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**THE EFFECTS OF *RHIZOBIUM* INOCULATION ON GROWTH PERFORMANCE,
FORAGE PRODUCTION, NUTRIENT AND ANTI-NUTRIENT CONTENT OF
LABLAB PURPUREUS CULTIVARS**

BY

S.P. SENTI (201201817)



**A dissertation submitted in partial fulfillment of the requirements for the degree of
Master of Science in Agriculture (Pasture Science)**

University of Fort Hare
Together in Excellence
Department of Livestock and Pasture Sciences

Faculty of Science and Agriculture

University of Fort Hare

Alice, Eastern Cape

South Africa

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2017

**RHIZOBIUM INOCULATION EFFECTS ON GROWTH PERFORMANCE,
FORAGE PRODUCTION, NUTRIENT AND ANTI-NUTRIENT CONTENT OF
LABLAB PURPUREUS CULTIVARS**

By

SENTI SIBONGILE PORTIA

**A dissertation submitted in fulfilment of the requirement for the degree of
MASTER OF SCIENCE IN AGRICULTURE (PASTURE SCIENCE)**

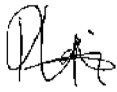
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2017

DECLARATION

I, **Sibongile Portia Senti**, declare that the dissertation hereby submitted for the degree of Master of Science in Agriculture (Pasture Science) at the University of Fort Hare is my work and all the reference materials contained in this dissertation have been acknowledged accordingly. This dissertation has not been previously submitted to another university for any other degree.



Sibongile Sibongile Portia

DEDICATION

I dedicate this work to my loving parents, my late father Moffat Senti and my dearest mother Nobetu Senti.



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The success and final outcome of this study required a lot of guidance and assistance from many people, and I am extremely privileged to have got this all along the completion of my research. All that I have done is only due to such supervision and assistance and I would not forget to thank them.

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Lastly, my special and most sincere gratitude goes to my family, especially my mom, for love, support and prayers.

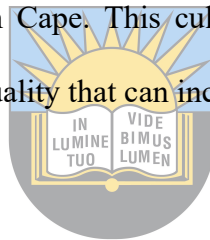
ABSTRACT

Declining soil fertility and inadequate low quality feed resources limit smallholder livestock production in the semi-arid regions of Eastern Cape in South Africa. Smallholder farmers are facing problems of high cost of artificial fertilisers limiting them in production of cereal crops and also fodder for their livestock. This leads to poor nutrition, particularly in the dry season, it affects livestock production. Planting *Lablab purpureus* and inoculating with *Rhizobium* would limit the necessity for buying expensive protein supplements and nitrogen fertilisers. *Lablab purpureus* can provide feed in the dry season for the improvement of livestock production. The objective of this study was to investigate the effect of *Bradyrhizobium japonicum* inoculation on growth performance, biomass production, nutrient and anti-nutrient content of the two *Lablab purpureus* cultivars (Rongai and Highworth). The experiment was conducted at Fort Hare Research Farm in Alice, South Africa. Data was collected on plant seedling emergence, plant height, stem diameter, plant vigour, chlorophyll content, nodule numbers as well as dry matter content, biomass yield, nutrient and anti-nutrient contents at 30, 60 and 90 DAP. Samples of *Lablab purpureus* were harvested at flowering stage and analysed for dry matter content, biomass yield, chemical, mineral and anti-nutrient composition.

The results showed that *Rhizobium* inoculation significantly increased ($p < 0.05$) seedling emergence, plant height and stem diameter of both *Lablab purpureus* cultivars. *Rhizobium* inoculation had no significant effect ($p > 0.05$) on chlorophyll content for both cultivars. Highworth had significantly higher ($p < 0.05$) plant vigour than Rongai on both inoculated and uninoculated. Varieties and inoculation effects were significant ($p < 0.05$) for number of nodules, nodulation rate, active nodules and nodule dry yield. *Rhizobium* inoculation significantly ($p < 0.05$) increased fresh matter yield, dry matter yield and dry matter content on both cultivars.

There were no significant differences ($p>0.05$) in NDF, ADIN, ADL, ADF, and Na between inoculated and uninoculated plants for both cultivars. The inoculation of *Rhizobium* improved CP (15.65%) in Highworth cultivar. The inoculation of *Rhizobium* significantly increased Ca (1.76-1.84%), Zn (191.91-2 8.33ppm) and Cu (3.50-6.16ppm) compared to un-inoculated plants. Varieties and *Rhizobium* had no interaction ($p>0.05$) with condensed tannin, hydrolysable tannin, total polyphenols and saponins . Therefore, the *Rhizobium* inoculation assisted in improving quality in terms of nutrient composition and decreasing anti-nutrients. It is recommended to use both cultivars of *Lablab purpureus*.

Based on the results of this study it is concluded that *Rhizobium* inoculation increases yield and forage quality of *Lablab purpureus*. It is recommended that Highworth cultivar can be used by communal farmers in the Eastern Cape. This cultivar showed that it needs *Rhizobium* inoculation inorder to produce high quality that can increase livestock production.

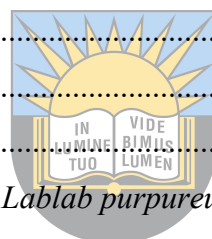


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Key words: *Lablab purpureus*, *Rhizobium* inoculation, forage quality, tannin content

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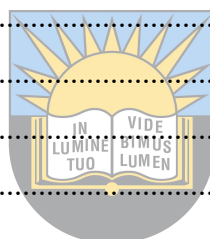
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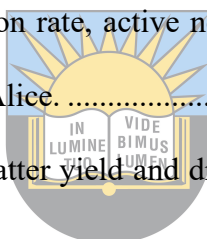
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CHAPTER 1: INTRODUCTION

1.1 Background of the study

Livestock production is an important agricultural activity in South Africa and significantly contributes to food security and clothing; it also provides many social and economic benefits to the country as a whole (Meissner *et al.*, 2013). Globally, livestock products contribute 33% to the world's agricultural Gross Domestic Product (GDP), and the percentage is increasing rapidly (Thornton, 2010). There is a huge demand for livestock foods especially meat due to the increase in world's human population (Scollan *et al.*, 2010; Meissner, 2012). Generally, climate, diseases and nutrition are the main factors limiting livestock production (Lamy *et al.*, 2012).

Poor livestock nutrition is a significant problem that smallholder farmers face, especially during the dry season (Bayble *et al.*, 2007). During this season, forage from natural pastures and crop residues is generally inadequate and is of low quality; and it provides insufficient nutrients to grazing livestock (Amole *et al.*, 2013). This poor quality results in animals losing body weight of approximately 20 – 40% at the onset of the dry season (Rojas-Downing *et al.*, 2017), which negatively affects reproduction and meat quality (Nardone *et al.*, 2010). Normally, in some areas crop residues are used in the dry winter season to improve the fodder flow (Macedo *et al.*, 2015; Molesworth, 2015), but poor quality limits their sole use and thus protein-rich leguminous forages are sometimes used to provide adequate nutrients to the animals (Amole *et al.*, 2013). The production of these leguminous forages in semi-arid communal areas is limited by low rainfall and infertile soils, and the threat of climate change has aggravated the situation (Orodho, 2006).

Most arable lands are now abandoned in South Africa's communal areas, especially those in Eastern Cape (Andrew and Fox, 2004). The estimation of unutilised land ranges from 20 - 100%, where parts of Ciskei recorded 100% of land abandonment (Andrew *et al.*, 2003). There is little maize cultivation because of less income compared to cash crops. People prefer to just buy maize meal because it is uneconomic to plant for consumption and that has reduced subsistence farming in Eastern Cape. Also soils are poor due to the history of monocropping, thus farmers are forced to use chemical fertilizers to promote growth and production. The practice of monocropping depletes nutrients in the soil (making the soil less productive over time), so that is the reason farmers use chemical fertilisers to treat these poor soils. According to Gulwa *et al.* (2017), most small-scale farmers do not have enough money to buy these chemical fertilizers for their crop and pasture lands, however, presence of legumes can be important in maintaining the fertility of marginal lands.

During the winter or dry season, farmers become affected by a shortage of good quality forage in South Africa. *Lablab purpureus* is a summer-growing legume, that can be used as an alternative feed for animals during the winter/dry season. According to Hassan *et al.* (2014), when fibrous crop residues and forage materials such as maize stover is supplemented with lablab forage then fed to ruminant animals, that usually improves their performance. Studies in SA (Makgoga, 2013; Mthembu *et al.*, 2018) evaluated the effect of intercropping *Lablab purpureus* with maize to improve fodder production and soil fertility. Mthembu *et al.* (2018) found that intercropping maize with *Lablab purpureus* improved the yield and quality of maize grain. Makgoga (2013) showed that intercropping with cultivar Rongai decreased grain yield of maize and soil fertility status. The problem was that during intercropping, fertilizer or manure application was excluded. Also, inoculation of legume seeds with specific bacteria to improve biological nitrogen fixation and plant growth also needs to be included. Inoculation of legumes with effective *Rhizobium* during planting can improve soil fertility and thus yield and

quality of the legume forage, and also provide a substitute to inorganic fertilisers (Chemining'wa *et al.*, 2007).

Hassan *et al.* (2014) mentioned that *Lablab purpureus* is one of the popular leguminous forage species in the world. The two common varieties of *Lablab purpureus* are Rongai and Highworth. According to Maass *et al.* (2010), *Lablab purpureus* can tolerate drought conditions compared with other legume species like *Phaseolus vulgaris* or *Vigna unguiculata*. In some regions *Lablab purpureus* also remains green during the dry season (Aganga and Tshwenyane, 2003) and when it is green, the mineral content is high. The benefit of this legume staying green is that it becomes a cover crop and thus conserving moisture. When it dies or when its leaves fall and decompose, this provide organic matter to improve soil fertility. Giller (2001) reports that legumes can fix nitrogen as much as 200 kg N ha⁻¹ year⁻¹ under optimal field conditions, while McDonald *et al.* (2001) mentioned that Lablab can fix 177 kg N ha⁻¹. Chemining'wa *et al.* (2007) mentioned that such fixation is attained in the presence of efficient natural rhizobial strains, or those introduced in the form of commercial inoculants.

The available cultivars in South Africa are the early-flowering Highworth and late-flowering Rongai. Highworth has high seed yielding potential and is appropriate for pulse production and forage use (Aganga and Tshwenyane, 2003). Rongai cultivar continues growing even in autumn season and provides good fodder in winter (Nichols *et al.*, 2007), and it is not good for hay making. This study was set up to to determine the effect of *Rhizobium* inoculation on growth performance, forage production, nutrient and anti-nutrient content of Rongai and Highworth in Alice, Eastern Cape.

1.2 Problem statement

Poor nutrition, especially during the dry season, affects livestock production for smallholder farmers (Simbaya, 2002). Furthermore, the threat of climate change severely worsened the plight of these poorly resourced smallholder farmers. During the dry season the grazing areas are characterised by low quantity and quality of forage. Farmers mitigate the situation by supplementing forage grazed from the rangeland with crop residue such as stover which is nutritionally enhanced with urea (Mudzengi *et al.*, 2014) or other expensive protein supplements. Affordable supplementary protein sources need to be developed for smallholder farmers in communal areas, and forage legumes can be that potential source.

Forage legumes fix nitrogen, and they are usually intercropped with cereal crops to boost the protein content of cereals (Eskandari *et al.*, 2009). Their inherent ability to fix nitrogen results in reduced cost associated with the use of inorganic fertilisers in infertile soils, typical of arable lands of smallholder farmers (Mabrouk *et al.*, 2018). Problems of high cost of inorganic fertilizers limit communal farmers in the production of cereal crops and also fodder for their livestock (Mia and Shamsuddin, 2010). Most of the arable soils in communal areas are less fertile due to the history of maize monoculture. In addition the high incidence of drought which might be due to climate change has exacerbated the situation. Climatic changes can also reduce the presence of the natural rhizobia in the soils. According to Simon *et al.* (2014), population of *Rhizobium* and *Bradyrhizobium* species native to the soil differ in their tolerance to major environmental factors. Therefore, use of bio-fertilisers such as *Rhizobium* and arbuscular mycorrhizal fungi can reduce such challenges.

Generally, grain and fodder plants require nitrogen for production of green yield and forage (Ayub *et al.*, 1999). However, for successful biological nitrogen fixation forage legumes need need phosphorus (Wagner, 2011; Mabrouk *et al.*, 2018), which is low in semi-arid soils. According to Tiaro and Ndakudemi (2013), the majority of African soils have low levels of nitrogen and phosphorus, hence they are less capable of supporting leguminous crop growth. The effect of *Rhizobium* on growth and forage production has been studied in some legumes under pot experiments, different soil types, moisture, and fertiliser regimes by Egamberdieva *et al.* (2010); Amel *et al.* (2014); and Tufail *et al.* (2018) among others. However, there have been no studies on the effect of *Rhizobium* on growth, yield and nutritional composition of different cultivars of *Lablab purpureus* under field conditions. Thus, this study will be of great help to smallholder farmers as they will get the information about the effect of *Rhizobium* inoculation on growth performance, forage production, nutrient, and anti-nutrient content of the cultivars Rongai and Highworth.



1.3 Justification

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Information gained from this research would help in identifying the best performing *Lablab purpureus* cultivar when inoculated with *Rhizobium*, and this cultivar can be recommended for use by communal farmers in Alice, Eastern Cape. The research findings would also be used by extension agents during their farmer advisory sessions on the benefits of using *Rhizobium* in forage production. The use of *Rhizobium* will be a great help to smallholder farmers who often farm in infertile soils and have little or no access to mineral fertilisers due to financial challenges. In addition, the knowledge produced in this research will open a gap for researchers to compare the performance of *Rhizobium* to other bacteria species used elsewhere.

Planting the drought tolerant *Lablab purpureus* crop would reduce the need for buying expensive protein supplements in winter as the crop can be stored as hay. Although *Lablab*

purpureus and other legumes are able to fix atmospheric nitrogen through *Rhizobium* species living in roots nodules, seed inoculation is practised to ensure optimum *Rhizobia* population (Ahmad *et al.*, 2008). Amel *et al.* (2014) reported that when symbiotic association of *Bradyrhizobium japonicum* and *Lablab purpureus* plants is achieved, *Lablab purpureus* can fix up to 400 kg N ha⁻¹ year⁻¹. This would improve fertility, soil ground cover as well as crude protein content of the plant. Biological nitrogen fixation can reduce the need for nitrogen fertilisers thereby alleviating environmental damage, resulting in an economy estimated in US\$ 3 billion per crop season (Nicolás *et al.*, 2006). *Rhizobium* inoculation can also reduce the need for expensive and environmentally damaging nitrogen fertilizer (Amel *et al.*, 2014). Use of the bio-fertilisers can also prevent the reduction of the soil organic matter (Mia and Shamsuddin, 2010).



1.4 Objectives

1.4.1 General objective

To investigate the effect of *Rhizobium* inoculation on growth performance, forage production, nutrient and anti-nutrient content of the *Lablab purpureus* cultivars.

1.4.2 Specific objectives

- To determine the effect of *Rhizobium* inoculation on growth performance and biomass production of Rongai and Highworth *Lablab purpureus* cultivars.
- To determine the effect of *Rhizobium* inoculation on nutrient and anti-nutrient content of Rongai and Highworth *Lablab purpureus* cultivars.

