



GROWTH, NODULATION AND YIELD RESPONSE OF COWPEA [*Vigna unguiculata* (L.) Walp.] VARIETIES TO *BRADYRHIZOBIUM* INOCULATION IN DALE AND HAWASSA, SIDAMA REGION, SOUTHERN ETHIOPIA

MSc. THESIS

LEMLEM YOHANNES GEZAHEGN

HAWASSA UNIVERSITY

COLLAGE OF AGRICULTURE

HAWASSA, ETHIOPIA

MAY, 2023

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MSc. THESIS

LEMLEM YOHANNES GEZAHEGN

MAJOR ADVISOR: TAREKEGN YOSEPH (Ph.D.)

CO-ADVISOR: TEWODROS AYALEW (Ph.D.)

A THESIS SUBMITTED TO THE SCHOOL OF PLANT AND HORTICULTURAL SCIENCES, COLLEGE OF AGRICULTURE, SCHOOL OF GRADUATE STUDIES, HAWASSA UNIVERSITY, HAWASSA, ETHIOPIA

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(Submission Sheet-2)

We, the undersigned, members of the Board of Examiners of the final open defense by **Lemlem Yohannes** have read and evaluated her thesis entitled “**Growth, nodulation and yield response of cowpea [*Vigna unguiculata* (L.) Walp.] varieties to *Bradyrhizobium inoculation* in Dale and Hawassa, Sidama Region, Southern Ethiopia**” and examined the candidate. This is, therefore, to certify that the thesis has been accepted in partial fulfillment for the requirement of the degree **Master of Science** in Plant Sciences with a specialization in **Agronomy**.

<u>Tarekegn Yoseph (Ph.D.)</u> Name of Major Advisor	_____	_____
	Signature	Date
<u>Tewodros Ayalew (Ph.D.)</u> Name of Co-advisor	_____	_____
	Signature	Date
_____ Name of Internal Examiner I	_____	_____
	Signature	Date
_____ Name of Internal Examiner II	_____	_____
	Signature	Date
_____ Name of External Examiner	_____	_____
	Signature	Date
_____ Name of SGS Approval	_____	_____
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Final approval and acceptance of the thesis is contingent upon the submission of the final copy of the thesis to the school of Graduate Studies (SGS) through the school of Graduate Committee (SGC) of the candidate's department.

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DEDICATION

I dedicate this thesis manuscript to my dear father, Yohannes Gezahegn, whom I lost before the completion of this research work. I also dedicate this piece of work to my wonderful and generous mother, Medhin Gebremariam, and my entire family for their devoted help and encouragement to make my life a success.

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DECLARATION

First, I declare that this is my original work and that all sources of materials used for this thesis have been duly or properly acknowledged. This thesis has been submitted in partial fulfillment of the M.Sc. degree at Hawassa University College of Agriculture and is deposited at the university library to be made available to borrowers under the rules of the library. I solemnly declare that this thesis is not submitted to any other institution anywhere for the award of any academic degree, diploma, or certificate.

Name: ----- **Signature** -----

Place: College of Agriculture, Hawassa University, south Ethiopia

Date of submission: -----

LIST OF ABBREVIATIONS AND ACRONYMS

ANOVA	Analysis of Variance
BNF	Biological Nitrogen Fixation
CEC	Cation Exchange Capacity
CIMMYT	International Maize and Wheat Improvement Center
CSAE	Central Statistical Agency of Ethiopia
FAO	Food and Agricultural Organization
FAOSTAT	Food and Agriculture Organization of the United Nations Statistics
LSD	Least Significance Difference
MARC	Melkassa Agricultural Research Center
MBI	Menagesha Biotech Industry
P.L.C	Private Limited Company
RCBD	Randomized Complete Block Design
SAS	Statistical Analysis System
SNNPR	Southern Nations, Nationalities and People's Region
SSA	Sub-Saharan Africa
TSP	Triple Super Phosphate

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Growth, nodulation and yield response of cowpea [*Vigna unguiculata* (L.) Walp] varieties to *Bradyrhizobium* inoculation in Dale and Hawassa, Sidama Region, Southern Ethiopia

Lemlem Yohannes (BSc), Tarekegn Yoseph (Ph.D.) and Tewodros Ayalew (Ph.D.) School of Plant and Horticultural Sciences, Hawassa University, P.O. Box 05, Hawassa, Ethiopia

ABSTRACT

*Cowpea [*Vigna unguiculata* (L.) Walp] is an important legume crop grown widely in lowland areas of Ethiopia. However, its yield remains low due to the lack of improved varieties and soil fertility decline. Therefore, the current study was conducted to evaluate the growth, nodulation, and yield response of cowpea varieties to *Bradyrhizobium* inoculation in Dale and Hawassa, Sidama Region, Southern Ethiopia during the 2022 main cropping season. The treatment combination consisting of four cowpea varieties (Bole, TVU, White Wonder Trailing, and Keti), with two levels of *Bradyrhizobium* inoculation (non-inoculated and inoculated with the strain: MBI-cowpea) was laid out in Randomized Complete Block Design in a factorial arrangement with three replications. The size of each experimental plot was 2 m in length x 4. 2 m in width with inter and intra-row spacing of 0.6 and 0.2 m respectively, as well as 1 and 1.5 m between two adjacent plots and replications, respectively. The representative soil samples were randomly taken from the experimental sites from 20 different spots at a depth of 0–20 cm and analyzed the soil texture, soil pH, total N, organic carbon, available P, and cation exchange capacity at Hawassa university soil science laboratory. Phenology, growth, nodulation and yield related parameter data's were collected and subjected to ANOVA using SAS software version 9.0. The economic analysis was carried out using partial budget procedure. The results revealed that the day to 50% flowering, day to 90% physiological maturity, nodule fresh and dry weight, plant height, number of primary branches, fresh and dry shoot weight, root fresh weight, number of pods plant⁻¹, number of seeds pod⁻¹, hundred seed weight, and grain yield were significantly influenced by the main effect of varieties and *Bradyrhizobium* inoculation. However, days to 50% emergence and harvest index were significantly affected only by varieties. The nodule number, effective nodule, leaf area, leaf area index, root dry weight, root length, above ground biomass, and straw yield were significantly influenced by the interaction effect of varieties and *Bradyrhizobium* inoculation. Grain yield was positively and strongly correlated with nodule number, nodule dry weight, plant height, leaf area, shoot and root dry weight, number of pods plant⁻¹, pod length, number of seeds pod⁻¹, above ground biomass, and straw yield. The results showed that the White Wonder Trailing variety with *Bradyrhizobium* strain MBI-cowpea produced the highest grain yield (3.5 t ha⁻¹), with a net benefit of 67171.3 ETB ha⁻¹ and a marginal rate of return of 1386.7%. Therefore, this combination could be recommended to increase the grain yield of cowpea and economic profit earned in the study areas, and areas having similar agroecologies. However, this experiment was conducted in a single season, a lower number of varieties and two levels of *Bradyrhizobium* strain, repeating over multiple seasons, using a large number of cowpea varieties, and more *Bradyrhizobium* strains will be demanding to come up with a plausible recommendation.*

Keywords: *Bradyrhizobium* inoculation, cowpea varieties, growth, nodulation, yield components

1. INTRODUCTION

Ethiopia's diverse agroecology allows for the production of various crops, especially grain legumes, which are essential for the livelihood of smallholder farmers (Kebede, 2020). Grain legumes are the most significant crops next to cereals in the country based on production of 3 million tons (Atnaf *et al.*, 2015) and area coverage of 1.7 million hectares (12.9%) year⁻¹ (Hailu and Gidey, 2022). Its provide foods and sources of income to more than 10 million households and contributor for the country's foreign currency earnings (Asrat *et al.*, 2021; CSA, 2021). Among leguminous crops, cowpea is an important food legume grown widely throughout the world, mainly in tropical and subtropical regions, including Ethiopia, and used as food (matured seed and vegetable), animal fodder, has medicinal use, and it fixes nitrogen to maintain soil fertility (Khan *et al.*, 2010).

Cowpea [*Vigna unguiculata* (L) Walp] (diploid, $2n=2x=22$) is an annual herbaceous and dicotyledonous legume plant belonging to the family Fabaceae and cultivated for its edible seeds or fodder (Chaudhary *et al.*, 2022; Urgesa, 2023). It is the most important edible legume in tropical Africa next to the common bean but it is the least cultivated and scarcely distributed in different geographical and cultivation regions of Ethiopia (Belay *et al.*, 2017). From a nutritional point of view, cowpea is an affordable source of carbohydrates, protein, essential minerals, vitamins, and folic acid, especially for poor people who do not have the means to subsist on animals (Biama *et al.*, 2020). Researchers sometimes refer to cowpea as "vegetable meat" or "poor man's meat" because of their high protein content (Mulugeta *et al.*, 2016). Its seeds comprise an average of 23–25% protein and 50–60% starch, as well as 63.6% carbohydrates (Akyaw *et al.*, 2014) and less anti-nutritional and bloating factors than common beans (Khan *et al.*, 2010). Furthermore,

cowpea is a good source of folic acid, and other micronutrients which is especially important for pregnant women (Abdul *et al.*, 2021).

In addition to being directly consumed, cowpeas have significant agronomic benefits. It contributes significantly to the sustainability of cropping systems, improves soil fertility by fixing the atmospheric nitrogen, since it has nodule roots where the *bradyrhizobia* reside, and acts as a good cover crop and thus helps to check soil erosion (Chaudhary *et al.*, 2022). It is highly drought tolerant, adaptable to different soil types, and grows well in multiple cropping systems (Aliyu *et al.*, 2022), especially in sites with low soil fertility, because of its high nitrogen fixation capacity (Sheahan, 2012).

According to the Food and Agriculture Organization database report (FAOSTAT, 2021), the majority (96.7%) of the global production of cowpea is in west Africa, with Nigeria being the largest producer and consumer producing 3.6 million tonnes in 2019. Although Ethiopia is a secondary center of cowpea diversity, unfortunately, however, data on cowpea production and productivity are limited (Beshir *et al.*, 2019). Nevertheless, some sources indicate that the national average yield is estimated to be 0.4 t ha⁻¹ (Beshir *et al.*, 2019) and the potential average yield was 1.5 to 6 t ha⁻¹ depending on genotype (Asiwe *et al.*, 2008). The average actual yield growth of cowpea between 2005/06 and 2020/21 was 118.8%, and the yield gap between the national average yield and the potential yield of the released cultivars under farmer management was 34.7% (Hailu and Gidey, 2022). As a result, the farmers' actual yields are substantially lower than their potential yields, which might be increased with better management.

According to the reports of Gothwal *et al.*, (2008), nitrogen is an important nutrient required for crop growth and productivity, and its deficiency in agricultural soil has a

negative impact on crop growth and yield. Its deficiency results in reduced growth, yellowing leaves, reduced branching of small trifoliolate leaves, and the onset of older, prematurely fading legume leaves (Simon *et al.*, 2014). Improving the N content of the soil through application of mineral fertilizer is important. However, mineral fertilizers are expensive, hurt the soil's fertility and ability to retain water, create nutrient imbalances, and contribute to intolerable levels of water contamination (Nosheen *et al.*, 2021). It is known to contaminate the environment by releasing greenhouse gases into the atmosphere, causing runoff to pollute the soil and water, and damaging the underground water systems through leaching (Nakei *et al.*, 2022). Therefore, using beneficial N-fixing microorganisms in place of chemical nitrogen (N) fertilizer through an integrated approach is a successful strategy to reduce the above challenges (Benson *et al.*, 2015).

According to Nosheen *et al.* (2021), biofertilizers are cost-effective, non-toxic, and easy to apply; they help maintain soil structure and are eco-friendly, serve as a good substitute for expensive and harmful chemical fertilizers and enhancement of crop production by their biological activity in the rhizosphere. Similarly, Rajesha and Sanjay (2020) stated that biofertilizers are one of the crucial elements of integrated nutrient management because they are affordable, renewable sources of plant nutrients that can be used in place of or as a supplement to mineral fertilizers in the production of sustainable agriculture. Therefore, microbial inoculants including beneficial bacteria were often known as the best bio fertilizer tools in sustainable agriculture (Kumari *et al.*, 2023). Its input consists of microorganisms that can mobilize significant nutritional components in the soil from inactive to active states through biological processes (Amit and Satish, 2015). Gothwal *et al.* (2008), biological nitrogen fixation (BNF) is a method of converting elemental inert

atmospheric nitrogen into a form available to plants as an organic compound through enzymatic reduction by N-fixing microorganisms such as rhizobia.

An economical and environmentally beneficial alternative to using mineral nitrogen fertilizers in legume crops is BNF via *Rhizobium*-legume symbioses (Mendoza-Suárez *et al.*, 2021). *Bradyrhizobium* inoculant is an alternative, 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 N fertilizer (Kebede, 2021). *Bradyrhizobium* inoculation is a substitute, less expensive source of nitrogen for grain legumes due to the legume-Rhizobium symbiosis' ability to fix atmospheric nitrogen (Ulzen *et al.*, 2020). Similar to this, Rhizobia are essential for the supply of N to ecosystems because they can fix N in symbiosis with legumes and encourage plant development (Nakei *et al.*, 2022). According to Allito *et al.* (2021), legume-Rhizobia symbiosis significantly increases legume productivity and contributes to atmospheric nitrogen fixation. In developing nations, this symbiosis can offer a low-cost method to increase soil fertility and increase crop yield (Samago *et al.*, 2018; Odori *et al.*, 2020).

According to Franco *et al.* (2002), the symbiosis of *Bradyrhizobium* bacteria through the process of BNF belongs to the yield-enhancing form of legumes and avoiding costs of soluble N fertilizers. Similarly, Erica *et al.* (2017) demonstrated that BNF in cowpeas results in great crop yields and optimal node development. Furthermore, Bambara and Ndakidemi (2010) reported that *Rhizobium* inoculation considerably increased yield and all other yield components of cowpea, such as the number of pods plant⁻¹, number of seeds pod⁻¹ and hundred seed weight as compared to uninoculated. Productivities of different cowpea varieties were improved due to inoculation of *Bradyrhizobia* strain (Ayalew *et al.*, 2021).

Currently, the yields of cowpea in Ethiopia as well as in the study areas are low as compared to other African countries for a variety of reasons (Asrat *et al.*, 2021). Such restrictions leading to low yield of cowpea are due lack of improved high-yielding cowpea varieties, poor soil fertility, and unavailability of alternative technologies like *Bradyrhizobium* inoculants strains, poor agronomic practice, limited research work and market (Kebede, 2020). Due to their high price, inorganic nitrogen fertilizers may not be affordable for the majority of farmers, and those who can apply them do so below the recommendation levels (Bitire *et al.*, 2022). Therefore, in order to achieve maximum productivity in legumes, seeds could be inoculated with the effective rhizobia bacteria as an alternative to using pricey commercial nitrogen fertilizers (Mulika *et al.*, 2022).

Bradyrhizobia inoculation of cowpeas is not a common agronomic practice among smallholders, according to Ayalew *et al.* (2021), who also found that there was lack of awareness on the inoculation's positive effects on the economy and the environment. Farmers need to be made aware of this new strategy as rhizobia inoculants and related technologies readily available in research institutes that needs to reach the farmers (Bitire *et al.*, 2022). Poor input and output markets and limited access to extension services are other significant production constraints for smallholder farmers. Therefore, for the impoverished farming community to increase the production of their cowpea and increase their profit, more efforts are needed to spread new inventions to farmers in rural settlements and promote this affordable and friendly technique. This study, therefore, was conducted to evaluate the growth, nodulation, and yield response of cowpea varieties to *Bradyrhizobium* inoculation in Dale and Hawassa, Sidama Region, Southern Ethiopia.

Specific objectives were:

- ❖ To assess the effect of *Bradyrhizobium* inoculation on the growth, nodulation and yield components of cowpea varieties
- ❖ To evaluate the interaction effect of varieties and *Bradyrhizobium* inoculation on growth, nodulation and yield response of cowpea
- ❖ To determine the economically feasible combination of varieties and *Bradyrhizobium* strain for the study areas

2. LITERATURE REVIEW

2.1. Cowpea: Origin, Description and Botany

Based on the oldest evidence of archaeological excavations; cowpea [*Vigna unguiculata* (L) Walp] was originated and domesticated in Africa, probably in both West and East Africa, and cultivated before 2500 BC (D'Andrea *et al.*, 2007; Herniter *et al.*, 2020). West African countries such as Benin, Burkina Faso, Northwest Cameroon, Niger, Nigeria, and Togo were thought to be centers of high diversity of cowpea (Lo *et al.*, 2021). However, it was probably brought to Europe around 300 B.C. and to Asia (India) around 200 B.C. (Madamba *et al.*, 2006). Further spread occurred as part of the Columbian Exchange, which brought African germplasm to the Caribbean, the southeastern United States, and South America, and Mediterranean germplasm to Cuba, the southwestern United States, and northwestern Mexico (Herniter *et al.*, 2020).

According to Herniter *et al.* (2020) and Urgesa (2023), cultivated cowpeas are annual herbaceous legume characterized either by erect, semi-erect, prostrate (trailing), or ascending growth patterns, depending on genotype and environmental conditions. Cultivated cowpea with improved varieties being either extra maturing in 60 days, early (65–75 days), medium (75–100 days), or late (more than 100 days) (Boukar *et al.*, 2020), although up to 240 days for last maturity of pods. The growth habits range from indeterminate to moderately determinate and non-vining types are more determinate (Ntombela, 2012).

Ntombela (2012) indicated that the seed of cowpea also varies in size, color, flavor, yield, and ripening time and a single pod can contain around 10 to 20 seeds per pod. Its height also varies from dwarf (15 cm) to tall (over 100 cm) depending on the growth habit

(Mulugeta *et al.*, 2016). It has a long tap root system with lateral branches with nodules responsible for nitrogen fixation (Urgesa, 2023), and it reaches a maximum effective rooting depth of about 2.4 m within eight weeks of planting, particularly as drought conditions prevail (Ntombela, 2012).

The pod is green in the early stage of growth and when mature it is usually yellow, light brown, pink, or purple in color and the length of the pod can vary from 11 cm to 100 cm (Mulugeta *et al.*, 2016). It is a self-pollinated crop, with natural cross-pollination of up to 1% and can grow up to 80 cm and up to 2 m for climbing cultivars (Urgesa, 2023). Their flowers are borne on raceme inflorescences at the end of stalks and each stalk bears two to three pods but may bear four or more depending on genotype and environmental conditions (Timko and Singh, 2008). Large straight keels, and come in a variety of flower colors (white, pink, cream, purple, dirty yellow, and blue) and open early in the morning and close before noon (Gómez, 2004; Timko and Singh, 2008).

2.2. Importance and Utilization of Cowpea

Cowpea is mainly grown in lowland sites of Ethiopia, where its seeds, pods, and leaves are grown for human consumption, animal feed, and income generation for smallholder farmers (Asrat *et al.*, 2021). It is a principal and multipurpose food legume in many African countries including Ethiopia, where tender leaves, fresh pods, and grains are used for either human consumption or livestock feed (Alemu *et al.*, 2016; Belay *et al.*, 2017).

Besides cowpeas' nutritional role, it improves soil fertility; it compensates for the loss of nitrogen absorbed by cereal crops and thus has a positive effect on soil properties through nitrogen fixation (Belay *et al.*, 2017; Asrat *et al.*, 2021; Ayalew *et al.*, 2022). According to Beshir *et al.* (2019); Asrat *et al.* (2021) it has a good ability to suppress weeds, especially

Striga hermonthica, a parasitic weed of crops such as sorghum and corn, and reduce the number of adherent *Striga hermonthica* plants per corn plant by at least 50% when corn post cowpea is grown reported by (Fatokun *et al.*, 2002).

Goa *et al.* (2022) reported that cowpea provides quick food access to food-insecure households, using short growing cycles to allow cowpea varieties to grow with minimal rainfall in drought-prone sites and grow a second crop in the same harvesting season. It is also a promising alternative for African farmers, including the Rift Valley and southern Ethiopia, where the majority of communities' dietary protein comes from crops (Hallensleben *et al.*, 2009). Therefore, its drought tolerance and adaptability to stressful environments make the cowpea an ideal crop under changing climate conditions (Bisikwa *et al.*, 2014). Economically, cowpea is also one of the most important sources of income; farmers use the harvest to generate income, including by selling kernels and leaves at local markets (Mulugeta *et al.*, 2016; Sisay *et al.*, 2019). As reported by Timko and Singh (2008), cowpea is a cash-generating commodity for farmers and small and medium-sized entrepreneurs in Africa.

In many African countries, including Ethiopia, it can be eaten as a vegetable at all growth stages and all parts of the plant can be used as nutritious food (Mulugeta *et al.*, 2016; Belay *et al.*, 2017). In Ethiopia, cowpea seeds are mainly used as cooked grains (Nifro), bread (kita), and as a component of various sauces such as Shiro or kike wet (split grain sauce), local soup (Shorba) and oatmeal (Genfo) and kollo in the diet of society at different sites (Walle *et al.*, 2018). Sisay *et al.* (2019) reported that different parts of the harvest (fresh leaves, young shoots, and grains) were used for home consumption in the form of traditional stew and sauce in Gambella and southern parts of the country's special districts of South Ari and Konso sites. All parts of the plant serve as nutritious, protein, and

vitamin-providing food (Islam *et al.*, 2006). According to Wakili (2013); Langyintuo and Lowenberg (2006), it plays an important role in meeting the protein needs of rural and urban residents. It is important to reduce protein deficiency in the human diet due to malnutrition among children and resource-poor rural households, where it is considered the meat of the poor (Owolabi *et al.*, 2012).

