2015). Oklahoma is the largest producer of mungbean in the United States with an estimated 100,000 acres produced for both food products and green manure (Jefferson Institute, 2003). While very little research has been carried out on varietal effects on nodulation and nitrogen fixation, research has shown that mungbean will perform best in conventionally tilled soil that allows for proper nodulation and increased root density (Imran et al., 2016).

Phosphorus applications of up to 80 kg ha^{-1} P_2O_5 and potash (K_2O) applications of up to 90 kg ha^{-1} were shown to significantly increase mungbean nodulation and various yield parameters across multiple varieties and locations (Hussain et al., 2011; Imran et al., 2015). Mungbean cultivar also has a significant effect on yield and nodulation, with hybrids typically out performing local varieties although this effect has not been studied much further (Hussain et al., 2011; Imran et al., 2015). Some research has shown that seed inoculation along with a P application of 50 kg ha^{-1} will be the most efficient use of resources and produce greatest grain yields although specific inoculant were not specified (Malik et al., 2002). Seed inoculation has proven to be superior to soil applications of inoculant in mung bean systems (Anjum et al., 2006).

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Chapter 2: The effect of glucoheptonate on the water holding capacity of soils

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Abstract

Freshwater resources for agricultural irrigation are becoming increasingly limited as our climate continues to change at rapid rates. The available water capacity (AWC) of a soil can be manipulated to increase conservation of water resources. This study aims to evaluate the effectiveness of glucoheptonate as a source of organic matter, in order to increase the AWC of a soil. The first trial in this study utilized a sandy-loam and clay-loam soil to measure AWC in response to 6 application rates of GH (0, 100, 200, 400, 800, & 1600 kg ha^{-1}). Half of the soil samples were sterilized via autoclave and each treatment was replicated 3 times. The second trial in this study used a sandy loam and a loamy sand soil in order to measure AWC response over time in a greenhouse setting. GH application rates were 0, 150, 300, 600, 1200 kg ha^{-1} along with 2 watering regimes (80% FC and 40% FC). Sterilized soil samples in trial 1, with GH additions, showed significant increases in AWC. GH additions at higher rates (800-1600 kg/ha) shows to be effective in slightly increasing the AWC in the Braddock soil when unsterilized (~3% AWC increase). The AWC of the sandy loam in our first trial showed no response to GH in any treatment. Results from the second study showed small decreases in water needed in order to maintain FC in the coarser textured soil over 7 days. The coarse soil treated with 1200 kg ha^{-1} GH, required 71 ml less water added to maintain 80% FC. Glucoheptonate additions showed no significant difference on the sandy loam soils, overall.

Introduction

When considering factors affecting plant production, the availability of water in a soil and how quickly that water moves through the soil is crucial to plant health. Approximately 70% of the world's freshwater supply is consumed by agricultural irrigation and it is currently estimated that a majority of irrigation systems have potential application efficiency of 70% (Barker, 2004; Irmak et al., 2011). Effectively, only about 50% of the water applied to irrigated crops is utilized by the plants in production. This inefficiency in plant water usage can be attributed to many factors such as climatic changes, soil water evaporation, cultural soil management practices (Irmak et al., 2011; Kirkham, 2005). Soil physical properties, such as texture, organic matter concentrations, and bulk density can also significantly affect the water use efficiency of plants. As our climate changes and freshwater sources become less available, it is important to investigate practices that affect soil available water.

The ability of a soil to store water that is available for plants to utilize is called the Available Water Capacity (AWC) and represents the difference between field capacity (FC) and wilting point (WP) (Eden et al., 2017; Sun & Yang, 2013). Field capacity is expressed as the amount of water held in the soil 24-72 hours after an irrigation event, depending on the soil type. (Sun & Yang, 2013). Even after a period without any irrigation events there will still be a relatively small amount of water adhering to the soil particle surfaces. This moisture left in the soil is no longer accessible to plants and is classified the wilting point of a soil. In recent literature it has been noted that the full range between field capacity and wilting point may only apply in areas that are able to resist drought (Wesseling et al., 2009).

By increasing a soils AWC, it is possible to reduce the need for excessive irrigation. Increasing the AWC of a soil by just 1% can reduce the need for irrigation by ~22,400 liters per

hectare. Sands, sandy loams and loamy sands are typically classified by fractions of AWC less than 10% (10g H_2O/100g of soil). Plants grown in soils with high sand fractions are highly susceptible to experiencing drought stress due to water leaching out of the root zone rapidly (Wesseling et al., 2009).

In a meta-analysis of studies aiming to find the extent of which we can change the AWC of a soil Hudson (1994), reported that the addition of organic matter to a mineral soil has shown to be an effective strategy. They reported that the largest differences in effect (organic matter concentrations) were observed in sandy/coarsely textured soils and saw no difference in finer textured soils (Hudson, 1994). Glucoheponate (GH) is an organic chelating agent, typically utilized in micronutrient fertilizer applications, that can be manufactured or derived from corn syrup or sugar beet (*beta vulgaris)* molasses (Clemens et al., 1990). This soil amendment has been anecdotally observed to increase the AWC of a soil for a temporary amount of time and help plants combat effects of plant stress. This is hypothesized to be due to increases in organic matter of a soil.

The aim of our first trial was to determine the effect of glucoheptonate, as an organic matter addition, on soil moisture retention, and determine the utility of GH to marginal/drought susceptible agricultural land. We hypothesized, given previous research and anecdotal evidence, the addition of glucoheptonate to a coarse textured soil will increase AWC compared to a soil of a finer texture or without. The objective of our second trial was to determine the persistence of glucoheptonate in the soil and how long any affects to AWC would be noticeable due to the GH additions. We also wanted to observe any differences in plant biomass composition affected by levels of GH additions to the soil.

Materials and Methods

Glucoheptonate fertilizer product

A 'novel' formulation of sodium glucoheptonate was formulated and provided by Whitehurst and Associates Inc to be used for this research. The percent content of the sodium glucoheptonate by weight is 25-60% with the exact percentages being withheld as confidential business information. Sodium Glucoheptonate was diluted with water to achieve the correct composition. Sodium glucoheptonate will henceforth be referred to as *GH.*

Soil Collection and Analysis

Two soils were collected for use in this study: a Braddock clay loam (Fine, mixed, semiactive, mesic Typic Hapludults) and Bojac sandy loam (coarse-loamy, mixed, semiactive, thermic Typic Hapludults). The soils will be referred to as *clay loam* and *sandy loam.* Soil texture was determined These two soils were picked based on differences in soil texture as well as previous nutrient analysis observations. (Table 2-1). The soils were collected at a depth of 15 cm, mixed to ensure uniformity and air dried for 5-7 d prior to use. Samples were extracted using Mehlich 1 extraction solution containing 0.05N HCl and 0.025N H_2SO_4 (Mehlich, 1953) at a ratio of 4cc's soil to 20 ml of solution. Extracts were then analyzed by Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES; CirOS VISION model, Spectro Analytical). For GH analysis in a greenhouse setting, we collected two soils from the costal and eastern region of Virginia. Bojac (coarse-loamy, mixed, semiactive, thermic Typic Hapludult) and Dragston fine sandy loam (fine-loamy, mixed, semiactive, thermic Aeric Endoqauult). Dragston will now be referred to as 'fine' soil and bojac soil in trial 2 will be referred to as 'coarse' soil. The fine soil was chosen in addition to previously used coarse soil due to its finer texture and

assumed differences to be had in water holding properties. The soils were collected to a depth of 15 cm, mixed to ensure uniformity and air dried prior to use. Soils were extracted using Mehlich 1 extraction solution containing 0.05N HCl and 0.025N H2SO4 (Mehlich, 1953) at a 1:1, solution: soil ratio. Extracts were then analyzed by Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES; CirOS VISION model, Spectro Analytical) (Table 2-2). The Bojac soil was deficient in P which was corrected by adding a solution of $(NH_4)_2HPO_4$ at a rate of 30 kg P/ha. Field capacity (FC) was determined using gravitation drainage method, where the dried soils were measured to a set weight, saturated, and then allowed to drain for 2 days via holes on the bottom of a disposable cup before being weighed again (Bond et al., 2006). Soil pH for all soils used in this study were determined by methods outlined by Maguire & Heckendorn, (2011), using a 1:1 soil-water mix.

Trial 1: Available Water Capacity

Six GH treatments (0, 100, 200, 400, 800, & 1600 kg ha^{-1}) were implemented, with 3 replicates of each. To determine if biological activity has an effect on the persistence of GH in on the soil surface, soil was sterilized in an autoclave at 121°C for 30 minutes (Sinegani & Hosseinpur, 2010). Available water capacity (AWC) was determined using the water retention procedure (Allison et al., 1954). Soil samples were mixed with the appropriate GH application rate in centrifuge tubes to ensure uniformity in each sample. Samples were then placed in retainer rings on a porous ceramic plate in a pressure-plate extractor. The plates were then covered with DI water to wet samples by capillarity for at least 4 hours. Plates with samples were then placed in extractors and ran until samples reach equilibrium at 1/3 bar and 15 bar moisture retention. Moisture retention at 1/3 bar was representative of the field capacity of the and took

about 2-3 days to reach equilibrium while 15 bar moisture retention was representative of the permanent wilting point of a soil which took about 14 days to reach equilibrium. Percent water retained at both 1/3 bar and 15 bar was determined by weighing the samples after coming out of the extractor, oven dried and weighed again. The difference between the 'wet' and dry soil was then calculated as a percentage or g H_20 $100g^{-1}$ soil. For our reports our measurements are in percentage format. The difference between 1/3 bar and 15 bar water retention measurements was used to determine the AWC of the samples.

Trial 2: Available Water Capacity

There were 5 GH treatments (0, 150, 300, 600, 1200 kg ha^{-1}) and 2 watering regimes (80% FC and 40% FC). Eighty percent FC is considered to be a typical irrigation regime in the United States while 40% FC is considered to be a drought stressed environment for most plants (Kapanigowda et al., 2014). Mid Atlantic corn cultivar MA 5069(3300) was planted in pots (15.24 cm x 16.51 cm) filled with soil until 7.5cm from the top of the pot. GH applications where applied in a trench and then cover with 5 cm of soil (approx. 1 kg of Dragston soil and 1.13kg of Bojac soil) along with granulated application of urea (46-0-0) at 45kg N ha^{-1}. Seeds were then planted 2.5cm deep at 4 seeds per pot. Within 2 weeks of planting, corn plants were thinned to 2 plants pot^{-1}. Each pot was lined with a coffee filter and placed over a styrofoam catch plate to eliminate nutrient leaching and soil loss, with any collected water being replaced into the pot from which it was lost. Pots were then saturated to correct field capacity by weight. Pots containing Bojac soil at 40% FC and 80% FC were saturated to a weight of 3.075 and 3.455kg respectively and pots containing Dragston soil at 40% at 80% were saturated to 2.785 and 3.175kg, respectively. Saturated weight was checked every 2-3 days and pots were brought back

up to initial saturated weight to replace any water loss. Water added to each pot was recorded. All pots were also covered in saran wrap with a 2.5cm hole in the center until seedlings appeared to reduce loss of water due to evaporation.

Greenhouse lights were set for 14 h day^{-1} and temperature was set to 23 ± 2 °C for 28 days. The amount of water added to bring each pot back to estimated field capacity (80% or 40%) was recorded for 28 days. At the end of the time period most plants were at V3 and harvested carefully to retain roots. Total above ground and below ground plant tissue, roots, and soil were collected from each pot. Roots and above ground biomass were carefully washed and weighed for a wet mass measurement. Plants were then dried at 50°C until a constant weight. Samples were weighed dry and ground for analysis on a vario MAX CNS Element Analyzer. Total N and C were collected from analyzer data. Soil samples were mixed and extracted using Mehlich 1 as described above.

Statistical Analysis

Statistical analysis was performed using JMP Pro 14 statistical software (SAS Institute Inc., 1989-2019). Two-way ANOVA was conducted by soil for each to investigate the significance of soil GH application rate and soil biological activity on AWC indicated by p-values < 0.05. All treatment differences were investigated using post-hoc Tukey's honestly significance difference (HSD) at a significance level of $p < 0.05$. Two-way ANOVA was conducted by soil for each to investigate the significance of soil GH application rate and irrigation regime on the water retention of soils while growing indicated by p-values < 0.05. Two-way ANOVA was conducted by soil and biomass location (above or below ground) to investigate the significance of GH application rate and irrigation regime on amount of total C

and N. All treatment differences were investigated using post-hoc Tukey's honestly significance difference (HSD) at a significance level of $p < 0.05$. A time series ANOVA was also preformed comparing each water added event to determine if GH had a significant effect on the amount of water added over time.

Results and Discussion

Trial 1: Pressure Plate Analysis

At close to 6.5, the pH of both soils was within the agronomic range for good crop production (Maguire and Heckendorn, 2019). According to soil test calibrations in Virginia, the sandy loam was very high in P and medium in K, while the clay loam was low in P and high in K (Table 2-1.) (Maguire and Heckendorn, 2019). The sandy loam had 10 g kg^{-1} OM, while the clay loam had over five times that amount at 52 g kg^{-1} OM (Table 2-1). For this study, the texture and OM were very important, as these are well known to affect water holding capacity. Vengadaramana (2012) and Minasny & McBratney, (2018), saw that additions of organic matter moderately influence the water holding capacity of that soil while showing increases in total porosity eg. 2.1 mm 100 mm^{-1} with a 1% (10 g kg^{-1}) increase in OM. Organic matter increases a soils ability to hold water by two modes of action: (1) organic matter being negatively charged, has a high affinity for water molecules and (2) contributes to enhanced soil structure and increased aggregate stability (Minasny & McBratney, 2018; Vengadaramana, 2012).

