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THE IMPACT OF INOCULATION AND SEED DRESSING ON DOUBLED-UP LEGUME TECHNOLOGY

by

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Submitted in partial fulfilment of the requirements for the degree

MSc (Agric) Agronomy

In the Department of Plant and Soil Sciences

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DECLARATION

I, Adon Phiri hereby declare and confirm that this Master's Research is the product of my own effort, except the information that has been duly acknowledged. I also admit that no plagiarism was committed in compiling this dissertation. Further, I confirm that this work has not been submitted elsewhere.

Signed: --------------------------------

ADON PHIRI
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LIST OF ABBREVIATIONS AND ACRONYMS

PP : Pigeon pea

GN : Groundnut

ARC : Agricultural Research Council

RCBD: Randomised Complete Block Design

LER : Land Equivalent Ratio

°C : Degrees Celsius

% : Percentage

ha : Hectare

kg : Kilogram

ABSTRACT

THE IMPACT OF INOCULATION AND SEED DRESSING ON DOUBLED-UP LEGUME TECHNOLOGY

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Supervisor: Dr. D. Marais

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Degree: Master of Science (Agric) Agronomy

Rhizobium inoculation and seed dressing are some of the agronomic practices that affect crop productivity in doubled-up legume technology. Doubled-up legume technology is the practice of intercropping two compatible grain legume crops that have different root and shoot growth systems in the same field in order to maximise land and crop productivity. A field study with groundnut and pigeon pea as test crops was conducted at Innovation Africa at the University of Pretoria (IA@UP), South Africa during the 2020/2021 crop growing season to investigate the effect of inoculation and seed dressing with a fungicide and pesticide on doubled-up legume technology. The study involved twelve treatment combinations namely; Untreated sole pigeon pea (PP), Untreated sole groundnut (GN), Untreated PP-GN intercrop, Inoculated sole PP, Inoculated sole GN, Inoculated PP-GN intercrop, Seed dressed sole PP, Seed dressed sole GN, Seed dressed PP-GN intercrop, Inoculated + Seed dressed sole PP, Inoculated + seed dressed sole GN, Inoculated + Seed dressed PP-GN intercrop. Each treatment was replicated three times using a randomized complete block design (RCBD). Parameters assessed included soil analysis, germination and survival %, *Rhizobium* and plant growth parameters, and yield and land equivalent ratios. Grain yield for both groundnut and pigeon pea indicated that treatments had a significant ($P < 0.05$) effect on grain yield. Furthermore, some sole treatments produced higher grain yield for both groundnut and pigeon pea than their respective intercropped treatments. The sole treatments for both groundnut and pigeon pea receiving both inoculation and seed dressing produced outstanding grain yields (2450.7 kg ha^{-1} and 2340.0 kg ha^{-1} respectively), as compared to the other sole crop treatments such as 1550.0 kg ha^{-1}, 1959.0 kg ha^{-1} and 2264.7 kg ha^{-1} for groundnut and 931.0 kg ha^{-1}, 1442.0 kg ha^{-1} and 1254.0 kg ha^{-1} for pigeon pea. For intercropped treatments, the inoculation and seed dressing treatments outperformed other treatments in terms of survival %, nodule efficiency and grain yield. Based on productivity, except for the untreated intercrop, all other intercropped treatments were more productive than their respective sole treatments. Groundnut–pigeon pea intercropping (doubled-up) with both *Rhizobium* inoculation and seed dressing was the best treatment since it resulted into higher land equivalent ratio (1.7054) and grain yields for both crops than any of the other intercropped treatments.

Key words: groundnut, land equivalent ratio, pigeon pea and rhizobium**.**

CHAPTER 1

GENERAL INTRODUCTION

1.1 INTRODUCTION

Doubled-up legume technology is an agronomic practice of intercropping two legume crops with different growth habits (Chiwoko et al. 2015). The term intercropping refers to the practice of growing two or more crops simultaneously in the same field. It intensifies the crop in both time and space dimensions as farmers manage more than one crop at a time in the same field (Mloza - Banda 1994). Intercropping in developing countries such as Malawi is being practiced by smallholder farmers in different ways; mixed intercropping, row intercropping and strip cropping. Mixed intercropping involves growing two or more crops simultaneously with no distinct row arrangement. Row intercropping is the system of growing two or more crops simultaneously where one or two crops are planted in rows, while in strip intercropping, two or more crops are grown simultaneously in different strips, wide enough to permit independent cultivation but narrow enough for the crops to interact agronomically.

Due to the ever-growing population of people in most of the developing countries, smallholder farmers lack enough land for agriculture. It is for this reason that smallholder farmers should start adopting farming systems that increase land productivity. Doubled-up legume technology is one of them. It involves growing two legume crops of different growing habits in the same field. The benefits of doubled-up legume technology are ‘double’ legume grain and ‘double’ soil fertility inputs that are realised from residues of the two legumes (Chiwoko et al. 2015). An intercrop of pigeon pea and groundnut is an example of a doubled-up legume technology. The groundnut-pigeon pea intercropping provides the best combination of crops due to their different growth habits and maturity dates. Pigeon pea growth starts out slow, followed by rapid growth and pod production at the stage when groundnut has already matured and been harvested. Although intercropping requires skills and a wide knowledge on different crops, this cropping system plays a big role in improving the living standards of smallholder farmers as they obtain multiple yields from different crops. Farmers also rely on the other crop when one crop fails to produce enough yield due to unfavourable conditions such as drought. Where legumes are involved in an intercropping system, they fix nitrogen into the soil while other nutrients can be introduced into soil when legume residues from groundnuts and pigeon pea are incorporated into the soil (Rao

1990). Smallholder farmers in Malawi intercrop crops from different families such as groundnuts and maize or crops of the same family such as legumes (groundnuts and pigeon pea).

The effectiveness of the doubled-up legume technology relies on inoculation and seed dressing. Inoculation is the practice of adding effective type of bacteria to the host plant seed before planting. The main purpose of inoculation is to ensure that there is enough and correct type of bacteria in the soil so as to have a successful legume-bacteria relationship in the soil. Effective inoculation results into good nodulation which in turn ensures good biological nitrogen fixation. On the other hand, seed dressing is a seed treatment whereby plant seeds are treated (coated) with a chemical so as to protect seed and seedlings from either diseases or pests or both. A common example of a seed dressing chemical is Monceren GT 390 FS. Monceren GT 390 FS (Imidacloprid + Pencycuron + Thiram) is a concentrated suspension for seed treatment which acts by contact and ingestion through systemic activity. It is applied to seeds at a rate of 1.5 L of Monceren GT 390 FS 100 kg^{-1} of seed. It works as both pesticide and fungicide. It should also be noted that both inoculation and seed dressing can be done simultaneously.

1.2 PROBLEM STATEMENT AND JUSTIFICATION

Doubled-up legume technology is a new farming practice that involves intercropping two legume crops with different growth habits. Among other advantages, it encourages land productivity since farmers grow more crops on limited land, hence diversifying income sources on the farm. In addition, the doubled-up legume technology helps to double soil fertility benefits as both legume crops add fertility to the soil through biological nitrogen fixation.

When groundnut is intercropped with pigeon pea, there is less competition for soil water, nutrients and light since these crops have different growth habits. They utilise soil water and nutrients at different soil depths and times in the same field and growing season. Pigeon pea has a slow initial growth, and only begins to grow rapidly when groundnut is approaching maturity. Groundnut matures first and pigeon pea continues to grow as a sole crop and is harvested subsequently. In addition, pigeon pea has a deeper root system than groundnut. The two crops are therefore compatible in the same field due to limited competition between them over soil water, soil nutrients and space for their growth. In this way, we ‘double’ the crop yields and ‘double’ soil fertility benefits as both legume crops add fertility to the soil through biological nitrogen fixation.

There are a number of factors that negatively affect the positive performance of doubled-up legume technology that involve the use of groundnut and pigeon pea. Some of them are notorious soil borne pests and diseases that attack the plants in their early stages of growth. The second factor that limits effective production of doubled-up legume technology is poor mutual relationship between rhizobia and host legume plant in the soil. Some research works involving doubled-up legume technology have been done in southern Africa to investigate the factors that can affect performance of the doubled-up legume technology. Njira et al. (2012) evaluated biological nitrogen fixation in sole and doubled-up legume cropping systems on the sandy soils of Kasungu in central Malawi. The authors found that pigeon pea-groundnut intercrop proved to be the most beneficial in terms of nitrogen fixation, while pigeon pea-soybean intercrop lowered the amount of nitrogen fixed by the pigeon pea. Mhango (2011) reported that groundnut-pigeon pea intercropping reduced vegetative growth of pigeon pea under limited soil moisture. So far, limited research work has been done to investigate productivity of doubled-up legume technology. Therefore, the research project assessed how inoculation and seed dressing could improve productivity of doubled-up legume technology.

1.3 RESEARCH HYPOTHESES

(a) Yield from sole cropped treatments would be higher than the respective intercropped treatments due to limited competition in pure stands.

(b) Pigeon pea and groundnut yield would be higher in treatments with seed dressing combined with *Rhizobia* inoculation due to initial plant immunisation against early pests and diseases through seed dressing and effective nodulation through inoculation.

(c) Land equivalent ratio (LER) for the treated (inoculated, seed dressed and inoculated plus seed dressed) treatments would be more productive than untreated ones.

1.4 OBJECTIVES OF THE STUDY

1.4.1 Main objectives

- To determine the performance of groundnut-pigeon pea intercropping as affected by inoculation and seed dressing.

1.4.2 Specific objectives

- To evaluate seed germination and seedling survival rate for seed dressed and the control
- To evaluate nodule effectiveness and leaf chlorophyll content for the inoculated and the control
- To estimate disease severity for the treated (seed dressed) and the control
- To estimate pest infestation in seed dressed and the control
- To evaluate the effect of treatments on yield and productivity (LER)

CHAPTER 2

LITERATURE REVIEW

2.1 INTERCROPPING

In developing countries, such as Malawi, groundnuts are commonly grown in intercropping systems, especially by smallholder farmers who traditionally use combinations involving up to 5 - 6 crops. Although intercropping leads to reduced yields due to interspecific competition among crop plants, intercropping of legumes with different growth habits (e.g. groundnuts – pigeon pea intercrop) has a higher potential to enhance soil nitrogen due to limited competition between them for space, soil water, nutrients and sunlight (Rao et al.1980). These legumes fix nitrogen into the soil through biological nitrogen fixation when their root systems get colonised by *Rhizobium* bacteria which cause the roots to form nodules to accommodate the bacteria hence improving the soil fertility status, while the soil structure also improves due to the presence of the organic matter from their leaves. Improvement in the soil fertility of crop fields helps crops to grow healthy due to the availability of essential plant nutrients. Farmers are therefore assured of bumper yields from such fields, hence they are food secured. Intercropping is a major method of crop production in tropical Africa, subtropical Asia, and central south America (Allen et al. 1983). Planting geometries directly affect crop yields in intercropping systems compared with pure cropping in which one species is planted. Intercropping involves planting two or more crops in the same field and season. Intercropping can be illustrated in different scenarios. For example; intercropping may involve annual crops with annual crops, annual crops with perennial crops and perennial crops with perennial crops (Mousavi et al. 2011). Generally, intercropping may be grouped into a number of categories. Row – intercropping: growing two or more crops simultaneously where one or more crops are planted in regular rows, and another crop or crops may be grown simultaneously in rows or randomly with the first crop. Mixed – intercropping: growing two or more crops simultaneously with no distinct row arrangement. This type of intercropping may be ideal for grass – legume intercropping in pasture production. Strip – intercropping: growing two or more crops simultaneously in different strips wide enough to permit independent cultivation but narrow enough for the crops to interact agronomically. Relay – intercropping: growing two or more crops simultaneously during part of the life cycle of each. A second crop is planted after the first crop has reached its reproductive stage but before it is ready for harvest. Many agricultural reports support intercropping over pure cropping. The advantages of intercropping systems over pure cropping include; improved soil fertility and increased nitrogen, reduced pest, disease and weed damage and increased crop production due to multiple harvests (Mousavi et al. 2011).

2.1.1 Principles of intercropping

Successful intercropping requires farmers to follow certain principles (Stomph et al. 2020). For example; crops of the same family should not follow one another in successive growing seasons to control pests and diseases by breaking life cycles of both pests and diseases. Growth habits of the crops should also be considered. Tall crop varieties should be intercropped with short crop varieties in order to avoid shading effect among crops. Pigeon pea intercropped with groundnut is a good example. Maturity dates of crops should also be considered. On this point, crops with different maturity days should be intercropped. Number of seeds to be sown or number of seedlings to be transplanted in a field, is also an important principle in intercropping as it determines crop density. High crop plant density intensifies interspecific competition among plants in a field. Time of planting component crops in an intercrop is also a vital principle in intercropping systems. Root systems of plants affect intercropping, as such; they also need to be considered in intercropping systems. Crops of the same root systems should not follow one another in successive growing seasons. Plants with tap root systems should be followed by plants with fibrous root systems in successive growing seasons. This ensures maximum use of soil nutrients, water and other resources from different soil depths.

2.1.2 Role of intercropping

Intercropping plays a number of roles in crop production. Through intercropping systems, farmers obtain multiple yields from different crops (crop diversification) and hence end up having enough yield for both food and sale to obtain income for buying other basic needs. Farmers also rely on the other crop when one crop fails to produce enough yields due to unfavourable conditions such as drought. Hence, farmers become food secure since they are assured of having enough food at all times. Intercropping may also be of importance when legume crops are included in intercropping systems. They fix nitrogen in soil through biological nitrogen fixation (BNF) (Rao et al. 1987). Leguminous plants such as groundnut and pigeon pea have the ability to fix nitrogen biologically from the atmosphere. This fixed nitrogen cannot only be important to the legumes themselves but also to any intercropped or subsequent crops, hence reducing the necessity of applying N fertilisers in fields. Chemical synthesis of alternative N fertilisers demands fossil fuel, while *Rhizobium* uses solar energy sourced from plant photosynthesis (Phillips 1980).

2.1.3 Doubled-up legume technology

Doubled-up legume technology is a form of intercropping in which legumes are grown in association with other legumes (Njira 2016). These legumes are always compatible in terms of their growth habits in order to minimize intraspecific competition between them for space, soil moisture, soil nutrients, etc. The system is best illustrated by a pigeon pea-groundnut intercropping system. The crops are planted simultaneously but the pigeon pea component has a much slower initial growth, resulting in little intraspecific competition. On the other hand, groundnut matures and is harvested 4 months after planting, leaving pigeon pea in the field to stand as a sole crop that matures 2 – 3 months later. Doubled-up legume technology involving groundnut and pigeon pea can result into 20 – 50% more biological N_2-fixation, compared with sole legume cropping (Chikowo et al. 2020).