Besides its nutritional value, it is also used for medicinal purposes (Sisay *et al.*, 2019). Mulugeta *et al.* (2016) also reported that the medicinal value of cowpea ranks fifth in cowpea utilization, where a quarter of the farmers were reported to have used the leaves and grains of cowpea for the treatment of gastric discomfort, malaria and liver diseases. According to Hall *et al.* (2003); Timko and Singh (2008), it contains high amounts of folic acid, and vitamin B to prevent birth defects in the brain and spine during pregnancy. Sisay *et al.* (2019) reported that varieties of Boho and Atera babile are mainly used for medicinal purposes in the Gambella, Oromia, and Dire Dawa regions, using the green leaf to treat liver pain in humans, and local farmers use cowpea seeds to treat malaria infections and stomach ailments.

2.3. Agro-Ecological Requirements of Cowpea

Cowpea is the main drought-resistant food crop for drought-prone areas, particularly in the lowland area of Ethiopia (Asrat *et al.*, 2021). According to Bashir *et al.* (2018); Sisay *et al.* (2019); Ayalew *et al.* (2021), it is an important drought and warm weather-tolerant legume, it adapts well to the drier region of the tropics, where other legumes do not perform well. Similarly, Khan *et al.* (2010), cowpea is a versatile crop that exhibits high adaptability to temperature and drought extremes, tolerates alkaline soil conditions, and has a high potential for biological nitrogen fixation. The deep-rooted system and less water loss

through the stomata, as well as early maturity, are some of the factors that make cowpeas adaptable to hostile environments (Gómez, 2004).

Cowpea is a warm-weather crop with a somewhat higher temperature requirement than maize and grows with less rainfall and under more adverse conditions than common bean (Asrat *et al.*, 2021). It requires minimum and maximum temperatures of 28 and 30°C (day and night) during the growing season (Dugje *et al.*, 2009; Mariam, 2021). It does not tolerate frost, and temperatures above 35°C cause flower and pod shedding (Rafaele *et al.*, 2023).

According to Dugje *et al.* (2009), it is performed well in agroecological zones with annual rainfall between 500 and 1200 mm/year and altitudes range between 350 and 2000 m.a.s.l. However, with the development of extra-early and early-maturing cowpea varieties, the crop can thrive with an annual rainfall of less than 500 mm (Madamba *et al.*, 2006). It can be grown in Ethiopia with an average annual rainfall of 400 – 1500 mm (Bilatu *et al.*, 2012). It is highly tolerant to waterlogging conditions, and well-distributed rainfall is important for normal growth and development (Mulugeta *et al.*, 2016).

According to Bilatu *et al.* (2012), it can be grown well in a variety of soil conditions; however, the best yields are achieved in well-drained sandy to clay loam soils with a soil pH in the range of 5.5 to 8.3, which tend to be less restrictive of root growth. Mariam (2021) stated that it requires well-drained, sandy or loamy soil that tolerates heat and drought, and is well-adapted to a variety of soils even poor soil. It also survives under poor, acidic soil but is reportedly less tolerant of cold soil (DAFF, 2011). The plant performs well under low N conditions due to a high N-fixing capacity (Gbaguidi *et al.*, 2013), and it grows well even in poor soils with more than 85% sand, less than 0.2%

organic matter (Bilatu *et al.*, 2012; Asrat *et al.*, 2021). In Southern Nations, Nationalities, and Peoples' Region (SNNPR) the cowpea is cultivated in low rainfall areas including Konso, Derashe, Humbo, Hammer Bako, Loka Abaya, Gofa, and Loma districts (Nandeshwar *et al.*, 2020).

2.4. Cowpea Production in the World

The global cowpea coverage area is estimated at 11 million hectares, producing 6 million tonnes of grain, with a share of Africa accounting for 96% of world cowpea grain production (FAOSTAT, 2018). According to FAOSTAT, (2019); Samireddypalle *et al.* (2017), about 95% of global production is produced in West Africa, with Nigeria being the largest producer and consumer of cowpea, producing 3.4 million tonnes in 2017. Langyintuo *et al.* (2003) reported that most of the world's cowpea production comes from West-Central Africa where countries such as Nigeria, Niger, Burkina Faso, Senegal, Ghana, Cameroon, and Mali are the major producers. Other important production sites include lower elevation sites of eastern and southern Africa and in South America (particularly in northeastern Brazil and Peru), parts of India, and the southeastern and southwestern regions of North America.

The major production with an average yield of 5, 564 kg ha⁻¹ comes from Western Africa (Mofokeng and Gerrano, 2021). In terms of the metric ton production levels of cowpea grain, Nigeria is the largest producer in the world (FAO, 2020), and accounts for 61% of the production in Africa and 58% worldwide (Baysah, 2013). Uganda and Kenya are also the largest cowpea-producing countries in East Africa (Ojiewo *et al.*, 2019). In South Africa, the production is about 4, 801 tonnes in an area of 10, 990 hectares and is mainly by small-scale farmers in marginal sites (FAO, 2019), Fifty-two percent of Africa's

production of cowpeas is used for food, 13% as animal feed, 10% for seeds, 9% for other uses, and 16% is wasted (Baysah, 2013). According to FAO, the US harvested 11,750 tons of cowpea cultivated on 5220 ha in 2019 (FAOSTAT, 2021).

2.5. Cowpea Production and Productivity in Ethiopia

In Ethiopia, cowpea is one of a few legumes crop which play a vital role in the livelihood of smallholder farmers and a source of cheaper protein in the dry sites of SNNPR, Oromia, Amhara, Tigray, Somali, and Gambella Regions (Beshir *et al.*, 2019). The average yield of the four varieties (Keti, TVU, Black eye bean, and White wonderer trailing) varied from 1.64 to 3.38 t ha⁻¹, indicating a 52% yield difference between the high- and low-yielding varieties (Ayalew *et al.*, 2021). In addition, the average grain yield for the seven cowpea varieties were 2.22 t ha⁻¹, and these values were significantly different among varieties (Kebede and Bekeko, 2020). A larger total area of cowpea production was allocated in Amhara covered with 0.25 ha, followed by SNNP (0.15 ha) regional states and Gambella (0.08 ha) (Beshir *et al.*, 2019). The average yield of cowpea recorded on farmers' fields was 1.7 to 2.1 t ha⁻¹ for the improved varieties, whereas on the research plot was 2.2 to 3.2 t ha⁻¹ have observed from improved varieties with appropriate cultivation and conservation practices under rain field conditions (Kebede and Bekeko, 2020). In a recent study, Beshir *et al.* (2019) indicated that the average grain productivity of cowpea was observed to be low (0.8 t ha⁻¹) on farmers' fields as compared to the yield-on-farm demonstration research station. Beshir *et al.* (2019) reported that the annual cowpea production is estimated to be 55,600 tons produced on 69,500 ha of land based on the survey conducted in five major producing regional states (Amhara, Gambella, Oromia, SNNPR) and Tigray).

2.6. Constraints of Cowpea Production

Numerous limitations restrict the improvement of cowpea yield and productivity in Africa. These limiting factors can be broadly referred to as abiotic or biotic stress and climatic variability and have a tremendous impact on the production and productivity of cowpea grains and forage vegetables produced in the various cowpea-producing nations of the world, particularly in Africa including Ethiopia (Omomowo and Babalola, 2021).

Among the abiotic factors, climate change is directly affecting cowpea production through irregular rainfall patterns and pest and disease infestations (Murtala and Abaje, 2019). Erratic rainfall affects plant populations, and flowering capacity, leading to overall reduction in grain yield and total biomass (Timko and Singh, 2008; Sariah, 2010). Cowpeas respond to severe moisture stress by restricting growth (particularly leaf growth) and reducing leaf area by changing leaf orientation and closing stomata (Timko and Singh, 2008).

Important production constraints related to biotic stress that limit cowpea productivity are expressed by a variety of organisms, including destructive pests, parasitic weeds, viral pathogens, bacterial pathogens, and fungal pathogens (Boukar *et al.*, 2019). Brisibe *et al.* (2011) point out that cowpeas have many limitations because disease and pest exploitation are among the main problems affecting cowpeas.

Other reasons for the decline in productivity in sub-Saharan African countries are the lack of improved varieties that can withstand these stresses and the lack of adequate production practices and inputs needed for greater productivity and profitability (Nkomo *et al.*, 2021). According to Mulugeta *et al.* (2016), limited information on the genetic resource and the main production challenges and social factors related to cowpea production.

2.7. Soil Fertility Decline and its Effect on the Productivity of Cowpea in Ethiopia

Soil fertility is a key factor for successful crop production and it is a measure of the capacity of soil to provide physical, chemical, and biological needs for the plant growth and productivity (Schoenholtz *et al.*, 2000). Soil fertility decline is a major constraint on agricultural Productivity and the livelihoods of millions of rural households in Ethiopia (Haile *et al.*, 2006); (Bejital *et al.*, 2021). It is also the fundamental cause of declining per capital food biomass (Mohammed *et al.*, 2021). The fertility status of Ethiopian soils has also declined and continued to decline posing a challenge to crop production (Kebede and Yamoah, 2009). Even though the consequence of soil fertility decline is very serious; it has not received as much research attention.

Different unmanageable land uses have led to a decline in agricultural productivity due to the loss of soil fertility as a result of poor farm management practices (Isgren *et al.*, 2020). Declining agricultural productivity has been a challenge worldwide and especially in Sub-Saharan Africa (SSA) including Ethiopia, with high rate of nutrient depletion caused by inadequate synthetic fertilizer input, limited return of organic residues, and leaching loss of nutrient elements (Belachew and Abera, 2010). Low agricultural productivity has been attributed to factors such as poor farm management practices, soil infertility, and no use of modern technologies to restore soil fertility (Belachew and Abera, 2010; Ndegwa *et al.*, 2023). Generally, crop production is highly dependent on the level of soil fertility.

This can be improved through the use of grain legumes, which improve soil fertility through biological nitrogen fixation, and reduce the use of commercial nitrogen fertilizer (Isgren *et al.*, 2020). It's also further improved by incorporating cover crops that add biofertilizer to the soil, which leads to improved soil structure and promotes a healthy, fertile soil (Ndegwa *et al.*, 2023). It exceeded most of the legumes to enhance soil fertility

due to high N-fixing capacity when inoculated with effective rhizobia (Ayanwuyi and Akintonde, 2012). Similarly, soil fertility can be improved by applications of organic materials, bio slurry and the inclusion of legumes in the cropping method (Khan et al., 2010); (Gautam *et al.*, 2022). In this regard, cowpea, as a legume, contributes a lot in soil nutrient cycling, because of its nature to form symbiosis with *Rhizobium* bacteria with a reported contribution of 70–350 kg N ha⁻¹ through BNF (Ayalew and Yoseph, 2022). This indicates the importance of legumes symbiosis to enhance crop yield with economic and ecological sustainability.

2.8. Varietal Responses of Cowpea

2.8.1. Phenological and growth parameters

The variation among cowpea varieties for days to 50% emergence, days to 50% flowering, and physiological maturity were significant (Manore, 2017; Ayalew *et al.*, 2021). Similarly, Belay *et al.* (2017) reported performance of five cowpea varieties had a significant difference in all the phenological and growth traits (days to 50% flowering, pod filling period, 50% maturity, and plant height). On the other hand, Miheretu and Addo (2017) reported that plant height and the number of branches plant⁻¹ were significantly affected by cowpea varieties. Similarly, Karikari *et al.* (2015); Ayalew *et al.* (2022) investigated that plant height was significantly affected by the cowpea variety. The tallest plant height 59.92 cm was observed for the variety Bekur, followed by the variety White Wonder Trailing with a height of 58.42 cm, while Ketu recorded the least plant height of 37.46 cm (Belay *et al.*, 2017). However, Augustine and Godfre (2019) revealed that cowpea varieties did not show a significant difference on plant height.

Shoot fresh and dry weight showed a marked difference among the cowpea varieties (Ayalew *et al.*, 2022). However, Manore (2017) reported non-significant differences among cowpea varieties for shoot dry weight. Moreover, tested cowpea varieties showed a significantly different in terms of leaf numbers, leaf area, and leaf area index plant⁻¹ during the 2018 and 2019 cropping seasons (Ayalew *et al.*, 2022). Leaf area index was significantly different between cowpea varieties also reported by (Nyamaizi *et al.*, 2020).

2.8.2. Nodulation parameters

Almost all the varieties (Bole, Black eye bean, TVU, Assabot and Wonder) were developed nodules on their root, the highest nodule number and nodule dry weight was recorded from the Black eye bean and Assabot varieties and the lowest from the Wonder and Bole varieties at Hawassa university research site (Manore, 2017). According to Tesfaye and Nebiyu (2021), nodule number plant⁻¹, effective nodule plant⁻¹, and nodule dry weight of cowpea was significantly affected by the genotype. Similarly, Augustine and Godfre (2019) observed significant varietal variations in nodules number plant⁻¹ and nodule dry weight of cowpea varieties. Moreover, Simms and Lee (2002) who reported that, the nodules occupied by the effective varieties were 2.5 times larger than the nodules occupied by the native varieties in clover. However, Olamide *et al.* (2021) reported non-significant difference in nodule number plant⁻¹, nodules fresh and dry weight of the two varieties.

2.8.3. Yield and yield components

Among the tested seven cowpea varieties (Bekur, Bole, TVU, Black eye bean, Kenkety, White wonder trailing and Adengur) Bekur and Bole had the highest seed yield of 1.49 and 1.36 t.ha⁻¹, respectively, while Black eye bean had the lowest seed yield of 0.67 t ha⁻¹ (Belay *et al.*, 2017). Similarly, Asrat *et al.* (2021) reported higher average grain yield

means of 1.53 t ha⁻¹ was obtained from white wonder and the lowest grain yield of 0.631 t ha⁻¹ obtained from Bole varieties. Furthermore, Bilatu *et al.* (2012) reported that the highest grain yield of 2.89 t ha⁻¹ which was recorded from Black-eyed bean. Additionally, Manore (2017) who had compared five cowpea varieties in southern Ethiopia, reported the highest grain yield was obtained in variety Black eye bean (2.88 t ha⁻¹) and Assabot (2.8 t ha⁻¹). Grain yields significantly difference between cowpea variety (Miheretu and Addo, 2017; Nyamaizi *et al.*, 2020).

A study conducted by Belay *et al.* (2017) in Abergelle on the effect of cowpea varieties on the number of seeds pod⁻¹, and thousand seed weight indicated that there is highly significance differences in both parameters among the varieties with the ranged of 10 (Black eye bean variety) to 14 (Bekur variety), and 130g (Black eye bean variety) to 67g (Adengur, local cultivar), respectively (Belay *et al.*, 2017). Similarly, Manore (2017) reported that there was statistically significant difference between varieties in hundred seed weight and the highest value was recorded from variety Black eye bean and lowest was from Wonder. According to Ayalew *et al.* (2021), number of seeds pod⁻¹, pod length, and hundred seed weight of cowpea was affected by varieties. Similarly, Augustine and Godfre (2019), who reported cowpea varieties significantly affected pod length, number of pods plant⁻¹ and grain yield but no influence on hundred seed weight. However, among the tested three cowpea varieties did not significantly difference in pods plant⁻¹ and pod length but differed in hundred seed weight (Giridhar *et al.*, 2020). The variation among the tested cowpea varieties for pod length BEB and Keti had the longest (15.62 cm) and the shortest (11.47 cm) pod length, respectively (Belay *et al.*, 2017).

According to Belay *et al.* (2017), the highest biomass yield was observed in Bole (3.39 t.ha⁻¹) followed by Bekur (3.27 t.ha⁻¹), whereas the lowest biomass yield was recorded

from BEB (1.8 t.ha⁻¹). Manore (2017) reported that cowpea varieties significantly affected the harvest index and varieties with lower total yields tended to have higher HI and varieties with higher total yields tended to have lower HI.

2.9. Effect of *Bradyrhizobium* Inoculations on Cowpea

2.9.1. Phenological and growth parameters

Bradyrhizobia inoculants significantly improved day to 50% flowering and a longer days were recorded from inoculated cowpea compared with the non-inoculated at Hawassa university research site (Manore, 2017). According to Ibrahim *et al.* (2010), legume plants inoculated with *Bradyrhizobia* strains resulted in higher plant height, fruiting branches, and number of pods compared to uninoculated treatments. Similarly, Nyoki and Ndakidemi (2013) revealed that *Bradyrhizobium japonicum* inoculation increased the number of branches plant⁻¹ by 28.8% in field trials compared to the non-inoculated treatment. Ayalew *et al.* (2022) reported that cowpea inoculated with *Bradyrhizobium* strains had higher leaf area and leaf area index and produced more leaves per plant compared to the not inoculated. Growth and shoot dry weight of cowpea plants also improved with *Bradyrhizobia* inoculants compared to non-inoculated plants (Kyei-boahen *et al.*, 2017; Mintah *et al.* 2020; Ulzen *et al.*, 2020).

2.9.2. Nodulation parameters

Inoculated plants produced a larger nodules number, nodule fresh and dry weight than uninoculated plants due to the effectiveness of the introduced rhizobia strain in nodule initiation and formation with cowpea roots (Ayalew *et al.*, 2022). Similarly, Ulzen *et al.* (2016), Manore (2017) and Kyei-boahen *et al.* (2017) found that plants inoculated with *Bradyrhizobium* strains had higher nodule numbers and effective nodules compared to

uninoculated plants. Furthermore, Nyoki and Ndakidemi (2013) observed an increased nodule count for the field experiment by 19.8% due to *Bradyrhizobium japonicum* inoculation compared to control treatments. Similarly, Ulzen *et al.* (2016) reported an increased cowpea nodule dry weight by 62% due to *Bradyrhizobium* inoculation compared to uninoculated treatments. Furthermore, Ayalew and Yoseph (2020) reported improved nodule activity and formation in cowpea due to *Bradyrhizobium* inoculations.

2.9.3. Yield and yield components

Emmanuel *et al.* (2021) reported improved grain yield of cowpea due to the ability of the rhizobia strain to establish efficient nodulation. Similarly, Ulzen *et al.* (2016); Kyei-boahen *et al.* (2017); Yoseph *et al.* (2017); Miheretu and Addo (2017) reported that seed inoculation by appropriate *Bradyrhizobium* strains produced significantly higher grain yields due to the inoculation with *Bradyrhizobium* inoculants. Rhizobial inoculation resulted in increased grain yield by 30% (Martins *et al.* 2003); 50% (Almeida *et al.*, 2010); 46% (Ulzen *et al.*, 2016); 25% (Kyei-boahen *et al.*, 2017); 63% (Mintah *et al.* 2020); 28.6% (Nyaga and Njeru ,2020), and 60% (Ayalew *et al.*, 2021), compared to non-inoculated.

Bradyrhizobia inoculation also resulted in increased yield and yield components such as pods plant⁻¹, hundred seed weight, seed yield, pod length, and seeds pod⁻¹ (Bambara and Ndakidemi, 2010; Ayalew *et al.* 2021). Similarly, Nyoki and Ndakidemi (2013) reported that *Bradyrhizobium japonicum* inoculation increased pods plant⁻¹ by 13.7%, seeds pod⁻¹ by 11.6%, hundred seed weight by 8.5%, and grain yield by 12.44%. Furthermore, Erica *et al.* (2017); Kyei-boahen *et al.* (2017) confirmed that increased pods plant⁻¹, seeds pod⁻¹, and hundred seed weight of cowpea crop due to the inoculation of *Rhizobium* strains.

Moreover, Mintah *et al.* (2020) indicated that seed inoculated with rhizobium was increased the number of pod plant⁻¹, number of seed pod⁻¹ and hundred seed weight of aerial bean crop.

According to Erica *et al.* (2017); Emmanuel *et al.* (2021), *Bradyrhizobium* inoculation significantly increased the aboveground biomass of inoculated cowpeas compared to control treatments. In addition, Kyei-boahen *et al.* (2017) observed that *Rhizobium* inoculation increased cowpea grain yield and aboveground dry matter production by an average of 25% and 22%, respectively. In contrast, Mintah *et al.* (2020) reported a non-significant effect on aboveground biomass yield due to *Bradyrhizobium* inoculation.

2.10. Biological Nitrogen Fixation and Factors Limiting N₂-Fixation

Biological Nitrogen Fixation (BNF) is essential for sustainable agriculture and to reduce soil fertility decline (Yadav *et al.*, 2022). It is a natural process in which atmospheric dinitrogen gas (N₂) is converted to ammonia under normal temperature and pressure (Lindström, 2010). According to Nag *et al.* (2022) and Maheshwari and Sankar (2023), nitrogen fixation by legume *rhizobium* symbiosis is of considerable agricultural importance, as it leads to very significant increases in combined nitrogen in the soil. Symbiotic nitrogen fixation is part of a mutualistic relationship in which plants provide a niche and fixed carbon to bacteria in exchange for fixed nitrogen (Mus *et al.*, 2016). Many leguminous plant species (family Fabaceae) can enter a symbiotic relationship with root-nodule bacteria, collectively called *rhizobial* inoculants (bacterial genera *Azorhizobium*, *Bradyrhizobium*, *Mesorhizobium*, *Rhizobium*, and *Sinorhizobium*) and convert the nitrogenous molecules into ammonia (Allito, 2022). Biological nitrogen fixation (BNF)

and nodulation are sensitive to environmental stressful conditions, which may cause inhibition of the initial steps of bacterial infection in roots (Bouhmouch *et al.*, 2005).