With no addition of GH, the clay loam unsterilized soil held 15.25 g H_2O 100 g $soil^{-1}$ and the sandy loam held 5.26 g H_2O 100 g $soil^{-1}$ (Figs. 2-1a and 2-2a). The sterilized soil samples showed similar AWC readings as the clay loam held 15.85 g H_2O 100 g $soil^{-1}$ and the sandy loam held 8.11 g H_2O 100 g $soil^{-1}$. As discussed above, the greater AWC of the clay loam was

expected as it had finer texture and higher OM as shown by Adamu et al., (2012). Although there were no significant AWC responses at the lower GH rates, the clay loam showed a statistically significant response to the highest GH application rate of 1600 kg GH ha^{-1} (18.4 g H_2O 100 g $soil^{-1}$) compared to the control (Fig. 2-1a). This is contrary to the findings of Minasny & McBratney (2018) who noted that sandy soils will often express a greater response to organic matter contributions.

When sterilized, AWC of the clay loam for GH application rates of 100, 200, 400, & 800 kg GH ha^{-1} (18.18, 17.08, 17.76, 18.87 g H_2O 100 g $soil^{-1}$ respectively) were higher than its corresponding unsterilized treatment (16.48, 16.82, 16.98, 16.19 g H_2O 100 g $soil^{-1}$ respectively). There was a statistically significant increase in AWC of the sterilized clay loam soil at 800 kg GH ha^{-1} when compared to the control, 15.85 g H_2O 100 g $soil^{-}$ vs. 18.87 g H_2O 100 g $soil^{-}$ (Fig. 2-1b). Within the unsterilized clay loam soil, there were numerical increases in AWC at 100, 200, 400, & 800 kg GH ha^{-1}, but these increases were not statistically significant. Naveed et al. (2014), found that additions of organic amendments increase biological activity and overall increase in soil porosity which would explain the general decrease in AWC for the unsterilized clay loam soil. The sandy loam showed no response to GH applications at any rate whether sterilized or not. Contrary to trends noticed for the clay loam soil, AWC responses to GH application rates for the sandy loam soil were sporadic and presented relatively low correlations for unsterilized and sterilized soil, r^2: 0.14 & 0.09, respectively (Fig. 2-2a & 2-2b).

While the use of GH on the clay loam soil showed a significant difference on AWC at the highest application rate, further research is needed explore causes of this trend. Applications of sodium glucoheptonate at 1600 kg ha^{-} and 800 kg ha^{-} could potentially cause further issues with soil salinity and micronutrient uptake. Along with that, other studies have shown that soil organic

matter amendments are most effective at increasing water holding capacity of soils at lower application rates of 1% of 2% on a weight/weight (w/w) basis (Demir & Demir, 2019). Demir and Demir (2019) found that farmyard manure, legume leaves, rice stalk, biochar, etc. were all effective in increasing water holding capacity and AWC but most of these methods are cost prohibitive.

Trial 2: Water Holding Capacity – Greenhouse

The average amount of water added to bring each pot back to FC for the coarse soil was about 410 mL per day to maintain a 40% FC and 688 mL per day to maintain 80% FC. The dragston soil soil received 555 mL per day to maintain 40% FC and 886 mL to maintain 80% FC, on average across all treatments. Shaddox et al. (2016b) observed that GH is readily biodegradable and only remains in soil solution for 4-7 days. Due to the biodegradability of GH it was hypothesized that any treatment effects would be most noticeable in the first 7 days after planting For the bojac sandy loam soil saturated to 80% FC, water added each day decreased slightly as application rate of GH increased for 1 day after planting (DAP) (72 mL water added at 0 kg GH ha^- compared to 43 mL water added at 1200 kg GH ha^-) and 4 DAP (63 mL water added at 0 kg GH ha^- compared to 42 mL water added at 1200 kg GH ha^-). The amount of water added on 7 DAP increased as application rate of GH increased (72 mL water added at 0 kg GH ha^- compared to 43 mL water added at 1200 kg GH ha^-) (Fig. 2-3a). This trend was statistically insignificant at a 95% confidence level. The decrease in water added over the 7 days was consistent with the findings of in Minasny & McBratney (2018) in that coarse sandy soils will show greater AWC responses to organic matter increases. None of these increases or decreases in water added were statistically significant when compared to any other application rate. For the

coarse soil set at 40% FC, no water was added to any pots on 1 DAP and we recorded a slight decrease in water added each day as application rate of GH increased for 4 DAP (60 mL water added at 0 kg GH ha^{-} compared to 0 mL water added at 1200 kg GH ha^{-}) and 7 DAP (105 mL water added at 0 kg GH ha^{-} compared to 88 mL water added at 1200 kg GH ha^{-}) (Fig. 2-3b). None of these decreases in water added were statistically significant when compared to any other application rate. A reason these results were not representative of other research may be due to the differences of pot capacity and field capacity. There were no underlying soil to enact gravimetrical forces on the water and/or replenish any soil moisture as we would observe in a typical agricultural field and so, FC does not always directly translate to pot capacity (Kirkham, 2005).

A similar trend was recorded in the fine soil. We observed an overall increase in the amount of water needed to reach 80% and 40% field capacity at every GH application rate compared to the coarse soil as noted above (Figs. 2-3c & 2-3d). We did not observe a statistically significant response in water added compared to GH application rate increases. For the fine soil set at 80% FC water added each day decreased slightly as application rate of GH increased for 1 DAP (138 mL water added at 0 kg GH ha^{-} compared to 73 mL water added at 1200 kg GH ha^{-1}) and there were no notable differences on 4 DAP (85 mL water added at 0 kg GH ha^{-1} compared to 83 mL water added at 1200 kg GH ha^{-1}) and 7 DAP (247 mL water added at 0 kg GH ha^{-1} compared to 230 mL water added at 1200 kg GH ha^{-1}) (Fig. 2-4a). At 1 DAP we observed an abnormally high addition of water of 645 ml at the application rate of 600 kg GH ha^{-1} for the 80% FC treatment factor (Fig. 2-4a). This had a huge influence on the statistical analysis of this sub sample. For the fine soil at 40% FC we did observe a general decrease in water added as application rate increased for 1 DAP (70 mL water added at 0 kg GH ha^{-1} compared to 33 mL

water added at 1200 kg GH ha^{-1}), 4 DAP (78 mL water added at 0 kg GH ha^{-1} compared to 66 mL water added at 1200 kg GH ha^{-1}), and 7 DAP (150 mL water added at 0 kg GH ha^{-1} compared to 138 mL water added at 1200 kg GH ha^{-1}), but none of these decreases were statistically significant when compared to the other treatment levels on the same day. According to Shaddox et al., (2016a), the ability of GH to remain in soil is severely limited which will give rise to a lack of noticeable AWC differences with increasing time.

Total Carbon and Nitrogen

There were no noticeable treatment effects of GH application rates or FC on the amount of carbon in above or below ground biomass or nitrogen in above or below ground biomass of the corn plants used in this experiment. The plants showed no visible signs of nitrogen deficiencies or any other nutrient deficiency. Total carbon remained relatively similar no matter the application rate of GH for both above (41g C kg^{-1}) and below ground (32 g C kg^{-1}) biomass measurements under 80% FC irrigation regime in the coarse soil. For 40% FC, we recorded a similar above ground C of 41 g kg^{-1} but a lower C in the roots of 26 g kg^{-1} . This would most likely be due to the lack of soil moisture for vigorous plant growth in the coarse soil. The difference in total C for below ground biomass between 80% and 40% FC was not statistically significant. Total carbon % remained relatively similar no matter the application rate of GH for both above (41 g C kg^{-1}) and below ground (29 g C kg^{-1}) biomass measurements under 80% FC irrigation regime in the fine soil as well. For 40% FC, we recorded a similar above ground C of 41 g C kg^{-1} but a lower C in the roots of 25 g C kg^{-1} of the fine soil.

Total nitrogen percentage followed a similar trend for the fine soil at 80% FC and 40% FC. The N (g N kg^{-1}) at 80%FC averaged at 22 g N kg^{-1} in the above ground biomass and 5 g

N kg $^{-1}$ in the roots. At 40% FC we observed the N to be averaged at 19 g N kg $^{-1}$ in the above ground biomass and 7 g N kg $^{-1}$ in the roots. Total nitrogen percentage showed a slightly different pattern for the coarse soil at 80% FC and 40% FC. The N at 80%FC averaged at 17 g N kg $^{-1}$ in the above ground biomass and 8 g N kg $^{-1}$ in the roots. There were some obersevred differences between application rate 0, 150, 300, 600, 1200 kg GH ha^{-1} and N (13.4 11.6, 10.0, 7.3, 0.8 g N kg $^{-1}$respectively) for both above and below ground measurements at 80% FC. Even with these differences, the results were not statistically significant at a 95% confidence level. Further research is needed to investigate the lack of total N at the application rate of 1200 kg GH ha^{-1} and why we would see a general decrease in N with the addition of GH amendments. At 40% FC we observed the N% to be averaged at 16% in the above ground biomass and 10% in the roots. This followed the same trend as the nitrogen and carbon percentages of the Dragston soil.

Conclusions

While glucoheptonate additions did increase the AWC of the clay soils, we cannot conclude that GH is a viable option for short term increases in AWC of soil. Results from these two trials show that any significant increases in AWC are not strongly correlated with the addition of GH. The addition of GH also showed no significant differences in total C and N in corn plants tissues or soil concentrations. A more sensitive analysis of the interaction of GH and water in soil is needed to confirm that GH does influence the AWC of soil. Soil particle size as well as frequency of wetting and drying cycles can affect the persistence OM in the soil and its adhesion to soil particle surfaces (Schmidt et al., 2011). Further research with special attention to these factors could provide clarity into GH persistence in the soil and its ability to practically influence the water holding capacity of a soil.

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Table 2-1. Chemical and physical characteristics of the Bojac soil and Braddock soil.

Name	Soil Texture	pH	P	K	OM
			----------g kg^{-1}----------		
Bojac	sandy loam	6.5	70.5	55.0	10.0
Braddock	clay loam	6.5	6.0	98.8	52.0

Note. pH: soil (1:1 soil: deionized water mixture on a volumetric basis); Plant available nutrients: soil (Mehlich 1 solution).

Table 2-2. Chemical and physical characteristics of the Bojac soil and Dragston soil.

Name	Soil Texture	pH	P	K	OM
			----------g kg^{-1}----------		
Bojac	sandy loam	5.6	9.3	145.0	15.0
Dragston	sandy loam	5.7	46.7	114.6	17.0

Note. pH: soil (1:1 soil: deionized water mixture on a volumetric basis); Plant available nutrients: soil (Mehlich 1 solution).

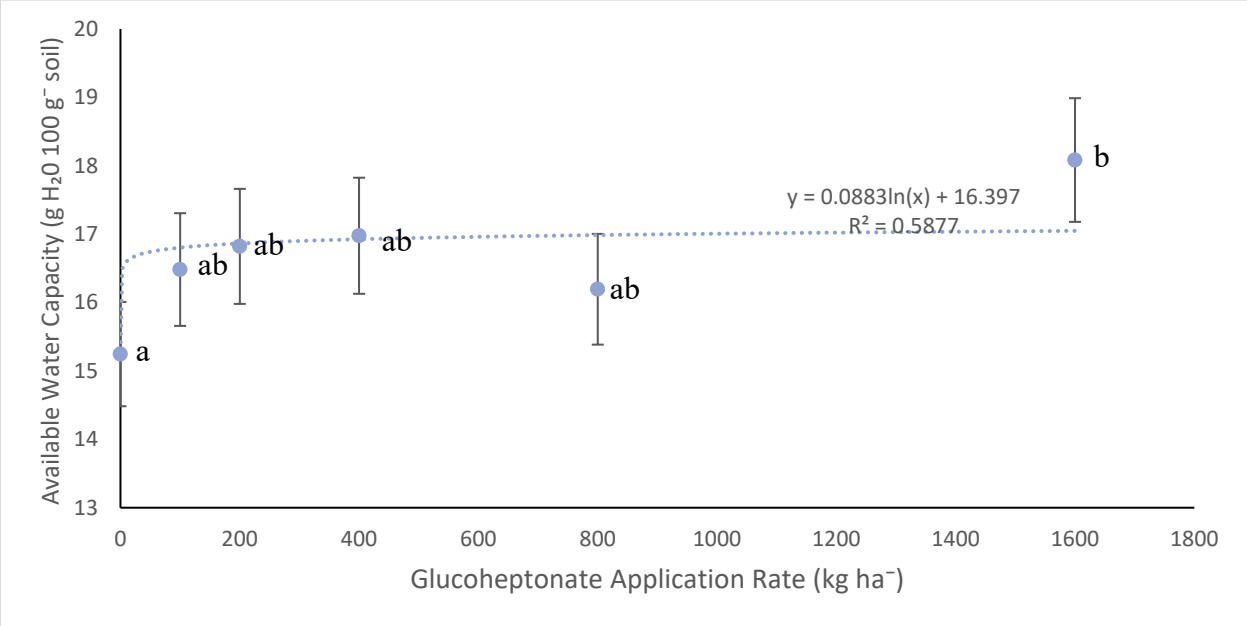

Figure 2-1a. Braddock clay loam available water capacity response to glucoheptonate. Soil was not sterilized before treatment was added. Average water held is shown as averages of replications. Means denoted by a different letter indicate significant differences between treatments ($p < 0.05$).