2.1.4 Rhizobium inoculation and seed dressing

Rhizobium inoculation in legume crops is the practice of coating legume seeds before planting in order to efficiently and conveniently introduce effective rhizobia to soil and subsequently the rhizosphere of legumes (Deaker et al. 2004). Inoculation of legumes with rhizobia is one of the success stories of world agriculture. It is important to note that nitrogen fixation by legumes does not just happen. Effective biological nitrogen fixation occurs in the presence of compatible and effective rhizobia in the soil in which the legume is growing before root nodulation and nitrogen fixation can occur (Drew et al. 2012). On the other hand, seed dressing is the practice of coating crop seeds with fungicides or pesticides in order to protect crop seeds or seedlings from being destroyed by early fungal diseases and / or pests. A good example of such a seed dressing chemical is Monceren GT 390 FS which protects the crop against both diseases and insects. Seed dressed seeds improve germination as well as seedling vigour compared with untreated seeds (Ansaah 2018). Therefore, combined use of inoculation and seed dressing brings about double positive effects in doubled-up legume technology.

2.2 PIGEON PEA

Pigeon pea (*Cajanus cajan*) (Figure 2.1) is one of the most common legume crops grown in the world. It is an erect legume shrub. Pigeon pea is commonly cultivated in India which accounts for 75% of the world's production. Pigeon pea has a fast growing and deep tap root system that enables it to be used in areas with moisture deficit problems, hence it is also called the crop of dry land agriculture. The root system also helps to control soil erosion. As a legume, it is capable of biological nitrogen fixation and it does well in most soil types, as long as the soil is not saline, shallow and waterlogged. Farmers incorporate pigeon pea in several cropping systems such as mixed cropping, intercropping or as a perennial crop (Chauhan 1990). The physiological characteristics of pigeon pea makes it possible to fit well with crops such as groundnuts, cotton and soybean. Limited competition for soil water and soil nutrients has been found between pigeon pea and these companion crops (Sharma 1980). It facilitates biological nitrogen fixation through nodules and microbes that live in the rhizosphere. The plants use the fixed nitrogen to grow and when its biomass is incorporated into the soil, it releases nutrients through decomposition that can be used by other crops in the following growing season. Incorporation of the pigeon pea residues into the soil is the best-bet for smallholder farmers to increase their yields of follow-up crops (Rao 1990).

Some specific conditions that favour pigeon pea production are: a soil pH of between 5.0 to 7.0, rainfall between 600 to 1000 mm year^{-1} and temperatures ranging from 18 to 38^{o}C. Pigeon pea has a very slow initial vegetative growth, with the seedlings emerging 2 to 3 weeks after sowing. Physiological growth starts to pick up when the plants are 2 to 3 months old. Flowering takes place within 56 to 210 days after sowing, while the time to maturity ranges from 95 to 256 days, depending on variety and environmental conditions.

Figure 2. 1: Pigeon pea plants growing at IA@UP in an open field (A Phiri, 2020)

Nitrogen contribution from fallen leaves of pigeon peas in Malawi has generally ranged from 30 to 90 kg ha^{-1} (Kumwenda et al. 1996). However, Kumwenda et al. (1996) reported that due to the high lignin (16%) and low nitrogen content (1.8%), pigeon pea leaves may immobilise nitrogen for up to two months.

Just as any other legume crop, pigeon peas have a wide range of pests and diseases which have an adverse impact on productivity. They also contribute to poor quality seed. In addition, pests and diseases of pigeon pea reduce plant stand (Sharma et al. 2010). Pigeon pea pests and diseases can be controlled by using cultivars that are resistant to pests and diseases, crop rotation, weed removal and intercropping with cereals. Some of the pests and diseases that attack pigeon pea are described in the following section.

2.2.1 Common pests of pigeon peas

(a) Aphids (*Acyrthosiphum pisum*)

These are small soft bodied insects that are found on the underside of leaves and/or stems of plants. They appear in different colours such as green, yellow, red, pink, brown or black, depending on the species and host plant. Heavy aphid infestation results into yellow leaves or distorted, necrotic spots on leaves and stunted shoots. The sticky sugary substances that are produced by aphids contribute to the growth of sooty mould on the plants. If the infestation is limited, the infested plants should be pruned to control the aphids. Reflective mulches such as silver coloured plastic paper help to deter aphids. Strong jet water application can also help to knock down the aphids from plant leaves or stems (Sharma et al. 2010). Chemically, aphids can be controlled by Monceren GT 390 FS due to the presence of Imidacloprid (active ingredient).

(b) Armyworms (*Spodoptera frugiperda*)

Armyworms cause singular or closely grouped circular to irregular shaped holes on leaves. Overfeeding of young larvae on leaves results into skeletonised leaves. Their egg clusters are covered in a whitish scale which provides the cluster a cottony or fuzzy appearance. Armyworms can be controlled biologically by using natural enemies which feed on the larvae (DAFF 2010). Chemically, army worms can be controlled by spraying Alpha-cypermethrin and Chlorpyrifos on the infested crop at the rate of 20 to 40 ml per 20 litres of water.

(c) Leaf miners (*Chromatomyia horticola*)

These are small black and yellow flies that lay their eggs in the mesophyll of leaves. Larvae hatch and feed on the interior part of leaves. Leaf miners cause thin, white, winding trails on leaves and leaves drop from plants prematurely. Mature larvae drop from the leaves into the soil to pupate. The whole lifecycle takes 2 weeks in warm weather. Insecticides such as Dynamec and Abalone 18 EC can be used to control leaf miners at the rate of 10 ml per 20 litres of water (Sharma 1980).

2.2.2 Common diseases of pigeon peas

(a) Alternaria blight (*Alternaria alternata*

This is a fungal disease that favours high humidity and warm temperatures. It commonly attacks crop plants that are grown in soils that are nitrogen and potassium deficient. It is characterized by small, irregular brown lesions on leaves which expand and turn grey-brown or dark brown with concentric zones. Older areas of lesions may dry out and drop from leaves causing a shot hole pattern. This disease can be controlled by using fungicides. Naturally the disease can be prevented by planting crops in fertile soils (DAFF 2010). Chemically, the disease can be controlled by seed dressing with Monceren GT 390 FS.

(b) Anthracnose (*Glomerella cingulate*)

Anthracnose is a fungal disease that is transmitted through infected seeds. It is characterised by small, dark brown to black lesions on stems that turn sunken and brown with purple to red margins. Stems may be weakened and break due to cankers. Anthracnose has a drying effect on pods. It also results in reddish brown spots on the pods, leading to circular sunken spots with a rust coloured margin. The use of certified disease-free seed and resistant varieties can help to control the disease. Watering plants at their base also helps to control this disease, hence sprinkler irrigation should be avoided. It is also important to keep the field clean and free from crop debris as they provide a host for disease survival and spreading (DAFF 2010).

(c) Cercospora leaf spot (*Cercospora canescens*)

Cercospora leaf spot is a fungal disease. Its characteristics are small light brown lesions on the upper leaves, angular brown spots on leaves, leaf death and lesions on stems and petioles. The leaf spots are triangular in outline and are raised above the surface of the leaf. The infected leaves may later start dying and in severe cases they defoliate. *Cercospora* leaf spots can be controlled by spraying fungicides (Sharma 1980).

(d) Fusarium Wilt (*Fusarium oxysporum*)

Fusarium Wilt is a fungal disease that can survive in soil for a number of years. The infected plants become yellow in colour, followed by dropping leaves and finally the whole plant dries up.

Symptoms of this disease may only appear on one side of the plant. Crop rotation and good watering regimes help to control the disease. Soil drenching with fungicides such as Benomyl and Chlorothalonil can also help to control the disease (DAFF 2010).

2.3 GROUNDNUTS

The cultivated groundnut (*Arachis hypogea*) (Figure 2.2) is a native of South America and the crop is now grown throughout the tropical and warm temperate regions of the world. Although groundnut is predominantly a crop of the tropics, the approximate limits of present commercial production are between 40^o N and 40^o S. Groundnuts favour temperatures which are high and frost-free for about 160 days. Otherwise cool environmental conditions are not ideal for groundnut production since they don't reach their optimum maturity for a marketable yield. This necessitates that the groundnuts should only be planted when the minimum temperature stabilises above 18^{o}C as this also improves germination % and vegetative plant growth. Groundnuts are therefore not grown in higher altitudes where climates are cool. Groundnut production is also affected by soil moisture content. Drier soils contribute to poor seed germination, while moist warm soils promote good seed germination. Rainfall of 500 to 700 mm annum^{-1} results into high groundnut yields. Clay soils are not ideal for groundnut production as they limit pegging. Soils should be well drained, fertile sandy to sandy loam soil with a pH range of 5.5 to 7.0. Groundnuts, just like any other legume, facilitate biological nitrogen fixation through microbes (DAFF 2010).

Figure 2. 2: Groundnut plants growing at IA@UP in an open field (A Phiri, 2020)

Groundnuts can fix 60 to 70% of their nitrogen requirement as long as the conditions are ideal for biological nitrogen fixation. The mutual relationship that exists between the rhizobia and the host legume plants determines the possibility and effectiveness of the biological nitrogen fixation that may exist. Inoculation therefore plays a big role in providing a good symbiotic relationship between *Rhizobium* bacteria and the legume crop. There are several constraints to increased groundnut production worldwide. Some of the factors responsible for low yield in groundnut production are use of the genetically poor yielding cultivars and pest and disease attacks. Yields from cultivars grown by farmers are generally low (500 -700 kg ha^{-1}), compared to over 3000 kg ha^{-1} at research stations. These low farmer yields are usually due to drought, unreliable rainfall, poor timing of cultural practices management, diseases and pest attacks (Sakala 1991).

2.3.1 Common pests and diseases of groundnuts

Just as any other crop, groundnut has a number of pests and diseases that affect its growth and potential yield. Some of the common pests and diseases that attack groundnut are mentioned in the following sections:

2.3.1.1 Pests of groundnuts

Groundnuts have a number of pests including aphids (*Acyrthosiphum pisum*), leaf eaters (*Spodoptera exempta*), leaf miners (*Chromatomyia horticola*) etc., which were already discussed earlier. Leaf miners rank as the most serious pest for groundnuts. This notorious pest can cause yield loss of more than 50%. Some of the control measures of the pest is by intercropping legumes with sorghum and chemical control.

2.3.1.2 Diseases of groundnuts

(a) Early Leaf Spot disease (*Mycosphaerella arachidis*) and Late Leaf Spot disease (*Mycosphaerella berkeleyi*)

These are severe fungal diseases for groundnut worldwide. Their major problems are reported in Burkina Faso, Malawi, Mali, Nigeria and Sudan. They are characterised by spots on leaves, stems and petioles, resulting in leaf fall and high pod yield losses. Some of the management practices for these diseases include the use of resistant and early yielding varieties, cultural control such as crop rotation, removal of volunteer plants and weeds, isolating crops from those that are infected and elimination of plant debris after harvest. Fungicides such as chlorothalonil are used to control leaf spot diseases (ASHC 2015).

(b) Stem and pod rot disease (*Sclerotium rolfsii*)

This is a fungal disease of groundnut and is also called southern blight. It occurs whenever groundnuts are grown. Losses due to this disease are not well recorded in Africa. It is a soil-borne disease, attacking groundnut stems just below soil level and causes leaves to yellow and wilt. Some agronomic measures such as crop rotation, early removal of affected plants, careful weeding and use of mulch are used to manage this disease (ASHC 2015).

(c) Groundnut rosette disease (*Groundnut rosette*)

This is a viral groundnut disease, which is common in sub-Saharan Africa. This disease causes stunted plant growth and severe loss of pod yield. The disease is spread through aphids. Using tolerant varieties play a big role in management of this disease. On the other hand, cultural measures such as removing volunteer and diseased groundnut plants and weeds, early planting, intercropping with other legumes and cereals and crop rotation can also help to control the disease (ASHC 2015).

(d) Groundnut rust (*Puccinia arachidis*)

This is a relatively new disease to Africa. It affects leaves, stems and pegs and produces a number of small red spots or pustules containing masses of spores. It is characterised by yellow foliage that dries up and plants die early. Early and late leaf spots create an infection point for the groundnut rust disease (ASHC 2015). Some important measures to consider when managing this disease are as follows; removing volunteer groundnut plants before planting, planting resistant varieties, crop rotation with cereals and possibly fungicides can be used.

CHAPTER 3

GENERAL MATERIALS AND METHODS

3.1 INTRODUCTION

The success of doubled-up legume technology relies on a number of agronomic practices. Some of the agronomic practices that may have great positive impact on doubled-up legume technology are inoculation and seed dressing. Inoculation is the process of adding effective bacteria to the host plant seeds prior to planting, while seed dressing is the practice of coating plant seeds with chemicals prior to planting so as to protect the seeds or / and seedlings from diseases and insect pests. The doubled-up legume technology trial that involved intercropping groundnut with pigeon pea was implemented during the 2020 – 2021 crop growing season in order to assess the impact of inoculation and seed dressing on doubled-up legume technology. The site description and procedures followed for the field trial are presented in this chapter.

3.2 EXPERIMENTAL SITE

The experiment was carried out at Innovation Africa at the University of Pretoria (IA@UP), South Africa from September 2020 to June 2021. The area has a unimodal summer rainfall pattern (October to April) and is located at an altitude of 1372 mm above sea level. The soil is a well-drained sandy clay loam.

3.3 CROP VARIETY DESCRIPTION AND PLANTING METHODS

During the experiment, CG 7 and ICEAP 00040 groundnut and pigeon pea varieties respectively were used. CG 7 is a bunch type of groundnut variety that is tolerant to drought. Maturity occurs at 130 – 150 days, with a potential yield of 2 500 kg ha^{-1} in a sole stand (Mhango 2011). ICEAP 00040 is a medium to long duration pigeon pea variety that matures between 190 to 240 days. It flowers between 140 to 180 days. ICEAP 00040 has a potential yield of 2 500 kg ha^{-1} if grown in a sole stand (Kamanga et al. 2019).

Depending on the nature of a specific treatment, some crop seeds were not treated, some seeds were only seed dressed, some seeds were only inoculated, while some seeds were seed dressed as well as inoculated. Seed dressing was done by using Monceren GT 390 FS (Imidacloprid, Pencycuron and Thiram) at the rate of 1.5 L of Monceren GT 390 FS 100 kg $^{-1}$ of seed for both crops. Proportionally, seed for both crops were put in separate pails where after the respective amounts of Monceren GT 390 FS were added and thoroughly mixed with seed and then put on a tarpaulin under shade for 10 to 20 minutes to dry. Inoculation for both crop seeds was done by using the specific inoculants for each crop at a rate of 100 g inoculant per 25 kg of seed. Proportionally, seeds for each crop were put in separate pails where after the specific inoculants for each crop were added, then gradual application of water was done to mix the seed thoroughly with the inoculant, without damaging the seed coats. The inoculated seed was spread on a tarpaulin under shade for 10 to 20 minutes to dry before planting. For treatments that involved both seed dressing and inoculation, the same procedures above were followed, but seed dressing was done first before inoculation of the same seed. Seed dressing and inoculation was done on the same day of planting.

