GROWTH, NODULATION, AND YIELD OF MUNGBEAN (*Vigna radiata* L. (Wilczek) AS AFFECTED BY THE COMBINED USE OF BIO-CHEMICAL FERTILIZERS AT HAWASSA, SOUTHERN ETHIOPIA

MSc THESIS

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DEDICATION

I dedicate this thesis to my beloved family for nursing me with affection and unconditional love.

STATEMENT OF AUTHOR

I declare that this thesis is a result of my real work and that all sources of materials used for this thesis have been duly acknowledged. This thesis has been submitted in partial fulfillment of the Degree of Master of science. The thesis is deposited at the library of the University to be made available to borrowers in accordance with the library rules. I also declare that this thesis is not submitted to any other institution anywhere for the award of any academic Degree, Diploma, or Certificate. Brief quotations from this thesis are allowable without special permission provided that accurate acknowledgment of the source is made. Request for this manuscript in whole or in part may be granted by the head of the major Department or the Dean of the School of Graduate Studies when in his or her judgment the proposed use of the material is in the interest of scholarship. In all other instances, however, permission must be obtained from the author.

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ABBREVIATION AND ACRONOMYS

ANOVA	Analysis of Variance
ATP	Adenosine Tri Phosphate
BNF	Biological Nitrogen Fixation
BS	Bio-slurry
CEC	Cation Exchange Capacity
CIMYT	International Maize and Wheat Improvement Center
CSA	Central Statistical Agency
EC	Exchangeable Cation
ECEX	Ethiopia Commodity Exchange
EEPA	Ethiopian Export Promotion Agency
EIAR	Ethiopian Institute of Agricultural Research
EMA	Ethiopian Meteorology Agency
EPP	Ethiopia Pulse Production
ETB	Ethiopian Birr
FYM	Farmyard Manure
LAI	Leaf Area index
LSD	Least Significant Difference
NMA	National Meteorology Agency
NP	Nitrogen and Phosphorus
NPK	Nitrogen, Phosphorus and Potassium
OM	Organic Matter

pH	Power of Hydrogen Ion Concentration
Pn	Photosynthesis
PSB	Phosphorus Solubilizing Bacteria
RCBD	Randomized Complete Block Design
SADAFF	South African Department of Agriculture, Forestry and Fisheries
SAS	Statistical Analysis of Software
SNNPR	Southern Nations, Nationalities and Peoples Region

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ABSTRACT

Mungbean (Vigna radiata L. (Wilczek) is a short duration, legume crop cultivated for consumption, industrial processing, with a potential for alleviation of climate change effects on crop production due to its biological nitrogen fixing ability. Integrated use of bio-chemical fertilizers is beneficial in achieving sustainable crop yield improvement and sole dependency on chemical fertilizer is not recommendable due to its unaffordable cost and contribution to climate change. Hence, this experiment was conducted under field condition in the 2019/20 main cropping season at Hawassa University experimental site to determine the combined effects of bio-chemical fertilizer sources on the growth, nodulation, and yield of mungbean. The factorial combinations of four bio-slurry levels (control, 50, 100, and 150%) and four inoculant/N fertilizer treatments (control, 23kg N ha^{-1}, strain MB-001, and 23kg N + strain MB-001) were laid out in a randomized complete block design with four replications. Data on growth, nodulation, gas-exchange, and yield parameters were collected following the standard procedures. The results revealed that the interaction effects of bio-slurry and inoculant/N fertilizer had a marked effect on phenology, nodulation, and yield parameters. Combined application of bio-slurry and inoculant/N fertilizer delayed days to flowering and days to physiological maturity. Rhizobium inoculation alone and applying with 100% bio-slurry resulted in significant improvement in nodule number, nodule dry weight, and nodule diameter of mungbean. Similarly combined application of bio-slurry and inoculant/N fertilizer had shown a significant impact on a hundred seed weight, above ground biological, grain, and straw yields. For instance, the application of 150% bio-slurry with 23 kg N ha^{-1} resulted in the highest biological and straw yield whereas the application of 100% bio-slurry with inoculant resulted in marked improvement of seeds pod^{-1}and grain yield. Pod number plant^{-1} and hundred seed weight were highest when 100% bio-slurry was applied. The highest net benefit with the highest marginal rate of return was obtained from the combined application of 50% bio-slurry with 23kg ha^{-1} N fertilizer followed by 100% bio-slurry with Rhizobium inoculation. From this result, it can be concluded that the application of 50% bio-slurry ha^{-1} in combination with 23kg ha^{-1} N fertilizer can be recommended for mungbean production in the experimental area and areas having similar agroecology and socio-economic status.

Keywords: Bio-slurry, Mungbean, Nitrogen fertilizer, *Rhizobium* inoculation.

1. INTRODUCTION

1.1. Background and Justification

Mungbean (*Vigna radiata* L. (Wilczek) is a short duration, self-pollinated diploid legume crop which belongs to the family Fabaceae and the genus *Vigna* subgenus Ceratotropis. It is one of the most important crops grown in several tropical and sub-tropical parts of the world (Kumari *et al*., 2012; Khan *et al*., 2012). It is Indian origin as evidenced by its occurrence at archeological sites in the continent and the world's largest production comes from India (Singh *et al*., 2011).

Mungbean is an interesting crop from the consumer, farmer, and processor point of view. For the consumer, mungbean is noted for its protein and lysine-rich grain, which supplements cereal based diets (Minh, 2014). The seeds, sprouts, and young pods are all consumed and provide a rich source of amino acids, vitamins, and minerals (Somta and Srinives, 2007). The grain contains 24.2% protein, 1.3% fat, and 60.4% carbohydrate (Hussain *et al.,* 2011). It improves a farmers' field soil fertility by fixing atmospheric N through symbiosis with bacteria and its green plants used as a source of animal feed. It has also a special importance in the intensive crop production system because of its short growing period. For the processor, mungbean provides many possibilities such as canned or frozen seeds and pods (Mogotsi, 2006).

In Ethiopia, the smallholder farmers grow mungbean in drier marginal environments. Locally, it is known as "*masho*", and grown two times in a year covering 27,085.92 ha and a production level of 27,898.5 t ha^{-1} per year with an average yield of 0.75 t ha^{-1} (CSA, 2016/2017). Studies and reports showed that the yield of mungbean is very low in Ethiopia as compared to other countries of the world especially concerning soil fertility apparently due to limited amendment through the application of fertilizers (Asrat *et al*., 2012; EEPA, 2004). Hence, there is a scope for improving the production potential of this crop by integrated fertilizer use including bio-slurry and *Rhizobium* inoculation.

The use of organic wastes such as crop residues, manures, and compost has a large potential for improving soil productivity and crop yield through the improvement of the physical, chemical, and microbiological properties of the soil as well as nutrient supply (Sinha *et al*., 2011). The fermented slurry, called bio-slurry, is the by-product of methane and heat production coming from organic wastes and it has the ability to deliver nutrients especially nitrogen (N) in the form of ammonium which is available for plants (Makadi, 2012; Karki, 2006). Hence, its application to the soil maintains a higher status of N in both organic and inorganic N- fractions. Mineralization of organic N was superior in liquid bio-slurry compared to dried bio-slurry (Möller and Müller, 2012). Lower N mineralization in dried bio-slurry could be due to poor diffusion of colloidal material increasing resistance following microbial decomposition (Gurung, 1997).

Bio-fertilizers, a component of integrated nutrient management and are considered to be a cost-effective, eco-friendly and renewable source of non-bulky, low cost plant nutrient supplementing fertilizers in sustainable agriculture systems in the world (Rana *et al*., 2013). Hence, the use of bio-fertilizers has currently attained a special significance in crop production, and tremendous success has been achieved in several crops (Seema *et al.,* 2018).

Nitrogen is a major essential element for all organisms, and a constituent of proteins, nucleic acids and other indispensable organic compounds. Although N is highly abundant (about 78% by volume) in the air, its concentrations in soil, crust rocks, and seawater are relatively low, and the availability of N is often a limiting factor for plant growth in natural habitats as well as agricultural crop production (Ohyama, 2010). In this regard, optimal use of N improves dry matter, especially the economic parts of the plant (i.e. flowers and fruits). However, N availability to plants depends on the source, soil type, and environmental conditions, which may affect crop performance.

Therefore, a crop's N supply should be synchronized to its demand. N losses are observed in every type of soil and management of such losses should always be a top priority when considering the study of N supply to crops (Salazar *et al*., 2011). Knowledge about the integrated use of bio-chemical fertilizers could enable the development of new agricultural approaches for improving N management and contribute to developing models of sustainable agriculture (Sáez *et al*., 2012).

1.2. Statement of the Problem

The major problems of developing countries in the area of crop production are low productivity which attributes to many factors. Among others use of a proper type of fertilizer as well as the combined use of organic and inorganic fertilizer for economic feasibility and environmental safety is low. The dependency on inorganic fertilizer should be minimized due to the high cost, the release of greenhouse gases and loss due to ineffective application (Haile, 2012; Ferguson and Boyd, 2002). The sole dependency on chemical fertilizer is not recommended particularly for small-scale farmers who have options of using organic sources of fertilizer. Also, the bulkiness and unavailability of organic fertilizers is a problem for the sole use of it (Warnars and Oppenoorth, 2014). The integrated use of organic wastes and chemical fertilizers is the most feasible option to smallholder farmers and beneficial in improving crop yield as well as soil physic-chemical proprieties such as soil pH, organic carbon, and available N, P, and K in sandy loam soil (Rathour *et al*., 2015). Moreover, optimizing the combined use of inorganic and organic fertilizers could be a better option to balance high productivity and environmental and economic sustainability.

The symbiosis between *Rhizobium* and legumes are a cheaper and usually more effective agronomic practice for ensuring an adequate supply of N for legume based crop and pasture production than the application of fertilizer-N. Since chemical fertilizers pollute soil, water and crops, conscientious agricultural producers seek alternatives to chemical fertilizers. Biological fertilizers are considered as a reliable alternative for improving soil productivity and plant growth in sustainable agriculture.

So far, several researches have been conducted dealing with the response of mungbean to chemical fertilizer application. However, the response of the crop either to singular or combined application of bio-chemical fertilizers and, its advantage for economic and environmental sustainability is scanty, and even for those practicing the same, the research recommended rate is lacking for the study area. Therefore, the overall aim of this study was to evaluate the growth, nodulation and yield response of mungbean to bio-chemical fertilizer application at Hawassa, southern Ethiopia.

1.3. Specific Objectives

- To assess the effect of bio-slurry and inoculant/N fertilizers and their interaction on growth, nodulation, and yield performance of mungbean.
- To examine the yield and yield components response of mungbean to the combined application of bio-slurry and inoculant/N fertilizer application.
- To determine the economic feasibility of the bio-slurry, inoculant and mineral N fertilizer application for mungbean production at Hawassa, Ethiopia.

2. LITERATURE REVIEW

2.1. Origin, Distribution and Botany of Mungbean

Mungbean (*Vigna radiata* L. Wilczek) is thought to have originated from the Indian subcontinent where it was domesticated as early as 1500 BC. Cultivated mungbean was introduced to southern and eastern Asia, Africa, Austronesia, the Americas, and the West Indies. It is now widespread throughout the Tropics and is found from sea level up to an altitude of 1850 m. It is known by different common names like green gram, golden gram, moong, Chickasaw, Oregon pea, and chop suey bean and this legume has a strategic position in Southeast Asian countries for nutritional security and sustainable crop production (Lambrides *et al*., 2006; Mogotsi, 2006).

Mungbean is an annual crop, cultivated mostly in rotation with cereals. It is an erect plant that is highly branched and is about 60 to 76 cm tall (Oplinger *et al.*, 1990). The optimum temperature range for growth is between 27 and 30 °C. This means that the crop is usually grown during summer. Seed can be planted when the minimum temperature is above 15 °C. Mungbean is responsive to daylight length. Short days result in early flowering, while long days result in late flowering. However, mungbean varieties differ in their photoperiod response. It is considered to be heat and drought tolerant. Adequate rainfall is required from flowering to late pod fill for purposes of ensuring good yield. Late plantings that result in flowering during the high temperature-low moisture periods will reduce yield. Mungbean does best on fertile, sandy loam soils with good internal drainage and a pH in the range of 6.3 and 7.2 (SADAFF 2010; Oplinger *et al.*, 1990).

2.2. Mungbean Production in Ethiopia

Mungbean is a recently introduced crop in Ethiopian pulse production and grown in drier and warmer areas of the country (Itefa, 2016; EPP, 2004). It has been produced mostly in the northeastern part of the Amhara region (North Shewa, Oromiya special zone, and Southern Wollo), SNNPR (Gofa, Konso, South Omo, Konta), pocket areas in the Oromiya region (Hararge), and in some woreda's of Benishangul Gumuz regional state (ECX, 2014; Keatinge *et al*., 2011). The average yield of the crop is limited to 0.6 – 0.8 t ha^{-1} due to different reasons (EPP, 2004).

Ethiopia Commodity Exchange (ECX) announces the debut of a new commodity, green mungbean, on its trade floor. Green mungbean is the sixth product that ECX is trading next to coffee, sesame, white pea beans, maize, and wheat. Despite its growing demand in the international market, there is a chronic supply gap in the country even if its export has grown slightly from time to time (EPP, 2004).