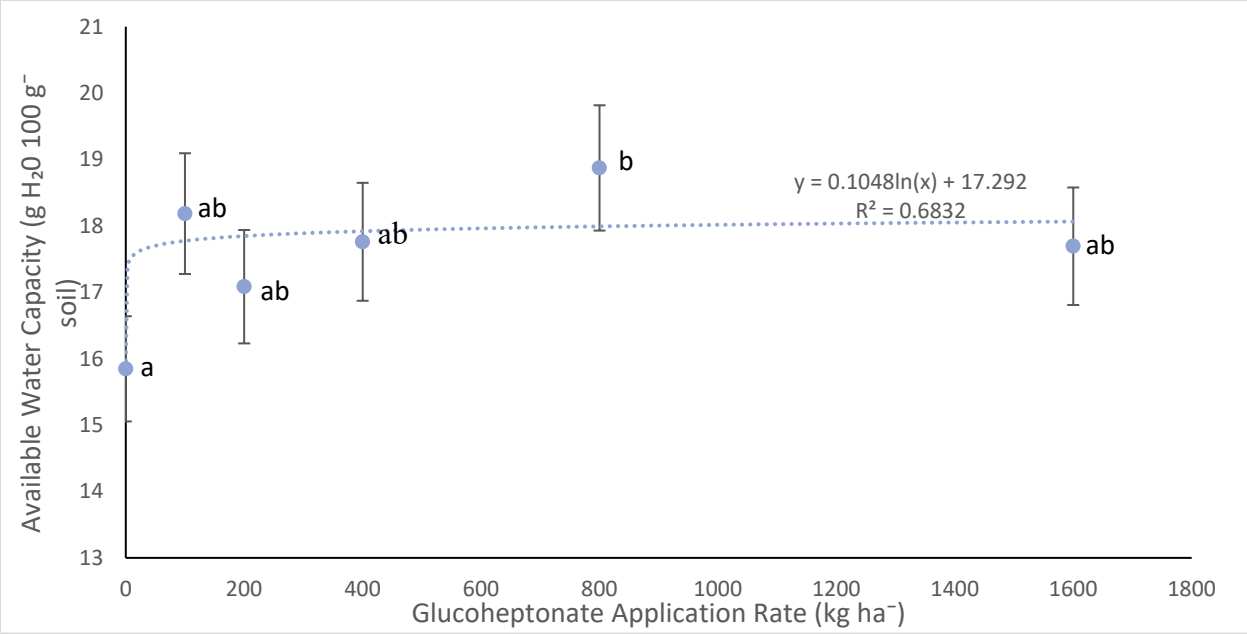

Figure 2-1b. Braddock clay loam available water capacity response to glucoheptonate. Soil was sterilized by autoclave before treatment was added. Average water held is shown as averages of replications. Means denoted by a different letter indicate significant differences between treatments ($p < 0.05$).

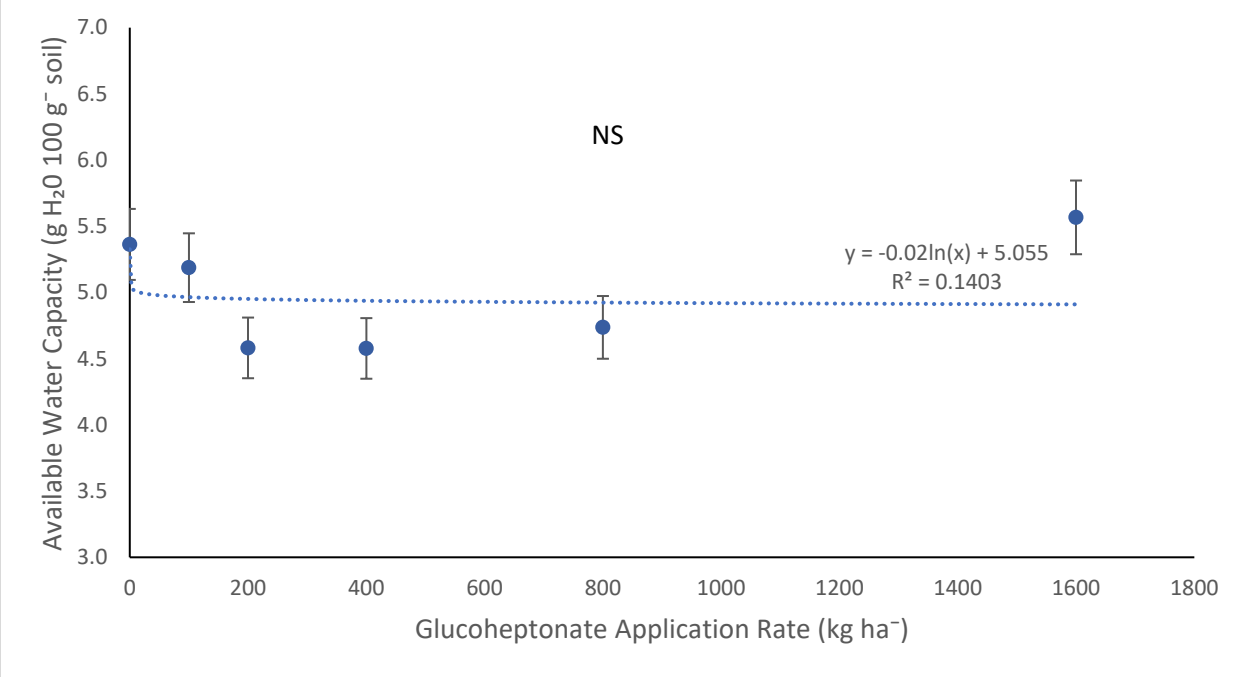

Figure 2-2a. Bojac sandy loam available water capacity response to glucoheptonate. Soil was not sterilized before treatment was added. Average water held is shown as averages of replications. Means denoted by a different letter indicate significant differences between treatments ($p < 0.05$).

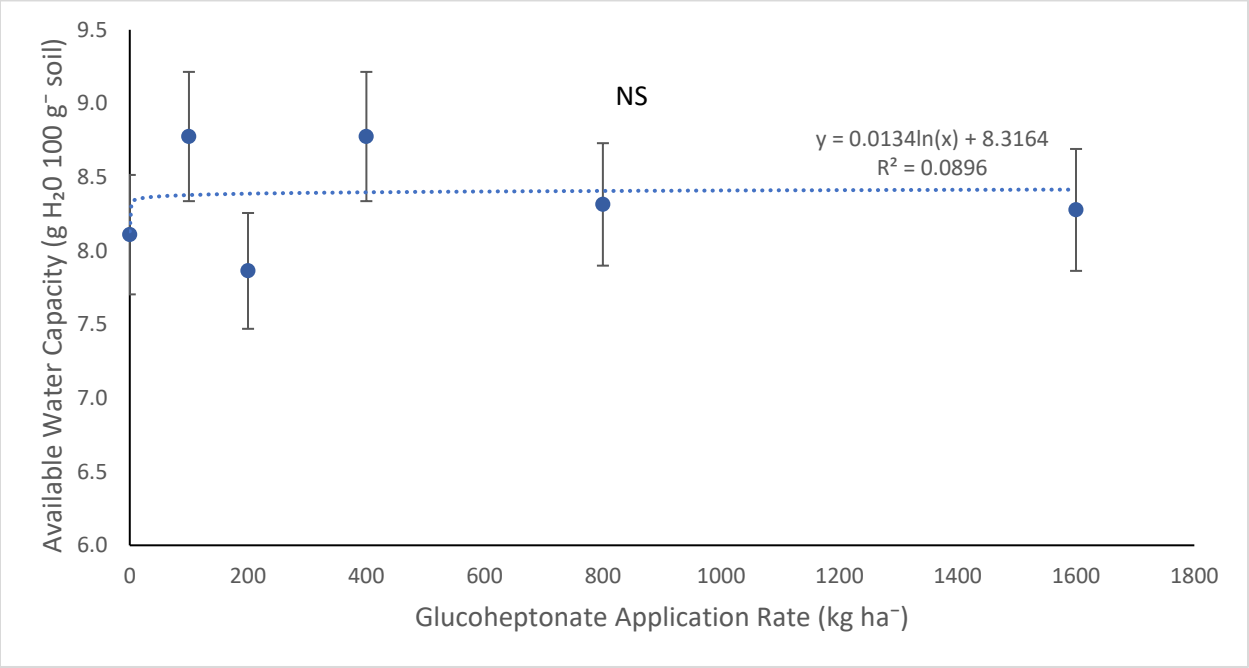

Figure 2-2b. Bojac sandy loam available water capacity response to glucoheptonate. Soil was sterilized by autoclave before treatment was added. Average water held is shown as averages of replications. Means denoted by a different letter indicate significant differences between treatments ($p < 0.05$).

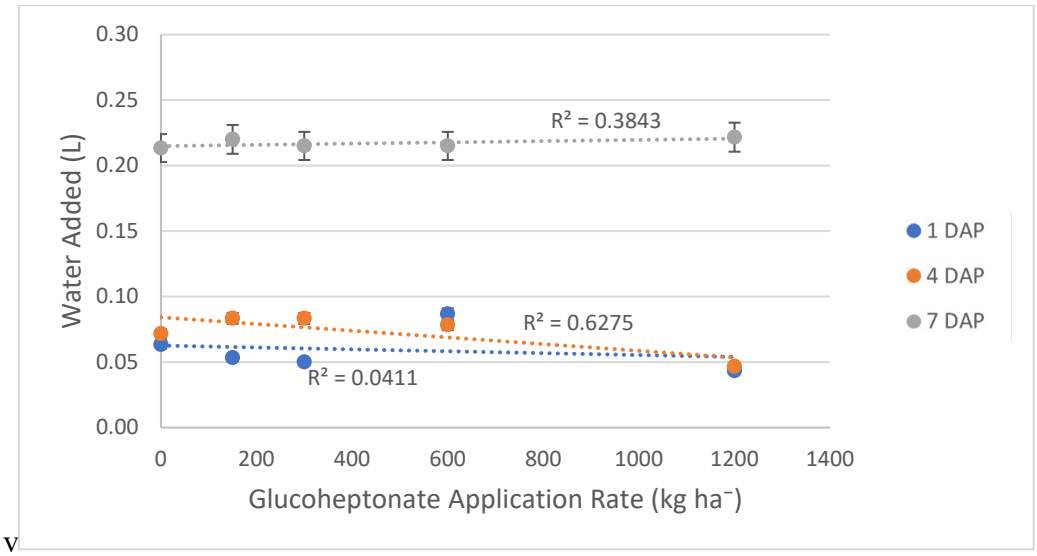

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Figure 2-3a. Amount of water used to bring soil moisture to 80% field capacity for the first 7 days after planting (DAP) in Bojac soil with varying rates of Glucoheptonate added. Water added was recorded on 3 separate days during the 7 day period after planting. 80% field capacity is representative of typical irrigations. 40% field capacity is representative of soils with relatively

low available water. The increase in water added (L) on 7 DAP is largely due to the removal of plastic wrap to reduce moisture loss.

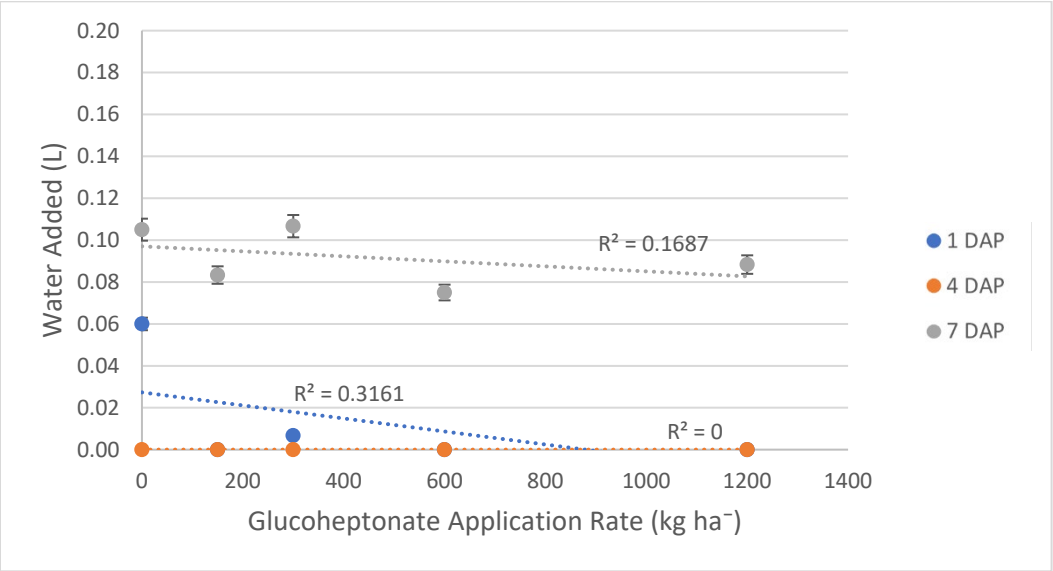

Figure 2-3b. Amount of water used to bring soil moisture to 40% field capacity for the first 7 days after planting in Bojac soil with varying rates of Glucoheptonate added. Water added was recorded on 3 separate days during the 7 day period after planting. 80% field capacity is representative of typical irrigations. 40% field capacity is representative of soils with relatively low available water. The increase in water added (L) on 7 DAP is largely due to the removal of plastic wrap to reduce moisture loss.

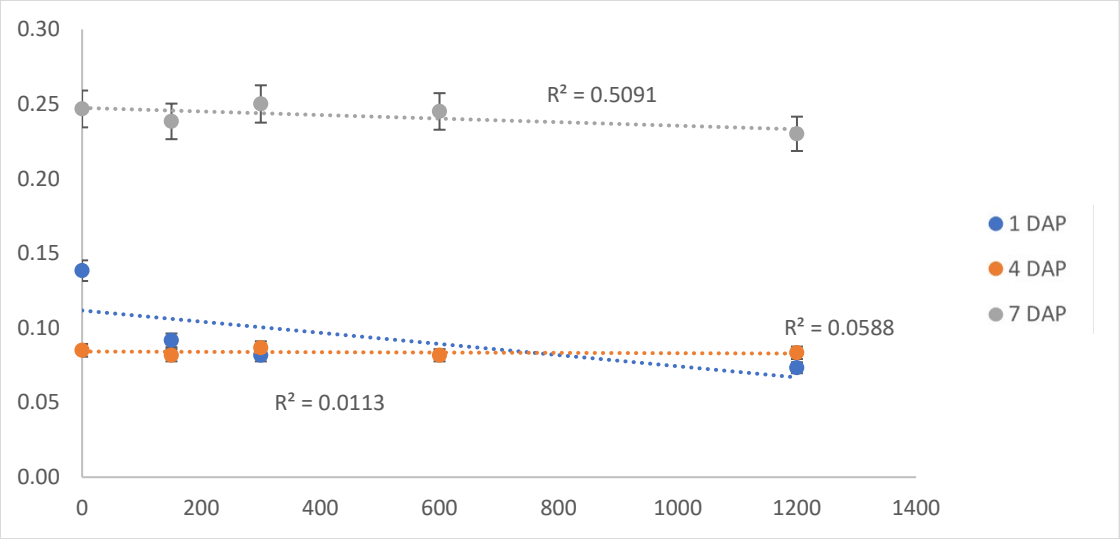

Figure 2 4a. Amount of water used to bring soil moisture to 80% field capacity for the first 7 days after planting in Dragston soil with varying rates of Glucoheptonate added. Water added was recorded on 3 separate days during the 7 day period after planting. 80% field capacity is representative of typical irrigations. 40% field capacity is representative of soils with relatively low available water. The increase in water added (L) on 7 DAP is largely due to the removal of plastic wrap to reduce moisture loss.

