Integrating Microdosing of Fertilizers with Biological Control Agents for Maize Production in the Eastern Cape, South Africa

By

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GENERAL ABSTRACT

Maize is an important staple crop in South Africa, and is also used for animal feed. In the Eastern Cape Province in South Africa a larger percentage of the farmers are small scale farmers and lack financial resources to apply the recommended levels of fertilizer inputs for optimal production. The currently promoted system of maize production in the Eastern Cape was designed specifically for commercial production, e.g., it is based on the use of agrochemicals to control plant diseases and pests, combined with the use of synthetic fertilizers to provide nutrients, and the application of large quantities of lime to solve soil acidity issues. The currently available mechanical equipment used to fertilize maize are only for row fertilization, whereas in between rows there may be losses of fertilizer due to the distance to the roots. Small scale farmers of this region do not apply lime. Consequently, maize yields are very low for small scale farmers in the Eastern Cape Province, relative to commercial farmers. Both biotic and abiotic factors combine to reduce maize yields. These include root diseases caused by *Rhizoctonia solani* Kühn and other root pathogens and poor soils (highly acidic soils with low nutrient content, especially of P and Mo). Given that the farmers do not treat the seed, lime the soil, and apply little or no fertilization, yields are consistently low.

One goal of this study was to control root rot on maize caused by *R. solani* using a biocontrol agent, and a potassium silicate fertilizer as a priming agent of plant disease resistance. A commercial biocontrol agent, Eco-T® (a.i. *Trichoderma harzianum* Strain kd), is known to control most pathogenic root fungi, including *R. solani*. This treatment was evaluated alone and in combination with potassium silicate (KSil) in field trials over two seasons. Two KSil formulations were tested, namely a liquid and a slow release formulation. All treatments significantly reduced damage by *R. solani,* with *T. harzianum* plus the liquid formulation of KSil resulting in the highest level of control in Season 1, and *T. harzianum* alone providing the highest level of disease control in Season 2 ($p = 0.018$). There was no significant difference in the levels of control provided by *T. harzianum* and KSil applications when they were applied individually. All treatments significantly increased the maize yield relative to inoculated control. The treatment that gave the highest percentage difference relative to the inoculated control was KSil liquid formulation combination

with *T. harzianum*, the combinations gave a significant 45% increased yield over the inoculated control. This means that this combination is an option for the farmers.

Small scale farmers in the Eastern Cape produce maize in poor soils that have low pH levels, very high levels of acid saturation and low nutrient levels, especially of P. The second part of this study was to investigate achievable approaches to liming and fertilization for small scale farmers in the Eastern Cape. These included fertilization and liming by micro-dosing of 2:3:2 (34) fertilizer, superphosphate fertilizer and dolomitic lime using a cap of a soda bottle to measure out approximately 5g to each maize plant, applied directly into the planting hole. In order to fix atmospheric nitrogen and to solubilize phosphates in these acidic soils, a nitrogen-fixing isolate of *B. megaterium* was drenched into the planting holes. Micro-dosing of 2:3:2 (34) fertilizer increased maize yields by 64.6% and 13.6%, over the two seasons of the study. Micro-dosing with superphosphate fertilizer also significantly increased the maize yield ($P = 0.001$) by 50.5% and 37.4%. The combination of *B. megaterium* and 2:3:2 (34) fertilizer significantly increased the maize yield ($P = 0.001$) by 54.7% and 48.1% in season 1 and 2, respectively. The combination of *B. megaterium*, 2:3:2 (34) fertilizer and lime significantly ($P = 0.018$) increased maize yield, maize plant height, and stem diameter in both seasons. The increases in both seasons were consistent as a result of this combination compared to the 2:3:2 (34) fertilizer and lime combination. Whenever *B. megaterium* was included in the treatment combination, yields were increased, although not significantly. It was therefore concluded that micro-dosing of fertilizers can have a significant role in improving the yields for small scale farmers that cannot afford to apply the recommended levels of fertilizer or lime. It was also concluded that the use of *B. megaterium* is beneficial when combined with NPK and P fertilizers.

Field experiments over three seasons were designed to evaluate the integration of the treatments applied in the field experiments mentioned above. The study was conducted in a field with a pH of 4.0 and an acid saturation of 54%. The methods included micro-dosing and spot application of fertilizers and lime. A strain of *B. megaterium* was used as a nitrogen fixer and phosphate solubilizer. For maize root disease control, the methods employed included the use of a biological

control agent, *T. harzianum* and potassium silicate as a plant defense activator. The aim of the study was to reduce input costs whilst still providing adequate fertilization and root disease management. *R. solani* significantly reduced maize yields, by up to 34%, but treatment of maize seed with *T. harzianum,* or *B. megaterium* reduced losses over the three seasons from 34% to 16% and to 10%, respectively. In Season 1, the integration of all treatments (*T. harzianum*, *B. megaterium* and potassium silicate) increased maize yields by 130% relative to the *R. solani* inoculated control. The plots with the highest yields in the presence of *R. solani* were treated with *T. harzianum* (216%), followed by *T. harzianum* plus potassium silicate (214%), and lastly plots treated with *T. harzianum* plus *B. megaterium* (178%). A similar trend was observed over the three seasons.

A cost benefit analysis of the integrated management of maize grown under acidic conditions and also in the presence of *R. solani* was undertaken after the three seasons of field experiments. The first experiment evaluated the control of *R. solani* using the *T. harzianum*, priming of plant resistance using potassium silicate, as well as the combination of *T. harzianum* and potassium silicate. The second experiment evaluated micro-dosing of 2:3:2 (34) fertilizer and lime, and the use of *B. megaterium* as a nitrogen fixer and phosphate solubilizer. The third experiment evaluated the integration of micro-dosing of 2:3:2 (34) fertilizer, superphosphate, lime and *B. megaterium.* The current retail prices were used. It was observed that the combination of *T. harzianum* and the potassium silicate liquid formulation consistently gave the highest returns on investment in controlling *R. solani* over the three seasons. Full fertilization consistently provided a negative return, with a mean loss of R3, 363 over three seasons, relative to the Untreated Control, which was not fertilized. Micro-dosing with lime plus 2:3:2 fertilizer gave the highest net return on investment. This was significantly different ($p = 0.001$) to both the Untreated Control and the Full Fertilization in Season 1. However in Season 2, the combination of *B. megaterium* plus 2:3:2 (34) fertilizer micro-dosed resulted in the highest net return that differed significantly from both the Untreated Control and the Full Fertilization. In the integration experiment all treatments in Season 1 gave a significantly higher yields and increased net returns on investment, relative to the *R. solani* inoculated control, with the lowest giving a 39% net return and the highest giving a 65% net return. In Season 2 none of the treatments resulted in significantly higher yields. In Season 3 a repeat of

Season 1 results was seen where all treatments resulted in a significantly higher yields and net returns relative to the *R. solani* inoculated control, with the exception of *B. megaterium* in the presence of the pathogen. Two treatments, namely *T. harzianum* only and *T. harzianum* plus *B. megaterium* were consistently among the top three treatments that significantly controlled *R. solani*. The combination that gave consistently higher return on investment in the control of *R. solani* and also in the provision of nutrients was the *T. harzianum* plus *B. megaterium* plus micro-dosed 2:3:2 (34) and lime.

It was therefore concluded that a cost effective method of fertilization and liming that will suit the Eastern Cape small scale farmers is micro-dosing rather than conventional method. Moreover incorporating *B. megaterium* improved yield consistently at little cost. For the concurrent control of root pathogens such as *R. solani*, it is recommended that the small scale farmers use *T. harzianum*, and possibly potassium silicate.

Key words: Biocontrol, nitrogen fixation, phosphorus solubilization, potassium silicate, micro-dosing, small scale farmers, acid soil

DECLARATION

I, Bongani Petros Kubheka, declare that the research reported in this thesis, except where otherwise indicated, and is my original work. This thesis has not been submitted for any degree or examination at any other University. This thesis does not contain other persons' data, pictures, graphs or other.

This thesis does not contain other persons' writing, unless specifically acknowledged as being sourced from other researchers. Where other written sources have been quoted, then their words have been re-written but the general information attributed to them has been referenced.

Signed:………………………………..
Bongani P. Kubheka

Signed:………………………………..	Signed:………………………………..
Prof. Mark D. Laing (Supervisor)	Dr Kwasi Sackey Yobo (Co-supervisor)

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DEDICATION

This thesis is dedicated to
my late mother and mother in law
for always
seeing the best in me

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GENERAL INTRODUCTION

Maize (*Zea mays L.*) is one of the major crops grown in South Africa. It contributes 13.2% to gross agricultural production, which makes it the 2nd most valuable after the poultry meat industry (Anonymous, 2013). Commercially, South Africa produced 11.8 million tons of maize in 2012/2013 season. The main provinces that contributed to the maize production were Free State (4 million tons), North West (1 million tons) and Mpumalanga (3.5 million tons). All other provinces contribute relatively little, with the Eastern Cape Province being the 2nd last Province for maize production, with 108,500 tons, which is 0.82% of the total commercial production. The area under maize production in the Eastern Cape Province was 263,700 ha, and only 7.09% of this was for commercial purposes (Dredge, 2014). The remaining 92.91% was maize production for domestic consumption, with a mean yield of 1.6 t ha^{-1} compared to 5.8 t ha^{-1} by commercial farmers (Anonymous, 2013). The gap in maize yield between commercial farmers and small scale farmers in the Eastern Cape is largely due to the low level of crop inputs applied to maize crops by small scale farmers due to the costs involved (Kibirige, 2014). As a result, small scale farmers do not control plant diseases and they do not fertilize for maximum yields. Compounding this problem is that most of the agricultural fields in the Eastern Cape have highly acidic soils, resulting in a number of soil nutrition problems (Buhmann *et al*., 2006). However, most small scale farmers there do not lime their soils. Affordable interventions are needed to mitigate the causes of the yield gap and lower regional food insecurity.

Food insecurity is also promoted by plant diseases. However, plants have their own defense mechanism that can be primed to enhance the ability of plants to defend themselves from plant diseases. For example, fertilization of many crops with silicon results in the priming of the defense mechanisms of the plants to biotic and abiotic stress (Gonzalo *et al*., 2013; Kurabachewa *et al*., 2013; Bekker *et al.,* 2014).

Biocontrol agents may be used to protect crops against plant pathogens. Some isolates of biocontrol agents also contribute to enhanced tolerance of abiotic stress (Hermosa *et al*., 2012).

However, these are living organisms and their efficacy may be affected by environmental factors (López-Mondéjar *et al.*, 2012; Steindorff *et al*., 2012).

The integrated use of both methods mentioned above, namely priming of plants' resistance and employing biocontrol agents are promising technologies that require more research before they can become mainstream practices. It has been shown in many studies that integrating two or more methods that have different mode of action have the potential to increase their efficacy against pathogens (Abo-Elyousr *et al*., 2009).

The costs of fertilizers, their transportation cost, and the costs of application itself at the correct soil depths make conventional fertilizer application too expensive for small scale farmers. A more affordable alternative may be offered by the application of free-living nitrogen fixing bacteria to enhance nitrogen fixation in non-legume crops. This includes microorganisms in the genera *Azotobacter*, *Bacillus* and many others (Adesemoye *et al.*, 2009). Some of these microorganisms, such as *Bacillus megaterium,* may not only fix atmospheric nitrogen but also solubilize phosphates and micronutrients such as molybdenum (Qureshi *et al.,* 2012). This is important in acidic soils because phosphates and some micronutrients bind strongly to clay particles and are therefore unavailable to crops.

The soils of the Eastern Cape have been well characterized by soil scientists. Their fertilizer recommendations for the lands used in this study were for a maize grain yield of 4.0 t ha^{-1}, as would be targeted by commercial farmers. They recommended the application of zz 9.4 bags ha^{-1} of 2:3:4(38), 3.8 bags ha^{-1} of KCl, 0.7 bags ha^{-1} of LAN or 0.4 bags ha^{-1} of urea. In informal discussions with small scale farmers, it was made clear that these farmers did not have the money or equipment to buy, transport and apply fertilizers on the scale recommended by the Department of Agriculture soils laboratory. Their responses to the recommendations were to express frustration, and to apply no fertilizers, because the recommendations were unaffordable and no intermediate alternatives were suggested. This research into microdosing was therefore to provide a novel alternative fertilizer recommendation for small scale farmers.

This study was novel in a number of areas:

a) It is the first study in South Africa and Africa focused on the integration of micro-dosing of fertilizers with the application of biological control agents.

b) It is the first research on micro-dosing fertilization of maize in South Africa, although the practice of microdosing of fertilizers is well established in the rest of Africa (ICRISAT, 2009; Sime and Aune, 2014).

c) It is the first study on microdosing of lime in South Africa, and possibly the second in the world at large, as only one other reference were found to research conducted on this practice (Kisinyo et al. 2009).

d) This is the first study globally on the use of a strain of *Trichoderma harzianum* versus *Rhizoctonia solani* root disease of maize in the field.

e) This is the first study on a strain of *Bacillus megaterium* used as a free living bacterium to fix nitrogen and solubilize phosphate for maize in South Africa.

f) Globally this is the first research on the integration of biological control agents, nitrogen fixation and phosphate solubilization with microdosing of lime and fertilizers.

g) This is the first study on the use of potassium silicate on maize in the field trials, integrated with *T. harzianum* and *B. megaterium* for enhanced yield in the presence of *R. solani*.

h) It is the first cost-benefit analysis of conventional fertilization and liming versus microdosing of fertilizer and lime, combined with root disease control, with the goal of providing affordable solutions to small scale maize farmers in the Eastern Cape of South Africa.

The overall aim of this study was to improve maize yields by integrating the use of selected isolates of *Trichoderma harzianum* and *Bacillus megaterium* with potassium silicate fertilization, and the micro-dosing of macronutrient fertilizers and lime.

The specific objectives were:

- To evaluate the efficacy of a commercial biocontrol strain of *T. harzianum* to control maize root rot caused by *Rhizoctonia solani* under field conditions;
- To evaluate the potential of a potassium silicate fertilizer to prime maize plants for enhanced resistance to *R. solani* under field conditions;

- To evaluate crop responses to biological nitrogen fixation and phosphorus solubilization by an isolate of *Bacillus megaterium;*
- To investigate the potential of micro-dosing of macro-nutrient fertilizers and lime in terms of crop responses in acid soils in the Eastern Cape;
- To evaluate the effects of integrating *T. harzianum, B. megaterium*, potassium silicate and micro-dosing of fertilizers and lime on maize yields;
- To conduct a cost benefit analysis comparing the costs of the researched crop inputs versus outputs, in order to determine which inputs generated the greatest returns on investments for small scale farmers, and the lowest risks.

The thesis is in the form of discrete research chapters, each following the format of a stand-alone research paper. This is an official thesis format adopted by the University of KwaZulu-Natal because it facilitates the publishing of research out of the thesis far more readily than the older monograph form of thesis. As such, there is an unavoidable repetition between chapters of some references, introductory information, and some materials and methods.

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CHAPTER ONE

LITERATURE REVIEW

1.1. Introduction

Food security is a global problem but it affects mostly low income countries (Poulsen *et al.,* 2015). Affordability plays a major role in vulnerability of communities to food insecurity. Globally agricultural input costs increase consistently, making them unaffordable for small scale farmers. These farmers therefore struggle to fertilize adequately. Most of the time they do not fertilize or lime their soils, which ensures a continued situation of poor soils with low crop productivity (Holden and Lunduka, 2013). Hence there is a need for researchers to conduct studies on the mitigation of poor soils by developing affordable fertilization technologies that result in increased crop yields.

Climate change has the potential to worsen the situation in poor and vulnerable communities (Maponya and Mpandeli, 2012). The increase in temperatures may result in drought problems, yet poor communities cannot afford to install proper irrigation systems to compensate for faster water loss. Moreover the shifts in seasons impact directly on agriculture, especially affecting poor and illiterate communities because they take longer to adapt their agricultural systems (Dumenu and Obeng, 2015).

Globally, agriculture is seen as a vehicle to reduce food insecurity. Increasing production per ha at a reduced cost would contribute to the fight against food insecurity (Ogundari, 2014). However, reduced yields are the result of many factors including: poor soil fertility (Rusinamhodzi *et al*., 2015), plant diseases, negative climate change effects, and the reduced effectiveness of chemical control options due to resistance by weeds, pests and pathogens (Fitt *et al*., 2015).

Biocontrol strategies are an option because regular use of effective biocontrol agents will provide stable competition to manage the pathogens present. However, biocontrol agents may be inconsistent in their performance due to the impact of environmental factors on their efficacy. Therefore integrated systems that are cost effective and efficient in improving crop production can be used to provide for stable crop management. This includes the use of plant growth promoting rhizobacteria (atmospheric nitrogen fixers and phosphorus solubilizers), biocontrol agents to manage plant diseases, chemical agents such as silicon for priming plant self-defense mechanisms, and spot fertilization and liming to reduce the quantities of applied fertilizers and lime needed to enhance crop yields.