2.3. Nitrogen Nutrition Requirement of Mungbean

Nitrogen is an important major nutrient element for the plant. For legumes, it is more useful because it is the main component of amino acids as well as proteins. An adequate supply of N is essential for normal growth and yield. Despite the 78% abundance of N in the atmosphere, plants are unable to use it directly because it is present in an inert form (N_2), and then in the soil, it is lost through microbial denitrification, soil erosion, leaching, chemical volatilization and removal of nitrogen containing crop residues from the land (Aziz *et al*., 2016).

But the use of biological nitrogen fixation (BNF) in the form of *Rhizobium* inoculants in grain legumes can be an alternative to nitrogenous fertilizer (Aziz *et al*., 2016; Chowdhury *et al*., 1998). Without N it is not possible to synthesize the necessary proteins, enzymes, DNA, and RNA required in virtually all plant cells for their initial development, sustained growth, and functioning to support other tissues of the plant (Mozumder *et al.,* 2003).

Mungbean can fix atmospheric N for its use and also improves soil fertility through residual carry over in association with *Rhizobial* strains. To achieve the maximum yield of legume crops and for soil improvement, the crop should be inoculated with suitable *Rhizobial* strain before planting. Thus inoculation of mungbean with *Rhizobium* increased plant height, leaf area, photosynthetic rate, and dry matter production in mungbean (Thakur and Panwar, 1995).

2.4. Biological Nitrogen Fixation

Biological nitrogen fixation (BNF) is one of the most important processes for the ecosystem to access available N for all living organisms. Although N_2 consists of 78% of the atmosphere, the triple bond between two N atoms is very stable, chemically inert, and cannot directly assimilate by plants. Plants absorb most of N in the NH_4+ and NO_3- forms (Dakora, 2008). The crop plants cannot utilize N in its elemental form (N_2) for their growth and development and hence need conversion to a usable form. Only limited numbers of prokaryotes can convert the N_2 molecule into a usable form of N through a process known as BNF (Allito *et al*., 2015).

Plants obtain N either from N-fixing bacteria when the bacteria die and release N to the environment, or when the bacteria live in close association with the plant. In the process of N_2-fixation legume plant supplies the carbohydrate for bacterial growth while the bacteria fix atmospheric N_2 into NH_4+, to convert into amino acids that can be used by the plant to synthesize proteins for its growth and development (Wondwosen *et al*., 2017). This interaction between plant and bacteria leading to the initiation and development of the root nodules.

In the global N cycle, BNF becoming more attractive and economically viable N inputs, substitutes for inorganic fertilizers of N for resource poor farmers and an environmentally friendly agricultural input (Workneh *et al*., 2012). Zahran (2009) described that the ability of many legumes to form associations with bacteria that fix atmospheric N (the symbiotic association that improves growth) is thus a big matter of ecological and economic interest. Besides, the symbiosis is initiated through the legume root infection by the *rhizobia* and formation of root nodules where BNF occurs through the action of a bacterial enzyme, called “Nitrogenase” (Masson-Boivin *et al*., 2009).

The nitrogenase enzyme is a biological catalyst, which is present in the bacteria and mediates the reaction. Nutrient uptake by plants depends largely on the amount, concentration, and activities in the rhizosphere as well as the capacity of the soil to replenish the nutrients in the soil solution (Makoi *et al*., 2013). In addition, *rhizobia* inoculation increased the uptake of P, potassium (K), calcium (Ca), and magnesium (Mg) in the plant parts attributed to the increased pH of the soil. Moreover, *rhizobia* inoculation have been reported to improve plant nutrient such as P by mobilizing inorganic and organic P from organic and inorganic sources in the soil rhizosphere (Saharan and Nehra, 2011).

Nodulation and N fixation are vital methods of providing N to the agricultural system. However, both processes are adversely affected due to environmental conditions. An environmental factor that negatively influences either the growth of *rhizobia* or the host plant itself has a dramatic impact on symbiotic N_2-fixation. It can negatively influence the nodulation process and indirectly affect N_2-fixation, or directly influence plant growth and vigor during post nodulation events and so affect the efficient functioning of the nitrogenase enzyme complex (Mulissa and Fassil, 2012). During the formation and emergence of root hairs, N_2-fixation affected by many factors such as the presence and density of nodulating bacteria in the root zone, the physical and chemical properties such as humidity, temperature, the salt concentration in the soil, pH levels and deficiencies of several mineral nutrients (AbdulJabbar and Saud, 2012).

2.5. Status of Bio-slurry Utilization as Fertilizer Source in Ethiopia

In Ethiopia, bio-slurry usage as alternative organic fertilizer is after biogas introduction as an energy source in about 1970; even if on a fragmented approach (Kidane *et al*., 2007). There is a little attempt in the utilization of bio-slurry as a fertilizer to improve crop production and maintain the physical and chemical properties of soil. However, the utilization of bio-slurry varies from region to region. Except in a few households, bio-slurry in urban areas is not mostly utilized but rather discarded to wastelands especially in Debre-Zeit and Butajira towns. However, in recent years the local energy expert in Meskan in collaboration with Butajira municipality has started to collect bio-slurry from these households for the greening project of Butajira town (Getachew, 2011).

Another story told by Worku Sima from Yetebon kebele in Meskan woreda expressed bio-slurry as ‘glucose’ for his khat (*Chata edulis*) plot. In the Oromiya region, the majority of farmers use bio-slurry for crop production after composting. However, some model farmers like Beyene Tadesse in Hitosa and Delelegn Girma in Lode Hitosa woreda use bio-slurry not only for crop production but also for horticulture, forage, and pasture. In crop production, Beyene was able to produce 4700 kg of wheat per hectare as compared to 3200 kg he produced on average using chemical fertilizer (Getachew, 2011).

Although there are some promising stories told by farmers there is still a lack of enough empirical data to substantiate the stories and preliminary results from a few trials. In southern nation's nationalities and peoples region (SNNPR) rural areas of Meskan and Arbaminch woreda, bio-slurry is used in liquid form predominantly for the production of fruit trees such as banana, mango, khat, orange, and other vegetables. Terefe Mekuriya, the resident of Lente kebele in Arba Minch Zuria woreda, informed that he tried to conduct a trial to compare the effect of bio-slurry and compost on his banana plot. According to the results he observed, bio-slurry was very effective in making the banana plant greenery within a short period as compared to compost (Geremew, 2017).

2.6. Nutrient Contents and Factors Affecting Nitrogen Availability in Bio-slurry

Bio-slurry is a by-product of the anaerobic digestion of cattle, pig, or poultry manure in a biogas digester and is rich in plant macro and micro-nutrients (Islam, 2006). Digested bio-slurry proved higher plant nutrients required for growth than the other organic nutrient sources. For instance, it contains 1.6, 1.6, and 1.0 % of N, P, and K, respectively. The composted slurry comprises 0.8, 0.7, and 1.1% of N, P, and K, respectively (Amrit, 2006). On the other hand, FYM contains 0.8, 0.7, and 0.7% of N, P, and K, respectively.

Factors that influence nitrogen availability from bio-slurry are its inorganic N content, digestion process (aerobic or anaerobic), C: N ratio, pH, the method and timing of application, and soil type and properties (Warman and Termeer 2005). Due to the decomposition and breakdown of its organic content, digested slurry provides fast-acting nutrients that easily enter the soil solution, thus becoming immediately available to plants. They simultaneously serve as primary nutrients for the development of soil organisms, e.g. the replenishment of microorganisms lost through exposure to air in the course of spreading the slurry over the fields.

2.7. Effect of Bio-slurry on Growth, Nodulation and Yield

Organic fertilization is a very important method of providing the plants with their nutritional requirements without having an undesirable impact on the environment (Adeoye *et al.,* 2001; Menon *et al.,* 2010). Bio-slurry is an efficient source of organic manure with the ability to deliver nutrients, especially N to crops. It was reported that the application of bio-slurry maintains the higher status of N in both organic and inorganic N fractions. Most of the hydrolyzable N fractions contributed to the N uptake by crop by first transforming into exchangeable NH_4+ and NO_3-N and thereby establish an equilibrium condition for maintaining the steady flow of N for plants (Sharma *et al.,* 2007).

Also, a high increment in crop yield was reported when bio-slurry was applied in combination with chemical fertilizer (Warnars and Oppenoorth, 2014; Islam, 2006) and when applied as a separate application (Islam, 2006; Karki and Expert, 2006). The field tests with biological sources of nutrients and spray Panchagavya evaluate the yield and economics of crops in the maize - sunflower - green gram cropping system was conducted. It was concluded that the grain Yield of green grams was higher among the recommended fertilizer treatments, but it was similar to biogas slurry Panchagavya (Somasundaram *et al.,* 2007).

Organic manures significantly increase the growth attributes of cowpea such as plant height and leaf area index and also significantly increased the yield attributes such as the number of pod plant^{-1}, number of seeds pod^{-1} (Joshi *et al*., 2016). It also provides increased growth, dry matter accumulation, and chlorophyll index of crops (Cavalcante *et al*., 2016). Generally, the application of bio-slurry has been shown to increase plant performance in these crops including mungbean (Warnars and Oppenoorth, 2014).

2.8. Advantage of Bio-Slurry over other Organic Fertilizer Sources

The bio-slurry has the following advantages over raw manure such as cow dung or poultry manure. It is odorless and does not attract flies. Manure attracts termites while bio-slurry repels them. Therefore, fertilizing crops with manure increases the risk of crops being attacked by the termites (Karki and Expert, 2006). Besides, bio-slurry could be considered as an alternative to chemical fertilizers because it is relatively cheap and safe and easily prepared from local material. Cow dung and poultry manure are reported to have higher fertilizer value when converted into bio-slurry after undergoing the fermentation process, leading to the production of biogas (Bonten *et al*., 2014). Bio-slurry could also potentially supplement commercial fertilizer, thus, reducing the amount of chemical fertilizer required by the farmer, hence, reducing production costs. One of the reports demonstrates the positive effects of the use of bio-slurry on increased crop yield, improved crop quality, and reduced dependence on chemical fertilizer use (Karki and Expert, 2006).

The use of bio-slurry as an organic mulch could potentially provide cheaper and alternative sources to the use of expensive mulching materials. Bio-slurry is reported to have some characteristics of mulching, which include suppressing weed growth and improving soil water holding capacity (No, 2012). Bio-slurry contains organic matter and fiber, and it also improves soil organic matter; all these features contribute to its ability to hold soil moisture (Karki and Expert, 2006). Possibly, the use of bio-slurry during dry spells could allow the soil to hold enough water for crop use and eventually improve crop yield.

2.9. Effect of Inoculation on Growth, Nodulation and Yield of Mungbean

The earth is made up of nearly 78 % nitrogen gas, which is unutilized by a majority of living things. Both animals and plants can die because of nitrogen deficiency (White, 2012). Therefore, it is important that nitrogen be made available to synthesize proteins, nucleic acids, amino acids, and other nitrogenous cell organelles that are essential for life (Hungaria *et al*., 2003). Biological nitrogen fixation occurs when the *rhizobia* bacteria invade the plant roots inducing a nodule (Dhar *et al*., 2015).

The bacteria in the nodule then reduce atmospheric nitrogen forming ammonia thus supplying the plant with nitrogenous compounds by either α-Proteobacteria or *Rhizobiaceae* nitrogen fixing bacteria (Hart *et al*., 2003). The use of appropriate strains of inoculants in N deficient soils may offer an excellent opportunity for improving legume growth and development (Mfilinge *et al*., 2014). As Delic *et al*. (2011) reported that all investigated characteristics of grain and shoot dry matter (above-ground biomass) yields of mungbean significantly increased due to seed inoculation with particular *Rhizobial* strains.

Nodule formed in leguminous plants shows a typical healthy and effectiveness by showing a pink or red color and is brought about by the formation of a protein called Leghemoglobin. This protein contains both iron (Fe) and molybdenum (Mo) which is responsible for binding oxygen. Besides, it creates a low oxygen environment within the nodule, which allows *Rhizobium* bacteria to live and fix N_2 easily, and in a comfortable environment (Chowdhury *et al.,* 1998).

Chatterjee and Bhattacharjee (2002) studied the effects of inoculation with *Rhizobium* sp. on the nodulation of mungbean and found that the plants inoculated with *Rhizobium* strains showed higher nodulation and N content. Navgire *et al.* (2001) conducted a field experiment with mungbean cultivars BM-4, S-8, and BM-86, and these were inoculated with *Rhizobium* strains M-l1- 7 85, M-6-84, GR-4, and M-6-65. They recorded the highest mean nodulation (16.66) in S-8, BM-4, and BM-86. Other findings also reported that inoculation of mungbean cultivars with *Rhizobium* strains and in combination with P has a significant effect on the nodulation of mungbean. BARI Mung-2, BARI Mung-3, BARI Mung-4, BARI Mung-5. Bina Moog-2 and BU Mung-1 to *Rhizobium* sp. strain TALI69 and TAL441 and found that bacterial inoculation of the seeds increased nodulation (Narendra and Chandra 2003; Solaiman *et al.,* 2003).

The legume plant gains the capability to grow in nitrogen deficiency soils while the bacteria obtain sugars and a protected niche in which they multiply and finally escape back to the nearby soil when the nodule bursts. In legumes, the nitrogen-fixing bacteria live in root nodules where nitrogen fixation occurs. This mutual association between the bean plant and the bacteria is symbiotic and contributes less than 5 tons of nitrogen per ha annually (Tambalo *et al*., 2015).