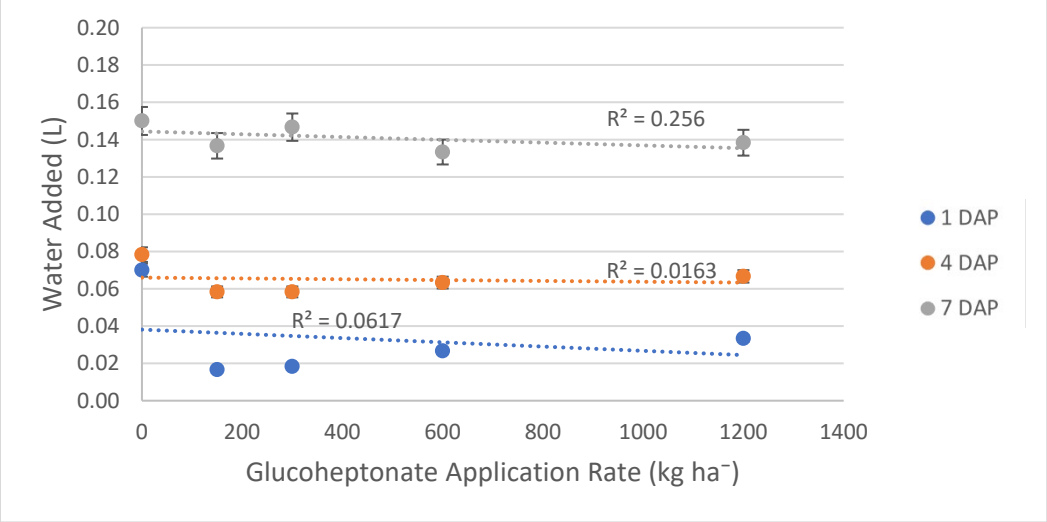

Figure 2-4b. Amount of water used to bring soil moisture to 40% field capacity for the first 7 days after planting in Dragston soil with varying rates of Glucoheptonate added. Water added was recorded on 3 separate days during the 7 day period after planting. 80% field capacity is representative of typical irrigations. 40% field capacity is representative of soils with relatively low available water. The increase in water added (L) on 7 DAP is largely due to the removal of plastic wrap to reduce moisture loss.

Chapter 3: Evaluating Effects of *Bradyrhizobium* and Arbuscular Mycorrhizal Fungi Inoculation on Yield Components of Mung Bean [*Vigna radiata* (L.) Wilczek] and Nitrogen Fixation.

Joshua Mott, A.O. Abaye, Mark Reiter, and Rory O. Maguire

Abstract

Mung beans [*Vigna radiata* (L.) Wilczek] are only inoculated in some production systems, but there is a current lack of knowledge on the best inoculants to use for effective nitrogen fixation (nodulation) and plant yields. The objectives of this study were to determine if dual inoculation of Arbuscular Mycorrhizal Fungi (AMF) and *Bradyrhizobium* (R) provide greater, a.) mung bean yield and quality b.) nitrogen fixation for mung bean and residual soil nitrogen for the following crop, and c.) determine if these effects are consistent across soil types. Field trials were conducted in Blacksburg, VA (sandy clay loam) and Painter, VA (sandy loam) over the summers of 2020 and 2021. There were 5 treatments replicated 5 times for each variety at each site; R, AMF, R+AMF, high Nitrogen (N) (100 kg ha $^{-1}$), and a control, for a total of 25 plots per site. Mung beans grown in Blacksburg in 2020 and 2021 averaged 53.8% more seeds per pod than mung beans grown at the Eastern Shore. Overall yield components (seeds per pod, pods per plant) are heavily influenced by soil type. Dual inoculation significantly increased grain yield (+33%) compared to a synthetic N fertilizer application, but did not significantly increase grain yield compared to the control (+22%). Dual inoculation may increase grain yields of mung beans compared to synthetic fertilizer regime, but does not show evidence of improving N fixation.

Introduction

Mung bean [*Vigna radiata* (L.) Wilczek], also known as green gram or moong, is a warm season pulse legume, with a short growing season (50-70 days) that is relatively drought tolerant and well acclimated to sandy soils with poor fertility (Baath et al., 2018; Kim et al., 2015). Mung bean is widely considered to be native to tropical and sub-tropical regions of India (Kim et al., 2015) but is grown in most tropical and sub-tropical regions as well as in temperate climates. In addition to being a rich source of protein, mung bean is also high in folate, which is an essential nutrient for pregnant women and nursing women (Nair et al., 2013) and is an excellent source of calcium and phosphorus containing 118 mg Ca per 100 g of seed and 340 mg P per 100 g of seed.

Most modern varieties of mung bean typically have an upright growth habit with trifoliate leaves. The seeds are often green but come in a variety of colors ranging from black to yellowish shades. Mung bean can be grown under rain fed conditions, in well drained soils (Al et al., 2011) with optimal temperatures between 20°-40°C (Baath et al., 2018). Phosphorus applications of up to 80 kg ha^{-1} and K applications of up to 90 kg ha^{-1} were shown to significantly increase mung bean nodulation and various yield parameters across multiple varieties and locations (Hussain et al., 2011; Imran et al., 2015). Applications of 60 - 110 kg N ha^{-1} provide enough nitrogen for early plant establishment (Oplinger et al., 1990). The optimal pH for mung bean growth is 6.3-7.2 (Oplinger et al., 1990). Karamany (2006), found that medium-high density planting (30-50 cm row spacing) produced greatest seed yield and harvest index. Row spacing for mung bean can vary from 18 to 100 cm with the highest overall yields being less than 50 cm (Karamany, 2006).

Mung bean cultivar also has a significant effect on yield and nodulation, with hybrids typically out performing local varieties although this effect has not been studied much further (Hussain et al., 2011; Imran et al., 2015). Many studies have (Anjum et al., 2006; Bhuiyan et al., 1970; Diatta et al., 2018) shown that *Rhizobium* inoculation can significantly increase yield components of mung bean such as number of pods, seed weight, and plant residue. Seed inoculation along with P application of 50 kg ha^{-1} has been shown to be the most efficient use of resources and produce greatest grain yields although specific inoculant is not explicitly addressed (Malik et al., 2002). Anjum et al. (2006), found that seed inoculation was more effective compared to soil inoculation when observing yield parameters in mung bean.

Field crop legumes that make up a majority of global production are inoculated by the genera *Rhizobium, Sinorhizobium, Mesorhizobium, Allorhizobium, or Bradyrhizobium* and are referred to as symbionts. Arbuscular mycorrhizal fungi (AMF) also form symbiotic relationships with legumes via similar mechanisms as Rhizobium, collecting mainly P and other nutrients for the plants to use. (Gage, 2009; Suzaki et al., 2019). AMF's are not limited to interactions with legumes, as they have been shown to colonize 80% of plant species (Harrison, 1997). AMF's will typically colonize and extend root systems of plants which could allow for increased overall nutrient uptake (Baird et al., 2010; Mukerji et al., 2012). "The symbiosis enhances the ability of the plant to become established and cope with stress situations (nutrient deficiency, drought, trace element imbalance, soil disturbance), which are typical in desert situations" (Havugimana et al., 2016). Plants that have been colonized by AMF have also shown to improve total plant chlorophyll levels, photosynthesis rates, and transpiration rates (Hayman, 1983).

In order to optimize legume production, producers must continue to encourage the use of plant growth-promoting rhizobacteria (PGPR) or proper 'inoculant'. Some factors affecting

proper nodulation include, soil acidity, water holding capacity of the soil, and soil texture (Diatta et al., 2018; Dommergues & Ganry, 1986). Soil transfer, or residual indigenous rhizobium, is largely considered to be the first method of legume inoculation and early experiments of exogenous soil inoculation saw yields 10 times that of the control (Miller & May, 1991). Applications of both *Rhizobium* and Arbuscular Mycorrhizal Fungi (AMF) have shown to significantly increase grain yield in mung bean production compared to a control (Havugimana et al., 2016).

With proper bio-fertilization (inoculation), nitrogen fixation is enhanced by leguminous crops. N_2 fixing pulse crops can acquire an average amount of 140 kg N ha^{-1}, with about 18-30% of that being left for the next crop depending on soil management, texture, and climate (Kakraliya et al., 2018; Nguyen, 2018). The use of biofertilizers in pulse crop production should be a cost effective and environmentally friendly alternative to synthetic fertilizer production (Mia & Shamsuddin, 2010).

Few field trials have investigated the effect of the dual-inoculation of Rhizobium and AMF on mung bean yield and yield components, while also observing residual soil fertility across two soil types. Legumes like soybean, alfalfa, and various clovers, have proven to be effective high-quality forages for animal production in Virginia. Mung bean has been explored as an alternative forage legume for its low input and stress tolerance. The life cycle of mung bean, being a warm season annual, could be a profitable solution to late summer forage quality gaps found in many cattle production systems that is enhanced by the proposed effects of the dual inoculation of mung bean. We hypothesize that the combination of AMF and *Rhizobium* inoculum in mung bean production will increase the yield components of mung bean while also adding residual N to soil, increasing overall efficiency of production systems.

Methods and procedures:

Experimental Design

Field plots were established at the Eastern Shore AREC and the College Farm in Blacksburg during the summers of 2020 and 2021 in portions of land that had no previous inoculum over the past 7 years. Each field site had an equal number of plots and replications (5) at each site. The variety of mung bean used was "Berken", purchased from Oklahoma Foundation Seed Stocks in Stillwater, OK. "Berken" is widely grown in the United States. Plots were established in a randomized complete block design (RCBD). The experiment utilized 5 treatments: R, F, R+F, high N 100 kg N ha^{-1} (N), and a control (C). Seeds were coated with treatments before planting. Thirty seeds were planted per row with 4 rows per plot application at 3.05m x 3.05m long.

Soil Description

The soil in Blacksburg, VA was well-drained Ross loam soil, with 2% slopes. The Eastern Shore AREC (Painter) site was Bojac Sandy loam soil. The Painter site was previously in tomato production and non-leguminous cover crops while the site in Blacksburg was under cover crop and melon rotation. We did not suspect the presence of foreign symbiotic bacteria due to no legumes in previous rotations and the soil was not tested for any naturally occurring bacteria that would affect results of this study.

Plant Nutritional Analysis

Five plants from each plot were randomly selected and hand harvested once to record grain yields (number of seeds, seed weight, and pods per plant). The same plots used for yield components were analyzed for crude protein content as well as dry-matter above-ground biomass yields, plant height, C:N analysis, and Leaf-N concentration (Karamany, 2006). For dry

matter determinations, plants selected for harvest were oven dried at 50°C to a constant weight and ground for further analysis.

Rhizobium Colonization Determination

Before sowing, mung bean seeds were inoculated with *Bradyrhizobium* inoculum, at a rate of 10 g inoculant kg^{-1} according to package instructions, purchased from Hancock Farm & Seed Co., Inc. (Dade City, FL, USA). Nodulation by *bradyrhizobium* were analyzed by direct observation. Selected plants were excavated and carefully washed at 30 days after sowing and maturity, approximately 100 days after sowing (DAS). Nodules were observed using a magnifying glass (Patterson et al., 1990).

Soil Fertility Analysis

Routine soil analysis was conducted pre-planting and post-harvest to measure N concentrations in the soil. The determination of soil ammonium (NH_4^+) and nitrate (NO_3^-) was made using Lachat QuikChem AE flow-injection autoanalyzer and ion chromatography. The soil samples were prepared using the methods outlined by Bremner & Keeney, 1966. Samples were air dried, ground and passed through a 10-mesh (2-mm) sieve. Mehlich 1 extracted P was determined at a ratio of 5 g soil: 20 ml of extraction solution containing 0.05N HCl and 0.025N H_2SO_4. (Mehlich, 1953). Soil pH was determined using a 1:1 (vol/vol) soil-water mixture (Maguire & Heckendorn, 2011).

Soil physical and chemical property data from locations and both years is displayed in Table 3-1. Soil pH for was considered to be optimal for successful mung bean growth. High soil phosphorus levels were observed at the Eastern Shore for both 2020 and 2021. Levels of soil K, Ca, and Mg were considered to be optimal for efficient mung bean growth.

Statistical Analysis

Statistical analysis was performed using RStudio software (version 3.2.2)(RStudio Team, 2015). Two-way ANOVA was conducted by plot location and cultivar for each of the treatments to investigate soil N and P availability in the soil with significance indicated by p-values < 0.05. Two-way ANOVA was also be performed by plot location and cultivar for plant tissue in order to investigate differences in N and P plant uptake, Nodules counts (30 DAS and 100 DAS), crude protein levels, forage yields, and grain yields across treatments. All treatment differences were investigated using post-hoc Tukey's honestly significance difference (HSD) at a significance level of $p < 0.05$. All yield data collected from the Eastern Shore site during 2020 was excluded due to excessive crop damage and delayed maturity.