1.2. Plant Growth Promoting Rhizobacteria

The plant growth promoting rhizobacteria (PGPR) are microorganisms in the rhizosphere that enhance plant growth and often suppress plant pathogens (Ahemad and Kibret, 2014). Microorganisms play a vital role in plant growth promotion. They promote plant growth directly and indirectly (Glick *et al*., 2007; Farajzadeh *et al.,* 2012). Indirect plant growth promotion is where the responsible microorganisms produce enzymes that lyse the cell wall of pathogens; or induce systemic resistance in host plants, resulting in the production of enzymes such as chitinase, β1,3-glucanase, lipase and protease; or they compete with pathogens for binding sites on host roots by preventing or restricting access of the pathogen to the roots; or they produce antibiotics against the pathogen; or produce siderophores to remove iron from the rhizosphere, thus limiting growth of the pathogen (Bhattacharyya and Jha, 2012). Direct plant growth promotion is where the responsible microorganisms promote plant growth by fixing atmospheric nitrogen and then making it available to the plant in a usable form (Baset Mia *et al*., 2010); or to solubilize the bound phosphorus and micronutrients, and thereby making them available for plant uptake (Ramírez and Kloepper, 2010); or they promote plant growth through the production of phytohormones such as auxins (Ahemad and Kibret, 2014).

1.2.1. Nitrogen fixation

Fertilizer consumption has increased exponentially throughout the world because it is important for the agricultural productivity and quality. This increase is a serious concern because nitrogenous fertilizers pose environmental and health risks as they may lead to soil, water and air pollution (Savci, 2012). Nitrogenous fertilizer production is energy intensive and it adds to atmospheric carbon dioxide (Jorquera *et al.*, 2014). Microorganisms play a vital role in making nutrients available to crops. Some microorganisms such as bacteria belonging to a genus *Azotobacter* and *Bacillus* are known to convert atmospheric nitrogen to usable forms for crops (Farajzadeh *et al.*, 2012). Nitrogen is important for growth and development of plants. For example, Peng *et al.* (2013) found that plants inoculated with *Azotobacter* grew significantly higher than plants that were not inoculated (Peng *et al.*, 2013). These microorganisms can be formulated and sold as biofertilizers (Hashemabadi *et al.*, 2012). The use of these microorganisms is an approach to produce crops with reduced inputs of chemical fertilizers.

In this study, *Bacillus megaterium* was used fix nitrogen, solubilize phosphorus, and therefore to enhance maize production in a marginal soil in the Eastern Cape, a novel approach in South Africa. Free-living *B. megaterium* has been used by others to fix atmospheric nitrogen for other crops (Liu *et al.*, 2006; Hassan *et al.*, 2012), but not for maize in marginal soils in Africa.

1.2.2. Phosphorus solubilization

Phosphorus is very important in plant growth and development (Barker, 2012). It is therefore important that it is available for utilization by plants. Sometimes its availability is hampered by the chemical reactions in the soil. These include P sorption through solid-phase adsorption and precipitation. This happens mostly in acidic soils, resulting in phosphorus being unavailable to plants (Reis *et al.*, 2011; Schefe *et al.*, 2011; Goh *et al.*, 2013). The pH of acidic soils is normally corrected by amending them with lime. However, liming does not make the clay-bound phosphorus available again (Barker, 2012; Cong and Merckx, 2005). Phosphorus solubilization is therefore very important in acidic soils because it releases the bound phosphorus into soil solution where it is available for uptake by plants. Some microorganisms such as *Azotobacter chroococcum*, *Azotobacter vinelandi* (Farajzadeh *et al*., 2012), *Bacillus megaterium* (Hassan *et al*., 2012; Xinxian *et al*., 2011) and others have been reported to solubilize phosphates (Singh *et al*., 2013, Ramírez and Kloepper, 2010).

The use of biofertilizers such as *B. megaterium* may reduce the levels of fertilizer applications, reducing nutrient leaching and run-off that may lead to eutrophication and death of aquatic life

(Adesemoye and Kloepper, 2009). *Bacillus megaterium* is reported to both mineralize and solubilize phosphorus (Tao *et al*., 2008; Hu *et al.,* 2013).

1.3. Biological Control of Root Diseases

It has been reported that efficient and sustainable plant production systems requires the use of chemical inputs in agriculture must be reduced. Moreover the increased pesticide use poses a threat to public health and environment (Spadaro and Gullino, 2005). This has moved researchers to alternative strategies such as biological control which involves the use of beneficial bacteria and fungi to control root diseases. There are many bacteria and fungi that have been reported to control root diseases. *Trichoderma* spp. are among the fungi that have been reported to control root diseases such as damping off and root rot of many crops (Mghalu *et al.,* 2007). *Bacillus subtilis* is one of the bacteria that control *R. solani*, the causal agent of damping off and root rots of many vegetable crops (Mizumoto *et al.,* 2007). In this study *Trichoderma harzianum* was used as a biocontrol agent of root diseases caused by *Rhizoctonia solani* of maize.

1.3.1. Use of *Trichoderma harzianum* for the biological control of root diseases

It has been reported widely that strains of *Trichoderma harzianum* can be effective biological control agents active against many pathogens (Table1.1). It uses various mechanisms to control the effect of the pathogen. These mechanisms include enzymatic activities, siderophore production, competition for nutrients and space, and direct mycoparasitism. *Trichoderma* species are also known to induce defense mechanisms in plants by releasing a number of elicitors that may induce different types of signals for expression of plant defense proteins (Nawrocka and Malolepsza, 2013).

Table1.1: Root diseases controlled by *Trichoderma harzianum* to improve plant growth

Disease or pathogen	**Crop**	**Comments**	**Reference**
Fusarium oxysporum f. sp. melonis	Muskmelon (*Cucumis melo L.* cv. Giotto)	Control by chitinolytic activity	López-Mondéjar *et al*., 2012
Root-knot nematode (*Meloidogyne javanica*)	Tomatoes (*Lycopersicon esculentum* var. Roma VF) and a wide variety of crops.	Penetrates nematode egg mass matrix and decreases nematode egg hatching level by chitinase and protease activities	Sahebani and Hadavi, 2008.
Downy mildew ((*Plasmopara viticola (Berk. and Curt.)* Berl. and de Toni))	Grape (*Vitis vinifera L.*)	Induces resistance against downy mildew by priming for defense without costs for grapevine	Perazzolli *et al*., 2011; Perazzolli *et al*., 2008.
Fusarium wilt (*Fusarium oxysporum f. sp. cucumerinum*)	Cucumber (*Cucumis sativus* L.)	Reduces the incidence	Zhang *et al*., 2013a; Zhang *et al*., 2013b
Dry root rot (*Rhizoctonia bataticola* (Taub) Butler)	Mungbean (*Vigna radiata (L.)* Wilczek)	Reduces the dry root rot incidence	Dubey *et al*. 2009.
Fusarium wilt (*Fusarium oxysporum f. sp. cubense*)	Banana (*Musa sp.*)	Reduces the incidence	Thangavelu *et al.* 2004
Early and late blight of tomato (*Alternaria solani* OTA 22 and *Phytophthora infestans* PIT 30)	Tomato (*Lycopersicon esculentum* Mill.)	Induction of systemic resistance	Chowdappa *et al*. 2013
Basal rot	Onion	Antifungal activity against the pathogen thus reducing the incidence	Cos-Kuntuna and Özer, 2008.
Root rot (*Fusarium solani* (Mart.) Sacc. f sp. *phaseoli*)	Common bean (*Phaseolus vulgaris* L.)	Mycoparasitism	Steindorff *et al*., 2012

In Table1.1 *T. harzianum* has been tested on many crops in the field, but not on maize against *Rhizoctonia solani.* This study therefore tested *T. harzianum* on maize grown on a marginal soil. It was also integrated with *B. megaterium* and potassium silicate to improve maize yields.

1.4. Silicon and Plant Disease Resistance

Silicon is a trace element found in the soil and it is one of the most abundant minerals in soils. It forms 27% of the soil minerals, which is the highest percentage of all soil minerals (Ma, 2005). It is regarded as beneficial for plant growth (Romero-Aranda *et al*., 2006). Silicon benefits the plant by priming the plant to defend itself against plant diseases and insects. Some plants treated with silicon fertilizers are known to increase the concentration of antimicrobial phenolic acids and flavonoids in response to infections (Shetty *et al*., 2011). Silicon accumulates in the epidermis of the cell wall in the leaves, stem and roots, thus modifying the cell wall structure by making it more rigid. Pathogens therefore are unable to infect the plant because of access restriction to the plant (Liang *et al.*, 2005). Different plants have different abilities to accumulate silicon in their tissues and this is due to different uptake abilities between different plants (Balakhnina and Borkowska, 2013). For example tomato plants (*Solanum lycopersicum* L.) that are susceptible to bacterial wilt increased their resistance to the disease when they were treated with silicon. The increase in resistance was between 38.1-100% resistance to bacterial wilt caused by *Ralstonia solanacearum* (Smith) Yabuuchi*,* while the tomato plants that were not treated with silicon could not resist infection by *R. solanacearum* (Diogo and Wydra, 2007).

1.4.1. Use of silicon to prime plants for enhanced disease resistance

Crops have multiple pest and disease resistance mechanisms, including systemically acquired resistance and induced systemic resistance against different diseases. Induced systemic resistance may be primed by either biotic or abiotic agents (Walters *et al.*, 2013). The abiotic agents may include hormones, citrate, fumarate and other defined chemicals (Pastor *et al.*, 2014). Silicon is one of the chemical compounds that are reported to prime plants to defend themselves from pathogens. Table 1.2 shows examples of silicon priming plants for enhanced disease resistance.

Table1.2: Examples of plant diseases controlled by the use of silicon to prime plants for enhanced disease resistance

Disease or Pathogen	Crop	Comments	Reference
Powdery mildew (*Podosphaera fusca* (Fr.) Braun and Shishkoff. synonym *Podosphaera xanthii)*	Cucumber (*Cucumis sativus* L.); zucchini (*Cucurbita pepo* L. cv. '*Rival'*)	Silicon significantly enhanced the activities of peroxidase, polyphenoloxidase and chitinase while it significantly decreased the activity of phenylalamine ammonia-lyase.	Liang *et al.* 2005; Savvasa *et al*. 2009
Rose powdery mildew (*Podosphaera pannosa* (Wallr.) de Bary*)*	Potted roses (*Rosa* hybrid)	Silicon primed the accumulation of phenolic compounds	Shetty *et al*. 2011
Bacterial wilt (*Ralstonia solanacearum* (Smith) Yabuuchi et al)*)*	Tomato (*Solanum lycopersicum* L.)	Silicon primed plant defense by gene activation	Kiirika *et al*. 2013; Kurabachewa *et al.* 2013
Powdery mildew (*Blumeria graminis* (DC.) Speer)	Wheat (*Triticum aestivum* L.)	Silicon primed the diseased plant to produce antifungal methylated forms of trans-aconitate	Re´mus-Borel *et al.* 2009; Côté-Beaulieu *et al.* 2009
Brown rust (*Puccinia melanocephala* Syd. and Syd.)	Sugarcane (*Saccharum* sp.)	Silicon primes for the production of phenolic compounds	Camargo *et al*. 2013
Tobacco ringspot virus	Tobacco (*Nicotiana tabacum* L)	Delays the symptoms	Zellnera *et al.* 2011
Bacterial blight (*Xanthomonas oryzae* pv. o*ryzae*)	Rice (*Oryza sativa* L.)	Activates multiple defense responses	Li *et al.* 2012

1.4.2. Mechanisms of silicon to enhance root disease resistance

The soluble form of silicon is absorbed by plant roots and deposited in the whole plant including the roots as amorphous silica (SiO_2-$n$$H_2O$). In the plant it is deposited mainly in the cell wall by interacting with polyphenols and pectin thus enhancing cell wall strength and rigidity (Pilon-Smits *et al*., 2009). The rigidity and strength of the cell wall is the one that prevents the pathogens from entering the plant. For example, fungal pathogens will struggle to penetrate the epidermis of the cell wall, thus impeding infection (Hayasaka *et al.*, 2008; Deepak *et al.*, 2008). The challenge with this phenomenon is that it needs a constant supply of soluble silicon; if the supply of silicon stops, then the cell wall strength is lost and it goes back to its original susceptible status (Pilon-Smits *et al*., 2009).

1.5. Fertilization as a Challenge for Small scale Farmers

Fertilization is important for the growth and development of crops in most soils. Most farmers are aware of this but many small scale farmers are unable to apply fertilizers to their crops for many reasons. Some farmers are unable to fertilize properly because they do not know the fertility status of their soils; others lack proper education on fertilization; others lack the equipment to apply fertilizers to all their fields; whereas other farmers who have the equipment to apply fertilizers do not apply a complete dose of fertilizer because of cost issues (Kibirige, 2013). There are various methods of fertilization in the field. Some broadcast the fertilizers, which is an expensive method because some reasonable quantity of the fertilizer is wasted. Others use a deep band application, which is an economical method because it reduces the fertilization of weeds between rows (Bordoli and Mallarino, 1988) and reduces wastage of fertilizer. This method also reduces nitrate contamination of surface and ground water, which is not the case with broadcast applications (Di Tomaso, 1995).

Regardless of the application method, some nutrients that are supplied by the fertilizers may become unavailable to crops because of the acidity of the soils. For example, phosphates are fixed by free aluminum ions in acidic soils, whereas nitrogen fertilizers may be leached because of the reduced activity of bacteria in acid soils, which affects the bacteria responsible for the nitrification process (Karaivazoglou *et al*., 2007; Mijangos *et al*., 2010).

1.5.1. Cost of fertilization

Fertilization is a major cost in crop production. Small scale farmers cannot afford to buy the recommended quantities of fertilizers required for their crops, so they apply reduced levels or none at all. Small scale farmers routinely disregard the recommendations from extension officers obtained from soil analyses because of the high cost of fertilizers and the feasibility of lime application as their soils require huge amounts such as 6 tons per ha of lime.(Kormawa *et al*., 2003). The first problem with the farmers is in the logistics of bringing 6 t ha^{-1} of lime to the field and its application. This study seeks to mitigate in this problem by reducing the quantities to an affordable and practical quantities, ten quantify how much increased yields can farmers get.

Furthermore, intensive chemical fertilization may have detrimental effects on soils in the long-term (Ghosh, 2004). This leads researchers to a search for alternative approaches to intensive chemical fertilization in order to make fertilization more affordable, and to make agriculture more sustainable. In this study therefore, microorganisms were used to enhance nitrogen fixation and phosphate solubilisation and therefore to indirectly fertilize maize crops. Conventional fertilizers and lime were also used but in micro-doses, in order to make their benefits more affordable, and their negative impacts to be reduced.

1.5.2. Effect of acid soils, and the importance of liming

Most of the soils in the eastern region of the Eastern Cape are highly acidic. This limits the growth and development of crops. Acid soils limit root growth. Roots of plants grown in acidic soils are consistently shorter than when they grow in neutral soils (Caires *et al*., 2008; Wright *et al.* 1988). In acidic soils aluminum ions dissolve from aluminium minerals and these aluminum ions are toxic to plant roots, which limits root elongation in crops such as maize (Eticha *et al*. 2005). Aluminium loss of phosphates in acid soil is promoted because soluble aluminum ions bind with phosphate ions to form insoluble aluminum complexes. Therefore, liming reduces phosphate losses and improves phosphate uptake by plants that have effective root systems (Karaivazoglou *et al*., 2007; Mengel, 1997). Some of the losses of major plant nutrients in acidic soils are due to the inhibition of microbial activities in soils, especially bacteria (Bolan *et al*. 2008; Mijangos *et al*., 2010). Liming also improves water use efficiency in maize, due to an enhanced root surface area and hence, enhanced water uptake (de Barros *et al*., 2007).

Farhoodi and Coventry (2008) showed that liming increases productivity and profitability in crops such as wheat (*Triticum aestivum* L.). It was also found that liming off-sets the acid inputs associated with soil acidification. In particular, fertilization with ammonium fertilizers or urea enhance soil acidity by releasing a proton as the ammonium ion is converted to the nitrate ion (Caires *et al*., 2006). Liming enhances the physical, biological and chemical properties of soils thus increase the productivity of the soil (Bolan *et al*., 2008).

Despite the many benefits derived from liming acid soils, it is difficult for small scale farmers to buy and transport lime in the necessary quantities. With the acid soils of the Eastern Cape (pH around 4.0 or below) and high levels of acid saturation (strong buffering capacity), levels of lime application are typically 6-16 tons per ha which is far outside the financial or logistic capabilities of small scale farmers to buy, transport or apply.

In this study micro-doses of lime, applied into planting holes instead of liming the whole field, was therefore tested as a means to improve the productivity of maize in acid soils.

1.6. Integrating Plant Growth Promoting Rhizobacteria, Biological Control, potassium silicate, Minimal Fertilization and Liming

Integrated disease management has been reported as an approach to disease management (Obradovic, *et al*., 2005). This system has been used in the control of bacterial spot on tomato where a biological control agent was used in conjunction with the systemic acquired resistance inducers, with an additive or synergistic effect when combined (Obradovic, *et al*., 2005).

In another study in Egypt, three biological control agents were used in conjunction with two resistance inducers, and the combination treatment was the most effective for the control of cotton root diseases (Abo-Elyousr *et al*., 2009). Another integration that has been reported is the synergy between two biological control agents (Xu *et al.,* 2011). It is also reported that biological control agents and potassium silicate may be integrated to control powdery mildew of zucchini caused by *Podosphaera xanthii* (Castagne) (Tesfagiorgis and Annegarn, 2013). There is no study in the literature on the integrated microdosing of fertilizers, biological control agents and priming of maize plant defences using potassium silicate against *Rhizoctonia solani*.