2.10. Effects of Combined use of Bio-Chemical Fertilizers on Growth, Nodulation and Yield

Nutrient combination to support and sustain crop growth is very important due to the rising cost of chemical fertilizers and the bulkiness and unavailability of organic manure. Complimentary use of organic and inorganic fertilizers has been proven to be a sound soil fertility management strategy in many countries of the world (Landon, 1991)

It is obviously understood that chemical fertilizers have helped to improve the yields of crops. However, farmers are reluctant to use sources of inorganic fertilizers because of the higher costs compared with organic fertilizers. Thus, farmers are now looking for alternatives to organic fertilizers. Organic fertilization is a very important method of providing the plants with their nutritional requirements without having an undesirable impact on the environment (Adeoye *et al.,* 2011; Menon *et al.,* 2010).

Nitrogen is supplied by the organic fertilizer favoring the higher chlorophyll content. According to Taiz and Zeiger (2013), plants with a high concentration of chlorophyll are potentially capable of achieving higher photosynthetic rates due to its light energy capture value per unit time. The application of organic and inorganic fertilizers enhanced the shoot biomass of plants. Hence, the improved biomass accumulation is attributed to the increased availability of plant nutrients which might have been enhanced the photosynthetic capacity of the plants (Furtini *et al.*, 2006). Different studies results show that organic fertilizer has a significant effect on vegetative growth (plant height, number of leaves and branches as well as fresh and dry weight of leaves), and favors the gas exchange performance of legume crops (Khan *et al*., 2017; EL-Desuki *et al*., 2010; EL-Etr *et al*., 2004).

An experiment conducted in South West Nigeria on the influence of organic manure and NPK fertilizer on yield and yield components of maize crops under different cropping systems revealed that the application of NPK and organic manure significantly increased grain yield as well as other parameters (Landon, 1991).

Srinivas *et al.* (2002) observed the effects of N (0, 20, 40, and 60 kg ha^{-1}) and P (0, 25, 50, and 75 kg ha^{-1}), along with seed inoculation with *Rhizobium* culture on the growth, yield and yield components of green gram. Plant height, number of seed per pods, number of pods per plant, phytomass per plant, generally increased with increasing rates of P as well as N up to 40 kg ha^{-1} further increase in N decreased the yield.

Jain *et al.* (2003) observed the effect of organic and phosphorus fertilizer on the growth and nutrition of chickpea. The organic treatments produced higher yield through improved plant height, dry matter accumulation, and the number of branches per plant respectively compared to the control treatment. Application of P at 50 kg ha^{-1} showed 9.99, 34.33, and 16.46 percent higher values for these growth parameters respectively, compared to the control.

A high increment in mungbean yield was reported when bio-slurry was applied in combination with chemical fertilizer (Warnars and Oppenoorth, 2014; Islam, 2006) and when applied as a separate application (Islam, 2006; Karki and Expert, 2006).

The combined application of *Rhizobium* inoculation and phosphorous fertilizer had a profound effect on the nodulation of mungbean varieties. The *Rhizobium* inoculation alone and along with the application of phosphorus fertilizer significantly increased all the parameters measured. Application of mungbean with *Rhizobium* 500g with P_2O_5 46 kg ha^{-1}, *Rhizobium* 500g with P 23 kg P_2O_5 ha^{-1} combinations and *Rhizobium* 500g alone, increased yield and yield component trait compared with other treatment combination (Geletu and Mekonnen, 2018).

The combined applications of bio-slurry and NP fertilizers have a remarkable effect on the growth, yield, and quality of cabbage. Based on the results of the study it was concluded that the combined application of 50 m^3, bio-slurry with 75% NP ha^{-1} was recommended for the production of head cabbage (Terefe *et al*., 2018).

Moreover, integrated use of bio-slurry, *rhizobium,* and chemical N fertilizer effect on crop production is well reported in different previous findings (Meesam *et al*., 2014; Sahu, 2014; Shah *et al*., 2007, and Irshad *et al*., 2002).

3. MATERIALS AND METHODS

3.1. Description of the Experimental Site

The experiment was conducted at the experimental field of Hawassa University, Hawassa, Ethiopia. The site is located 270 km south away from the capital city Addis Ababa. Geographically the area lies at 7° 03' 05.7" N and 38° 30' 21.1" E (Figure 1) with a mean altitude of 1712 m above sea level. It receives a mean annual rainfall of 952 mm with a mean minimum and maximum temperature of 13.9 and 26.5°C, respectively (EMA, Hawassa Meteorological Center, 2019).

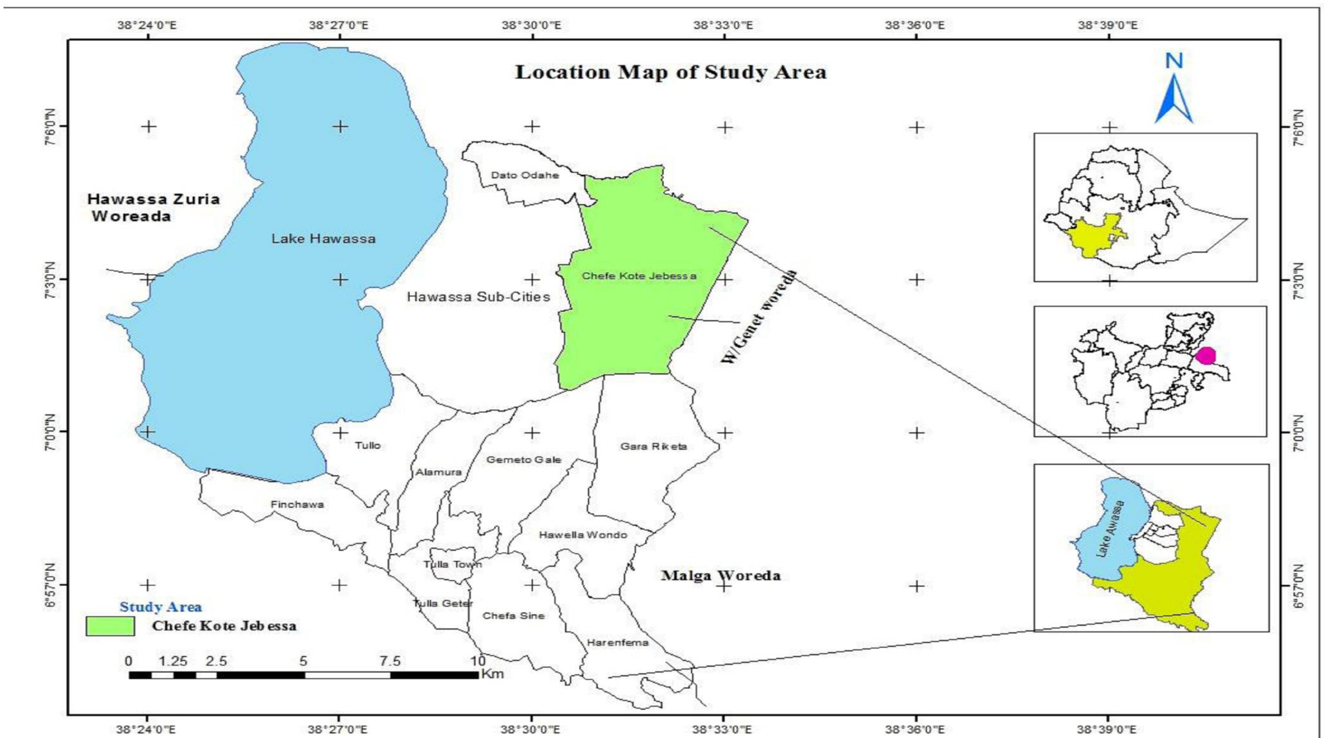

Figure 1: Geographical location of study area Source; Ethio GIS 2015

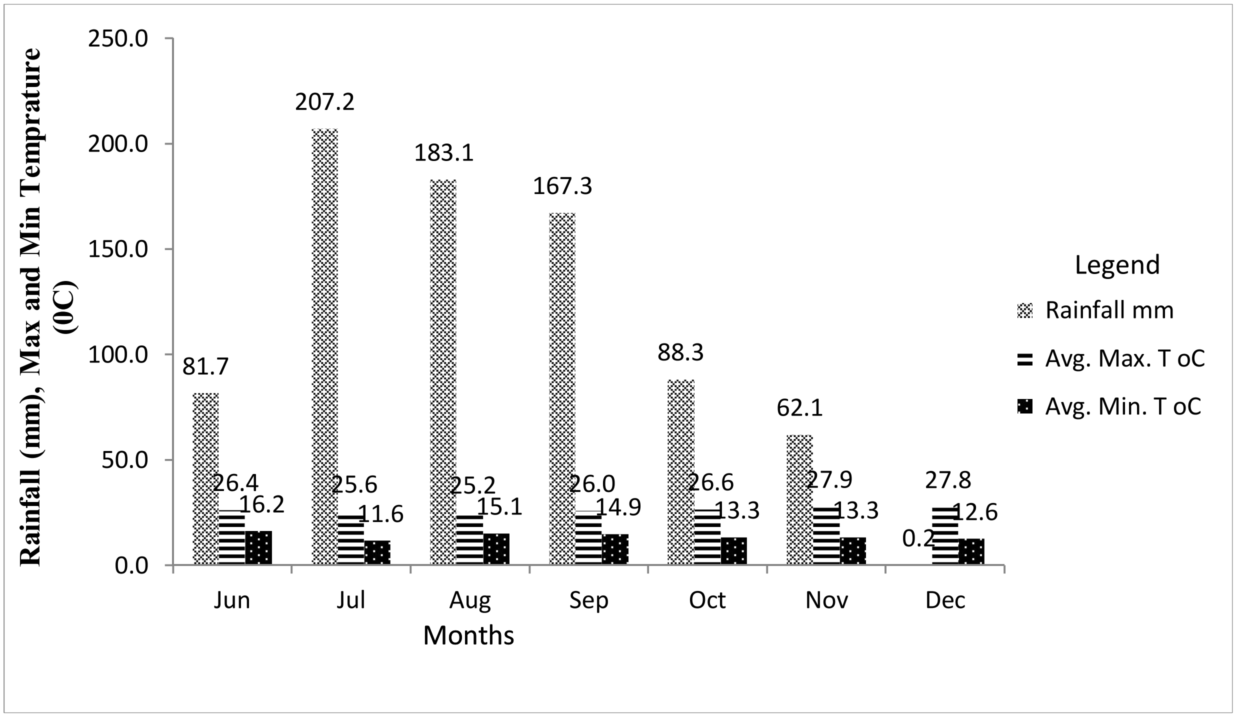

Figure 2: Meteorological data of the study area; (NMA, Hawassa Meteorological center, 2019)

Physico-Chemical Characteristics of the Experimental Soil and Bio-slurry

Before planting soil samples were taken randomly from the experimental field 0-20 cm depth using augur. The samples were mixed well in a plastic bag, sieved and one composite representative sample was taken for analysis of physical and chemical properties (pH, total N, available P, exchangeable K, OM, C: N ratio and CEC) of the soil. The composite soil sample was sent to Debrezeit Horticoop Ethiopia (Horticulture) soil and water analysis laboratory and analysis were done following the standard procedure for each parameter. The bio-slurry was characterized for the same at Hawassa University, Agriculture College soil laboratory.

The soil texture was separated following the Bouyoucos Hydrometer method (Day, 1965). Total nitrogen was determined according to the Micro-Kjeldahl method with sulphuric acid

(Dewis and Freitas, 1975). The pH of the soil was determined by a glass electrode pH meter using soil: water suspension of 1:2.5 ratios as described by Page (1982). Analysis of organic carbon content of the soil in a laboratory was determined by Walkley and wet oxidation method as described by Jackson (1958). The determination of available phosphorous was carried out according to the methods of Olsen and Dean (1965). Exchangeable potassium was extracted using 1N neutral ammonium acetate at pH 7 (Pratt, 1965) and determined by the atomic absorption spectrophotometer. CEC was determined using Kjeldhal procedure as described by (Ranist *et al*., 1999).

Before using the bio-slurry for the experiment the nutrient contents were analyzed. The liquid bio-slurry in the tank of the bio-digester was stirred in a circular movement with a stick. The preventive measure was taken to avoid scraping off the bottom and corners of the tank. Then five representative liquid bio-slurry samples were collected with 2 L sampling plastic. Then mixed in the plastic container and 1 L representative sample was oven-dried and subjected for analysis to determine water content, pH, CEC, organic carbon, EC, organic matter, total nitrogen, available P, exchangeable K, and exchangeable bases. The oven-dried 1kg bio-slurry sample was digested instead of a 1 kg soil sample for manure analysis. The N, P, K, pH, OM content, and CEC were determined by using the procedures stated for soil analysis in the above section (Table 1).

Table 1 The physico-chemical characteristics of the experimental soil and bio-slurry

Description of the soil		Description of the bio-slurry	
Profile code	***Concentration***	***Profile code***	***concentration***
Sand (%)	40	Water (%)	93.93
Clay (%)	32	Dry matter (%)	6.07
Silt (%)	28	Organic matter (%)	30.6
Textural class	Clay loam	Organic carbon (%)	17.7
pH	7.17	pH-H_20(1:2.5)	7.33
Avai. P (mg kg^{-1})	51.73	Total N (%)	1.53
Exch. K^+ (mg kg^{-1})	946.85	Avai. P (mg P_2O_5 kg^{-1} BS	301.4
Exch. Ca^{2+} (mg kg^{-1})	2,641.79	Avai. K (mg K_2O kg^{-1} BS)	715.25
Exch. Mg^{2+} (mg kg^{-1})	320.30	Exch. Ca^{2+} (cmol (+) kg^{-1} BS)	114.4
Exch. Na^+ (mg kg^{-1})	222.73	Exch. Na (cmol (+) kg^{-1} BS)	33.2
S (mg kg^{-1})	16.54	Exch. K^+ (cmol (+) kg^{-1} BS)	28.6
OC (%)	1.69	Exch. Mg^{2+}(cmol (+) kg^{-1} BS)	17.4
Total N (%)	0.15	CEC (cmol (+) kg^{-1} BS)	64
C/N (%)	11.27		
CEC (Meq/100g soil)	22.79		

Avai = available, BS= bio-slurry, Exch. = exchangeable.