Results and Discussion:

Plant Height

The comparison of location on plant height showed that plants grown on the Eastern Shore, were on average taller than plants grown in Blacksburg, in both 2020 and 2021 (Table 3-2). Mungbean plants grown at the Eastern Shore in 2021 (96.9 cm) were on average 16.9% taller than plants grown in Blacksburg during 2020 (83.54 cm) and 24.6% taller than plants grown in Blacksburg during 2021 (77.2 cm) (Table 3-2). These results are consistent with Diatta et al. (2018), who reported differences on mungbean plant height when grown in different soil textures (sandy loam and a loam soil). The significant differences in height, as related to soil texture, may also be attributed to seasonal temperature differences as well as precipitation during the season. Average monthly temperatures in Blacksburg were consistently lower than average monthly temperatures on the Eastern Shore (4-5 ◦C lower). Consistent differences in mung bean physiology were observed by Hanif et al. (2019), noting significant decreases stem length as temperature decreased. Ntukamazina et al., (2017) found that a reduction of precipitation led to

accelerated maturity and decreased plant height. While total precipitation at both locations during both seasons was relatively similar (Table 3-3), the timing of that precipitation may have influenced the overall height of the plants each year. The plants grown at the Eastern Shore during 2021 received 56% more precipitation during July compared to plants grown during 2020 in Blacksburg (150 mm vs 66 mm) and 52% more precipitation than plants grown during June 2021 (121 mm vs 58 mm). Significant differences in precipitation at planting may have influenced overall plant height differences reflected at each location despite soil texture differences.

The location by treatment interaction was statistically significant for plant height (Table 3-4). Plants grown in Blacksburg during 2020 and at the Eastern Shore in 2021 showed no significant difference in plant height when considering treatment, alone (Table 3-3). The height of plants treated with the N fertilizer were, on average, taller than plants grown with the *Bradyrhizobium spp.* inoculant (85.7 cm vs 70.7 cm). These results are contrary to what was found by Uddin (2009), reporting that *Bradyrhizobium spp.* inoculant increased plant height when compared to an N fertilizer treatment. This may be explained by the different rate and source of N fertilizer used in their study and optimal soil nutrient levels when considering soil P, K, Ca, & Mg (Table 3-1) in the Blacksburg 2021 soil.

Dry Biomass

While the overall ANOVA for above ground dry biomass was not significant, treatment effects were observed in 2021 at both the Eastern Shore and Blacksburg locations (Table 3-4). In Blacksburg, the AMF treatment produced, on average, 39.1% more biomass than the control (115.5 g plant^{-1} vs 160.9 g plant^{-1}) and the *Bradyrhizobium* + AMF dual inoculation treatment

produced 38.2% more biomass than the control (159.8 g plant^{-1} vs 115.5 g plant^{-1}). This is consistent with findings from Uddin (2009), reporting that overall plant biomass of mung beans was significantly increased by the use of bio-fertilizers when compared to a control treatment. At the Eastern Shore (2021) location, the *Bradyrhizobium* treatment produced 44.8% more biomass, on average than the nitrogen fertilizer treatment (171.2 g plant^{-1} vs 118.2 g plant^{-1}). While there are significant differences between treatments in some cases, it should be noted that these differences were not consistent across years or location.

Pod Number of seeds/pod

The number of seeds per pod was significantly affected by location (Table 3-4). Mung beans grown in Blacksburg (sandy clay-loam) in 2020 and 2021 averaged 53.8% more seeds per pod than mung beans grown at the Eastern Shore (sandy loam) in 2021, regardless of treatment (Table 3-2). This finding is consistent with results that were reported by Oke & Eyitayo (2010), considering soil texture and fallow effects on cowpea (*Vigna unguiculata*) pod development. They reported an increase in overall pod development in a sandy clay loam over a sandy loam. The difference across soil type may be due to several factors including planting date, higher pH, and greater percentage of exchangeable cations, as seen on table 2. Higher percentages of water retention on the sandy clay loam may also be a factor in the increase in seeds per pod. When considering temperature differences (Table 3-3), Chikukura et al., (2017) reported consistent results noting that a 2-3 degree increase in temperature can significantly decrease overall yield (-13.6%) and inhibit pod formation due physiological stress.

The number of pods per plant was significantly affected by the location of the mung beans (Table 3-4). Mung beans planted in Blacksburg in both 2020 and 2021 produced 68.3% to

490% more pods per plant compared to those planted at the Eastern Shore in 2021 (Table 3-2). The overall increase in pods per plant by location can be attributed to several factors such as planting date, higher pH, and greater percentage of exchangeable cations, as seen on table 1. These results are consistent with previous findings from Oke & Eyitayo (2010) with soil texture and location greatly influencing yield components such as pods per plant.

Soil Nitrogen

Differences in residual soil nitrate at harvest were significantly affected by the location and treatment. Among the treatments at the Blacksburg locations in 2020 and 2021 only the R+F treatment during 2021 produced a significantly smaller amount of nitrate as compared to the rest of the treatments (Figure 3-1). Similar results were found in Australian mung bean production systems, where inoculation resulted in little change to residual soil nitrate differences at harvest as well as %Ndfa (Nitrogen Derived From the Atmosphere) derived from the atmosphere (Herridge et al., 2005). The study suggest that current inoculation practices are not effective enough resulting in poor nodule activity. On average, plots treated with R or R+F, had less residual nitrate in the soil across locations, even though this difference was not statistically significant. Soil nitrate levels at the Blacksburg locations for both years were significantly higher than plots at the Eastern Shore during 2021.

Differences in residual soil ammonia at harvest were only significant when comparing locations (Figure 3-2). Overall levels of soil ammonia were significantly lower in Blacksburg during 2021. Differences in soil texture, nutrient holding capacity, and volatilization rates may have led to this difference in ammonia concentration in the soil. The treatments did not have a clear effect on the ability of mung beans N fixation capabilities.

Grain Yield

Figure 3-3a shows the combined yields from each year and location combined. The dual inoculation treatment produced a similar amount of grain compared to the control, AMF, and R solo treatments. The dual inoculation and *Bradyrhizobium* solo treatment yielded approximately 45% more grain than the urea fertilizer treatment. These results are consistent with previous studies (Dommergues & Ganry, 1986; Ju et al., 2009; Wang et al., 2020) finding that excess N fertilizer does not correlate to increased grain yields and can inhibit the nodulation within legume crops.

The comparison of location on the average grain yield of mungbean was significant. Our data showed that plants grown in Blacksburg in both 2020 and 2021 produced more grain than plants grown at the Eastern Shore in 2021 (Figure 3-3b). The R+F treatment in Blacksburg during the 2020 season produced the max grain yield (3728 kg ha^{-1}) which was 25.9% more than the control and that same year 67.4% more than the AMF treatment alone in that same year. These findings are similar to the grain yield trends for mung bean reported by Havugimana et al (2016) when comparing dual inoculation of rhizobia and AMF to a control and solo treatments. They reported a 24-44% increases in grain yield with the dual inoculation treatment compared to the control and AMF solo treatments.

Conclusions

Overall mungbean yield components (plant height, number of seeds per pod, pods per plant, and grain yield) were heavily influenced by location. Dual inoculation of mungbean with *Bradyrhizobium* + AMF or just *Bradyrhizobium* seems to be a promising agronomic practice for increasing grain yield compared to using a synthetic nitrogen fertilizer but is not statistically an improvement compared to the control. Nitrogen fertilizer amendments seem to inhibit pod

formation and reduce residual soil ammonium at harvest while increasing total biomass. *Bradyrhizobium* inoculation decreased plant height which could reduce lodging and disease formation on plants. These results indicating significant effects of location and treatment on mungbean yield and yield components need to be further analyzed through more field experiments. AMF colonization studies and nodulation studies could add more clarity to these preliminary results.

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Table 3-1. Summary of soil chemical properties at Blacksburg and Eastern Shore sites in 2020 and 2021.

Year	Location	pH	NO_3^-	NH_4^+	P	K	Ca	Mg
			----mg kg^{-1}----		----------mg kg^{-1}----------			
2020	Blacksburg	7.1	14.9	8.7	10.0	88.0	680	180
	Eastern Shore	6.5	16.6	5.0	46.2	136	392	44
2021	Blacksburg	6.9	17.4	5.2	15.2	150	744	189
	Eastern Shore	6.4	5.6	7.1	44.0	130	372	54.0

Table 3-2. Mungbean plant height, dry biomass, seeds per pod, and pods per plant by treatments at Blacksburg 2020, Blacksburg 2021, & Eastern Shore 2021

Location	Control	Arbuscular mycorrhizal fungi	Nitrogen fertilizer	*Bradyrhizobium*	*Bradyrhizobium* + AMF
Plant height (cm)					
Blacksburg 2020	84.7a	83.1a	82.7a	86.4a	80.7a
Blacksburg 2021	73.5ab	77.4ab	85.7a	70.7b	78.5ab
Eastern Shore 2021	97.5a	101.6a	89.1a	91.4a	104.8a
Dry biomass (g)					
Blacksburg 2020	131.8a	141.4a	121.2a	118.6a	124.2a
Blacksburg 2021	115.5b	160.9a	132.9ab	152.4ab	159.8a
Eastern Shore 2021	128.5ab	133.8ab	118.2b	171.2a	134.2ab
Seeds pod $^{-1}$					
Blacksburg 2020	10.8a	10.7a	10.9a	11.2a	11.9a
Blacksburg 2021	9.7a	10.5a	10.2a	10.2a	9.5a
Eastern Shore 2021	7.0a	8.1a	6.5a	6.9a	6.6a
Pods plant $^{-1}$					
Blacksburg 2020	61.8ab	47.2b	49.3ab	62.7ab	70.8a
Blacksburg 2021	20.2a	29.4a	21.9a	37.6a	30.4a
Eastern Shore 2021	12.0a	13.5a	8.2a	16.2a	12.7a

Means within each row followed by different letters are significantly different according to Fisher's protected LSD (α=0.10)

Table 3-3. Monthly rainfall (mm) and temperature (◦C), between June and November at Blacksburg and Eastern Shore sites in 2020 and 2021.

Year	Location	Parameter	June	July	Aug	Sept	Oct	Nov	Total
2020									
	Blacksburg	Rainfall (mm)	127	66	183	117	100	137	729
		Temperature (◦C)	20	24	22	17	13	8	
	Eastern Shore	Rainfall (mm)	84	81	188	163	112	141	768
		Temperature (◦C)	23	28	26	22	17	13	
2021									
	Blacksburg	Rainfall (mm)	58	178	118	184	81	24	643
		Temperature (◦C)	20	22	23	18	15	5	
	Eastern Shore	Rainfall (mm)	121	150	181	84	78	-	613
		Temperature (◦C)	24	26	26	22	19	-	

Table 3-4. Analysis of variance of site, treatments, their interactions, and block for yield and yield components of mung bean and difference in soil N as nitrate and ammonia, from planting to harvest.

Source		Plant Height	Dry Biomass	Seeds	Number Pods	Soil Nitrate	Soil Ammonia	Grain Yield
	df	cm	g plant^{-1}	pod^{-1}	plant^{-1}	mg kg^{-1} plot^{-1}		Kg ha^{-1}
Location	1	0.0001	0.1477	0.0001	0.0001	0.0001	0.0001	0.0001
Treatment	4	0.4583	0.1049	0.9441	0.1045	0.0698	0.5223	0.1484
Location*Treatment	4	0.0734	0.2087	0.9345	0.6030	0.2063	0.2904	0.4107
Block	4	0.3638	0.0624	0.0008	0.5410	0.2578	0.3322	0.3607
Model	13							
Error	61							
C. Total	74							

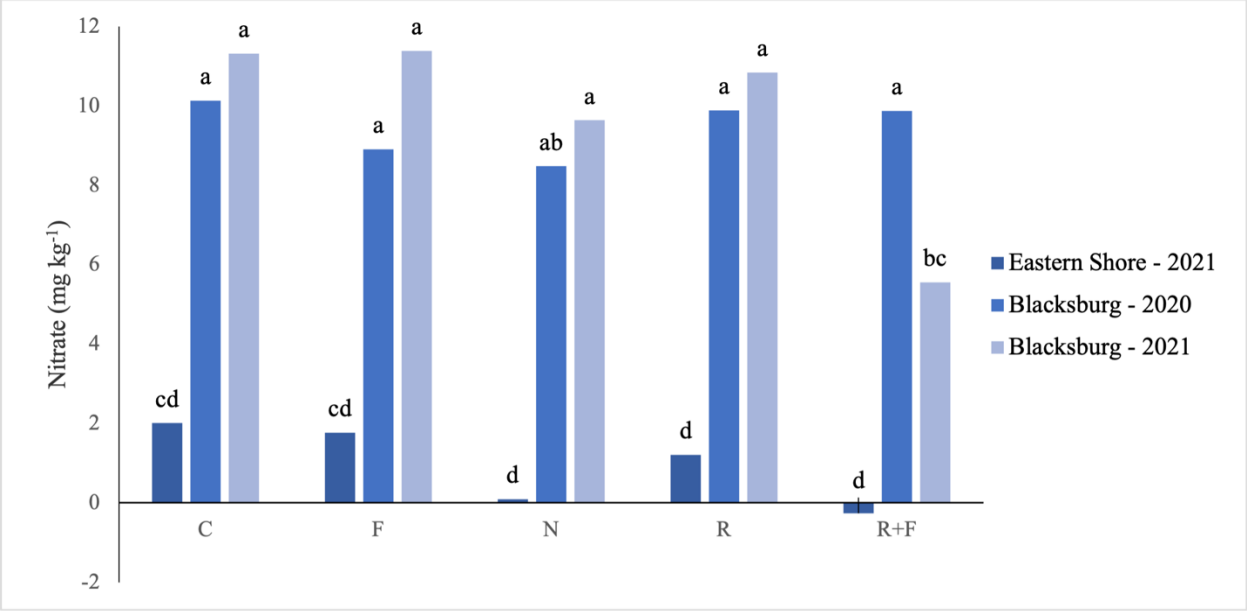

Figure 3-1. Average difference in soil nitrate, from planting date until harvest, across treatments at each location. Treatments are labeled as follows: C = control, F = *Arbusuclar Mycorrhizal Fungi*, N = Urea fertilizer, R = *Bradyrhizobium spp.,* R+F = Dual inoculation with *Bradyrhizobium spp & Arbusuclar Mycorrhizal Fungi*. Treatments connected by different letters are significantly different at α=0.10 according to Fishers protected LSD. (n=75)