1.5 Hypotheses

H₀: *Rhizobium* inoculation has no effect on growth performance and biomass production of the lablab cultivars Rongai and Highworth.

H₀: *Rhizobium* inoculation has no effect on nutrient and anti-nutrient content of the lablab cultivars Rongai and Highworth cultivars.



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CHAPTER 2: LITERATURE REVIEW

2.1 Introduction

Lablab purpureus is an annual tropical and subtropical legume belonging to the family *Fabaceae* under subfamily *Faboideae* (Tainton, 2000; Pengelly and Maass, 2001). In more temperate climates, its growth is challenged and does not produce pods so well, but it can still be rewarding to grow it. Murphy and Colluci (1999) mentioned that the crop grows well in areas receiving 600-2500 mm annual rainfall. *Lablab purpureus* grows in a range of soils from deep sands to heavy clays (Madzonga and Mogotsi, 2014) and it performs well in low fertility soils (Mugambi, 2013). This legume does not tolerate prolonged waterlogging, and it is less vulnerable to root diseases compared to other legumes (Valenzuela and Smith, 2002).

According to Macedo *et al.* (2015), *Lablab purpureus* is an annual legume capable of producing large quantities of high quality above ground biomass for livestock feed. This leguminous crop has an ability to be an outstanding resource for tropical agricultural systems and is also capable of improving livestock fodder (Kimani *et al.*, 2012). Furthermore, as other forage legumes it improves soil fertility through nitrogen fixation (Peoples and Baldock, 2001) which results in improved yields when intercropped with cereals as commonly done in tropical farming systems. *Lablab purpureus* leaves contain an average of 24% crude protein (Ogundipe *et al.*, 2003), its high yield and protein content makes the plant a valued forage for livestock especially during dry seasons (Heuzé *et al.*, 2016). Furthermore, this legume can also be used in other different forms such as silage and hay (Aganga and Autlwetse, 2000). In several experiments, results showed that lablab increased livestock weight and milk production during the dry season (Malami and Suleiman, 2007; Amole *et al.*, 2013). *Lablab purpureus* is also used in ethnoveterinary medicine such as in the treatment of eye problems in sheep, as well as lung problems in sheep, cattle and goats (Adebisi and Bosch, 2004). The

benefits of this legume in livestock nutrition and health is significant, therefore, its improved production will immensely contribute to livestock productivity and ultimately food security.

2.2 *Lablab purpureus*

2.2.1 Rongai cultivar

The Rongai cultivar is the oldest cultivar, which was introduced in New South Wales, Australia in 1962 (Pitman and Sotomayor-Rios, 2000). It was derived from plant materials collected in the Rongai district of Kenya (Murphy and Colucci, 1999). Rongai has a vigorous and leafy growth, and it has white flowers and two to four seeds per pod. The seed colour varies from buff to pale brown, while some are light brown and they have a linear white hilum (Fairey *et al.*, 1997). Rongai is a late flowering cultivar that grows until it is damaged by frost (Aganga and Tshwenyane, 2003). According to Ayisi *et al.* (2004), presently Rongai, which is a long season forage type (greater than 150 days to maturity), is the only commercial lablab cultivar in South Africa.. The inoculation of Rongai seeds with host-specific bacteria did enhance biological nitrogen fixation and plant growth (Makgoga, 2013). The experiment that was conducted by Ayisi *et al.* (2004) in Limpopo showed that Rongai produced yield of less than 50 kg ha⁻¹ biomass and the following inoculation with an appropriate commercial Bradyrhizobium inoculant. Odhiambo (2004) also conducted a field research in Limpopo and he found that the Rongai cultivar constantly produced more than 2200 kg ha⁻¹ of biomass without seed inoculation.

2.2.2 Highworth cultivar

The Highworth cultivar originates is Coimbatore, South India and is morphologically similar to Rongai (Venkatesha *et al.*, 2013). According to Cameron (2013), the similarity is in vegetative growth, it however differs from Rongai because of the purple flowers, smaller seeds that are black when mature, or dark brown when ripened rapidly. Aganga and Tshwenyane (2003) further mentioned that Highworth starts to bloom flowers earlier than Rongai and matures earlier in May. Forage yield of Highworth is lower, but seed yield is higher than for

Rongai (Cameron, 2013). The study by Kawonga *et al.* (2005) showed mean average yield of Highworth of 1270 kg ha⁻¹ biomass following phosphorus fertilization.

2.3 Factors affecting the growth of *Lablab purpureus*

2.3.1 Rainfall

Shortage of rainfall is one of the major factors that limit the growth and yields of legumes, especially during the critical period of blooming and pod setting stage (Podlesny and Podlesna, 2011). Therefore, rainfall is regarded as one of the major limiting climatic factors affecting plant growth (Bewket, 2009). Rainfall produces the water that serves as a medium through which nutrients are transported for crop development (Ndamani and Watanabe, 2015). Monthly rainfall of approximately 100mm produced 977 kg leaf ha⁻¹ and 2123 kg stem ha⁻¹ yield of Highworth variety (Amole *et al.*, 2013). It was observed that a 10% decrease in seasonal rainfall from the long-term average generally translates into a 4.4% decrease in forage production (Ndamani and Watanabe, 2015). Podlesny and Podlesna (2011) reported that rainfall shortage in the growing season decreased the height of *Lablab purpureus* plants at blooming by 30%, while high rainfall in the following growing season significantly improved height at blooming. Rainfall variability from season to season significantly affects soil water availability to crops, and thus poses crop production risks (Woo, 2010).

Shortage of rain in spring and summer negatively affected height of *Lablab purpureus* and restricted the leaf area (Podlesny and Podlesna, 2011). On the other hand, heavy rainfall after planting can also negatively influence crop productivity in several ways, including direct physical damage, restricted root development, oxygen deficiency and nutrient loss (Rootman *et al.*, 2004). According to Li *et al.* (2019), heavy rainfall can reduce the yield more in cooler areas and the effect is worsened in areas that have poor drainage. Generally rainfall has a positive effect on crop production, but heavy and light rainfalls have negative effects on production.

The study conducted by Ayisi *et al.* (2004) in Dalmada, where Rongai cultivar was used revealed that *Lablab purpureus* is likely to grow well in semi-arid areas. Hassan *et al.* (2014) planted *Lablab purpureus* in semi-arid regions of Nigeria during the dry season but under irrigation, and the results showed that at 15 weeks after sowing this plant can produce the highest dry matter yield which can be used as hay or silage.

2.3.2 Soil properties

Lablab purpureus is well known as being drought tolerant (Valenzuela and Smith, 2002). This leguminous crop grows well at soil pH values between 4.5 and 6.5, it tolerates infertile and acidic soils better than most legumes (Mthembu *et al.*, 2018). Legumes are significant in agriculture as they accommodate bacteria in their root nodules that fix atmospheric nitrogen (Delfin *et al.*, 2008) in the soil. Valenzuela and Smith (2002) state that *Lablab purpureus* is a good N-fixer, it is rich in nitrogen and is thus used as green manure to increase soil organic matter. The availability of macro-elements (Ca, K, Mg, Na and S) as well as most of the micro-nutrients is directly influenced by soil pH (Donaldson, 2001). Xu *et al.* (2003) reported that a high level of soil acidification is a major factor that is limiting crop production. Low pH is corrected by adding lime. Most soils of the Eastern Cape (EC) Province of South Africa (SA) have poor agricultural sustainability due to soil erosion and fertility deterioration (Mukumbareza, 2014). Low soil fertility is considered a significant cause for the low productivity of many soils.

One of the primary interests of agriculturalists is the use of soil as a medium for plant production (Benjamin *et al.*, 2003). According to Mugambi (2013), nitrogen-fixing legumes improve soil structure through practices such as green manuring and crop rotation. *Lablab purpureus* is able to grow in a wide range of soil types from deep sands to heavy black clays (Madzonga and Mogotsi, 2014). Washaya *et al.* (2018) conducted a study in Alice, Eastern

Cape where there are deep and alluvial soils and 300 kg ha⁻¹ of single superphosphate fertiliser was applied in that soil which then produced *Lablab purpureus* plants with the highest nutrient concentrations at flowering stage.

2.3.3 *Rhizobium* Inoculation

Rhizobium is a symbiotic bacteria that causes the development of root nodules that fix nitrogen in leguminous plants (Long, 1989), and this results in improved yield with little nitrogen fertilizer application (Amole *et al.*, 2001). *Rhizobium* inoculation is an important technology for improving crop productivity and soil fertility (Fuskhah and Darmawati, 2019). Leguminous crops are usually inoculated with selected strains of *Rhizobium* with the expectation that inoculation will increase nitrogen fixation and crop yield (Singleton and Tavares, 1986). Association of leguminous plants and *Rhizobium* have the greatest quantitative impact on the nitrogen cycle (Zahran, 2001). Other genera, such as *Azorhizobium* and *Bradyrhizobium* can also nodulate leguminous plants, and together with *Rhizobium*, they are referred to as rhizobia (Mugambi, 2013). It is important to inoculate legume seed at planting with the correct strain of *Rhizobium* to ensure adequate plant growth.

According to Chemining'wa *et al.* (2007), *Rhizobium* inoculation improves nodule biomass, nodulation and biomass production. Rhizobia are very sensitive to salt stress and therefore, the effect of *Rhizobium* inoculation on the growth and yield of a plant also differs depending on the salinity of the soil (Tufail *et al.*, 2018). Inoculation is, however, not common and it does not always produce positive responses (Chemining'wa *et al.*, 2007). Thus, inoculation of legumes is essential in the absence of well-matched *Rhizobium* and when rhizobial populations are low or unproductive in fixing nitrogen in the soil (Catroux *et al.*, 2001). A study conducted by Amel *et al.* (2014) showed that *Bradyrhizobium* inoculation improved dry weights of shoots and nodule number in each *Lablab purpureus* plant compared to uninoculated plants. Similar

findings were established by Ahmed *et al.* (2011), where *Rhizobium* inoculation increased *Lablab purpureus* shoot dry weight relative to the uninoculated plants.

2.4 Growth performance of cultivars

2.4.1 Seed emergence

The factors that affect seed emergence, are obviously many starting from soil properties (moisture, fertility, texture, structure, soil temperature), depth of planting, and seed quality. Butler *et al.* (2014) reported 82% *Lablab purpureus* germination. These results were similar to those of Karuma *et al.* (2011) who observed 80% *Lablab* seed germination. Niewenhuis and Nieuwelink (2002) mentioned that the seed must first at least absorb 50% of its weight in water. Generally, germination and emergence of *Lablab purpureus* seeds take about 4 to 10 days (Maass, 2006). The emergence occurred within 5-7 days after planting under annual rainfall amounts of 519.40 mm.

Minta and Tsige (2014) noted that seed inoculation and application of 18 kg ha⁻¹ phosphorus positively enhanced vigour of legume seeds, resulting in the early formation of some plants. Pawar *et al.* (2014) reported that seeds coated with *Rhizobium* and plants grown in soil that was inoculated with *Rhizobium* had high seed germination compared with those not inoculated and showing that *Rhizobium* can be successfully used as a bio-fertiliser to improve the seed germination.

The study by Brandon and Jones (1998) recorded 93% of Highworth emergence at 14 days after planting. Similarly, McDonald *et al.* (2002) observed that inoculated Highworth germination was greater than 80 and 90% at 14 days after planting. Nworgu and Ajayi (2005) used Rongai cultivar where 95% was obtained in 2002 and 97% in 2001 was recorded at 4 weeks after planting. Similar results were found by Whitbread *et al.* (2011), who observed that

germination of Rongai Lablab cultivar was greater than 80% at 4 weeks after planting. Thus, both *Lablab purpureus* cultivars generally have good seed emergence or germination.

2.4.2 Plant ground cover

Ground cover is defined as any material on or near the soil surface that protects the soil against raindrops and overland flow (Lang and McDonald, 2005). Legumes are able to provide good ground cover, just by minimizing soil erosion through limiting runoff and raindrop effect (Mureithi *et al.*, 2003). Lang and McDonald (2005) mentioned that where the soil is left exposed or bare, up to 100 t ha⁻¹ of valuable topsoil can be lost in a year. Cover crops prevent leaching of nitrogen and potassium and possibly other elements from soils (Torstensson, 2006). Moreover, there are conservative methods for measuring ground cover comprise point, plot, and transect methods (Booth *et al.*, 2006).

Field results showed that legume cover crops produced less effective nodules (Suharyanto *et al.*, 2005). Widiastuti (2016) mentioned that the legume cover crops should be inoculated with *Rhizobium* in order to improve *Rhizobium* population and nodule formation. Ground cover is one of the most easily evaluated key indicators of leaf area index (Booth and Tueller, 2003). Leaf area index is important in measuring growth and vigour of *Lablab purpureus*. According to ECHO Staff (2017), legumes with dense leaf canopies destroy weeds by shading the ground. Normally, legumes reach the highest leaf area index at flowering time (Grotelüschen, 2014). According to Poorter and Remkes (1990), early vigour and rapid growing rates might be connected to improved root growth to handle nutrient shortages. Hassan *et al.* (2014) reported that leaf area index of uninoculated Rongai ranged from 0.35 to 0.95 when intercropped with maize.

2.4.3 Plant height and stem diameter

Lablab purpureus normally grows 1 to 6 meters high with long stems depending on the type of variety. Most studies on grazing strategies aim to improve forage production and animal performance and are based on pasture height (Delevatti *et al.*, 2019). Gasim (2001) indicated that the increase in plant height after applying nitrogen fertilizer occurs because nitrogen promotes plant growth, it increases the number of internodes and length of the internodes which results in progressive improvement in plant height.

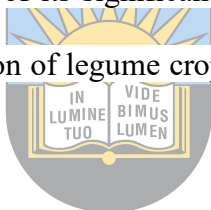
Murtagh and Dougherty (1968) showed that as the plant grew, there was a decline in the proportion of leaf material in the total dry matter yield. Hassan *et al.* (2014) noted that delaying the harvest beyond 18 weeks of age could result in a decline of forage quality. Results of Kim *et al.* (1992) experiment show that the increase in plant height after exposure to more than 13 hour day lengths is attributable to the growth in the number of internodes and not to the increase of internode lengths, particularly those of the lower internodes. Hassan *et al.* (2014) recorded a plant height of 160.58 cm using Rongai cultivar. Then, Amole *et al.* (2015) planted both cultivars and the highest plant height of 88.4 cm was recorded on Highworth cultivar while the shortest plant height (87.3 cm) was recorded on Rongai cultivar.

Lablab purpureus has strong stems that are normally trailing upright to 3-6 m in length and it has a basal stem of perennials that may reach up to 4 cm diameter. Murtagh and Dougherty (1968) established that in ungrazed plants there were more stems than leaf material. Abdallah *et al.* (2015) in Sudan found *Lablab purpureus* stem diameter ranging from 1.493 to 2.083 mm. Thinner stems were observed in plants with the lowest sowing rate compared with that of highest sowing rates and thicker stems have been reported to have higher NDF and ADF values compared with thinner stems (Berti and Samarappuli, 2018). The stem is less palatable compared to the leaf (Abdallah *et al.*, 2015).

2.5 The importance of Forage Legume Nodulation

Root nodules originate from the roots of legume plants, they then form a symbiotic relationship with nitrogen-fixing bacteria. As nitrogen is the most limiting nutrient for the growth of leguminous plants, its absence in the soil has a negative effect on plant growth and development (Simon *et al.*, 2014). Nitrogen-fixing rhizobia fix the nitrogen in the nodules, which then becomes available for plant development and this is a major benefit in sub Saharan farming systems where most soils are less fertile because of continuous cropping (N’Zi *et al.*, 2016).