3.2. Treatments and Experimental Design

The experiment was laid down using a randomized complete block design (RCBD) with a factorial arrangement of bio-slurry and inoculant/N fertilizer sources. Hence, four rates of bio-slurry (0, 50, 100, and 150%) were combined with four inoculant/N fertilizer application including control (0, 23kg N, strain MB-001 and 23kg N + strain MB-001). The total numbers of treatments were 16 replicated four times with a total of 64 plots.

Each experimental plot had an area of 1.8 x 1.5m (2.7m^2), a total area of 326.7m^2 with a spacing of 0.5 and 1m between plots and blocks, respectively. The treatments were randomly assigned to each plot. Planting was done 0.3m x 0.1m between rows and plants respectively.

There were six rows in each plot and data were collected from plants of the middle four rows, excluding plants in the two border rows as well as those at both ends of each row to avoid border effects.

3.3. Experimental Materials Used

Experimental bio-slurry was obtained from a private hotel (Gebrekiristos hotel) in Hawassa, Ethiopia. Its rate was adjusted based on the recommended rate of inorganic N for legumes, respective to its N content. Representative 1 L liquid bio-slurry sample was oven-dried and subjected for analysis to determine N amount in it and the rate was adjusted based respective to it. Urea was used as the source of N and 23kg ha^{-1} was used. Commercial *Rhizobium* strain MB-001 obtained from Menagesha Biotech, priv. plc was used at the rate of 500g ha^{-1} for legume production. Mungbean variety MH-97-6 (Boreda) was used for this study which was obtained from Hawassa Agricultural research center.

3.4. Management of Experiment

Land preparation: the experimental field was prepared following the conventional tillage practice which includes three times plowing before sowing of the mungbean. As per the specifications of the design, a field layout was prepared; the land was cleared, leveled, and made suitable for crop establishment.

Sowing and field management:

A week before planting, prepared bio-slurry as per treatment was applied to the plots to reduce its burning effects on the crop. Sowing was done in August 2019 with row spacing 0.3m and between plants 0.1m and covered slightly with soil at the depth of 3–5 cm.

At the time of planting, *Rhizobium* inoculant (strain MB-001) was mixed with a sugar solution in which the seed is soaked. The sugar solution ensures the strain adheres to the coating of the seed and that the *Rhizobia* quickly colonize the interior of the plant after germination. The seed inoculation was done just before planting under shade to maintain the viability of cells at the rate of 10 g/kg of seed. Seeds were allowed to air dry for a few minutes before planting to avoid fungal growth. The uninoculated seed was planted first to avoid bacterial contamination with inoculated seeds.

3.5. Data Collection and Measurements

The data for the experiment were collected and the measurement of every parameter was done following the standard procedure for each parameter.

3.5.1. Phenological parameters

Days to flowering*:* It was recorded by counting the number of days that were taken from emergence to about 50% of the plant population in the plot reached flowering.

Days to physiological maturity: it was recorded by counting the number of days from the date of sowing till when 90% of the pod in plants was changed from a green color to yellowish.

3.5.2. Nodule determination

Sampling for nodulation was performed by excavating the roots of five randomly selected plants from each plot of rows next to border rows at the mid-flowering stage. Uprooting was done using a spade and then the soil was removed from the root manually. The adhering soil was removed by washing the roots gently with water over a metal sieve. Nodules remaining in the soil were picked up by hand.

Number of nodules: it was taken from five randomly selected plants at the mid-flowering stage from each plot and counted carefully to determine the average number of nodules plant^{-1}.

Nodules diameter: from nodules collected in each plot six nodules were randomly selected and the diameter was measured and the average data was recorded as nodule diameter.

Nodules fresh weight: after recording nodule number, nodules were weighed immediately with the sensitive balance and recorded as nodule fresh weight.

Nodules dry weight: after recording the fresh weight, the same nodules were oven-dried at 70°C for 48hr to determine nodule dry weight.

3.5.3. Growth parameters

Plant height (cm): The average height of plants was determined by measuring the height of five randomly selected plants from the ground surface to the top of the main stem in centimeter at maturity.

Shoot fresh weight and dry weight (g): it was recorded by measuring the above ground biomass at the mid flowering stage from plants that were sampled for nodulation. The samples were placed in labeled perforated paper bags and oven-dried for 48 hours at 70^{o}C to constant weight. The averages were recorded as shoot dry weight plant^{-1}.

Leaf area: Leaf area was measured from plants harvested for shoot weight determination from each plot at mid flowering stage. The average of the five plants leaf area was taken as the leaf area of the plant. It was measured by using portable leaf area meter LI-3000A.

Leaf area index: the leaf index was determined by the ratio of leaf area to the ground area covered.

$$\mathrm{lAI} = \frac{\text{leaf area}}{\text{grand area}}$$

3.5.4. Gas-exchange parameters

Photosynthetic rates and transpiration rates were measured from 10 young fully expanded leaves per plot using a portable infrared gas analyzer (ADC Bio Scientific LCi-SD System). The measurement was made from 9-11:00 am and 2-4:00 pm.

3.5.5. Yield and yield components

Number of pods plant^{-1}: it was determined from ten plants harvested from three central rows of each plot and the average was taken as the number of pods plant^{-1}.

Number of seeds pod^{-1}: the plants harvested for pod number determination were threshed and then total seeds were divided by total pods to calculate the average seed number pod^{-1}.

Hundred seed weight (g)*:* it was determined by weighing randomly sampling hundred seeds threshed for seeds pod^{-1} determination from each plot. Selected seeds were weighed using sensitive balance and their average weight was taken as the weight of hundred seeds.

Biological yield (t ha^{-1}): it was measured from plants manually harvested from the three central rows of each plot. The harvested plants were sun dried in an open air four days and the average total biological yield was reported in t ha^{-1}.

Grain yield (t ha^{-1}): the harvested plants from three central rows for biological yield determination were threshed and weighed, then converted to t ha^{-1} to determine the grain yield. The seed yield was adjusted at 10% moisture level using seed moisture tester.

Straw yield (t ha^{-1}): it was calculated by subtracting grain yield from the corresponding total above ground biomass yield.

Harvest index (%): it was calculated as the ratio of grain yield to above ground biomass

$$\text{Harvest index (\%)} = \frac{\text{grain yield}}{\text{above ground dry biomass}} \times 100$$

3.6. Partial Budget Analysis

The potential response of crop towards the added fertilizer and price of fertilizers during planting ultimately determines the economic feasibility of fertilizer application (CIMMYT, 1988). To estimate economic parameters, seeds were valued at an average open market price of 40.00 Birr kg^{-1} for seed.

The economic analysis was based on the formula developed by CIMMYT (1988) and given as follows:

Gross average yield (kg ha^{-1} or ton ha^{-1}): is the average yield of each treatment.

Adjusted yield: is the average yield adjusted downward by 10% to reflect the difference between the experimental yield and yield of farmers.

Adjusted yield = Gross average yield - (Gross average yield * 0.1)

Gross field benefit: was computed by multiplying field/farm gate price that farmers receive for the crop when they sale it as adjusted yield. Gross field benefit= Adjustable yield * field/farm gate price of a crop. Total cost: mean current prices of urea (20 birr kg^{-1}), bio-slurry compost (0.4 birr l^{-1}), the wage for bio-slurry application and urea application were considered per hectare.

Net benefit: was calculated by subtracting the total costs from the gross field benefit for each treatment. Net benefit = Gross field benefit – Total cost

Marginal rate of return (%) = (Marginal benefit/ Marginal Cost)*100

3.7. Statistical Analysis

The data were subjected to analysis of variance (ANOVA) using a General Linear Model in SAS software version 9.0 (SAS Institute, 2002) and mean separation was made based on LSD at 5% (P<0.05) level of significance.

4. RESULTS AND DISCUSSION

4.1. Physico-Chemical Properties of Soil and Bio-slurry

Before sowing, the Physico-chemical properties of an experimental soil, and bio-slurry were analyzed for its nutrient contents. The results revealed that the soil was clay loam in texture (40% sand, 32% clay, 28% silt) with a pH value of 7.17, which is within the range of optimum soil pH for mungbean production (Ryczkowski, 2018).

The total N, available P, OC, C/N ratio and CEC of the soil before planting were 0.15%, 51.7 mg kg^{-1}, 1.69%, 11.2 and 22.7 cmol (+) kg^{-1}, respectively (Table 2). The total N content of the soil was within the range of medium according to Tekalign, (1991), who classified the range of total N < 0.1, 0.1 – 0.15, 0.15 – 0.25 and > 0.25% as very low, low, medium and high, respectively. Olsen *et al*. (1954) classified available P content as: < 5 as low, 5 – 10 as medium and > 10 mg kg^{-1} as high. Hence the available P of the soil before planting lies under the high range. According to Landon (1991), the soil OC content ranges of 1 – 2, 2 – 4, and 4 – 6% are rated as low, medium and high, respectively while cation exchange capacity (CEC) ranges of 5 – 15, 15 – 25 and 25 – 40 cmol kg^{-1} are rated as low, medium and high, respectively. Based on these ratings, the OC (1.69%) and CEC (22.7 cmol kg^{-1}) of the soil before planting were within the low and medium ranges, respectively.

Generally, the nutrient content of the study site was rated as good in terms of the availability of major plant nutrients. However, the moderate alkalinity of soil may limit the availability of other nutrients. Hence, applying appropriate soil amendment strategies including the application of OM is very essential to neutralize soil solution (Musse *et al.,* 2020).

Regarding to the current result of the experimental bio-slurry analysis revealed a slightly alkaline pH (7.3) value, medium amount of total N (1.53%), low amount of OC (17.7%), very high amount of available P (301.4 mg kg^{-1}), medium CEC 64.0 (cmol kg^{-1}), as noted by Singh *et al*.(2007).

4.2. Effects of Bio-slurry and Inoculant/N Fertilizer on the Crop Phenology

Discussions on significant main effects (bio-slurry and inoculant/N fertilizer) are presented only when their interaction is not significant. Interaction effects are discussed when resulted in a significant effect on the parameter considered.

4.2.1. Days to 50% flowering and days to 90% physiological maturity

The analysis of variance revealed that the main effects of bio-slurry and inoculant/N fertilizer had significant (P<0.001) on both days to 50% flowering and days to 90% physiological maturity. Similarly, the interaction of treatments had also a highly significant (P<0.001) effect on those phenological parameters (Appendix 2).

Application of bio-slurry at the rate of 150% with both the inoculant/N fertilizer treatments resulted in late flowering and maturity, which is statically similar with combination of 150% bio-slurry with both strain MB-001 and 23kg ha^{-1} N. Whereas, the early flowering and maturity was recorded from the control treatment which is statically similar with combined application of nil bio-slurry with both MB-001 and with 23kg N ha^{-1}(Table 2). For instance, the shortest days required for 50% flowering (38.0 days) were recorded from the control, which was 7.5 days earlier than the longest (45.5) days recorded from the application of 150% bio-slurry + 23 kg N ha^{-1}. Similarly, the shortest days (9 less days) to physiological maturity were recorded from the control treatment compared to the application of bio-slurry at the rate of 150% in combination with 23 kg N ha^{-1}.

Early flowering and maturity of mungbean in a control treatment might be attributed due to a nutrients deficiency or stress conditions that force a plant to complete its life cycle within a short period of time. However, the delay in flowering and maturity due to applied treatments might be attributed to the availability of N from applied bio-slurry and inoculant/N fertilizer which favors vegetative growth.

In line with this finding Terefe *et al*. (2018) reported earlier flowering and maturity of cabbage crop at the lowest rate of bio-slurry and nitrogen and phosphorus (NP) fertilizer application. Similarly, Geremew (2017) reported that the application of dry bio-slurry and chemical fertilizers on tomato increased days to flowering and days to maturity compared to the control. Similar effects of combined application of organic and N fertilizers on phenological parameters were also reported by Solomon *et al*. (2019). Moreover, the N taken up by plant roots in the form of nitrate from the root nodule could be used for an increased cell division and synthesis of carbohydrates, resulting in plants with a luxurious foliage growth (Marschner, 2002).

Table 2 Interaction effects of treatments on phenological parameters of mungbean grown at Hawassa, Ethiopia, during 2019/20.

Treatments		Phenological parameters	
Rate of bio-slurry (%)	Inoculant/N fertilizer	Days to 50% flowering	Days to 90% maturity
0	0	38.0^{e}	65.0^{e}
	23	40.0^{cd}	67.0^{d}
	Strain MB-001	38.3^{e}	65.2^{e}
	23+MB-001	39.0^{de}	65.0^{e}
50	0	40.0^{cd}	67.0^{d}
	23	43.0^{b}	70.0^{c}
	Strain MB-001	43.0^{c}	70.0^{c}
	23+MB-001	40.5^{c}	67.5^{d}
100	0	42.0^{b}	69.0^{c}
	23	42.3^{b}	69.2^{c}
	Strain MB-001	42.3^{b}	69.2^{c}
	23+MB-001	42.5^{b}	69.5^{c}
150	0	45.3^{a}	73.2^{ab}
	23	45.5^{a}	74.0^{a}
	Strain MB-001	44.5^{a}	72.5^{b}
	23+MB-001	45.0^{a}	73.5^{ab}
CV%		2.07	1.43
LSD (0.05)		1.23	1.37

Means followed by the same letter (s) within the column are not significantly different at (P ≤0.05).