In spite, BNF is one of the important soil microbial activities it is affected by all ongoing processes in soil as well as other soil microorganisms. N₂-fixation was affected by many different physiological and environmental factors in soil, such as extreme temperature conditions, unfavorable soil pH, low or extremely high levels of soil moisture, pests and diseases, salinity, and nutrient deficiencies among other factors greatly influence the growth, development, survival, and metabolic activity of N₂-fixation bacteria and plants, and their ability to enter into symbiotic interactions (Katulanda, 2011; Ndungu, 2017). Additionally, N₂-fixation by legumes in agricultural systems is influenced by the plant genotype, genotype of rhizobia, and farm management practices (Salvagiotti *et al.*, 2008).

2.10.1. Soil moisture status

Soil moisture content can affect the growth and survival of soil rhizobia as well as the plant itself (Nigatu, 2017). A prolonged lack of moisture in the soil causes drought and affects plant growth and development as well as microorganisms (Goyal *et al.*, 2021). According to Yadav *et al.* (2010), drought is among the most harmful abiotic constraints to BNF, mainly due to its effect on soil's physical and biological characteristics. It also has a pronounced effect on N₂-fixation because nodule initiation, nodule growth, and nodule activity are all more sensitive to water stress than general root and shoot metabolism (Fatma *et al.*, 2012). Symbiotic N₂-fixation of legumes is also very sensitive to soil water deficits, especially in temperate and tropical legumes (Nigatu, 2017). Marquez-Garcia *et al.* (2015) reported that the number of nodules, nodule weight, and nodule-specific activity decreased under drought conditions, which in turn resulted in a significant reduction in

biologically derived nitrogen acquisition. For all legumes, exposure to drought leads to an inhibition of nodule N₂-fixation and a breakdown of symbiosis that limits yields in many parts of the world (Ladrera *et al.*, 2007).

On the other hand, excessive moisture and/or waterlogging negatively affect both the growth and survival of soil rhizobia and plants (Nigatu, 2017). Excess soil moisture significantly restricted N₂-fixation by reducing the supply of oxygen to nodulated roots or indirectly by reducing the availability of photosynthesis. Similarly, Mohammadi *et al.* (2012) stated that soil-water content directly influences the growth of rhizosphere microorganisms through processes of diffusion, mass flow, and nutrient concentration, like rhizobia, by decreasing water activity below critical tolerance limits and indirectly by altering plant growth, root architecture, and exudations. According to Manore (2017), at the condition of waterlogging, the occupation of all soil pores results in limited O₂ availability for both rhizobia and plant roots, thereby causing reduced respiration.

2.10.2. Soil temperatures

Temperature plays an important role in the success of BNF due to its effect on the survival, growth, nodulation, and persistence of rhizobial strains in the soil (Mohammadi *et al.*, 2012; Kabahuma, 2013; Simon *et al.*, 2014; Nigatu, 2017). Both very high and low soil temperatures affect BNF. Relatively, high root temperature has been shown to influence infection and N₂-fixation ability and has a strong influence on the survival and persistence of rhizobial strains (Mohammadi *et al.*, 2012). However, Wang *et al.* (2018); Nandanwar *et al.* (2020) and Goyal *et al.* (2021) reviewed that low temperatures reduce nodule formation and N₂-fixation in temperate legumes, which may contribute to poor N₂-fixation by

rhizobia. Furthermore, most strains thrive better at low temperatures than at high temperatures (Laranjo and Oliveira, 2011).

For most rhizobia, depending on their type, Nandanwar *et al.* (2020); Rodrigues *et al.* (2006) and Florentino *et al.* (2010) point out that the optimum soil temperature range is 25 – 35 °C is preferred for nodulation and N₂-fixation and many are unable to grow above 37°C. However, Odori *et al.* (2020) suggested that high-temperature tolerance can be considered an important candidate to develop inoculates as biofertilizers and isolates grow at extreme temperatures (45°C). According to Dwivedi *et al.* (2015), the strains are fixed N between 30 and 42°C in arid and semiarid conditions. Furthermore, the suitable temperatures for the cowpea seed inoculation strain suggested by Florentino *et al.* (2010), are the only soils with temperatures below 40°C.

2.10.3. Soil pH

Soil pH affects N₂-fixation and is the key determinant of soil nutrient availability to plants (Ndungu, 2017). Odori *et al.* (2020) reported that soil pH influences nodulation or the biology of *Rhizobium* and considers its influence on N metabolism as a whole. According to Mohammadi *et al.* (2012), soil pH may affect nodulation and/or N₂-fixation either directly or indirectly; directly by affecting plants growth and yield of legumes and indirectly through the depression of nodulation and symbiotic nitrogen fixation, especially in plants depending exclusively on symbiosis to acquire nitrogen.

According to Katulanda (2011) and Kawaka *et al.* (2014), and most leguminous plants require a neutral or only a slightly acid soil pH, especially when they rely on symbiotic N₂-fixation. Optimum soil pH for rhizobia growth was between pH values ranging between 5.0 and 11.0 (for cowpea and mungbean isolates (Odori *et al.*, 2020). However, Ali *et al.*

(2009) and Boakye *et al.* (2016) revealed that few cowpea test strains can grow and survive at a pH value as low as 4.0 and can nodulate cowpea. Whereas, Katulanda (2011); Nigatu (2017); Odori *et al.* (2020) and Goyal *et al.* (2021) reported that highly acidic soils (pH < 5) increase the solubility of phosphorous, calcium, and molybdenum with toxicity of aluminum and manganese, which in turn affects plants growth and survival of rhizobia. Moreover, Lin *et al.* (2012) indicated that in low pH conditions, nodule number and dry weight decreased by 90 and 50% in cowpea and soybean varieties, respectively.

2.10.4. Nutrient availability

Nutrients have a significant impact on the process of BNF (Ndungu, 2017). The growth of legume and *Rhizobium* symbiosis might be affected by a multitude of nutrient disorders, including both deficiencies and toxicities (Chalk *et al.*, 2010). Rhizobia have a primarily positive effect on plant growth, but the strength of the effect depends on environmental factors, such as N availability (Gibert *et al.*, 2019).

According to Kimutai (2017), N availability in the soil is one of the major environmental factors that influence the performance of the symbioses. Ereso (2017) stated that the shoot dry matter of the legumes grown in N-free sand is a good indicator of the symbiotic effectiveness of the rhizobial symbionts. Application of large quantities of N fertilizer inhibits N₂-fixation, but low doses (<30 kg N ha⁻¹) of fertilizer N can stimulate the early growth of legumes and increase their overall N₂-fixation (Donald, 2016). However, deficiency in mineral N often limits plant growth, and as such, symbiotic relationships have evolved between plants and a variety of N₂-fixing organisms.

2.10.5. Genotypes of *Rhizobium* and plant

The establishment of effective symbiosis between rhizobia and the host plant primarily requires optimal conditions that are necessary for the growth of the host plants. N₂-fixation in legume-*Rhizobium* symbiosis depends on the genotype of legumes, rhizobium strain, and the interactions of these with the bio-physical environment (Vanlauwea *et al.*, 2019). Therefore, the use of a compatible host–rhizobia association may lead to better optimization of the host nutrition, leading to superior plant growth and yield and enhancing N₂-fixation efficiency. According to Almeida *et al.* (2010), the diversity of rhizobia species along with the variable edaphic-climatic conditions and the genetic specificities of cowpea varieties also effective for BNF. Similarly, Florentino *et al.* (2010), the symbiotic efficiency of rhizobia in fixing N in the soil is also influenced by the cowpea genotype. In addition, the relationship between the host and environmental factors plays a significant role in the distribution of rhizobia (Odori *et al.*, 2020). Goss *et al.* (2002) noted that each legume species requires a specific *Rhizobium* strain for effective nodulation and N₂-fixation. However, Fauvart and Michiels (2008) revealed that most legume genotypes are non-promiscuous and nodulate with specific rhizobial strains in the rhizosphere.

2.10.6. Salinity

Both bacteria and plants experience adverse effects of salinity, reducing thereby the availability of water and causing water deficiency or desiccation (Goyal *et al.*, 2021). Salinity stress has significant consequences on the survival, colonization, and nodule activity of rhizobia (Vriezen *et al.*, 2007; Brígido *et al.*, 2012). Symbiosis may be affected by salt in different steps: growth and survival of rhizobia in soil, root colonization, infection and nodule development processes, or nodule functioning (Laranjo and Oliveira,

2011). According to Tulu *et al.* (2018); Odori *et al.* (2020), decrease the growth of rhizobia with increasing NaCl concentrations through direct toxicity and osmotic stress. Thus, for cowpea, the tolerant isolates decreased from 83 to 3% when the NaCl concentration increased from 1.5 to 5.5% (Tulu *et al.*, 2018).

2.10.7. Pests, diseases, and agronomic practice

According to Goyal *et al.* (2021), pests and diseases can potentially cause substantial yield losses, and such harmful effects extend beyond crops to nitrogen-fixing rhizobia. Farm management practices have a profound influence on both the soil and the crop under consideration. Yadav *et al.* (2010); Ronner and Franke (2012) showed that farm management practices influence BNF by affecting both the crop and the microbial activity in the rhizosphere including tillage practices, selection of effective or responsive crops, appropriate cropping systems, method of sowing, time of sowing, use of agrochemicals, use of Rhizobium cultures and its frequency, the way of handling the inoculants and the method of inoculation.

2.11. Role of Biological Nitrogen Fixation

According to Kebede *et al.* (2022), strong symbiotic association with rhizobia and N₂-fixation could be a nitrogen supply source for the crop production systems, that is economically attractive and environment benign to the environment and soil system. Nature has an alternative method of providing N to plants and enriching soil N resources through biological nitrogen fixation. The most important nitrogen-fixing symbiotic association in agricultural systems is taking place between legumes and rhizobia (Peoples *et al.*, 2009). Mendoza-Suárez *et al.* (2021) indicated that biological nitrogen fixation by *Rhizobium*-legume symbioses represents an environmentally friendly and inexpensive

alternative to the use of chemical nitrogen fertilizers in legume crops. Biological nitrogen fixation (BNF), a key source of N for farmers using little or no fertilizer, constitutes one of the potential solutions and critical for sustained grain legume production (Herridge *et al.*, 2008; Chianu *et al.*, 2011).

Under favorable conditions, biological nitrogen fixation by legume crops like cowpea in cropping systems have been considered as one of the affordable options to increase not only soil N levels but also soil productivity (Pule-Meulenbergh *et al.*, 2010). Nyaga and Njeru (2020) reported that the ability of cowpea to fix nitrogen through biological nitrogen fixation is a cheap and sustainable alternative to inorganic fertilizers. In this regard, BNF is seen as a sustainable alternative to using of nitrogen fertilizers, which are mostly inaccessible to smallholder farmers due to their high cost (Gopalakrishnan *et al.*, 2015).

According to Kebede *et al.* (2022), cowpea can fix about 240 kg ha⁻¹ of atmospheric nitrogen and make about 60–70 kg ha⁻¹ nitrogen available for subsequent crops grown. Similarly, Simon *et al.* (2014), root nodule Rhizobia approximately reduce 20 million tons of atmospheric nitrogen to ammonia which is 50–70% of the world biological nitrogen fixation. Moreover, Herridge *et al.* (2008); reported that grain legumes contribute more than 20 million tonnes of fixed N each year, implying that legume BNF capacity is a critical process for maintain an effective and efficient management of arable land and source of N supply to plants under favorable atmospheric and environmental conditions. Chemining'wa *et al.* (2007) reported that legumes can fix about 200 kg organic N per year under optimal field conditions and, play an important role in climate preservation and sustainability of agriculture (Peoples *et al.*, 2009).

3. MATERIALS AND METHODS

3.1. Description of the Study Sites

The experiment was conducted at the two experimental sites at farmer station in Dale and Hawassa university research station of Sidama regional state (Figure 1) under rain fed from March-September, 2022 main season. Dale district is bordered by Aleta Wendo and Chuko in the south, Loko Abaya in the west, Boricha in the northwest, Shebedino in the north, and Wensho in the east. Geographically, it is located at latitude of 6°39' to 6°50' N, and a longitude of 38°18' to 38°31' E at altitudes ranging from 1650 to 2800 m.a.s.l. The soil texture is clay with a pH value of 6.3 with a moist to humid, warm subtropical climate agroecologies. Mean annual rainfall and temperature of the district is ranged from 1000 to 1800 mm and 15 to 20 °C, respectively with the total land area coverage of 1,411 km² and 320 km far from Addis Ababa, the capital of Ethiopia to the southern direction.

Annual crop like maize (*Zea mays* L.), barley, teff, wheat, and vegetable crops as well as perennial crops such as enset, coffee (*Coffea arabica*), and chat (*catha edulis*) are grown in these areas (Alemu *et al.*, 2022).

Hawassa is located in the Great Rift Valley, and it is bordered on the south by Shebedino and Boricha, on the west and north by the Oromia Region, and on the east by Wondo Genet. It is located at a latitude of 7°3' N and longitude of 38° 28' E with an average altitude of 1700 m a.s.l. The soil texture is sandy loam with a pH value of 7.0 with moist-to-sub-humid, warm-to-humid, subtropical climate agroecologies. The mean annual minimum and maximum temperature of the area is 12 °C to 27 °C, respectively. The area receives a mean annual maximum and minimum rain-fall of 900–1100 mm, respectively,

with total land area coverage of 157.2 km², and about 275 km far from Addis Ababa; (the capital of Ethiopia) to the southern direction.

Annual crops like maize (*Zea mays* L.), tomato (*Solanum lycopersicum*), sweet potato (*Ipomea batatas*), and haricot bean (*Phaseolus vulgaris* L.) as well as perennial crops like "enset" and "chat" (*Catha edulis*), coffee (*Coffea arabica*), and avocado (*Persea americana*) are grown extensively in these areas (Kibreselassie *et al.*, 2018).

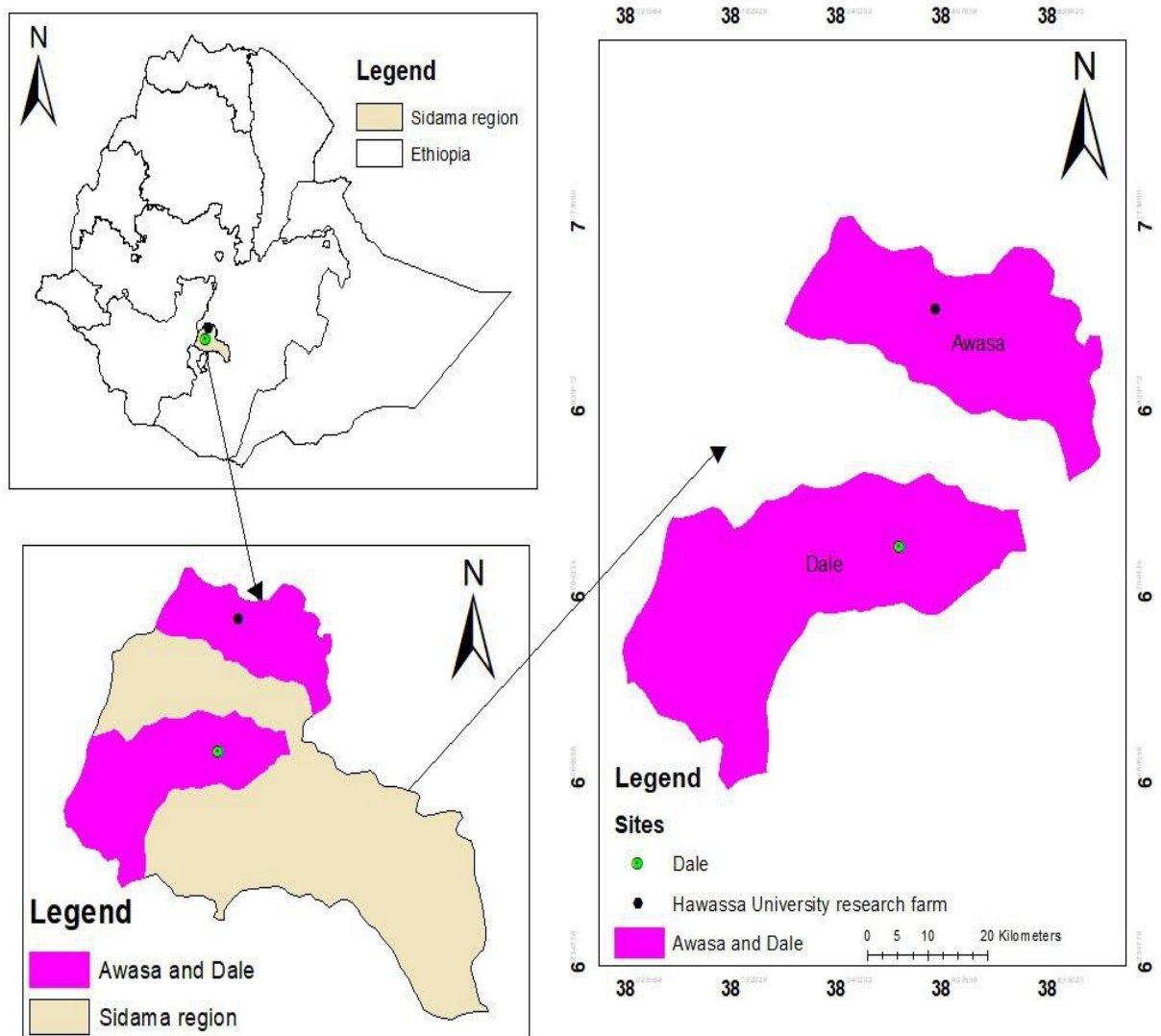


Figure 1: Map of the study areas in Dale and Hawassa, Sidama Region, Southern Ethiopia (Source: Google map from internet)

3.2. Experimental Materials

3.2.1. Source of planting materials

The seeds of the four cowpea varieties (Bole, TVU, White-wonder-trailing, and Keti) (Table 1) used for both experimental sites were obtained from Melkassa Agricultural Research Center, Ethiopia. These varieties were chosen based on their yield potential, acceptability by farmers, and seed availability. Triple Super Phosphate (TSP) fertilizer was obtained from Hawassa Agricultural Research Center, Ethiopia. *Bradyrhizobium* strain (MBI-cowpea) was obtained from Menagesha Biotech Industry P.L.C. (MBI), Addis Ababa, Ethiopia, already known for its nodulation and agronomic performance for cowpea crops.

Table 1: Description of cowpea varieties used for the field experiments

Cowpea genotype	Status	Years of release	Seed size	Agro-ecology	Source
Bole	Released	2005	Medium	Lowland	MARC
TVU	Released	1978	Small	Lowland	MARC
White Wonder Trailing	Released	1976	Small	Lowland	MARC
Keti	Released	2012	Small	Lowland	MARC

Note: MARC = Melkassa Agricultural Research center

3.2.2. Soil sampling and analysis

Before land preparation, soil samples were randomly taken from the experimental sites in a zigzag manner from 20 different spots at a depth of 0–20 cm using an auger as described by Munson and Nelson (1993), and the samples were mixed thoroughly to produce 1.0 kg of a representative composite soil sample. Then air-dried and crushed soil samples were thoroughly mixed and packed in a polyethylene bag, labeled, and stored in the laboratory for analysis at Hawassa University Soil Science Laboratory, Hawassa, Ethiopia.

The analyzed parameter includes soil particle size distribution soil pH, total N, organic carbon, available P, cation exchange capacity (CEC). The particle size distribution was determined using the Bouyoucous hydrometer method (Day, 1965). Soil pH was determined in soil-water suspension 1:2.5 with glass electrode pH meters (Jackson, 1967). Total N was analyzed by the Kjeldhal method, as described by Dewis and Freitas (1975). Organic carbon content was determined using the Walkley and wet oxidation method as described by Jackson (1958). Available P was determined according to the methods of (Olsen *et al.*, 1954). The CEC was determined by the ammonium acetate using the Kjeldhal method (Ranist *et al.*, 1999).

3.3. Treatments and Experimental Design

The treatments studied at each sites consisted of four cowpea varieties (Bole, TVU, Ketu, and White Wonder Trailing) and two inoculation levels (non-inoculated, inoculated with *Bradyrhizobial* strains: MBI-cowpea) (Appendix Table 1) with three replicates. The experiment was laid out in a Randomized Complete Block Design with a factorial arrangement (Appendix Figure 1). Inter and intra row spacing of 0.6 and 0.2 m, respectively as well as 1 and 1.5 m between two adjacent plots and between blocks respectively were used. The size of each experimental plot was 2 m in length x 4. 2 m in width (8.4 m²) with 7 rows plot⁻¹, ten plants row⁻¹, and 70 plants plot⁻¹ on a total experimental area of 410.4 m². Phosphorus in the form of triple super phosphate (TSP) was applied at a rate of 20 kg ha⁻¹ uniformly to all treatments. Sampled data including growth and nodulation, and yield related parameters were collected from the middle five and three rows respectively, leaving border rows.

3.4. Experimental Procedures and Field Management

Before sowing the land of both sites were prepared for cowpea production by the majority of farmers falls within the research recommendation of 2–3 times tillage (Beshir *et al.*, 2019). Accordingly, fine seedbeds were prepared and leveled manually and rows were made across each plot. After the layout, each treatment was assigned randomly to the experimental plots within a block. The planting was done on April 24 and 26, 2022, for the Dale and Hawassa sites, respectively. Seeds were inoculated with the *Bradyrhizobium* strain at the recommended rate (10g inoculant per 1 kg of seed) (Rice *et al.*, 2001) and inoculation was done prior to planting in the shade to preserve the viability of the bacterial cells. In order to ensure that the applied inoculum stick to the seed, the required quantity of inoculant was suspended in 1:1 ratio in 10% sugar solution (Getachew and Abeble, 2021). The seeds and thick slurry of inoculants were mixed carefully until the seeds were coated with a black film of inoculants. The inoculated seeds are then allowed to air dry for 15 minutes before planting to avoid fungal growth. Planting was done manually by dropping two seeds hole⁻¹ (3-4 cm deep) for both inoculated and non-inoculated treatments, using the recommended spacing of 60 cm between rows and 20 cm between plants. As a precaution against cross-contamination, non-inoculated treatments were planted first, followed by inoculated treatments. The seeds were covered with soil using a rake. Ridges were made to prevent the movement of bacteria through rainwater between plots and blocks. Thinning one of the seedlings was carried out two weeks after the emergence of the seedlings to maintain the population density of the plants in each plot. After seed germination, the agronomic management practices such as weeding, hoeing, cultivating, pest control, harvesting, threshing, etc. were uniformly performed on all cowpea varieties in both experimental fields.