Root nodulation and nitrogen fixation in legumes are affected by environmental factors (Kucuk and Cevheri, 2014), such as temperature, soil acidity and drought. However, phosphorus has a major role because of its significance in the formation of the root system (Vasileva and Athar, 2018). Inoculation of legume crops with *Rhizobium* can improve forage yield (Chemining’wa *et al.*, 2007).



In order to ensure optimal *Rhizobium* population in the rhizosphere, seed inoculation of legumes with an efficient *Rhizobium* strain is required. Cheming’wa *et al.* (2007) noted that *Rhizobium* inoculation improved nodule number by 30% in a short period. Similar findings were earlier observed by Ali *et al.* (2004) that seed inoculation improved nodule numbers by 21% compared uninoculated plants. Legume inoculation is important because not all soils have the specific bacteria essential to produce nodules and fix atmospheric nitrogen symbiotically (Dawson, 1962).

2.6 Forage production of Lablab cultivars

Plant growth and productivity of crops are usually constrained by the absence of certain minerals, especially nitrogen and phosphorus in the soil (Bano and Iqbal, 2016). Therefore, *Lablab purpureus* is usually intercropped with cereal crops to improve the fertility of the soil

and thus crop production (Amel *et al.*, 2014). Hassan *et al.* (2014) indicated that *Lablab purpureus* is also used as a forage for animal feed, and it produces high-quality conserved feed (4 t DM ha⁻¹ and 120 g CP kg⁻¹ DM). For improved forage and crop production bio-fertilisers are important as they promote plant growth and productivity and are internationally acknowledged as an alternative source of nitrogen fertiliser (Mia and Shamsuddin, 2010). Bio-fertilisers are microorganisms that improve the nutrient quality of the soil and help promote the growth of plants. Bano and Iqbal (2016) mentioned that inoculation enhance crop growth and yield.

Amel *et al.* (2014) reported that *Bradyrhizobium* inoculation of *Lablab purpureus* seeds significantly increased dry matter yield to 37.12 g/plant compared to the un-inoculated control (10.78 g/plant). Hassan *et al.* (2014) found dry matter yield that falls within the range of 5-14 t ha⁻¹ and Mullen (1999), reported that to be adequate for a good forage legume. However, Hassan *et al.* (2014) recorded 3.68 to 7.22 t DM ha⁻¹ of lablab which was higher than the values (1.4 to 7.07 t DM ha⁻¹) that were reported in previous studies of Agyemang *et al.*, 2000; Omokanye *et al.*, 2004; Amodu *et al.*, 2004; Anthony and John, 2007.

Clem and Cook (2004) in Queensland (annual rainfall of 700 -1000 mm), reported different yields of the two *Lablab purpureus* cultivars, namely Rongai and Highworth. Rongai produced more forage yield of 4.55 t ha⁻¹ and Highworth 3.86 t ha⁻¹. Ewansiha *et al.* (2007) noted that higher dry matter yields were related with late flowering plants (Rongai) and lower dry matter yields with early flowering plants (Highworth). The study conducted by Kawonga *et al.* (2005) showed that Rongai is more productive compared to Highworth under the rainfall range of 326.7 - 512.4 mm. In Table 2.2 below Rongai produced bigger plants and ultimately more D.M than Highworth in all the three places. Environmental conditions have an effect on the growth *L. purpureus*. Variety also has an influence the growth because they have different adaptations. O'Mara (2005) shows that Rongai grows well in semi- arid areas.

Table 2. 1: Effect of variety on *Lablab purpureus* production in three areas of Botswana

Variety	Yield (t/ha)		
	Maun	Morale	Panda
Rongai	1.53	0.97	1.64
Highworth	1.28	0.90	1.61

Source: O'Mara (2005)

2.7 Nutrient content of *Lablab cultivars*

2.7.1 Crude protein

The potential of any feed to support animal production depends on the quality consumed by the animal and the extent to which the feed meets energy, protein, minerals and vitamin requirements. Valenzuela and Smith (2002) noted that *Lablab purpureus* produces about 21 to 38% crude protein and that makes it a good forage plant to ruminants. The nutritive quality of *Lablab purpureus* varies from area to area, and between seasons and also with the growing stage (Amole *et al.*, 2013). *Lablab purpureus* is highly palatable at the vegetative stage of growth. It was identified that after flowering, the leaf was mostly preferred by cattle, since it had much higher protein content than the stem and it was regarded as a valuable part of the lablab crop (Ewansiha *et al.*, 2007). Generally, the herbage is more nutritious when it is young than when older (Mokoboki *et al.*, 2002).

The use of protein and other energy rich feeds in the form of concentrates to supplement the poor quality forage and crop residues is an option for the farmers in meeting the nutrient needs of their ruminant livestock (Hassan *et al.*, 2014). Mokoboki *et al.* (2002) reported a crude protein content of 24.4% of *Lablab purpureus* species. This is more than what has been proposed as the minimum requirements for lactation (12% CP) and growth (11.3% CP) in

ruminants (ARC, 1984). Several authors (Amole *et al.* (2013), Ngongoni *et al.* (2008) and Mupangwa *et al.* (1997)) are in agreement with Mokoboki *et al.* (2002) in terms of the nutrient content of lablab meeting the requirements for animal production and growth. Hassan *et al.* (2014) recorded crude protein range of 10.1-17.2%, that met the requirements of growing goats (10-31% CP; Alemu, 2008) and lambs (9.1-15.1% CP; NRC, 1985).

2.7.2 Acid detergent fibre and Neutral detergent fibre

Acid detergent fiber (ADF) is a quantity of the plant components in forages that are the least digestible by livestock, including cellulose and lignin (Beauchemin, 1996). When ADF increases, digestibility decreases, so forages with high ADF concentrations are usually lower in energy. The total plant cell wall material is measured by neutral detergent fiber (NDF), and it contains mainly hemicellulose, cellulose and lignin (Beauchemin, 1996). NDF relates to free-choice by livestock because it provides low-calorie filler. There are two main components that regulate forage quality, namely, fiber [acid detergent fiber (ADF) and neutral detergent fiber (NDF)] and crude protein (CP). According to Horrocks and Vallentine (1999), both are correlated to the age or stage of development of the crop, as the crop ages, fiber increases and protein decreases (on a dry-matter basis). Thus, forage quality then decreases with age because fiber becomes a main component.

Results obtained by Murphy and Colucci (1999) indicated that *Lablab purpureus* crop contains average crude fiber of 27% with an average of 43% NDF and 38.6% ADF. Washaya *et al.* (2018) observed that *Lablab purpureus* had higher ADF (33.7%), NDF (46.5%) and hemicellulose (12.9%) at flowering stage. High ADF and NDF concentrations reduce digestibility of plants and hence can affect the availability of protein even in forages with a high concentration of protein. The table below (Table 2.3) below shows nutrient content of *Lablab purpureus* cultivars observed in different parts of the plant and also in different studies.

Table 2.2: The chemical composition (CP, CF, ADF, NDF, and ADL) of *L. purpureus* in different parts of the plant (% DM).

Cultivar	Sample	CP	CF	ADF	NDF	ADL	Reference
Rongai	Leaves	18.1-30.6	–	22.2-36	26-51.5	4.8-12	Karachi (1997)
	Stems	9.4-16.9	–	41.5-62.7	50-69.2	7.6-15.5	
Highworth	Leaves	20.4- 22.6	29.2-37.1	29.5-36.3	66.1-69.1	–	Amole <i>et al.</i> (2013)
	Stems	18.1-20.4	30.4-42.2	35.8-36.9	65.6-70.1	–	
	Whole plant	24.5	–	37	48.7	–	Mokoboki <i>et al.</i> (2002)
Highworth	Whole plant	15.4	–	27.5	54.9	–	Ngongoni <i>et al.</i> (2008)
	Whole plant	23.3	–	29.9	55.1	8.8	Ajayi and Babayemi (2008)
Rongai	Whole plant	10	18.9	20.6	59.7	–	Hassan <i>et al.</i> (2014)
Highworth	Whole plant	25.4	–	28.2	32.8	9.6	Mupangwa <i>et al.</i> (1997)

Source: Murphy and Colucci (1999); CP: crude protein; CF: crude fibre; ADF: acid detergent fibre; NDF: nutrient detergent fibre; ADL: acid detergent lignin.

2.7.3 Anti- nutrient contents

The nutritive value of grain legumes depends mainly on their nutrient content, and availability of anti-nutritional or toxic factors (Ramakrishna *et al.*, 2008). *Lablab purpureus* plant contains anti-nutritional factors including tannins, phytate and trypsin inhibitors. Mokoboki *et al.* (2002) stated that polyphenolic concentration increases with age. Processing methods, such as removing the seed coat, soaking and cooking, are effective in alleviating the effects of these factors (Lambourne and Wood, 1985; Deka and Sarkar, 1990). The higher the tannin content in feed, the lower the digestibility as it reduces the population of fibre degrading bacteria in the rumen and hence low activity (Jayanegara *et al.*, 2010).

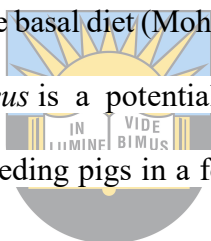
Lablab purpureus leaves are a good feed for monogastric animals because they are free of tannins (Murphy *et al.*, 1999). According to Mokoboki *et al.* (2002), the climate and soils of the area in which the crop is grown influence the amount and type of tannins present in forage legumes. The presence of tannins in plants has the power to reduce the nutritional value of forages (Washaya *et al.*, 2018). Phenolics and tannins in *Lablab purpureus* leaves decrease digestibility and reduce feed consumption (Sonali *et al.*, 2015). The value of phytate (2.4%) obtained in the study of Ajayi *et al.* (2009) was lower than the phytate range of 9-50% for monogastric nutrition (Martens *et al.*, 2012). These values illustrate that some important minerals such as Ca, Mg, Fe and Zn would be made available from the forages for animals. Soetan (2012) recorded saponin levels of Rongai seed (1.13%) and Highworth (1.21%). Saponins do not delay growth in ruminants because they undergo bacterial degradation in the rumen (Ajayi *et al.*, 2009).

2.8 Response of animals to Lablab cultivars

Legume pastures are the centre of pasture and animal production systems in many parts of the world including South Africa, and so, they act as an alternative and supplementary source of nutrients for sheep and cattle in numerous agro ecological areas (Gulwa *et al.*, 2017). *Lablab*

purpureus is seen as the legume of the future for both ruminants and monogastric nutrition (Ogundipe *et al.*, 2003). The level of animal production attained in any environment is connected to quantity, quality and continuity of supply of feed throughout the year (Mokoboki *et al.*, 2002). *Lablab purpureus* hay is a good forage for goats and sheep and it can supplement low quality forage-based diets. Its high yield and protein content make lablab a good source of forage for growing animals during hot and dry periods (Fribourg *et al.*, 1984).

According to Kabirizi *et al.* (2007) and Hassan *et al.* (2014) when ruminant animals were fed with fibrous crop residues and forage materials such as maize stover, then supplemented with *Lablab purpureus* forage, their performance generally improved. In a study where this plant was used as a supplement to oat hay, the average daily gain in sheep fed the supplement was almost double that of sheep fed only the basal diet (Mohamed, 2008). Nworgu and Ajayi (2005) also mentioned that *Lablab purpureus* is a potential forage legume for the sub-Saharan environment. It is normally used in feeding pigs in a form of leaf meal in Kenya (Beckmann and Clements, 2002).



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Mohamed (2008) noted that in small scale dairy production, milk produced by cows fed *Lablab purpureus* was higher than those fed grass only in Sudan. An edible flavour in milk was also attributed to be a result of lablab feeding (Evans, 2002). Studies with *Lablab purpureus* cv. Rongai by Thurbon *et al.* (1970); Hendricksen and Myle (1980) verified the acceptable level of animal production as well as the nutritive value of Rongai herbage. *Lablab purpureus* can also be conserved as a silage and fed to ruminant animals during the dry season when feed availability and quality are low (Ojo *et al.*, 2013). Iyeghe-Erakpotobor and Muhammad (2008) compared *Lablab purpureus* to other tropical legume forages reported it to be palatable, and high feed intakes were found when it was fed in equal parts with grass forages such as elephant grass (*Pennisetum purpureum*) or Rhodes grass (*Chloris gayana*). *Lablab purpureus* is a

suitable forage for animal feeding even when used as sole feed, but it is better when included in a balanced diet, as it improves the production and growth of animals.

The addition of *Lablab purpureus* to cereal silage improved protein concentration that results in higher feed intakes and superior liveweight performance (Ngongoni *et al.*, 2008). Singh *et al.* (2010) mentioned that *Lablab purpureus* fed at 3 or 6 g/kg liveweight does not affect growth rate, however, when fed at 12 g/kg liveweight level, the growth rate of sheep declined. Hendricksen and Myles (1980) noted that cattle grazing Rongai vegetation had low growth rates, they performed better when Rongai was fed as 'chaff'.



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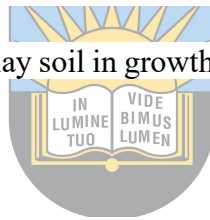
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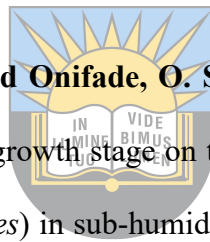
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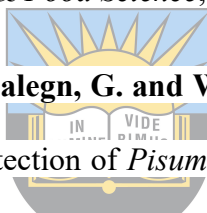
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CHAPTER 3: THE EFFECTS OF *BRADYRHIZOBIUM JAPONICUM* INOCULATION ON GROWTH PERFORMANCE AND BIOMASS PRODUCTION OF *LABLAB PURPUREUS* CULTIVARS.

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Abstract

During the dry season semi-arid grazing areas are characterised by low quality and quantity of forage. Planted forage legumes, including lablab, can be used in alleviating the resultant feed shortage. A field experiment was conducted to determine the effect of *Rhizobium* inoculation on growth performance and forage production of Rongai and Highworth cultivars of *L. purpureus* at the University of Fort Hare Research Farm. The treatments were laid in a randomized complete block design with four replications. The treatments were each lablab cultivar with or without rhizobium inoculation. Data was collected on plant seedling emergence, plant height, stem diameter, plant vigour, chlorophyll content, nodule numbers, dry matter content and biomass yield at 14, 28, 30, 60, 90 DAP. The results showed that *Rhizobium* inoculation significantly increased ($p < 0.05$) seedling emergence by 96% and 97%. For Rongai

and Highworth, respectively; plant height (113.8 cm, 136.4 cm) and stem diameter (1.1 mm, 1.2 mm). *Rhizobium* inoculation had no significant effect ($p>0.05$) on chlorophyll content for both cultivars. Highworth had significantly higher plant vigour than Rongai on both inoculated and uninoculated. The number of nodules, nodulation rate, active nodules and nodule dry yield were significantly different ($p<0.05$) between varieties and inoculation. *Rhizobium* inoculation significantly ($p<0.05$) increased fresh matter yield, dry matter yield and dry matter content on both cultivars. It is concluded that the Highworth cultivar performed very well under *Rhizobium* inoculation because it is best suited to warm areas with rainfall.

Keywords: Forage legume yield, chlorophyll content, nodule numbers.