Primary and secondary soil cultivation was followed by construction of ridges, 75 cm apart and 40 cm wide. Two rows of groundnuts plants, 30 cm apart were planted per ridge, with one seed per planting station spaced at 15 cm apart. For the sole stands of pigeon pea, one row of plants was planted per ridge, with three seeds placed per planting station spaced 90 cm apart. For the pigeon pea- groundnut intercrop, a row of pigeon pea plants was panted between the two rows of groundnut per ridge (Figure 3.1).

Figure 3. 1: Two rows of peanut with one row of pigeon pea in between, per ridge for an intercropped plot (A Phiri, 2020)

3.4 EXPERIMENTAL DESIGN, TREATMENTS AND PLOT LAYOUT

The experiment had 12 treatments (Table 3.1); Untreated sole PP, Untreated sole GN, Untreated PP-GN intercrop, Inoculated sole PP, Inoculated sole GN, Inoculated PP-GN intercrop, Seed dressed sole PP, Seed dressed sole GN, Seed dressed PP-GN intercrop, Inoculated + Seed dressed sole PP, Inoculated + seed dressed sole GN, Inoculated + Seed dressed PP-GN intercrop. The experiment had one intercrop control (Untreated PP-GN intercrop). This treatment was used as a control since it is the common growing practice that farmers do in areas where doubled-up legume technology is done. Therefore, it was the treatment against which all other treatments were compared.

The experiment was set out as a Randomized Complete Block Design (RCBD), with each treatment replicated three times (Table 3.2). Each experimental plot had 6 ridges of 6 m long and spaced at 75 cm apart. The gross plot area therefore covered 27 m^2 (0.75 m x 6 x 6 m). The net plot covered an area of 12 m^2 (0.75 m x 4 x 4 m) with 4 ridges of 4 m long each and spaced at 75 cm apart. The net plot was demarcated by excluding one border ridge from both sides of the gross plot as well as one meter from both ends of the 4 x 4 m ridges. That helped to avoid border effects during data collection and analysis.

Table 3. 1: Field experiment treatment description

TREATMENT CODE	TREATMENT NAME	DESCRIPTION OF THE TREATMENT
T1	Untreated sole PP	Pigeon pea pure stand without using inoculation and seed dressing chemicals
T2	Untreated sole GN	Groundnut pure stand without using inoculation and seed dressing chemicals
T3	Untreated PP-GN Intercrop	Groundnut intercropped with pigeon pea without using inoculation and seed dressing chemicals
T4	Inoculated sole PP	Inoculated pigeon pea pure stand
T5	Inoculated sole GN	Inoculated groundnut pure stand
T6	Inoculated GN-PP Intercrop	Inoculated groundnut intercropped with inoculated pigeon pea
T7	Seed dressed sole PP	Seed dressed pigeon pea pure stand
T8	Seed dressed sole GN	Seed dressed groundnut pure stand
T9	Seed dressed GN-PP intercrop	Seed dressed groundnut intercropped with seed dressed pigeon pea
T10	Inoculated + Seed dressed sole PP	Inoculated and seed dressed pigeon pea pure stand
T11	Inoculated + seed dressed sole GN	Inoculated and seed dressed groundnut pure stand
T12	Inoculated+ Seed dressed GN-PP Intercrop	Inoculated and seed dressed groundnut intercropped with inoculated and seed dressed pigeon pea

Table 3. 2: Field experiment layout

BLOCK I	BLOCK II	BLOCK III
T2	**T3**	**T12**
T8	**T6**	**T3**
T4	**T9**	**T5**
T12	**T12**	**T9**
T5	**T10**	**T11**
T1	**T2**	**T7**
T11	**T7**	**T4**
T7	**T11**	**T1**
T10	**T4**	**T8**
T3	**T8**	**T10**
T9	**T5**	**T6**
T6	**T1**	**T2**

Where;

T1= Untreated sole PP, **T2**= Untreated sole GN, T3= Untreated PP-GN intercrop, **T4**= Inoculated sole PP, **T5**= Inoculated sole GN, **T6**= Inoculated PP-GN intercrop, **T7**= Seed dressed sole PP, **T8**= Seed dressed sole GN, **T9**= Seed dressed PP-GN intercrop, **T10**= Inoculated + Seed dressed sole PP, **T11**= Inoculated + seed dressed sole GN, **T12**= Inoculated+ Seed dressed PP-GN intercrop.

3.5 DATA COLLECTION PROCEDURES

3.5.1 Soil sample collection and analysis

Soil sampling and analysis was done before the trial was implemented.

Soil type determination procedure

The experimental project covered a total area of 972 m^2 which was demarcated into three blocks and each block had twelve experimental plots, each covered an area of $27m^2$. From the total experimental area, 12 soil samples were collected at random by using a soil auger. Figure 3.1 illustrates how the soil sampling was being done at the experimental site.

Figure 3. 2: Soil sampling at the experimental site (A Phiri, 2020)

The soil samples consisted of 6 topsoil samples and 6 subsoil samples. The topsoil samples were collected at the depth of 0 – 30 cm while the subsoil samples were collected at the depth of 30 - 80 cm at the same point. Each set of soil samples (topsoil and subsoil) was kept separately, but in a set the soil was thoroughly mixed in order to obtain a composite soil sample using the quartering method of sampling. The two sets of soil samples were then transported to the Soil Science laboratory at the University of Pretoria for analysis.

At the soil laboratory, the hydrometer method was used to determine soil type. Gavlack et al. (2005) stated that this method is of lower precision than the pipette or sedimentation methods, and has detection limit of 2% sand, silt and clay (dry basis). A 400 ml beaker was weighed and the mass was noted. Then 50 g of soil from one soil sample (e.g. top soil) was added to the weighed beaker. The soil was then thoroughly transferred to a mixing flask where 10 ml of Calgon and enough de-ionised water were added to the flask so as to cover the blades of the mixer. The contents were mixed for 5 minutes and then transferred to a clear 2 L measuring cylinder through a 0.054 mm sieve. The sieve was then rinsed with water until only sand particles remained on the sieve. Water was then used to transfer sand particles from the sieve into weighed beaker. The above procedures were repeated for the other soil sample (sub soil). The two 400 ml beakers that contained sand particles were then placed in an oven at 105°C to dry. The dry masses for sand soil particles were noted. The 2 L cylinders were made up to a predetermined mark with water. The cylinder contents were mixed and 2 hydrometer readings were taken at 40 seconds and 6 hours 35 minutes and were used to calculate the clay and silt soil masses. The top soil layer sample contained 64% sand particles, 24% clay particles and 12% silt particles while the deeper layer sample contained 56% sand particles, 28% clay particles and 16% silt particles. A soil textural triangle (Figure 3.2) was used to determine the soil types, showing that both layers types were sandy clay loam soil.

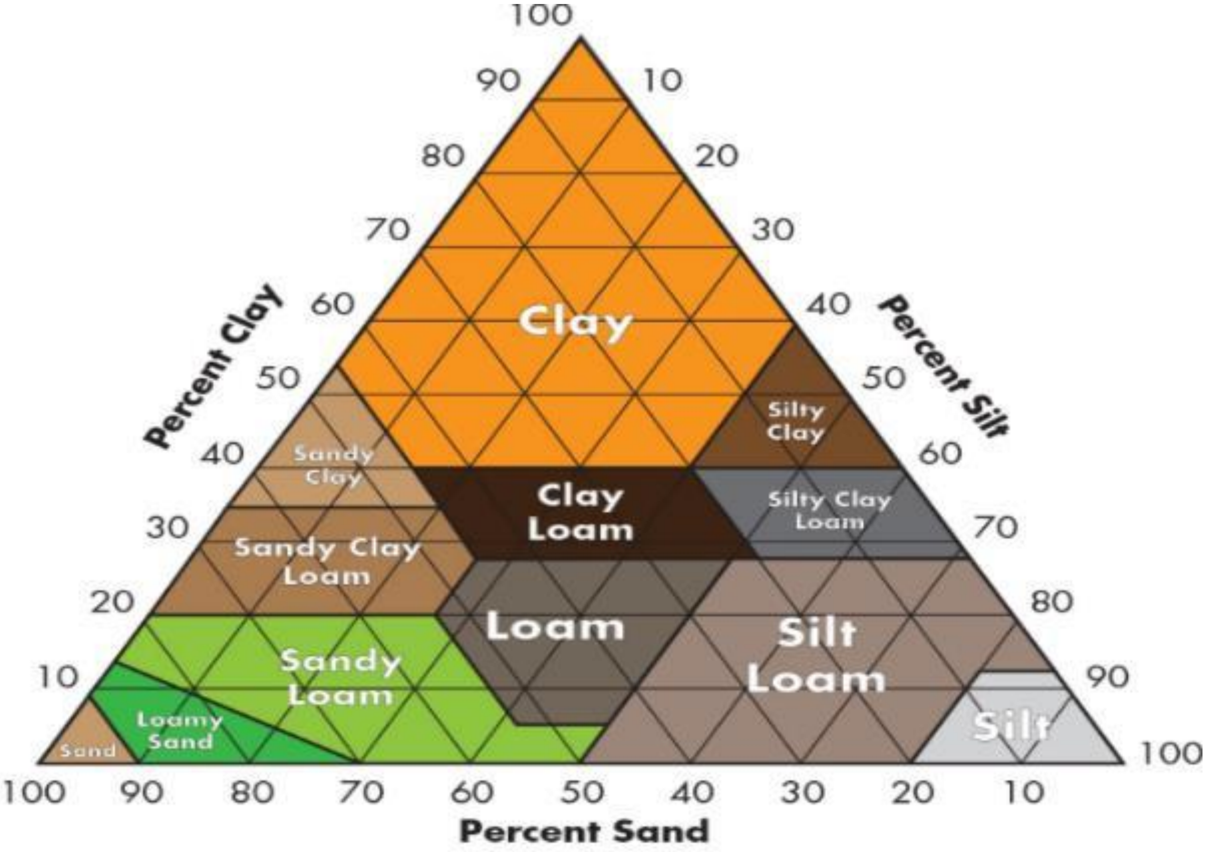


Figure 3. 3: Soil textural triangle (https://www.trugreen.com/lawn-care-101/learning-center/grass-basics/dig-deeper/soil-texture)

<u>Soil Nutrient analysis</u>

Soil nutrient analysis was done on the samples collected before the research project commenced. The results of the analysis are presented in Table 3.3.

<u>Soil pH and nutrient assessment procedures</u>

Soil pH for both sets of soil samples were determined by using a Calibrated pH meter. From each soil sample, 10 g of dried soil were weighed into two 50 ml tubes to which 25 ml of de-ionised water was added. The contents were rapidly shaken for 5 minutes and then allowed to stand for 30 minutes. The contents were then shaken again for 10 minutes before taking the pH reading. Soil sample that was collected from the top 30 cm had a pH of 5.47 while the soil sample that was collected from 80 cm indicated a pH of 5.88 (Table 3.3).

Soil nutrients (P, K, Mg, Na and Ca) were determined by using Mehlich 3 (M3) method. This is an appropriate and economical method because it is suitable for a wide range of soils and can serve as a universal soil test extractant (Ziadi et al. 2008). The following procedures were followed during the analysis:

3 g of dry soil were weighed into 50 ml tube. 15 ml tubes and filter papers (Whatman # 42) were arranged, ready for filtration. 30 ml of Mehlich 3 solution were added to the 3 g of dry soil in a 50 ml tube and shaken immediately for exactly 5 minutes on a reciprocating shaker. The contents were then filtered through M3-rinsen Whatman #42 filter papers into 15 ml tubes and stored at 4^{o}C until analysis. The analysis was done by an automated method known as Inductively Coupled Plasma (ICP).

Soil nitrogen was determined through Spectrophotometric analysis. This is a fast and direct method for the sequential determination of nitrate and nitrite at low concentrations in small volumes (Garcia-Robledo et al. 2014). The following procedures were used:

2.5g of soil were weighed into 50 ml tube where 25 ml of 1 M KCl were added. The contents were then shaken for 60 minutes and filtered into a 15 ml tube using Whatman # 2 filter paper. The contents were then analysed by Spectrophotometer.

Table 3. 3: Soil pH and nutrient analysis results before planting

Soil layer	**pH**	**P**	**K**	**Mg**	**Na**	**Ca**	**NH4**	**NO2 + NO3**	**Total N**
	*	**	**	**	**	**	***	***	
					mg kg^{-1}				
Top soil	5.47	61.7	84.7	126.2	43.5	353.4	13.7	38.3	52.0
Sub soil	5.88	6.0	34.1	144.9	50.8	613.0	10.9	24.8	35.7

*Water (H_2O), **Mehlich 3 (M3), *** KCl extract

3.5.2 Meteorological and soil moisture content data

(a) Rainfall and Temperature

Data for rainfall and temperature for the whole growing season were collected by an automated weather station located within 100 m of the experimental site. Average monthly and annual temperature and total rainfall over the growing season were recorded as illustrated in Table 3.4. The data show that the total annual rainfall over the growing season was 855.4 mm and an average maximum annual temperature of 26.2^{o}C was observed during the 2020/2021 crop growing season.

Table 3. 4: Temperature (^{o}C) and rainfall (mm) data over the 2020/2021 cropping season

	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	**Avg**
Max temp (^{o}C)	22.9	27.1	28.6	27.9	28.5	29.0	28.1	27.8	27.0	23.6	21.1	22.3	26.2
Min temp (^{o}C)	7.6	11.5	14.8	15.2	17.0	17.6	16.5	14.1	11.2	7.1	5.3	5.8	12.0
													Total
Rainfall (mm)	0.0	7.8	83.2	222.0	121.4	206.8	96.4	73.0	37.8	3.0	4.0	0.0	855.4

(b) Soil Moisture

Soil moisture content was monitored and measured on weekly basis starting from day of planting to day of maturity by using a neutron probe meter whereby access tubes were installed at the centre of each and every treatment plot. A hand soil auger was used to prepare holes in the plots for the installation of access tubes. Soil moisture content was being observed at 20 cm depth intervals up to a depth of 100 cm in order to check any necessity of supplementary water supply through irrigation but soil moisture was enough for both crops throughout the growing season such that irrigation was not used.

3.5.3 Disease assessment

During the research all possible diseases for groundnut such as early and late leaf spot, stem and pod rot disease, groundnut rosette disease and groundnut rust were assessed. Diseases for pigeon pea such as *Alternaria*, anthracnose, *Cercospora* leaf spot, white mold and *Fusarium* wilt were also assessed. Prof. J. Van der Waals, a pathologist from the Department of Plant and Soil Sciences at the University of Pretoria helped with disease assessments. The disease assessment was done manually by checking their presence in all plants within the net plot for all the treatments at one-week intervals. The symptoms for the diseases per treatment were categorised and scored, depending on the severity of the diseases. Disease severity was determined by physically counting the affected plants per treatment. A score range of 1 to 3 was used to categorise the severity of the diseases. There were disease scoring charts for all possible diseases. For example, a score of 1 was assigned to treatments with 0 to 9 % plants with disease infection. A score of 2 was assigned to treatments with 10 to 49 % plants with disease infection. A score of 3 was assigned to treatments with 50% or more plants with disease infection.