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CHAPTER TWO

Integration of *Trichoderma harzianum* and potassium silicate to improve growth and yield of maize in the presence of *Rhizoctonia solani*

Abstract

Maize (*Zea mays*) is important in South Africa as a staple crop for most people, and as an animal feed. Small scale farmers struggle to achieve optimum yields due to biotic and abiotic factors. Eco-T® (a.i. *Trichoderma harzianum*), a commercial biocontrol agent, is known to control most pathogenic fungi including *Rhizoctonia solani*, a causal agent of root rot that reduces maize yield. In this study its effect was evaluated when it was applied together with silicon fertilizer. Two silicon formulations were tested, namely a liquid and a slow release formulation. All plots were inoculated with *R. solani* except the positive control plots. Some plots were treated with *T. harzianum,* others with the different formulations of potassium silicate (KSil) and others with the combinations. All treatments significantly increased the maize yield relative to inoculated control. The treatment that gave the highest percentage difference relative to the inoculated control was KSil liquid formulation combination with *T. harzianum*, the combinations gave a significant 45% increased yield over the inoculated control. This means that this combination is an option for the farmers.

2.1. Introduction

Plant diseases that affect maize production include root diseases. Maize root rot is one of the diseases that most farmers do not attempt to control. It is caused by fungi such as *Rhizoctonia solani* Kühn, *Pythium* and *Fusarium* species (Govaerts *et al*., 2007). *Rhizoctonia solani* has a wide host range which makes it difficult to control. If not controlled, it has the potential to reduce yields because it reduces crop root volume, reducing uptake of water and nutrients by plants.

Farmers are uncertain about the severity of root diseases in a given cropping season. This is because maize root diseases may be sub-lethal and do not kill infected maize plants. However, they can reduce maize yields considerably, although the level of losses are not quantified or seen (Anees *et al.,* 2010; Bennett, 2012).

Agrochemicals may be used to control many diseases. However, there are few fungicides that control root diseases effectively. Other means of control include crop rotation, biological control and priming of crop resistance to the pathogen (Pettie *et al.*, 2012). These control methods vary in terms of efficiency, sustainability, application and costs.

Biological control of root diseases involves the use of microorganisms that may live in the rhizosphere of the host plant. These microorganisms compete for root colonization because roots exudates sustain their growth. They compete in various ways. Some produce compounds that are toxic to pathogens such as antibiotics, phenazines, siderophores, pyrrolnitrin, and pyoluteorin, as well as other enzymes that are lethal to the pathogens (Babalola, 2010).

Among the microorganisms that have been used as biological control agents is *Trichoderma harzianum* Rifai (Tan *et al*., 2015). Some strains are competitive including the mechanism of hyperparasitism, to control crown, stem and root rot diseases caused by *Rhizoctonia sp.*, *Sclerotinia sp.* and *Pythium sp.* in many crops (Marzano *et al*., 2013). It is widely used as biological control agent against many plant diseases. *Trichoderma harzianum* also induces plant systemic resistance by the release of compounds such as salicylic acid to trigger the plant defense mechanisms, either induced systemic resistance or systemic acquired resistance (Nawrocka and Małolepsza, 2013).

The defense mechanisms in a plant may also be primed by chemical substances in the soil such as silicon (Kurabachew and Wydra, 2014). However, soluble forms of silicon are not readily available in the soil for plant use. Silicon is an abiotic elicitor that primes the plant's defense mechanism to enhance resistance to abiotic and biotic stresses (Gonzalo *et al*., 2013; Kurabachewa *et al*., 2013).

Integrating disease control methods has been reported as one of the methods that improve disease control. It has been suggested that integrating two or more different strategies of disease control may result in better control than employing each disease control option separately (Rather *et al.*, 2012). This may be because they have different modes of action, which prolongs the time for the pathogen to develop resistance or coping mechanism (Abo-Elyousr, 2009).

In this study the effects of independent and combined treatments of maize with a biological control agent using *T. harzianum,* and priming for resistance using potassium silicate fertilizers, were evaluated for their ability to improve maize production in the presence of *R. solani* under field conditions.

2.2. Materials and Methods

The field trial was conducted in loamy soils at the Mgwalana Village, Elliot, Eastern Cape, South Africa (31° 28' 0" South, 27° 18' 0" East). The soils are acidic (pH 3.98); approximately 5g of dolomitic lime (0.026t.ha^{-1}) was applied per planting station together with approximately 5g of 2:3:2 (34) fertilizer[1]. The soils needed 6 t ha^{-1} but microdosing was employed. The seed was then placed into the planting hole after the fertilizer was covered with soil. The seeds were treated as per the requirement of the plot as per the experimental design.

2.2.1. Seed Treatment

Maize seeds PAN14[2]were treated with the Eco-T® (2×10^9 conidia g^{-1} of *Trichoderma harzianum Rifai*) or were not treated. One kilogram of maize seeds was treated with 5g of Eco-T® as per the instruction manual of the Eco-T® producer (Plant Health Products (Pty) Ltd[3]). Eco-T® is a registered biocontrol product which has *T. harzianum* Strain kd as the active ingredient. The seeds were treated on the day of planting.

2.2.2. Silicon Fertilizer Application

Silicon fertilizer[2] was applied in two ways. Some plots were treated with potassium silicate (KSil), a liquid formulation of potassium silicate (21% Si) and others with a slow release formulation of silicon [Solid potassium silicate (50%)]. The liquid formulation of KSil was drenched (5ml of 400mg L^{-1} solution per hole where the seed was planted). Approximately 5 g of the slow release potassium silicate powder was placed in the planting hole and covered before the maize seed was planted.

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[1] Omnia Fertilizers, Sasolburg, South Africa

[2] Pannar Seeds, Greytown, South Africa

[3] Plant Health Products (Pty) Ltd, P. O. Box 207, Nottingham Road, KwaZulu-Natal, South Africa

2.2.3. *Rhizoctonia solani* Inoculum Preparation and Application

The pathogen that was used in the experiment was an isolate of *Rhizoctonia solani* obtained from Dr K.S. Yobo[4]. It was subcultured on Potato Dextrose Agar and then transferred to double autoclaved barley seeds in 500 ml conical flasks. It was allowed to grow at room temperature on the barley seeds until it colonized all the barley seeds. During planting in the field, one infected barley seed was placed 40 mm away from one maize seed and covered with soil.

2.2.4. Experimental Design and Analysis

The experiment had two controls namely: a control with fertilization only but no pathogen, and a control with the inoculum of the pathogen applied and fertilization. The treatments were Eco-T®, liquid potassium silicate, slow release potassium silicate, Eco-T® plus liquid potassium silicate, Eco-T® plus slow release potassium silicate. Each treatment was applied on maize seeds planted in a 3 m x 3.6 m plots. In each plot there were 55 plants with a 0.9 m spacing between rows and a 0.3 m spacing between plants. The spacing between plots was 1 m as well as between replicates. The trials were conducted in the 2012/2013 and 2013/2014 seasons in the same plots with the same treatments.

A Complete Randomized blocks design with 4 replicates was employed. The parameters measured were stem diameter, plant height, root dry weight, 1000 kernel weight, number of rows per cob, number of kernels per row, and grain yield (at 12% moisture).

Factorial analysis of variance was performed using the General Linear Model of ANOVA, using Genstat® 14th edition. An F value for main treatment effects and their interaction were considered significant at the $P \leq 0.05$ level. Treatment means were separated using Fisher's unprotected LSD test at the 5% probability level.

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[4] Dr K.S. Yobo, Plant Pathology, SAEES, UKZN, Private bag X1, Scottsville, 3309, South Africa.

2.3. Results

The application of *T. harzianum* only or KSil liquid formulation only or KSil solid formulation plus *T. harzianum* or KSil liquid formulation combination with *T. harzianum*, increased the maize yield significantly, when compared to the Inoculated Control by 39%, 41%, 37% and 45%, respectively. When the same treatments were compared to the Untreated Control the difference in yields of 10 - 18% were not significant. The treatment that gave the lowest yield was the application of KSil slow release formulation alone, which yielded 31% higher than the inoculated control (Table 2.1)

When *T. harzianum* was applied with the KSil slow release (Solid) the maize plants were significantly taller than the Inoculated Control by 12.58%. The KSil liquid application also resulted in significantly taller maize plants than the Inoculated Control (Table 2.1).

The stem diameters of maize plants treated with both formulations of KSil were significantly bigger than the Inoculated Control, by 8% for liquid formulation and 17.5% for the slow release formulation for the stem diameter, respectively.

Application of *T. harzianum* alone significantly enhanced root dry weight by 81.28% relative to the Inoculated Control. Moreover its mixture with KSil slow release also increased the root dry weight, by 55.7% although not significantly.

All treatments resulted in maize cobs with significantly more kernels per row and significantly more rows per cob, relative to the Inoculated Control. The following (Table 2.1) outlines the interactions between treatments.

Table 2.1: Efficacy of potassium silicate formulations, *T. harzianum* Strain T.kd and their combinations on maize yield components under field infected with *R. solani* in the 2012/2013 season

Treatment	**KSil liquid**	**KSil Slow release**	***T. harzianum***	**Yield t ha^{-1}**	**1000 kernel Weight (g)**	**Stem diameter (mm)**	**Plant Height (cm)**	**Kernels row^{-1}**	**Root dry weight (g)**	**Rows cob^{-1}**
KSil liquid	Yes	No	No	5.480a	197.1abc	24.81b	75.98a	36.15a	24.70b	15.00a
KSil Slow release	No	Yes	No	4.973ab	202.7a	27.00a	62.40c	36.45a	28.62ab	14.25a
T. harzianum	No	No	Yes	5.878a	175.9d	24.29bc	62.38c	35.40a	36.60a	14.70a
T. harzianum+KSil liquid	Yes	No	Yes	5.890a	191.5abc	24.33bc	71.62ab	37.75a	22.70b	14.70a
T. harzianum+KSil Slow release	No	Yes	Yes	5.402a	185.5bcd	24.04bc	72.92a	36.80a	31.43ab	14.45a
Untreated Control	No	No	No	4.628ab	197.9ab	24.10bc	72.18ab	29.00b	27.29ab	13.25b
Inoculated Control	No	No	No	3.669b	181.8cd	22.97c	64.77bc	27.45b	20.19b	12.50b
Treatment effects				P value	P value	P value	P value	P value	P value	P value
KSil liquid				0.178	0.397	0.135	0.022	0.421	0.050	0.245
KSil Slow release				0.306	0.491	0.147	0.232	0.808	0.520	0.170
T. harzianum				0.459	0.003	0.009	0.786	0.723	0.081	0.979
T. harzianum +KSil liquid				0.802	0.082	0.045	0.078	0.342	0.098	0.355
T.harzianum +KSil Slow release				0.728	0.707	0.037	0.006	0.972	0.725	0.453
F value				3.50	4.02	6.89	4.92	10.36	2.76	7.59
CV%				16.5	5.1	3.8	7.3	7.5	24.3	4.7

*****Numbers with different letters in a column are significantly different at p=0.05 according to Duncan's multiple range test

The presence of the liquid formulation of KSil contributed significantly to an increase in maize height and root dry weight. Treatment with *T. harzianum* only resulted in a significant increase of the kernel weight and the stem diameter. The combination of the KSil liquid formulation and *T. harzianum* resulted in significant increases to kernel weight, stem diameter, height and root dry weight. The combination of the KSil slow release and *T. harzianum* resulted in a significant increase of maize plant height and stem diameter (Table 2.1).

In Season 2 the same scenario was observed as shown in Table 2.2 where all treatments resulted in significantly higher yields than the Inoculated Control. The root dry weight for all treatments caused higher root dry weight than the Inoculated Control and the differences were significant, as in Season 1. The 1000 kernel weights for all treatments were not significantly different relative to the Inoculated Control. The KSil liquid formulation increased the maize plant height significantly in both seasons relative to both Untreated Control and Inoculated Control (Table 2.2).

The interactions of the treatments used, shows clearly that the combination of *T. harzianum* and the liquid formulation of silicon significantly increased the yield more than any other treatment, followed by the individual treatments; and then *T. harzianum* and liquid formulation of KSil (Table 2.2).

Table 2.2: Efficacy of potassium silicate formulations, *T. harzianum* Strain T.kd and their combinations on maize yield components under field infected with *R. solani* in the 2013/2014 season

Treatment	**KSil liquid**	**KSil Slow release**	***T. harzianum***	**Yield t ha^{-1}**	**1000 kernel Weight (g)**	**Stem diameter (mm)**	**Plant Height (cm)**	**Kernels Row^{-1}**	**Root dry weight (g)**	**Rows Cob^{-1}**
KSil liquid	Yes	No	No	12.84ab	0.3190a	24.68bc	1.518a	40.95a	4.2:3:2a	14.1ab
KSil Slow release	No	Yes	No	12.31ab	0.3170a	21.45d	1.027c	41.05a	4.306a	13.40bc
T. harzianum	No	No	Yes	14.04a	0.3240a	25.67ab	1.443ab	38.30ab	4.054a	14.35a
T. harzianum+KSil liquid	Yes	No	Yes	8.27c	0.3060a	27.90a	1.387ab	35.70b	3.754a	14.1ab
T. harzianum+KSil Slow release	No	Yes	Yes	11.51b	0.3140a	25.38abc	1.264b	41.55a	4.568a	13.45abc
Untreated Control	No	No	No	8.12c	0.3205a	22.90cd	1.278b	35.20b	2.527b	13.65ab
Inoculated Control	No	No	No	5.38d	0.3155a	24.52bc	1.314b	30.40c	1.370c	12.65c
Treatment effects				P value	P value	P value	P value	P value	P value	P value
KSil liquid				0.011	0.450	0.007	0.032	0.220	0.230	0.303
KSil Slow release				0.869	0.918	0.031	0.081	0.067	0.148	0.027
T. harzianum				0.032	0.665	0.002	0.720	0.085	0.595	0.425
T. harzianum +KSil liquid				0.004	0.346	0.678	0.267	0.212	0.407	0.488
T.harzianum +KSil Slow release				0.728	0.714	0.952	0.421	0.159	0.169	0.295
F value				20.24	0.35	5.78	5.32	8.49	17.99	3.56
CV%				13.5	6.1	7.0	10.4	7.5	15.5	4.5

*****Numbers with different letters in a column are significantly different at p=0.05 according to Duncan's multiple range test

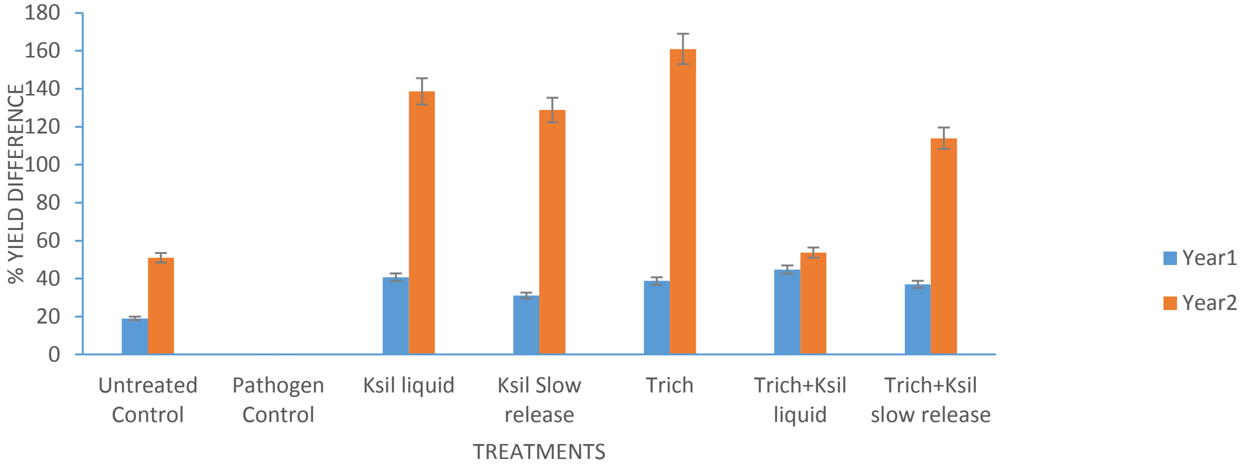

Figure 2.1: Yield differences relative to the Pathogen Control over the seasons

In Figure 2.1 above all treatments caused significant increases in yields compared to the Inoculated Control (CP). It was also observed that all treatments increased markedly the percentage yield difference in Season 2, relative to Season 1.

2.4. Discussion

In Season 1 the grain yield from the Untreated Control was 19% higher than the Inoculated Control, although the difference was not significant at 95% confidence level. In Season 2 the Untreated Control had a yield increase of 50.93%, which was significant. The increase in the difference between the two seasons may have been caused by the difference in the rainfall pattern, the second seasons was a wet season compared to the first season. Moreover, a build-up of inoculum in the soil as the same plots were used in both seasons was expected (Govaerts *et al.*, 2006; Govaerts *et al.*, 2007). This links very well with the increase of root dry weight observed between the Untreated and Inoculated Controls by 35.2% in Season 1 and 84.6% in Season 2. This reduction reflects the sub-lethal loss of roots caused by the pathogen treatment with inoculum of *R. solani*. The reduction in root mass made a big contribution to the reduced crop yields as observed in these trial. This therefore confirms that the strain of *R. solani* used in these trials was able to decrease crop yield considerably when not controlled and the extent of disease attack determined the yield losses (Anees *et al*., 2010).