3.2 Introduction

Rhizobia are aerobic rod-shaped, motile, gram-negative, heterotrophic, and non-spore forming bacteria (Saha and Haque, 2005). These bacteria are able to fix atmospheric nitrogen after becoming established inside the root nodules of legume plants. This consequently does not only improve production in the inoculated plants but also leave a fair amount of nitrogen in the soil for the benefit of the succeeding crops. According to Soto *et al.* (2006), soil bacteria belonging to numerous genera of the order Rhizobiales (collectively called rhizobia) are able to invade legume roots in nitrogen-limited environments, leading to the formation of a highly specialized organ, the root nodule. *Rhizobium* symbiosis has a positive influence on plant growth (Saia *et al.*, 2014).

Lablab purpureus is a legume known to fix atmospheric nitrogen and improve soil fertility, soil organic matter content, and soil structure. It is an annual legume that is capable of producing large quantities of high-quality, above-ground biomass for livestock feed (Murungweni *et al.*, 2004; Peters *et al.*, 2010). During the dry season, low yield and quality of forage is a problem in communal areas of South Africa. At this time, low-quality crop residues are used to feed livestock (Macedo *et al.*, 2008). The addition of a protein supplement which

can be easily cultivated by resource-limited communal farmers in their abandoned former arable lands can improve the quality of the feed. A drought tolerant crop which can thrive in less fertile soils in the form of a legume such as lablab (Macedo *et al.*, 2015) can be a best fit. Although lablab grows well in dry and less fertile areas, the quantity and quality of its forage decreases in the dry season. Use of rhizobia can increase the quality and yield of *Lablab purpureus* in the dry season (Saia *et al.*, 2014). Amel *et al.* (2014) reported that forage legumes have produced when treated with rhizobia inoculants. There have been no studies on the effect of *Rhizobium* on growth and yield of different cultivars of *Lablab purpureus* under field conditions in poor soils of South Africa. Therefore, this study aims to determine the effect of *Rhizobium* inoculation on growth performance and biomass production of two *Lablab purpureus* cultivars.



3.3 Materials and Methods

3.3.1 Ethical Clearance

- Ethical principles were considered in this study to conform to the national and international standards governing the usage of legumes.
- Consent to carry out the study was obtained from the University of Fort Hare Ethical Clearance Committee.

3.3.2 Description of the study site

The experiment was conducted at the University of Fort Hare Research Farm in the Eastern Cape Province of South Africa, during the November 2017 summer season. The farm is located at an altitude of 535 metres above sea level (m.a.s.l.), at a latitude of 32° 46' S and longitude of 26° 50' E. This area has a warm-temperate climate with an average annual rainfall of about 575 mm received mainly during the summer months of November to April. The average

maximum temperature is 24° C and the average minimum temperature is 11° C for November to April. The soils are deep and are of alluvial origin and are classified as the Oak leaf form (Oa), belonging to the Ritchie family, according to the South African system of soil classification (Soil Working Group, 1991; Mukumbareza, 2014). According to the soil map of the world, the soils are Eutric fluvisols (Fle) (FAO, 1988). The vegetation is dominated by grasses such as *Themeda triandra* and *Cynopogon plurinodis* with woody plants such as *Vachellia karroo* and shrubs encroaching some parts of the grazing lands (Mucina and Rutherford, 2006).

3.3.3 Experimental design

The four treatments were laid out in a randomized complete block design. Each plot was 3m x 3m to give a gross plot size of 9m². A distance of 1m was kept between the plots and a pathway of 2m was created between the blocks. The treatments were a 2 x 2 factorial arrangement of two rhizobia inoculation levels (with or without inoculation) and two lablab cultivars (Rongai and Highworth; Table 3.1) which were replicated four times.

Table 3. 1: Treatments combination in the factorial set up

Treatments	Combination
1	Highworth seed without <i>Rhizobium</i> inoculation (R ⁰ L ^H)
2	Highworth seed with <i>Rhizobium</i> inoculation (R ⁺ L ^H)
3	Rongai seed without <i>Rhizobium</i> inoculation (R ⁰ L ^R)
4	Rongai seed with <i>Rhizobium</i> inoculation (R ⁺ L ^R)

3.3.4 Forage legume establishment

The site for the trial was ploughed twice before planting using a tractor-drawn plough and disked to a fine tilth. Soil samples were taken just before planting for analysis (to check the number of *Rhizobium* bacteria, pH and nutrients that were already available in the soil). Thereafter, *Lablab purpureus* cv. Rongai and Highworth seeds were planted in rows using hand hoes. Each plot consisted of four rows of legumes with inter-row spacing of 0.5 m and intra-row spacing of 0.3 m and two seeds were planted per station. The seeds for the treatments of *Rhizobium* inoculation were inoculated with a *Bradyrhizobium japonicum* strain. The seeds were sown by hand at 4-6 cm depth. The average seeding rates for Lablab was 50 kg/ha (FAO, 1999), at least two seeds were put in each hole. Single super phosphate fertiliser was broadcasted at 40 kg P ha⁻¹ to each plot in a block. The plots were kept free of weeds to avoid competition. The weeds between the plots were removed using hoe, mower or slasher to keep the area clear. This was all done after the first effective rains during the 2017/2018 growing season (November to April).



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3.3.5 Rainfall and Temperature *Together in Excellence*

During the 2017/2018 season the University of Fort Hare Research Farm received 413.75 mm of rainfall in warmer season/summer and 159.5 mm in cooler season/winter (Figure 3.1). There was little rainfall at planting stage, the rainfall increased through pre-anthesis stage, it then declined during December then increased again at anthesis and decreased a bit at post-anthesis. The highest rainfall of 62.23 mm was received in April and dropped after that to the lowest rainfall of 14.47 mm in May (Figure 3.1).

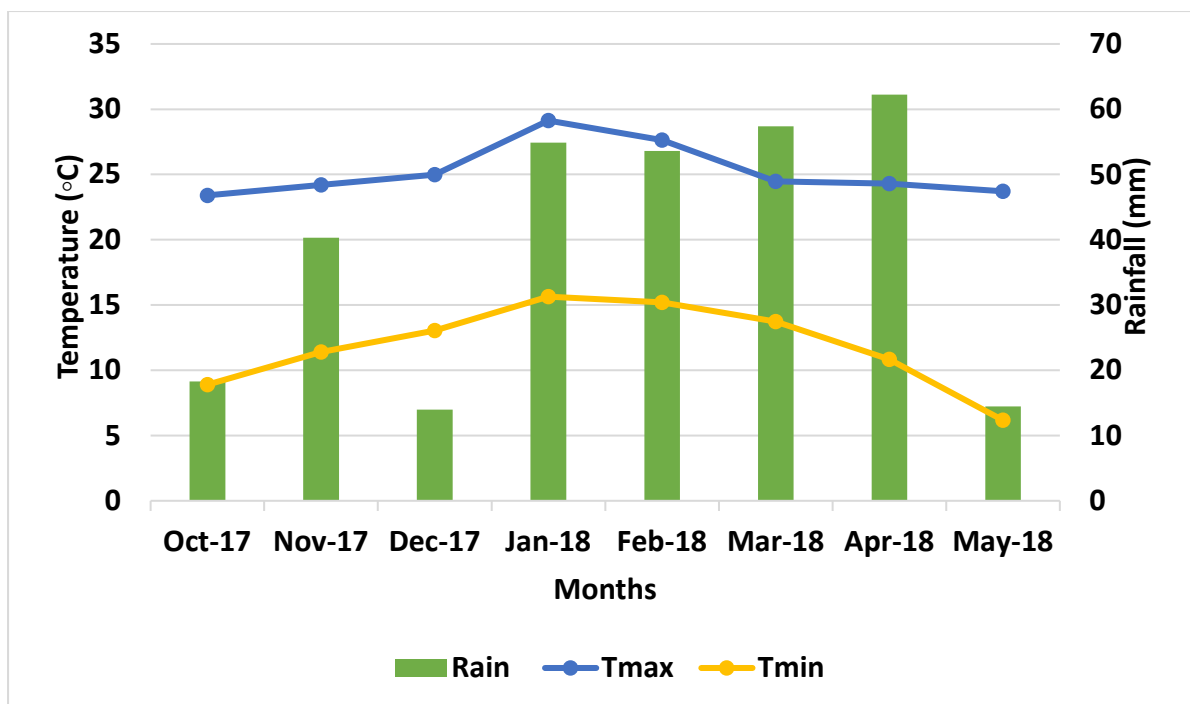


Figure 3.1: Temperature ($^{\circ}\text{C}$) and Rainfall (mm) for the experimental period (October 2017 to May 2018) at University of Fort Hare Research Farm.



3.3.6 Soil physical and chemical properties

Soil analysis of the experimental field showed that the soil had a sandy loam texture with a pH of 5.6 (Table 3.2), which was rated as slightly acidic (Tekalign, 1991). Soil total N (0.84 g.kg^{-1}) at the experimental site was low. Low organic carbon (OC) and organic matter (OM) might be accredited to the intensive cultivation. This is also regarded as being caused by the low nitrogen (N) content of the soil. The analysis further showed that the soil had low available phosphorus (P) (0.32%). Elemental analysis showed that the soil was generally high to very high in its total content of nutrients, except for Nitrogen (N), Phosphorus (P) and Sodium (Na). There was a high level of Manganese (398 mg kg^{-1}) available in this soil.

Table 3. 2: Initial physicochemical properties of the experimental soil

Soil physical properties	Value at a depth of 0-30 cm
Sand	64.2%
Silt	16.0%
Clay	19.8%
Soil texture class	Sandy loam
Soil chemical properties	
pH (water)	5.6
Cation Exchange Capacity (CEC)	6.2 cmol/kg
Soil Organic Carbon (SOC)	6.4 g kg ⁻¹
Soil Organic Matter (SOM)	9.6 g kg ⁻¹
Total Nitrogen (N)	0.84 g kg ⁻¹
Total Phosphorus availability (P)	0.32 g kg ⁻¹
Potassium (K)	6.02 g kg ⁻¹
Calcium (Ca)	5.35 g kg ⁻¹
Magnesium (Mg)	1.48 g kg ⁻¹
Sodium (Na)	0.58 g kg ⁻¹
Sulfur (S)	16.92 g kg ⁻¹
Iron (Fe)	21.22 g kg ⁻¹
Manganese (Mn)	398 mg kg ⁻¹
Zinc (Zn)	26 mg kg ⁻¹
Copper (Cu)	52 mg kg ⁻¹
Boron (B)	44 mg kg ⁻¹
Molybdenum (Mo)	58 mg kg ⁻¹

3.3.7 Growth performance

3.3.7.1 Seed emergence

Emergence measurements were done using a 1 m² quadrat per plot, at 14 and 28 days after planting (DAP) to give an estimation of plant seedling emergence. Plant seedling emergence was calculated using the formula below:

$$• E\% = \frac{\text{number of emerging seedling at 14 DAP}}{\text{Total number of seeds planted}} \times 100$$

Source: Mokoboki *et al.* (2002)

Where:

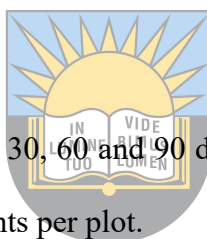
DAP is days after planting, and E% is the emergence percentage.

3.3.7.2 Plant height

Plant height (stretched) was measured in centimetres (cm) using a tape measure. Five plants were randomly selected per plot and plants were measured from the base to the tip of the plant. The measurements were taken at 30, 60 and 90 days after planting to measure crop growth rate (Malami and Suleiman, 2007).

3.3.7.3 Stem diameter

Stem diameter (mm) was measured at 30, 60 and 90 days after planting using vernier sliding caliper by randomly selecting five plants per plot.



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3.3.7.4 Plant vigour

Plant vigour was evaluated at 30, 60 and 90 days after post-germination using a formula prescribed by Abdul-Baki and Anderson (1973) as follow:

$$\text{Seedling vigour (SV)} = \sum \left\{ \frac{\text{Seedling emergence percentage} \times \text{Seedling length (mm)}}{100} \right\}$$

3.3.7.5 Chlorophyll content

Total chlorophyll content was estimated quantitatively following Arnon's (1949) method and SPAD leaf chlorophyll meter (SPAD-502, Minolta Camera Co., Osaka, Japan). Chlorophyll meter reading were taken from the forage legume at 30, 45, 60, 75 and 90 days post-germination using SPAD meter. Five plants were selected in each plot and three fully expanded

leaves per plant were selected randomly at vegetative, flowering, pod filling and maturity stages to determine the chlorophyll content.

3.3.8 Forage legume harvesting

Forage legumes were harvested at flowering to determine biomass production. A 0.25 m² quadrat was used, it was tossed 3 times within the 3m x 3m net plot, and forage was cut at a stubble height of 10 cm. The harvested forage was put into khaki bags and weighed immediately for fresh weight. The fresh samples were put into the oven for 48 hours at 65 °C and then the dry matter content was measured. The formula that was used to calculate the dry matter content is as follows:

$$\% \text{Dry matter content} = \frac{\text{Dry weight}}{\text{fresh weight}} \times 100$$

Dry matter (DM) yield was measured using the following formula.

$$\text{DM yield (kg/ha)} = \text{Fresh yield} \times \% \text{DM}$$



3.3.9 Forage Legume Nodulation

In each plot, five plants were randomly selected in the net plot and they were uprooted with their soils for measurement of nodules. The roots were put in clean water to carefully remove the soil on each plant. Nodule numbers were recorded. Fresh and dry weights of nodules were also determined. Nodulation rating was done by examining nodules that are available in primary roots and in secondary roots close to the taproot. Thus, it was essentially done by examining the entire root system and even those plants that showed no root nodulation were examined (Otieno *et al.*, 2009). The rating of the plants for nodulation was done according to a scale of 1-10. The number of plants that have developed nodules on the taproot, close to the taproot or scattered over the entire roots were identified. Plants with no nodules on their roots were identified and counted, then their total number was subjected to the following formula adapted from NifTAL (1979).

$$\text{Nodulation rating} = \frac{(10 \times \text{NPTRN}) + (5 \times \text{NPNCTR}) + (1 \times \text{PSN}) + (0 \times \text{PPN})}{n} \times 100$$

Where,

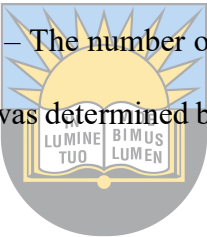
NPTRN: Number of plants with taproot nodulation,

NPNCTR: Number of plants with nodules close to taproot,

PSN: Number of plants with scattered nodulation,

PNN: Number of plants without nodulation and,

n: Total number of plants

- 
Number of nodules per plant – The number of nodules per plant was taken from the same uprooted plants, and this was determined by counting the number of nodules from each of the five plants.
- Nodule dry weight** - The nodules collected from the five plant samples from each plot were pooled and their dry weight was measured after oven drying at 70 °C to a constant weight.
- Active numbers of nodules** - Ten nodules were randomly taken from the same sample plants and dissected with a blade to observe their colour in the centre. The colour score was on a 1- 4 scale as: 1 = white (non-active), 2 = pink (moderately active), 3 = slightly dark red (active) and 4 = deep dark red (highly active) as adopted from Tekalign and Asgelel (1994).

3.4 Statistical analysis

Data was subjected to analysis of variance using SAS 9.1 GLM procedures (SAS Institute, Inc., Cary, NC, USA) for a randomized complete block design. Means of all treatments were calculated and the differences were tested for significance using the Least Significant Difference (LSD) test at $p=0.05$.