3.5. Data Collection and Measurements

3.5.1. Phenological parameters

Days to 50% emergence: The number of days from the date of planting to the date when 50% of the seedling had emerged in a plot above the ground was recorded.

Days to 50% flowering: It was determined as the number of days from emergence to the date when 50% of the plants in a plot produced at least one flower.

Days to 90% physiological maturity: It was counted from the date of emergence to 90% of the pods per plot had turned to yellow, black or brown in color depending on the varieties considered. Black or brown and yellowing of pods and drying of leaves are used as indicators of physiological maturity.

3.5.2. Nodulation and growth parameters

Nodule number plant⁻¹: It was recorded at the mid (50%) flowering stage from the second inner rows at both sides of the plot and one plant from both ends of each row were considered as a border and carefully hoeing and uprooting five plants randomly from each plot using prong weeder hoe tools. The plants were separated into roots and shoots. Soil adhering to the roots was removed by gentle washing with tap water over a metal sieve. The nodules from each plant were removed and spread separately on the sieve for a few minutes until the water drained from the surface of the nodules. The nodules were counted and their average was taken as the number of nodules per plant.

Effectiveness of the nodules: To evaluate the nodules for effectiveness the nodules were cutted with a snap-Off Sharp Blade and checked for its color. Those with pinkish, brown, and reddish color due to leghemoglobin presence were considered effective nodules while

nodules with green, yellow, and white color were classified as ineffective in N₂-fixation (Tajima *et al.*, 2015; Ayalew and Yoseph, 2020). The scores for nodule coloration were made on a 1–3 scale bases: 1 scored for white, yellow, and green nodules, ineffective in N₂-fixation, 2 scored for pink and slightly red nodules it is moderately effective in N₂-fixation, 3 scored for red and dark red nodules which are effective in N₂-fixation.

Nodule fresh weight (g): It was measured after recording the nodule number and effective nodule, and immediately weighed both effective and non-effective with the sensitive balance. Then the average values of five plants were recorded as nodule fresh weight plant⁻¹.

Nodules dry weight (g): Nodules were oven-dried at 70°C for 48 hours for determination of the nodule dry weight. Then, the average of five plants was taken as the nodule dry weight plant⁻¹.

Plant height (cm): Five plants from the middle rows of each plot were randomly selected from short, tall and medium plants for measuring plant height using meter tape steel 5 meter at the physiological maturity. Then their average values were recorded as plant height plant⁻¹ and expressed in centimeters.

Number of primary branches: It was determined by counting the total number of primary branches from the main stem from the five randomly selected plants plot⁻¹ at the physiological maturity. The mean of five plants was taken as the number of primary branches plant⁻¹.

Leaf area (cm²) and leaf area index: The leaf area of the cowpea was measured using an automatic leaf area meter (model LI 31000A Li-Cor, Lincoln, USA), and the leaf area index was determined as the ratio of the leaf area to the corresponding ground area.

$$LAI = \frac{\text{Leaf area}}{\text{Ground Area}}$$

Root length (cm): It was determined from the five randomly selected plants pot⁻¹ for nodulation determination by measuring the central tap root using a standard ruler. Then the average values of these plants were recorded as root length of the cowpea crop plant⁻¹ and expressed in centimeters.

Shoot fresh and dry weight (g): Shoot samples (all plant parts above ground) from five plants plot⁻¹, dug up for nodule determination, were subject to shoot fresh biomass determination and oven dried at 70 °C for 48 hours to determine the shoot dry weight.

Root fresh and dry weight (g): It (whole taproot and its branches) was measured by taking the five plants collected for nodule determination and oven dried at 70 °C for 48 hours to determine root dry weight

3.5.3. Yield and yield components

Plants within the 1.8 m x 1.6 m (2.88 m²) net plot, in the three central rows, were harvested at harvest maturity to determine the number of pods plant⁻¹, pod length, number of seeds pod⁻¹, hundred seed weight, above ground biomass yield, grain yield, straw yield, and harvest index for each experimental sites.

Number of pods plant⁻¹: The total number of pods was counted from ten randomly taken plants from the sampled yield data at the end of harvesting time in each plot, and the average number of pods was recorded as the number of pods plant⁻¹.

Pod length (cm): It was measured using a standard ruler from the petiole of the pod to the pod apex from ten randomly collected pods from the bottom, middle and upper part of the plant from the number of pods taken plants and the average was recorded.

Number of seeds pod⁻¹: The total number of seeds was counted from ten randomly taken plants for pod length during harvesting time and these pods were threshed. The threshed seeds were manually counted by hand and total number of seeds was divided by the total number of pods to compute an average number of seeds pod⁻¹.

Hundred seed weight (g): It was determined by randomly taking 100 seeds from the plants sampled for yield data and weighing them with a sensitive balance and seed weight was adjusted to moisture content 11% moisture content using grain moisture meter (model GMM mini, DRAMINISKI, Poland).

Above ground biomass yield (t ha⁻¹): The total biomass yield of the harvested plants plot⁻¹ was weighed using metal salter (model 235-M metal body scales) to estimate the above ground biomass yield plot⁻¹ before threshing, i.e. while both the yield component and the straw are on the plant. The plot-based value has been converted to a hectare basis and reported as a t ha⁻¹.

Grain yield (t ha⁻¹): At harvesting maturity, the harvested plants from the three middle rows of the plot were threshed and the grain yield was adjusted to 11% moisture content using a grain moisture meter (model GMM mini, DRAMINISKI, Poland). Thereafter, the

grain from each plot was weighed using a sensitive balance to determine grain yield. Finally, yield plot⁻¹ was converted to a hectare basis and reported as a t ha⁻¹.

$$\text{Moisture content} = \frac{(\text{Harvested yield}) (100 - \text{Harvest moisture \%})}{(100 - \text{Standard moisture \%})}$$

Straw yield (t ha⁻¹): It was calculated by subtracting the total grain yield from the corresponding total above ground biomass yield.

Harvest index (%): From the above ground biomass yield and grain yield data, the crop harvest index plot⁻¹ was calculated using the formula given by Donald (1962).

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

3.6. Statistical Analysis

All the data collected in the study areas were entered in excel spreadsheets and subjected to analysis of variance (ANOVA) appropriate to factorial experiment in an RCBD using the General Linear Model (GLM) of the Statistical Analysis System software version 9.0 (SAS Institute, 2012). Before performing combined analysis over the two sites, the homogeneity of error variances for all parameters was evaluated using the Hartley (1950) F_{\max} test.

$$F_{\max} = \frac{\text{Larger mean square error}}{\text{Smaller mean square error}}$$

Cowpea varieties and *Bradyrhizobium* inoculation were considered as fixed factors and site was as random variables. A combined analysis of variance (ANOVA) was performed on the data from the two sites because the $F_{\max} \leq 3.00$, test indicated that all parameter data were homogenous across the sites. The mean separations were performed using the Least

Significant Differences (LSD) test at 5% probability of significance. Correlation analysis was performed using simple Pearson's correlation coefficient for preferred parameters (SAS version 9.0).

3.7. Partial Budget Analysis

The economic analysis was carried out to evaluate the profitability of bio-fertilizers in order to determine the agronomic efficiency using partial budget procedure described by CIMMYT (1988) The net returns (benefits) and other economic analysis was based on the formula developed by CIMMYT (1988) and given as follows:

Unadjusted Grain Yield (UGY) ($t\ ha^{-1}$): was the average yield of each treatment.

Adjusted grain yield (AGY) ($t\ ha^{-1}$): was the average yield adjusted downward by 10% reflect the difference between the experimental yield and the yield that a farmer could expect from the same treatment without the researchers' involvement as described by CIMMYT (1988).

Gross field benefit (GFB) ($ETB\ ha^{-1}$): it was computed by multiplying the field/farm gate price (25. 50 $ETB\ kg^{-1}$) that farmers receive for the crop when they sell it as an adjusted yield. $GFB = AGY \times \text{field/farm gate price for the crop}$.

Total variable cost (TVC) ($ETB\ ha^{-1}$): it was included the current price of TSP fertilizer cost (275 $ETB\ kg^{-1}$), cowpea seed (38.45 $ETB\ kg^{-1}$), and *Bradyrhizobium* inoculation (260.61 $ETB\ ha^{-1}$), cost as well as the labor cost for all activities (land preparation, planting, weeding, harvesting, threshing, and transportation) were considered as the sum of all costs was variable or specific to treatment against the control.

Net benefit (NB) (ETB ha⁻¹): was calculated by subtracting the total variable costs (TVC) from gross field benefits (GFB) for each treatment as $NB = GFB - TVC$.

The **dominance analysis** was carried out by first listing all the treatments in their order of increasing costs that vary (TVC) and their net benefits (NB) were then put aside. Any treatment that has higher TVC but net benefits that are less than or equal to the preceding treatment (with lower TVC but higher net benefits) is dominated treatment (marked as “D”). The dominance analysis illustrates that to improve farmers' income; it is important to pay attention to net benefits rather than yields because higher yields do not necessarily mean high net benefits.

Marginal rate of return (MRR) (%): was calculated as the change in net benefits ($\Delta NB = NB \text{ Treatment} - NB \text{ Control}$) per the change in total variance costs ($\Delta TVC = TC \text{ Treatment} - TC \text{ Control}$) and then multiplied by 100:

$$MRR (\%) = \frac{\Delta NB}{\Delta TVC} \times 100$$

Finally, among the non-dominated treatments, a treatment having a marginal rate of return greater than 100% with the highest net benefit was considered to be economically best and it was recommended for farmers.

4. RESULTS AND DISCUSSION

4.1. Soil Physico-Chemical Properties at Dale and Hawassa Experimental Sites

Cowpea thrives on a variety of soil types, including sandy and heavy clays soils (provided the soil is well-drained) (Shyam *et al.*, 2020). The pre-planting soil analysis result indicated that the textural class of soil of farmer stations in Dale is clay loam containing 42% sand, 38% clay, and 20% silt whereas the textural class of soil of research stations in Hawassa is sandy clay loam containing 52% sand, 28% clay, and 20% silt (Table 2). Clay particles have a greater capacity to hold soil nutrients and are thought to shield some easily degradable organic compounds from fast microbial degradation through encrustation and trapping. It is also directly related to the soils' ability to hold water, to be permeable, and to be workable, since loam and clay soils do so better than sandy ones (Dou *et al.*, 2016; Fang and Su, 2019).

Soil pH affects BNF by directly influencing the host legumes, and the microbe (Ferguson *et al.*, 2013). According to Tekalign (1991), soil pH values classified as < 4.5 very strongly acidic, 4.5-5.2 strong acid, 5.3-5.9 moderately acidic, 6.0-6.6 slightly acidic, 6.7-7.3 neutral, 7.4-8.0 moderately alkaline, and >8.0 strongly alkaline. Thus, the soil pH of 5.8 and 6.5 obtained at farmer's station in Dale and at research station in Hawassa, respectively are related as moderately acidic and slightly acidic, respectively (Table 2), which might partially explain the positive response to inoculation (Nyoki and Ndakidemi, 2014). Cowpea is more tolerant to infertile and acid soils than many other crops (Mulugeta *et al.*, 2016) and grows best in slightly acid to alkaline soils (pH 5.5 – 8.5) (Shyam *et al.*, 2020). The isolate thrives in acidic, neutral, and alkaline soil conditions, growing well at pH levels between 6.0 and 8.5 with an optimal growth density found at pH around neutral

(7.0) (Kebede *et al.*, 2021). This isolate can also increase cowpea yield and encourages sustainable agricultural production methods.

The amount of N in the soil also influences N₂-fixation. According to the study by Havlin *et al.* (1999), soils with total N levels in the ranges of <0.1, 0.1 – 0.15, 0.15 – 0.25, 0.25 – 0.5 and >0.5% can be classified as very low, low, medium, high, and very high, respectively. Total N of the soils of both experimental sites are rated as moderate with values 0.2% and 0.15% in Dale and Hawassa sites, respectively (Table 2), inoculating legume seeds with effective Bradyrhizobia strains may increase N₂-fixation and help replenish soil N for higher crop yields. Due to Legumes' preference for using the majority of the available soil N before to starting to fix atmospheric N, but high soil N levels decrease N fixation.

According to Olsen *et al.* (1954), the available P concentrations (mg kg⁻¹) of soils can be categorized as extremely low (5), low (5 – 15), medium (15 – 25), and high (> 25). As a result, the experimental soil at Dale and Hawassa sites were evaluated as having low levels of available P (5.9 and 9.5 mg kg⁻¹, respectively). Phosphorus is essential for nodule formation and provides the energy needed for the rhizobial strain to convert atmospheric N to NH₄ in BNF. Due to low soil P levels, the growth of legume roots and the rhizobial populations are constrained, which in turn negatively affects BNF (Yakubu *et al.*, 2011). Therefore, it is known that TSP fertilizer can improve N₂-fixation and growth, nodulation, and yield components of cowpea in areas with P-deficient soil.

According to Jackson (1958) and Herrera (1995), organic carbon contents (OC %) are rated as very low (< 2), low (2 – 4), medium (4 – 10), high (10 – 20) and very high (>20). One of the key components of long-term agricultural production systems is the increase and preservation of soil productivity through the improvement of soil organic matter and

mineral elements. Thus, the organic carbon of 2.3% and 1.74% obtained at farmer's stations in Dale and at research station in Hawassa, respectively are related as low and very low, respectively indicating that the production of grain legumes influenced by the richness of organic matter content.

Cation exchange capacity (CEC) is a critical parameter of soil because it reveals the type of clay mineral that occurs in soil and its ability to protect nutrients from leaching. According to Landon (1991), CEC (cmol (+) kg⁻¹) of top soils were classified as extremely high (>40), high (25-40), medium (15 – 25), low (5 – 15), and very low (< 5). The CEC of the soils in the study sites, which were 35.2 and 31.6 (cmol (+)/kg⁻¹) at Dale and Hawassa sites, respectively, indicates that they have a higher potential to retain the cations at both study sites (Table 2).

Table 2: Soil physico-chemical properties at Dale and Hawassa experimental sites before planting during the 2022 main cropping season

Sites	Dale site		Hawassa site	
	Value	Rating	Value	Rating
Particle size distribution				
Sand (%)	42		52	
Silt (%)	20		20	
Clay (%)	38		28	
Textural class		Clay loam		Sandy clay loam
Chemical analysis				
Soil pH (1:2.5) (H ₂ O)	5.8	Moderately acidic	6.5	Slightly acidic
Total N (%)	0.2	Moderate	0.15	Moderate
Available P (mg kg ⁻¹)	5.9	Low	9.5	Low
Organic C (%)	2.3	Low	1.7	Very low
CEC (cmol(+)/kg ⁻¹)	35.2	High	31.6	High

4.2. Meteorological Data of the Experimental Sites

4.2.1. Total rainfall

The annual total rainfall, and maximum and minimum temperature received at the study sites was listed in Appendix Table 2. During 2022 main season, monthly average temperature and total rainfall received in study sites were indicated in Figure 2. Ayalew *et al.* (2021) reported that the recommended average monthly rainfall for cowpea varieties is approximately 91 mm.

During the main season 125.4 and 72.79 mm of rainfall was recorded at the Dale and Hawassa experimental sites, respectively. The total rainfall varied between the sites was 52.6 mm. The higher rainfall was recorded at Dale area with 34.4 mm more than the average recommendation for the cowpea crop particularly at the stages of planting (202 mm in April) and harvesting (179.4 mm in September). Hence, due to the higher amount of rainfall there was a challenging to harvest the seed, since the crop in this study region was late matured and produced new flowers and pods at the time of harvest, which also could be due to high amount of rainfall received at Dale than Hawassa. When compared to average recommendations for cowpea crops, rainfall in the Hawassa site was lower (18.2 mm less) particularly at the early stage of the plant. Cowpea is an important legume that can withstand drought and warm weather. It thrives in the drier portion of the tropics, where other legumes do not perform well (Bashir *et al.*, 2018; Sisay *et al.*, 2019; Ayalew *et al.*, 2021). Overall, the mean annual rainfall for the crop in both study sites was acceptable (Appendix Table 2). Cowpeas may grow in Ethiopia even with a mean annual rainfall of 400 to 1500 mm (Bilatu *et al.*, 2012).

4.2.2. Average temperature

The average temperature during the 2022 main season from April to June was slightly higher, especially in April and May at the Hawassa site (Figure 2). Cowpea is a warm-weather crop adapted to high temperatures (20 – 35°C) than maize and grows under more adverse conditions than haricot bean (Shyam *et al.*, 2020; Asrat *et al.*, 2021). For most rhizobia, depending on their soil type, Florentino *et al.* (2010); Nandanwar *et al.* (2020), Mariam (2021), reported that the optimum soil temperature range is 25 – 35 °C is preferred for nodulation and N₂-fixation and many are unable to grow above 37°C. Similarly, rhizobial isolates grew and were able to develop a colony at a temperature range between 20 and 35°C (Kebede *et al.*, 2021). Moreover, Wang *et al.* (2018); Nandanwar *et al.* (2020) and Goyal *et al.* (2021) reviewed that, low temperatures reduce nodule formation and N₂-fixation in temperate legumes, which could contribute to poor N₂-fixation by the rhizobia.

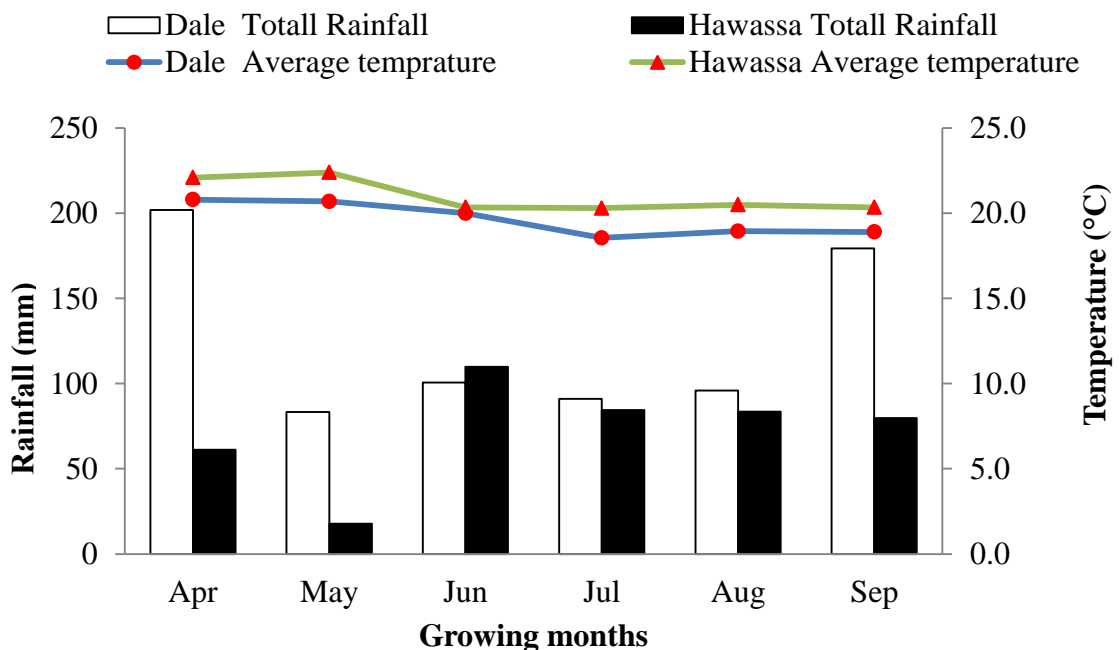


Figure 2: Monthly total rainfall and the average temperature of the two experimental sites during the 2022 cropping season.

4.3. Effects of Varieties and *Bradyrhizobium* Inoculation on Phenology of Cowpea

4.3.1. Days to 50% emergence

The results of combined data from the two sites revealed that days to 50% emergence were significantly ($P < 0.001$) affected only by the main effect of the varieties. However, the main effect of *Bradyrhizobium* inoculation and their interaction did not show a significant ($P > 0.05$) difference on days to 50% emergence (Appendix Table 3).

Among the four varieties, the White Wonder Trailing variety required the longest days to reach 50% of emergence, followed by the TVU variety. However, the shortest days to 50% emergence were recorded from the Bole variety. But, there were no statistically significant differences between TVU and White Wonder Trailing, and TVU and Keti varieties (Table 3). The observed differences in seedling emergence dates among cowpea varieties might be due to seed size because genotypes with larger seeds produced larger seedlings that emerge earlier than smaller seeds (N'Danikou *et al.*, 2022). This result is consistent with Manore (2017), who recorded a significant difference between the cowpea cultivars for days to 50% emergence. Similarly, Eshetu *et al.* (2018) reported that days to 50% emergence of faba bean were highly influenced by varieties, and seed germination was largely dependent on stored nutrients rather than external inputs.