3.5.4 Pest infestation

Data for pest infestation in all treatments were collected manually by checking the presence of pests in all plants within the net plot for all treatments at one-week intervals. This process is known as scouting. The following pests for groundnut were assessed: aphids, leaf eaters and leaf miners while the following pests for pigeon pea were assessed: aphids, armyworms, corn earworm, cutworms and leaf miners. The degree of pest presence in treatments was indicated by scores ranging from 1 to 3. All treatments with 0 to 9 cumulative pests per net plot were assigned a score of 1. All treatments with 10 to 19 cumulative pests per net plot were assigned a score of 2. A score of 3 was assigned to all treatments with 20 or more cumulative pests per net plot.

3.5.5 Stand count, Germination % and Survival %

Stand count for all treatments were done manually by counting all plants in net plots after seedling emergence and again prior to harvesting so as to calculate germination % and survival % respectively. For example: germination % and survival % were calculated as follows:

Germination % = (Seeds germinated / Total seeds planted) × 100%.

Survival % = (Number of plants survived / Total number of plants that germinated) ×100%

Plant growth rates were assessed by manually measuring both groundnut and pigeon pea plant heights for sampled plants per net plot at two-week intervals. Four sampled plants for each treatment were selected at random, tagged and used to assess plant growth rate throughout the research period. The measurements were collected by vertically putting a measuring ruler close to the stems of sampled plants and the measurement reading were being taken at the highest point of the plants.

3.6 DATA ANALYSIS

GenStat, Excel and RStudio were used to analyse data that were collected from the research project and to create Analysis of Variance (ANOVA) tables. Means were compared using the Fisher's least significance difference (LSD) test to test probability level at 5% (P = 0.05).

CHAPTER 4

GROUNDNUT AND PIGEON PEA SEED GERMINATION AND SEEDLING SURVIVAL

4.1 INTRODUCTION

Groundnut and pigeon pea seed germination and seedling survival are some of the most important and vulnerable phases of the crop cycle. Under field conditions, seed germination and seedling survival are affected by a number of abiotic and biotic factors (Lamichhane et al. 2018). Some examples of abiotic factors are soil temperature, soil pH, soil moisture, light, salinity and seed planting depth, while examples of biotic factors are soil pests. Farmers lose some crop yields due to these factors. It is therefore important to treat seeds (seed dressing) before planting to minimise poor seed germination and seedling survival that come due to some biotic factors. It is also important to note that a good seed treatment before planting discourages the development of soil borne diseases such as Fusarium wilt and smuts among others. Fungicide seed dressing significantly reduces seedling mortality (Muthomi et al. 2007). A good example of a fungicide that is used in seed dressing is Monceren GT 390 FS. Seed dressing is the seed treatment whereby plant seeds are treated (coated) with a chemical so as to protect seed and seedlings from either diseases or pests or both. Monceren GT 390 FS is a concentrated suspension for seed treatment which acts by contact and ingestion through systemic activity. It is applied to seeds at a rate of 1.5 L of Monceren GT 390 FS 100 kg^{-1} of seed. It works as both a pesticide and fungicide. Therefore, the main objective of this chapter is to quantify the effect of different treatments on the germination and survival of the two crops.

4.2 MATERIALS AND METHODS

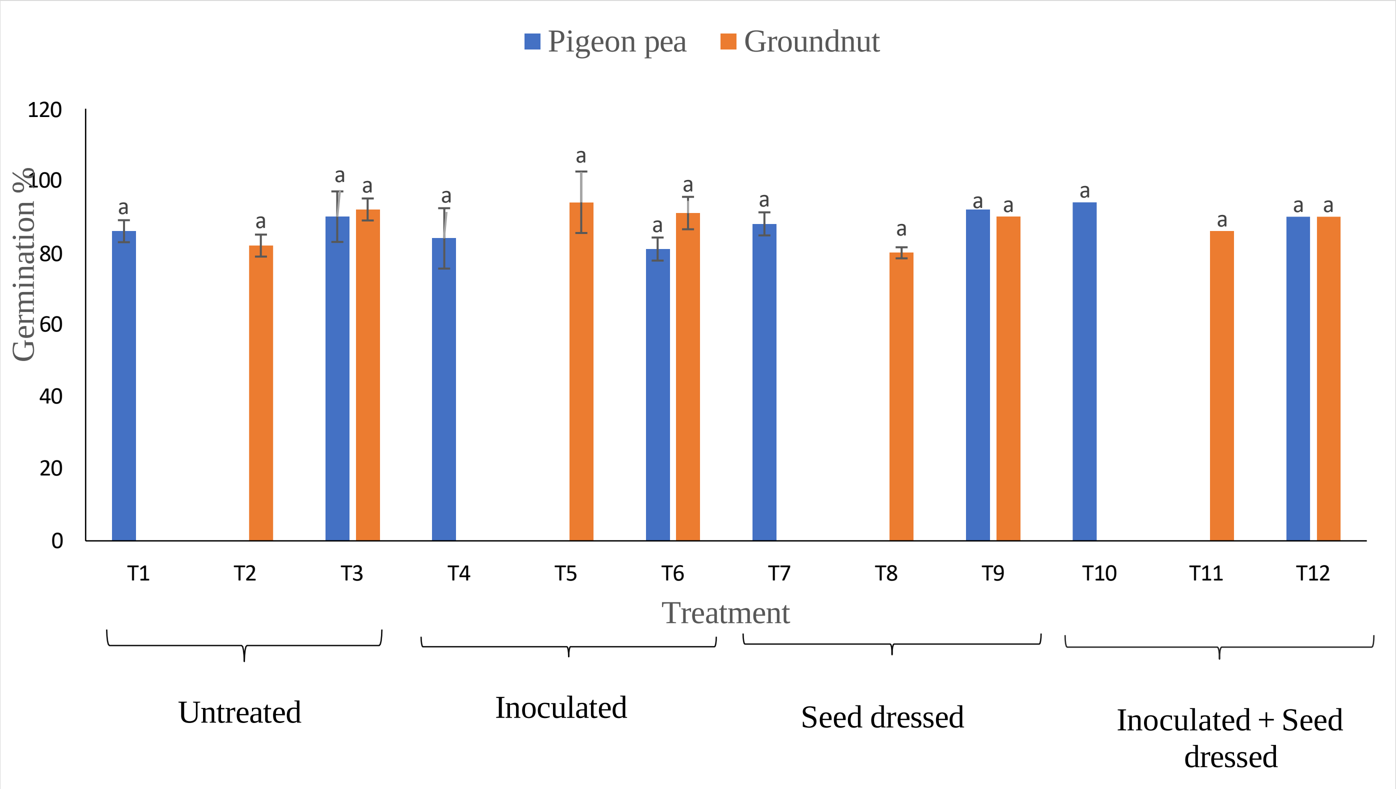
The general procedures for the doubled-up legume technology trial can be accessed from chapter 3. This section will briefly explain the materials and methods that are relevant to this chapter. The trial had twelve treatments in total of which some were seed dressed while others were not.

The seed germination and seedling survival were established by physical counting the plants on the net plots (Figure 4.1). Germination % was calculated at 14 days after planting. Survival rates for groundnut and pigeon pea were determined at 140 and 174 days respectively after planting.

Figure 4. 1: Stand count for seed germination and seedling emergence

4.3 RESULTS AND DISCUSSION

Results from the trial shown in the ANOVA tables (Appendix 1, 2) and Figure 4.2 indicate that there was no significant difference in germination percentage among treatments. This is evidenced by the P- values of 0.73 and 0.49 for pigeon pea and groundnut respectively. Germination percentage ranged from 80% to 94%.



Bars with the same colour and letter do not differ significantly from each other ($P<0.05$), T1, 2, 4, 5, 7, 8, 10 and 11 are sole crops while T3, 6, 9 and 12 are intercropped

Figure 4. 2: Germination % for groundnut and pigeon pea observed at week 2 after planting

Groundnut does well in temperatures ranging from 20^{o}C to 30^{o}C, while pigeon pea requires temperatures ranging from 18^{o}C to 35^{o}C (Cilliers 2017). Sandy loam to sandy soil types with pH of 5.3 to 6.8 are ideal for groundnut production. Pigeon pea does well in almost all types of soils varying from sandy to heavy loam soils with a pH range of 5.0 to 7.0. Both crops require rainfall amount ranging from 600 to 1000 mm annum^{-1}. The site where the trial was carried out, received total rainfall of 855.4 mm over the growing season (Table 3.4). The soil type was sandy clay loam soil with pH ranging from 5.47 to 5.87 (Table 3.3) while the average annual maximum temperature was 26.2 ^{o}C (Table 3.4). Thus the environmental conditions were ideal for their growth and survival of both crops. After seed germination, the seedlings were therefore able to cope with the environmental conditions it was grown under.

Soil analysis further indicated that except P, all other nutrients (K, Mg, Na, Ca and N) (Table 3.3) were within requirements for both crops. P was observed to be slightly higher (top soil) than expected in the soil. It could be due to residual P from the previous crops.

Despite the fact that almost all environmental conditions were quite suitable for both crops, it was observed that most of the treatments that were not seed dressed were attacked by diseases such as Fusarium wilt (Figure 4.3) in pigeon pea and leaf spot (Figure 4.4) in groundnut. This resulted in poor survival rates in plots without seed dressing, as compared to those plots in which the seed were seed dressed (Figure 4.5). The ANOVA table (Appendix 3) indicates p-values of 9.12E-08 and 6.34E-09 for pigeon pea and groundnut respectively. The p-values show that treatment had an effect on survival rate for both pigeon pea and groundnut. P-value of 9.57E-15 (Appendix 4) is the combined analysis for both crops that also shows that treatment had an effect on survival rate among all treatment combinations for both crops.

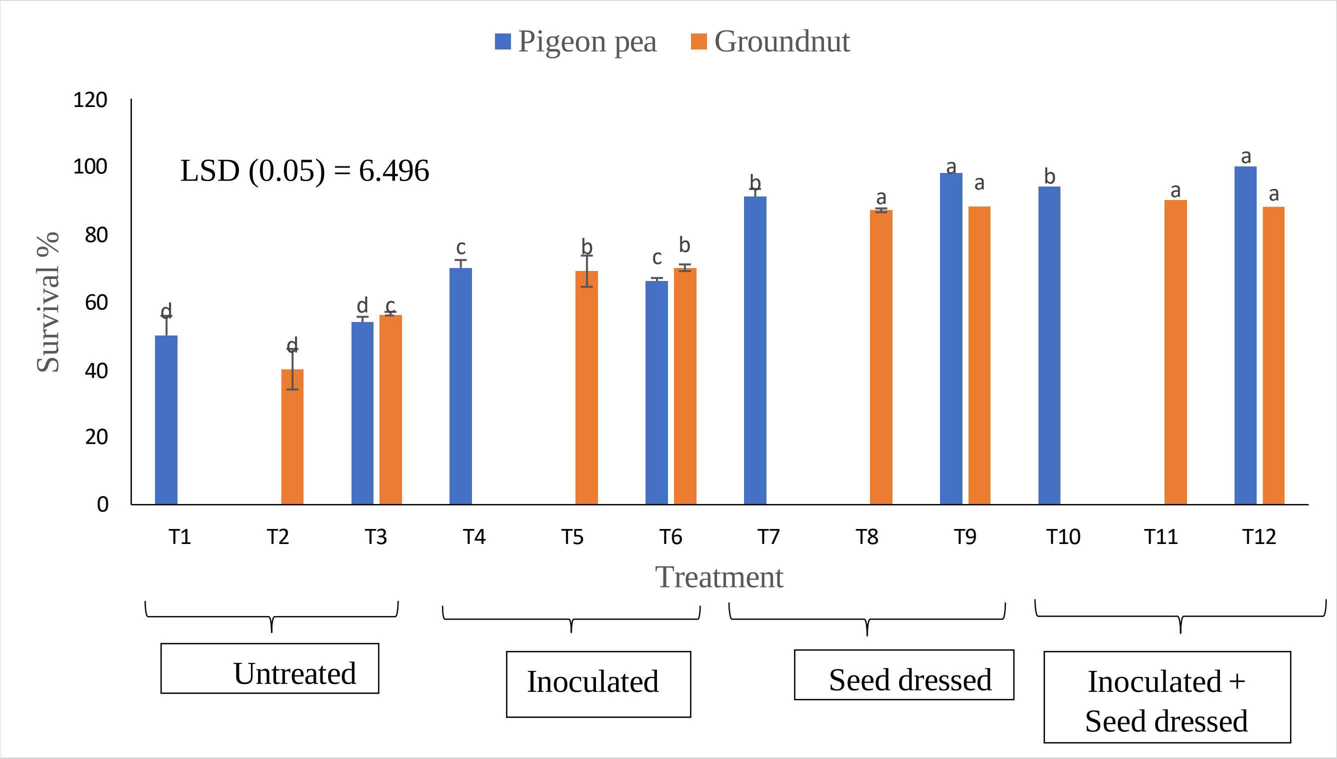
Figure 4. 3: Fusarium wilt observed in untreated pigeon pea (T1) 18 days after planting (A Phiri, 2020).

Figure 4. 4: Leaf spot disease in untreated groundnut plot (T2), 86 days after planting (A Phiri, 2020)

Among all sole treatments (for both crops), significant difference in terms of survival rate was observed, with untreated treatments performing the worst (Figure 4.5). More pests, diseases and plant deaths were observed in groundnut than pigeon pea. As for intercropped treatments, significant difference in terms of survival rate was also observed among intercropped treatments. Treatments which were seed dressed performed better than treatments which were not seed dressed.

Page et al. (2002) state that in order to reduce the impact of blight on groundnuts that are caused by soil bacteria and fungi and also other fungal diseases, a fungicide seed dressing is recommended. Thiram gives good protection and can be applied as a dust at 120g of thiram per 100 kg of seed. The argument raised by Page et al. (2001) is in line with applying Monceren GT 390 FS as seed dressing in the trial. Seed dressing improved survival rate of both crops. Results of the trial that was done by Liu et al. (2011) also explains that seed dressing of peanut played an important role in full stand and achieving high and stable yield. He evaluated the effects of applying four different types of seed dressing agents (Trichoderma harzianum (biological antifungi agent), Thiophanate-methyl (chemical fungicide, TM), Celest (chemical fungicide), and Carbosulfan (chemical insecticide)) to peanut seed, by measuring the growth and development, yield, quality of peanut and soil microorganisms. One of the major results that Liu et al. (2011)

found was that all antifungi agents increased seedling survival rate. Monceren GT 390 FS had the same impact on both crops during the trial.