The statistical model below was used:

$$Y_{ijk} = \mu + B_i + C_j + R_k + (CR)_{jk} + e_{ijk}$$

Where;

- Y_{ijk} is the dependent variable (e.g. height, dry matter, yield)
- μ is the overall mean
- B_i is the block effect ($i= 1,2,3$)
- C_j is the effect of lablab cultivar
- R_k is the effect of rhizobia inoculation
- $(CR)_{jk}$ is the treatment interaction effect of lablab cultivar and rhizobia inoculation
- E_{ijk} is the random error



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3. 5 Results

Table 3. 3: Plant Seedling emergence, plant height and stem diameter of *Lablab purpureus* at UFH Research Farm in Alice.

Treatments		Seedling emergence (%)		Plant height (cm)			Stem diameter (mm)		
Variety	Inoculation	14 DAP	28 DAP	30 DAP	60 DAP	90 DAP	30 DAP	60 DAP	90 DAP
Highworth	R ⁺	96.750 ^a	93.750 ^a	78.550 ^a	107.980 ^a	136.355 ^a	0.851 ^a	1.064	1.216
Highworth	R ⁻	91.250 ^c	87.000 ^d	65.600 ^c	88.135 ^c	112.955 ^b	0.652 ^b	0.903	0.969
Rongai	R ⁺	95.500 ^b	92.500 ^b	71.200 ^b	96.420 ^b	113.805 ^b	0.904 ^a	1.086	1.119
Rongai	R ⁻	91.500 ^c	87.750 ^c	59.450 ^d	79.965 ^d	99.725 ^c	0.683 ^b	0.809	0.912
S.E.D		0.215	0.258	1.017	1.199	1.516	0.020	1.779	2.549
Significant level	V	*	n.s	***	***	***	*	n.s	n.s
	R	***	***	***	***	***	***	n.s	n.s
	V×R	***	***	n.s	n.s	***	n.s	n.s	n.s

^{abc} Means with different superscripts within columns differ significantly (p<0.05). S.E.D= standard error of difference; *=p<0.05; **= p<0.01; ***= p<0.001; n.s= not significant; V= Variety; R= *Rhizobium*; V×R interaction between Variety and *Rhizobium*

3.5.1 Seedling emergence

At 14 DAP, there was a significant difference ($p < 0.05$) between the inoculated Highworth and the uninoculated Highworth. Treatments Rongai R⁺ and Rongai R⁻ were also significant different ($p < 0.05$). When comparing both varieties, there was a significant difference between Highworth R⁺ and Rongai R⁺ while treatments Highworth R⁻ and Highworth R⁻ were not significant different ($p > 0.05$). The highest (96.75%) seedling emergence occurred in treatment Highworth R⁺ whereas the lowest number (91.25%) of seedling emergence was found in treatment Highworth R⁻ (Table 3.3)..

At 28 DAP, treatment Highworth R⁺ was significantly different ($p < 0.05$) from treatment Highworth R⁻. Also, Rongai R⁺ and Rongai R⁻ showed a significant difference ($p < 0.05$). Table 3.3 showed that Highworth R⁺ and Rongai R⁺ had a significant difference ($p < 0.05$). Treatment Highworth R⁻ and treatment Rongai R⁻ were also significant different amongst each other. The inoculated Highworth had the highest (93.75%) seedling emergence while the uninoculated Highworth had the lowest emergence (87%) at 28 DAP. However, there was a significant interaction ($p > 0.001$) between variety and *Rhizobium* inoculation.

3.5.2 Plant height

At 30 DAP there was a significant difference ($p < 0.05$) in plant height amongst treatments Highworth R⁺ and Highworth R⁻. Furthermore, treatments Rongai R⁺ and Rongai R⁻ were also significantly different ($p < 0.05$) at 30 DAP. When comparing both varieties, inoculated Highworth and inoculated Rongai had a significant difference ($p < 0.05$) on plant height. There was also a significant difference ($p < 0.05$) between uninoculated Highworth and uninoculated Rongai at 30 DAP. The tallest plant was found in treatment Highworth R⁺ with a value of 78.55cm while the shortest plant height was recorded in Rongai R⁻ (59.45cm). Table 3.3 shows that there was no significant interaction ($p > 0.05$) between variety and *Rhizobium* inoculation.

At 60 days after sowing (DAP), there was a significant difference ($p < 0.05$) between Highworth R^+ and Highworth R^- . There was also a significant difference ($p < 0.05$) between Rongai R^+ and Rongai R^- at this stage. Variety had a significant effect on plant height because Highworth R^+ and Rongai R^+ were significant different ($p < 0.05$) and Highworth R^- and Rongai R^- were also significant different ($p < 0.05$) from each other. There was no significant interaction ($p > 0.05$) between *Rhizobium* inoculation and variety (Table 3.3). The tallest plant was found on treatment Highworth R^+ (107.98 cm) while the shortest was found at Rongai R^- (79.97 cm) at 60 DAP.

At 90 DAP, there was a significant ($p < 0.05$) between the two treatments (Highworth R^+ and Highworth R^-). There was also a significant difference when comparing plant height in Rongai R^+ and Rongai R^- . Treatments Highworth R^+ and Rongai R^+ had a significant difference ($p < 0.05$) and also Highworth R^- and Rongai R^- had a significant difference ($p < 0.05$). There was a significant ($p < 0.001$) interaction between *Rhizobium* inoculation and variety (Table 3.3). The highest plant was found on treatment Highworth R^+ with the value 136.36 cm and the shortest was at treatment Rongai R^- (99.73 cm).

3.5.3 Stem diameter

Rhizobium inoculation affected ($p < 0.001$) the stem diameter at 30 DAP. There was a significant ($p < 0.05$) difference between Highworth R^+ and Highworth R^- at 30 DAP. Treatment Rongai R^+ and Rongai R^- were significant different ($p < 0.05$) from each other. Similarly, the varieties had a significant effect ($p < 0.05$) on stem diameter at this stage. There was no significant difference ($p > 0.05$) between Highworth R^+ and Rongai R^+ . Similarly uninoculated Highworth and Rongai were also not significant different ($p > 0.05$). There was a significant interaction ($p > 0.05$) between variety and *Rhizobium* inoculation. However, thicker stems were found for treatment Rongai R^+ (0.9 mm) while thinner stem was recorded at Highworth R^- (0.65 mm) at 30 DAP.

Rhizobium inoculation had no significant difference ($p>0.05$) on stem diameter at 60 and 90 DAP. Variety also had no significant difference ($p>0.05$) on stem diameter at both stages. As shown in Table 3.3 below biggest stem diameter (1.09 mm) at 60 DAP was observed on treatment Rongai R⁺ while the smallest was recorded at treatment Rongai R⁻ (0.81 mm). At 90 DAP treatment Highworth R⁺ gradually improved with a thicker stem diameter of 1.216 mm. However, the treatment Rongai R⁻ was having lower values of stem diameter (0.91 mm) compared to all other treatments at 90 DAP but did not differ significantly ($p > 0.05$) with all of them (Table 3.3). There was no significant interaction ($p>0.05$) on between *Rhizobium* inoculation and both varieties at 60 and 90 DAP.



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Table 3. 4: Plant vigour and chlorophyll content of *Lablab purpureus* at UFH Research Farm in Alice.

Treatments		Plant vigour (mm)			Chlorophyll content (°SPAD)				
Variety	Inoculation	30 DAP	60 DAP	90 DAP	30 DAP	45 DAP	60 DAP	75 DAP	90 DAP
Highworth	R ⁺	75.959 ^a	101.115 ^a	127.705 ^a	55.567 ^a	51.982 ^a	54.247 ^{ab}	41.430	43.598
Highworth	R ⁻	59.863 ^c	76.680 ^c	98.273 ^c	52.490 ^{bc}	49.053 ^b	54.953 ^{ab}	40.887	44.403
Rongai	R ⁺	68.083 ^b	89.336 ^b	105.357 ^b	51.347 ^c	48.980 ^b	53.550 ^b	43.435	43.927
Rongai	R ⁻	54.399 ^d	70.192 ^d	87.522 ^d	53.095 ^b	49.865 ^b	55.725 ^a	41.233	43.460
S.E.D		0.943	1.064	1.258	0.588	0.462	0.707	0.761	0.756
Significant	V	***	***	***	**	*	n.s	n.s	n.s
level	R	***	***	***	n.s	*	*	n.s	n.s
	V×R	n.s	n.s	***	***	***	n.s	n.s	n.s

^{abc} Means with different superscripts within columns differ significantly (p<0.05). S.E.D= standard error of the difference; *=p<0.05; **= p<0.01; ***= p<0.001; n.s= not significant; V= Variety; R=*Rhizobium*; V×R interaction between Variety and *Rhizobium*

3.5.4 Plant vigour

At 30 DAP, there were significant differences ($p < 0.05$) between Highworth R^+ and Highworth R^- and between Rongai R^+ and Rongai R^- (Table 3.4). Both varieties had a significant effect on plant vigour at this stage. Treatments Highworth R^+ and Rongai R^+ were significantly different ($p < 0.05$) at 30 DAP; Highworth R^- and Rongai R^- had a significant difference ($p < 0.05$) (Table 3.4). The highest plant vigour at this stage was found in treatment Highworth R^+ (75.96 mm) while the lowest plant vigour was recorded in Rongai R^- (54.39 mm). The interaction ($p > 0.05$) between variety and *Rhizobium* inoculation was not significant effect for plant vigour at 30 DAP (Table 3.4).

As shown in Table 3.4, at 60 DAP the inoculated Highworth and uninoculated Highworth were significantly different ($p < 0.05$), and also for inoculated Rongai and uninoculated Rongai. However, Highworth R^+ (101.12 mm) and Rongai R^+ (89.34 mm) had a significant difference ($p < 0.05$). Treatment Highworth R^- and Rongai R^- were significant different ($p < 0.05$) amongst each other. There was no significant interaction ($p > 0.05$) between *Rhizobium* inoculation and variety on plant vigour at 60 DAP. The treatment that had the highest plant vigour at 60 DAP was Highworth R^+ (101.12 mm) whereas Rongai R^- had the least plant vigour of 70.19 mm (Table 3.4).

Similarly, Highworth R^+ and Highworth R^- had a significant difference ($p < 0.05$) at 90 DAP, and so was in treatments Rongai R^+ and Rongai R^- . Even for variety, Highworth R^+ and Rongai R^+ were significantly different ($p < 0.05$) from each other. In Table 3.4, Highworth R^- and Rongai R^- were significantly different ($p < 0.05$). At this stage plant vigour was highest with treatment Highworth R^- (127.71 mm) and was least with Rongai R^- (87.52 mm). There was a significant interaction ($p < 0.001$) between *Rhizobium* inoculation and variety at 90 DAP.

3.5.5 Chlorophyll content

There was a significant difference ($p < 0.05$) on chlorophyll content of leaves from inoculated plants compared to those plants which were not inoculated at 30 and 45 DAP. The inoculated Highworth and inoculated Rongai were significantly different ($p < 0.05$) at both above mentioned stages. Table 3.4 shows that uninoculated Highworth and uninoculated Rongai were not significantly different ($p > 0.05$) at 30 and 45 DAP. Chlorophyll content was highest with Highworth R^+ (55.57) while it was lowest with Rongai R^+ (51.35) at 30 DAP. At 45 DAP, highest chlorophyll content was found on Highworth R^+ (51.98) while the lowest chlorophyll content was recorded for Rongai R^+ (48.98). There was significant interaction ($p < 0.001$) between *Rhizobium* inoculation and variety at 30 and 45 DAP (Table 3.4).

Table 3.4 shows that at 60 DAP the inoculated Highworth and uninoculated Highworth had no significant difference ($p > 0.05$) while inoculated Rongai and uninoculated Rongai had a significant difference ($p < 0.05$). Highworth R^- and Rongai R^+ had a significant difference ($p < 0.05$) but Highworth R^- and Rongai R^- had no significant difference ($p > 0.05$). The highest chlorophyll content was found with Rongai R^- (55.73) and the lowest was recorded for Rongai R^+ (53.55). There was no significant interaction ($p > 0.05$) between *Rhizobium* inoculation and variety at 60 DAP.

Variety and *Rhizobium* inoculation had no significant ($p > 0.05$) effect on chlorophyll content at 75 and 90 DAP. At 75 DAP, the highest chlorophyll content was recorded at treatment Rongai R^+ (43.44) while the lowest was recorded at Highworth R^- (40.89). The chlorophyll content gradually improved at 90 DAP, the highest value was found at treatment Highworth R^- (44.40). There was no significant interaction ($p > 0.05$) between *Rhizobium* inoculation and variety at 75 and 90 DAP.

Table 3. 5: Nodule number, nodulation rate, active nodules and nodule dry yield of *Lablab purpureus* at UFH Research Farm in Alice.

Treatments		NSN	NTN	Nodule numbers	Nodulation rate	Active nodules	Nodule dry yield
		(n)	(n)	(n)	(%)	(n)/plant??	(mg/plant)
Variety	Inoculation						
Highworth	R ⁺	33.800 ^a	19.750 ^a	53.550 ^a	81.850 ^a	4.000 ^a	0.019 ^a
Highworth	R ⁻	24.050 ^b	11.450 ^c	35.650 ^c	65.000 ^c	3.050 ^b	0.011 ^c
Rongai	R ⁺	34.150 ^a	16.000 ^b	50.150 ^b	78.750 ^b	4.000 ^a	0.015 ^b
Rongai	R ⁻	16.250 ^c	6.200 ^d	22.450 ^d	51.350 ^d	1.750 ^c	0.009 ^c
S.E.D		0.701	0.715	0.967	0.928	0.089	0.001
Significant level	V	***	***	***	***	***	***
	R	***	***	***	***	***	***
	V×R	***	n.s	***	***	***	n.s

^{abc} Means with different superscripts within columns differ significantly (p<0.05). S.E.D= standard error of the difference; *=p<0.05; **= p<0.01; ***= p<0.001; n.s= not significant; V= Variety; R=*Rhizobium*; V×R interaction between Variety and *Rhizobium*; NSN=Number of scattered nodules; NTN=Number of tap root nodules

3.5.6 Nodule parameters

As presented in Table 3.5, there was a significant difference ($p < 0.05$) between Highworth R^+ and Highworth R^- , and also Rongai R^+ and Rongai R^- , for number of scattered nodules. Treatment Highworth R^+ and Rongai R^+ were not significantly different ($p > 0.05$) while Highworth R^- and Rongai R^- were significantly different ($p < 0.05$) for NSN. The highest number of scattered nodules was recorded with treatment Rongai R^+ (34) while the lowest number was recorded with Rongai R^- (16). There was a significant interaction ($p < 0.001$) between *Rhizobium* inoculation and variety for NSN.

There was a significant difference ($p < 0.05$) between Highworth R^+ and Highworth R^- whereas Rongai R^+ and Rongai R^- were not significantly different ($p > 0.05$) for tap root nodules. Highworth R^+ and Rongai R^+ were significantly different ($p < 0.05$). Similarly, Highworth R^- and Rongai R^- were significantly different ($p < 0.05$). There was no significant interaction ($p > 0.05$) for tap root nodules (Table 3.5). The highest number of tap root nodule was found with Highworth R^+ (19) while the lowest number was found with Rongai R^- (6).