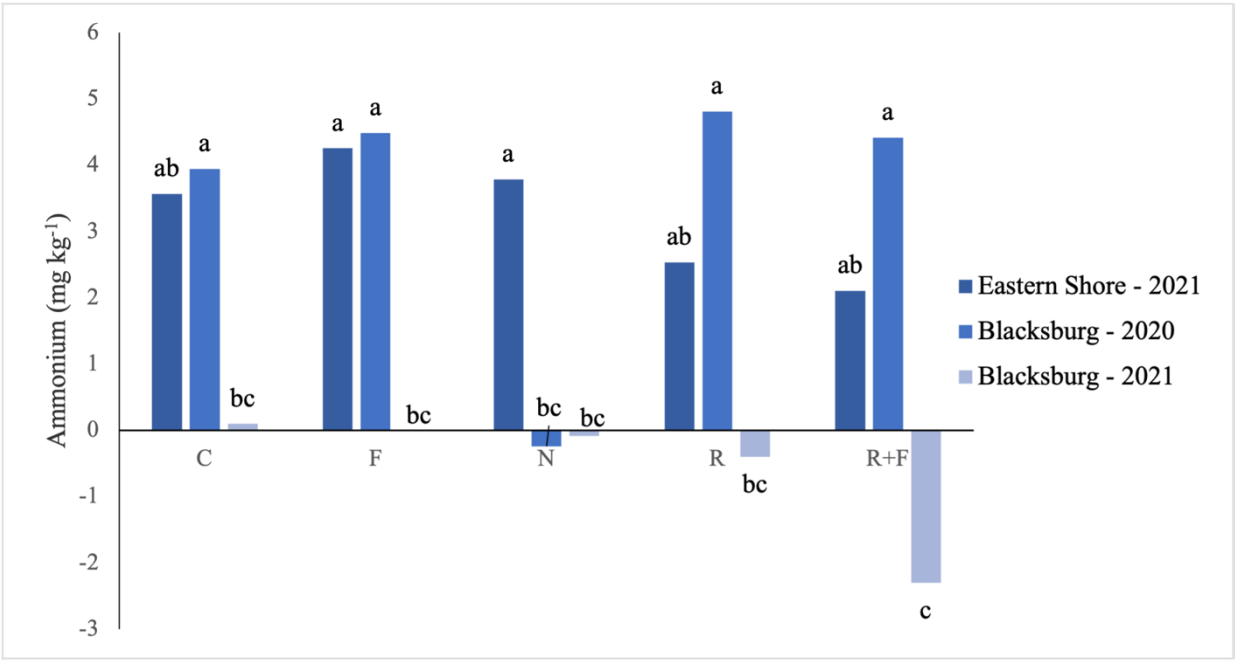

Figure 3-2. Average difference in soil ammonia across treatments at each location, at harvest. . Treatments are labeled as follows: C = control, F = Arbusuclar Mycorrhizal Fungi, N = Urea fertilizer, R = Bradyrhizobium spp., R+F = Dual inoculation with Bradyrhizobium spp &

Arbusuclar Mycorrhizal Fungi. Treatments connected by different letters are significantly different at α=0.10 according to Fishers protected LSD. (n=75)

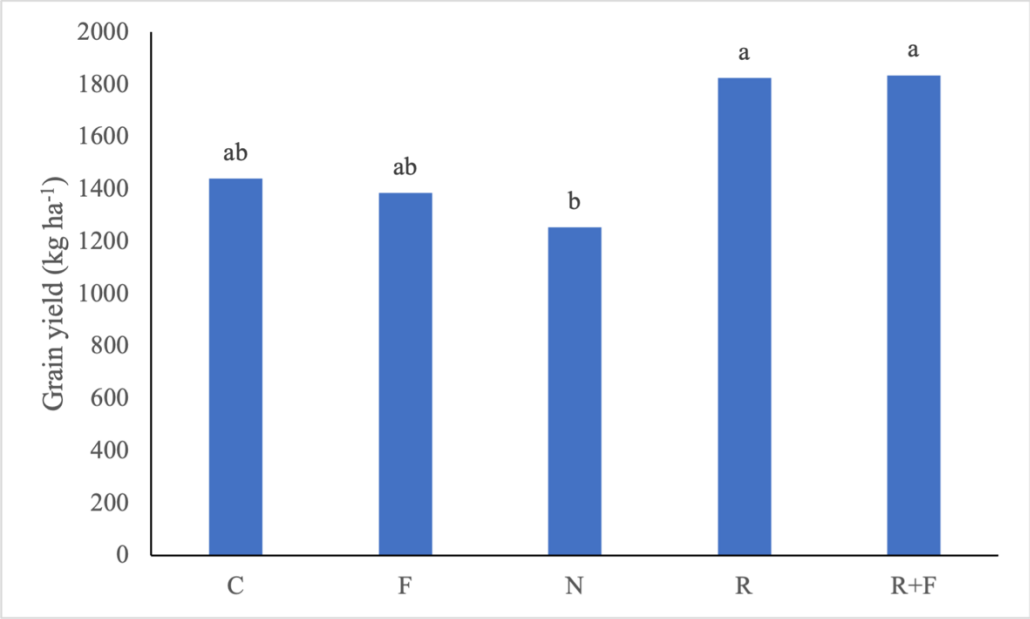

Figure 3-3.1 Average grain yields from both locations and years by treatment. Treatments are labeled as follows: C = control, F = Arbusuclar Mycorrhizal Fungi, N = Urea fertilizer, R = Bradyrhizobium spp., R+F = Dual inoculation with Bradyrhizobium spp & Arbusuclar Mycorrhizal Fungi. Treatments connected by different letters are significantly different at α=0.10 according to Fishers protected LSD. (n=75)

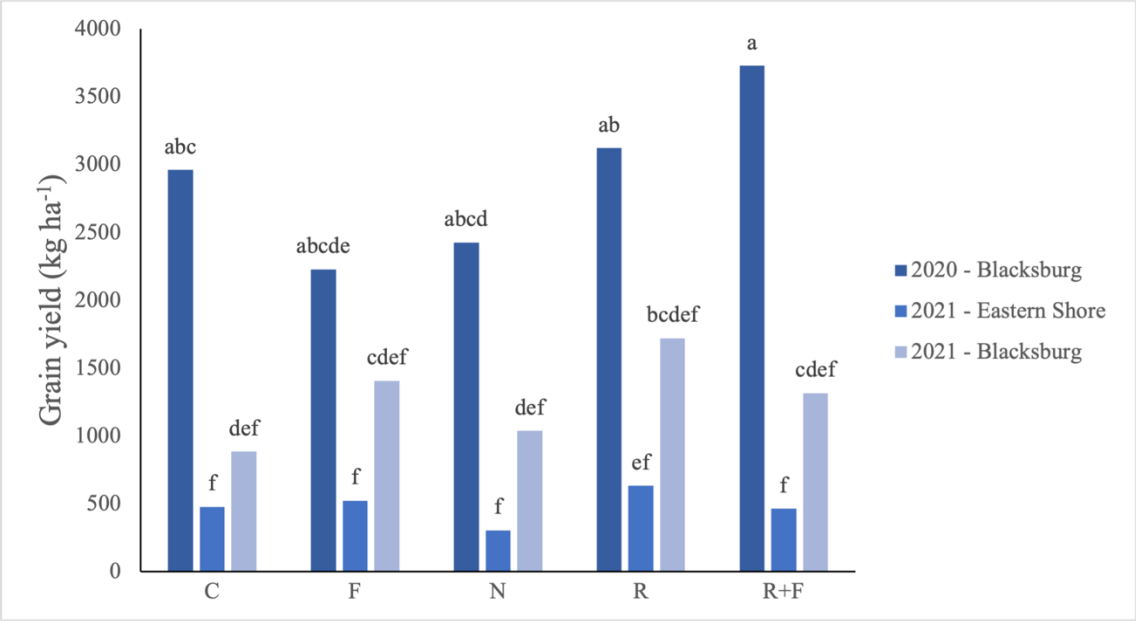

Figure 3-3.2. Grain yields from each location by treatment. . Treatments are labeled as follows: C = control, F = Arbusuclar Mycorrhizal Fungi, N = Urea fertilizer, R = Bradyrhizobium spp., R+F = Dual inoculation with Bradyrhizobium spp & Arbusuclar Mycorrhizal Fungi. Treatments connected by dissimilar letters are significantly different at α=0.10 according to Fishers protected LSD. (n = 75)

Chapter 4: Soil Salinity Effects on Mung Bean Cultivars Inoculated by Brady-Rhizobium spp.

Joshua Mott, A.O. Abaye, and Rory O. Maguire

Abstract

Soil salinization is an ever-increasing issue as climatic changes disrupt global precipitation patterns and general temperature regimes. Mung bean, a popular crop grown in areas subject to saline soils is particularly sensitive to saline soils. The objectives of this study were to evaluate some mung bean cultivars to determine their susceptibility to salt stress and evaluate the effect of inoculation to alleviate this abiotic stress. A greenhouse experiment in Blackburg, VA was conducted, involving 4 cultivars of mung bean, four concentrations of NaCl solution (0, 1.5, 3 and 6 dS m^{-1} NaCl) and two inoculation treatments (inoculated vs uninoculated). Measurements were taken for germination (%), plant height, total biomass, seeds per pod, pods per plant, and days to flowering. Germination was significantly decreased by about 36% at 6 dS/m in all cultivars when compared to the control treatment (0 dS/m). Seed yields, pods per plant and seeds per plant, increased as salt concentration increased. Results indicated that inoculation by *Brady-Rhizobium* spp does not have an effect on any of the variables measured in this study of mung beans subject to moderately saline soil conditions.

Introduction

Of cultivated lands across the globe, 23% (0.34 x 10^9 ha) are considered to have saline soils, having an electrical conductivity of 4.0 mmhos/cm and a pH less than 8.5 (Yang et al., 2007). Salt stress in plants effects physiological and morphological traits such as overall plant growth & yield potential, and can lead to plant mortality (HanumanthaRao et al., 2016; Yang et al., 2007). The accumulations of Na^+, Mg^{2+}, and Cl^- in soil can prohibit adequate water uptake required for proper growth and results in plant injury (Stoytcheva & Zlatev, 2013; Yang et al., 2007). The application of gypsum is a common and efficient method of reclaiming salt affected soils by replacing the sodium ions with calcium ions (HanumanthaRao et al., 2016). Nodulation of legumes is significantly affected by soil salinity, with some plants showing no signs of nodulation at 6 dS m^{-1} (Hanumantha Rao et al., 2016; Zurayk et al., 1998). At higher concentrations, germination, root growth, and shoot length are all detrimentally affected for many plants (Roychoudhury & Ghosh, 2013).

Mung bean (*Vigna radiata L.*) is a leguminous crop commonly grown in India, Southeast Asia, the Middle East, and East Africa. With projected changing climatic conditions, these areas are expected to see major increases in saline soils due to intermittent periods of drought (Corwin, 2021). Mungbean is considered to be a relatively salt sensitive plant and so, much of mung bean breeding research in recent years has been dedicated to exploring cultivars that can tolerate or combat the negative effects of salt stress as well as many other abiotic stressors (HanumanthaRao et al., 2016a; Hapsari & Trustinah, 2018; Salim & Pitman, 1988; Ullah et al., 2016).

Some efforts have been focused on the biological nitrogen fixation ability of some legumes in order to counteract nutrient deficits caused by salt stress in plants. Padilla et al.

(2016) reported that, when inoculated with native strains of *Bradyrhizobium,* cowpea (*Vigna unguiculata*) can see increases in nodulation, plant uptake of N, and plant biomass in a saline soil.

The objectives of this experiment were to evaluate the performance of various mung bean cultivars under saline conditions in order to help increase mung bean production on salt-affected lands. We also wanted to determine if inoculation with *Brady-Rhizobium spp* could alleviate any of the negative effects caused by salt stress in the plants.

Methods and Procedures

Experimental Design

A total of 4 cultivars of mung bean were used in this experiment consisting of one hybrid variety ('Berken') and 3 open-pollenated lines; PLM-869 (L16), TUA-TAWNG (L17), and DES-M-1 (L26). Berken was obtained from the Oklahoma Foundation Seed Stocks (Stillwater, OK, USA) and is commonly grown in the United States. The 3 open pollinated lines were obtained from the U.S. National Plant Germplasm System and have accession origins in India (PLM-869 PI *364044*), Taiwan (TUA-TAWNG PI *371816*), and the Philippines (DES-M-1 PI *425239*). Berken is a hybrid variety with a medium to large green seed, and a 100-seed weight of 7.51 g. L-16 is characterized by a small brown seed and a 100-seed weight of 3.24 g. L-17 is characterized by a medium yellow-brown seed and a 100-seed weight of 6.42g. L-26 is characterized by a medium seed that varies in color and a 100-seed weight of 5.18g.

To study the effect of salinity, soil was saturated with four concentrations of NaCl solution (0, 1.5, 3 and 6 dS m^{-1} NaCl). To study the effect of inoculation upon the cultivars at each salt concentration each cultivar was used either inoculated or without inoculation at each of

the 4 salt concentrations. The treatments were replicated 3 times for a total of 96 pots (4 cultivars x 2 [inoculated/ uninoculated] x 4 salt concentrations x 3 reps). The experiment was setup in a randomized complete block design (Table 4-1).