Table 3: Effect of *Bradyrhizobium* inoculation on the phenology of cowpea varieties averaged across the two sites during the 2022 main cropping season

Treatments	Day to emergence	Day to 50% flowering	Day to 90% physiological maturity
<u>Varieties (V)</u>			
Bole	6.00 ^c	85.00 ^a	117.00 ^a
TVU	7.50 ^{ab}	82.00 ^b	114.00 ^b
White Wonder Trailing	8.00 ^a	79.00 ^c	111.00 ^c
Keti	7.00 ^b	74.00 ^d	102.00 ^d
LSD _(0.05)	0.98	1.63	1.93
<u>Bradyrhizobium (BR)</u>			
Inoculated	7.00	82.0 ^a	113.00 ^a
Non-inoculated	7.00	78.0 ^b	109.00 ^b
LSD _(0.05)	0.51	0.85	1.01
CV (%)	8.13	1.22	1.04
<u>F-values</u>			
Varieties	14.74 ^{***}	125.8 ^{***}	198.27 ^{***}
<i>Bradyrhizobium</i> (BR)	0.00 ^{ns}	78.03 ^{***}	75.37 ^{***}
V*BR	2.95 ^{ns}	0.53 ^{ns}	0.43 ^{ns}

Means sharing the same letter(s) within a column of treatment is not statistically significant at 5% level of significant. ^{ns}= non-significant, ^{***} significant probability level at ($P < 0.001$)

4.3.2. Days to 50% flowering

The analysis of variance for combined data from the two sites revealed that the main effects of varieties and *Bradyrhizobium* inoculation were highly significantly ($P < 0.001$) affected days to 50% flowering. However, the interaction effect of varieties and *Bradyrhizobium* inoculation did not show a significant ($P > 0.05$) effect on days to 50% flowering (Appendix Table 3).

Among the varieties, the longest days to reach 50% flowering were recorded from the variety Bole. While the Keti variety recorded the shortest days to 50% flowering compared to TVU and White Wonder Trailing varieties. The difference in the number of days between the earliest and the latest varieties to reach 50% flowering was 11 days (Table 3).

The difference in days required for flowering among the varieties could be due to genetic differences in parental traits and the plant's tendency to respond differently to agro-climatic conditions. This suggests that depending on their genetic makeup and level of adaptability, various plants react to environmental variables in different ways. Furthermore, it may be due to the effective use of available environmental conditions such as light, water, and nutrients by some genotypes during the first growth stage of the experiment. This result is inconsistent with Goa *et al.* (2022), who reported a significant difference in days to 50% flowering among the cowpea varieties mainly due to varietal differences. Similarly, Eshetu *et al.* (2018) reported faba bean varietal differences in days to 50% flowering under rain-fed conditions. Moreover, Mulika *et al.* (2022) reported a significant difference in days required to reach 50% flowering among green gram varieties.

Likewise, plants inoculated with *Bradyrhizobium* strain MBI-cowpea showed the longest days to 50% flowering, while the shortest days to 50% flowering were recorded from the control treatment (Table 3). The possible reason for delayed flowering in inoculated plants could be increased N₂-fixation and, consequently, increased N uptake by plants, which may improve the crop's vegetative growth. In line with this result, Bediru (2022) reported that plants with *Rhizobium* inoculation had the longest days of flowering and delayed onset of flowering and fruiting due to the supplied N through symbiotic N₂-fixation. Similarly, Mulika *et al.* (2022); Manore (2017); Bitire *et al.* (2022) reported that *Bradyrhizobium* inoculation increased the number of days to reach 50% flowering of legume plants. However, this finding contradicts the results of Macil *et al.* (2017), who reported a non-significant effect of *Rhizobium* inoculation on days to 50% flowering of chickpeas.

4.3.3. Days to 90% physiological maturity

The maturity trait represents the number of days required for varieties to complete their life cycle from seed to full harvest in dry conditions (Goa *et al.*, 2022). Analysis of variance for combined data from the two sites revealed that the main effects of varieties and *Bradyrhizobium* inoculation were highly significantly ($P < 0.001$) affected days to 90% of physiological maturity. However, the interaction effects of varieties and *Bradyrhizobium* inoculation did not show a significant ($P > 0.05$) effect on days to 90% of physiological maturity (Appendix Table 3).

Days to physiological maturity among the varieties ranged between (102 to 117 days) for Ketu and Bole varieties, respectively with (15 days) differences between early and late maturing varieties (Table 3). The differences in days to physiological maturity among cowpea varieties may be attributed to the effect of climatic conditions or the genetic makeup of the genotypes tested. This result is consistent with Ayalew *et al.* (2021), who reported significant variation in the number of days required for the physiological maturity of cowpea varieties over two years and three sites. Similarly, Mulika *et al.* (2022) reported significant differences in varieties on the number of days to maturity in green gram. Moreover, the number of days required to reach physiological maturity was influenced among the cowpea varieties (Manore, 2017).

Likewise, *Bradyrhizobium* inoculated plants recorded the longest (113 days) days to physiological maturity, while uninoculated plants showed the shortest (109 days) to physiological maturity. The delay in physiological maturity with *Bradyrhizobium* inoculation may be caused by improved N supply from BNF and enhanced vegetative growth through the conversion of atmospheric N_2 to ammonium (NH_4^+), a plant-useable

form. The result is in line with Bediru (2022); Ayalew *et al.* (2021), who found that the photosynthetic performance of plant was prolonged when inoculated with *Rhizobium* because high N fixation from inoculation promoted vegetative growth, thereby taking advantage of the extended days to maturity. In contrast, Ndlovu (2015) reported that the number of days required for the common beans to reach physiological maturity was extended over two seasons in non-inoculated treatments. On the other hand, Mulika *et al.* (2022) reported a non-significant difference in days to maturity on green gram between inoculated and non-inoculated treatments at Kiboko and Ithookwe sites.

4.4. Effects of *Bradyrhizobium* Inoculation on Nodulation Parameters of Cowpea Varieties

4.4.1. Number of nodules plant⁻¹

The results from combined data of the two sites revealed that the number of nodules plant⁻¹ was significantly ($P < 0.001$) affected by the main effects of varieties and *Bradyrhizobium* inoculation. Similarly, the treatment interaction effect had a significant ($P < 0.05$) effect on the number of nodules plant⁻¹ (Appendix Table 4).

The maximum and the minimum number of nodules plant⁻¹ were recorded from the White Wonder Trailing variety inoculated with the strain MBI-cowpea and the Bole variety without *Bradyrhizobium* inoculation, respectively. However, there were no statistically significant differences between Keti and TVU varieties of *Bradyrhizobium* inoculated and non-inoculated treatments, respectively. The number of nodules in inoculated plants was increased by 63.75% compared to the control (Table 4). The increment in the number of nodules in the root of cowpea varieties might be due to the synergetic effect of the two treatments, which increase rhizobial activities and, consequently, the plant's ability to

absorb N. In line with this result, Shumet *et al.* (2022) reported that inoculation treatment increased nodules, possibly due to the effectiveness of the introduced *Bradyrhizobium* strain against native bacteria in the soil. Similarly, Emmanuel *et al.* (2021) reported that the number of nodules on different cowpea varieties increased in *Bradyrhizobia*-inoculated plants compared to uninoculated plants. Moreover, Ayalew and Yoseph (2020) also reported an improvement in the nodulation performance of cowpea cultivars when inoculated with *Bradyrhizobium* strains. Several other relevant findings also revealed an increased nodulations due to *Bradyrhizobium* inoculation (Yoseph *et al.*, 2017; Ndungu, 2017; Mulika *et al.*, 2022; Bediru 2022). In contrast, Mintah *et al.* (2020); Nyaga and Njeru (2020) found that rhizobia inoculation had no significant effects on nodule number of cowpea crop as compared to the non-inoculated treatments.

4.4.2. Effectiveness of nodules

The main objective of rhizobial inoculation is to provide sufficient quantities of effective rhizobia to colonize the legume rhizosphere and increase N₂-fixing efficient of the nodules. Nodules are effective when they have a pinkish color, while white nodule color is categorized as ineffective (Ayalew and Yoseph, 2020). Analysis of variance for combined data from the two sites revealed that the main effects of varieties and *Bradyrhizobium* inoculation, as well as their interaction effect, were highly significantly ($P < 0.001$) affected the number of effective nodules plant⁻¹ (Appendix Table 4).

The highest number of effective nodule plant⁻¹ was obtained from the White Wonder Trailing variety inoculated with *Bradyrhizobium* strain MBI-cowpea, while the lowest number of effective nodule plant⁻¹ was obtained from the Bole variety without inoculation (Table 4). However, Keti variety did not influenced by *Bradyrhizobium* inoculation as they

respond the same values in number of effective nodule. The number of effective nodules was increased by 82.86% in inoculated plants compared to the non-inoculated plants. The increment in size and the number of effective nodules with *Bradyrhizobium* inoculation might be due to the compatibility of the strain and the host plant. The current study in agreement with Bala *et al.* (2010) who reported that nodules with strong pink color had good N₂-fixing ability due to the presence of leghemoglobin, which is responsible for active N₂-fixation while the light pink color indicates average N₂-fixation. Similarly, Ayalew and Yoseph (2020); Yohane (2016) reported that the cowpea genotype inoculated with rhizobia had a strong pink color nodule. Moreover, Yusif *et al.* (2016) revealed that the number of effective nodules was significantly higher with inoculated plants than with non-inoculated ones on groundnut plants. Conversely, Mulika *et al.* (2022) stated that the number of effective nodules showed non-significant differences between the inoculated and non-inoculated treatments on green gram plants.

Table 4: Interaction effect of varieties by *Bradyrhizobium* inoculation on nodule number and effective nodules of cowpea averaged across the two sites during the 2022 main season

<i>Bradyrhizobium</i>	Varieties	Nodule number plant ⁻¹	Effective nodule plant ⁻¹
Inoculated	Bole	24.50 ^f	13.00 ^d
	TVU	46.93 ^b	27.00 ^b
	White Wonder Trailing	50.81 ^a	35.00 ^a
Non-inoculated	Keti	38.52 ^d	18.00 ^c
	Bole	18.42 ^g	6.00 ^e
	TVU	36.29 ^d	17.00 ^{cd}
	White Wonder Trailing	42.37 ^c	19.00 ^c
	Keti	28.50 ^e	18.0 ^{cd}
LSD _(0.05)		3.26	5.5
CV (%)		3.16	10
F-value		4.82 [*]	15.12 ^{***}

Means sharing the same letter(s) within a column of treatment is not statistically significant at 5% level of significant, ^{*}, ^{***} significant probability level at ($P < 0.01$), ($P < 0.001$), respectively

4.4.3. Nodule fresh weight

The results of combined data from the two sites revealed that the nodule fresh weight of cowpea was highly significantly ($P < 0.001$) affected by the main effects of variety and *Bradyrhizobium* inoculation. However, the interaction of variety by *Bradyrhizobium* inoculation showed non-significant ($P > 0.05$) differences in nodule fresh weight plant⁻¹ (Appendix Table 4).

The highest nodule fresh weight was recorded from the White Wonder Trailing variety. While the Bole variety was found to have the lowest nodule fresh weight (Table 5). The variation among the tested varieties in term of nodule dry weight was 0.28 g or 50.9%. The differences in nodule fresh weight among the cowpea varieties might be attributed to the sizes and quantities of nodules. Nodule fresh weight was strongly and positively correlated with nodule number and effective nodules, indicating that an increase in this parameter could lead to an increase in nodule number and effective nodules (Appendix Table 9). The result is in line with Ayalew and Yoseph (2020), who reported a significant effect of varieties on the nodule fresh weight of cowpea. Similarly, Judith *et al.* (2021) reported a substantial impact of varieties on the fresh weight of nodules in chickpeas. However, the result contradicts the finding of Olamide *et al.* (2021), who reported a non-significant difference in the fresh weight among cowpea varieties.

On the other hand, the highest nodule fresh weight was recorded from *Bradyrhizobium* inoculated plants, while the lowest nodule fresh weight was recorded from the control (Table 5). *Bradyrhizobium* inoculation significantly increased nodule fresh weight by 15.9% compared to the non-inoculated treatments. Improved nodule number and size may account for the significant variation in the fresh weight of inoculated cowpea plants. This

result is consistent with Ayalew and Yoseph (2020), who recorded that cowpea inoculated with *Bradyrhizobium* strains produced significantly higher nodule fresh weight at the sand filled-pot trail. Similarly, Korir *et al.* (2017) found that *Rhizobia* inoculation produced higher nodule fresh weight of common beans than the control treatment. Moreover, Tarekegn and Kibret (2017); Argaw (2012) reported that seeds inoculated with *Bradyrhizobium japonicum* significantly increased soybean nodule fresh weight plant⁻¹ compared to the non-inoculated plants.

Table 5: Effects of *Bradyrhizobium* inoculation and varieties on nodulation of cowpea averaged across the two sites during the 2022 main season

Treatments	Nodule fresh weight (g)	Nodule dry weight (g)
<u>Varieties (V)</u>		
Bole	0.27 ^d	0.17 ^d
TVU	0.46 ^b	0.25 ^b
White Wonder Trailing	0.55 ^a	0.29 ^a
Keti	0.35 ^c	0.21 ^c
LSD _(0.05)	0.023	0.016
<u>Bradyrhizobium (BR)</u>		
Inoculated	0.44 ^a	0.27 ^a
Non-inoculated	0.37 ^b	0.19 ^b
LSD _(0.05)	0.01	8.46
CV (%)	3.38	4.20
<u>F- value</u>		
Varieties	471.35 ^{***}	169.83 ^{***}
<i>Bradyrhizobium</i> (BR)	163.32 ^{***}	402.39 ^{***}
V*BR	0.97 ^{ns}	0.04 ^{ns}

Means sharing the same letter(s) within a column of treatment is not statistically significant at 5% level of significant. ^{***}, ^{ns} Significant probability level at ($P < 0.001$), non-significant probability level at ($P < 0.05$) respectively

4.4.4. Nodule dry weight

Nodulation, specifically nodule dry weight, is a vital parameter in determining the ability of rhizobia to fix N (Nakei *et al.*, 2023). The analysis of variance for combined data from the two sites revealed that the main effects of varieties and *Bradyrhizobium* inoculation

were highly significantly ($P < 0.001$) affected nodules dry weight plant⁻¹. However, the interaction effect of varieties and *Bradyrhizobium* inoculation did not show a significant ($P > 0.05$) effect on nodule dry weight plant⁻¹ (Appendix Table 4).

Among the varieties, the White Wonder Trailing variety produced the highest nodule dry weight. However, the lowest nodule dry weight plant⁻¹ was recorded from the Bole variety (Table 5). The variation among the tested varieties in term of nodule dry weight was 41.4%. Such significant differences among varieties in nodule dry weight may be due to the differences in the size of nodules and the total number of nodules plant⁻¹. This result is consistent with the findings of Ayalew *et al.* (2022); Tesfaye and Nebiyu (2021); Mintah *et al.* (2020); Nyaga and Njeru (2020); Manore (2017) and Yoseph *et al.* (2017), who reported the significant variation among cowpea varieties for nodule dry weight. Similarly, a significant difference among green gram varieties on nodules dry weight plant⁻¹ was reported by Mulika *et al.* (2022). On the other hand, Olamide *et al.* (2021) reported non-significant difference in nodule dry weight between the two cowpea varieties.

Regarding the *Bradyrhizobium* inoculation, the highest nodule dry weight was recorded from *Bradyrhizobium* strain MBI-cowpea inoculated plants compared to non-inoculated plants, which shows the role of inoculation in the performance of nodule dry weight (Table 5). *Bradyrhizobium* inoculation significantly increased nodule dry weight by 29.63% compared to the non-inoculated treatments. This could be due to the fact that inoculated plants produced larger nodules than uninoculated plants due to the effectiveness of the introduced rhizobia strain to initiate nodule formation with cowpea roots (Yoseph *et al.*, 2017). This is further reflected in the direct relationship between nodule dry weight and nodule number, effective nodule, and nodule fresh weight (Table 4 and 5). In harmony with this result, Ayalew and Yoseph (2020) reported that *Bradyrhizobium* resulted in

increased nodule dry weight. Similarly, Kyei-boahen *et al.* (2017); Ndungu (2017); Nyaga and Njeru (2020); Emmanuel *et al.* (2021) reported that seed inoculated with *Bradyrhizobium* strains considerably increased nodule dry weight of cowpea due to their better performances than the native rhizobial strains.

4.5. Effects of *Bradyrhizobium* Inoculation on Growth Parameters of Cowpea Varieties

4.5.1. Leaf area plant⁻¹ and leaf area index

The analysis of variance for combined data from the two sites revealed that the main effect of varieties was highly significantly ($P < 0.001$) and *Bradyrhizobium* inoculation and their two factor interaction significantly ($P < 0.05$) influenced leaf area and leaf area index, respectively (Appendix Table 5).

Regarding the interaction effect, the highest leaf area and leaf area index were measured from the White Wonder Trailing variety inoculated with the *Bradyrhizobium* strain. However, the smallest leaf area and leaf area index were recorded from the Bole variety without inoculation, even though the varietal difference of the non-inoculated White Wonder Trailing variety showed statistical similarity with inoculated Ketu and Bole varieties (Figure 3). *Bradyrhizobium* inoculation significantly increased leaf area and LAI of cowpea varieties by 56% compared to non-inoculated treatment. According to Bediru (2022), increased nutrient content through N₂-fixation improves leaf area expansion and higher leaf number growth prolongs the photosynthetic performance of plants, which in turn results in enhanced vegetative growth. In line with this result, Ayalew *et al.* (2022); Kaviraja (2017) found that cowpea inoculation with *Bradyrhizobium* strains significantly improved leaf area and leaf area index than the control treatments. Similarly, Bitire *et al.*

(2022) obtained that rhizobial inoculated strains considerably increased the leaf area and leaf area index and expected for N₂-fixation increment with varietal positive responses to rhizobia inoculation, which also improves vegetative expansion. Moreover, Nyamaizi *et al.* (2020) reported that cowpea inoculated with *Bradyrhizobium* significantly increased LAI by 32.9% over non-inoculated treatments, which might be attributed to the differences originating from varietal phenotypic traits.

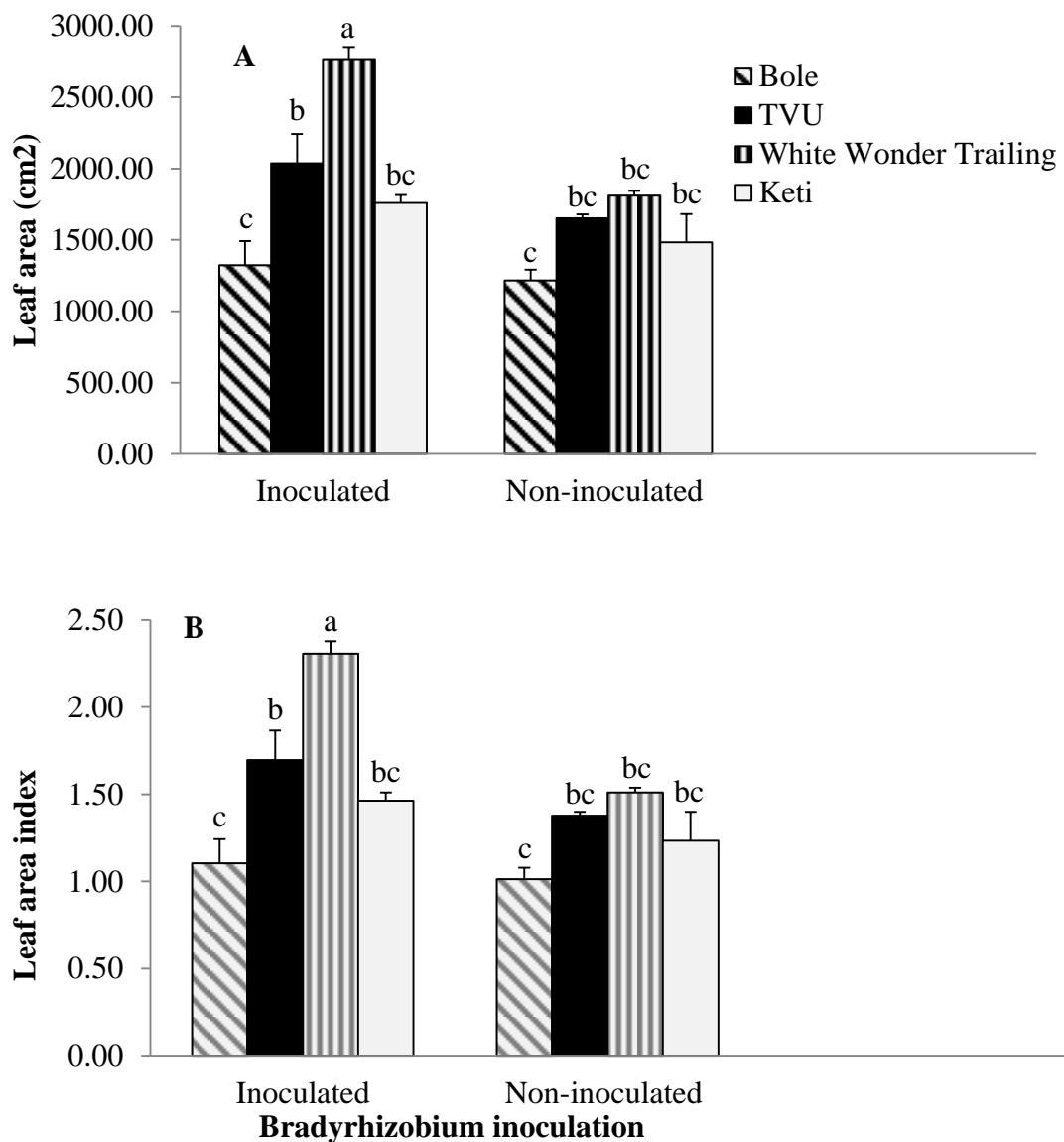


Figure 3: Interaction effect of varieties and *Bradyrhizobium* inoculation on (A) leaf area and (B) leaf area index of cowpea averaged across the two sites during the 2022 main season

4.5.2. Plant height

The analysis of variance for combined data from the two sites revealed that the main effects of varieties and *Bradyrhizobium* inoculation were highly significantly ($P < 0.001$) affected plant height of cowpea. However, the treatment interaction effect was not significant ($P > 0.05$) on plant height (Appendix Table 5).