Table 3.5 also shows that for nodule numbers inoculated Highworth and uninoculated Highworth had a significant difference ($p < 0.05$) and the same was true for inoculated Rongai and uninoculated Rongai. For variety, there was a significant difference ($p < 0.05$) between Highworth R^+ and Rongai R^+ , and also between Highworth R^- and Rongai R^- . Treatment Highworth R^+ had the highest (53) nodule numbers while Rongai R^- had the lowest (22) nodule numbers. There was a significant interaction ($p < 0.05$) between *Rhizobium* inoculation and variety for nodule numbers.

For nodulation rate treatments Highworth R^+ and Highworth R^- had a significant difference ($p < 0.05$), similar results were found for Rongai R^+ and Rongai R^- . The inoculated Highworth and inoculated Rongai showed significant difference ($p < 0.05$), also uninoculated Highworth

and uninoculated Rongai had a significant difference ($p < 0.05$) on nodulation rate. The highest nodulation rate was 81.85% for Highworth R^+ and the lowest rate was 51.35% for Rongai R^- . There was a significant interaction ($p < 0.001$) between *Rhizobium* inoculation and variety on nodulation rate (Table 3.5).

There was significant difference ($p < 0.05$) between Highworth R^+ and Highworth R^- . Rongai R^+ had a significant difference ($p < 0.05$) for active nodules. Meaning, inoculation of *Rhizobium* makes the plant to have high number of nodules. However, Highworth R^+ and Rongai R^+ had no significant difference ($p > 0.05$) but Highworth R^- and Rongai R^- had a significant difference ($p < 0.05$). Table 3.5 above shows that there was a significant interaction ($p < 0.001$) between *Rhizobium* inoculation and variety for active nodules. The highest number of active nodules was 4/plant and it was recorded for both Highworth R^+ and Rongai R^+ . The lowest value of active nodules was recorded for Rongai R^- (Table 3.5).

The highest nodule dry yield was recorded for Highworth R^+ (0.019 mg/plant; Table 3.5) followed by Rongai R^+ (0.015) and lastly Highworth R^- and Rongai R^- whose yields were not significantly different. However, the interaction between variety and *Rhizobium* inoculation had no significant effect on nodule dry yield (Table 3.5).

Table 3. 6: Fresh matter yield, dry matter yield and dry matter content of *Lablab purpureus*.

Treatments		Fresh Matter Yield (kg/ha)	Dry Matter Yield (kg/ha)	Dry Matter Content (%)
Variety	Inoculation			
Highworth	R ⁺	5533.333 ^b	1916.667 ^b	34.075 ^b
Highworth	R ⁻	6293.333 ^a	1766.667 ^b	28.008 ^b
Rongai	R ⁺	5433.333 ^b	1850.000 ^b	34.105 ^b
Rongai	R ⁻	5056.667 ^c	2466.667 ^a	50.341 ^a
S.E.D		309.494	545.318	11.233
Significant	V	*	n.s	n.s
level	R	n.s	n.s	n.s
	V×R	n.s	n.s	n.s

^{abc} Means with different superscripts within columns differ significantly (p<0.05). S.E.D= standard error of the difference; *=p<0.05; **= p<0.01; ***= p<0.001; n.s= not significant; V= Variety; R=*Rhizobium*; V×R interaction between Variety and *Rhizobium*

3.5.7 Fresh matter yield, dry matter yield and dry matter content

There was a significant difference ($p < 0.05$) in fresh matter yield between inoculated plants and uninoculated ones for both cultivars. There was a significant difference ($p < 0.05$) in fresh matter yield between inoculated Highworth compared to inoculated Rongai, and the same trend was observed between the uninoculated Highworth and uninoculated Rongai. The highest fresh matter yield was observed for uninoculated Highworth (6293.3 kg/ha) and the least for uninoculated Rongai (5056.7 kg/ha) (Table 3.6). Rongai R⁻ is different to all other treatments for dry matter content and dry matter yield. Table 3.6 above shows that there was no significant difference ($p > 0.05$) in Highworth R⁺, Highworth R⁻ and Rongai R⁺.



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3.6 Discussion

3.6.1 Plant Seedling emergence

Plant seedling emergence ranged from 87 to 97% in this study. The highest plant seedling emergence was recorded in inoculated Highworth (97%) and Rongai (96%) at 20 DAP and the lowest was observed in uninoculated Highworth (87%) at 40 and 60 DAS. These results were similar to the results of McDonald *et al.* (2002), who observed that Highworth germination was over 90 at 14 days after inoculation (DAI). Similar results were reported by Brandon and Jones (1998) where 93% of Highworth emergence was recorded at 14 days after planting. However, Nworgu and Ajayi (2005) obtained 95% in 2002 and 97% in 2001 at 3 weeks after planting using Rongai cultivar. Whitbread *et al.* (2011) recorded that germination of Rongai cultivar was greater than 80% at 20 days after planting. Despite the difference in number of days after planting the results of this study were similar to the results of other researchers. For both inoculated cultivars, the emergence was high. This might have been due to the effect of *Rhizobium* releasing phosphorus needed for plant growth, therefore both varieties performed very well under *Rhizobium* inoculation. There was no significant difference in effect of variety because Highworth and Rongai are similar in vegetative growth (Cameron, 2013), seed emergence can be affected by field conditions. There was a significant interaction at 14, 28 DAP, seedling emergence in both cultivars was declining with time. This may be caused by the poor quality of seed and sowing conditions.

3.6.2 Plant Height

Generally, plants need macro-nutrients, particularly nitrogen and phosphorus in order to grow fast and become tall. Inoculation showed a great impact on *Lablab purpureus* height as shown in Table 3.3. The inoculation of *Rhizobium* increased the plant height in both cultivars. Amel *et al.* (2014) reported that the inoculated plants have a better growth rate than the uninoculated plants. *Rhizobium* inoculation had an effect on the plant height in this study, as all the tallest

(136 cm) plants were in the inoculated Highworth plots while the shortest (59 cm) plants were found in the uninoculated Rongai plots. The positive effect of inoculation on plant height was also observed by Hassan *et al.* (2014) on Rongai cultivar where a higher plant height of 160 cm was recorded. Lower plant heights were observed in uninoculated Highworth (88 cm) and Rongai (87 cm) cultivars (Amole *et al.*, 2015). The inoculated plants were always taller than uninoculated plants at 30, 60 and 90 DAP in both *Lablab purpureus* cultivars. There was a significant difference in plant height in all the treatments. This showed that *Rhizobium* inoculation was significant for lablab growth.

3.6.3 Stem Diameter

Lablab purpureus has strong stems that are normally trailing upright to 3-6 m in length and it has basal stem of perennials which may reach up to 4cm in diameter. The stem diameter in this study varied from 0.652 mm to 6.216 mm. *Rhizobium* inoculation increased stem sizes, inoculated crops had thicker stems than uninoculated crops at 30, 60 and 90 DAP. This occurs because *Rhizobium* inoculation increase nitrogen fixation and accelerates plant growth. Rongai cultivar performed better when inoculated while Highworth cultivar grew well when plants were uninoculated in most stages. Rongai is morphologically similar to Highworth, thus, *Rhizobium* was more effective to Rongai cultivar than Highworth. Contrary results were found by Kawonga *et al.* (2005) where stem diameter ranged from 2.38 mm to 2.69 mm. This must have been caused by the fact that one of their treatments was application of different levels of phosphorus fertiliser, Kawonga *et al.* (2005) mentioned that phosphorus fertiliser slightly increased the stem size of both varieties (Rongai and Highworth). Abdallah *et al.* (2015) used different cultivars in their study and they found that there was no significant difference in the stem diameter (1.49 mm).

3.6.4 Plant Vigour

The four most important factors that influence plant strength and healthy growth are soil fertility, availability of water, climate, and diseases or pests. The seed vigour index is an important parameter that includes seed germination and seedling growth properties. Highworth had significantly higher vigour values in both its inoculated and uninoculated state. Highworth

is an early flowering plant with high seed ability, hence seed inoculation positively enhanced the vigour of this cultivar. Minta and Tsige (2014) reported that seedling vigour was not significantly ($p>0.05$) affected by *Rhizobium* inoculation but higher vigour index in inoculated plants showed that their seeds were better developed than un-inoculated plant seeds. In both varieties inoculation resulted in higher plant vigour. This shows that the association of leguminous plants and *Rhizobium* have a good impact on plant vigour.

3.6.5 Chlorophyll Content

At 30 DAS there was a significant difference between inoculated and uninoculated for both cultivars tested. Furthermore at 45 DAP there was a difference in Highworth, and at 60 DAP there was a difference in Rongai. The results reported that the maximum chlorophyll content was noticed in the leaves of inoculated *Lablab purpureus* cultivars in most stages (table 3.4). The reason behind might be high nitrogen presence in the soil, it can cause high chlorophyll content to the inoculated crops. Hossain and Lundquist (2016) also showed that inoculation enhanced (37.3 °SPAD readings) the leaf chlorophyll content of the legume. Similar study by Arumugam *et al.* (2010) reported a significant increase (2.43 mg/g fresh weight) in chlorophyll content of *Rhizobium* inoculated *Lablab purpureus* at 45th day after inoculation. Highworth had significantly higher chlorophyll content at 30 and 45 DAP while there was no significant difference between uninoculated plants.

3.6.6 Nodule parameters

The highest number of nodules and nodulation rate were found in Highworth with *Rhizobium* (53.55n) and (81.85%) whereas the lowest nodule number and nodulation rate were found in treatment Rongai without *Rhizobium*, (23.45m) and (51.35%). The main trend in this section was that for both Highworth and Rongai, inoculation resulted in high values of nodule number, nodule rate, active nodules and nodule dry yield. Therefore, this shows that *Rhizobium* inoculation had a great impact on all parameters measured. Similar results were found by

Massawe *et al.* (2016) that *Rhizobium* inoculation improved number of nodules in both glasshouse and field experiment. The results also revealed that few nodules were detected on uni-nodulated plots. The maximum value of active nodules was recorded in both inoculated plants while minimum number of active nodules was found in uninoculated plants. Also the nodule dry yield had the highest value of 0.019 mg/plant in inoculated Highworth and the lowest nodule dry yield was recorded in un-inoculated Rongai cultivar. This may be caused by the inoculation of *Rhizobium* which fixes the nitrogen in the nodules and avail it for plant growth. Cheming'wa *et al.* (2007) noted that *Rhizobium* inoculation improved nodule by 30% in light rain areas. Amel *et al.* (2014) reported that *Bradyrhizobium* inoculation significantly ($P < 0.05$) increased dry weight of nodules, nodulation rate and number of nodule compared to the uni-nodulated control. These results are in agreement with those reported by Ali *et al.* (2004).



3.6.7 Dry matter content

As shown in Table 3.6, dry matter content was higher in Rongai R⁻ with the value of 50.3% while it was found low in treatment Highworth R⁻ with the value of 28.0%. This showed that *Lablab purpureus* cultivars had no significant difference on dry matter content between inoculated and uninoculated plants for both cultivars. Similarly within inoculated plants there was no significant difference on dry matter content between varieties, the same applies to uninoculated plants. Kiflewahid and Mosimanyana (1987) reported the dry matter content of 25.3% for whole *Lablab purpureus* plant which was in the same range with the results of this study. Amel *et al.* (2014) recorded that *Bradyrhizobium* inoculation of *Lablab purpureus* seeds significantly increased dry matter yield to 37.12 g/plant compared to the un-inoculated control (10.78 g/plant). Through all the growing season the Highworth cultivar showed higher values in dry matter yield as compared to Rongai cultivar. The difference is probably due to the genetic differences between the two cultivars.

3.6.8 Fresh matter yield

Fresh biomass yield depends on many factors such as soil fertility, soil pH, rainfall and application of phosphorus and inoculation. Generally, leaf fresh biomass production is influenced mostly by stage of harvest and variety of plant, particularly the soil moisture. Ewansiha *et al.* (2016) recorded fresh biomass of *Lablab purpureus* (L.) Sweet of 3210.0 kg/ha at 8 weeks after planting. There were no significant differences due to inoculation. There was only a significant difference in uninoculated varieties where Highworth had a significantly higher fresh matter yield compared to Rongai. This may be caused by the fact that seed yield of Highworth is higher than in Rongai. Ogedegbe *et al.* (2011) used Highworth cultivar without inoculation in their study and obtained the highest fresh matter yield of 33.0 t/ha, respectively. Abusuwar and Omer (2011) reported that *Lablab purpureus* fresh yield should range from 9180 to 12580 kg ha⁻¹ when inoculated of which that is higher than the values obtained in this study.

3.6.9 Dry matter yield

Total dry matter yield recorded in this study ranged from 1766.667 to 2466.667 kg ha⁻¹. The lowest dry matter yield was found for Highworth R⁻ and the highest for Rongai R⁻ (Table 3.6). There was no significant effect of inoculation and variety on dry matter yield. This might be due to that Highworth and Rongai are genetically similar to each other and the *Lablab purpureus* had already fixed the nitrogen in the soil that is needed by the plant. Mokoboki *et al.* (2002) reported that *Lablab purpureus* species had the highest DM yield of approximately 8 tons/ha, which is between 5 to 14 tons/ha indicated by Mullen (1999) as acceptable yield for a good forage legume. In this study dry matter yield was below these recommended values. Similarly results by Malami and Abdullahi (2007) who reported that yield increased by 58% when *Rhizobium* was applied in the seed. Hassan *et al.* (2014) found values that were within the range of 5-14 t ha⁻¹. The range 1.8 to 2.5 t ha⁻¹ reported in this study are on the lower end of this range but comparable to the range 1.4 to 7.07 t ha⁻¹ reported in some previous studies (Agyemang

et al., 2000; Omokanye *et al.*, 2004; Amodu *et al.*, 2004; Anthony and John, 2007). Contrary to the results of this study Kawonga *et al.* (2005) state that higher dry matter yields were associated with late flowering (Rongai) plants and lower dry matter yields with very early flowering (Highworth) plants.

3.7 Conclusion

In the present study, inoculation with *Rhizobium* improved seedling emergence, nitrogen fixation, plant growth and biomass production in both cultivars. The interaction between variety and *Rhizobium* inoculation had no significant effect ($p > 0.05$) on plant vigour and seedling emergence to nitrogen fixation parameters such as number of nodules and nodulation rate.



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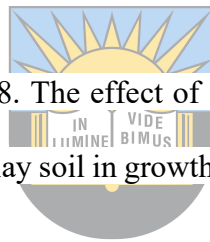
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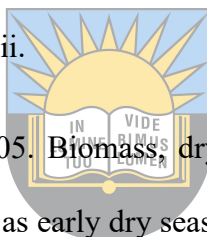
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CHAPTER 4: THE EFFECTS OF RHIZOBIUM INOCULATION ON NUTRIENT AND ANTI-NUTRIENT CONTENT OF LABLAB PURPUREUS CULTIVARS.