4.3. Effects of Bio-Slurry and Inoculant/N Fertilizer on Growth Parameters of Mungbean

4.3.1. Plant height

The analysis of variance indicated that the main effect of bio-slurry and inoculant/N fertilizer application had highly significant ($P<0.001$) effects on the height of mungbean. Likewise, their interaction effects had also a highly significant ($P<0.001$) effect on the plant height of mungbean (Appendix 2).

The tallest plant was recorded from the combined application of 150% bio-slurry + 23 kg N ha^{-1} (54.5 cm) which is followed by combined application of 150% bio-slurry with both stain MB-001 and 23kg N ha^{-1} statically similar each other. Whereas, numerically the shortest plant (34.0 cm) was recorded from the control which is statically similar with nil bio-slurry combination with both strain MB-001 and 23kg N ha^{-1}, 50% bio-slurry with 23kg N ha^{-1} (Table 3). Increasing of bio-slurry rates from 0 – 150% resulted in an increasing trend in plant height of mungbean.

The increment in plant height might be due to the fact that organic fertilizer improves the soil structure and aggregation; hence improve the availability of nutrients as well as encourage the plant to have good root development by improving the aeration in the soil, which leads to higher plant growth (Warnars and Oppenoorth, 2014; Widowati *et al*., 2012). Such increase in plant height due to the increasing bio-slurry rates with N fertilizer application might be also attributed due to the increasing availability of N in the soil for uptake by plant roots that enhanced vegetative growth through increasing cell division and elongation (Warnars and Oppenoorth, 2014).

The result agrees with Krishnan (2016), who reported an increased plant height in green gram due to the combined use of vermicompost + NPK. The increase in plant height due to the combined application of bio-slurry and chemical fertilizer was also reported by Yalemtsehay (2019). The result of the current experiment was also in close conformity with Meesam *et al*. (2014), who reported an increment in plant height due to the integrated use of *Rhizobium*, compost, and mineral N. Similar findings have also been reported by previous studies (Shweta, 2014; Shah *et al*., 2007; Irshad *et al*., 2002).

4.3.2. Leaf area and leaf area index

There were highly significant (P<0.001) differences in leaf area and leaf area indices among applied bio-slurry and inoculant/N fertilizer. The interaction among applied bio-slurry and inoculant/N fertilizer was also significant (P<0.001) (Appendix 2).

Maximum leaf area and leaf area index were recorded from combined application of 150% bio-slurry + 23 kg N ha^{-1} + strain MB-001 (1533.1cm^2 and 5.1); followed by plots received 150% bio-slurry + 23 kg N ha^{-1} (1447.5 cm^2 and 4.8), 150% bio-slurry + strain MB-001 (1440.6 cm^2 and 4.8), and 100% bio-slurry + strain MB-001 (1439.5cm^2 and 4.7), statically similar to each other respectively. On the other hand, the control treatment showed the lowest leaf area and leaf area index (510.4 cm^2 and 1.7), Table (3).

The increment of the leaf area and leaf area index of mungbean to applied N sourced treatments might be due to the fact that N is the most essential nutrient element and its adequate supply increases the growth and yield of a crop. Similar results were reported previously that the integrated use of the recommended dose of chemical fertilizer (NPK) in combination with decaying organic manures and bio-fertilizer was significantly produced higher leaf area at all growth stages of mungbean (Al-Owied, 2016). This increment can be because of the release of more macro and micronutrients due to effective microorganisms in the soil root zone and followed by plant vigor as described by Ashour (1998).

Thus, the application of bio-slurry in combination with inoculant/N fertilizer might have supplied the required amount of plant nutrients for vegetative growth due to the availability of micro-nutrients and that can support the crop during and the later growth stages favored by the slow and continuous decomposition and release of nutrients. Similarly, findings of increased leaf area and leaf area index due to the integration of nutrient management were reported by Hussain *et al*., (2019); Larimi *et al*., (2014); Islam *et al*., (2010); Garg *et al*., (2005).

4.3.3. Shoot fresh and dry weight

The results revealed that the main effects of bio-slurry and inoculant/N fertilizer had highly significant (P<0.001) effects on shoot fresh and dry weight of mungbean. Similarly, their interaction effects had shown a highly significant (P<0.001) effect on shoot fresh and dry weight of mungbean (Appendix 2).

Maximum shoot fresh and dry weight were recorded from the integrated application of 150% bio-slurry + 23 kg N ha^{-1} + strain MB-001 (131.5 and 33.2 g) which were closely followed by

150% bio-slurry with both inoculant/N fertilizer, whereas minimum shoot fresh and dry weight (55 and 8 g) was recorded from the control treatment (Table 3).

The increase in shoot fresh and dry weight of mungbean to the application of bio-slurry (a component of several essential macro and micronutrients) might be due to the effect of these nutrients, which are an integral component of many essential plants compounds like chlorophyll, proteins, and amino acids. Nutrients obtained from supplied treatments enhance increased vegetative growth and enables plants to produce good quality foliage. These, in turn, promote carbohydrate synthesis through photosynthesis and ultimately increased the yield of plants (Brady and Weil, 2002).

Anwar *et al.*(2018) reported similar results, stated that treated plots with organic and inorganic fertilizers produced maximum (834 gm^{-2}) fresh weight, while control plots produced a minimum (781 g m^{-2}) fresh weight of mungbean. According to Argaw (2017), manure, compost, and DAP applied with *Bradyrhizobium* caused a significant positive influence on the shoot dry weight of peanut at Fedis site eastern Ethiopia. Ashenafi and Tewodros (2018) also reported that the fresh biomass of kale is significantly influenced by the application of liquid bio-slurry and inorganic N. Moreover, bio-slurry treatment of 10 t ha^{-1} + 25kg N ha^{-1} resulted in the highest fresh weight and dry weight of soybean plants (Yafizhan and Sutarno, 2018).

Table 3 Interaction effects of treatments on the growth parameters of mungbean grown at Hawassa, Ethiopia, during 2019.

Treatments		Parameters				
Rate of bio-slurry (%)	Inoculant/N fertilizer	Plant height (cm)	Leaf Area (cm^2)	LAI	Shoot fresh weight(g)	Shoot dry weight(g)
0	0	36.5^{e}	510.4^{h}	1.7^{h}	55.0^{i}	8.0^{j}
	23	35.0^{e}	605.5^{h}	2.0^{h}	77.3^{de}	12.2^{i}
	Strain MB-001	34.0^{e}	574.8^{h}	1.9^{h}	74.0^{def}	15.5^{gh}
	23+MB-001	42.2^{bcde}	756.1^{g}	2.5^{g}	113.8^{b}	17.7^{fg}
50	0	39.7^{de}	739.0^{g}	2.4^{g}	71.5^{efg}	14.5^{hi}
	23	34.2^{e}	904.4^{f}	3.0^{f}	72.7^{efg}	16.2^{gh}
	Strain MB-001	42.2^{bcde}	943.4^{ef}	3.1^{ef}	58.0h^{i}	18.2^{fg}
	23+MB-001	48.7^{abc}	1076.8^{d}	3.5^{d}	67.5^{fg}	21.5^{de}
100	0	40.0^{de}	1005.3^{de}	3.3^{de}	74.5^{def}	16.2^{gh}
	23	40.7^{cde}	1277.3^{c}	4.0^{c}	64.5^{gh}	19.7^{ef}
	Strain MB-001	48.7^{abc}	1439.5^{ab}	4.7^{ab}	82.5^{d}	23.7^{d}
	23+MB-001	48.0^{bcd}	1396.6^{b}	4.6^{b}	74.0^{def}	27.5^{c}
150	0	42.0^{bcde}	1383.0^{b}	4.6^{b}	101.7^{c}	28.7^{bc}
	23	54.5^{a}	1447.5^{ab}	4.8^{ab}	112.5^{b}	30.5^{ab}
	Strain MB-001	48.2^{abcd}	1440.6^{c}	4.8^{ab}	115.7^{b}	31.3^{ab}
	23+MB-001	50.0^{ab}	1533.1^{a}	5.1^{a}	131.5^{a}	33.2^{a}
CV%		14.1	6.4	6.5	7.8	11.6
LSD (0.05)		8.6	97.9	0.3	9.3	3.4

Means followed by the same letter (s) within the column are not significantly different at (P ≤0.05).

4.4. Effects of Bio-slurry and Inoculant/N Fertilizer Application on Nodulation Parameters

4.4.1. Number of nodules plant^{-1}

The statistical analysis of results revealed that there were highly significant (P<0.001) differences in bio-slurry rates and inoculant/N fertilizer on nodule numbers. Likewise, the combined effect of bio-slurry and inoculant/N fertilizer resulted in significant differences on the number of nodule plant^{-1} (Appendix 3).

Concerning the main effects among inoculant/N fertilizer, the maximum nodule number plant^{-1} was obtained from *Rhizobium* (strain MB-001) inoculation whereas; the lowest nodule number was obtained from 150% rate of bio-slurry.

Regarding the interaction effect, maximum nodule numbers plant^{-1} (26.15) were recorded from *Rhizobium* inoculation with nil bio-slurry, which is followed by strain MB-001 inoculation with 100% bio-slurry and control (20.00 and 19.95) statistically at par. The minimum mean nodule number (12.7) was recorded from 150% bio-slurry + 23 kg N ha^{-1}. Numerically the lowest number of nodules per plant was observed due to the application of 150% bio-slurry with 23kg N ha^{-1}compared to the control (Table 4).

The nodule formation might be related to the ability of mungbean to form a symbiotic association with bacteria introduced. In the rhizosphere, the number of bacterial colonizes increased along with the plant roots due to the use of biofertilizer (Ahmad *et al.,* 2014; Meunchang *et* *al.,* 2005), which thereby increase the nodule formation. It has been documented that in the rhizosphere, growth promotion activities of rhizobacteria depend on effective colonization ability and survival in the changing environment (Lugtenberg *et al.,* 2001).

Increasing of nodule number when the seeds treated by *Rhizobium* strain indicated that the native *Rhizobia* population density nodulating the legume might not be adequate and this, in turn, brought the response of the plant to inoculation of the *Rhizobium* strains (chemining'wa *et al*., 2007). Similarly, it was well reported that inoculation of *Rhizobium* strains increased the number of nodules significantly for haricot bean varieties (Habte and Buraka, 2016; Otieno *et al*.,2007). Another report also shows that inoculation with organic and inorganic fertilizer improved nodulation and yield of peanut. Thus the nodule number and nodule dry weight of peanut showed a significant response to organic (compost and manure) and inorganic fertilizer (Urea and DAP) integrated with *Bradyrhizobium* inoculation (Argaw, 2017). The nodule number was found to be increased by 141, 142.8, and 143.6% due to the applied compost *Bradyrhizobium*, Manure *Bradyrhizobium*, and DAP *Bradyrhizobium*, respectively, compared to the control check.

The lowest number of nodule plant^{-1} was observed due to the highest rate application of bio-slurry with mineral N fertilizer. Thus it reduced the activity of bacteria; the plant did not form an association with the bacteria and did not cost further energy due to the availability of enough N for plant roots from the soil solution (Zelalem, 2018). This result is supported by the findings of Tarekegn and Serawit (2017) on growth, symbiotic, and yield response of mineral N-fertilizer and *Rhizobium* inoculated common bean indicated that the application of the recommended N fertilizer rate resulted in decreased nodule numbers and dry weight. Another study which was conducted by Hussien *et al*., (2015) revealed that the application of mineral N fertilizer significantly reduced the nodule numbers and dry weight per plant in snap bean.

Thus, nodulation was inhibited when a higher rate of N was applied as compared to lower rates. It may reduce the activity of inoculated *Rhizobium* to fix biological N in the presence of a high amount of N. Actually the NO_3-N does not allow the legumes to fix atmospheric N in the existence of optimal N nutrition, because the plant needs greater energy to fix atmospheric N than to utilize NO_3 (Zahran, 1999). Moreover, the presence of excess NO_3/NH_4 level affects *Rhizobium* activity in soil by inhibiting the lectin compounds that attract *Rhizobium* towards legume. Nodulation was decreased due to a higher rate of N application (Habte and Buraka, 2016; Otieno *et al*., 2007; and Mozumder *et al*. 2003). The exogenous application of N may raise the soil N to a level that may reduce nodulation (Gao *et al*., (2020); Zhang *et al., (*2011); Wang *et al., (*2006); Laws and Graves, (2005).

4.4.2. Nodule diameter

Analysis of variance showed that the main effect of bio-slurry and inoculant/N fertilizer had a significant (P<0.05) effect on the nodule diameter. Interaction among treatments was also significant (P<0.001) (Appendix 3).