Bars with the same colour and letter do not differ significantly from each other ($P<0.05$), T1, 2, 4, 5, 7, 8, 10 and 11 are sole crops while T3, 6, 9 and 12 are intercropped

Figure 4. 5: Survival variations among treatments at maturity

4.4 CONCLUSION

Based on the results and observations on seed germination and survival among the treatments, seed dressing had no effect on seed germination but did improve seedling survival. Seed dressing protects crop seedlings from early pests and diseases during tender stages of their growth, hence improving survival rates. It can therefore be concluded that farmers should seed dress their groundnut and pigeon pea seeds in doubled-up legume technology to avoid early pests, diseases and subsequent yield losses.

CHAPTER 5

NODULE EFFECTIVENESS AND CHLOROPHYLL CONTENT

5.1 INTRODUCTION

Inoculation is one of the crucial agronomic practices required for successful legume crop production. Inoculation is the practice of adding an effective type of bacteria to the host plant seed before planting. The main purpose of inoculation is to ensure that there is enough and the correct type of bacteria in the soil so as to have a successful legume-bacteria relationship in the soil. Inoculation with rhizobia provides success stories of agriculture and it is considered as the most cost-effective of all agricultural practices. *Rhizobia* are a group of common soil bacteria that form small growths (nodules) on the roots of legumes. *Rhizobia* are important because they convert nitrogen gas from the atmosphere into a form of nitrogen that is readily used by plants (Flynn 2015). Effective inoculation results into good nodulation which in turn ensures good biological nitrogen fixation. Legume inoculants consist of live bacteria known as *Rhizobia* which are perishable in nature (Drew et al. 2012). These microorganisms are very sensitive to a wide range of environmental factors such as high temperatures and desiccation, which in turn decrease their viability. The process of inoculation provides a host-legume plant with a large number of effective nitrogen- fixing bacteria to optimise nodulation and nitrogen fixation. Inoculation is crucial for nodulation in a field where the host-legume has not previously been grown. The nodule development on roots of the host-legume plant is as a result of the regulated infection process by the *Rhizobia*. The compatibility between the rhizobia and the host-legume determines the success of the nodule formation and nitrogen fixation. The rhizobia therefore play a big role in the development of nodules on roots or/ and stems of the host plants (legumes). The nodules on the roots or stems of the host plants fix nitrogen that is directly used by the host plants. This enables the host plants to accommodate nitrogen fixation due to the availability of required energy source. Cereal crops, such as wheat and rice among others do not have a mutual relationship between them and rhizobia, hence these species do not take part in biological nitrogen fixation. Nitrogen is one of the essential nutrients and plays a big role in plant production. It forms part of proteins, nucleic acids and other nitrogen compounds (Arora 2013). This implies that nitrogen supports life for all organisms. Biological nitrogen fixation therefore supports sustainable ecologies.

5.1.1 Factors that enhance biological nitrogen fixation

Biological nitrogen fixation, along with photosynthesis, interrupt the stability of the soil environment due to continuous state of change of some soil factors and as such they create a stressful environment for both macro- and micro-organisms in the soil. For example, changes in soil water status, soil nutrient availability, differences in soil pH and soil temperature, to mention a few, affect the growth, survival and metabolic activity of rhizobia and plants (Mohammadi et al. 2012). With time, soil organisms mutate or evolve in order to suit the hostile soil environment. Generally, soil factors influence symbiotic nitrogen fixation in one way or the other.

(a) Soil water status

Soil micro-organisms are affected by soil water due to the process of diffusion, mass flow and nutrient concentration. Soils that contain larger pore spaces retain less water than soils with smaller internal pore spaces. Therefore, soils with smaller internal pore spaces tend to be more inhabitable environments for most soil microbes (Mohammadi et al. 2012). It should also be noted that too much soil water negatively affects soil rhizosphere micro-organisms like rhizobia and plants. As a mechanism of survival, rhizobia evolved to a variety of species that adapt osmotic stress through intercellular build-up of inorganic and organic solutes.

(b) Soil nutrients

Soil nutrient availability has a great impact on symbiosis, growth and survival of both micro-organisms such as rhizobia and plants. Fixation decreases in legumes due to concomitant increase in soil nitrogen. Application of organic matter and biofertilizers enhances nodule development, nodule number and effective nitrogen fixation. Calcium deficiency in the soil also limits rhizobia from getting attached to plant root hairs. Effective nodulation and nodule development may also be jeopardised. Thus, calcium influences rhizobia- legume symbiotic interactions more especially at molecular point (Njira et al. 2012). Apart from nitrogen and calcium, the growth and perseverance of micro-organisms such as rhizobia is also supported by number of nutritional factors. The rhizobia which are metabolically diverse, use a wide range of plants and soil derived compounds for growth. Soil supplementation and inoculation enhances persistence and number of rhizobia. It also influences both early onset of nodulation and effective nitrogen fixation.

(c) Soil pH

Low soil pH serves as a signal for the presence of conditions under which some other soil properties may negatively affect plant growth and development rather than as a principle cause of poor plant growth. Worldwide acid soils negatively affect crop production and as much as 25% of the crop lands have problems related to soil acidity (Mohammadi et al. 2012). Acid stress leads to stunted root growth and development in legumes and in turn affect nodule development and nitrogen fixation (Mohammadi et al. 2012). An example of such acids is abscisic acid. Only a limited number of *rhizobia* species survive below soil pH of 4.5 to 5.0. Low soil pH also negatively affects both survival and infection process of the legume plants.

(d) Soil temperature

Temperature plays a remarkable role with regard to endurance and perseverance of rhizobial strains in soils. Every strain grows and survives well at a specific temperature. A specific example is of cowpea rhizobia from West Africa, which grows well in hot- savannah regions at the temperature of 30^{o}C, while other rhizobial strains in the same region grow well at temperatures of up to 40^{o}C. This implies that temperature affects the growth and survival of rhizobia in relation to strain and soil type. *Bradyrhizobium* spps are actually less susceptible to high soil temperature than *R. leguminosarum* bv trifolii. The scenario changes when montmorillonite and illite (soil types) are added to sandy soils. They modify soil temperature in sandy soils. High temperature results into high infection, nitrogen fixation capability and legume growth and development. Temperature also dictates the strain and legume combinations. For biological nitrogen fixation to take place, both rhizobium and legume plant should have a common specific optimum temperature that accommodates both of them (Mohammadi et al. 2012).

5.1.2 Impact of nitrogen on plant growth and development

Nitrogen is an influential macro- nutrient that controls all vegetative biological processes in plants. This calls for a proper management of nitrogen so as to utilize it effectively and efficiently. An optimum nitrogen rate improves photosynthetic processes and vegetative growth of plants. For plants to use nitrogen, it should be converted into usable forms such as nitrate (NO_3^-) and ammonia NH_4^+ (Leghari et al. 2016). It is the most imperative element that controls biochemical and physiological functions in plants. This implies that nitrogen controls vital chemical processes in plants. All crops with insufficient amount of nitrogen result into poor crop production in terms of yield and quality. For crops to have a vigorous growth they need a balanced amount of nitrogen.

5.1.3 Role of nitrogen in crops

- Growth and development

Nitrogen promotes growth and development in plants. Without nitrogen plants suffer from stunted growth. Amino acids in plant structures rely on nitrogen in the formation of plant proteins that are used for growth and development of vital plant tissues and all cells (Leghari et al. 2016).

- Chlorophyll and Photosynthesis

Nitrogen helps the formation of chlorophyll in plants. This is a special pigment that is found in green plants and in Cyanobacteria which plays a big role in absorption of light to ensure the presence of energy for photosynthesis. Photosynthesis is the chemical system that allows green plants and/ or Cyanobacteria to use carbon dioxide and water to obtain nutrients with the aid of sunlight. The major chemical pathway in photosynthesis is the conversion of carbon dioxide and water to carbohydrates and oxygen (Hall et al. 1999). The reaction can be represented by the equation:

$$CO_2 + H_2O \xrightarrow[\boxed{\text{Plants}}]{\boxed{\text{Sunlight}}} CH_2O + O_2$$

Effective photosynthesis relies on the availability of nitrogen in plants. Sufficient presence of nitrogen in plants leads to high rates of photosynthesis which in turn results in vigorous growth and development of plants.

- DNA formation

The word DNA refers to Deoxyribonucleic acid. This is the carrier of genetic material. Nearly all living organisms contain DNA. It is actually a self- replicating material. Nitrogen helps the formation of DNA, the genetic material that plays a big role in transferring certain crop traits and characteristics for plant survival (Leghari et al. 2016).

Inoculation has a great impact on nitrogen formation in crop plants such as legumes which in turn controls the amount of their chlorophyll content and yield. Therefore, the main objective of this chapter is to unpack the effect of different treatments on nodule development and chlorophyll formation of the two crops.

5.2 MATERIALS AND METHODS

The general procedures on how the doubled-up legume technology trial was implemented can be accessed from Chapter 3. This section will briefly focus on the materials and methods that are relevant to this chapter. During the trial, some groundnut and pigeon pea seeds were inoculated with specific inoculants for each crop before planting.

5.2.1Inoculation process

Inoculation for both crop seeds was done by specific inoculants for each crop at the rate of 25 kg of seed per 100 g of inoculant. Proportionally, seeds for each crop were put in separate pails to which specific inoculants for each crop were added, then gradual application of water was done to mix the seeds with the inoculant thoroughly, without damaging the seed coats. The inoculated seeds were spread out on a tarpaulin under shade for 10 to 20 minutes to dry before planting.

5.2.2 Nodule assessment

Nodule evaluation was done during vegetative growth (80 days after planting), before maturity in order to find out the effectiveness of the nodules. In this assessment 2 sample plants from each net plot were collected at random. The roots for the collected sample plants were cleaned by water to remove soil particles. Five well-developed nodules from each sample plant were selected for assessment. That gave a to a total of 10 nodules per net plot. The 10 selected nodules were then sliced into halves by using a razor blade to check internal colour. Red or pink colour indicated nodule involvement in biological nitrogen fixation. White or green colour indicated nodule inactiveness in biological nitrogen fixation. A score range of 1 to 3 was used to categorise nodule effectiveness per treatment. For example; a score of 1 was assigned to treatments with 0 to 3 red or pink nodules. Treatments with 4 to 6 red or pink nodules were assigned a score of 2. A score of 3 was assigned to all treatments with at least 7 red or pink nodules (Figure 5.1).

Figure 5. 1: Nodule assessment in a weighing room at University of Pretoria (IA@UP), South Africa

5.2.3 Chlorophyll assessment

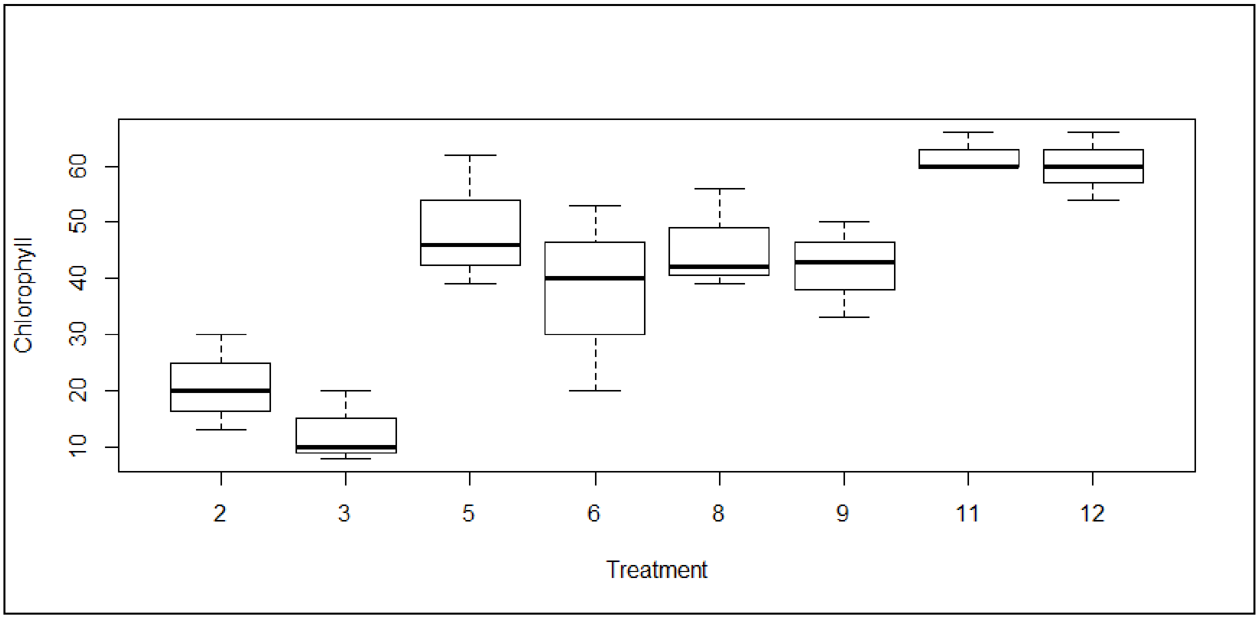
Chlorophyll assessment was done at week number six in order to determine the chlorophyll content of leaves in each treatment. During the process, a SPAD meter (Figure 5.2) was used.

Figure 5. 2: SPAD meter which was used for leaf chlorophyll determinations during the research

The trial had twelve treatments in total and out of them some were inoculated, while others were not (Table 3.2).

5.3 RESULTS AND DISCUSSION

Figure 5.3 and 5.4 show that there were some differences of chlorophyll concentration among treatments during the trial and the differences were significant as evidenced by p-values of <0.001 (Tables 5.1 and 5.2) for both groundnut and pigeon pea respectively. Another observation was that sole treatments had higher chlorophyll concentrations than their respective intercropped counterparts. Among the sole treatments, the treatment that was not inoculated and seed dressed had the lowest chlorophyll concentration level, while the treatment that was both inoculated and seed dressed had the highest chlorophyll concentration level. The same trend of chlorophyll concentration levels was observed among intercropped treatments. The treatment that was not inoculated and seed dressed had the lowest level of chlorophyll concentration, while intercropped treatments that were both inoculated and seed dressed had the highest level of chlorophyll concentration level for both crops. The same relationship between inoculation and chlorophyll was also observed by Nyoki et al. 2017 who explained that there is a great relationship between inoculation and chlorophyll content in plants in the sense that inoculation increases chlorophyll content in plants. The more effective biological nitrogen fixation due to inoculation, the higher the concentration of chlorophyll in plants. Another observation was the presence of diseases such as early and late leaf spot diseases in groundnut and fusarium wilt in pigeon pea. More such diseases were observed in untreated treatments. Therefore, inoculation also helped to boost resistance of inoculated plants for both crops. This observation corresponds well with the argument that was made by Volpiano et al. (2019) who stated that rhizobia should also be considered an alternative method to agricultural pesticides use in plant disease management. Several rhizobial strains have been reported leading to diseases resistance while also promoting plant yield and biomass increases.