Abstract

Dry season nutritional stress is a major constraint to livestock production in the Eastern Cape. During the winter season forage from natural pastures and crop residues is reduced in quality and quantity; and thus provide inadequate nutrients to grazing livestock. One way of improving the quality of such crop residues is by supplementing with protein-rich leguminous forages to provide adequate nutrients to the animals. There is a need to explore cultivation of drought tolerant legumes in less fertile soils under rain-fed conditions for use as a dry season protein supplement. This study evaluated the effect of *Rhizobium* inoculation on nutrient and anti-nutrient content of Rongai and Highworth cultivars of *Lablab purpureus*. The forage samples were harvested at flowering stage, dried and then milled to pass through a 1 mm sieve. The milled samples were analysed for chemical, nutrient and anti-nutrient content. There were no significant difference ($p>0.05$) in NDF, ADIN, ADL, ADF, and Na between inoculated and uninoculated plants in both cultivars. The inoculation of *Rhizobium* improved CP (15.65%) in Highworth cultivar. The inoculation of *Rhizobium* significantly increased Ca (1.76-1.84%), Zn (191.91-2 8.33ppm) and Cu (3.50-6.16ppm) compared to un-inoculated plants. Therefore, the *Rhizobium* inoculation assisted in improving quality in terms of nutrient composition and decreasing anti-nutrients. It is recommended to use Highworth cultivar as it had most minerals increased and reduced tannins.

Keywords: legume forage, NDF, Highworth, Rongai, tannin content.

4.1 Introduction

Livestock farming systems depend mostly on grazing natural pastures and crop residues for dietary energy and protein in most African countries (Hassan *et al.*, 2014). During the dry season, the quality and quantity of feed in natural rangelands decrease and farmers with financial resources supplement their animals with commercial supplements. Poorly resourced farmers use crop residues which are low in protein. Some farmers intercrop legumes with cereals to maximise the benefits of some legumes such as the use of some leaves as vegetables and pods for human consumption, and also as cover crops to conserve moisture, and also for their nitrogen fixation capability. *Lablab purpureus* has been intercropped with maize (Hassan *et al.*, 2014) and dry beans (Makgoga, 2013). According to Macedo *et al.* (2015), *Lablab purpureus* is an annual legume that is capable of producing large quantities of high quality above ground biomass for livestock feed. Chemical analysis showed that *Lablab purpureus* contained about 24% crude protein in its leaf (Ogundipe *et al.*, 2003). In the Eastern Cape Province where most former arable lands have been abandoned due to various reasons, including depleted soils due to monocropping, lablab can be grown under rainfed conditions to supply forage for protein supplement during the dry season.

Most of the abandoned former arable lands are less fertile, and therefore inoculation of legumes with *Rhizobium* bacteria can improve production and nutrient content. *Rhizobium* bacteria assist plants by extending their hyphae towards the nutrient zone for continuous nutrient absorption and transferring them to plants (Arumugam *et al.*, 2010). Soil micro-organisms such as *Rhizobium* represent a key link between plants and soil mineral nutrients (Berruti *et al.*, 2016).

Legumes can be part of the sustainable agricultural development without destroying the environment and thus, introducing selected microorganisms helps improve the conditions for plant development (Bano and Iqbal, 2016). Recent studies show that inoculation with

Rhizobium and phosphorus supplementation enhance the macro- nutrient uptake (N, P, K) in different organs of the legumes (Zoundji *et al.*, 2015), and thus result in increased forage quality and yield. There has been no studies done on the effect of *Rhizobium* on nutritional composition and anti-nutrient content of different cultivars of *Lablab purpureus* under field conditions in the Eastern Cape. Therefore, the objective of this study was to determine the effect of *Rhizobium* inoculation on nutrient content and anti-nutrient content of two *Lablab purpureus* cultivars.



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4.2 Methods and Materials

4.2.1 Description of the study site in Chapter 3

The study site description is as described in Chapter 3

4.2.2 Laboratory analysis

4.2.2.1 Chemical composition of *Lablab purpureus*

The forage samples were harvested as described in Chapter 3. The plant samples that were harvested at flowering stage were milled to pass through a 1 mm sieve and stored in plastic bags at room temperature before laboratory analysis (Matizha *et al.*, 2001). The samples were analysed for crude protein (N % x 6.25), ash, moisture, and fat according to AOAC (1995). The method of Van Soest *et al.* (1991) was used to determine neutral detergent fibre (NDF) and acid detergent fibre (ADF), while hemicellulose was calculated as the difference between NDF and ADF. The samples for macro and micro elements were digested by nitric and perchloric acids mixture (ratio = 4.1 v/v) and the concentrations of the minerals (Calcium (Ca), Potassium (K), Phosphorus (P) and Magnesium (Mg), Zinc (Zn), Copper (Cu), Iron (Fe) and Manganese (Mn)) in the samples were determined by an Atomic Absorption Spectrophotometer procedure using the methods described by Van Soest *et al.* (1991) and Fritz and Schenk (1979).

4.2.2.2 Anti-nutrient contents of *Lablab purpureus*

Tannin content was determined according A.O.A.C., (1995) using methanol/paraffin impregnated double layered Whatman number 41 filter paper, and Folin-Denis reagent. The colorimetry method was used to measure total saponin content, using gin- senoside Rb1 as standard. Total phenols was determined with the Folin-Ciocalteau reagent (Makkar, 1993).

4.3 Statistical analysis

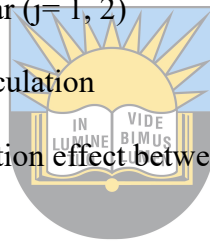
The data from laboratory analysis was subjected to analysis of variance as as described in Chapter 3.

The statistical model below was used:

$$Y_{ijk} = \mu + B_i + C_j + R_k + (CR)_{jk} + e_{ijk}$$

Where;

- Y_{ijk} is the dependent variable (e.g. nutrient and anti-nutrient content)
- μ is the overall mean
- B_i is the block effect ($i=1, 2, 3$)
- C_j is the effect of lablab cultivar ($j= 1, 2$)
- R_k is the effect of rhizobia inoculation
- $(CR)_{jk}$ is the treatment interaction effect between cultivar and *Rhizobium* inoculation
- e_{ijk} is the random error



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Ethical issues

All procedures that were used in the production and management of legumes in this study followed all ethical protocols of the Ethics Committee as stipulated by the University of Fort Hare Animal Research Ethics Committee (AREC). Plant welfare issues were practiced in this study.

4.4 Results

4.4.1 Chemical composition

Table 4.1 below presents the effect of *Rhizobium* inoculation on chemical composition of *Lablab purpureus* forage harvested at flowering stage. There was no significant ($p>0.05$) difference in the NDF, ADL, ADIN, crude protein, cellulose and hemicellulose between inoculated and uninoculated plants in both cultivars. There was no significant difference ($p>0.05$) between inoculated Highworth and inoculated Rongai. Inoculation resulted in significantly ($p<0.05$) higher levels of Ash content in the Highworth cultivar, while the levels of ADF, NDN, and TNC were significantly reduced (Table 4.1). In Rongai inoculation significantly reduced the Ash and Fat content which were 9.5 % and 2.5 %, respectively.

When comparing inoculated cultivars, Highworth had significantly higher levels of ash (11.6%) and fat (2.6%), while Rongai had high levels of ADF (32.4%), NDIN (1.7%), and TNC (11.9%). On the other hand in uninoculated cultivars Rongai had significantly higher levels of ash (12.2) and fat (2.8), while Highworth has high levels of NDIN (2%), and TNC (12.8%). There was a non-significant interaction for NDF ($p>0.05$), ADL ($p>0.05$), ADIN ($p>0.05$), crude protein ($p>0.05$), cellulose ($p>0.05$) and hemicellulose ($p>0.05$). Then, a significant 2-way interaction of variety $\times$ *Rhizobium* inoculation was observed for ash ($p<0.001$), fat ($p<0.001$), ADF ($p<0.05$), NDIN ($P<0.001$) and TNC ($p<0.01$) (Table 4.1).

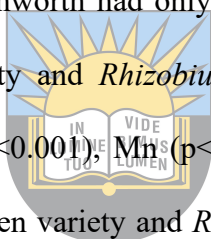
Table 4.1: Chemical composition of *Lablab purpureus* at UFH Research Farm in Alice.

Treatment		Ash	Fat	ADF	NDF	ADL	ADIN	NDIN	Crude	TNC	Cellulose	Hemi
		(%)	(%)	(%)	(%)	(%)	(%)	(%)	Protein	(%)	(%)	(%)
		(%)										
Variety	Inoculation											
Highworth	R ⁺	11.573 ^a	2.598 ^b	29.808 ^b	39.792	9.473	0.906	1.492 ^c	11.925	9.263 ^c	19.590	10.598
Highworth	R ⁻	9.872 ^b	2.599 ^b	32.688 ^a	41.856	12.953	1.323	2.036 ^a	14.662	12.812 ^a	19.734	9.168
Rongai	R ⁺	9.477 ^b	2.463 ^c	32.372 ^a	46.093	10.667	0.918	1.748 ^b	15.646	11.902 ^{ab}	21.705	13.722
Rongai	R ⁻	12.185 ^a	2.798 ^a	30.807 ^{ab}	42.979	10.843	0.918	1.628 ^{bc}	14.677	9.999 ^{bc}	19.964	12.173
S.E.D		0.331	0.043	0.765	2.104	0.949	0.020	0.084	0.924	0.861	0.836	1.686
Significant Level	V	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s
	R	n.s	***	n.s	n.s	n.s	n.s	*	n.s	n.s	n.s	n.s
	V × R	***	***	*	n.s	n.s	n.s	***	n.s	**	n.s	n.s

^{abc} Means with different superscripts within columns differ significantly (p<0.05). S.E.D= standard error of difference; *=p<0.05; **= p<0.01; ***= p<0.001; n.s= not significant; V= Variety; R=*Rhizobium*; V×R interaction between Variety and *Rhizobium*.

4.5.2 Nutrient composition

The mineral composition of lablab forage is presented in Table 4.2. *Rhizobium* inoculation and variety had no significant effect on the concentration of Na in the forage, while variety had no significant effect on the concentration of Cu. *Rhizobium* inoculation significantly increased levels of Ca (1.8%), Mg (0.4%), Cu (6.2 ppm), Fe (353.6 ppm), Mn (68.9 ppm) and Zn (28.3 ppm) in Highworth forage. Uninoculated Highworth had significantly higher levels of K/Ca⁺Mg, P and K. On the other hand in Rongai inoculation significantly increased the levels of K/Ca⁺Mg and Cu, while uninoculated Rongai had high levels of Ca, P, Fe and Mn. On comparing the inoculated varieties, Highworth had higher levels of Ca, Mg, Fe, and Mn, while Rongai had higher levels of cations, P, and K. In uninoculated plants, Rongai had high levels of Ca, P, Fe, Mn, and Zn, while Highworth had only higher levels of cations. There was a significant interaction between variety and *Rhizobium* inoculation for Ca ($p < 0.001$), Mg ($p < 0.05$), K/Ca⁺Mg ($p < 0.001$), Fe ($p < 0.001$), Mn ($p < 0.01$) and Zn ($p < 0.01$). There was no significant interaction ($p > 0.05$) between variety and *Rhizobium* inoculation for Na, P, K and Cu.



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Table 4.2: Nutrient compounds of *Lablab purpureus* at UFH Research Farm in Alice.

Treatment		Ca	Mg	Na	K/Ca ⁺ Mg	P	K	Cu	Fe	Mn	Zn
		(%)	(%)	(%)	(%)	(%)	(%)	(ppm)	(ppm)	(ppm)	(ppm)
Variety	Inoculation										
Highworth	R ⁺	1.841 ^a	0.406 ^a	0.046	0.413 ^b	0.236 ^c	1.658 ^b	6.167 ^a	353.583 ^a	68.917 ^a	28.333 ^a
Highworth	R ⁻	1.212 ^b	0.317 ^b	0.045	0.564 ^a	0.281 ^b	1.863 ^a	3.667 ^b	272.167 ^b	54.167 ^b	19.917 ^b
Rongai	R ⁺	1.198 ^b	0.303 ^b	0.048	0.595 ^a	0.293 ^b	1.877 ^a	5.583 ^a	239.833 ^b	50.333 ^b	26.667 ^a
Rongai	R ⁻	1.764 ^a	0.348 ^{ab}	0.029	0.435 ^b	0.348 ^a	1.972 ^a	3.500 ^b	326.250 ^a	69.750 ^a	26.083 ^a
S.E.D		0.123	0.029	0.008	0.031	0.012	0.062	0.655	23.157	3.049	1.242
Significant Level	V	n.s	n.s	n.s	n.s	***	*	n.s	n.s	n.s	n.s
	R	n.s	n.s	n.s	n.s	***	*	**	n.s	n.s	***
	V × R	***	*	n.s	***	n.s	n.s	n.s	***	**	**

^{abc} Means with different superscripts within columns differ significantly (p<0.05). S.E.D= standard error of difference; *=p<0.05; **= p<0.01; ***= p<0.001; n.s= not significant; V= Variety; R=*Rhizobium*; V×R interaction between Variety and *Rhizobium*

4.5.3 Anti-nutritional factors

Anti-nutritional factors of *Lablab purpureus* are presented in Table 4.3. Inoculating Highworth seeds only significantly ($p < 0.05$) reduced the level of condensed tannins, while the other anti-nutrients were not significantly ($p > 0.05$) different in all the treatments. In Rongai inoculation significantly ($p < 0.05$) reduced the level of all the measured anti-nutritional factors. In inoculated plants, the level of condensed tannins and total polyphenols was significantly higher in Highworth than Rongai, while there was no significant ($p > 0.05$) difference in the levels of hydrolysable tannins. Saponins were significantly ($p < 0.05$) higher in the uninoculated cultivars. The level of condensed tannins was also significantly higher in uninoculated Highworth. Thus, there was a significant ($p < 0.05$) interaction between variety and *Rhizobium* inoculation of condensed tannins, total polyphenols, hydrolysable tannins and saponins.



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Table 4.3: Anti-nutritional factors of *Lablab purpureus* at UFH Research Farm in Alice.

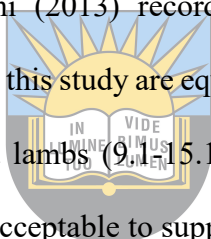
Treatments		Condensed Tannins	Total Polyphenols	Hydrolysable Tannins	Saponins
		(mg/g)	(mg/g)	(mg/g)	(%)
Variety	Inoculation				
Highworth	R ⁺	0.489 ^b	0.835 ^a	1.466 ^b	3.181 ^b
Highworth	R ⁻	0.598 ^a	0.906 ^a	1.615 ^a	3.413 ^a
Rongai	R ⁺	0.377 ^c	0.729 ^b	1.410 ^b	3.078 ^c
Rongai	R ⁻	0.489 ^b	0.881 ^a	1.644 ^a	3.433 ^a
S.E.D		0.036	0.029	0.052	0.088
Significant Level	V	**	*	n.s	n.s
	R	**	***	***	**
	V × R	n.s	n.s	n.s	n.s

^{abc} Means with different superscripts within columns differ significantly (p<0.05). S.E.D= standard error of difference; *=p<0.05; **= p<0.01; ***= p<0.001; ns= not significant; V= Variety; R=*Rhizobium*; V×R interaction between Variety and *Rhizobium*

4.5 Discussion

4.5.1 Chemical composition

The potential of any feed to support animal production depends on the quality consumed by the animal and extent to which the feed meets energy, protein, minerals and vitamin requirement. Mahala *et al.* (2012) indicated that the CP of forage legumes usually varies from 14–20%, while Valenzuela and Smith (2002) noted that *Lablab* produced about 21 to 38% crude protein and that makes it a good forage legume to ruminants. The main finding of this study was that inoculation did not change or increase the crude protein in both cultivars (there was no significant difference). Results of crude protein (10.1- 17.2%) were found by Hassan *et al.* (2014) reported crude protein levels of 10.1 = 17.2% for uninoculated Rongai cultivar. The study by Abusuwar and Al-Solimani (2013) recorded 14.5% CP in inoculated *Lablab purpureus*. The CP values perceived in this study are equivalent to the requirements of growing goats (10-31% CP; Alemu, 2008) and lambs (9.1-15.1%CP; NRC, 1985). The crude protein values recorded in table 4.2 could be acceptable to supply ruminant livestock.