The application of *T. harzianum* increased maize yield by 38.8% when compared to the Inoculated Control and 16.6% when compared to the Untreated Control. The yield increase was also confirmed in Season 2. This confirmed that *T. harzianum* can control *R. solani*, as reported by Mghalu *et al.* (2007); López-Mondéjar *et al.* (2012) and Steindorff *et al*. (2012).

The liquid formulation of KSil caused a significant yield increase of 40.7%, suggesting that KSil was able to protect the maize roots from the pathogen. Potassium silicate has been used to control other pathogens such as *Phytophthora cinnamomi* Rands (Bekker *et al.*, 2014) and also damping-off caused by *Pythium ultimum* Trow 1901 (Deliopoulos *et al*., 2010). In some studies it has been reported that silicon inhibits the mycelial growth of pathogenic fungi, and their spore germination and germ tube elongation (Bekker *et al.*, 2014). Other studies reported that silicon control plant diseases by priming the plant for enhanced resistance to the pathogen (van Bockhaven, 2013; Zhang *et al*., 2013).

The slow release formulation of KSil also caused a significant yield increase of 38.8%. The difference in effects between the two formulations of KSil was not significant, which means that either formulation may be used.

The application of *T. harzianum* caused a yield increase of 31%. This confirmed that *T. harzianum* may be used as a biological control agent to control various diseases including root rots as reported by others (López-Mondéjar *et al.*, 2012; Steindorff *et al*., 2012).

The mixtures of KSil and *T. harzianum* did not perform consistently. In Season 1, application of the mixtures increased yields compared to KSil alone and *T. harzianum* alone. In Season 2, the performance of the mixtures were not better than the single treatments. This may have been because the KSil and the *T. harzianum* levels were enhanced in Season 2.

It is therefore concluded that KSil and *T. harzianum* both controlled maize root rot when applied individually, thus increasing maize yields. As mixtures they gave inconsistent results. There was no difference in the performance of the liquid formulation and the slow release formulation of KSil.

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CHAPTER THREE

Integration of a Biofertilizer with Micro-dosed Chemical Fertilizers on Maize Grown in a Marginal Soil

Abstract

Maize yields in the Eastern Cape of South Africa are lower for small scale farmers relative to commercial farmers due to uncontrolled root diseases, unlimed acidic soils, and nutrient deficient soils, due to low levels of fertilization of crops. The soils that were used in this study had a pH of 3.98 and an acid saturation of 54%. The study aimed to develop cost effective strategies on liming, fertilization, and plant growth promotion through phosphate solubilization and nitrogen fixation by *Bacillus megaterium* to increase maize yields for small scale farmers. Micro-dosing of 2:3:2 (34) fertilizer and superphosphate fertilizer as well as lime was employed. A soda bottle cap was used to measure out approximately 5g of fertilizer or lime to fertilize each maize plant, applied directly into the planting hole. A selected isolate of *B. megaterium* was applied through drenching. Micro-dosing of 2:3:2 (34) fertilizer increased maize yields by 64.6% and 13.6% when compared to the control treatment over two seasons. Micro-dosing with superphosphate fertilizer also increased maize yield significantly (P = 0.001) in both the first and second seasons by 50.5% and 37.4%, respectively. The combination of *B. megaterium* and 2:3:2 (34) fertilizer increased maize yield significantly (P = 0.001) in both seasons by 54.7% and 48.1%, respectively. The combination of *B. megaterium*, 2:3:2 (34) fertilizer, and lime significantly (P = 0.018) increased maize yield, maize stalk height, and maize stem diameter in both seasons. The increases were consistent as a result of this combination as compared to the 2:3:2 (34) fertilizer, and lime combination. Whenever *B. megaterium* was included in the treatment combination the increased yield was increased further, but not significantly. It was therefore concluded that micro-dosing fertilizers could play a significant role in improving maize yields for small scale farmers who cannot not afford full field fertilization and liming. It was also concluded that the use of *B. megaterium* is beneficial when combined with low levels of 2:3:2 (34) fertilizer.

3.1. Introduction

Maize (*Zea mays* L.) production in South Africa is important for export and consumption by humans and livestock. Maize is exported to Lesotho, Mozambique, Swaziland, Namibia, Botswana, and Mexico. In the 2013/2014 season, South Africa started to export maize to Japan and Taiwan (Grain SA, 2013). South Africa produces a mean maize yield of 4.25 t ha^{-1}. In the Eastern Cape the average maize yields are 5.8 t ha^{-1} on commercial farms. However, on non-commercial farms, maize yields are 1.6 t ha^{-1} on the 92.91% of the land used for maize production (Dredge, 2014). There are many factors contributing to this poor yield including root diseases, poor soils, and the reality that most of the small scale farmers in this province cannot afford to buy the recommended agricultural inputs (Kibirige, 2013).

Most agricultural land in the Eastern Cape has acidic soils (Mandiringana *et al.*, 2005). Acidic soils limit agricultural production by promoting the binding of phosphorus to clay, making it unavailable to plants. Soil acids also dissolve aluminium and manganese from mineral complexes, which in turn poison the crops, especially their roots (Kochian *et al.* 2004; Reis *et al*. 2011; Schefe *et al*. 2011; Goh *et al*. 2013). The use of microorganisms to solubilize the bound phosphorus is another option to make phosphorus available to the crop. Moreover the effectiveness of beneficial microorganisms in the soils is reduced in acidic soils because most bacteria are negatively affected by low pH (Bolan *et al.* 2008).

Most crops tolerate acidity up to a certain level. Maize, which is the most grown crop in the Eastern Cape, may tolerate up to 20% of acid saturation. However, the acid saturation is often over 40% in unlimed soils. These soils are also short of Ca and Mg due to the leaching of base salts that created the acid soils. This can be reversed by the application of lime, ideally dolomitic lime with a Ca:Mg ratio of approximately 4:1. However, the application of lime to acidic soils poses a challenge to the Eastern Cape small scale farmers because they need to apply the lime 2 to 3 months before planting and also there must be a degree of mixing in the soil to shorten the time to correct subsoil acidity (Tisdale *et al.*, 2003). This means that they need to till their land and apply the lime up to 3 months before planting of the maize crop. Therefore they will need to till the land again because weeds would have come up, which makes it a costly exercise, especially in a region where there is a chronic shortage of labour. This study therefore seeks to reduce the burden of high input costs by employing microdosing of fertilizer and lime. Secondly the study seeks to change the norm of applying lime 3 months before planting to reduce the cost of mechanization by applying lime during planting and directly in the planting hole rather than broadcasting it. Thirdly the study seeks to employ the use of nitrogen and fixers and phosphorus solubilizers together with the micro-doses of fertilizer and lime to improve maize yield at low cost. Some strains of *Bacillus megaterium* have been reported to solubilize phosphorus and fix atmospheric nitrogen (Xinxian *et al*., 2011; Hassan *et al*. 2012). The bacterium is easy to apply to maize seeds and it may be more sustainable as it forms spores hence may overwinter and continue fixing nitrogen and solubilize phosphates in the next season when conditions are favorable. Because of that, *B. megaterium* may provide a cost effective source of N and P to maize for small scale farmers.

About 93.0% of the agricultural land in the Eastern Cape is used by small scale farmers (Dredge, 2014). Most small scale farmers cannot afford to buy the recommended levels of agricultural inputs to produce high maize yields. This research therefore aimed to develop strategies that would give the small scale farmers better yields from fertilization practices that they could afford.

3.2. Materials and Methods

The field trial was conducted in loamy and marginal soil at the Mgwalana Village, Elliot, Eastern Cape, South Africa (31° 28' 0" South, 27° 18' 0" East). According to the soil test, soil pH was 3.98 and acid saturation was 54%, which meant that to grow maize for a maximum yield, the soils needed to be limed by 4.5 tons.ha^{-1} of dolomitic lime. The seeds were treated as per the requirement of the plot as per the experimental design. The experiment was conducted over two seasons in the same field, in the same plots. This was done to monitor the build-up of treatments over seasons and to mimic the reality of small scale farming as they don’t have enough land to do proper crop rotations. The test crop was maize and the cultivar used was PAN14[5].

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[5] Pannar Seeds, Greytown, South Africa

3.2.1. Fertilizer Application

The fertilizers were applied as per the experimental design in Section 3.2.4 below. Where fertilizers were applied, approximately 5g were applied into each planting hole, using the cap of a soda drink bottle. Overall fertilizer application was approximately 185kg ha^{-1} for each of the two fertilizers used, which were 2:3:2 (34) and single superphosphate (10.5)[1]. The amount of fertilizer applied was not what the soil required but it was what the small scale farmers can afford hence microdosing technology was tested for the small scale farmers.

3.2.2. Lime Application

Approximately 5 g of dolomitic lime[1] was applied per planting hole instead of applying lime to the whole field. The lime applied was buried before fertilizer was applied, which was also covered by the soil before the seed was planted. Again, the micro-dosing translates into approximately 185kg ha^{-1} of lime being applied.

3.2.3. *Bacillus megaterium* Application

The *B. megaterium* application was done three weeks after planting. It was drenched onto the seedling. The formulation of *Bacillus megaterium* used was obtained from Plant Health Products (Pty) Ltd[6]. It was in a tea bag formulation. Each "tea bag" had approximately 10^8 spores of *B. megaterium*. The tea bag was soaked in a 500 ml of water for 10 minutes to release the bacterium from a water soluble matrix. Exactly 10 ml of the supernatant was added to 5 liters of water and approximately 10ml of the suspension was drenched onto the base of seedlings using a watering can. This was done early in the morning.

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[6] Plant Health Products (Pty) Ltd, P. O. Box 207, Nottingham Road, KwaZulu-Natal, South Africa

3.2.4. Experimental Design and Analysis

The experiment had one control: Untreated Control (a plot with no fertilization). The treatments were the microdose (all the fertilizers and lime were applied using a soda bottle cap full of each fertilizer or lime) of: Superphosphate only; Fertilizer 2:3:2 only; Lime only; *Bacillus megaterium* only; Superphosphate plus Fertilizer 2:3:2; Superphosphate plus Lime; Superphosphate plus *B. megaterium*; Fertilizer 2:3:2 plus Lime; Fertilizer 2:3:2 plus *B. megaterium*; Lime plus *B. megaterium*; Superphosphate plus Fertilizer 2:3:2 plus Lime; Superphosphate plus Fertilizer 2:3:2 plus *B. megaterium*; Fertilizer 2:3:2 plus Lime plus *B. megaterium*; Lime plus *B. megaterium* plus Superphosphate; Superphosphate plus Fertilizer 2:3:2 plus Lime plus *B. megaterium*.

Each treatment was applied to a 3 m x 3.6 m plot. In each plot there were 55 plants with 0.9m spacing between rows and 0.3 m spacing between plants (approx. 37,000 plants per ha). The spacing between plots and between replicates was 1m.

A Complete Randomized block design with four replicates was employed. The parameters that were measured were stem girth, plant height, 1000 kernel weight, number of rows per cob, number kernels per row, and maize yield.

A factorial analysis of variance was performed using the General Linear Model of ANOVA, using Genstat® 14th edition. An F test value for main treatment effects and their interaction were considered significant at $P \leq 0.05$ level. Treatment means were separated using Fisher's unprotected LSD test at the 5% probability level.

3.3. Results

3.3.1. Effect of micro-dosing fertilizer on maize yield components and yield

Micro-dosing of 2:3:2 (34) fertilizer significantly increased maize yield by 64.6%, relative to the Untreated Control treatment in Season 1 (Table 3.1). However, in Season 2 the yield increase was not significant and maize yield increase was 13.6% relative to the Untreated Control (Table 3.2). Micro-dosing with the superphosphate fertilizer significantly increased maize yield in both the first and second seasons by 50.5% and 37.4%, respectively (Table 3.1; Table 3.2).

The maize stalk heights were significantly greater with the treatments that received a micro-dose of 2:3:2 (34) fertilizer in both first and second seasons by 42.2% and 89.5%, respectively. Micro-dosing with superphosphate increased the stalk height in Season 1 and second season by 10% and 84.7%, respectively, but the difference was significant only in Season 2

Table 3.1: Effect of *Bacillus megaterium and* microdosing of fertilizers and lime on maize yield components in the 2012/2013 season

Treatment	BM	Lime	SP	2:3:2(34)	Yield t ha^{-1}	1000 KM (g)	SG (mm)	PH (cm)	Kernels Row^{-1}	Rows cob^{-1}
BM	Yes	No	No	No	1.401a	157.1ab	19.84a	82.8a	17.05a	12.95ab
Lime	No	Yes	No	No	2.010bcde	158.7ab	19.82a	90.6abc	23.45bcde	13.10ab
SP	No	No	Yes	No	2.490efgh	176.8abc	21.17ab	106.0bcd	26.70de	14.25cd
2:3:2(34)	No	No	No	Yes	2.723gh	214.5d	22.80bcd	136.9ef	24.60bcde	13.90bcd
Lime+ *BM*	Yes	Yes	No	No	1.571ab	151.3a	19.90a	86.2ab	20.55abc	12.05a
SP + *BM*	Yes	No	Yes	No	1.895bcd	175.7abc	21.23ab	110.0cd	19.25ab	12.80ab
2:3:2(34)+ *BM*	Yes	No	No	Yes	2.558fgh	213.7d	22.86bcd	136.3ef	25.65cde	13.65bcd
Lime SP	No	Yes	Yes	No	2.132cdef	189.5bcd	20.18a	102.6abc	21.50abcd	12.75ab
2:3:2(34)+lime	No	Yes	No	Yes	2.891h	223.2d	23.55cd	148.8f	27.40e	13.65bcd
2:3:2(34)+ SP	No	No	Yes	Yes	2.705gh	223.9d	23.81d	156.2f	25.85cde	13.85bcd
SP +lime+ *BM*	Yes	Yes	Yes	No	2.283defg	203.1cd	21.02ab	125.3de	22.55bcde	13.45bc
2:3:2(34)+ SP +lime	No	Yes	Yes	Yes	2.496efgh	196.6cd	23.45cd	149.3f	26.35de	14.40cd
2:3:2(34)+lime+ *BM*	Yes	Yes	No	Yes	2.623fgh	219.4d	23.55cd	150.4f	25.50cde	14.30cd
2:3:2(34)+ SP + *BM*	Yes	No	Yes	Yes	2.293defg	210.0cd	22.90bcd	145.6ef	22.10abcde	13.50bc
2:3:2(34)+ SP +lime+ *BM*	Yes	Yes	Yes	Yes	2.635fgh	221.8d	24.06d	146.5f	26.10cde	14.70d
Control	No	No	No	No	1.654abc	151.5a	21.57abc	96.3abc	19.55abc	13.00ab
Treatment effects					P value	P value	P value	P value	P value	P value
BM					0.001	0.540	0.554	0.208	0.003	0.141
Lime					0.528	0.675	0.848	0.325	0.168	0.809
SP					0.327	0.049	0.038	0.001	0.548	0.035
2:3:2(34)					0.001	0.001	0.001	0.001	0.001	0.001
Lime+ *BM*					0.018	0.238	0.333	0.267	0.072	0.045
SP + *BM*					0.119	0.279	0.621	0.337	0.848	0.544
2:3:2(34)+ *BM*					0.512	0.844	0.636	0.259	0.160	0.133
Lime SP					0.885	0.786	0.565	0.184	0.632	0.083
2:3:2(34)+lime					0.300	0.578	0.127	0.891	0.336	0.003
2:3:2(34)+ SP					0.003	0.279	0.417	0.009	0.301	0.897
SP +lime+ *BM*					0.075	0.050	0.430	0.263	0.311	0.775
2:3:2(34)+ SP +lime					0.877	0.415	0.685	0.196	0.389	0.342
Fertilizer2:3:2+lime+ *BM*					0.317	0.462	0.886	0.895	0.068	0.408
2:3:2(34)+ SP + BM					0.456	0.904	0.524	0.103	0.141	0.046
CV%					13.7	11.4	6.0	10.8	14.3	5.1

*Numbers with different letters in a column are significantly different at p=0.05 according to Duncan's multiple range test

* SP= Superphosphate * BM = B. Megaterium * KM= Kernel mass * SG= Stem girth.

3.3.2. Effect of *Bacillus megaterium* on maize yield components and yield

Inoculation with *B. megaterium* did not increase the yield in Season 1 but in Season 2 it increased it significantly (p = 0.001) by 34.4%. All the other measured yield components such as 1000 kernel weight, height, number of rows per cob and number of kernels per row were not increased significantly by the presence of *B. megaterium* (Table 3.1 and Table 3.2).

3.3.3. Effect of micro-dosing of dolomitic lime on maize yield components and yield

Micro-dosing of dolomitic lime alone did not significantly increase maize yield and yield components except for the maize stalk height that was increased significantly by 61.9% in Season 2 only (Table 3.2).