Regarding the varieties, the tallest plant height was measured from the White Wonder Trailing variety, while the shortest plant height was from the Keti variety. However, there was no statistically significant difference between the Keti and Bole varieties (Table 6). The range difference between the heights of the plants was 30.2 cm. Such differences among the varieties may be due to nature of genetic differences and environmental variations (Miheretu and Addo, 2017). In line with this result, Ayalew *et al.* (2022); Aryal *et al.* (2021); Nyaga and Njeru (2020) and Manore (2017) reported a significant variation among cowpea varieties in plant height. Similarly, Mulika *et al.* (2022) stated that plant height was significantly affected by the green gram varieties at all the sampling stages from three to seven weeks after sowing in Kiboko and Ithookwe sites. Moreover, Eshetu *et al.* (2018) reported a significant difference among faba bean varieties on plant height.

Likewise, inoculation of cowpea with *Bradyrhizobium* strain resulted in the tallest plant height compared to non-inoculated plants. *Bradyrhizobium* inoculation increased plant height by 10.33% over the control (Table 6). The increase in plant height due to inoculation may be because the *Bradyrhizobium* inoculation increases nutrient solubility and make it more available to plants from BNF, which in turn results in an increment in plant height. In addition, rhizobial bacteria can increase the stimulating compounds (phytohormones) and solubilize phosphate, which may not always be available for plant use (Bediru, 2022). Similarly, Eshetu *et al.* (2018) reported that seeds inoculated with an

effective *Rhizobium* strain resulted in increased atmospheric N-fixing activity and bacterial numbers, which in turn increased crop growth. The results reported by Mulika *et al.* (2022); Hannan *et al.* (2022); Matkarimov *et al.* (2019); Yohane (2016); Ibrahim *et al.* (2010) showed that legume seeds inoculated with *Bradyrhizobia* had higher plant height compared to the control. However, Yoseph *et al.* (2017) found that the response of cowpea varieties to *Bradyrhizobia* inoculation was not different in plant height between the inoculated and control treatments.

4.5.3. Number of primary branches plant⁻¹

The results of combined data from the two sites revealed that the number of primary branch plant⁻¹ highly significantly ($P < 0.001$) influenced by the main effects of varieties and *Bradyrhizobium* inoculation. However, their interaction effect was not significant ($P > 0.05$) on the number of primary branch plant⁻¹ (Appendix Table 5).

Among the varieties, the maximum number of primary branches plant⁻¹ was counted from the Bole variety followed by the White Wonder Trailing variety. However, the minimum number of primary branches plant⁻¹ was counted from the Keti variety, but statistically, it was similar with TVU varieties (Table 6). The difference among the maximum and minimum varieties in term of number of primary branches was 24.92%. This result is in line with Mulika *et al.* (2022), who reported a significant variation in branch formation between the tested varieties, and suggested that this variation may be caused by the inheritance of genetic divergence. Similar results were also reported by Miheretu and Addo (2017) and Manore (2017) regarding the varietal differences in the number of branches plant⁻¹, which may be due to the differences in genetic composition among the varieties.

The result contradicts the finding of Aryal *et al.* (2021), who reported a non-significance effect of cowpea varieties on the number of branches plant⁻¹.

Regarding *Bradyrhizobium* inoculation, the maximum number of primary branches plant⁻¹ was counted from plants inoculated with the *Bradyrhizobium* strain. However, the minimum number of primary branches plant⁻¹ was counted from non-inoculated plants (Table 6). The increment in the number of primary branches plant⁻¹ due to inoculation was 14.58% over the control. The increase in the number of primary branches plant⁻¹ through *Bradyrhizobium* inoculation might be due to higher vegetative growth of the plants with higher N supply through BNF since cowpea produces most of its primary and secondary branches during the early vegetative growth period when there are high soil N content or effective nodules (Hannan *et al.*, 2022). The result is in harmony with Bitire *et al.* (2022) and Mulika *et al.* (2022), who indicated that legume seeds when inoculated with effective rhizobial strains significantly increased the number of primary branch plant⁻¹ over control. Similarly, Nyoki and Ndakidemi (2013) observed that *Bradyrhizobium japonicum* inoculation improved the number of branches plant⁻¹ of cowpea by 28.8% in field trials compared to the uninoculated treatment.

Table 6: Effects of *Bradyrhizobium* inoculation and varieties on growth parameters of cowpea averaged across two sites during 2022 main season

Treatments	Plant Height (cm)	Number of primary branches	Shoot fresh weight (g)	Shoot dry weight (g)	Root fresh weight (g)
<u>Varieties (V)</u>					
Bole	64.43 ^c	8.79 ^a	239.57 ^c	35.16 ^b	25.93 ^{ab}
TVU	83.00 ^b	7.35 ^{bc}	266.32 ^a	43.82 ^a	23.85 ^{bc}
White Wonder Trailing	89.73 ^a	8.28 ^{ab}	252.96 ^b	44.11 ^a	27.10 ^a
Keti	59.55 ^c	6.60 ^c	227.58 ^d	30.95 ^c	21.82 ^c
LSD _(0.05)	5.16	1.02	5.81	1.39	2.28
<u>Bradyrhizobium (BR)</u>					
Inoculated	78.22 ^a	8.37 ^a	252.17 ^a	41.66 ^a	27.32 ^a
Non-inoculated	70.14 ^b	7.15 ^b	241.04 ^b	35.36 ^b	22.03 ^b
LSD _(0.05)	2.70	0.53	3.03	0.73	1.19
CV (%)	4.15	7.82	1.40	2.15	5.51
<u>F- value</u>					
Varieties	132.84 ^{***}	15.44 ^{***}	140.06 ^{***}	371.76 ^{***}	17.66 ^{***}
<i>Bradyrhizobium</i> (BR)	41.33 ^{***}	24.29 ^{***}	61.96 ^{***}	346.85 ^{***}	90.74 ^{***}
V*BR	0.36 ^{ns}	1.23 ^{ns}	0.42 ^{ns}	1.38 ^{ns}	1.21 ^{ns}

Means sharing the same letter(s) within a column of treatment is not statistically significant at 5% level of significant, ^{ns} = non-significance, ^{***} significant probability level at (P<0.001).

4.5.4. Shoot fresh and dry weights

The analysis of variance for combined data from the two sites revealed that the main effects of varieties and *Bradyrhizobium* inoculation were highly significantly ($P < 0.001$) affected shoot fresh and dry weights plant⁻¹ of cowpea. However, their interaction effect was not significant ($P > 0.05$) on shoot fresh and dry weights plant⁻¹ (Appendix Table 6).

Among the varieties, the TVU variety recorded the highest shoot fresh weight, while the Keti variety recorded the lowest shoot fresh weight than the rest of the varieties. The difference between the highest and lowest shoot fresh weight between the cowpea varieties was 38.74g (Table 6). The observed differences in shoot fresh weight may be attributed to genetic variations in plant height, branch count, and N₂-fixation capacity among cultivars which determines the shoot's fresh weight. This result is consistent with Shyam *et al.* (2020), who obtained that there was significant variation among the cowpea genotypes in terms of shoot fresh weight.

The highest shoot dry weight was obtained from White Wonder Trailing, followed by TVU varieties which are statistically at par. The lowest shoot dry weight was also obtained from the Keti variety (Table 6). The variation among cowpea varieties in shoot-dry weight could be due to the variation in shoot-fresh weight. The result is in agreement with Ayalew and Yoseph (2020), who showed a significant variation in shoot dry weight formation among the tested five cowpea varieties. Similarly, Mulika *et al.* (2022) showed that significant difference between the green gram varieties in shoot dry weight. In contrast, Nyaga and Njeru (2020) reported no significant difference in shoot dry weight among the tested cowpea genotypes during the second cropping season.

Likewise, *Bradyrhizobium* inoculated plants produced the highest fresh and dry shoot weight than non-inoculated plants. The maximum fresh and dry shoot weight was produced by *Bradyrhizobium* inoculation, which increased by 4.41% and 15.12% over non-inoculated treatments, respectively (Table 6). The differences in the shoot-fresh weight of cowpea due to *Bradyrhizobium* inoculation might be attributed to the availability of N to the crop through symbiotic N₂-fixation. Similarly, the increased shoot fresh and dry weight due to *Bradyrhizobium* inoculation might be attributed to the effectiveness of the *Bradyrhizobia* inoculants (Yoseph *et al.*, 2017). This result is in agreement with Ayalew and Yoseph (2020), who observed a significant variation in shoot fresh weight cowpeas. Similarly, Mintah *et al.* (2020) obtained the highest shoot fresh weight with inoculated rhizobial strains on groundnut and cowpea crops. *Bradyrhizobium* isolates enhanced shoot dry weight of cowpea and the relatively the higher shoot dry weight was obtained from inoculated treatment (Kyei-boahen *et al.*, 2017; Korir *et al.*, 2017; Mintah *et al.* 2020; Nyaga and Njeru, 2020). Similarly, Ulzen *et al.* (2020), found that cowpea inoculated with effective rhizobium significantly increased shoot dry weight by 38% compared to the uninoculated treatments. Likewise, Matkarimov *et al.* (2019) and Yusif *et al.* (2016) reported that seeds inoculated with *Rhizobium* bacteria resulted in increased shoot dry weight of mung bean and groundnut, respectively.

4.5.5. Root fresh weight

The combined data results from the two sites revealed that root fresh weight was significantly ($P < 0.001$) affected by the main effects of varieties and *Bradyrhizobium* inoculation. However, the interaction effect of varieties by *Bradyrhizobium* inoculation was not significant ($P > 0.05$) difference on root fresh weight (Appendix Table 6).

Among the varieties, the highest root fresh weight was recorded from the White Wonder Trailing variety, followed by Bole. However, the lowest root fresh weight was measured from the Keti variety but statistically similar to TVU and Keti varieties on root fresh weight (Table 6). The observed differences among cowpea varieties could be related to genetic variation in root growth and development. In line with this result, Syafriani *et al.* (2022) reported a significant difference between cowpea and long-bean varieties on root fresh weight.

Regarding inoculation, the highest and the lowest root fresh weight was obtained from inoculated and uninoculated treatments, respectively (Table 6). The root fresh weight of the cowpea was increased by 19.36% due to *Bradyrhizobium* inoculation compared with the control. Such improvement in root fresh weight may be due to increased root hair, length, and nodule number of the plants due to the introduced *Bradyrhizobium* strain. In line with this result, Bediru (2022) reported that inoculating common beans with *Rhizobium* enhanced root growth and development.

4.5.6. Root length

Root length is typically a good predictor of seed vigor, which may help seedlings establish more successfully (Shyam *et al.*, 2020). The analysis of variance for combined data from the two sites revealed that the main effects of varieties and *Bradyrhizobium* inoculation and the two factors interaction significantly ($P < 0.01$) affected the root length of cowpea (Appendix Table 6).

The longest root length was measured from the Bole variety with *Bradyrhizobium* inoculation, and the shortest root length was from the Keti variety without inoculation treatment. However, the differences in root length between White Wonder Trailing and

TVU with *Bradyrhizobium* inoculation were statistically similar. Likewise, statistical similarities between Bole with TVU and TVU with White Wonder Trailing without *Bradyrhizobium* inoculation and un-inoculated White Wonder Trailing with inoculated Keti varieties were recorded. However, the Keti variety did not show a significant difference in response to *Bradyrhizobium* inoculation (Table 7). The increment in root length may be due to *Bradyrhizobium* inoculation produces a suitable environment for the root to develop freely and hunt for various nutrients that are crucial for the crop's growth and compatibility of the varieties. The result is in agreement with Bediru (2022), who reported that *Rhizobium* inoculation significantly influenced taproot length and increased root length in leguminous plants. Similarly, Eshetu *et al.* (2018) reported a significant effect of *Rhizobium* inoculation on the taproot length of faba bean varieties.

Table 7: Interaction effect of varieties and *Bradyrhizobium* inoculation on root length (cm) of cowpea averaged across the two sites during the 2022 main season

<i>Bradyrhizobium</i>	Varieties	Root length (cm)
Inoculated	Bole	34.497 ^a
	TVU	29.717 ^b
	White Wonder Trailing	30.173 ^b
	Keti	19.547 ^{de}
Non-inoculated	Bole	24.993 ^c
	TVU	23.140 ^{cd}
	White Wonder Trailing	20.057 ^d
	Keti	15.983 ^e
LSD _(0.05)		3.82
CV (%)		5.35
F-value		7.75 ^{**}

Means sharing the same letter(s) within a column of treatment is not statistically significant at 5% level of significance, ^{ns} = non-significance, ^{**} significant probability level at ($P < 0.01$).

4.5.7. Root dry weight

The analysis of variance for combined data from the two sites revealed that the main effects of varieties and *Bradyrhizobium* inoculation as well as their interaction effect were significantly ($P < 0.001$) influenced root dry weight plant⁻¹ of cowpea (Appendix Table 6).

The larger root dry weight was obtained from White Wonder Trailing variety inoculated with *Bradyrhizobium* strain MBI-cowpea followed by Bole and TVU varieties, whereas the smaller root dry weight was recorded from Keti variety without inoculated (Figure 4). The *Bradyrhizobium* inoculation increased the root dry weight of the White Wonder Trailing variety by 27.2% as compared to the non-inoculated treatment. The increment in root dry weight may be due to effective nodulation and N₂-fixation of *Bradyrhizobium* isolate on the root growth, development, and compatibility with cowpea genotypes. It could also be attributed to improved root length and fresh weight due to *Bradyrhizobium* strain MBI-cowpea inoculation. In line with this result, Kaviraja (2017) reported a significant increase in root dry weight and nodulation following inoculation of cowpea with rhizobia strains. Similarly, Yusif *et al.* (2016) and Ibrahim *et al.* (2011) reported higher root dry weights of groundnut and soybean due to rhizobia inoculation compared to the control treatment. Moreover, Matkarimov *et al.* (2019) found that seed inoculation with Rhizobium bacteria increases the root dry weight of mung beans.

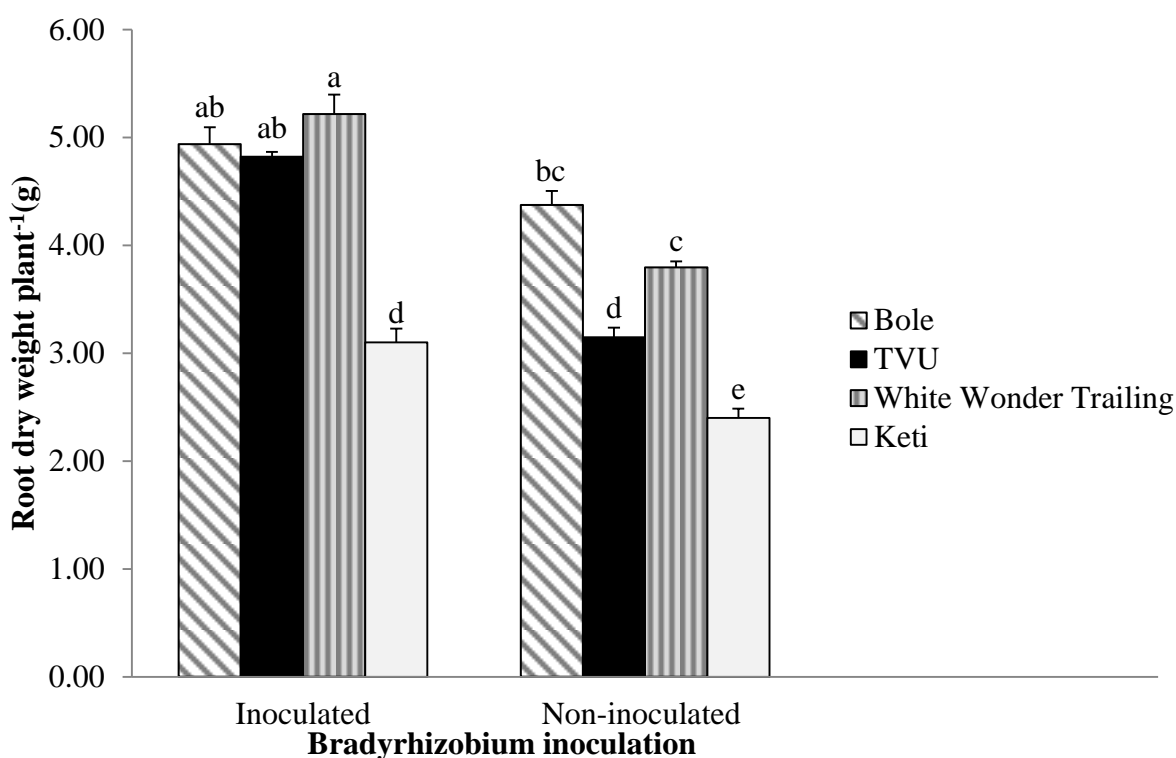


Figure 4: Interaction effects of *Bradyrhizobium* inoculation and varieties on root dry weight of cowpea averaged across the two sites during the 2022 main season

4.6. Effects of *Bradyrhizobium* Inoculation on Yield and Yield Components of Cowpea Varieties

4.6.1. Pod length

The analysis of variance for combined data from the two sites revealed that the main effect of varieties, *Bradyrhizobium* inoculation and their interaction effects were significantly ($P < 0.001$) influenced pod length of cowpea (Appendix Table 7).

The longest pod length was recorded from the White Wonder Trailing varieties with *Bradyrhizobium* inoculation and the shortest pod length was recorded from the Bole variety without inoculation. However, the difference between the other treatments was statistically similar except between the inoculated Keti and non-inoculated white wonder

trailing varieties (Figure 5). Seed inoculation with *Bradyrhizobium* could be related to an increased symbiosis between the host and the bacteria, which might ultimately, resulted in more supply of N due to N₂-fixation that leads to increased pod length. In general, inoculation with *Bradyrhizobium* strain increased pod length by 22.91% over uninoculated treatment. This might be due to the genetic potential of each variety to bear the different sizes of pods and the compatibility of bacteria with the variety. The result is consistent with Ayalew *et al.* (2021), who reported improved pod length in cowpea due to inoculation of the *Bradyrhizobium* strain. Similarly, Mulika *et al.* (2022) reported that the pod length of green bean varieties was significantly improved by inoculation treatments at both sites, and inoculated pods were longer than non-inoculated pods. Moreover, Matkarimov *et al.* (2019) reported an increased pod length by 32% due to *Rhizobium* inoculation on mung beans compared to the non-inoculated.

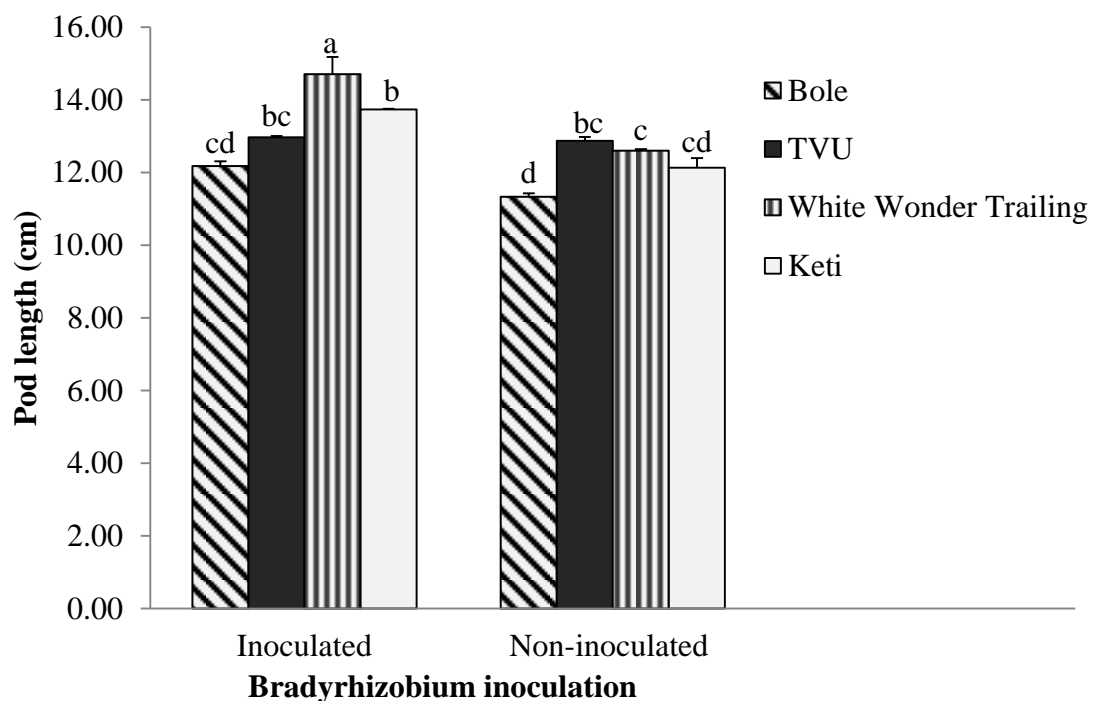


Figure 5: Interaction effects of *Bradyrhizobium* inoculation and varieties on pod length (cm) of cowpea averaged across the two sites during the 2022 main season

4.6.2. Number of pods plant⁻¹

The analysis of variance for combined data from the two sites revealed that the main effects of varieties and *Bradyrhizobium* inoculation were highly significantly ($P < 0.001$) affected the number of pods plant⁻¹ of cowpea. However, their interaction effects were not significant ($P > 0.05$) on the number of pods plant⁻¹ (Appendix Table 7).