The experiment was conducted in Blacksburg, VA at Virginia Tech in the greenhouse. Bojac sandy-loam soil (coarse-loamy, mixed, semiactive, thermic Typic Hapludult) was excavated from the Eastern Shore Agricultural Research and Extension Center in Painter, VA at a depth of 20 cm, mixed to ensure uniformity and air dried after collection. The soil was collected from an area that had not been in agricultural production for more than 7 years, to ensure the absence of residual inoculants. Pots (15.24 cm x 16.51 cm) were filled with 4 kg of soil and saturated with NaCl solutions of different concentrations one week before sowing as described by Hapsari & Trustinah, 2018 & Panwar et al., 2016. Each pot was lined with a coffee filter, before filling with soil, and placed over a catch plate to eliminate salt leaching and soil loss, with any collected water being replaced into the pot from which it was lost. Pots were saturated to 70% field capacity and maintained at that level for the duration of the experiment. Greenhouse lights were set for 14 h day^{-1} and the temperature was set to maintain 23 ± 2 °C from planting until maturity.

Two seeds per pot were hand sown at a depth of 2.5 cm on April 16th 2021. Seeds were inoculated with *Bradyrhizobium* inoculum, at a rate of 10 g inoculant kg^{-1} according to package instructions, purchased from Hancock Farm & Seed Co., Inc. (Dade City, FL, USA). Seedlings were thinned to one seedling per pot, 10 days after sowing. If a pot had no germination, then a mung bean seedling of the same treatment from a different block was transplanted into the pot missing a seedling. Total germination percentages by cultivar and treatment were collected. All plants were harvested on the same day, July 6th, 2021, 81 days after sowing. Plant height was

recorded by stretching the plant out straight and measuring from soil level to terminal node. Plants were harvested for seed pods and number of pods per plant was recorded. Days to flowering was recorded throughout the growing period. Roots and above ground biomass were carefully washed and weighed for a wet mass measurement with nodulation being observed. Due to the fragile nature of nodules on the roots many were lost in the washing process and reliable count of nodulation could not be obtained. Plants were then dried at 50°C until a constant weight was reached.

Soil Analysis and Description

Soil analysis was conducted pre-planting (Table 4-2). Soil pH was determined using a 1:1 (vol/vol) soil-water mixture as described by Maguire & Heckendorn (2019). Soil electrical conductivity (EC) was measured, pre-planting, by placing a conductivity meter in to a 1:2 (vol/vol) soil-water ratio (Maguire & Heckendorn, 2019). The EC measurements were taken throughout the growth period by a direct soil EC meter to ensure salinity levels were maintained. The determination of soil ammonium (NH_4^+) and nitrate (NO_3^-) were made using Lachat QuikChem AE flow-injection autoanalyzer and ion chromatography. The soil samples were prepared using the methods outlined by Bremner & Keeney, 1966. Samples were air dried, ground and passed through a 10-mesh (2-mm) sieve. Mehlich 1 extracts for P, K, Ca, and Mg analysis, were analyzed by Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES; CirOS VISION model, Spectro Analytical). Field capacity was determined using gravitation drainage method, where the dried soils were measured to a set weight, saturated, and then allowed to drain for 2 days via holes on the bottom of a disposable cup before being weighed again (Bond et al., 2006).

Statistical Analysis

Statistical analysis was performed using RStudio software (version 3.2.2)(RStudio Team, 2015). Three-way ANOVA was conducted by inoculation, cultivar, and NaCl level to investigate the effects of soil salinity, inoculation and genotype on yield and some morphological factors of mung bean with significance indicated by p-values < 0.05. All treatment differences were investigated using post-hoc Tukey's honestly significance difference (HSD) at a significance level of $p < 0.05$. Fisher's exact test was utilized to determine the relationship between main effects, and their interactions, on germination (%) variance with a significance level of $p < 0.05$. L16 was excluded from analysis due to extremely poor germination regardless of salt concertation and/or inoculation, and no data could be recorded. Multivariate imputation by chained equations (MICE) was utilized to complete missing data variables in plant height, yields, biomass, and days to flowering (Buuren & Groothuis-Oudshoorn, 2011).

Results and Discussion

The chemical and physical properties of the Bojac soil used in this study are shown in Table 4-2. The pH of the soil and levels of plant available macronutrients, K, Ca, & Mg, were considered to be optimal for average mung bean yield goals (Yin et al., 2018) Plant available P in the soil was determined to be optimal for average yield production in mung beans and would not have inhibited proper nodulation in mung beans inoculated by *Brady-rhizobium* spp. (Bhuiyan et al., 2022).

Germination

Salt concentration significantly affected germination percentages of mung bean (Table 4-3). Mung beans grown in a soil with a salt concentration of 6 dS/m had a germination rate of 54% compared to 75% at 0 dS/m, 79% at 1.5 dS/m, and 83% at 3 dS/m (Figure 4-1). Our findings showed that there was not a significant difference there was no significant difference

between cultivars and there was no significant interaction between cultivars and salinity upon seed germination (Table 4-3). Similar rates of germination decline at increasing salt concentrations for mung bean was reported by Ashraf & Rasul (1988). In another germination study between two mung bean cultivars under salt stress, Misra (2004) found differences between cultivar germination rates due to Na^+ ion accumulation in the embryo. Promila & Kumar (2000) reported that this decline in germination may be caused by Na^+ accumulation and K^+ ion leakage, which is an indicator of declining embryo membrane integrity (Cocucci & Cocucci, 1977).

Plant Height at Maturity

Differences in plant height between cultivar was significant (Table 4-3). The maximum height (17.8 cm) was observed for L26, followed by L17 at 13.8 cm and Berken at 11.9 cm. A greenhouse experiment conducted by Diatta et al, (2018) observed differences in plant heights between different cultivars. The main effect of salt concentration on plant height was not significant (Table 4-3). Contrary to our study, several salinity experiments that focused on mung bean morphology recorded significant decreases in plant height due to salt concentration increases, but salt concentrations used vary drastically from 0-30 dS/m. This difference between our findings and previous findings could possibly be due to an increase in the accumulation of osmolytes (metabolites that protect against ion loss under stressed conditions) in certain cultivars as shown by Syeed & Mehar (2011). Syeed & Mehar (2011) found that certain cultivars of mung bean with a higher affinity of osmolytes were not harmed by the salt stress and showed not difference compared to mung beans of the same cultivar grown in a non-saline soil.

Number of Pods per Plant, Seeds per Plant

The number of pods per plant was considerably lower than reported in previous references. The main effect of salt concentration on number of pods per plant was significant (Table 4-3). We recorded an increase in average number of pods per plant as salt concentration increased. At 0 dS/m average number of pods per plant was 1.0 while plants grown in a salt concentration of 6 dS/m produced 1.5 pods per plant. The interaction of cultivar and salt concentration on pods per plant was also significant. L26 did not produce any mature pods for salt concentrations of 0, 1.5, and 3 dS/m but did produce approximately 2 pods per plant at 6 dS/m. For L17, we recorded an increase in pods per plant as salt concentration increased, contrary to previous studies, although these increases were not statistically significant. The cultivar Berken, recorded an average decrease in pods per plant but this trend was not statistically significant (Figure 4-2). Looking at all the metrics collected, the main significant difference between cultivars was the number of pods per plant.

As a result of poor pod formation, the total number of seeds on the plants was low relative to other studies. The interaction of cultivar and salt concentration on seeds per plant was significant. For the cultivar Berken, an increase in salt concentration resulted in an overall decrease in total seeds (Figure 4-3).

Biomass

Plant biomass was significantly affected by cultivar (Table 4-3). Berken (16.9 g) and L17 (16.8 g) produced the same amount of biomass compared to L26 (22.7 g) (Figure 4-4).Similar results were reported by Diatta et al. (2018), HanumanthaRao et al. (2016), Syeed & Mehar, (2011), and Ullah et al., (2016), when using different cultivars. The interaction effect of salt concentration and cultivar on total biomass was not significant. The only significant difference was at 1.5 dS/m between L26 (25 g) and Berken (15 g) (Figure 4-4).

Days to flowering

On average, L26 took the most days to flower (73 days) compared to L17 (56 days) and Berken (59 days) (Figure 4-5). We recorded a decrease in average number of days to flowering at 3 dS/m (58 days) compared to 0, 1.5, & 6 dS/m (64, 65, & 63.5 Days; respectively) (Figure 4-6). In many mung bean varieties, prolonged maturity may lead to a decrease in overall crop yields. In a field experiment conducted by Mondal et al., (2012), reported that mung bean varieties with higher growth rates often showed increases in dry biomass and leaf area, leading to higher yields due to increased photosynthesis and reduction of pest pressures (weed competition, and diseases reduction). The decrease in days to flowering could be physiological response to stressful conditions for the plant as reported by Takeno (2016). In their review, they show how some plants respond to salt stress and other abiotic stresses by switching from vegetative growth to reproductive growth in order to increase success of reproduction. Why this response may be seen in plants grown in in a salt concentration of 3 dS/m and not at 6 dS/m suggests that another unaccounted-for variable may be influencing plant growth.

Conclusion

Our results indicated that mung bean cultivars are susceptible to decreased gemination rates at salt concentrations above 3 dS/m. The findings of this experiment, in contrast to previous experiments, do not suggest that there are significant differences in germination rate when considering cultivar and the salt concentration of soil. Mungbean production systems subject to saline soils should expect to see a decrease in germination regardless of the cultivar planted. Our study indicates, maturity (days to flowering) is not inherently prolonged due to salt concentration and or cultivar. The largest and only significant decrease in days to flowering was seen at 3 dS/m

but not at 6 dS/m suggesting a possible physiological response in mung bean at critical EC levels. This interaction will need to be explored further.

Further experimentation on the physiological response of mung bean to salt stress is needed. This study would improve with collection of data on salt accumulation in the biomass, Na:K ratio analysis, and a more specified germination study, to understand any unaccounted-for effects. Irrigation frequency may have also counteracted the salt stress affects even while the concentrations of salt in the soil remained the same throughout the experiment. Also, the use of multiple imputation for the data set may be inflating the realized values of our variables.

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Table and Figures

Table 4-1. Cultivars, inoculation treatments, and salt concentrations used in this experiment.

No.	Treatments		
	Cultivar	Inoculation	NaCl (dS m^{-1})
1	Berken	Inoculated	0, 1.5, 3, 6
2	Berken	Uninoculated	0, 1.5, 3, 6
3	L16	Inoculated	0, 1.5, 3, 6
4	L16	Uninoculated	0, 1.5, 3, 6
5	L17	Inoculated	0, 1.5, 3, 6
6	L17	Uninoculated	0, 1.5, 3, 6
7	L26	Inoculated	0, 1.5, 3, 6
8	L26	Uninoculated	0, 1.5, 3, 6

Table 4-2. Chemical and physical properties of the Bojac soil.

Soil Property	Bojac Soil
Soil Texture	Sandy Loam
pH	6.45
Field Capacity (%)	11.22
Electrical Conductivity (dS/m)	0.08
Bulk Density (g/cm^3)	1.62
NO_3^-(mg/kg)	2.60
NH_4^+(mg/kg)	9.51
P plant available (mg/kg)	6.40
K plant available (mg/kg)	170.70
Ca plant available (mg/kg)	448.79
Mg plant available (mg/kg)	75.86

Table 4-3. Analysis of variance of the effects of cultivar, inoculation, and salt concentration and their interactions on morphology and yield of mung bean.

Treatments	Germination	Plant Height	No. Pod	No. Seeds	Biomass	Days to Flowering
	%	---cm---	-----plant $^{-1}$-----		g plant $^{-1}$	
	Prob >ChiSq		*Prob > F*			
Cultivar	1.0000	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
Inoculation	0.9999	0.9759	0.8937	0.9781	0.6592	0.663
Cultivar*Inoculation	1.0000	0.7568	0.9993	0.5666	0.8397	0.661
Salt Concentration	**0.0080**	0.8974	**0.0448**	**0.0571**	0.647	**0.0048**
Cultivar*Salt Concentration	0.9878	0.8893	**0.0002**	**<0.0001**	0.7872	**0.0139**
Inoculation*Salt Concentration	0.8490	0.7775	0.8595	0.7998	0.2925	0.2512
Cultivar*Inoculation*Salt Con	0.4858	0.6669	0.9947	0.998	0.4805	0.1063

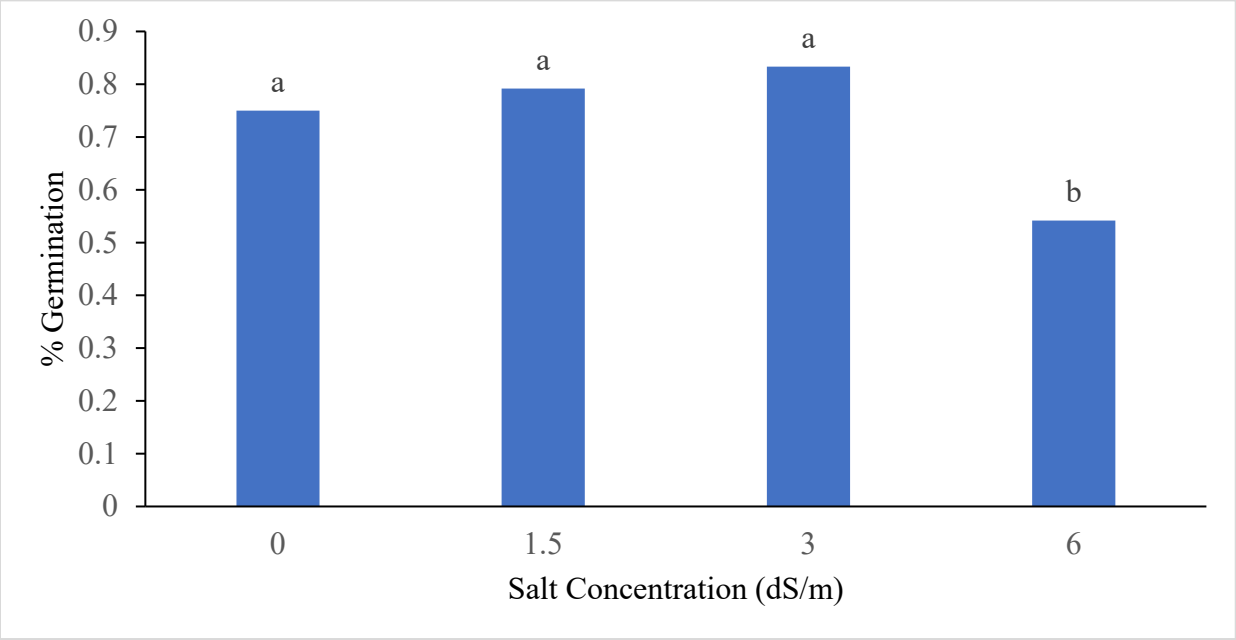

Figure 4-1. Percent (%) germination of all mung bean cultivars tested as related to salt concentration of soil. Treatments with the same letter are not significantly different according to Tukey's HSD (α = 0.05) n = 72