3.3.4. Effect of various combinations micro-dosed fertilizers [2:3:2 (34) and superphosphate] and lime on maize yield components and yield

The combination of 2:3:2 (34) fertilizer and lime increased maize yield significantly by 74.8% and 38.8% in the first and second season, respectively. When the combination was compared to the 2:3:2 (34) fertilizer treatment alone, it increased the maize yield by 6% and 22% in the first and second seasons, respectively, but the differences were not significant. When comparing the combination to the treatment with lime only, the combination increased the maize yield significantly by 53% in Season 1, but in Season 2 the increase was 31%, which was not significant. This combination also significantly increased the 1000 kernel mass, number of kernels per row on a cob and the maize stalk height in Season 1. In Season 2 only height was increased significantly (Table 3.1 and Table 3.2).

The combination of superphosphate and lime increased the maize yield by 28.9% and 18.5% in the first and second seasons, respectively, although the differences were not significant.

Table3.2: Effect of *Bacillus megaterium and* micro-dosing of fertilizers and lime on maize yield components in the 2013/2014 season

Treatment	BM	Lime	SP	2:3:2(34)	**Yield t ha^{-1}**	**1000 KM(g)**	**SD (mm)**	**PH (cm)**	**Kernels row^{-1}**	**Rows cob^{-1}**
BM	Yes	No	No	No	2.060abcd	0.3240a	20.40ab	0.738ef	34.55c	13.05a
Lime	No	Yes	No	No	1.653de	0.3075a	21.35ab	0.923de	36.05bc	13.35a
SP	No	No	Yes	No	2.106abcd	0.3155a	20.60ab	1.053cd	40.95abc	13.15a
2:3:2(34)	No	No	No	Yes	1.742cde	0.3240a	22.42ab	1.080bcd	39.60abc	12.65a
Lime+ BM	Yes	Yes	No	No	1.807bcde	0.3303a	21.27ab	0.920de	34.35c	13.05a
SP + BM	Yes	No	Yes	No	1.892abcde	0.3355a	22.62ab	1.110bcd	41.10abc	13.45a
2:3:2(34)+ BM	Yes	No	No	Yes	2.271ab	0.3040a	24.25a	1.286abc	41.50abc	13.45a
Lime+ SP	No	Yes	Yes	No	1.817bcde	0.3285a	22.62ab	1.205abcd	40.55abc	13.55a
2:3:2(34)+lime	No	Yes	No	Yes	2.127abcd	0.3070a	22.15ab	1.337ab	41.25abc	13.65a
2:3:2(34)+ SP	No	No	Yes	Yes	2.069abcd	0.2995a	23.52a	1.223abcd	41.95ab	13.10a
SP +lime+ BM	Yes	Yes	Yes	No	2.285ab	0.3233a	24.05a	1.258abc	42.75ab	13.15a
2:3:2(34)+ SP +lime	No	Yes	Yes	Yes	2.173abc	0.3223a	24.02a	1.248abc	42.20ab	12.80a
2:3:2(34)+lime+ BM	Yes	Yes	No	Yes	2.372a	0.3343a	23.55a	1.433a	43.80a	13.16a
2:3:2(34)+ SP + BM	Yes	No	Yes	Yes	1.865bcde	0.3003a	22.00ab	1.013cde	40.30abc	13.60a
2:3:2(34)+ SP +lime+ BM	Yes	Yes	Yes	Yes	2.276ab	0.3335a	21.75ab	1.500a	37.95abc	13.20a
Control	No	No	No	No	1.533e	0.3460a	18.62b	0.570f	37.05abc	13.35a
Treatment effects					P value	P value	P value	P value	P value	P value
BM					0.221	0.273	0.890	0.209	0.453	0.587
Lime					0.139	0.247	0.557	0.059	0.996	0.824
SP					0.205	0.806	0.427	0.711	0.034	0.695
2:3:2(34)					0.209	0.328	0.131	0.018	0.033	0.787
Lime+ BM					0.317	0.458	0.781	0.360	0.871	0.070
SP + BM					0.152	0.932	0.448	0.561	0.710	0.502
2:3:2(34)+ BM					0.774	0.589	0.546	0.349	0.927	0.147
Lime+ SP					0.835	0.382	0.955	0.171	0.328	0.135
2:3:2(34)+lime					0.576	0.210	0.280	0.387	0.768	0.751
2:3:2(34)+ SP					0.010	0.540	0.104	0.008	0.001	0.413
SP +lime+ BM					0.093	0.183	0.937	0.068	1.000	0.147
2:3:2(34)+ SP +lime					0.630	0.599	0.980	0.902	0.282	0.827
Fertilizer2:3:2+lime+ BM					0.663	0.260	0.693	0.727	0.922	0.989
2:3:2(34)+ SP + BM					0.123	0.608	0.129	0.779	0.133	0.452
F value					3.00	0.98	1.26	6.72	1.80	0.87
CV%					14.4	8.8	12.3	17.0	10.9	4.5

*Numbers with different letters in a column are significantly different at p=0.05 according to Duncan's multiple range test

* SP= Superphosphate * BM = B. Megaterium * KM= Kernel mass * SG= Stem girth.

The combination of 2:3:2 (34) and superphosphate fertilizers significantly increased maize yield, 1000 kernel mass, stem diameter, maize stalk height and number of kernels per row in a cob. In Season 2 the combination significantly increased the maize yield, stem diameter and maize stalk height. When comparing the combination and the treatments on their own, different situations were observed. Except for maize yield and number of kernels per row in a cob, the yield components such as 1000 kernel mass, stem diameter and height were significantly higher for the combination than for superphosphate alone in Season 1; yet in Season 2, none of the parameters were significantly different between the combination and the superphosphate alone. However, when the combination of 2:3:2 (34) and superphosphate fertilizers was compared to the 2:3:2 (34) fertilizer treatment alone, in both seasons there was no significant difference (Table 3.1 and Table 3.2).

The combination of all these treatments, 2:3:2 (34) fertilizer, superphosphate, and lime significantly increased maize yield, 1000 kernel mass, maize stalk height, number of rows per cob and number of kernels per row in Season 1. However, in Season 2 the combination only significantly increased maize yield, stem diameter and maize stalk height (Table 3.2).

3.3.5. Effects of combinations of *B. megaterium* and micro-dosing fertilizers and lime on maize yield and its components

The combination of *B. megaterium* and 2:3:2 (34) fertilizer increased the maize yield significantly in Season 1 and Season 2 by 54.7% and 48.1%, respectively (Fig. 3.1). When this combination was compared to the treatment with 2:3:2 (34) fertilizer alone, it still produced a maize yield increase of 30.4% in Season 2. The same combination increased the maize stalk height by 41.6% and 125.6% in the first and second seasons, respectively, which were highly significant (Fig. 3.1). When compared to the 2:3:2 (34) alone, this combination still had a positive effect in Season 2 because it increased the maize stalk height by 36%. The other yield components did not show significant differences with this combination.

The combination of *B. megaterium* and superphosphate fertilizer increased the yield by 14.6% and 23.4% in the first and second seasons, respectively, although the difference was not significant. This combination also increased the maize stalk height in the first and second season by 14% and 94.7%, respectively. When the combination of *B. megaterium* and superphosphate fertilizer is compared to the treatment with superphosphate only, it did not increase the maize yield as much as the superphosphate on its own did but it did increase the maize stalk height in Season 2 by 10% (Fig. 3.1).

The combination of *B. megaterium* and lime increased the maize yield in Season 2 by 17.9%, which was not significant. It also increased the maize stalk height in Season 2 by 61%. When the combination was compared to the performance of each treatment separately there was no significant difference except that *B. megaterium* performed better alone by increasing the maize yield more than the combination by 16.5%. The combination increased the maize yield by 10% when compared to lime alone as a treatment (Table 3.2).

The combination of *B. megaterium*, 2:3:2 (34) fertilizer, and lime significantly increased the maize yield, 1000 kernel mass, maize stalk height, maize stem diameter, number of rows per cob and number of kernels per row in Season 1 (Table 3.1). In Season 2 maize yield, stem diameter and maize stalk height were increased significantly (Table 3.2).

The combination of *B. megaterium*, superphosphate fertilizer, and lime significantly increased the maize yield, 1000 kernel mass and height in Season 1 (Table 3.1). In Season 2 this combination increased significantly the maize yield, stem diameter and maize stalk height (Table 3.2).

3.3.6. Effect of the combination of all treatments [*B. megaterium*, Superphosphate, 2:3:2(34) fertilizer and lime] on maize yield components

The combination of all treatments significantly increased all parameters, namely maize yield, 1000 kernel mass, stem diameter, maize stalk height, number of rows per cob and number of kernels per row in Season 1. However, in Season 2 only maize yield and height were significantly increased.

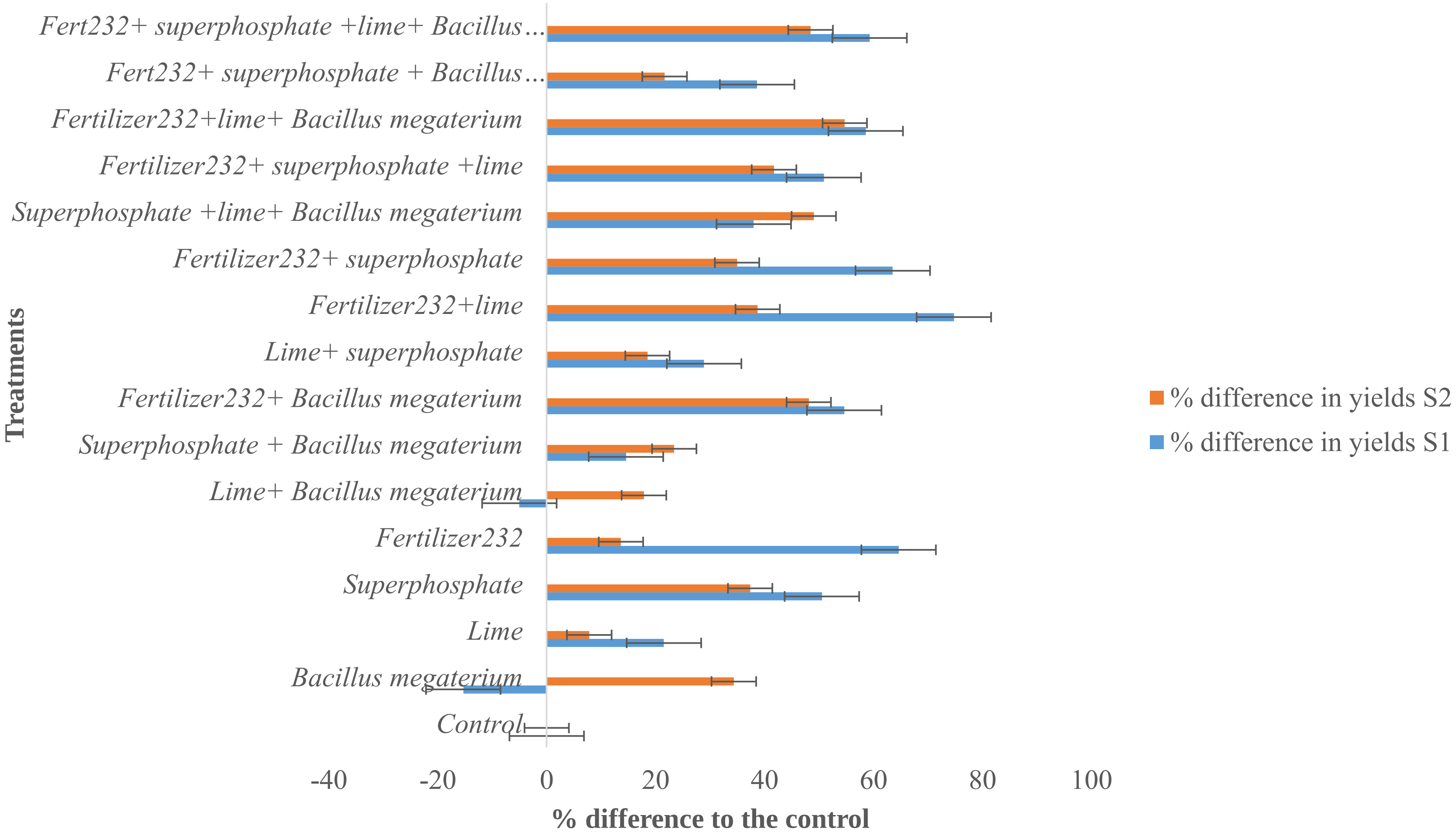

Figure 3.1: Comparing maize yields as a result of treatments and the Untreated Control treatment, as a percentage of the Control

3.4. Discussion

Micro-dosing of fertilizers and lime was an effective way of applying these crop inputs, and increased most maize yield components. Micro-dosing of 2:3:2 (34) fertilizer increased maize yield significantly by 64.6% in Season 1 and by 13.6% in Season 2. Micro-dosing with superphosphate also increased the maize yield significantly in both the first and second seasons by 50.5% and 37.4%, respectively. This is in agreement with Ousman and Aune (2011), and Camara *et al.* (2013), who both found that micro-dosing of fertilizers increases productivity and yields, especially for small scale farming. This is important for the Eastern Cape where the majority of farmers are small scale farmers and usually cannot afford to buy the recommended quantities of fertilizers or lime.

The maize stalk heights were significantly greater in the first and second season by 42.2 %% and 89.5%, respectively, for the treatments with a micro-dose of 2:3:2 (34) fertilizer. Micro-dosing with superphosphate increased the stalk height in Season 1 and second season by 10% and 84.7%, respectively, but the difference was only significant in Season 2. This reflects the fact that phosphorus increases plant growth (Barker, 2012). Cai *et al.* (2012) also found a strong relationship between maize plant height and phosphorus supply. The significant increase of the plant height in Season 2 may be attributed to an accumulation of phosphorus over two seasons.

The effect of phosphorus in increasing maize plant height was also confirmed by the results observed when micro-dosing dolomitic lime. The plots that were treated with a micro-dose of dolomitic lime significantly increased the maize stalk height by 61.9%. This was probably because the soils used were highly acidic, and therefore the phosphorus present in that soil was probably unavailable for plant uptake (Reis *et al.* 2011). The liming of the soil therefore increases the dehydrogenase activity, which positively correlates with the microbial activity (Mijangos *et al.*, 2010). Microbial activity involves the solubilization of phosphates by microorganisms such as *Azotobacter chroococcum*, *Azotobacter vinelandii* (Farajzadeh *et al.*, 2012), and *Bacillus megaterium* (Hassan *et al.* 2012; Xinxian *et al.*, 2011). It is therefore assumed that the bound phosphorus was solubilized making it available to the maize crop, resulting in an increase in maize plant height. A second factor was that lime would have reduces aluminium and manganese solubilization in the acidic soils, and these metallic cations cause stunting of maize (Abate *et al.*, 2013)

B. megaterium inoculation also had a positive effect on maize yield, as found previously by (Wu *et al.,* 2005). Inoculation with *B. megaterium* did not increase maize yield in Season 1 but in Season 2 it increased it significantly by 34.4%. This shows the accumulative effect of *B. megaterium* in the soil and its effect on solubilizing phosphates and nitrogen fixation as reported by Liu *et al*. (2006), Xinxian *et al*. (2011) and Hassan *et al*. (2012).

The combination of *B. megaterium* and micro-dosing of 2:3:2 (34) fertilizer increased the maize yields significantly in the first and second seasons by 54.7% and 48.1%, respectively. The increase was higher than the increase in the plots that were inoculated by *B. megaterium* alone and 2:3:2 (34) fertilizer alone. This means that the nutrients supplied by micro-dosing 2:3:2 (34) fertilizer, or applying *B. megaterium* alone were less than optimal by themselves. However, the combination supplied more nutrients in the long term, resulting in higher yields in Season 2. This is because in the second season the combination of 2:3:2 (34) fertilizer and *B. megaterium* was reapplied which means that the concentration of *B. megaterium* was higher as there was a build-up from the previous season. Higher concentrations of *B. megaterium* fix more nitrogen and solubilize more phosphorus hence the yield increased more in the second season than the first season.

The combination of *B. megaterium* and superphosphate fertilizer increased the yield by 14.6% and 23.4% in the first and second seasons, respectively, although the difference was not significant. This combination also increased the maize stalk height in the first and second season by 14% and 94.7%, respectively. When the combination of *B. megaterium* and superphosphate fertilizer is compared to the treatment with superphosphate only, it did not increase the maize yield but it increased the maize stalk height in Season 2 by 10%.

The combination of *B. megaterium* and lime increased the maize yield only in Season 2 by 17.9%, which was not significant. It also increased the maize stalk height only in Season 2 by 61%. This may have been due to an accumulative effect because the same plots were used in both seasons. When the combination is compared to the performance of each treatment separately, there was no significant difference except that *B. megaterium* performed better on its own by increasing the maize yield more than the combination by 16.5%. The combination increased the maize yield by 10% when compared to lime alone as a treatment. Again, the shortage of nitrogen and potassium was probably limiting.

The combination of *B. megaterium*, 2:3:2 (34) fertilizer, and lime significantly increased the maize yield, maize stalk height, maize stem diameter in both seasons. The increase was consistent as a result of this combination when compared to 2:3:2 (34) fertilizer, and lime combination. Whenever *B. megaterium* was in the combination, the yield increased, although not significant. This means that *B. megaterium* made enough phosphates available to the plant yet micro-dosing of lime was too little to make conditions conducive for maize growth. The combination of *B. megaterium*, superphosphate fertilizer, and lime resulted in a significant increase in maize yield, stem diameter and maize stalk which is line with the previous hypothesis. The addition of *B. megaterium* to the superphosphate fertilizer plus lime increased the maize yield in both seasons.