Among the four cowpea varieties, the maximum number of pods plant⁻¹ was counted from the White Wonder Trailing variety, followed by the TVU variety. However, the minimum number of pods plant⁻¹ was recorded from the Keti variety but statistically similar to TVU and Bole varieties (Table 8). Such differences may be attributed to the variation in number of primary branches, and plant height of the plant. In agreement with this result, Yoseph *et al.* (2017); Augustine and Godfre (2019); Aryal *et al.* (2021) reported marked differences in the number of pods plant⁻¹ among the cowpea varieties, which may be due to genetic differences in partitioning photosynthesis into pods development. However, this finding contradicts the results of Giridhar *et al.* (2020), who reported a non-significantly difference among three cowpea varieties on the number of pod⁻¹.

Likewise, inoculation with *Bradyrhizobium* strain MBI-cowpea resulted in higher pods plant⁻¹ compared to the control treatment (Table 8). On average, inoculation with *Bradyrhizobium* strain MBI-cowpea increased the number of pods by 17% compared to uninoculated plants. The increases in pod plant⁻¹ due to *Bradyrhizobium* inoculation may be related to improved growth and higher assimilate concentrations due to better N nutrition from BNF. In addition, adequate availability of N might have facilitated the production of more primary and secondary branches, leaf area, and plant height which might, in turn, have contributed to the production of the higher number of total pods. In

line with these results, Shumet *et al.* (2022); Mulika *et al.* (2022); Hannan *et al.* (2022) observed an increase in the number of pods plant⁻¹ due to *Rhizobium* inoculation. Furthermore, *Bradyrhizobium japonicum* inoculation resulted in a 13.7% increase in the number of cowpea pods plant⁻¹ compared to uninoculated treatments, according to (Nyoki and Ndakidemi, 2013).

4.6.3. Number of seeds Pod⁻¹

Seeds pod⁻¹ is an important yield component that directly mediates exploiting potential yield recovery in leguminous crops. Moreover, it is an important yield component contributing to the final yield of cowpea (Belay *et al.*, 2017). The analysis of variance for combined data from the two sites revealed that the main effects of varieties and *Bradyrhizobium* inoculation were highly significantly ($P < 0.001$) affected the number of seeds pod⁻¹ of cowpea. However, their interaction effect was not significant ($P > 0.05$) on the number of seeds pod⁻¹ (Appendix Table 7).

Regarding the varietal response, the highest number of seeds pod⁻¹ was recorded from the White Wonder Trailing variety. The lowest value for the number of seeds pod⁻¹ was counted from the Bole variety (Table 8). Moreover, the differences in the number of seeds pod⁻¹ between TVU and Keti were statistically similar. This might be due to inherent genetic differences among the cowpea varieties for seed set, pod development, seed production, and adaptation to the different environments. The result is in agreement with the previous research work by Ayalew *et al.* (2021), who reported variation among four cowpea varieties in terms of seeds pod⁻¹ at three trail fields. Similarly, Belay *et al.* (2017) and Manore (2017) observed differences among tested cowpea varieties in the number of seeds pod⁻¹.

On the other hand, the highest number of seeds pod⁻¹ was obtained from plants inoculated with *Bradyrhizobium* strain MBI-cowpea. However, the lowest value was recorded for non-inoculated treatment. An overall increase in the number of seeds pod⁻¹ due to *Bradyrhizobium* inoculation was 15.47% compared to the control treatment (Table 8). Seed inoculation might increase the availability of N to the plants through BNF, which ultimately resulted in more seeds pod⁻¹. Moreover, the increased number of seeds pod⁻¹ with inoculation might be due to more availability of nutrients to more root development, stem vigor, flower initiation of a seed, and pod length formation. This finding is consistent with Yoseph *et al.* (2017); Kyei-boahen *et al.* (2017); Erica *et al.* (2017); Ayalew *et al.* (2021), who reported an increased number of seeds pod⁻¹ of cowpea with *Bradyrhizobium* inoculation compared to non-inoculated.

4.6.4. Hundred seed weight

The analysis of variance for combined data from the two sites revealed that the main effects of varieties and *Bradyrhizobium* inoculation were highly significantly ($P < 0.001$) affected the hundred seed weight of cowpea. However, their interaction effect was not significant ($P > 0.05$) on hundred seed weight (Appendix Table 7).

The heavier hundred seed weight was measured from the Bole variety. Whereas the lighter hundred seed weight was measured from the TVU variety, the difference between TVU and White Wonder Trailing varieties were statistically at par in term of the hundred seed weight (Table 8). The significant variation in hundred seed weight among cowpea varieties could be due to the difference in genetic makeup to the seed size and difference in translocation and partitioning more efficiency of assimilates from source to sink and ultimately gained more seed weight and better growth and development of the crop (El

Naim and Jabereldar, 2010). The result is in agreement with Belay *et al.* (2017); Manore (2017); Nyaga and Njeru (2020); Giridhar *et al.*(2020) who showed statistically significant differences between cowpea varieties in terms of hundred seed weights. Furthermore, Mulika *et al.* (2022) reported a marked difference in the varieties for hundred seed weight under field conditions. However, this result contradicts Augustine and Godfre (2019), who reported non-significant differences among the cowpea varieties in hundred seed weight.

Regarding the inoculation, the heavier hundred seed weight was recorded from inoculated treatment while the lighter seed weight was recorded from the control treatment (Table 8). This may be related to increased nodulation due to symbiosis between cowpea and *Bradyrhizobium*, which resulted in increased N₂-fixation leading to increased hundred seed weight. On average, hundred seed weight was increased by 10.56% due to *Bradyrhizobium* inoculation over uninoculated treatment. The result is in harmony with Kyei-boahen *et al.* (2017); Mintah *et al.* (2020); Ayalew *et al.* (2021) who reported that *Bradyrhizobium* inoculation significantly improved hundred seed weights of cowpeas compared to control treatments. The study conducted by Erica *et al.* (2017) also showed an increased hundred-seed weight due to rhizobia inoculation.

Table 8: Effect of *Bradyrhizobium* inoculation on yield and yield components of cowpea varieties averaged across the two sites during the 2022 main season

Treatments	Number of pod plant ⁻¹	Number of seed pod ⁻¹	Hundred seed weight (g)	Grain yield (t ha ⁻¹)	Harvest index (%)
<u>Varieties (V)</u>					
Bole	42.68 ^b	10.11 ^c	17.45 ^a	2.20 ^c	43.44 ^{bc}
TVU	47.41 ^{ab}	12.60 ^b	13.40 ^c	2.68 ^b	39.95 ^c
White Wonder Trailing	50.71 ^a	13.57 ^a	14.02 ^c	2.98 ^a	49.91 ^a
Keti	31.65 ^c	12.42 ^b	15.80 ^b	1.79 ^d	46.85 ^{ab}
LSD (0.05)	7.63	0.96	0.88	0.26	5.62
<u>Bradyrhizobium (BR)</u>					
Inoculated	47.12 ^a	13.19 ^a	16.01 ^a	2.81 ^a	46.17
Non-inoculated	39.10 ^b	11.15 ^b	14.32 ^b	2.01 ^b	43.90
LSD (0.05)	3.98	0.50	0.46	0.14	2.93
CV (%)	10.55	4.68	3.45	6.49	7.44
<u>F- value</u>					
Varieties	20.07 ^{***}	39.66 ^{***}	73.06 ^{***}	67.74 ^{***}	9.87 ^{***}
<i>Bradyrhizobium</i> (BR)	18.64 ^{***}	76.62 ^{***}	62.49 ^{***}	156.42 ^{***}	2.74 ^{ns}
V*BR	0.47 ^{ns}	0.81 ^{ns}	1.31 ^{ns}	3.21 ^{ns}	2.06 ^{ns}

Means in column followed by the same letter/s are not significantly different at 5% level of significance. *** Very highly significant at 1% probability level, ^{ns} = non-significant at 5% probability level

4.6.5. Grain yield

The analysis of variance for combined data from the two sites revealed that the main effects of varieties and *Bradyrhizobium* inoculation were highly significantly ($P < 0.001$) affected grain yield of cowpea. However, their interaction effect was not significant ($P > 0.05$) on grain yield (Appendix Table 8).

Among the varieties, the highest grain yield was obtained from the White Wonder Trailing variety, while the lowest grain yield was obtained from the Keti variety. The difference between the high and low-yielding varieties ranges from 1.79 to 2.98 t ha⁻¹, scoring 39.93% yield differences averaged across the two sites (Table 8). Accordingly, in this study, White Wonder Trailing variety recorded the highest grain yield and was identified as the superior cowpea variety in the study sites. The reason for the higher grain yield measured from the White Wonder Trailing variety might be due to the ability of a genotype to produce more number of pod and longer pods as well as the higher number of seeds pod⁻¹, which increased its economic yield and profitability of the crop. Moreover, the difference in grain yield among cowpea varieties is largely due to differences in the inherent yielding potential of the varieties (Yoseph *et al.*, 2017). In line with this result, Mulika *et al.* (2022) reported a significant difference in seed yield and showed a positive correlation with seeds pod⁻¹, seed weight, plant height, and pod length. Similarly, several previous research findings showed a significant difference in grain yield among cowpea varieties (Augustine and Godfre, 2019; Nyamaizi *et al.*, 2020; Asrat *et al.*, 2021; Ayalew *et al.*, 2021).

Inoculation with *Bradyrhizobium* significantly increased seed yield compared with the uninoculated treatments. *Bradyrhizobium* inoculation increased grain yield by 28.47% compared to the non-inoculated treatment (Table 8). Such an increase in cowpea grain yield due to *Bradyrhizobium* inoculation could be attributed to an increased number of branches, which, in turn, increased the number of productive nodes and the number of pods plant⁻¹. It may also be related to better N availability at the early growth stage of the crop, which in turn may result in more nodules and adequate N fixation during growth, especially during pod and seed sets all these could contribute to the observed increase in seed yield (Tarekegn and Kibret, 2017). Similarly, Nyoki and Ndakidemi (2013) reported that the increase in grain yield due to *Bradyrhizobium* inoculation might be related to the effectiveness of the inoculation by fixing N and meeting the nutritional needs of the plant. According to Samson *et al.* (2020); Ayalew *et al.* (2021); Emmanuel *et al.* (2021); Ayalew *et al.* (2022), higher grain yield could also be associated with a higher number of pods plant⁻¹ and seeds pod⁻¹.

4.6.6. Harvest index

The harvest index is very useful in measuring nutrient and dry matter partitioning in crop plants, and indicates how efficiently the plant utilized acquired nutrients for grain production. The analysis of variance for combined data from the two sites revealed that the main effect of varieties was highly significantly ($P < 0.001$) affected the harvest index of cowpea. However, the main effect of *Bradyrhizobium* inoculation and the interaction effect of variety and *bradyrhizobium* inoculation were not significant ($P > 0.05$) on the harvest index (Appendix Table 8).

The highest harvest index value was obtained from the White Wonder Trailing variety, followed by the Keti variety. However, the lowest harvest index value was observed from the TVU variety, and the difference between the lowest and highest harvest index value was 19.96% (Table 8). This result is in agreement with Manore (2017), who reported that a significant differences among the tested cowpea varieties in terms of harvest index and the highest harvest index was obtained from the highest seed-yielding varieties. Similarly, Mulika *et al.* (2022) reported a significant difference among green gram varieties on harvest index. On the other hand, Belay *et al.* (2017) reported non-significant differences in cowpea varieties in term of the harvest index.

4.6.7. Above ground biomass yield

The analysis of variance for combined data from the two sites revealed that the main effects of varieties, *Bradyrhizobium* inoculation, and the interaction of both factors significantly influenced above ground biomass yield of cowpea (Appendix Table 8).

The highest above-ground biomass yield was recorded from the TVU varieties inoculated with *Bradyrhizobium* strain MBI-cowpea. However, the lowest above-ground biomass yield was recorded from the Keti variety without inoculation. Statistically significant differences were not observed among the uninoculated TVU variety with inoculated Bole variety and inoculated Keti variety with uninoculated Bole variety (Table 9). Generally, inoculation with *Bradyrhizobium* strain MBI-cowpea increased the aboveground biomass of cowpea by 56.6% compared to the control treatment. The increased above-ground biomass yield with *Bradyrhizobium* inoculation could be due to sufficient N supply, mainly from BNF, because N is a key element in many biological compounds and plays an important role in photosynthetic activity and chlorophyll synthesis, which ultimately

results in vigorous vegetative growth and more biomass accumulation. Moreover, the increase in aboveground biomass yield due to *Bradyrhizobium* inoculation could also be attributed to the increased primary branches, pod plant⁻¹, and seeds pod⁻¹. Similarly, Erica *et al.* (2017); Shumet *et al.* (2022); Emmanuel *et al.* (2021); Mulika *et al.* (2022) reported that rhizobial inoculation increases the above-ground biomass yields of several legume crops compared with non-inoculated treatment. In contrast, Mintah *et al.* (2020) reported a non-significant difference in aerial bean biomass yield between *Bradyrhizobium* inoculated and control treatments.

4.6.8. Straw yield

The analysis of variance for combined data from the two sites revealed that the main effects of varieties and *Bradyrhizobium* inoculation were highly significantly ($P < 0.001$) affected the straw yield of cowpea. Likewise, their interaction effect was significantly ($P < 0.05$) different on straw yield (Appendix Table 8).

The highest straw yield was recorded from the TVU variety inoculated with the *Bradyrhizobium* strain, whereas the lowest straw yield was recorded from the variety Keti without inoculation (Table 9). However, the variation among inoculated Bole and White wonder trailing varieties were statistically similar. The straw yield was higher in inoculated varieties compared to uninoculated ones by the range of 58.25%. The improvement in straw yield due to the *Bradyrhizobium* inoculation can be explained by increased nutrient availability, which in turn plays an important role in influencing growth-related parameters such as above-ground biomass yield, plant height, and shoot fresh and dry weight (Singh *et al.*, 2007). In line with this result, El-sherbeny *et al.* (2022) reported significantly increased straw yield of peanut crops due to *Bradyrhizobium* inoculation compared to the

uninoculated plants. Similarly, Yadav *et al.* (2023) reported that inoculation of biofertilizers had a significant effect on the straw yield of urdbean as compared to uninoculated.

Table 9: Interaction effect of varieties and *Bradyrhizobium* inoculation on above ground biomass yield (t ha⁻¹) and straw yield (t ha⁻¹) of cowpea averaged across the two sites during the 2022 main season

<i>Bradyrhizobium</i>	Varieties	Above ground biomass yield (t ha ⁻¹)	Straw yield (t ha ⁻¹)
Inoculated	Bole	5.89 ^c	3.41 ^b
	TVU	7.26 ^a	4.12 ^a
	White Wonder Trailing	6.83 ^b	3.33 ^b
	Keti	4.44 ^e	2.30 ^c
Non-inoculated	Bole	4.31 ^e	2.37 ^c
	TVU	6.10 ^c	3.84 ^{ab}
	White Wonder Trailing	5.10 ^d	2.60 ^c
	Keti	3.15 ^f	1.72 ^d
LSD _(0.05)		0.32	0.57
CV (%)		2.09	6.66
F-value		8.31 ^{**}	3.79 [*]

Means in column followed by the same letter/s are not significantly different at 5% level of significance. * ** significant at ($P < 0.05$), ($P < 0.01$), respectively.

4.7. Correlation between Yield and Nodulation, Growth, and Yield Components

The correlation values explain the association between different analyzed parameters and indicate the magnitude of their relationships (Appendix Table 9). The correlation analysis revealed that the grain yield of cowpea was positively and highly significantly ($P < 0.001$) correlated with nodule number, nodule dry weight, and physiological maturity (Table 10). The result is consistent with Nakei *et al.* (2023), who indicated that nodulation (nodule numbers and dry weight) influenced different plant growth and yield components and interactions between various components. Similarly, Argaw (2012) reported that nodule number positively correlated with grain yield of the soybean.

Likewise, grain yield was strongly and positively correlated with plant height, leaf area, shoot dry weight, root dry weight, and number of primary branches and the better growth and development performances of the crop give the highest grain yield (Table 10). The result is in line with Ayalew *et al.* (2022), who reported grain yield was significantly and positively correlated with leaf area and leaf area index, indicating the importance of leaf growth performance for grain yield improvement.

The grain yield was positively and strongly significantly ($P < 0.001$) correlated with number of pod plant⁻¹, pod length, number of seed pod⁻¹, above ground biomass, and straw yield which in return to increase the total grain yield. In line with this result, Sheidu and Igyuve (2023); Kavyashree *et al.* (2023); Walle *et al.* (2018); Diriba *et al.* (2014) reported a significant correlation between yield and yield-related parameters.

Table 10: Correlation among yield and nodulation, growth and yield attributes of cowpea

Parameters	Significance	
	R values	P value
Grain yield vs. nodule number	0.7819	***
Grain yield vs. nodule dry weight	0.8549	***
Grain yield vs. day to 90% physiological maturity	0.5602	**
Grain yield vs. plant height	0.8709	***
Grain yield vs. number of primary branches	0.5411	**
Grain yield vs. leaf area	0.7536	***
Grain yield vs. shoot dry weight	0.9331	***
Grain yield vs. root dry weight	0.785	***
Grain yield vs. number of pod plant ⁻¹	0.8238	***
Grain yield vs. Pod length	0.6368	***
Grain yield vs. number of seed pod ⁻¹	0.6811	***
Grain yield vs. above ground biomass yield	0.8868	***
Grain yield vs. straw yield	0.6588	***

4.8. Partial Budget Analysis

From the result of this study, the average yield of all treatments tested was obtained. The average yield of cowpea was adjusted downward by 10% to reflect the difference between

the experimental yield and the yield that a farmer could expect from the same treatment without the researchers' involvement as described by CIMMYT (1988).

Based on the partial budget procedure described by CIMMYT (1988), the total variable (TVC) included the current price of TSP fertilizer cost (5500 ETB kg⁻¹), cowpea seed (38.45ETB kg⁻¹), and *Bradyrhizobium* inoculation (260.61 ETB ha⁻¹) cost as well as the labor cost for all activities (land preparation, planting, weeding, harvesting, and transportation) in the different treatments.

The result indicated that in the economic analysis of cowpea varieties as influenced by *Bradyrhizobium* inoculation the highest net return of 67171.3 ETB ha⁻¹ was obtained from White Wonder Trailing variety with *Bradyrhizobium* inoculation with a marginal rate of return of 1386.7%, followed by the net benefit of 59322.3 ETBha⁻¹ with MRR 1226.5 % for variety TVU with inoculated treatments (Table 11). On the other hand, the lowest net benefit of 21400.0 ETB ha⁻¹ was for the variety Keti without inoculation. In order to use the marginal rate of return (MRR %) as a basis for cowpea varieties with *Bradyrhizobium* inoculation recommendation, the minimum acceptable marginal rate of return has to be 100% (CIMMYT, 1988). Therefore, the combination of White Wonder Trailing varieties with *Bradyrhizobium* inoculation was found to be economically feasible at the study sites and areas similar agroecologies. The result is consistent with Allito (2022), who reported rhizobia inoculation had a positive and significant effect on growth, yield, and economic sustainability for faba bean production.

Table 11: Summary of partial budget analysis for cowpea production averaged across the two sites (Dale and Hawassa), during the 2022 main season

Treatment	BR (ETB ha ⁻¹)	SC (ETB ha ⁻¹)	TSP (ETB ha ⁻¹)	LC (ETB ha ⁻¹)	UGY (t ha ⁻¹)	AGY (t ha ⁻¹)	GFB (ETB ha ⁻¹)	TVC (ETB ha ⁻¹)	NB (ETB ha ⁻¹)	MRR (%)
TVU BR-	0	730.55	5500	4464.5	2.22	2.00	51000	10695.1	40305.0	D
Wwt BR-	0	730.55	5500	5184.5	2.45	2.21	56355	11415.1	44940.0	643.8
Keti BR-	0	730.55	5500	5264.5	1.43	1.29	32895	11495.1	21400.0	D
Bole BR-	0	730.55	5500	5348.5	1.94	1.75	44625	11579.1	33046.0	D
TVU BR+	260.61	730.55	5500	6096.5	3.13	2.82	71910	12587.7	59322.3	1226.5
Wwt BR+	260.61	730.55	5500	6662.5	3.5	3.15	80325	13153.7	67171.3	1386.7
Keti BR+	260.61	730.55	5500	6692.5	2.14	1.93	49215	13183.7	36031.3	D
Bole BR+	260.61	730.55	5500	6724.5	2.46	2.21	56355	13215.7	43139.3	D

Where, TSP= Triple super phosphate; SC= Seed cost; LC= Labour cost; BR= Bradyrhizobium inoculation UGY= unadjusted grain yield; AGY = adjusted grain yield; GFB = gross field benefit; TVC = total variable costs; NB = net benefit; MRR% = marginal rate of return; ETB ha⁻¹ = Ethiopian Birr hectare⁻¹; D = dominated treatments.

5. SUMMARY AND CONCLUSION

5.1. Summary

Cowpea [*Vigna unguiculata* (L) Walp] is an important legume crop grown widely throughout the world, mainly in tropical and subtropical regions, including Ethiopia. However, its yield in Ethiopia and the study area is low compared to African countries due to a lack of improved varieties, high cost of mineral fertilizers, insufficient knowledge of *Bradyrhizobium* inoculants technologies and use, and poor agronomic practices. With this background, the present study was conducted to evaluate the growth, nodulation, and yield response of cowpea varieties to *Bradyrhizobium* inoculant and economically feasible combination of cowpea varieties and inoculant in Dale and Hawassa, Sidama region, Southern Ethiopia, during the 2022 main season.