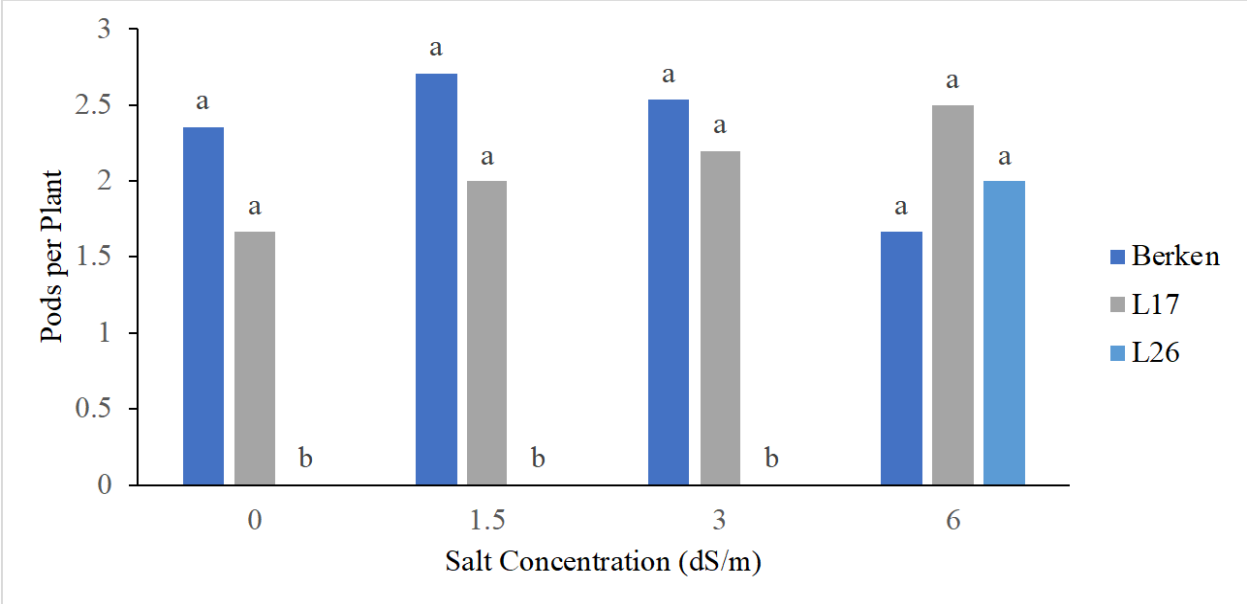

Figure 4-2. Effect of salt concentration and cultivar on the number of pods per plant of mung bean. Treatments with the same letter are not significantly different according to Tukey's HSD (α = 0.05). n = 72

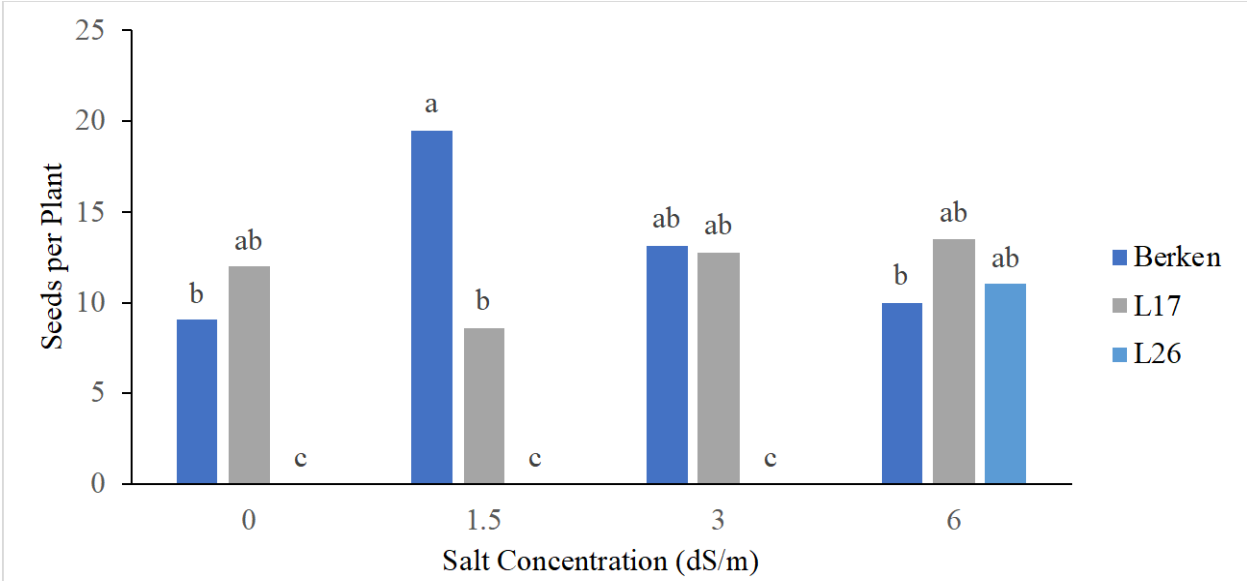

Figure 4-3. Effect of salt concentration and cultivar on the number of seeds per plant of mung bean. Treatments with the same letter are not significantly different according to Tukey's HSD ($\alpha = 0.05$). n = 72

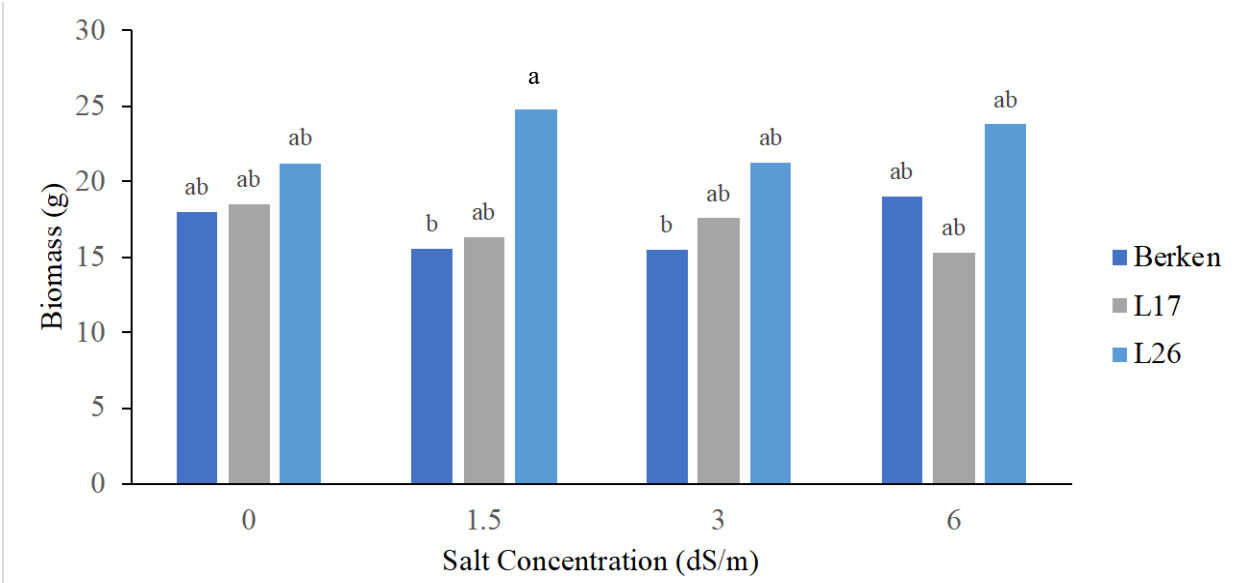

Figure 4-4. Effect of salt concentration and cultivar on total biomass (above and below ground) of mung bean plants. Treatments with the same letter are not significantly different according to Tukey's HSD ($\alpha = 0.05$). n = 72

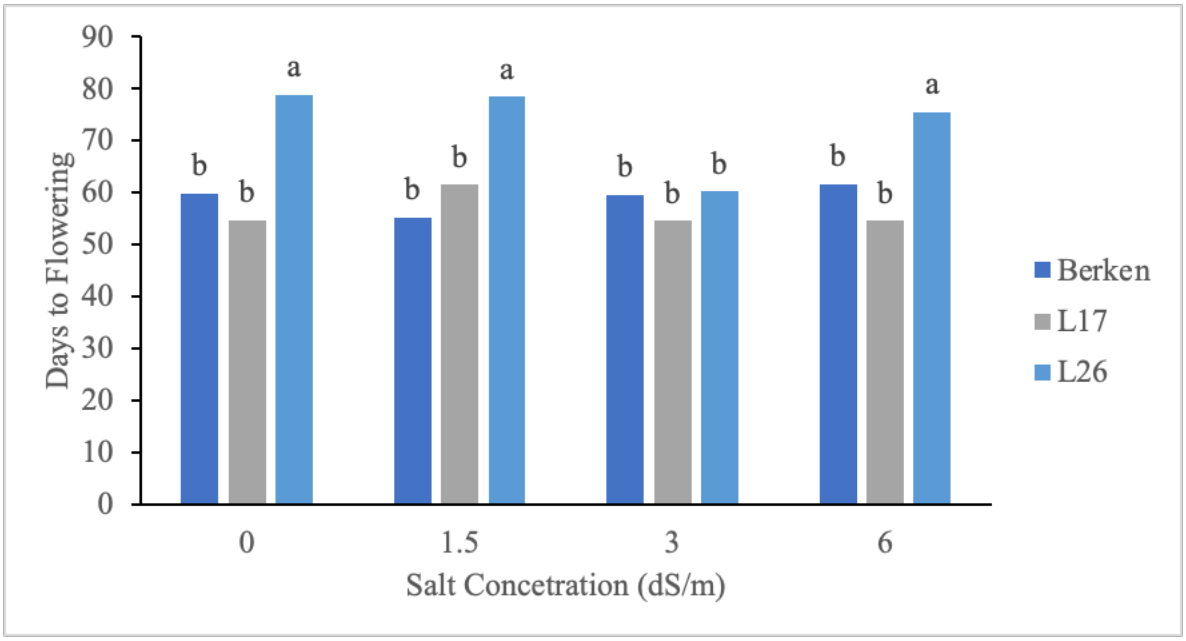

Figure 4-5. Effect of salt concentration and cultivar on number of days to flowering of mung bean. Treatments with the same letter are not significantly different according to Tukey's HSD (α = 0.05). n = 72

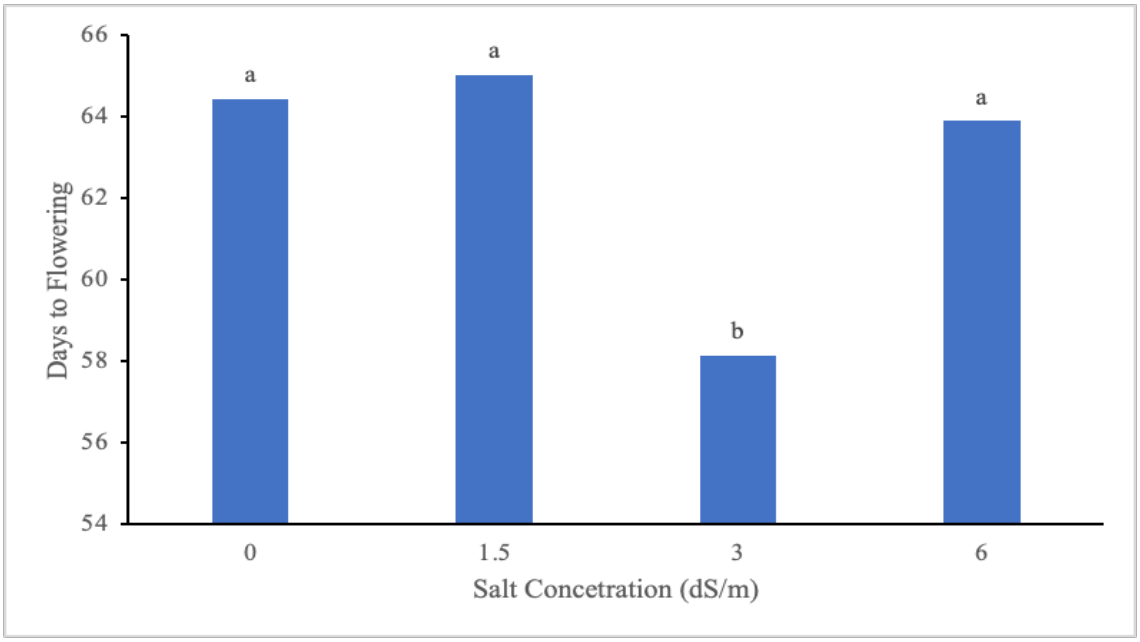

Figure 4-6. Effect of salt concentration on number of days to flowering of mung bean. Treatments with the same letter are not significantly different according to Tukey's HSD (α = 0.05). n = 72