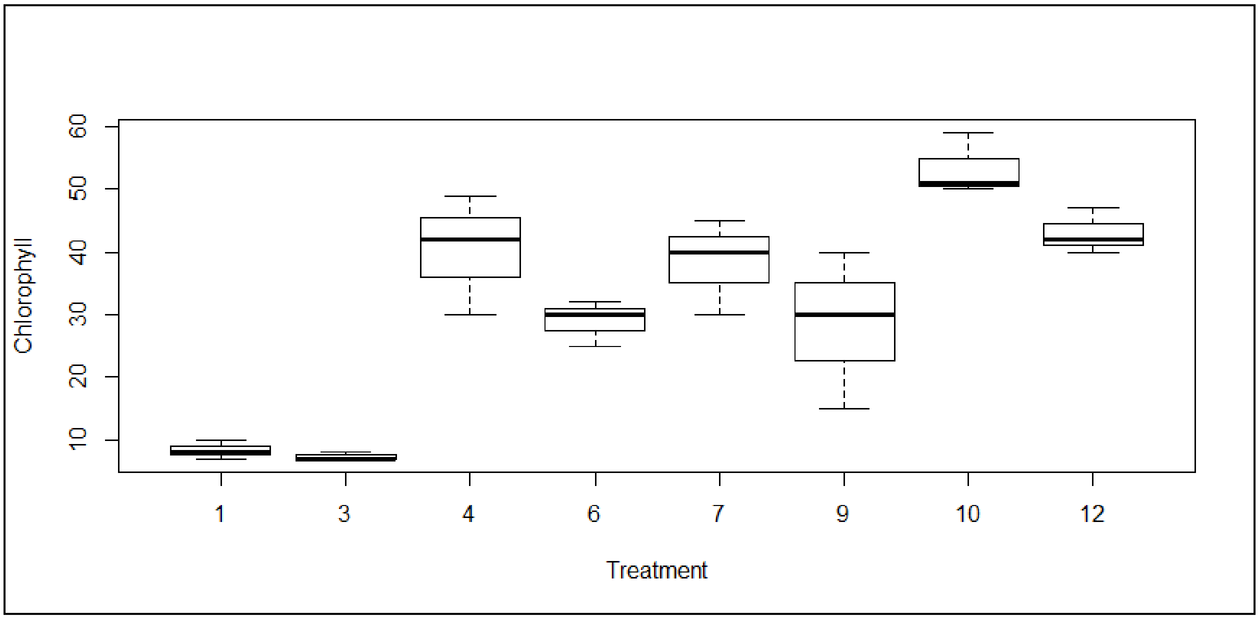
T2, 5, 8, and 11 are sole crops while T3, 6, 9 and 12 are intercropped

Figure 5. 3: Chlorophyll concentration variations for untreated (T2 and T3), inoculated (T5 and T6), seed dressed (T8 and T9) and inoculated + seed dressed (T11 and T12) groundnut at week 6 after planting

Table 5. 1: Groundnut chlorophyll content at week 6 after planting

Treatment	Chlorophyll content (SPAD Units)
Untreated sole gnut	21.0^{c}
Untreated gnut-pp intercrop	12.7^{c}
Inoculated sole gnut	49.0^{b}
Inoculated gnut - pp intercrop	37.7^{b}
Seed dressed sole gnut	45.7^{b}
Seed dressed gnut- pp intercrop	42.0^{b}
Inoculated + Seed dressed sole gnut	62.0^{a}
Inoculated + Seed dressed gnut- pp intercrop	60.0^{a}
Multiple R- Squared	0.8114
LSD (0.05)	16.6
P- Value	8.999e-5

Key: gnut = Groundnut
pp = Pigeon pea



T1, 4, 7, and 10 are sole crops while T3, 6, 9 and 12 are intercropped

Figure 5. 4: Chlorophyll concentration variations for untreated (T1 and T3), inoculated (T4 and T 6, seed dressed (T7 and T9) an inoculated + seed dressed (T10 and T12) pigeon pea at week 6 after planting

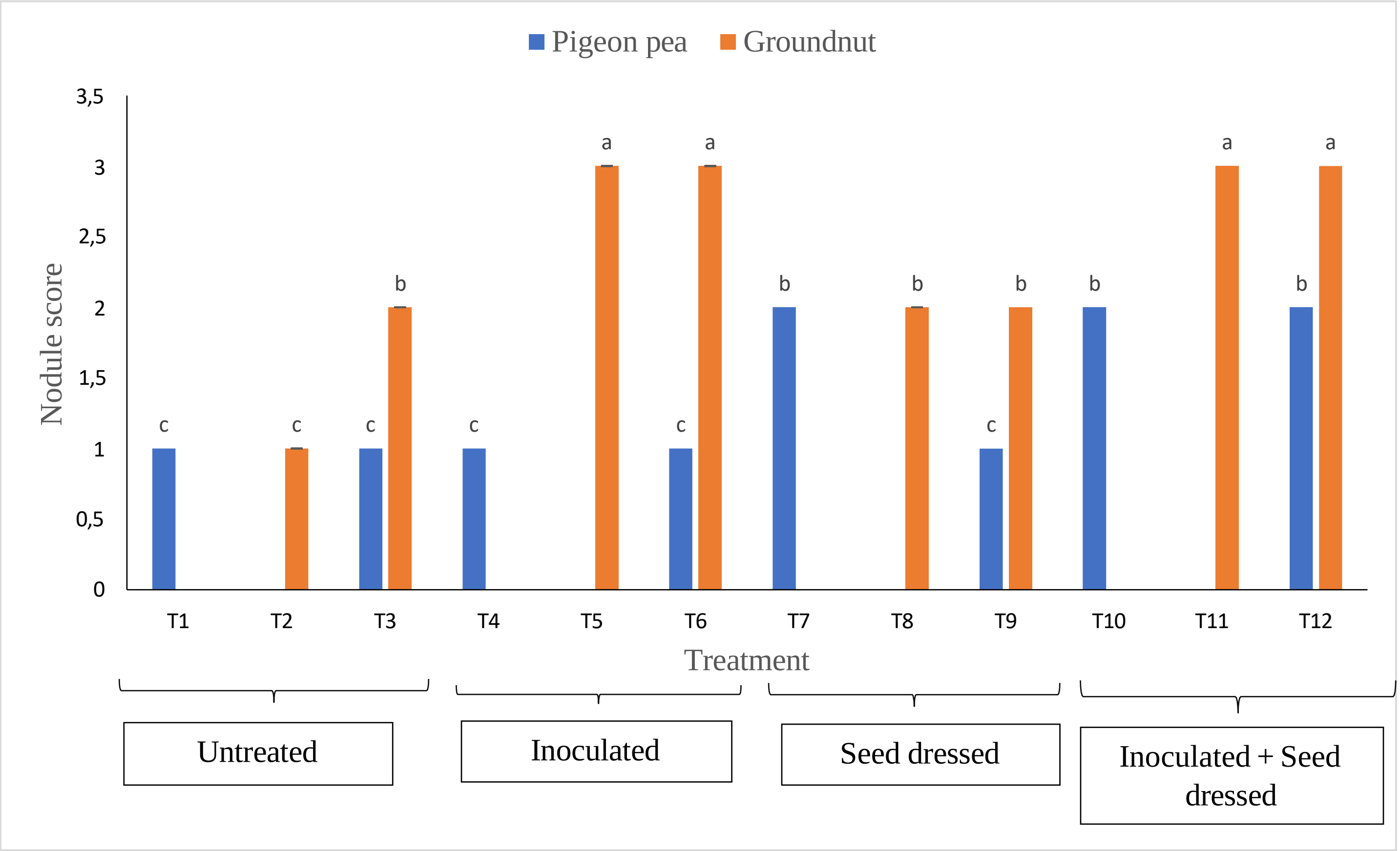
Table 5. 2: Pigeon pea chlorophyll content at week 6 after planting

Treatment	Chlorophyll content (SPAD Units)
Untreated sole pp	8.3^{d}
Untreated gnut-pp intercrop	7.3^{d}
Inoculated sole pp	40.3^{b}
Inoculated gnut - pp intercrop	29.0^{c}
Seed dressed sole pp	38.3^{c}
Seed dressed gnut- pp intercrop	28.3^{c}
Inoculated + Seed dressed sole pp	53.3^{a}
Inoculated + Seed dressed gnut- pp intercrop	43.0^{b}
Multiple R- Squared	0.8856
LSD (0.05)	11.6
P- Value	1.99e-6

Key: gnut = Groundnut
pp = Pigeon pea

ANOVA tables (Appendix 5 and 6) indicate that there were significant differences for nodule effectiveness among treatments. The tables further indicate that there were significant differences among all treatments for both crops (combined analysis). Treatments that were not inoculated did not have effective biological nitrogen fixation while those that were inoculated, had effective biological nitrogen fixation (Figure 5.5). It was clearly observed that inoculation had positive impact on biological nitrogen fixation for both crops. The observation corresponds with the results from the trial that was carried out by Sharma et al. (2011). The study that was carried out by Sharma et al. 2011 demonstrated that rhizobium inoculation in groundnut had a positive impact on nodulation. The study assessed the response of groundnut to rhizobium inoculation and it was found out that inoculation of rhizobium (IRG-6) enhanced number of pink coloured nodules (biological nitrogen fixation), nitrate reductase activity and leghaemoglobin content at 50 days after sowing groundnut.

It was also observed that most pigeon pea treatments had poorer nodule development compared to their respective groundnut treatments. There were no significant differences in terms of nodule effectiveness between untreated sole treatments (T1 and T2) and seed dressed sole treatments (T7 and T8). The rest of the treatments indicated significant differences between respective treatments in favour of groundnut treatments. A similar study that was carried out by Ahiabor et al. (1994) also indicated poor nodulation on pigeon pea compared to cow pea and groundnut. The study evaluated characteristic responses of three tropical legumes (cow pea, pigeon pea and groundnut) to the inoculation of two species of VAM fungi (Glomus sp) in Andosol soils with different fertilities. It was observed that percent fungal root colonisation was high in cow pea and groundnut but relatively low in pigeon pea in both soils.



Bars with the same colour and letter do not differ significantly from each other (P<0.05), T1, 2, 4, 5, 7, 8, 10 and 11 are sole crops while T3, 6, 9 and 12 are intercropped

Figure 5. 5: Nodule effectiveness observed during vegetative growth

5.4 CONCLUSION

Based on the statistical results and observations, inoculation generally improved nodule effectiveness and leaf chlorophyll content for both groundnut and pigeon pea plants. It can therefore be concluded that farmers should inoculate their groundnut and pigeon pea seeds in doubled-up legume technology to enhance biological nitrogen fixation, which in turn increases the production of chlorophyll contents in crop plants.

CHAPTER 6

GROUNDNUT AND PIGEON PEA DRY VEGETATIVE ABOVEGROUND MASS AND GRAIN YIELD

6.1 INTRODUCTION

Crop yields are affected by a number of environmental factors that can be classified into abiotic and biotic constraints. Groundnut and pigeon pea yields are no exception. Abiotic factors that affect crop yields are soil properties (soil components, soil pH, soil physicochemical and soil biological properties) and climatic stresses (drought, cold, flood, heat stress etc). On the other hand, biotic constraints that affect crop yields include beneficial organisms (pollinators, decomposers and natural enemies), pests (arthropods, pathogens, weeds, vertebrate pests) and anthropogenic factors (Liliane 2020). Most of these crop yield constraints affect yields in the tender stage of plant growth. The main objective of this chapter was to investigate the detailed effect of different treatments on dry vegetative aboveground mass, grain yield and productivity of cropping systems (land equivalent ratio).

6.2 MATERIALS AND METHODS

The general procedures for using inoculation and seed dressing in groundnut – pigeon pea doubled-up legume technology trial can be accessed from Chapter 3. In this section, only the materials and methods relevant to this chapter have been referred to.

6.2.1 Dry vegetative aboveground mass and moisture content

During harvesting time (21 weeks after planting), groundnut plants were dug manually from the net plots. Samples consisting of ±200 g groundnut haulms and leaves (excluding roots) picked at random from each net plot were collected, weighed and put in a drying oven set at 65^{o}C for 72 hours. The samples were reweighed after drying to determine the moisture content. Moisture content was determined by subtracting the weight after drying from the weight before drying. In order to assess the dry vegetative aboveground mass, groundnut haulms from net plots for all treatments were sun- dried separately in a drying room for 2 weeks and then weighed. The masses for dry vegetative aboveground material for all treatments were converted to yield per hectare.

The same procedures were also applied to determine pigeon pea moisture content and dry vegetative aboveground mass. During harvesting time (181 days after planting), all pigeon pea plants from net plots had their pods removed and cut manually at soil level. Samples consisting of pigeon pea stems and leaves (excluding roots) weighing ±200 g were picked at random from each net plot, weighed and put in a drying oven set at 65^{o}C for 72 hours. The samples were reweighed after drying to determine the moisture content. Moisture content was determined by subtracting the weight after drying from the weight before drying. In order to assess the dry vegetative aboveground mass, pigeon pea plants from net plots for all treatments were sun- dried separately in a drying room for 2 weeks and then weighed. The masses for dry vegetative aboveground mass for all treatments were converted to yields per hectare.

6.2.2 Yield (grain weight) for groundnut and pigeon pea

Groundnut pods were harvested and sun-dried for two weeks, then shelled with an electric groundnut sheller (Figure 6.1) and the grain weight per net plot determined. A sample of 100 groundnut seeds from each net plot were first weight and then oven dried at 65^{o}C for 72 hours. The seeds were reweighed after drying in order to determine the moisture content of the seeds, thereafter the economic yield (grain weight) of each treatment was adjusted to standard moisture content of 8% by using the following formulae:

Yield (at 8% grain moisture) = Grain yield × (100 – actual grain moisture %) / 92

Figure 6. 1: Electric groundnut sheller which was used to shell groundnuts at the ARC-Potchefstroom during the research

For pigeon pea, pods were harvested and sun- dried for two weeks, then shelled manually and the grain weight per net plot determined. A sample of 100 pigeon pea seeds from each net plot were first weighed and then oven dried at 65ºC for 72 hours. The seeds were reweighed after drying in order to determine the moisture content of the seeds, thereafter the economic yield (grain weight) of each treatment was adjusted to standard moisture content of 15% by using the following formulae: *Yield (at 15% grain moisture) = Grain yield × (100 – actual grain moisture %) / 85*

6.2.3 Productivity of sole and intercrops

Productivity of sole and intercropped groundnut and pigeon pea was assessed by calculating the land equivalent ratio. Land equivalent ratio is the ratio of the area needed under sole cropping to that of intercropping at the same management level required to give the same yield. When the land equivalent ratio is greater than 1, it indicates that the intercropping is more productive than sole cropping and the opposite is true for the land equivalent ratio of less than 1 (Willey 1979). Land equivalent ratio was calculated as follows:

LER = (Inter YA / Sole YA) + (Inter YB / Sole YB)

Where YA and YB are yields of crop A and B respectively. Inter YA and Inter YB are yields of crop A and B under intercropping. Sole YA and Sole YB are yields of crop A and B in sole stands. Land equivalent ratio was calculated for each treatment combination.