Maximum nodule diameter was recorded from *Rhizobium* inoculation with nil bio-slurry (4.44mm^2) followed by *Rhizobium* inoculation with 100% bio-slurry, whereas the lowest nodule diameter (2.1mm^2) was recorded from 150% bio-slurry + 23 kg N ha^{-1} (Table 4). The bigger nodule diameter attributed to the inoculation of *Rhizobium* improved the effectiveness of the nodule formation. The lowest nodule diameter due to the application of the highest amount of N from both sources, enough N to the soil from both organic and inorganic sources the plant and *Rhizobia* do not make association for further N_2 fixation.

This accommodates the report of Agele *et al*. (2017) that *Rhizobium* inoculation has increased nodule diameter in legumes compared to uninoculated treatments. Another study reported by Chiamaka (2014) indicated that inoculated plants with slurry and strain formed bigger nodules than uninoculated plants due to the effectiveness of the introduced *Rhizobia* strain to initiate nodulation with mungbean roots.

4.4.3. Nodule fresh and dry weight

Analysis of variance indicated that the main effects of bio-slurry and inoculant/N fertilizer had a highly significant (P<0.001) effect on the nodule fresh and dry weight. Likewise, interaction among treatments was also significant (P<0.05) (Appendix 3).

Therefore, the present study indicated that maximum nodule fresh and dry weight was recorded from *Rhizobium* inoculation with 100% bio-slurry (0.63g and 0.29g) respectively, followed by *Rhizobium* inoculation with nil bio-slurry, (0.57g) which was at par. Statically similar results with the highest dry weight was recorded from application of 100% and 150% bio-slurry with both strain MB-001 and 23kg ha^{-1} N. The lowest nodule fresh and dry weight was obtained from the control treatment (Table 4). The increase in nodule fresh and dry weight might be related to the ability of the bio-slurry to serve as a source of C, N, P, and micronutrients for the inoculated *Rhizobia (*Gao *et al., 2020)*

This study is consistent with a previous study suggesting that maximum nodule dry weight was obtained from the combined application of compost, *Rhizobium* inoculation, and 75% of recommended mineral N (Meesam *et al*., 2014). Mozumder *et al*. (2003) noticed that nitrogen applied in combination with *Rhizobium* increased nodule weight.

Table 4 Interaction effects of treatments on the nodulation parameters of mungbean grown at Hawassa, Ethiopia, during 2019.

Treatments		**Nodule parameters**			
Rate of bio-slurry (%)	Inoculant/N fertilizer	Number of Nodules $plant^{-1}$	Nodule diameter (mm^2)	Nodule fresh weight (g)	Nodule dry weight (g)
0	0	20.00^{b}	3.48^{bc}	0.13^{f}	0.06^{d}
	23	16.90^{c}	$2,68^{cde}$	0.14^{f}	0.07^{d}
	Strain MB-001	26.15^{a}	4.44^{a}	0.58^{ab}	0.27^{a}
	23+MB-001	13.70^{def}	2.20^{e}	0.21^{ef}	0.10^{d}
50	0	16.10^{cd}	2.83^{cde}	0.13^{f}	0.06^{d}
	23	13.40^{ef}	2.93^{cde}	0.14^{f}	0.07^{d}
	Strain MB-001	21.05^{b}	3.10^{cd}	0.29^{de}	0.16^{c}
	23+MB-001	13.20^{ef}	2.82^{cde}	0.17^{f}	0.09^{d}
100	0	15.10^{cdef}	2.18^{e}	0.39^{cd}	0.24^{ab}
	23	13.95^{def}	2.74^{cde}	0.40^{cd}	0.21^{bc}
	Strain MB-001	19.95^{b}	4.15^{ab}	0.63^{a}	0.29^{a}
	23+MB-001	14.00^{def}	2.79^{cde}	0.57^{ab}	0.29^{a}
150	0	13.90^{def}	2.65^{cde}	0.48^{bc}	0.28^{a}
	23	12.75^{f}	2.12^{e}	0.54^{ab}	0.26^{a}
	Strain MB-001	15.70^{cde}	2.76^{cde}	0.48^{bc}	0.27^{a}
	23+MB-001	13.17^{ef}	2.54^{de}	0.51^{abc}	0.28^{a}
CV%		11.46	20.92	24.19	17.65
LSD (0.05)		2.64	0.86	0.12	0.04

Means followed by the same letter (s) within the column are not significantly different at (P ≤0.05).

4.5. Effects of Bio-slurry and Inoculant/N Fertilizer Application on Gas-exchange Parameters

Analysis of variance revealed that the main effect of bio-slurry had a significant (P<0.05) effect on transpiration rate whereas, the interaction of bio-slurry and inoculant/N fertilizer had a significant effect on net photosynthesis.

4.5.1. Photosynthesis rate (Pn)

The results revealed that non-significant effects of bio-slurry and inoculant/N fertilizer application on the net photosynthesis rate. However, their interaction had a significant (P< 0.05) effect on the photosynthetic rate (Appendix 3).

Numerically the highest net photosynthesis rate (14.3µmolm^{-2}s^{1}) was recorded from the application of 100% bio-slurry + strain MB-001, whereas, the lowest photosynthesis rate (9.9µmolm^{-2}s^{1}) was recorded from the control treatment (Table 5). The increasing net photosynthesis rate might be attributed by the increase in leaf area due to applied treatments. Further addition of bio-slurry rate with both inoculant/N fertilizer does not show an increasing trend in net photosynthesis and the interaction effect is too small which is statistically similar to the treatment of highest net photosynthesis. Nitrogen supplied by the organic fertilizer favoring the higher chlorophyll content. Plants with a high concentration of chlorophyll are potentially capable of achieving higher photosynthetic rates due to its light energy capture value per unit time (Taiz and Zeiger 2013). Similarly, previous studies (Furtini *et al.*, 2006), showed that the application of organic and inorganic fertilizers enhanced the shoot biomass of plants. Hence, the improved biomass accumulation is attributed to an increased availability of plant nutrients which might have been enhanced the photosynthetic capacity of the plants.

This result is in line with Vazin, (2012), who stated that the application of inoculant and organic fertilizer sources improved all physiological attributes of the plant. Similarly, Al-Owied (2016), reported that the application of biofertilizers in combination with either the recommended dose of synthetic fertilizers or with organic manures mitigated the reduction detected in the application of each sole and recorded an increase in photosynthesis parameters as compared to control treatments. Such effect might be the improvement due to growth performance and higher plant vigor over plant growth periods through the increasing number of leaves and number of branches per plant, leaf area which directly affected on plant nutrient uptake.

Nitrogen is an integral component of chlorophyll, the primary molecule that absorbs the light energy needed for photosynthesis. The assimilates obtained through photosynthesis are stored only for a certain period of time in the plant but very soon consumed in maintenance respiration and growth respiration to provide the energy for the growth and development of structural components of plants (Alves *et al*., 2018; Jackson *et al.*, 2018).

Hence, increased net photosynthesis might be associated with increased duration of functional leaf area and the increased accumulation of biomass in the leaf (Islam *et al*., 2010). Similarly, the supplied N through organic fertilizer favoring the higher chlorophyll content and plants with a high concentration of chlorophyll is capable of achieving higher photosynthetic rates due to its light energy capture per unit time (Taiz and Zeiger, 2013). Furthermore, Sahu (2014) reported an increased rate of net photosynthesis due to the integrated application of organic and inorganic fertilizer.

Table 5 Interaction effects of treatments on the photosynthesis rate of mungbean grown at Hawassa, Ethiopia, during 2019.

Treatments		**parameter**
Rate of bio-slurry (%)	Inoculant/N fertilizer	Photosynthesis rate (μmolm^{-2}s^{1})
0	0	9.9^{c}
	23	11.5^{abc}
	Strain MB-001	11.6^{abc}
	23+MB-001	13.6^{a}
50	0	12.5^{ab}
	23	12.6^{ab}
	Strain MB-001	12.7^{ab}
	23+MB-001	13.2^{a}
100	0	12.6^{ab}
	23	11.4^{abc}
	Strain MB-001	14.3^{a}
	23+MB-001	9.92^{bc}
150	0	13.2^{a}
	23	14.0^{a}
	Strain MB-001	11.5^{abc}
	23+MB-001	13.1^{a}
CV%		18
LSD (0.05)		3.18

Means followed by the same letter (s) within the column are not significantly different at (P ≤0.05).

4.5.2. Transpiration rate (E)

Analysis of variance revealed that only the main effect of bio-slurry had a significant (P<0.05) effect on the transpiration rate. Whereas, the main effect of inoculant/N fertilizer as well as the interaction effect between bio-slurry and inoculant/N fertilizer was not significant on transpiration rate (Appendix 3).

The maximum transpiration rate (5.4 $mmolm^{-2}s^{-1}$) was recorded from the application of 100% bio-slurry, which is statically similar with 50% and 150% bio-slurry application whereas, the lowest transpiration rate (4.6 $mmolm^{-2}s^{-1}$) was recorded from the control treatment (Table 6). The rate of transpiration shown an increasing trend from 0 levels to 100% and did not increase further due to applied bio-slurry. Increasing doses of bio-fertilizer with organic fertilizer (20% earthworm hummus + 80% soil) increase the gas exchange in tomato plants, while high doses together with more concentrated organic fertilizers, decrease these characteristics (Jackson *et al*., 2018).

Table 6 Main effects of bio-slurry and inoculant/N fertilizer on transpiration rates (E) of mungbean at Hawassa, Ethiopia during 2019.

Treatments	**Parameter**
Bio-slurry (%)	**E ($mmolm^{-2}s^{-1}$)**
0	4.6^{b}
50	5.2^{a}
100	5.4^{a}
150	5.2^{a}
LSD (0.05)	0.6
Inoculant/N fertilizer	
0	4.9
23 kg N ha^{-1}	5.1
strain MB-001	5.2
23 kg N ha^{-1} + MB-001	5.3
CV%	16.3
LSD (0.05)	ns

Means followed by the same letter (s) within the column are not significantly different at (P ≤0.05).

4.6. Effects of Bio-slurry and Inoculant/N Fertilizer Application on Yield and Its Components

4.6.1. Number of pods plant^{-1}

The analysis of variance revealed that the main effect of bio-slurry had a significant (P< 0.05) effect on the number of pods plant^{-1}. However, the main effect of inoculant/N fertilizer and the interaction were not significant on pod number (Appendix 4).

The highest mean pod number (22.9) was recorded from the application of 100% bio-slurry, numerically followed by bio-slurry rates of 50% and 150% which are statically at par. The lowest mean pod number (17.5) was recorded from the control (Table 7). N is a fundamental component of protoplasm, chlorophyll particles, amino acids, and is involved in improving pod filling by insuring more survival of flowers **(**Brady and Weil, 2002**).** Furthermore, the administration of N on fertilized soil will further dissolve P, so that P is more available and can be utilized by plants for metabolism including photosynthesis, especially in CO_2 fixation so that carbohydrates are formed and translocated for pod formation (Lakitan *et al*., 2007). The increased pod number due to applied bio-slurry might be due to the availability of N in and it's enough release eases the uptake and enhances the pod formation of plants. Similar results were reported by Songkrit and Nukoon (2017) who found that an addition of 5 and 10% bio-chart each alone gives the highest 34.3 and 39 pods plant^{-1} of green bean respectively as compared to the lowest number of pods (29) from the control treatment.

Table 7 Main effects of bio-slurry and inoculant/N fertilizer on pod number of mungbean at Hawassa, Ethiopia during 2019.

Treatments	Yield parameters
Bio-slurry (%)	**Number of pods plant^{-1}**
0	17.5^{b}
50	20.6^{ab}
100	22.9^{a}
150	20.5^{ab}
LSD (0.05)	3.7
Inoculant/N fertilizer	
0	20.1
23kg N	19.8
Strain MB-001	20.9
23kg N + strain MB-001	20.7
CV%	25.8
LSD (0.05)	ns

Means followed by the same letter (s) within the column are not significantly different at (P ≤0.05).

4.6.2. Hundred seed weight

The result revealed the main effects of bio-slurry and inoculant/N had significant ($P< 0.05$) effects on hundred seed weight. Likewise, interaction among treatments was also highly significant ($P< 0.001$) on hundred seed weight (Appendix 4).

The heaver seed weight (5.1g) was recorded from the application of 100% bio-slurry with nil inoculant/N fertilizer whereas; the lowest hundred seed weight (3.1g) was recorded from the control treatment (Table 8). The increased seed weight due to the bio-slurry application might be attributed to the high P content in the bio-slurry, which is part of the ATP and involved in seed development (Amanullah and Khalid, 2020).

In agreement with the current result, Yafizham and Sutarno (2018) reported that an increased seed weight due to combined application of bio-slurry with mineral N. Sahu (2014) additionally detailed higher seed weight was due to the application of suggested rates of NPK with 4 t. ha^{-1} farmyard excrement compared to the control treatment. Moreover, Krishnan (2016) reported a maximum of 1000 grains weight under the combined application of inorganic and organic fertilizers.

4.6.3. Number of seeds pod^{-1}

The analysis of variance revealed that the main effect of bio-slurry and the combined effect of bio-slurry rates and inoculant/N fertilizer resulted in significant (P<0.05) differences in the number of seeds per plant (Appendix 4). Numerically the highest number of seeds pod^{-1} was obtained from 100% bio-slurry + strain MB-001 (13.49) which is statistically similar with an integrated application of 50% bio-slurry + 23 kg N ha^{-1} (13.21) and 100% bio-slurry with nil N (12.51) whereas the lowest seed number pod^{-1} (9.08) recorded from the control treatment (Table 8).