The treatments studied consisting of four cowpea varieties (Bole, TVU, White Wonder Trailing, Keti), and two levels of *Bradyrhizobium* inoculation (non-inoculated and inoculated with strain: MBI-cowpea) were tested in a factorial arrangement in Randomized Complete Block Design (RCBD) in three replications. Recommended P, at the rate of 20 kg ha⁻¹, was applied in the form of Triple Super Phosphate at the planting time.

The results of this study confirmed that day to 50% flowering, day to 90% physiological maturity, nodule fresh and dry weight, plant height, number of primary branches, fresh and dry shoot weight, root fresh weight, number of pods plant⁻¹, number of seeds pod⁻¹, hundred seed weight, and grain yield were influenced by the main effect of varieties and *Bradyrhizobium* inoculation. However, days to 50% emergence and harvest index were affected only by varieties. The nodule number, effective nodule, leaf area, leaf area

index, root dry weight, root length, above ground biomass yield, and straw yield were influenced by the interaction effect of varieties and *Bradyrhizobium* inoculation. Significantly the highest mean value of nodule number, effective nodule, leaf area, LAI, and root dry weight was obtained from White Wonder Trailing variety with inoculated *Bradyrhizobium* strain MBI-cowpea. The maximum above-ground biomass and straw yields were observed from inoculated TVU varieties. Whereas the minimum value of nodule number, effective nodule, leaf area, leaf area index, and pod length was obtained from non-inoculated Bole variety. Moreover, the non-inoculated Keti variety was the least in root dry weight, above ground biomass and straw yield.

Correlation analysis also revealed that grain yield was positively and highly significantly ($P < 0.001$) correlated with nodule number, nodule dry weight, plant height, leaf area, shoot and root dry weight, number of pod plant⁻¹, pod length, number of seed pod⁻¹, above ground biomass yield and straw yield. The partial budget analysis of the studied treatments revealed that the highest net benefit (67171.3 ETB ha⁻¹) with a marginal return of 1386.7% was gained from White Wonder Trailing varieties with inoculated *Bradyrhizobium* strain MBI-cowpea, followed by inoculated TVU varieties with a net benefit of 59322.3 ETB ha⁻¹ and marginal return of 1226.5 % where the White Wondered Trailing variety was superior to production in the study areas.

5.2. Conclusion

Based on the result of this study, it can be concluded that among the four varieties tested, White Wonder Trailing was the superior variety compared to the TVU, Bole, and Keti varieties, as it had better performance on most of the tested parameters. Variety Keti performed least in terms of grain yield.

Hence, the highest (3.5 t ha^{-1}) grain yield with a net benefit of ($67171.3 \text{ ETB ha}^{-1}$) with a marginal rate return of 1386.7% was obtained from the White Wonder Trailing variety with *Bradyrhizobium* strain MBI-cowpea inoculation. Therefore, *Bradyrhizobium* inoculation with White Wonder Trailing variety could be recommended to increase cowpea productivity and economic profit in the study areas, and areas having similar agro-ecologies.

However, this investigation was conducted in a single season, a lower number of varieties, and a single *Bradyrhizobium* strain, it is suggested that the effect of *Bradyrhizobium* inoculation on cowpea varieties should be tested using a large number of cowpea varieties with different types of *Bradyrhizobial* strains and repeating the experiment over multiple seasons will be demanding to come up with a plausible recommendation.

6. REFERENCES

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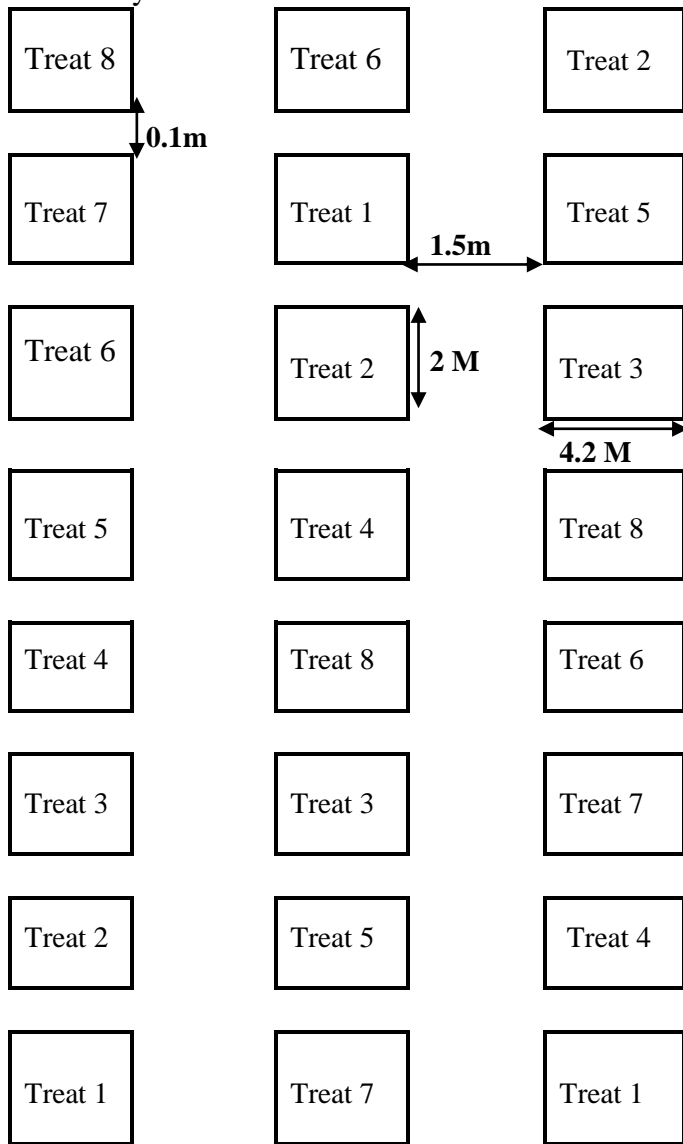
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APPENDICES

Appendix figure 1: Field layout and treatment randomization with block (replication) at each study sites



Block (rep) 1 Block (rep) 2 Block (rep) 3

Total area = $24\text{m} \times 17.1\text{m} = 410.4\text{m}^2$

Planting date = 24/08/2014 E.C Dale field

Planting date = 26/08/2014 E.C Hawassa field

Appendix Table 1: List of treatment and their combinations for cowpea varieties averaged across the two sites during the 2022 main season

Cowpea varieties	<i>Bradyrhizobium</i> inoculation	Treatment combination and code	Replication
1.Bole	Non-inoculated (-)	T1 Bole -	3
2.White Wonder Trailing	Inoculated (+)	T2 Bole +	3
3. Keti		T3 Wwt -	3
4.TVU		T4 Wwt +	3
		T5 Keti -	3
		T6 Keti +	3
		T7 TVU-	3
		T8 TVU+	3
Total			24

Appendix Table 2: Annual total rainfall and maximum and minimum temperature received at the two experimental sites during the 2022/2023 season

Months	Total rainfall (mm)		Max. Temperature (°C)	Min. Temperature	Max. Temperature	Min. Temperature
	Dale	Hawassa	Dale	Dale	Hawassa	Hawassa
Jan	40.2	56.7	28.2	10.4	28.6	13.4
Feb	15.7	15.1	29.7	10.3	30.0	13.3
Mar	78.1	50.3	30.7	11.1	30.3	13.9
Apr	202	61.2	30.2	11.4	29.5	14.7
May	83.32	17.8	30.0	11.4	29.8	15.0
Jun	100.7	109.9	28.7	11.3	26.1	14.6
Jul	91.1	84.5	26.4	10.7	25.2	15.4
Aug	96	83.5	25.2	12.7	25.0	16.0
Sep	179.4	79.8	24.9	12.9	25.5	15.2
Oct	269.1	40	25.4	12.4	27.1	14.8
Nov	63.1	37	26.5	10.9	28.5	12.2
Dec	19	20.3	27.7	10.7	28.5	13.4
AM	103.1	54.7	27.8	11.4	28.8	14.3
GMT	1237.7	656.1				

Source: Ethiopian Meteorology Agency, Hawassa branch (2023), Note. MA = monthly average, GMT = total for the growing months.

Appendix Table 3: Mean square values for phenology of cowpea as influenced by varieties, *Bradyrhizobium* inoculation and their interaction effects averaged across the two sites during the 2021 main season

Source of variation	Df	Day to emergence	Day to 50%flowering	Day to 90% maturity
Replication	2	0.29167	0.656	0.792
Varieties (V)	3	5.00 ^{***}	118.50 ^{***}	263.18 ^{***}
<i>Bradyrhizobium</i> (BR)	1	0.00 ^{ns}	73.50 ^{***}	100.04 ^{***}
V*BR	3	1.00 ^{ns}	0.50 ^{ns}	0.57 ^{ns}
Error	14	0.33929	0.942	1.327
Total	23			
CV (%)		8.13	1.22	1.04

Note: Df= degree of freedom, CV= coefficient of variance ^{ns}=non-significant , * , ** and *** indicates; significant probability level at (P<0.05), (P<0.01), and (P<0.001), respectively

Appendix Table 4: Mean square values for nodulation parameters of cowpea as influenced by varieties, *Bradyrhizobium* inoculation and their interaction effects averaged across the two sites during the 2021 main season

Source of variation	Df	Nodule number	Effective nodule	Nodule fresh weight	Nodule dry weight
Replication	2	3.259	5.448	0.0003	1.92E-04
Varieties (V)	3	722.25 ^{***}	332.75 ^{***}	0.09 ^{***}	0.02 ^{***}
<i>Bradyrhizobium</i> (BR)	1	464.02 ^{***}	408.21 ^{***}	0.03 ^{***}	0.04 ^{***}
V*BR	3	6.19 [*]	55.14 ^{***}	0.0002 ^{ns}	4.167E-06 ^{ns}
Error	14	1.283	3.646	0.0002	9.345E-05
Total	23				
CV (%)		3.16	10.00	3.38	4.20

Note: Df= degree of freedom, CV= coefficient of variance ^{ns}=non-significant , * , ** and *** indicates; significant probability level at (P<0.05), (P<0.01), and (P<0.001), respectively

Appendix Table 5: Mean square values for growth attribute of cowpea as influenced by varieties, *Bradyrhizobium* inoculation and their interaction effects averaged across the two sites during the 2021 main season

Source of variation	Df	Leaf area	Leaf area index	Plant height	Branches number
Replication	2	28738	0.02005	2.21	0.48129
Varieties (V)	3	1095038 ^{***}	0.76 ^{***}	1257.47 ^{***}	5.68 ^{***}
<i>Bradyrhizobium</i> (BR)	1	1119688 ^{***}	0.77 ^{***}	391.23 ^{***}	8.93 ^{***}
V*BR	3	202824 [*]	0.14 [*]	3.37 ^{ns}	0.45 ^{ns}
Error	14	50470	0.04	9.47	0.376
Total	23				
CV (%)		12.79	12.83	4.15	7.82

Note: - Df= degree of freedom, CV= coefficient of variance ^{ns}=non-significant ^{*}, ^{**} and ^{***} indicates; significant probability level at (P<0.05), (P<0.01), and (P<0.001), respectively.

Appendix Table 6: Mean square values for growth attribute of cowpea as influenced by varieties, *Bradyrhizobium* inoculation and their interaction effects averaged across the two sites during the 2021 main season

Source of variation	Df	Shoot fresh weight	Shoot dry weight	Root fresh weight	Root dry weight	Root length
Replication	2	0.67	0.148	1.767	0.0361	0.895
Varieties (V)	3	1680.79 ^{***}	255.65 ^{***}	32.59 ^{***}	4.50 ^{***}	153.38 ^{***}
<i>Bradyrhizobium</i> (BR)	1	743.60 ^{***}	238.52 ^{***}	167.48 ^{***}	7.13 ^{***}	332.12 ^{***}
V*BR	3	5.02 ^{ns}	0.95 ^{ns}	2.23 ^{ns}	0.44 ^{***}	13.59 ^{**}
Error	14	12.0	0.69	1.85	0.042	1.756
Total	23					
CV (%)		1.4	2.15	5.15	5.15	5.35

Note: - CV= coefficient of variance ^{ns}=non-significant ^{*}, ^{**} and ^{***} indicates; significant probability level at (P<0.05), (P<0.01), and (P<0.001), respectively.

Appendix Table 7: Mean square values for yield and yield components of cowpea as affected by varieties, *Bradyrhizobium* inoculation and their interaction effect averaged across the two sites during the 2021 main season.

Source of variation	Df	Pod length	Number of pod plant ⁻¹	Number of seed pod ⁻¹	Hundred seed weight
Replication	2	0.251	15.7	0.275	0.993
Varieties (V)	3	3.71 ^{***}	415.36 ^{***}	12.89 ^{***}	20.06 ^{***}
<i>Bradyrhizobium</i> (BR)	1	8.05 ^{***}	385.84 ^{***}	24.91 ^{***}	17.15 ^{***}
V*BR	3	1.15 ^{***}	9.62 ^{ns}	0.26 ^{ns}	0.36 ^{ns}
Error	14	0.103	20.70	0.33	0.275
Total	23				
CV (%)		2.5	10.55	4.68	3.45

Note: - CV= coefficient of variance ^{ns}=non-significant *, ** and *** indicates; significant probability level at (P<0.05), (P<0.01), and (P<0.001), respectively.

Appendix Table 8: Mean square values for yield and yield components of cowpea as affected by varieties, *Bradyrhizobium* inoculation and their interaction effect averaged across the two sites during the 2021 main season.

Source of variation	Df	Above ground biomass	Grain yield	Straw yield	Harvest index
Replication	2	0.0023	0.006	0.001	7.526
Varieties (V)	3	9.11 ^{***}	1.66 ^{***}	3.90 ^{***}	110.96 ^{***}
<i>Bradyrhizobium</i> (BR)	1	12.64 ^{***}	3.83 ^{***}	2.57 ^{***}	30.85 ^{ns}
V*BR	3	0.10 ^{**}	0.08 ^{ns}	0.15 [*]	23.17 ^{ns}
Error	14	0.01	0.02	0.04	11.24
Total	23				
CV (%)		2.09	6.49	6.66	7.44

Note: - CV= coefficient of variance ^{ns}=non-significant *, ** and *** indicates; significant probability level at (P<0.05), (P<0.01), and (P<0.001), respectively.

Appendix Table 9: Pearson Correlation coefficient(r) among parameters

	DF	DPM	NN	NDW	PH	NPB	LA	SDW	RDW	NPP	PL	NSP	AGB	GY	SY	HI
DF	1															
DPM	0.95 ^{***}	1														
NN	-0.13 ^{ns}	-0.04 ^{ns}	1													
NDW	0.09 ^{ns}	0.14 ^{ns}	0.93 ^{***}	1												
PH	0.29 ^{ns}	0.41 [*]	0.82 ^{***}	0.80 ^{***}	1											
NPB	0.73 ^{***}	0.68 ^{***}	0.03 ^{ns}	0.24 ^{ns}	0.33 ^{ns}	1										
LA	-0.05 ^{ns}	0.03 ^{ns}	0.85 ^{***}	0.84 ^{***}	0.75 ^{***}	0.18 ^{ns}	1									
SDW	0.49 [*]	0.57 ^{**}	0.77 ^{***}	0.82 ^{***}	0.94 ^{***}	0.46 [*]	0.68 ^{***}	1								
RDW	0.82 ^{***}	0.84 ^{***}	0.23 ^{ns}	0.43 [*]	0.56 ^{**}	0.80 ^{***}	0.37 ^{ns}	0.65 ^{***}	1							
NPP	0.57 ^{**}	0.66 ^{***}	0.56 ^{**}	0.63 ^{***}	0.82 ^{***}	0.62 ^{**}	0.54 ^{**}	0.87 ^{***}	0.75 ^{***}	1						
PL	-0.16 ^{ns}	-0.12 ^{ns}	0.81 ^{***}	0.84 ^{***}	0.62 ^{**}	0.07 ^{ns}	0.80 ^{***}	0.58 ^{**}	0.21 ^{ns}	0.44 [*]	1					
NSP	-0.13 ^{ns}	-0.13 ^{ns}	0.89 ^{***}	0.92 ^{***}	0.64 ^{***}	0.08 ^{ns}	0.84 ^{***}	0.63 ^{**}	0.23 ^{ns}	0.43 [*]	0.82 ^{***}	1				
AGB	0.65 ^{***}	0.71 ^{***}	0.62 ^{**}	0.77 ^{***}	0.84 ^{***}	0.49 [*]	0.56 ^{**}	0.95 ^{***}	0.72 ^{***}	0.84 ^{***}	0.49 [*]	0.51 [*]	1			
GY	0.50 [*]	0.56 ^{**}	0.73 ^{***}	0.85 ^{***}	0.87 ^{***}	0.54 ^{**}	0.75 ^{***}	0.92 ^{***}	0.79 ^{***}	0.82 ^{***}	0.64 ^{***}	0.68 ^{***}	0.89 ^{***}	1		
SY	0.66 ^{***}	0.72 ^{***}	0.43 [*]	0.48 [*]	0.68 ^{***}	0.37 ^{ns}	0.32 ^{ns}	0.82 ^{***}	0.56 ^{**}	0.72 ^{***}	0.30 ^{ns}	0.29 ^{ns}	0.93 ^{***}	0.66 ^{***}	1	
HI	-0.27 ^{ns}	0.26 ^{ns}	0.33 ^{ns}	0.39 ^{ns}	0.16 ^{ns}	0.12 ^{ns}	0.47 [*]	0.04 ^{ns}	0.18 ^{ns}	0.04 ^{ns}	0.34 ^{ns}	0.41 [*]	0.14 ^{ns}	0.32 ^{ns}	-0.48 [*]	1

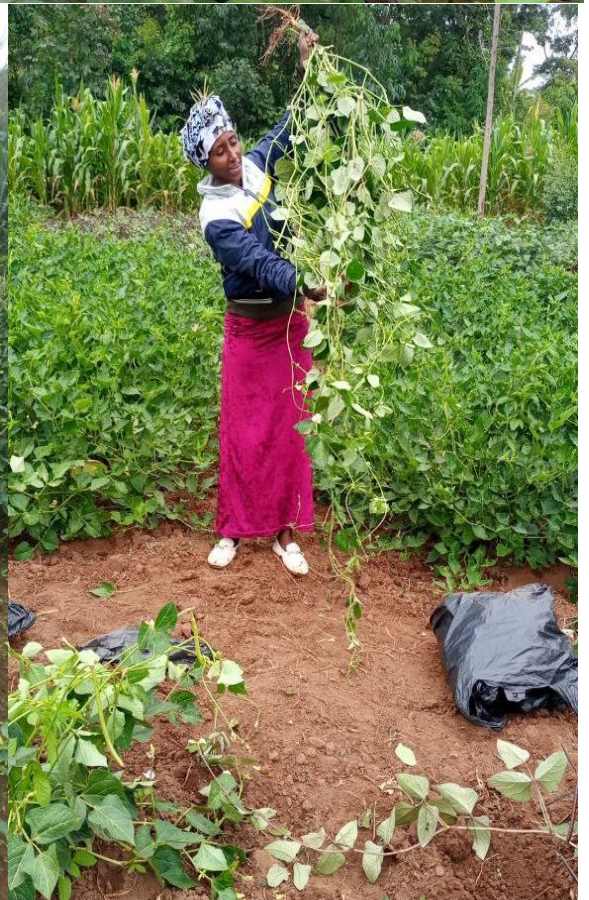
Whereas; *DF*= day to flowering, *DPM*= day to physiological maturity, *NN*=nodule number, *NDW*=nodule dry weight, *PH*= plant height, *NPB*=number of primary branch, *LA*=leaf area, *SDW*= shoot dry weight, *RDW*=root dry weight, *NPP*=number of pod per plant, *PL*=pod length, *NSP*=number of seed per pod, *AGB*= above ground biomass, *GY*=grain yield, *SY*=straw yield, *HI*=harvest index. A sign ^{***}Very highly significant, ^{**}highly significant, ^{*}highly significant, ^{ns}=non-significant at 0.1%, 1%, 0.5% probability level, respectively,



Appendix figure 2: Cowpea treatments in the experimental field



Appendix figure 3: Physiological maturity of cowpea



Appendix figure 4: Measuring of plant height and collected the distractive data



Appendix figure 5: Measuring leaf area and shoot fresh weight of cowpea



Appendix figure 6: Measurement of Nodulation and root parameters of cowpea



Appendix figure 7: Pod length and number of seed pod⁻¹ of cowpea



Appendix figure 8: Threshing and cleaning of cowpea



Appendix figure 9: Grain yield, hundred seed weight and seed moisture content measurement and test, respectively of cowpea

BIOGRAPHICAL SKETCH

The author, **Lemlem Yohannes** was born on August 21, 1996, at Debre Mear, Tigray Regional state, Northern Ethiopia from her father Mr. Yohannes Gezahegn, and her mother Mrs. Medhin Gebremariam. She attended her Primary school in Debre Mear and Secondary and preparatory school in Wukro, Megabit-30 secondary and Wukro preparatory schools, respectively. After completing the university entrance exam, she joined Adigrat University in 2015 to pursue her first Degree and graduated with BSc Degree in Plant Science in July 2018. After graduation, she was employed as a graduate assistant at Adigrat University for a year and then she joined Hawassa University Graduate School of Plant Science in 2020 for her Master's degree in Agronomy specialization.