In conclusion, micro-dosing of fertilizers could have a significant role to play in improving maize yields of small scale farmers who cannot afford to apply the recommended quantities of fertilization and lime. The application of *B. megaterium* can supplement micro-dosing with fertilizers and lime.

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CHAPTER FOUR

Integrated Management of *Rhizoctonia solani* on Maize

Abstract

Maize production in the Eastern Cape is limited by the lack of affordable inputs, poor soils and root diseases caused by *Rhizoctonia solani* and other pathogens. The currently promoted extension recommendations for maize production in the Eastern Cape are specifically for commercial production, e.g., based on the use of chemicals to control plant diseases. In this study over three seasons, low cost methods were tested. The study was conducted in a field with acidic soils with an acid saturation of 54%. A bacterium with the capacity to fix atmospheric nitrogen and solubilize phosphates and stimulate plant growth, *Bacillus megaterium*, was also applied. For maize root disease control, the methods employed included the use of *Trichoderma harzianum*, a biological control agent, and the use of potassium silicate to prime the plant's self-defense mechanism. The aim of the study was to reduce input costs whilst still providing adequate fertilization and root disease management. The root pathogen, *R. solani*, was previously shown to limit the maize yields significantly, by up to 34%. However, the losses decreased over seasons from 34% to 16% to 10% over 3 years. In Season 1, the integration of all treatments (*T. harzianum*, *B. megaterium* and potassium silicate) increased maize yields relative to the control by 130%. The plots with the highest yield in the presence of *R. solani* were treated with *T. harzianum* (216%) followed by *T. harzianum* plus potassium silicate (214%) and lastly plots treated with *T. harzianum* plus *B. megaterium* (178%). A similar trend was observed over the three seasons of this experiment. However in Season 3, treatments with only *T. harzianum* did not result in the highest yield as in the previous seasons. Instead, *T. harzianum* plus potassium silicate treatment resulted in the highest yields in the Season3.

4.1 Introduction

Maize (*Zea mays* L.) is a staple food crop on the African continent. In the Eastern Cape of South Africa small scale farmers grow maize, mainly for human and animal feed. Maize production is limited by the lack of affordable inputs, plant diseases, and poor soils. The current systems of maize production being promoted in the Eastern Cape were developed for commercial farmers, e.g., requiring the use of chemicals to control plant diseases and the use of synthetic fertilizers to provide nutrients.

The utilization of biological control agents or bio-fertilizers is not popular in the Eastern Cape. Farmers are still using chemical fertilizers. However, the use of bio-fertilizers is a possible alternative to the use of synthetic fertilizers (Grace and Peter 2004). In comparison to bio-fertilizers, synthetic fertilizers leach into ground water or are used up by plants whereas the bio-fertilizers remain in the soil as long as the soil conditions are suitable for their survival. Moreover bio-fertilizers multiply in the soils allowing small quantities to be applied, making these products more affordable than fertilizers, which is beneficial for small scale farmers.

Biocontrol agents are used for the control of plant diseases such as damping off and root rots of many crops by selected strains of *Trichoderma* (Mghalu *et al.* 2007; Dubey *et al*. 2009). Due to cases where biological control agents have given inconsistent results in the fields, integrated approach such as integrating plant disease control with agronomic aspects of crop production to improve the efficacy of the biocontrol agent has been used (Spadaro and Gullino, 2005).

Biocontrol agents can be integrated with compounds that prime crop resistance against plant diseases. These compounds are plant resistance inducers and include compounds such as silicon (Balakhnina and Borkowska, 2013). Inducers and biocontrol agents may be used on their own but are more effective when combined (Abo-Elyousr *et al*., 2009).

Integrated approach has greater advantages over the use of single strategy to control plant diseases. It may be selected according to their different mode of actions to enhance effectiveness against the pathogen. Mode of action may include the chitinolytic activity (López-Mondéjar *et al.*, 2012), protease activities (Sahebani and Hadavi, 2008), induction of systemic resistance (Chowdappa *et al*. 2013), mycoparasitism (Steindorff *et al*., 2012) and many others.

In this study an integrated approach was used to control maize root rot and improve maize yield. This included the use of *T. harzianum*, a biocontrol agent; *B. megaterium*, a bio-fertilizers to fix atmospheric nitrogen and to solubilize phosphates in acidic soils; and potassium silicate, to prime maize plant resistance against *R. solani*. The objective was to investigate whether an integrated approach has greater impact on maize yield than individual treatments, using unameliorated soils typical of those used by small scale farmers in the Eastern Cape, South Africa.

4.2 Materials and Methods

The field trial was conducted in loamy and acidic soils at the Mgwalana Village, Elliot, Eastern Cape, South Africa (31° 28' 0" South, 27° 18' 0" East). The land had been laying fallow with no recent cropping history. According to the soil tests, the soil pH was 3.98 and the acid saturation was 54% (Table 4.1), which meant that to target 4 t ha^{-1} in that field, 4.5 t ha^{-1} of dolomitic lime must be applied as per the soil analysis recommendations. The seeds were treated as per the requirement of the plot as per the experimental design outlined in section 4.2.7. The experiment was conducted over two seasons in the same field. The test crop was maize and the cultivar used was PAN14[7], which is the cultivar that is normally used in the area.

4.2.1 Rainfall and temperature

The rainfall and temperature varied among the three seasons. Figures 4.1, 4.2, and 4.3 shows the differences among the three seasons.

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[7] Pannar Seed Co, Greytown, South Africa

Table 4.1: Soil Analysis

Your sample ID	Lab No.	Sample Density mg/mL	P mg/mL	Ca mg/mL	K mg/mL	Mg mg/mL	Exch. cmol/L	Total cation cmol/L	Acid sat. %	pH (KCL)	Zn mg/mL	Mn mg/mL	Cu mg/mL	NIRS org. C%	NIRS Clay%
K	D1252	1.23	5	50	92	103	1.70	3.13	54	3.98	0.6	0	0.0	-	-

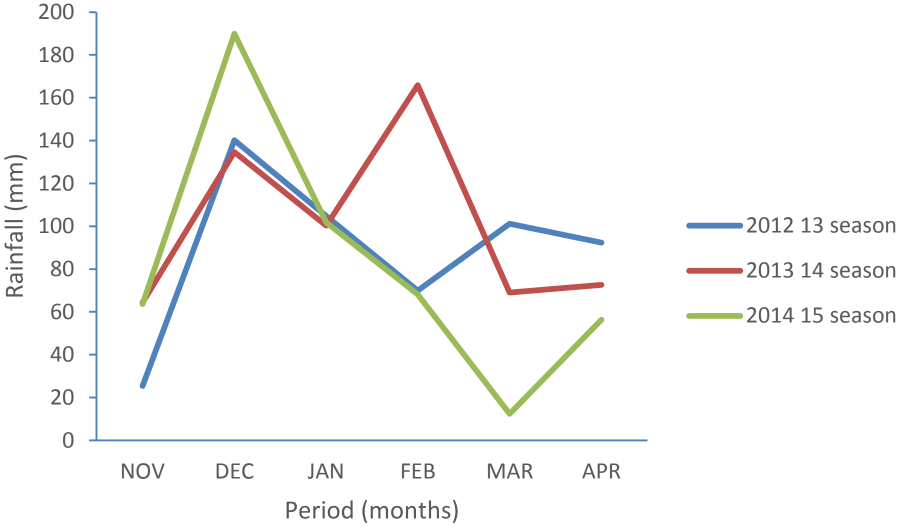

Fig.4.1 Rainfall pattern over the three seasons of the study, South African Weather Service (SAWS)

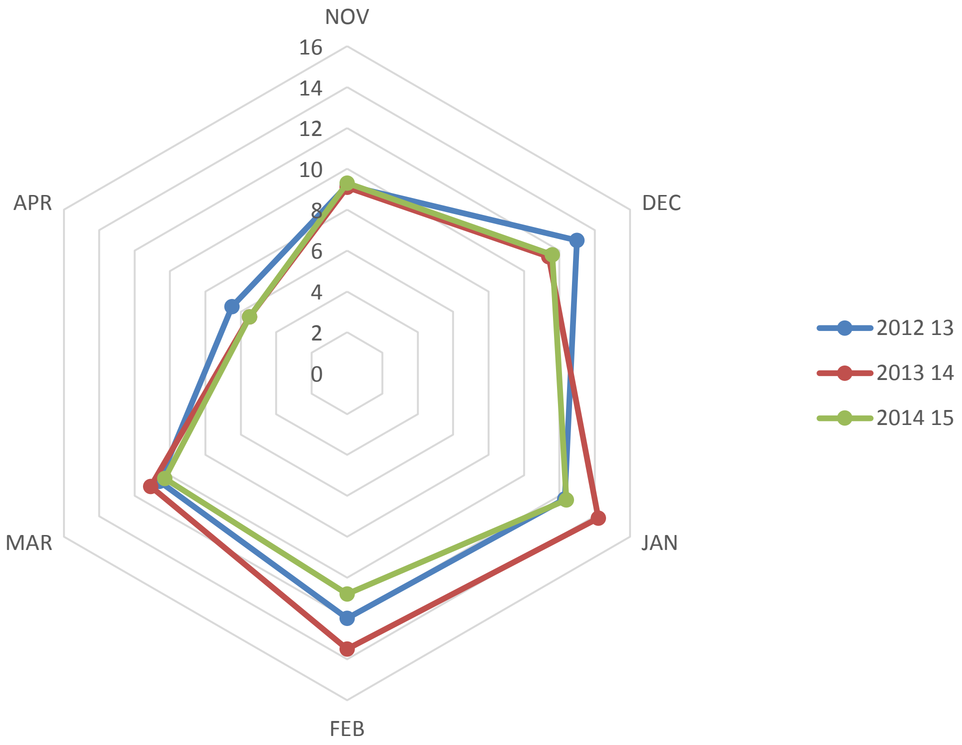

Fig.4.2 Minimum temperatures over the three seasons of the study, SAWS

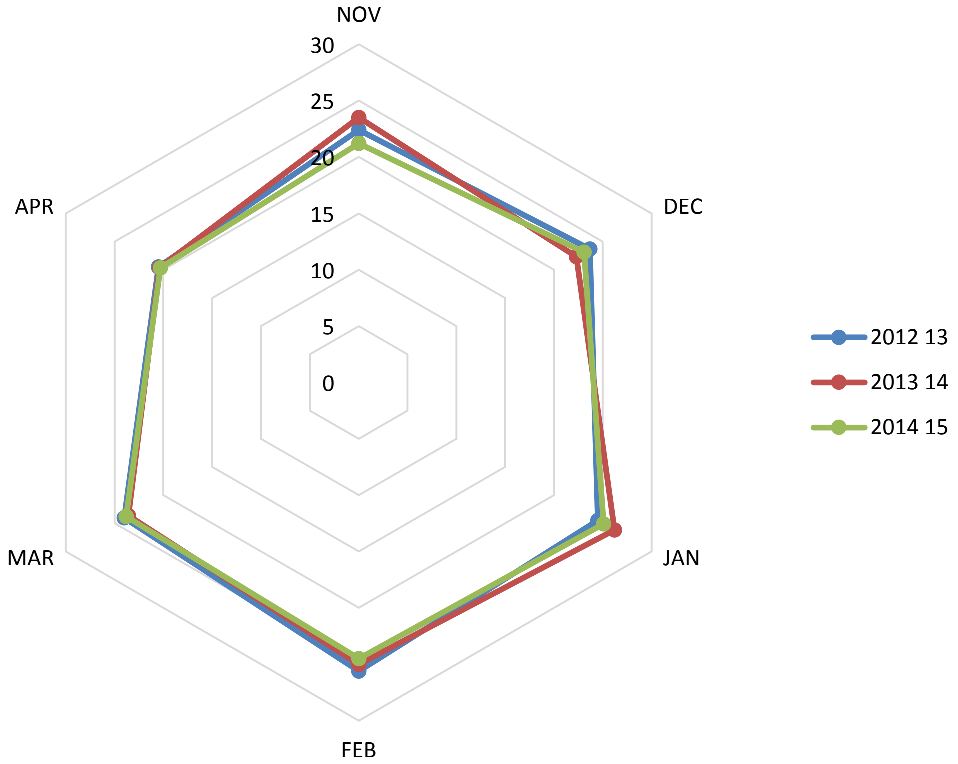

Fig.4.2 Maximum temperatures over the three seasons of the study, SAWS

4.2.2 Fertilizer and Lime Applications

The fertilizer used was a 2:3:2 (34) compound fertilizer[8]. The lime used was a dolomitic lime[7]. Both the fertilizer and lime were applied directly on each planting hole using the cap of a 2L cool drink bottle, with a capacity of approximately 5 g, a micro-dose translating to 185 kg ha^{-1} for each product, which is lower than the recommended fertilizer and lime but it is better than the current practice of small scale farmers. After this application a thin layer of soil was used to cover the lime and fertilizer before planting the seed.

4.2.3 Seed Treatment

Maize seeds (*Zea mays* L.) were treated with the Eco-T® (2×10^9 conidia g^{-1} of *Trichoderma harzianum*). One kilogram of maize seeds was treated with 5 g of Eco-T® as per the instruction manual of the Eco-T® manufacturer (Plant Health Products (Pty) Ltd[9]). Eco-T® is a registered biocontrol product which has *Trichoderma harzianum* Strain kd as an active ingredient. The seeds were treated on the day of planting.

4.2.4 Silicon Application

Some plots were treated with the potassium silicate, a slow release formulation of silicon [solid potassium silicate (50%)] as per the trial design. Approximately 5 g of the slow release potassium silicate powder was placed in the planting hole and covered before the maize seed was planted.

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[8] Omnia Fertilizers, Sasolburg, South Africa

[9] Plant Health Products (Pty) Ltd, P O Box 207, Nottingham Road, KwaZulu-Natal, South Africa

4.2.5 *Rhizoctonia solani* Inoculum Preparation and Application

The pathogen that was used in the experiment was *Rhizoctonia solani* obtained from Dr KS Yobo[10]. It was sub-cultured on potato dextrose agar and then transferred to double autoclaved barley seeds in 500 ml conical flasks. It was allowed to grow at room temperature on the barley seeds until it completely colonized all the barley seeds. During planting in the field, one barley seed infested with mycelium of *R. solani* was placed 40 mm away from the maize seed and covered with soil.

4.2.6 *Bacillus megaterium* Application

The *B. megaterium* application was done three weeks after planting by drenching. The strain of *B. megaterium* used was obtained from Plant Health Products (Pty) Ltd[11] in a "tea-bag" formulation. One "tea-bag" had 10^8 spores of *B. megaterium*. The "tea-bag" was soaked in a 500 ml of water for 10 minutes. Ten ml of the suspension was put into 5 liters of water and was drenched on seedlings using a watering can, early in the morning.

4.2.7 Experimental Design and Analysis

The experiment had two controls namely: a control with fertilization only (Fertilized Control), and a control with the pathogen (*Rhizoctonia solani*) inoculum applied (Inoculated Control). The treatments were *Trichoderma harzianum* only; *Trichoderma harzianum* plus *R. solani*; slow release potassium silicate only; slow release potassium silicate plus *R. solani*; *Bacillus megaterium* only; *Bacillus megaterium* plus *R. solani*; *Trichoderma harzianum* plus slow release potassium silicate; *Trichoderma harzianum* plus slow release potassium silicate plus *R. solani*; *Trichoderma harzianum* plus *B. megaterium*; *Trichoderma harzianum* plus *B. megaterium* plus *R. solani*; slow release potassium silicate plus *B. megaterium*; Slow release potassium silicate plus *B. megaterium* plus *R. solani*; *Trichoderma harzianum* plus *B. megaterium* plus slow release potassium silicate;

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[10] Discipline of Plant pathology, University of KwaZulu-Natal, Scottsville, South Africa

[11] Plant Health Products (Pty) Ltd, P. O. Box 207, Nottingham Road, KwaZulu-Natal, South Africa

Trichoderma harzianum plus *B. megaterium* plus slow release potassium silicate plus *R. solani*. Each treatment was applied to maize planted in a 3 m x 3.6 m plots. In each plot there were 55 plants with 0.9 m spacing between rows and 0.3 m spacing between plants. The spacing between plots were 1 m and between replicates it was also 1 m.

A Complete Randomized block design with four replicates was employed. The parameters that were measured were stem girth, plant height, root dry weight, 1000 kernel weight, number of rows per cob, number of kernels per row, number of kernels per cob, yield, soil analysis after harvest for fertility per plot (the soils were augured from the position where the plant was), and grain analysis for micro elements per plot.

Factorial analysis of variance was performed using the General Linear Model of ANOVA, of Genstat® 14th edition. An F value for main treatment effects and their interaction were considered significant at $P \leq 0.05$ level. Treatment means were separated using Fisher's unprotected LSD test at the 5% probability level.