6.3 RESULTS AND DISCUSSION

6.3.1 Grain yield and vegetative aboveground mass

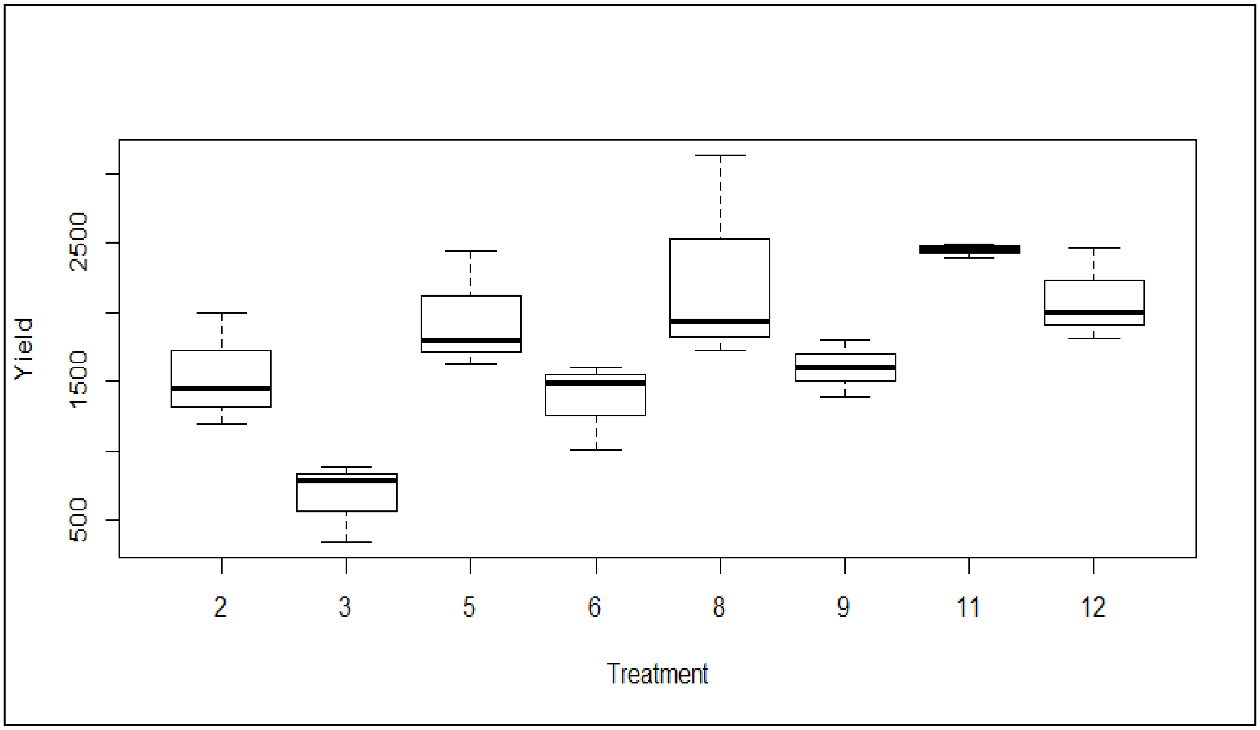
Groundnut and pigeon pea yield performance was assessed by using grain yield, dry vegetative aboveground mass and productivity (land equivalent ratio). Results from figure 6.2 and figure 6.3 show some variations in terms of grain yield and dry vegetative aboveground mass respectively for groundnut while Figures 6.4 and 6.5 show some variations in terms of grain yield and dry vegetative aboveground mass respectively for pigeon pea. It was observed that some sole treatments resulted in higher grain yields and dry vegetative aboveground mass than their respective intercropped treatments. Mhango (2011) reported a potential yield of 2500 kg ha^{-1} for groundnut in sole cropping system, while Kamanga et al. (2019) reported a potential pigeon pea yield of up to 2500 kg ha^{-1} in a sole cropping system. The yields for both crops during the trial were very similar to the potential yields in the literature. This could be due to favourable conditions for both crops. Almost all soil nutrients (Table 3.3) were within their requirements. P was observed to be slightly higher (top soil) than expected in the soil. It could be due to residual P from the previous crops. The site for the trial received a total annual rainfall amount of 855.4 mm. The total annual rainfall was within requirements for the production of both crops as evidenced by Phiri et al. 2013 who explained that the mean rainfall amount of 875 mm is suitable for the production of maize, pigeon pea and groundnut. Neither crop does well in water logged soil conditions. Unlike groundnut, pigeon pea does well in drought conditions due to its deeper root system.

The P-values (0.001 and 0.012) from Table 6.1 indicate that treatment had an effect on both groundnut grain yield as well as dry vegetative aboveground mass respectively, the P- values (0.0002 and <0.001) from Table 6.2 also show that treatment had an effect on both pigeon pea grain yield and dry vegetative aboveground mass respectively. P-values (0.0015 and 0.00095) from Appendix 7 and P-values (0.0028 and <0.001) from Appendix 8 indicate that both cropping systems and treatment had effect on yield for groundnut and pigeon pea respectively while the interaction between cropping system and treatment did not have an effect on yield for both crops as evidenced by p-values (0.7303 and 0.7186) respectively.

Some sole treatments outperformed their respective intercropped treatments in terms of both grain yield and dry vegetative aboveground mass for groundnut and pigeon pea. Similar results were also observed from a study that was carried out by Njira et al. (2021). The study assessed productivity of pigeon pea, cow pea and maize under sole cropping, legume-legume and legume-cereal intercrops on Alfisols in central Malawi. Results showed significantly higher ($p < 0.05$) grain yield per plant for sole cropped pigeon pea and cow pea than their intercrops. Sole cropping encourages limited interspecific competition among crop plants over space, soil moisture and soil nutrients. Such a competition is intense in intercropped treatments more especially during their earlier growth stages. Njira (2016) also argued that intercropping practices unlike sole cropping have challenges such as crop plant interactions that lead to suppression of component crops' yields and nutrient management. The presence of the limited interspecific competition in sole cropping enabled crop plants to have adequate soil moisture, soil nutrients and sunlight (radiation) for physiological processes such as photosynthesis and respiration. The sole crop plants were therefore able to carry out photosynthesis efficiently and produced higher grain yield and dry vegetative aboveground mass than in intercropped treatments. It was also observed that the treatments without inoculation and seed dressing produced relatively lower grain yields and dry vegetative aboveground mass than their respective inoculated /seed dressed treatments. Untreated treatments resulted into poor nodule development that contributed to low chlorophyll production (Figure 5.3 and 5.4). Low chlorophyll production resulted into low grain yield and dry vegetative aboveground mass for both groundnut and pigeon pea. On the other hand, the treatments that were both inoculated as well as seed dressed outperformed their respective treatments. Therefore, the treatments that were both inoculated and seed dressed benefited from both inoculation and seed dressing hence had highest grain yield and dry vegetative aboveground mass among all respective treatments for groundnut and pigeon pea.

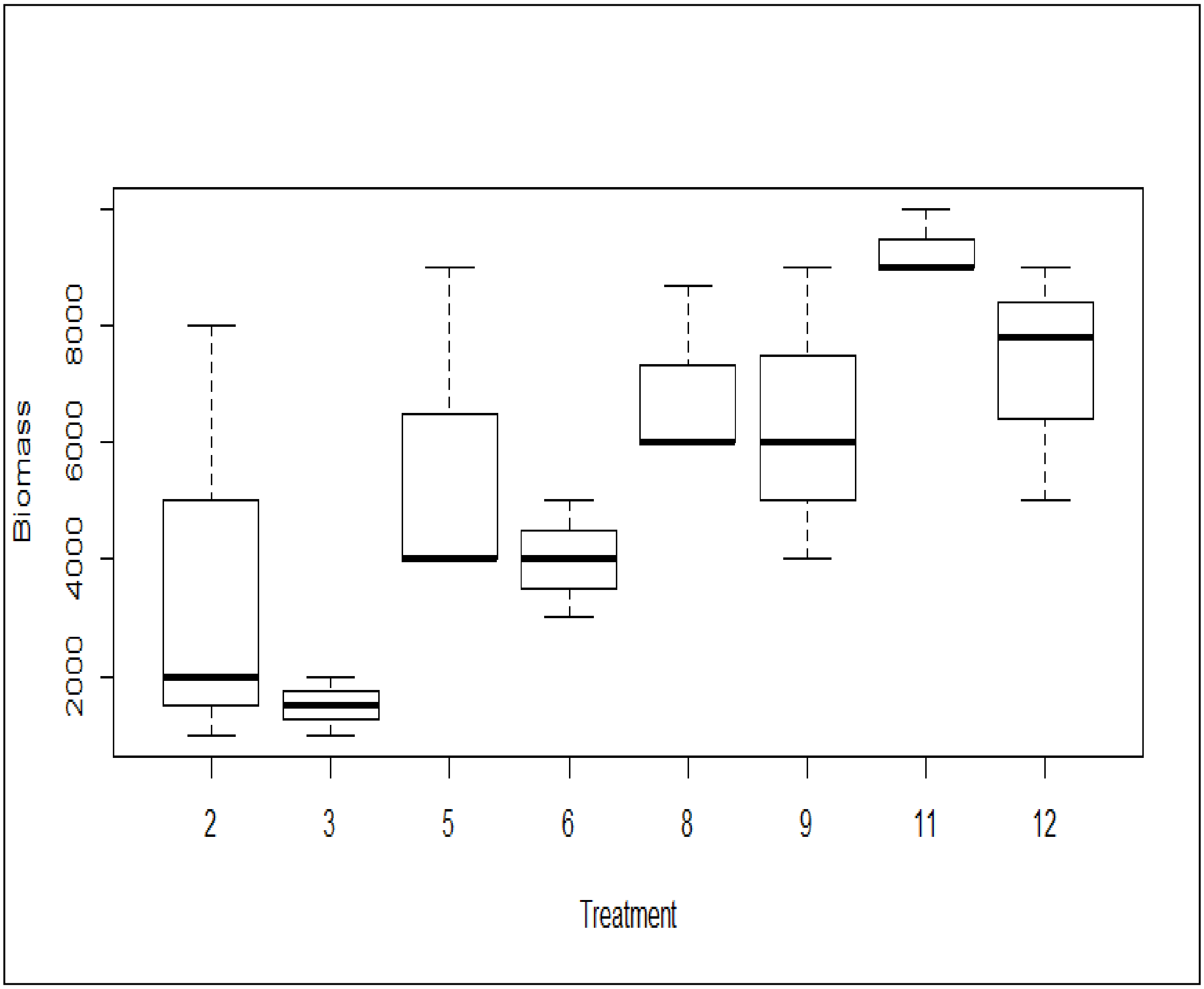
6.3.2 Productivity of sole and intercrops

Land equivalent ratio results from the trial indicated that untreated groundnut-pigeon pea intercrop had a LER of 0.8662, inoculated groundnut – pigeon pea intercrop had a LER of 1.0885, seed dressed groundnut- pigeon pea intercrop had a LER of 1.2648 and inoculated + seed dressed groundnut- pigeon pea intercrop had a LER of 1.7054. Willey (1979) explained that when the land equivalent ratio is greater than 1, it indicates that the intercropping is productive (advantageous) and the opposite is true for the land equivalent ratio of less than 1. Therefore, all intercropped treatments except untreated groundnut-pigeon pea intercrop were productive since their LER were all more than 1. Among the productive intercropping systems, inoculated + seed dressed groundnut-pigeon pea intercrop had the highest LER (1.7054), which indicates that it was the most productive intercrop system.



T2, 5, 8, and 11 are sole crops while T3, 6, 9 and 12 are intercropped

Figure 6. 2: Groundnut grain yield for untreated (T2 and T3), inoculated (T5 and T6), seed dressed (T8 and T9) and inoculated + seed dressed (T11 and T12) treatments observed during harvesting



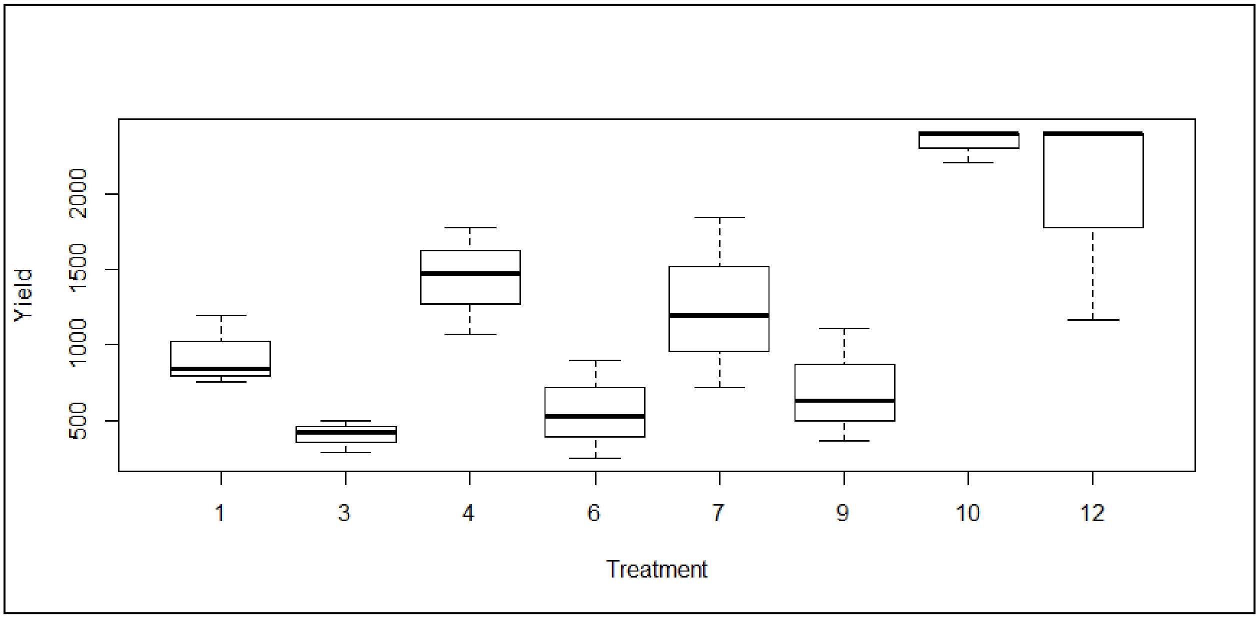
T2, 5, 8, and 11 are sole crops while T3, 6, 9 and 12 are intercropped

Figure 6. 3: Groundnut dry vegetative aboveground mass for untreated (T2 and T3), inoculated (T5 and T6), seed dressed (T8 and T9) and inoculated + seed dressed (T11 and T12) treatments observed during harvesting

Table 6. 1: Groundnut grain yield, dry vegetative aboveground mass and land equivalent ratio