This result is in line with Anwar and Saeed (2018); Mehraj *et al,* (2014), reporting a significant effect on mungbean seed number pod^{-1} of the combined application of organic and synthetic fertilizers. Anwar and Saeed (2018) reported that the seed number of mungbean significantly varied and the maximum number of seeds (12) were counted in treated plots as compared to control which produced minimum seeds pod^{-1} (10). Mehraj *et al,* (2014), showed on the report maximum number of seeds pod^{-1} (12.1) was found in combined application of 10

tha^{-1} Vermicompost + 75 % of recommended dose of inorganic fertilizer whereas minimum from control (10.1). Another study conducted by Meena *et al*. (2015) revealed that bioinorganic combination of fertilizer with *Rhizobium* inoculation improved number of seeds per pod of mungbean.

4.6.4. Biological yield

Analysis of variance indicated that the main effects of treatments had highly significant ($P < 0.001$) on biological yield. Similarly, interaction among treatments was also shown a significant ($P < 0.05$) effect on biological yield (Appendix 4).

The maximum biological yield was recorded from the integrated application of 150% bio-slurry + 23 kg N ha^{-1} (5.24 $t.ha^{-1}$), whereas the lowest (1.90 $t.ha^{-1}$) was obtained from the control treatment (Table 8). The improvement in biological yield might be related to progressed vegetative growth backed by the higher availability of nutrients in organic fertilizer. Thus the increased amount of N from both organic and inorganic sources attributed to the higher above ground biomass accumulation.

This result is in line with Sahu (2014), who reported a maximum biological yield from the application of inorganic NPK with organic farmyard manure over the remaining treatment. Meesam *et al.* (2014) also reported the number of pods, grain, and biological yields increases in mungbean due to the use of compost, mineral N, and *Rhizobium* inoculation. A comparable finding was detailed by Singh and Agrawal (2007), affirming the biological yield improvement due to the use of organic sources of fertilizers. It was also reported that the highest total biomass yield (kg ha^{-1}) of peanut was recorded by combined application of manure, compost and *Bradyrhizobium* (Argaw, 2017)

Increasing the level of bio-slurry presumably increased the availability of soil N, and that of other macro-and micronutrients, which might have enhanced meristematic growth and resulted in higher biological yield (Musse *et al.,* 2020; Islam *et al*., 2010). Nitrogen is a vitally important plant nutrient involved in protein and enzyme synthesis and enzymes control metabolic plant processes (Soetan *et al*., 2010).

Moreover, nitrogen is an integral component of chlorophyll, the primary molecule that absorbs the light energy needed for photosynthesis. The observed increase in biological yield in response to bio-slurry nitrogen might have been due to its positive effect on cell elongation, cell division, the formation of nucleotide, and coenzyme in meristematic activity, and increasing photosynthetic surface, resulting in more production and accumulation of photosynthetic compounds. The high P content of the bio-slurry might also have positively contributed to the biomass yield of mungbean. Phosphate compounds act as energy currency in plants, and play an important role in photosynthesis and the metabolism of carbohydrates; they are also stored for subsequent growth and reproductive processes.

4.6.5. Grain yield

The statistical analysis result revealed that the main effects and interaction of treatments had significant ($P< 0.001$) effects on grain yield (Appendix 4).

The highest grain yield was recorded from 100% bio-slurry + strain MB-001 (1.20 $t.ha^{-1}$), followed by 100% bio-slurry (1.16 $t.ha^{-1}$), and 50% bio-slurry + 23 kg N ha^{-1} (1.15 $t.ha^{-1}$) which did not differ statistically among each other. On the other hand, the lowest grain yield (0.70$t.ha^{-1}$) was obtained from the control treatment (Table 8).

When the rate of bio-slurry increases from 0% to 100 % in combination with both inoculant/N fertilizers, the grain yield was increased and when higher amount of bio-slurry (150%) in combination with inoculant/N fertilizer increased the biological yield rather further increasing the grain yield. The improved grain yield with the supplied bio-slurry and *Rhizobium* inoculation might be attributed to the improved net photosynthesis which resulted in maximum net grain product. It was reported that the grain yield of mungbean was significantly enhanced by the combined application of compost, *Rhizobium* and 75% recommended dose of N fertilizer (Meesam *et al*., 2014).

Additionally, an increase in the nutrient content of the soil due to organic fertilizer application led to the increase in the nutrient content of the plant that ultimately resulted in a higher rate of synthesis and assimilation of photosynthetic and finally higher grain yield. The other reason for the increase is might be due to the role of organic matter in the processing and supply of good nutrient for plants, which appears in the growth characteristics of the crop and these results are consistent with the findings of the revealed achievement of significantly high yield in leguminous crops due to application of organic materials (El-Desuki *et al*., 2010).

According to Tana and Woldesenbet (2017) grain yield of barley was significantly affected by the combined application of organic FYM and inorganic NP fertilizer. Another study by Meena *et al*. (2015) revealed that the application of organic fertilizer with inorganic fertilizer increased the seed yield of mungbean. There noted that application of 75% of the recommended dose of inorganic fertilizer + vermicompost + *Rhizobium* inoculation with PSB produced the highest seed yield of mungbean. Moreover, Krishnan (2016) indicated that the combined treatment of NPK with vermin-compost recorded the maximum grain yield of green gram.

4.6.6. Straw yield

The statistical analysis of results revealed that the main effects of treatments had a highly significant ($P< 0.001$) effect on straw yield. Interaction among treatments had also shown a significant ($P< 0.05$) effect on straw yield of mungbean (Appendix 4).

The maximum straw yield was recorded from the combined application of 150% bio-slurry + 23 kg N ha^{-1} (4.18 $t.ha^{-1}$), whereas, the lowest straw yield (1.20 $t.ha^{-1}$) was recorded from the control treatment (Table 8). The significant interactive effect because of bio-slurry and mineral

N fertilizer application was attributed to the favorable nutritional status of the soil resulting in increased biomass production of the crop and resulted in higher straw yield.

This result is in agreement with the data reported by Krishnan (2016), who revealed that the applied treatment NPK with vermin-compost brought about significant improvement in straw yield followed by farmyard manure in comparison to the control. The straw yield was significantly affected by the interactive application of organic and inorganic fertilization with *Rhizobium* inoculation and the highest straw yield was recorded from 75% of the recommended dose of inorganic + 2.5t vermicompost + *Rhizobium* +PSB compared to the minimum value recorded from the control treatment (Meena *et al*., 2015)

Table 8 Interaction effects of treatments on the yield of mungbean grown at Hawassa, Ethiopia, during 2019.

Treatments		**parameters**				
Rate of bio-slurry (%)	Inoculant/N fertilizer	Number of seed pod^{-1}	Hundred seed weight (g)	Biological yield ($t\ ha^{-1}$)	Grain yield ($t\ ha^{-1}$)	Straw yield ($t\ ha^{-1}$)
0	0	9.08^{d}	3.1^{d}	1.90^{e}	0.70^{f}	1.20^{d}
	23	10.11^{cd}	4.6^{ab}	2.62^{cde}	0.80^{ef}	1.82^{cd}
	Strain MB-001	10.76^{cd}	4.4^{bc}	3.41^{bc}	0.86^{de}	2.55^{bc}
	23+MB-001	11.04^{bc}	4.3^{bc}	3.27^{bcd}	0.82^{def}	2.45^{bc}
50	0	10.41^{cd}	4.3^{bc}	2.36^{e}	0.83^{def}	1.52^{d}
	23	13.21^{ab}	4.8^{ab}	2.63^{cde}	1.15^{ab}	1.47^{d}
	Strain MB-001	11.11^{bc}	4.0^{c}	2.35^{e}	1.03^{bc}	1.31^{d}
	23+MB-001	11.11^{bc}	4.6^{ab}	2.59^{de}	0.95^{cd}	1.64^{d}
100	0	12.51^{ab}	5.1^{a}	3.65^{b}	1.16^{ab}	2.49^{bc}
	23	10.75^{cd}	4.6^{ab}	3.85^{b}	0.93^{cde}	2.91^{b}
	Strain MB-001	13.49^{a}	4.3^{bc}	3.76^{b}	1.20^{a}	2.55^{bc}
	23+MB-001	11.07^{bc}	4.4^{bc}	3.96^{b}	0.91^{cde}	3.04^{b}
150	0	11.31^{bc}	4.4^{bc}	3.52^{b}	0.81^{def}	2.71^{b}
	23	11.30^{bc}	4.6^{ab}	5.24^{a}	1.05^{bc}	4.18^{a}
	Strain MB-001	11.00^{bc}	4.3^{bc}	3.84^{b}	0.88^{de}	2.96^{b}
	23+MB-001	9.76^{cd}	4.5^{bc}	3.85^{b}	0.88^{de}	2.97^{b}
CV%		11.07	9.3	17	10.46	23
LSD (0.05)		1.75	0.5	0.80	0.13	0.77

Means followed by the same letter (s) within the column are not significantly different at (P ≤0.05).

4.6.7. Harvest index

There was a significant difference ($P \leq 0.05$) among bio-slurry on harvest index of mungbean, while the main effect of inoculant/N fertilizer as well the interaction among bio-slurry and inoculant/N fertilizer did not have any significant effect on harvest index (Appendix 4).

The higher harvest index value of 40.0% was recorded from 50% bio-slurry application (Table 9). When the amount of bio-slurry increased to the rate of 100% and 150% the harvest index value showed decrement. This might be due to the increased release of N from high amount of bio-slurry increased above ground biomass and decreased the economic yield of crop, hence harvest index is the result of dividing grain yield to above ground biomass. Therefore, this is due to the positive correlation between grain yield and harvest index. Harvest index indicates that the physiological ability of a crop to convert a proportion of dry matter into economic yield (Tarekegn *et al*., 2018). Bio-slurry is known to have the ability to supply both macro and micronutrients required for crop growth, development and final economic yield (Parry *et al*., 2005).

Table 9 Main effects of bio-slurry and inoculant/N fertilizer on harvest index of mungbean at Hawassa, Ethiopia during 2019.

Treatments	**Parameter**
Bio-slurry (%)	**Harvest index (%)**
0	30.8[b]
50	40.0[a]
100	26.6[bc]
150	21.9[c]
LSD (0.05)	5
Inoculant/N fertilizer	
0	30.3
23kg N	29.7
Strain MB-001	31.9
23kg N + strain MB-001	27.5
CV%	23.5
LSD (0.05)	ns

Means followed by the same letter (s) within the column are not significantly different at (P ≤0.05).

4.7. Correlation Analysis

Simple correlation coefficient values (r) are computed to display the relationships between and within the growth, nodulation, and yield parameters of mungbean (Appendix 5). The correlation values showed the apparent association of the parameters of the crop with each other. Most of the growth and nodulation parameters are positively correlated with yield parameters. Nodulation was significantly and positively correlated with days to flowering (r=0.96***), days to maturity (=0.97***), plant height (r=0.95***), leaf area (r=0.86***), shoot fresh weight (r=0.91***), shoot dry weight (r=0.88***).

Also grain yield was significantly and positively correlated with plant height (r=0.98), leaf area (r=0.93***), shoot fresh weight(r=0.95***), shoot dry weight (r=0.94***), nodule number per plant (r=0.97***), pod number(r=0.97***), seeds per pod (r=0.99***), seed weight (r=0.99***), above ground biomass (r=0.96***). This could be therefore generalized that those factors positively influenced growth, nodulation parameters also influenced grain yield of the crop. The increments in these parameters have a direct relationship with the yield of mungbean. This implies that improving any parameter of these may lead to the improvement in the yield of mungbean.

4.8. Partial Budget Analysis

The economic analysis revealed that most treatments with marginal rate of return gives high net benefit than the control (Table 10). Thus, the combined application of 50% bio-slurry with 23 kg ha^{-1} N fertilizer gave the highest net benefit (35437.6 ETB) with marginal rate of return 7097%. This indicated that for every 1.00 birr invested, farmers can obtain an additional 70.97 Birr (CIMMYT, 1988).

All un-dominated treatments or application rates could be acceptable for mungbean producers or small scale farmers in the study area except the dominated one's. Because the marginal rate return below 100% is low and unacceptable to farmers (CIMMYT, 1988). Therefore, the most economical rate for producers resulting in the higher benefit was the combined application of 50% bio-slurry with 23kg N ha^{-1} fertilizer followed by 100% bio-slurry with *Rhizobium* inoculation which was the next most promising economically feasible treatment (Table 10).

Table 10 Partial budget analysis

Treatments		Parameters of economic analysis			
Rate of bio-slurry (%)	Inoculant/N fertilizer	ADMY kg ha^{-1}	TVC	NB ETB ha^{-1}	MRR %
0	0	630.54	0	25221.6	
	23	721.8	1000	27872	265
	Strain MB-001	774.09	1360	29603.6	481
	23+MB-001	743.76	2360	27390.4D	
50	0	755.28	6130	24081.2D	
	23	1043.19	6290	35437.6	7097
	Strain MB-001	933.12	7130	30194.8D	
	23+MB-001	858.06	7290	27032.4D	
100	0	1046.25	11060	30790	99
	23	842.76	11220	22490.4D	
	Strain MB-001	1087.83	12060	31453.2	1067
	23+MB-001	825.21	12220	20788.4D	
150	0	735.21	15990	13418.4D	
	23	946.8	16150	21722	5189
	Strain MB-001	792.36	16990	14704.4D	
	23+MB-001	791.19	17150	14497.6D	

ADMY= Adjusted marketable yield; TVC=Total variable cost, NB = net benefit; D= dominated and MRR=Marginal rate of return

5. SUMMARY AND CONCLUSION

The study was conducted at Hawassa University experimental site under field condition during the 2019/20 main cropping season to determine the combined effects of bio-chemical fertilizer sources on growth, nodulation and yield of mungbean. The factorial combinations of four bio-slurry levels (control, 50, 100, 150%,) and four N source treatments (0, 23kg N ha^{-1}, strain MB-001, 23kg N + strain MB-001) were laid out in a randomized complete block design with four replications.