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There are two main components that influence forage quality, namely, ADF and NDF. *Rhizobium* inoculation reduced levels of ADF, NDIN and TNC in Highworth. This could be due to a climatic condition and amount of rainfall that was experienced during the study period. However, inoculation increased ash and fat in Highworth. This means that *Rhizobium* was more effective in Highworth cultivar. Furthermore, Rongai cultivar had high levels of ADF, NDIN and TNC. High ADF and NDF concentrations reduce digestibility of plants and can affect the availability of protein even in forages with high concentration of protein. The levels of ash and fat in Rongai had been reduced and this may be caused by the rainfall in that study area. Murphy and Colucci (1999) reported *Lablab* average crude fiber, NDF and ADF levels of 27, 43 and 38.6%, respectively, which are higher than the results of this present study. Washaya *et al.* (2018) reported ADF and NDF values of 36.22 and 48.78%, respectively, which are in

agreement with results of this study. Hassan *et al.* (2014) recorded ADF (20.63%) and NDF (59.70%) in Rongai cultivar at 18 weeks after sowing (WAS). The ADF was low and NDF was high compared to the results mentioned by Murphy and Colucci (1999). High ADF and NDF concentrations reduce digestibility of plants and hence can affect the availability of protein even in forages with a high concentration of protein. This study recorded 12.19% fat of and 2.8% ash of similar to those reported by Washaya *et al.* (2018). It has been reported that high fat and NDF content limit feed intake (Washaya *et al.* 2018). Therefore, high NDF content is a symbol of high forage degradability (Mahala *et al.*, 2012).

4.5.2 Nutrient composition

Calcium is a constituent of the skeletal system and is also involved in vital functions such as blood clotting, transmission of nerve impulse, muscle contractions and cardiac regulation (NRC, 2001). Calcium (Ca) values recorded in this study ranged from 1.198 to 1.841%. In this study inoculation resulted in increased levels of calcium in both cultivars. *Rhizobium* inoculation is an important technology for improving nutrients of a crop. Highworth inoculation resulted in more calcium compared to Rongai and Rongai cultivar produces more calcium when it was not inoculated. This may be caused by the fact that Rongai adapted easily to environmental conditions of the study area, while Highworth needed to be nurtured by *Rhizobium* in order for it to perform well.

The calcium requirement for young cattle has been reported to be 3.5% (NRC, 1985). Thus, this shows that farmers need to supplement calcium to their young steers using Lablab especially during the dry season for better performance (Hassan *et al.* (2014).The calcium content in this study is less than the calcium required by young cattle (3.5%; NRC, 1985) meaning lablab is not satisfactory source of the mineral for young cattle. Nworgu and Ajayi (2005) reported Ca levels of 1.16 to 1.15% which are similar to calcium content in this study while Ajayi *et al.* (2009) reported lower values of 0.50 to 0.86% Ca. The results of Ca in this

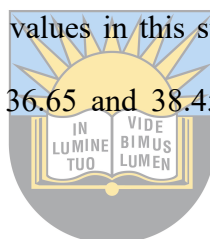
study were higher than the recommended Ca for growing animals; (0.31% and 0.58%) for lactating cattle (NRC, 2000). Therefore, this can be fed to lactating cattle to avoid Ca deficiency especially in dry seasons.

Only the inoculation of Highworth increased Mg content, there was no effect on inoculation in Rongai. This might be that the *Rhizobium* inoculation became more effective in Highworth cultivar. The magnesium (Mg) levels for inoculated *Lablab purpureus* cultivars ranged from 0.30 to 0.40% and were higher than 0.01% reported by Abusuwar and Al-Solimani (2013) after inoculation. For variety, the result is that inoculation significantly increased the levels of Mg in Highworth, while for the other to uninoculated cultivars there was no significant difference. The interaction between inoculation and cultivar is significant for Mg because when the two varieties were not inoculated there was no difference, but when inoculated Highworth performed far much better than Rongai. However, Mg content levels in this study were lower than 0.62% observed by Hassan *et al.* (2014) using Rongai cultivar and 0.8 to 1.4% reported by Mahala *et al.* (2012). Amole *et al.* (2013) mentioned that *Lablab purpureus* can assist in enhancing low quantity and quality forage during the dry season for ruminants in terms of weight gain, reproduction, growth rate, and productivity by adequately supplying required amounts of Mg.

Inoculation significantly reduced the amount of K in Highworth and there was no significant effect of inoculation in Rongai. This could be because of the environmental conditions of Alice, where the *L. purpureus* was planted. Nworgu and Ajayi (2005) found 0.24 to 0.028% of potassium in uninoculated *Lablab purpureus*. Similar results by Amole *et al.* (2015) reported 1.18 and 1.71% of potassium content in inoculated *Lablab purpureus* plants. When comparing uninoculated varieties there was no significant difference in K, then after inoculation the level of K was significantly less reduced in Rongai compared to Highworth. Therefore, Highworth can be used as K supplement for livestock feeding as it had high K content. However, Lablab

K content in this study was lower than 4.3 to 5.6% reported by Mahala *et al.* (2012). The K content in this study was higher than K required by livestock ruminants which ranges from 0.60 to 0.70% K (NRC, 2000). Therefore, *Lablab purpureus* forage can be used as K supplement for livestock feeding as it is high in K content.

Inoculation significantly reduced the level of phosphorus in both cultivars. This might be attributed by the age and stage of the plant. The values of phosphorus (P) content found in this study for uninoculated plants were 0.28-0.35%, different the 0.33 – 0.44% reported by Nworgu and Ajayi (2005) for uninoculated *Lablab purpureus* plants. Whether Highworth was inoculated or uninoculated, it was always having lower levels of phosphorus. Amole *et al.* (2013) observed that the uninoculated Highworth cultivar had phosphorus range of 0.25-0.31%. The manganese (Mn) content values in this study are higher than the values of Mn reported by Shaahu *et al.* (2015) of 36.65 and 38.45 mg/kg, respectively, on Rongai and Highworth cultivars.



4.5.3 Anti-nutritional factors

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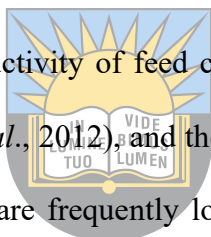
There are two forms of tannins in the plant kingdom: hydrolysable tannins (HTs) and condensed tannins (CTs). The results in this study showed that there was no significant effect of inoculation on hydrolysable tannins and there was also no significant effect of cultivar on hydrolysable tannins.

Significant effect for both cultivar and inoculation were observed for condensed tannins. Inoculation significantly reduced the level of condensed tannins in both cultivars. Tannins have also shown to reduce enteric CH₄ production in ruminants (Makkar 2003). For both inoculated and uninoculated varieties, Highworth was always had higher levels of condensed tannins. According to Mokoboki *et al.* (2002), the climate and soils of the area in which the crop is grown are the ones that influence the amount and type of tannins present in forage legumes.

Mueller-Harvey (2005) indicated that HTs are usually detrimental, in comparison with CTs, which are considered safe if they account for less than 5% of DM in the feed.

Total polyphenols in this study ranged from 0.729 – 0.906 mg/g. Mokoboki *et al.* (2002) stated that polyphenolic concentration increases with age. Inoculation did not reduce the levels of total polyphenols in Highworth, but it did it in Rongai. Kilonzi *et al.* (2017) noted that anti-nutritional factor's content in legume grains may differ as a result of species, cultivars, climatic conditions, soils, locations, seasons and seed germination.

Saponin percentage was low (3.08%) in Rongai with *Rhizobium* and it was high (3.44%) in Rongai without *Rhizobium*. Inoculation significantly reduced the levels of saponins in Rongai. It is reported that plants rich in saponins increase the flow of microbial protein from the rumen (Das *et al.*, 2012), enhance the productivity of feed consumption (Jayanegara *et al.*, 2012), reduce protozoal populations (Das *et al.*, 2012), and therefore decrease CH₄ production (Goel and Makkar, 2012). Saponin levels are frequently low in forages (Gestetner *et al.*, 1966). Saponin does not delay growth in ruminants because it undergoes bacterial degradation in the rumen (Ajayi *et al.*, 2009).



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4.6 Conclusion

Rhizobium inoculation increased levels of Ca, Mg, Cu, Fe, Mn, and Zn and it decreased level of cations, P and K in Highworth cultivar. However, in Rongai inoculation increased levels of Ca, cations and Cu, and decreased levels of P, Fe, and Mn. This means that *Rhizobium* inoculation positively affected the nutrient content of *Lablab purpureus*. When inoculated Highworth performed better than Rongai in levels of Ca, Mg, Fe and Mn, while Rongai had higher cations, P and K. In addition, it was reported in the present study that Highworth was having higher levels of tannins when uninoculated, then inoculation of *Rhizobium* reduced the tannins. Higher tannin diets are always aligned with poor palatability. Therefore, it is recommended that Highworth cultivar can be used by communal farmers in the Eastern Cape.



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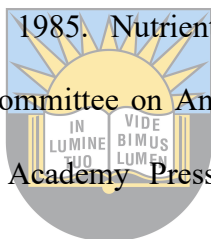
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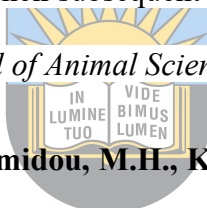
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CHAPTER 5: GENERAL DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1 General discussion

This study has shown the potential of *Lablab purpureus* to improve plant growth and limit the use of chemical fertilizers. *Lablab purpureus* is known as the forage legume which performs well even under poor conditions because it is a good nitrogen fixer and green manure that increases soil organic matter and improves soil structure and quality (Valenzuela and Smith, 2002). Inoculating with *Rhizobium* has been proved to be beneficial in improving productivity under dryland farming conditions. This study was conducted to investigate the effect of *Rhizobium* inoculation on two *Lablab purpureus* cultivars and to provide information on which cultivar of *Lablab purpureus* would be suitable for growing under poor soil conditions.

According to the results in Chapter 3, *Rhizobium* inoculation of *Lablab purpureus* played a very significant role in improving plant seedling emergence, plant ground cover, plant height, nodule numbers, nodulation rate, active nodule, nodule dry yield and biomass production yield. Mabrouk *et al.* (2018) noted that the expected benefits of the nitrogen fixing bacteria may be positive or negative depending on *Rhizobium* species and its interaction with the environment. *Rhizobium* inoculation can also reduce the need for expensive and environmentally damaging nitrogen fertilizer (Amel *et al.*, 2014). Use of the *Rhizobium* can also prevent the reduction of the soil organic matter (Mia and Shamsuddin, 2010). This study showed that *Lablab purpureus* inoculated with *Rhizobium* produced good quality forage which can be used to improve animal production.

Plant seedling emergence ranged between 87 to 97% in this study. Pawar *et al.* (2014) discovered that seeds coated with *Rhizobium* and plant grown in the soil indicated high seed germination and encouraged development over the control, these findings shows that *Rhizobium* can be successfully used as a bio-fertilizer to improve the yield of crops.

Total dry matter yield recorded in this study ranged from 1766.667 to 2466.667 kg/ha. Highworth had the best biomass yield in plants that were inoculated compared to un-inoculated whilst Rongai had the highest dry matter yield in plants that were not inoculated. Plant growth and productivity of crops is usually held due to the absence of certain minerals especially nitrogen and phosphorus in the soil (Bano and Iqbal, 2016). The nutritive value of grain legumes depends mainly on their nutrient contents, and availability of anti-nutritional or toxic factors (Ramakrishna *et al.*, 2008). There was no significant effect ($p>0.05$) in terms of NDF, ADIN, ADL, ADF, Na of the forage among the treatments. The inoculation of *Rhizobium* improved CP (15.65%) in Highworth cultivar. The inoculation of *Rhizobium* significantly increased Ca (1.76-1.84%), Zn (191.91-2 8.33ppm) and Cu (3.50-6.16ppm) compared to un-inoculated. Variety and *Rhizobium* had no significant ($p>0.05$) interaction on condensed tannin, hydrolysable tannin, total polyphenols and saponins. Legume pastures are the centre of pasture and animal production systems in many parts of the world including South Africa, and, they act as an alternative and/or supplementary source of nutrients for sheep and cattle in numerous agro ecological areas (Gulwa *et al.*, 2017).

5.2 Conclusion

The capability of forage feed for livestock production depends on the quality consumed by ruminant animals and extent to which the feed meets energy, protein, minerals and vitamins required. *Rhizobium* can be important to smallholder farmers in the Eastern Cape, who often farm on infertile soils and have little or no access to mineral fertilizers. From the findings of

this study inoculated Highworth performed the best. Also inoculated Highworth had high nutrient content and reduced tannins making the cultivar more palatable. Highworth can therefore be grown and used to improve the quality of crop residues in smallholder systems.

5.3 Recommendations for future studies

- There is a need to investigate the effect of *Rhizobium* inoculation on chemical composition at different stages of growth (pre-anthesis, anthesis and post-anthesis) on *Lablab purpureus* cultivars
- Studies need to be conducted to generate information on the effect of supplementation with *Lablab purpureus* and on animal performance in different areas around Eastern Cape.



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APPENDICES

Appendix 1: Ethical clearance certificate



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ETHICAL CLEARANCE CERTIFICATE **AREC-150311-008**

Certificate Reference Number: MOP021SSEN01

Project title: **The effect of rhizobia inoculation on growth performance, forage production, nutrient and anti-nutrient content of Lablab cultivars.**

Nature of Project MSc in Pasture Science

Principal Researcher: Sibongile Portia Senti

Supervisor: Dr. K Mopipi

Co-supervisor: Dr. B Moyo; Prof JF Mupangwa

On behalf of the University of Fort Hare's Animal Research Ethics Committee (AREC) I hereby give ethical approval in respect of the undertakings contained in the above-mentioned project and research instrument(s). Should any other instruments be used, these require separate authorization. The Researcher may therefore commence with the research as from the date of this certificate, using the reference number indicated above.

Please note that the AREC must be informed immediately of

- Any material change in the conditions or undertakings mentioned in the document;
- Any material breaches of ethical undertakings or events that impact upon the ethical conduct of the research.

The Principal Researcher must report to the AREC in the prescribed format, where applicable, annually, and at the end of the project, in respect of ethical compliance.

The AREC retains the right to

- Withdraw or amend this Ethical Clearance Certificate if
 - Any unethical principal or practices are revealed or suspected;
 - Relevant information has been withheld or misrepresented;
 - Regulatory changes of whatsoever nature so require;
 - The conditions contained in the Certificate have not been adhered to.
- Request access to any information or data at any time during the course or after completion of the project.
- In addition to the need to comply with the highest level of ethical conduct principle investigators must report back annually as an evaluation and monitoring mechanism on the progress being made by the research. Such a report must be sent to the Dean of Research's office.

The Animal Research Ethics Committee wishes you well in your research.

Yours sincerely



Dr. Craig Tambling
AREC Chairperson

20 August 2018

MSc dissertation

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