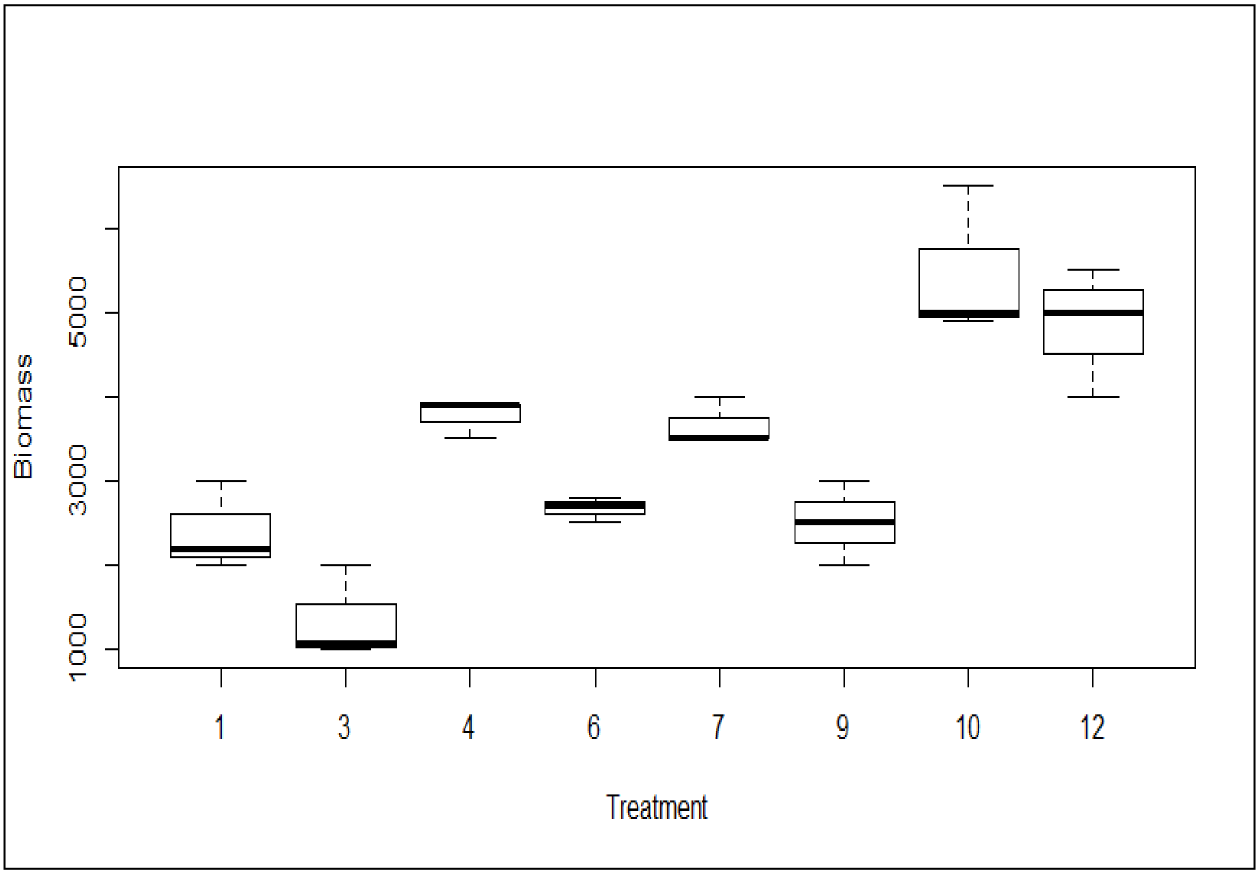
Treatment	Grain yield (kg ha^{-1})	Dry vegetative aboveground mass (kg ha^{-1})	LER
Untreated sole gnut	1550.0^{b}	3666.7^{b}	
Untreated gnut-pp intercrop	676.7^{c}	1500.0^{b}	0.8662
Inoculated sole gnut	1959.0^{b}	5666.7^{a}	
Inoculated gnut - pp intercrop	1371.7^{b}	4000.0^{b}	1.0885
Seed dressed sole gnut	2264.7^{a}	6900.0^{a}	
Seed dressed gnut- pp intercrop	1600.3^{b}	6333.4^{a}	1.2648
Inoculated + Seed dressed sole gnut	2450.7^{a}	9333.4^{a}	
Inoculated + Seed dressed gnut- pp intercrop	2096.3^{a}	7266.7^{a}	1.7054
Multiple R- Squared	0.7298	0.6289	
LSD (0.05)	688.1	3733.8	
P- Value	0.001	0.012	

Key: gnut = Groundnut
pp = Pigeon pea



T1, 4, 7, and 10 are sole crops while T3, 6, 9 and 12 are intercropped

Figure 6. 4: Pigeon pea yield for untreated (T1 and T3), inoculated (T4 and T6), seed dressed (T7 and T9) and inoculated + seed dressed (T10 and T12) treatments observed during harvesting



T1, 4, 7, and 10 are sole crops while T3, 6, 9 and 12 are intercropped

Figure 6. 5: Pigeon pea dry vegetative aboveground mass for untreated (T1 and T3), inoculated (T4 and T6), seed dressed (T7 and T9) and inoculated + seed dressed (T10 and T12) treatments observed during harvesting

Table 6. 2: Pigeon pea grain yield, dry vegetative aboveground mass and land equivalent ratio

Treatment	Grain yield (kg ha^{-1})	Dry vegetative aboveground mass (kg ha^{-1})	LER
Untreated sole pp	931.0^{c}	2393.7^{c}	
Untreated gnut-pp intercrop	400.0^{c}	1350.7^{d}	0.8662
Inoculated sole pp	1442.0^{b}	3766.7^{b}	
Inoculated gnut - pp intercrop	560.0^{c}	2666.7^{c}	1.0885
Seed dressed sole pp	1254.0^{b}	3666.7^{b}	
Seed dressed gnut- pp intercrop	700.0^{c}	2500.0^{c}	1.2648
Inoculated + Seed dressed sole pp	2340.0^{a}	5466.7^{a}	
Inoculated + Seed dressed gnut- pp intercrop	1989.0^{a}	4833.3^{a}	1.7054
Multiple R- Squared	0.7954	0.8909	
LSD (0.05)	696.3	947.6	
P- Value	0.0002	1.3809e-06	

Key: gnut = Groundnut
pp = Pigeon pea

6.4 CONCLUSION

Significant differences among treatments indicate that inoculation and seed dressing had an effect on grain yield and dry vegetative aboveground mass of sole and intercropping systems. Combination of inoculation and seed dressing improved grain yield, dry vegetative aboveground mass and land equivalent ratio. Since treatments (sole and intercrop) for both groundnut and pigeon pea that received both inoculation and seed dressing outperformed their respective treatments in terms of grain yield, farmers should be encouraged to be using inoculated seed dressed seeds during doubled-up legume technology. Secondly, considering the fact that LER for inoculated seed dressed treatment was more than 1, farmers should further be encouraged to be intercropping groundnut with pigeon pea, rather than applying sole cropping.

Considering the fact that the site for the trial received an ideal total annual rainfall amount of 855.4 mm which was within requirements for the production of both crops, the researcher suggests follow-up research work over multiple seasons to assess what would happen in wetter and dryer seasons. Further, the researcher suggests a follow-up research work to assess the carry-over of the fixed N with grain crops.

CONCLUSIONS

This study was carried out at Innovation Africa situated at University of Pretoria (IA@UP), South Africa. Through doubled-up legume technology trial, the effect of different treatments on the following aspects were evaluated:

- Germination rate
- Survival %
- Nodulation effectiveness
- Dry vegetative aboveground mass
- Grain yield
- Productivity (Land equivalent ratio)

From the findings, the following conclusions were made;

In **Chapter 4,** the effect of different treatments on germination and survival of the two crops were investigated. The investigation involved a trial with 12 treatments. Some treatments were seed dressed while others were not. The type of a treatment did not have any significant effect on seed germination but there were significant differences among treatments in terms of seedling survival. Seed germination assessment was done two weeks after planting and it was observed that all treatments indicated seed germination % of at least 80%. Survival rate was assessed one week before harvesting. There were significant differences among treatments on survival rates (plants that survived). All treatments that were seed dressed indicated higher survival rates than treatments that were not seed dressed. Hence, farmers should always consider seed dressing crop seeds before planting in groundnut – pigeon pea intercropping as this contributes to high survival rates of seedlings.

In **Chapter 5,** the effect of different treatments on nodule effectiveness and leaf chlorophyll content were investigated. The investigation involved a trial with 12 treatments. Some treatments were inoculated while others were not. The type of a treatment had effect on both nodule effectiveness and leaf chlorophyll content among treatments. Both nodule effectiveness and leaf chlorophyll content were examined during plant vegetative growth stage. All treatments where seeds were inoculated indicated both higher nodule effectiveness rates and leaf chlorophyll content. Inoculation generally improved nodule effectiveness and leaf chlorophyll content for both groundnut and pigeon pea plants. Inoculation encouraged good nodule development that in turn resulted into effective biological nitrogen fixation. Effective biological nitrogen fixation resulted into availability of enough leaf chlorophyll that plants need for plant physiological processes. Hence, farmers should always be encouraged to inoculate crop seeds before planting in groundnut – pigeon pea intercropping as this improves both nodule effectiveness as well as leaf chlorophyll content in crop plants.

In **Chapter 6,** the effect of different treatments on grain yield, dry vegetative aboveground mass and land equivalent ratio were investigated. The investigation involved a trial with 12 treatments. Significant differences among treatments in terms of grain yield and dry vegetative aboveground mass were observed. Treatments that involved both inoculation and seed dressing outperformed their respective treatments in both sole and intercrops in terms of grain yield. The combined use of inoculation and seed dressing contributed to high yields on treatments that used both inoculation and seed dressing due to high plant survival rate, high levels of leaf chlorophyll and effective biological nitrogen fixation. Thus, the combination of inoculation and seed dressing improved grain yield and land equivalent ratio. Since treatments (sole and intercrop) that used both inoculation and seed dressing outperformed their respective treatments, farmers should be encouraged to be using inoculated seed dressed seeds in doubled-up legume cropping. In addition, considering the fact that LER for the inoculated seed dressed treatment was more than 1 and highest among all intercropped systems, farmers should further be encouraged to be intercropping groundnut with pigeon pea against sole cropping.

SUMMARY

To investigate the impact of inoculation and seed dressing on doubled-up legume technology, an experiment was conducted at Innovation Africa situated at the University of Pretoria (IA@UP) in South Africa. A field trial with 12 treatments was used to determine the effect of inoculation and seed dressing on doubled-up legume technology in the 2020/2021 growing season. It was hypothesised that (i) yield from sole cropped treatments would be higher than the respective intercropped treatments due to limited competition in pure stands (ii) Groundnut and pigeon pea yield would be higher in treatments with seed dressing combined with *Rhizobia* inoculation due to initial plant immunisation against early pests and diseases through seed dressing and effective nodulation through inoculation and (iii) Land equivalent ratio (LER) for the treated (inoculated, seed dressed and inoculated + seed dressed) treatments would be more productive than untreated treatments.

A set of objectives were used to either accept or reject those hypotheses. The objectives included evaluation of seed germination and survival rate for seed dressed and the control, evaluation of nodule effectiveness and leaf chlorophyll content for the inoculated and the control, estimation of disease severity for the treated (seed dressed) and the control, estimation of pest infestation in seed dressed and the control and evaluation of the effect of treatment on yield and productivity (LER). Considering the results from the trial, all hypotheses were accepted except that not all yields from sole cropped treatments were higher than their respective intercropping treatments.

Germination rate among the treatments was not significantly different but the treatments significantly affected the seedling survival rate. Treatments that were seed dressed had higher seedling survival rate than untreated treatments. Treatments that were inoculated indicated higher nodulation effectiveness as well as leaf chlorophyll content than untreated (uninoculated) treatments. Significant differences in terms of grain yield and dry vegetative aboveground mass were observed among treatments. Treatments (sole and intercrop) that were both inoculated and seed dressed outperformed their respective treatments in terms of grain yield and land equivalent ratio.

The researcher recommends the following:

- Follow-up research work over multiple seasons to assess what would happen in wetter and dryer seasons.
- Follow-up research work to assess the carry-over of the fixed N with grain crops.

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APPENDICES

Appendix 1: Germination rate for all treatments per crop observed at 2 weeks after planting

Source of Variation	SS	Df	MS	F	P-value	F crit
Pigeon pea						
Between Groups	386.625	7	55.23214	0.623212	**0.729876**	2.657197
Within Groups	1418	16	88.625			
Groundnut						
Between Groups	518.625	7	74.08929	0.968487	**0.48567**	2.657197
Within Groups	1224	16	76.5			

Appendix 2: Germination rate for all treatments observed at 2 weeks after planting

Source of Variation	SS	Df	MS	F	P-value	F crit
Between Groups	905,25	15	60,35	0,730961	**0,736453**	1,99199
Within Groups	2642	32	82,5625			
Total	3547,25	47				

Appendix 3: Survival rate for all treatment combinations per crop observed at the time of harvesting

Source of Variation	SS	Df	MS	F	P-value	F crit
Pigeon pea						
Between Groups	7008	7	1001.14	27.4286	**9.12092E-08**	2.6572
Within Groups	584	16	36.5			
Groundnut						
Between Groups	7503.29	7	1071.9	39.4564	**6.33665E-09**	2.6572
Within Groups	434.667	16	27.1667			

Appendix 4: Survival rate for all treatment combinations observed at the time of harvesting

Source of Variation	SS	Df	MS	F	P-value	F crit
Between Groups	14616,31	15	974,4208	30,61008	**9,57E-15**	1,99199
Within Groups	1018,667	32	31,83333			
Total	15634,98	47				

Appendix 5: Nodule effectiveness for all treatments per crop observed during vegetative growth

Source of Variation	SS	Df	MS	F	F crit
Groundnut					
Between Groups	11.625	7	1.660714	**65535**	2.657197
Within Groups	0	16	0		
Pigeon pea					
Between Groups	5.625	7	0.803571	**65535**	2.657197
Within Groups	0	16	0		

Appendix 6: Nodule effectiveness for all treatments observed during vegetative growth

Source of Variation	SS	Df	MS	F	F crit
Between Groups	29,25	15	1,95	**65535**	1,99199
Within Groups	0	32	0		
Total	29,25	47			

Appendix 7: Interactions between groundnut cropping systems and treatments

	Sum Sq	Df	F value	Pr(>F)
Cropping system	2305160	1	14.5849	0.0015109 **
Treatment	4318876	3	9.1086	0.0009462 ***
Cropping system: Treatment	206683	3	0.4359	0.7303122
Residuals	2528822	16		

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Appendix 8: Interactions between pigeon pea cropping systems and treatments

	Sum Sq	**Df**	**F value**	**Pr(>F)**
Cropping system	2014921	1	12.4498	0.002791 **
Treatment	7831587	3	16.1299	4.283e-05 ***
Cropping system: Treatment	220081	3	0.4533	0.718568
Residuals	2589498	16		

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1