4.3 Results

The pathogen, *R. solani*, reduced the maize yield by 34% relative to the Untreated Control while all other treatments increased the yield significantly when compared to both the Untreated Control and the Inoculated Control (Table 4.1). *Trichoderma harzianum* controlled *Rhizoctonia solani* as a single treatment and gave the highest yield, which was 216% more than the Fertilized Control and 250% more than the Inoculated Control. Moreover, among the treatments that were not inoculated with *R. solani*, the *T. harzianum* treatment recorded the highest yield, which was 199% higher than the Untreated Control. The *T. harzianum* plus *R. solani* plus silicon gave the second highest yield of 214% more than the Untreated Control and 248% more than the Inoculated Control.

Potassium silicate treatment on its own controlled *R. solani*, increasing maize yield by 176% relative to the Inoculated Control. In the absence of *R. solani*, potassium silicate increased the yield by 129%. When integrated with *B. megaterium,* potassium silicate controlled *R. solani* and increased yield by 131% relative to the Inoculated Control. However, when *R. solani* was not inoculated, the yield was increased by 152%, relative to the Untreated Control. The *B. megaterium* as a treatment alone controlled *R. solani* increasing yield by 134%, relative to the Inoculated Control. The integration of all three treatments: potassium silicate, *T. harzianum* and *B. megaterium*, increased maize yield by 164% in the presence of *R. solani* relative to the Inoculated Control.

For the 1000 kernel weight, the *R. solani* treatment had 15% less kernel weight relative to the Untreated Control. All the other treatments had significantly higher 1000 kernel weights relative to both the Control and the Inoculated Control. The same trend was noted for the 1000 kernel weight as noted above for the maize yield, and *T. harzianum* caused the highest increase in 1000 kernel weight. The *T. harzianum* treatment in the presence of *R. solani* increased the 1000 kernel weight by 49% relative to the Fertilized Control and 64% relative to the Inoculated Control. The treatment that gave the least increase in 1000 kernel weight was potassium silicate in the presence of *R. solani*, increasing it by 34% relative to the Inoculated Control.

The Inoculated Control stalk height was 60% less than the Untreated Control, which was significant ($p < 0.001$) (Table 4.2). All the treatments had an increased stalk height relative to the Inoculated Control, with *T. harzianum* plus potassium silicate giving the highest increased stalk height of 30%, relative to the Fertilized Control and 90% relative to the Inoculated Control.

Table 4.1: Efficacy of *T. harzianum*, *B. megaterium*, potassium silicate and their combinations on *R. solani* control and maize plant growth promotion under field conditions and micro-dosed fertilizers and lime in 2012/2013 season

Treatment	*R. solani*	*T. harzianum*	KSil	BM	**Yield t ha^{-1}**	**Increased yield%**	**1000 KM (g)**	**Increased 1000KM**	**Height (cm)**	**Increased height%**
R.solani+ *T.harzianum*	Yes	Yes	No	No	5.498a	216%	198.8a	49%	64.33bc	5%
R.solani+KSil+BM	Yes	No	Yes	Yes	3.421cd	97%	197.5ab	48%	75.32a	23%
R.solani + KSil+ *T. harzianum*	Yes	Yes	Yes	No	5.468a	214%	187.0abc	40%	76.98a	26%
R.solani+*T.harzianum*+BM	Yes	Yes	No	Yes	4.839ab	178%	184.7abc	38%	74.01ab	21%
T. harzianum + BM	No	Yes	No	Yes	3.308cd	90%	184.5abc	38%	73.74ab	21%
KSil + *T. harzianum*	No	Yes	Yes	No	4.409abc	154%	184.2abc	38%	79.05a	30%
KSil + BM	No	No	Yes	Yes	4.374abc	152%	181.6abc	36%	71.73ab	18%
T. harzianum	No	Yes	No	No	5.205ab	199%	181.6abc	36%	75.20a	23%
R. solani + KSil+ *T. harzianum* + BM	Yes	No	No	Yes	3.997bcd	130%	177.6abcd	33%	73.42ab	20%
R. solani + BM	No	No	Yes	No	3.475cd	100%	175.9bcd	31%	69.27abc	14%
KSil	No	Yes	Yes	Yes	3.985bcd	129%	171.7cd	28%	78.52a	29%
KSil+ *T. harzianum* + BM	No	No	No	Yes	3.266cd	88%	170.3cd	27%	73.47ab	20%
BM	Yes	No	Yes	No	2.924d	68%	169.0cd	26%	73.32ab	20%
R. solani + KSil	Yes	No	No	No	4.208bc	142%	159.5d	19%	69.75abc	14%
Fertilized Control	No	No	No	No	1.739e	0%	133.8e	0%	61.02c	0%
Inoculated Control (*R. solani*)	Yes	No	No	No	1.155e	-34%	113.5f	-15%	24.62d	-60%
Treatment Effect					P value		P value		P value	
R. solani+ *T. harzianum*					0.003		0.060		0.074	
R. solani + KSil + BM					0.010		0.230		0.155	
R. solani + KSil + *T. harzianum*					0.552		0.941		0.345	
R. solani + *T. harzianum* + BM					0.920		0.041		0.042	
T. harzianum + BM					0.001		0.001		0.001	
KSil + *T. harzianum*					0.001		0.001		0.001	
KSil + BM					0.081		0.101		0.001	
T. harzianum					0.001		0.001		0.001	
R. solani + BM					0.191		0.007		0.001	
KSil					0.040		0.034		0.001	
KSil+ *T. harzianum* + BM					0.051		0.092		0.001	
BM					0.010		0.010		0.002	
R. solani + KSil					0.705		0.085		0.003	
R. solani					0.915		0.249		0.001	
F value					10.62		11.03		19.09	
Cv%					19.6		7.7		8.5	

*Numbers with different letters in a column are significantly different p=0.05 according to Duncan's multiple range test * BM= *Bacillus megaterium* *KM=Kernel mass

Table 4.2: Comparisons of Maize Grain Yields over seasons as Affected by Different Treatments

Treatment	**Season 1**		**Season 2**		**Season 3**	
	Yield t ha^{-1}	**Increased yield %**	**Yield t ha^{-1}**	**Increased yield %**	**Yield t ha^{-1}**	**Increased yield %**
Rhizoctonia solani+ *Trichoderma harzianum*	5.498	216%	4.927	21%	7.744	96%
R. solani + KSil Slow release + *Bacillus megaterium*	3.421	97%	3.894	-4%	5.855	48%
R. solani + KSil Slow release + *T. harzianum*	5.468	214%	4.247	4%	10.579	167%
R. solani + *T. harzianum* + *B. megaterium*	4.839	178%	4.362	7%	6.672	68%
T. harzianum + *B. megaterium*	3.308	90%	3.289	-19%	7.24	83%
KSil Slow release + *T. harzianum*	4.409	154%	3.246	-20%	9.047	128%
KSil Slow release + *B. megaterium*	4.374	152%	4.666	14%	5.628	42%
T. harzianum	5.205	199%	4.228	4%	9.492	140%
R. solani + KSil Slow release + *T. harzianum* + *B. megaterium*	3.997	130%	4.315	6%	6.313	59%
R. solani + *B. megaterium*	3.475	100%	3.655	-10%	5.398	36%
KSil Slow release	3.985	129%	4.234	4%	6.767	71%
KSil Slow release + *T. harzianum* + *B. megaterium*	3.266	88%	3.352	-18%	10.488	165%
B. megaterium	2.924	68%	4.004	-2%	4.946	25%
R. solani + KSil Slow release	4.208	142%	3.996	-2%	6.767	71%
Control	1.739	0%	4.076	0%	3.96	0%
R. solani	1.155	-34%	3.429	-16%	3.551	-10%

Inoculation with *R. solani* consistently reduced yields relative to the Untreated Control by 34% in Season 1, 16% in Season 2 and 10% in Season 3 (Table 4.2). Compared to all other treatments, other than the Untreated Control, the Inoculated Control had the least yields showing the negative effect of the pathogen on maize production.

The *T. harzianum* treatment consistently increased yield among all treatments in the presence or absence of *R. solani* (Table 4.2). The differences among treatments in Season 2 were not significant but *T. harzianum* application again resulted in the highest yields. In Season 3 the treatment that resulted in the highest yield was *T. harzianum* plus potassium silicate.

4.4 Discussion

The pathogen, *R. solani*, reduced the maize yield by 34%, 16% and 10% relative to the Untreated Control in Seasons 1, 2 and 3, respectively, confirming that root diseases are economically important for most crops including maize (Anees *et al.,* 2010; Bennett, 2012). This clearly shows that it is important for researchers to embark in developing new technologies that will help farmers to control root disease, especially *R. solani*. For small scale farmers the impact is more as they can’t afford expensive chemicals that could be effective, hence in this study the focus was on the biological control as it may provide sustainable control. Moreover the focus was on integrating biological control and priming of the crop’s self defense.

Yield losses to *R. solani* diminished over seasons from 34% to 16% and then to 10% in the first, second and third season, respectively. This was probably due to the natural increase in antagonists in the soil and the persistence of *T. harzianum* and *B. megaterium* from the previous seasons (Cook and Baker, 1983). This reveals sustainability of biological control agents as their population grows as long as conditions are favorable unlike the chemical control that must be used year by year as they finishes instead of multiplying.

All the treatments employed in this study increased the yield significantly when compared to both the Untreated Control and the Inoculated Control. This indicates that all the treatments selected as part of the integrated approach to control root diseases provided some level of control of *R. solani*.

This confirms the observations in Chapter 2, that potassium silicate and *T. harzianum* demonstrated a level of control of *R. solani*. It also confirms the findings of Malanicheva *et al.* (2012) that some strains of *B. megaterium* produce antimicrobials that can control some fungal pathogens in the soil. All these treatments have different mode of action, enhancing the value of integrated approach to disease control.

Where *R. solani* was not inoculated, maize yields also increased relative to the Untreated Control and Inoculated Control. This indicates that treatments had plant growth stimulation effect. Where other root pathogens were present, these were also suppressed by the treatments applied. There are many other root pathogens that may be found in the soils. These may include *Pythium* species (Sugawara *et al.*, 2011), *Fusarium* species (Barros *et al.*, 2014), and *Phytophthora* species (Sid-Ahmed *et al.*, 2003).

The *T. harzianum* treatment controlled *R. solani* and other root pathogens as a single treatment and gave the highest yield, which was 216% more than the Untreated Control. This confirms what has been observed by many researchers that some strains of *T. harzianum* can control *R. solani* (Ganesan and Sekar, 2011). It was also reported in Chapter 2 that the *T. harzianum* treatment controlled *R. solani*.

Moreover, among the treatments that were not inoculated with the *R. solani*, the *T. harzianum* treatment resulted in the highest yield, which was 199% higher than the Untreated Control. This may mean that there were other root pathogens other than the inoculated *R. solani* that were present in the soils, thus limiting the growth of the plant. The *T. harzianum* treatment controlled them as well (Sugawara *et al.*, 2011; Barros *et al.*, 2014; Sid-Ahmed *et al.*, 2003) or possibly provided plant growth stimulation or both.

The *T. harzianum* plus *R. solani* plus potassium silicate treatments resulted in the second highest yield of 214% more than the Untreated Control and 248% more than the Inoculated Control. This was probably because both *T. harzianum* and potassium silicate controlled root rot, using different modes of action. Silicon induces or primes the plant's resistance to the pathogen (Huang *et al.* 2011) while *T. harzianum* attacks the mycelium of the pathogen and also stimulate plant growth

(Ganesan and Sekar, 2011). The combination was therefore expected to provide excellent control. This trend was observed overall three seasons, when this combination performed consistently well.

Potassium silicate treatment on its own controlled *R. solani*, increasing yield by 176%, 14, and 81% relative to the Inoculated Control in the Seasons 1, 2 and 3, respectively. Potassium silicate fertilization may control *R. solani*, as reported in Chapter 2, by restricting the pathogen from entering the plant cells and also by priming the plant's resistance against the pathogen, resulting in the enhanced production of antifungal methylated forms of trans-aconitate and accumulation of phenolic compounds (Côté-Beaulieu *et al.* 2009; Re´mus-Borel *et al.* 2009; Walters *et al.*, 2013). Potassium silicate on its own provided less control of *R. solani* than when it was combined with *T. harzianum*.

In the absence of *R. solani*, silicon increased the yield by 129%, 4% and 71% in Seasons 1, 2 and 3, respectively, when compared to the Untreated Control. This also confirms that *R. solani* was not the only pathogen in the soil. Potassium silicate treatment would have primed the plant's defense mechanism to control these pathogens or stimulated plant growth, thus increasing the yields (Habtom, 2008).

When the *B. megaterium* treatment was combined with potassium silicate they controlled *R. solani* and increased yield by 131%, 12% and 58% in the first, second and third seasons, respectively. The combination also increased the maize yields by 97% in Season 1 and 48% in the last season, relative to the Untreated Control. The *B. megaterium* treatment on its own controlled *R. solani* increasing the yield by 100%, which was more than the combination. Moreover, potassium silicate on its own also increased yield more than the combination of the two treatments. In this case, therefore, the combination of *B. megaterium* and potassium silicate was not better than the treatments applied individually. However, when *R. solani* was not inoculated, the increased yield was 152%, 14% and 42% in the first, second and third seasons, respectively. This confirms again that *R. solani* was not the only yield limiting pathogen in the field or the treatment provided plant growth stimulation effect.

The integration of all three treatments: potassium silicate, *T. harzianum* and *B. megaterium* increased maize yields by 164%, 22% and 69% in the presence of *R. solani* relative to the

Inoculated Control in the first, second and third seasons, respectively. Thus the combination of all three treatments inconsistently controlled *R. solani*. When the *R. solani* is not inoculated, the yield increase was 88% in Season 1 and 165% in Season 3. The combination of all three treatments is not a viable option because they were not significantly better than the combinations of two treatments and, in some cases, yielded less than plots where *T. harzianum* was applied alone.

For the 1000 kernel weight data, the *R. solani* treatment resulted in 15% lesser kernel weight relative to the Untreated Control, which implies that *R. solani* had a negative impact on kernel weight, as expected. All the other treatments had significantly higher 1000 kernel weights relative to both Untreated Control and Inoculated Control. This confirms that all the selected treatments controlled *R. solani*, thus preventing it from reducing the kernel weight as it is recorded that *R. solani* reduces the kernel weight, making it chaffy (Rani *et al.*, 2013). The same trend that was noted on the effects of all treatments to maize yield was noted for the 1000 kernel weight, where *T. harzianum* treatment resulted in the highest increase of 1000 kernel weight. The *T. harzianum* treatment in the presence of *R. solani* resulted in the 1000 kernel weight increasing by 64% relative to the Inoculated Control. The treatment that resulted in the lowest increase of 1000 kernel weight was potassium silicate in the presence of *R. solani*, by 34% relative to the Inoculated Control.

The Inoculated Control had a stalk height that was significantly less than the Fertilized Control by 60%. All the other treatments had significantly increased stalk height relative to the Inoculated Control, with *T. harzianum* plus potassium silicate causing the highest increased stalk height of 90%, reflecting yield trends. This confirms the fact that height is a good measure of the efficacy of the biocontrol agent or plant growth stimulation (Gerber, 2010).

Overall the combination of *T. harzianum* and potassium silicate has a greater and positive influence to the control of *R. solani* and maize yield. It is therefore recommended for the control of *R. solani* on maize.

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CHAPTER FIVE

Cost-Benefit Analysis of Integrated Management of Maize

Abstract

A cost benefit analysis was undertaken of the integrated management of maize grown under acidic conditions, in the presence of *Rhizoctonia solani*, the causal organism of root diseases in many crops. The data used for the financial calculations came from prior research that had focused on the agronomic outcomes. The first study evaluated the control of *R. solani* using *Trichoderma harzianum*, priming of plant self-defense using potassium silicate, and the combination of both *T. harzianum* and potassium silicate. The second study evaluated micro-dosing of 2:3:2 (34) NPK fertilizer and lime, and the use of *Bacillus megaterium*. The third study evaluated integrated treatments for the control of *R. solani*, micro-dosing of 2:3:2 (34) NPK fertilizer, superphosphate and lime, and the use of *B. megaterium* to solubilize phosphates and fix nitrogen. The current retail prices were employed for calculations purposes. In the first study, it was observed that the combination of *T. harzianum* and the liquid formulation of potassium silicate consistently resulted in the highest returns on investment in controlling *Rhizoctonia solani* over two seasons. In the second study, full fertilization as per official recommendations consistently provided a negative return, with a mean loss of SA rand R3, 363 over two seasons relative to the Untreated Control, which was not fertilized. Micro-dosing with lime and 2:3:2 (34) NPK fertilizer gave the highest net return on investment that was significantly different to both the Untreated Control and the Full Fertilization in Season 1. However in Season 2, *B. megaterium* and 2:3:2 (34) NPK fertilizer micro-dosed resulted in the highest net return that was significantly different to both the Untreated Control and the Full Fertilization. In the third study, in Season 1 all treatments gave a significantly higher yield and net return on investment, relative to the Inoculated Control, with the lowest giving 39% net return and the highest giving 65% net return. In Season 2 none of the treatments resulted in significantly higher yield. In Season 3 the same scenario as in Season 1 where all treatments resulted in a significantly higher yields and net return relative to the Inoculated Control, with the exception of *B. megaterium* in the presence of the pathogen. The treatments that were consistently in the top three treatments that significantly controlled *R. solani* were *T. harzianum* only and *T.*

harzianum plus *B. megaterium*. The combination that gave consistently higher return on investment in the control of *R. solani* and also in the provision of nutrients was the *T. harzianum* plus *B. megaterium* plus 2:3:2 (34) NPK fertilizer and lime micro-dose. This means that this combination may be an appropriate alternative for small scale farmers in the Eastern Cape.