The result of the study revealed that the application of bio-slurry in combination with inoculant/mineral N fertilizer had a significant ($P<0.05$) effect on phenology, and most of the measured growth, nodulation, and yield parameters of mungbean. The combined application of bio-slurry and chemical N delayed both days to flowering and physiological maturity. Plant height, leaf number, leaf area, leaf area index; shoot fresh and dry weight per plant improved due to the combined application of bio-slurry and inoculant/N fertilizer.

Among the growth parameters, the plant height, leaf area, leaf area index, shoot fresh and dry weight showed a significant difference for the main effects of bio-slurry and inoculant/N fertilizer as well as their interaction effect. The highest nodule number, nodule dry and fresh weight and nodule diameter, were recorded from the Rhizobium inoculation and combined application of 100% bio-slurry with *Rhizobium* inoculation. Bio-slurry with *Rhizobium* inoculation had shown a significant effect on net photosynthesis and application of 100% bio-slurry demonstrated a marked effect on transpiration rate.

The main effects and interaction effect of bio-slurry and inoculant/N fertilizer supplement significantly affected the yield and yield components of mungbean. Applied bio-slurry and inoculant/N fertilizer had a significant influence on a number of seeds per pod, hundred seed weight, biological yield, grain yield, and straw yield whereas, pod number and harvest index had resulted in a marked improvement due to the main effect of bio-slurry treatment. The combined application of 150% bio-slurry with 23kg N ha^{-1} resulted in the highest biological and straw yield whereas, the application of 100% bio-slurry with *Rhizobium* inoculation resulted in marked improvement of seeds pod^{-1} and grain yield. Pod number per plant and 100 seed weight were highest from the application of 100% bio-slurry with nil N fertilizer. Therefore, significant responses in phenology, nodulation, gas-exchange, and yield of mungbean were obtained from the combined applications of bio-slurry and inoculant/N fertilizer. Besides, the study identified that the highest net benefit with the highest marginal rate of return could be obtained from the combined application of bio-slurry and inoculant/N fertilizer. Thus, the application of 50% bio-slurry with 23kg N ha^{-1} fertilizer has a higher net benefit (35437.6 ETB) followed by the application of 100% bio-slurry with *Rhizobium* inoculation which is (31453.2ETB).

Based on the results of this study, it can be concluded that the application of 50% bio-slurry in combination with 23kg N ha^{-1} fertilizer can be recommended for mungbean production in the experimental and other areas having similar agro-ecology and socio-economic status. However, verification of the result on farmers' fields across season and location could be required to put the recommendation on firm ground.

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7. APPENDICES

Appendix 1: List of treatments and its combinations.

Rate of Bio-Slurry (%)	Inoculant/N fertilizer	Treatment Combination & Code	
0 (BS0)	0 (N0)	T_0 = Control	T_8 = 100% BS
50 (BS1)	23kg N (N1)	T_1 = 23kg N	T_9 = 100% BS +23kg N
100 (BS2)	*Rhizobium* strain MB-001 (N2)	T_2 = MB-001	T_{10} = 100% BS + MB-001
150 (BS3)	23kg N+MB-001 (N3)	T_3 = 0BS +23kg N+ MB-0001	T_{11} = 100%BS +23kg N+ MB-001
		T_4 = 50% BS	T_{12} = 150% BS
		T_5 = 50%BS +23kg N	T_{13} = 150% BS +23kg N
		T_6 = 50%BS + MB-001	T_{14} =150%BS + MB-001
		T_7=50% BS +23kg + MB-001	T_{15}=150%BS +23kg N+ MB-001

Rate of bio-slurry; 50%BS = 12.325m^3, 100%BS = 24.65m^3, 150%BS = 36.975m^3

Appendix 2: Mean square values for phenology and growth parameters of mungbean.

Source of variation	DF	DF	DPM	PH	LA	LAI	SFW	SDW
Rep	3	0.37	0.29	198.70	6075.60	0.06	152.42	39.00
BS	3	105.20***	153.12***	394.20***	2227171.72***	24.77***	7342.61***	904.29***
N	3	5.29***	6.12***	177.87***	221288.34***	2.45***	1265.29***	193.12***
BS*N	9	3.02***	3.02**	71.50*	23431.88***	0.26***	716.39***	7.92*
Error	45	0.75	0.98	26.04	4652.67	0.05	36.42	3.742795

Where, ***, ** and * significant at 0.1%, 1% and 5% levels respectively, BS=Bio-slurry, N=inoculant/N fertilizer, ns= no significant, DF = days to 50% flowering, DM = days to 90% physiological maturity, PH = plant height, LN = leaf number, LA = leaf area, LAI = leaf area index, SFW = shoot fresh weight, SDW = shoot dry weight.

Appendix 3: Mean square values for nodule and gas-exchange parameters of mungbean.

Source of variation	DF	NN	NFW	NDW	ND	E	Ps
Rep	3	6.95	0.01	0.002	0.34	0.24	3.58
BS	3	77.72***	0.42***	0.13***	1.27*	2.04*	6.81ns
N	3	167.25***	0.14***	0.03***	3.73***	0.49ns	0.72ns
BS*N	9	12.78***	0.04***	0.007***	1.09**	0.27ns	10.62*
Error	45	3.44	0.007	0.001	0.36	0.71	5.12

Where, ***, ** and * significant at 0.1%, 1% and 5% levels respectively, DF= Degree of freedom, BS=Bio-slurry, N=inoculant/N fertilizer, ns= no significant, NN = nodule number, NFW = nodule fresh weight, NDW = Nodule dry weight, ND = nodule diameter, E = transpiration, Ps = photosynthesis.

Appendix 4: Mean square values for yield components of mungbean.

Source of variation	DF	PN	SNPP	SW	BY	GY	SY	HI
Rep	3	154.26	5.30	0.08	718905.17	14712.20	855992.27	181.27
BS	3	79.30*	8.81**	0.68**	9772928.0***	203696.40***	9373693.13***	1072.80***
N	3	3.91ns	2.63ns	0.61*	1560356.56**	59649.03**	1227476.75*	67.89ns
BS*N	9	44.33ns	5.00**	0.71***	952428.49**	54692.48***	824643.33*	68.22ns
Error	45	27.93	1.51	0.17	316249.70	9647.98	296542.69	48.74

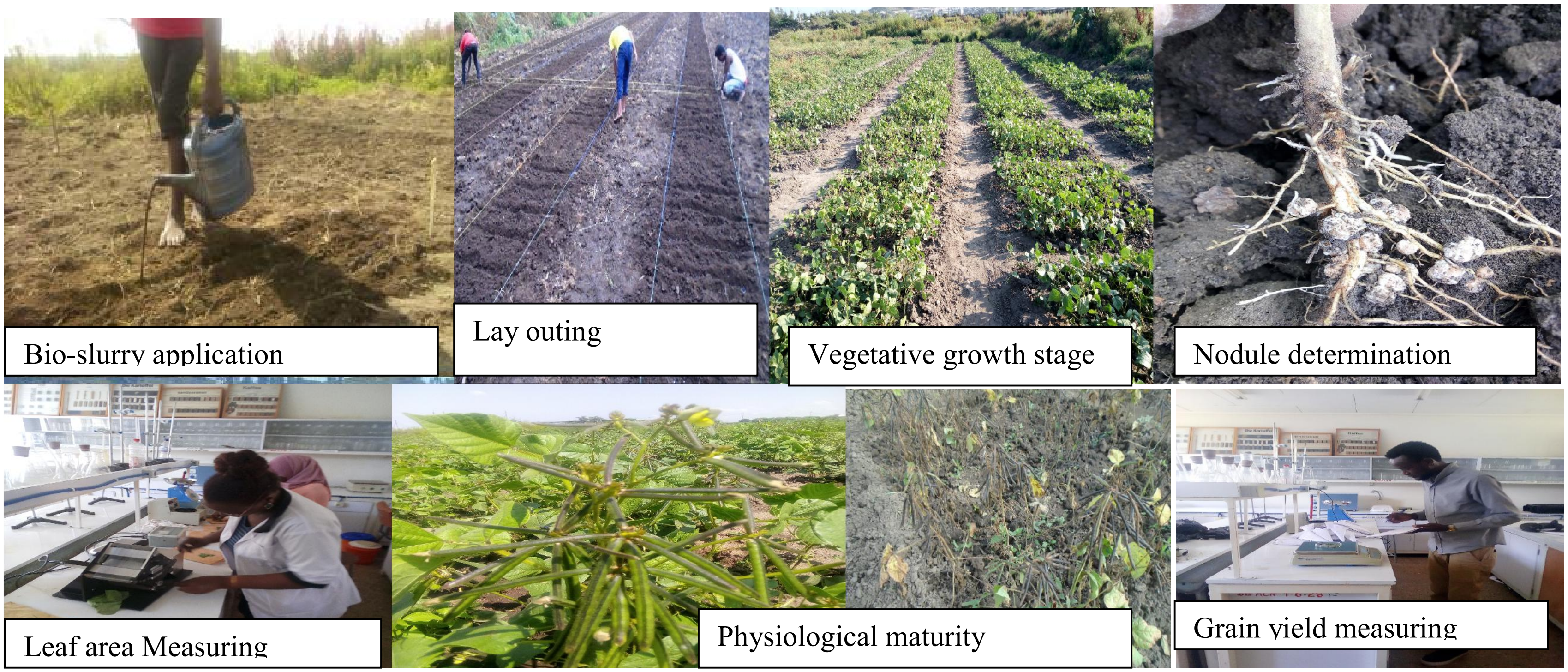
Where, ***, ** and * significant at 0.1%, 1% and 5% levels respectively, DF= Degree of freedom, BS=Bio-slurry, N=inoculant/N fertilizer, PN = pod number, SW = seed weight, BY = biological yield, GY = grain yield, SY = straw yield, HI = harvest index.

Appendix 5: Linear correlation coefficient (r) for selected parameters of mungbean

	DF	DPM	PH	LA	LAI	SFW	SDW	NN	NFW	NDW	ND	PN	SPP	SW	BY	GY	HI
DF																	
DPM	1.00																
PH	0.99	0.99															
LA	0.95	0.94	0.96														
LAI	0.95	0.94	0.96														
SFW	0.97	0.97	0.97	0.96	0.96												
SDW	0.95	0.95	0.96	0.99	0.99	0.97											
NN	0.96	0.97	0.95	0.86	0.86	0.91	0.88										
NFW	0.89	0.89	0.90	0.92	0.92	0.89	0.93	0.87									
NDW	0.90	0.90	0.91	0.94	0.94	0.91	0.94	0.87	0.99								
ND	0.96	0.96	0.95	0.87	0.87	0.90	0.88	0.97	0.88	0.87							
PN	0.96	0.96	0.96	0.91	0.91	0.93	0.92	0.92	0.87	0.89	0.92						
SPP	0.99	0.99	0.98	0.93	0.93	0.95	0.93	0.96	0.88	0.89	0.95	0.96					
SW	0.99	0.99	0.98	0.94	0.94	0.96	0.94	0.96	0.88	0.90	0.95	0.97	0.99				
BY	0.97	0.97	0.97	0.96	0.96	0.96	0.96	0.92	0.93	0.93	0.91	0.94	0.96	0.96			
GY	0.99	0.99	0.98	0.93	0.93	0.95	0.94	0.95	0.89	0.90	0.95	0.97	0.99	0.99	0.96		
SY	0.94	0.94	0.95	0.95	0.95	0.95	0.95	0.89	0.92	0.93	0.88	0.91	0.93	0.93	0.99	0.92	
HI	0.94	0.94	0.91	0.83	0.83	0.87	0.84	0.93	0.77	0.77	0.93	0.90	0.94	0.94	0.84	0.95	0.78

DF = days to 50% flowering, DPM = days to 90% physiological maturity, PH = plant height, LN = leaf number, LA = leaf area, LAI = leaf area index, LDW = leaf dry weight, SFW = shoot fresh weight, SDW = shoot dry weight, RFW = root fresh weight, RL = root length, RV = root volume, NN = nodule number, NFW = nodule fresh weight, NDW = Nodule dry weight, ND = nodule diameter, E = transpiration, Ps = photosynthesis, PN = pod number, SW = seed weight, BY = biological yield, GY = grain yield, SY = straw yield, HI = harvest index.

Filed and laboratory pictures during experimentation



8. BIOGRAPHICAL SKETCH

The author Tamirat Tadewos was born on the 17^{th} of May 1993 in Gamo Zone, Kucha District and from 1999 to 2011. He attended his junior and high school at Woide Woshe Primary School, and Selamber Secondary and Preparatory School, respectively. After successfully passing the Ethiopian School Leaving Certificate Examination, he joined Dilla University College of Agriculture in the 2012 academic year and graduated with a B.Sc degree in Plant Science in 2014. After graduation, he was recruited by the Gamo Gofa zone, Kucha District to serve as an Agronomist expert. In October 2018, he joined the Graduate School of Hawassa University to pursue studies for the Degree of Masters of Science majoring in the field of Agronomy.