5.1. Introduction

The relative high costs of agricultural inputs affects food security in rural communities because most of these farmers are small scale farmers. They cannot afford to fertilize their crops as per the soil recommendations or control plant diseases as is required. As a result they usually do not fertilize their crop at all and do not try to control plant diseases.

The constant rising costs of agricultural production undermine profit margins in agriculture. In the 2012/13 season the combined index of prices of intermediate production input and services increased by 11%, yet the volume of agricultural production increased by only 1.6% in the same season (Anonymous, 2013). Research on the reduction of the input costs is therefore imperative.

Maize production in the Eastern Cape is threatened by poor soils (Mandiringana *et al.,* 2005) and root diseases. The small scale farmers produce an average of 1.6 t ha^{-1} compared to 5.8 t ha^{-1} by commercial farmers in the same province. This yield gap of 72% results in food insecurity (Godfray *et al.,* 2010). The major causal problem of this gap is that small scale farmers cannot afford to access essential crop inputs such as fertilizers and pesticides. This is a major regional problem because small scale farmers occupy 92.9% of the land that is used for maize production in the Eastern Cape (Anonymous, 2013).

The cost of fertilizers is on the rise globally which creates a huge challenge for small scale farmers who have to choose whether to fertilize or use the money to buy food (Holden and Lunduka, 2013). Some small scale farmers do not adopt high yielding technologies because of their high costs relative to the net return (Suri, 2011). This is a critical choice for them because they have to choose whether to buy fertilizer and chemicals to possibly enhance crop yields, control plant diseases, or to buy food. However, without nutrient inputs or rotation, the soils become more acidic and depleted of nutrients, reducing yields further.

5.2. Materials and Methods

The field trial was conducted in loamy and acidic soils at the Mgwalana Village, Elliot, Eastern Cape, South Africa (31° 28' 0" South, 27° 18' 0" East). The land has been laying fallow with no recent cropping history. According to the soil tests, the soil pH was 3.98 and the acid saturation was 54%, which meant that to grow maize for maximum yields, the soils needed 4.5 t ha^{-1} of dolomitic lime. The seeds were treated as per the requirement of the plot as per the experimental design. The experiment was conducted over two seasons in the same field. The test crop was maize and the cultivar used was PAN14[12].

5.2.1. Fertilizer and Lime Application

The fertilizers used were 2:3:2 (34) N.P.K. and Superphosphate granular fertilizers[13]. Both fertilizers were applied directly into each planting hole using the cap of a 2L cool drink bottle, with a capacity of approximately 5 g, a micro-dose translating to 185 kg ha^{-1} for each as required by the trial design. The lime used was a dolomitic lime applied directly into the planting hole using a 2 L cool drink cap with approximately 9 g, a micro-dose translating to 333 kg ha^{-1}. After this application a thin layer of soil was used to cover the lime and fertilizer before planting the seed.

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[12] Pannar Seed Co, Greytown, South Africa

[13] Omnia Fertilizers, Sasolburg, South Africa

5.2.2. Potassium Silicate Fertilizer Application

Some plots were treated with the potassium silicate, a slow release formulation of silicon [Solid potassium silicate (50%)] as per the trial design. Approximately 5 g of the slow release potassium silicate powder was placed in the planting hole and covered before the maize seed was planted.

5.2.3. *Bacillus megaterium* Application

The *B. megaterium* application was done three weeks after planting by drenching application. The strain of *B. megaterium* used was obtained from Plant Health Products (Pty) Ltd[14]in a tea-bag formulation. One tea-bag formulation had 10^8 spores of *B. megaterium*. The tea-bag was soaked in a 500 ml of water for 10 minutes. Ten ml of the suspension was put into 5 L of water and was drenched on seedlings using a watering can, early in the morning.

5.2.4. *Rhizoctonia solani* Inoculum Preparation and Application

The pathogen that was used in the experiment was *Rhizoctonia solani* obtained from Dr K.S. Yobo[15]. It was subcultured on potato dextrose agar and then transferred to double autoclaved barley seeds in 500 ml conical flasks. It was let to grow at room temperature on the barley seeds until it colonized all the barley seeds. During planting in the field, one barley seed inoculum of *R. solani* was placed 40 mm away from the maize seed and covered with soil.

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[14] Plant Health Products (Pty) Ltd, P. O. Box 207, Nottingham Road, KwaZulu-Natal, South Africa

[15] Discipline of Plant pathology, University of KwaZulu-Natal, Scottsville, South Africa

5.2.5. *Trichoderma harzianum* Application

Maize seeds were treated with the Eco-T® (2×10^9 conidia g^{-1} of *Trichoderma harzianum* Rifai). One kilogram of maize seeds was treated with 5g of Eco-T® as per the application instructions (Plant Health Products (Pty) Ltd). The seeds were treated on the day of planting.

5.2.6. Experimental Design and Analysis

The first experiment was repeated over two seasons, it had two controls namely: a control with fertilization only, and a control with the pathogen inoculum only. The treatments were Eco-T®, liquid potassium silicate, slow release potassium silicate, Eco-T® plus liquid potassium silicate, Eco-T® plus slow release potassium silicate. Each treatment was applied on maize seeds planted in a 3 m x 3.6 m plots. In each plot there were 55 plants with a 0.9 m spacing between rows and a 0.3 m spacing between plants. The spacing between plots and replicates was 1 m. The same plots were used for each treatment in both seasons.

The second experiment was repeated over two seasons, it had one control: Control (a plot with no fertilization). The treatments were: Superphosphate only; 2:3:2 (34) NPK fertilizer only; Lime only; *Bacillus megaterium* only; Superphosphate plus Fertilizer 2:3:2; Superphosphate plus Lime; Superphosphate plus *B. megaterium*; 2:3:2 (34) NPK fertilizer plus Lime; 2:3:2 (34) NPK fertilizer plus *B. megaterium*; Lime plus *B. megaterium*; Superphosphate plus 2:3:2 (34) NPK fertilizer plus Lime; Superphosphate plus 2:3:2 (34) NPK fertilizer plus *B. megaterium*; 2:3:2 (34) NPK fertilizer plus Lime plus *B. megaterium*; Lime plus *B. megaterium* plus Superphosphate; Superphosphate plus 2:3:2 (34) NPK fertilizer plus lime plus *B. megaterium*. Each treatment was planted in a 3 m x 3.6 m plots. In each plot there were 55 plants with 0.9 m spacing between rows and 0.3 m spacing between plants. The spacing between plots were 1 m and between replicates it was also 1 m. The same plots were used for each treatment in both trials.

The third experiment was repeated over three seasons, it had two controls namely: a control with fertilization only, and a control with the pathogen (*R. solani*) inoculum applied. The treatments were *T. harzianum* only; *T. harzianum* plus *R. solani*; slow release potassium silicate only; slow release potassium silicate plus *R. solani*; *B. megaterium* only; *B. megaterium* plus *R. solani*; *T. harzianum* plus slow release potassium silicate; *T. harzianum* plus slow release potassium silicate plus *R. solani*; *T. harzianum* plus *B. megaterium*; *T. harzianum* plus *B. megaterium* plus *R. solani*; slow release potassium silicate plus *B. megaterium*; Slow release potassium silicate plus *B. megaterium* plus *R. solani*; *T. harzianum* plus *B. megaterium* plus slow release potassium silicate; *T. harzianum* plus *B. megaterium* plus slow release potassium silicate plus *R. solani*. Each treatment was applied on maize planted in a 3 m x 3.6 m plots. In each plot there were 55 plants with 0.9 m spacing between rows and 0.3 m spacing between plants (equivalent to 37 000 plants per ha). The spacing between plots and replicates were 1 m. The same plots were used for each treatment in all three seasons.

A randomized block design with four replicates was employed in all experiments each season. The parameter measured was the yield. Factorial analysis of variance was performed using the General Linear Model of ANOVA, of Genstat® 14th edition. An F value for main treatment effects and their interaction were considered significant at $P \leq 0.05$ level. Treatment means were separated using Fisher's unprotected LSD test at the 5% probability level.

5.2.7. Cost Benefit Analysis

In all the experiments conducted, the cost benefit analysis was done. An average of true prices of inputs in the Eastern Cape during the 2012 to 2015 maize seasons were used for calculations. As for the maize price, R2,015 per ton for the yellow maize was employed, which was a true price for the 2012 / 2013 season. This is the maize that is commonly grown in the Eastern Cape by small scale farmers, PAN14, which was used in this study. For the cost / benefit calculations, it was assumed that the maize price remained constant during the test seasons. Labour and other cost of production were kept constant as small scale farmers do not hire people instead they themselves work the land. Among treatments

5.3. Results

The cost benefit analysis was done for 3 different studies, the ones reported in chapter 2, chapter 3 and chapter 4. Following are the results for the cost benefit analysis per chapter.

5.3.1. Integration of *Trichoderma harzianum* and potassium silicate (KSil) to improve growth and yield of maize in the presence of *Rhizoctonia solani*

The combination of *T. harzianum* and the liquid formulation of potassium silicate gave the highest returns on investment in both seasons, which was 37% and 23% in the first and second season respectively. All treatments gave a positive return on investment (Table 5.1). The same scenario was observed in Season 2 (Table 5.2), where all treatments gave a positive return on investment. The net return per ha was significantly higher than the Inoculated Control for the *T. harzianum* treatment alone, the liquid formulation of potassium silicate, and lastly the combination of *T. harzianum* and liquid formulation of potassium silicate. This was observed in both seasons. The combination of *T. harzianum* and the slow release of potassium silicate treatment showed inconsistent results because in Season 1 it resulted in a significantly higher net return whereas in Season 2 it resulted in insignificantly higher net return relative to the Inoculated Control.

Table 5.1: Cost Benefit Analysis for the control of *Rhizoctonia solani* in the 2012/2013 season on maize

Treatment	**Yield ($t\ ha^{-1}$)**	**Gross Income ha^{-1}**	**Increased cost of treatments ha^{-1}**	**Net return ha^{-1}**	**Increased return relative to the Inoculated Control**	**Net return relative to the Inoculated Control (%)**
Inoculated Control	3.2c	R 6,519c	R 0	R 6,519c	R 0	0%
Untreated Control	4.8bc	R 9,702bc	R 0	R 9,702bc	R 3,184	20%
KSil liquid	6.3ab	R 12,729ab	R 42	R 12,687ab	R 6,169	32%
KSil Slow release	5.3ab	R 10,669ab	R 1,574	R 9,095bc	R 2,577	17%
T. harzianum	6.5ab	R 13,079ab	R 25	R 13,054ab	R 6,536	33%
T. harzianum+KSil liquid	7.15a	R 14,256a	R 223	R 14,033a	R 7,515	37%
T. harzianum +KSil Slow release	6.6ab	R 13,251ab	R 1,599	R 11,652ab	R 5,133	28%
F value	4.67	4.67		4.63		
P value	0.005	0.005		0.005		
CV%	21.7	21.7		22.7		

*Numbers with different letters in a column are significantly different at $p \leq 0.05$

Table 5.2: Cost Benefit Analysis for the control of *Rhizoctonia solani* in the 2013/2014 season on maize

Treatment	Yield ($t\ ha^{-1}$)	Gross Income ha^{-1}	Increased cost of treatments ha^{-1}	Net return ha^{-1}	Increased return relative to the Inoculated Control	Net return relative to the Inoculated Control (%)
Inoculated Control	3.7b	R 7,393b	R 0	R 7,393c	R 0	0%
Untreated Control	4.6ab	R 9,325ab	R 0	R 9,325abc	R 1,932	12%
KSil liquid	5.5a	R 11,042a	R 42	R 11,001ab	R 3,608	20%
KSil Slow release	5.0ab	R 10,021ab	R 1,574	R 8,447bc	R 1,053	7%
T. harzianum	5.9a	R 11,846a	R 25	R 11,821a	R 4,428	23%
T. harzianum+KSil liquid	5.9a	R 11,868a	R 67	R 11,802a	R 4,409	23%
T. harzianum +KSil Slow release	5.4a	R 10,885a	R 1,599	R 9,286abc	R 1,893	11%
F value	3.50	3.50		4.05		
P value	0.018	0.018		0.010		
Cv%	16.5	16.5		17.2		

*Numbers with different letters in a column are significantly different at p=0.05 according to Duncan's multiple range test

5.3.2. Integration of a Biofertilizer with Micro-dosed Chemical Fertilizers on Maize Grown in Marginal Soils

In Season 1 the Untreated Control yielded 2.8 t ha^{-1}, with a value of R5,642, with no risk or requirement for labour to apply the fertilizers. In Season 2 almost the same scenario was observed where the control plot yielded 3.0 t ha^{-1}, with a value of R 6,045 as a return on investment, which was only 6.7% higher than the previous season (Table 5.3 and Table 5.4).

Full fertilization as per the soil recommendations gave a yield of 4 t ha^{-1} yet the costs of full fertilization were high at R11,525 per ha because the land was a fallow land with 56% acid saturation and a large quantity of lime needed to be applied. This cost exceeded the value of the maize yield by -R3,463 per ha. Relative to the Untreated Control crop, a loss of R9,105 per ha and R9,308 per ha in the first and second seasons, respectively, was realized by employing full fertilization. All the other treatments gave positive net returns relative to fertilized control ranging from R7,669 to R16,380 and R7,496 to R12,529 in the first and second seasons, respectively (Table 5.3 and Table 5.4).

Lime micro-dosing gave a 16% net return increase in Season 1 and a 7% decrease in net return in Season 2 relative to the Untreated Control. In both seasons the difference with the Untreated Control was not significant. Treatments with lime, *B. megaterium* and superphosphate increased the yield significantly in both seasons by 16% in Season 1 and 13% in Season 2, relative to the Untreated Control. However, the increase in the net return was not significant in either season, the net returns being R2,191 and R1,787 in the first and second seasons, respectively, relative to Untreated Control (Table 5.3 and Table 5.4).

Micro-dosing lime and 2:3:2 fertilizer significantly increased the maize yield in both seasons. However, the net return on investment was significantly higher in Season 1 only, by R8,479 relative to the Untreated Control and R17,584 relative to the Fertilized Control. When *B. megaterium* was combined with 2:3:2 (34) NPK fertilizer plus lime micro-dose treatment, the yield increases were significant in both seasons as were the returns on investment (Table 5.3 and Table 5.4).

Table 5.3: Cost Benefit Analysis for Micro-dosing Fertilizers and Lime, and Applying a Biofertilizer, *Bacillus megaterium* in the 2012/2013 season on maize

Treatment	**Yield (t ha^{-1})**	**Gross income per ha**	**Increased cost of treatment per ha**	**Net return per ha**	**Increased return relative to Untreated Control**	**Increased return relative to Untreated Control (%)**	**Increased return relative to Fertilized Control**
Untreated Control	2.80fg	R 5,642	R 0	R 5,642fg	R 0	0%	R 9,105
Fertilized Control plus lime as per soil test	4.00efg	R 8,062	R 11,525	-R 3,463h	-R 9,105	-418%	R 0
B. megaterium	2.10g	R 4,2:3:2	R 25	R 4,207g	-R 1,436	-15%	R 7,669
Lime	4.10efg	R 8,262	R 408	R 7,853cdefg	R 2,211	16%	R 11,316
Superphosphate	6.30bcd	R 12,695	R 2,212	R 10,483abcd	R 4,841	30%	R 13,945
Fertilizer2:3:2	7.60ab	R 15,314	R 2,396	R 12,918ab	R 7,276	39%	R 16,380
Lime + *B. megaterium*	2.70fg	R 5,441	R 433	R 5,007fg	-R 635	-6%	R 8,470
Superphosphate + *B. megaterium*	3.70efg	R 7,456	R 2,237	R 5,219fg	-R 423	-4%	R 8,681
Fertilizer2:3:2 + *B. megaterium*	6.60abcd	R 13,299	R 2,421	R 10,878abcd	R 5,236	32%	R 14,340
Lime + Superphosphate	4.60def	R 9,269	R 2,620	R 6,649defg	R 1,007	8%	R 10,111
Fertilizer2:3:2 + lime	8.40a	R 16,926	R 2,805	R 14,121a	R 8,479	43%	R 17,584
Fertilizer2:3:2 + Superphosphate	7.30abc	R 14,710	R 4,608	R 10,102abcde	R 4,460	28%	R 13,564
Superphosphate +lime+ *B. megaterium*	5.20cde	R 10,478	R 2,645	R 7,833cdefg	R 2,191	16%	R 11,295
Fertilizer2:3:2 + Superphosphate +lime	6.40abcd	R 12,896	R 5,016	R 7,880cdefg	R 2,238	17%	R 11,342
Fertilizer2:3:2+lime + *B. megaterium*	7.00abc	R 14,105	R 2,830	R 11,275abc	R 5,633	33%	R 14,738
Fertilizer2:3:2+Superphosphate+*B. megaterium*	5.30cde	R 10,680	R 4,633	R 6,047efg	R 405	3%	R 9,509
Fertilizer2:3:2+Superphosphate+lime+ *B.megaterium*	7.00abc	R 14,105	R 5,041	R 9,064bcdef	R 3,422	23%	R 12,526
F value	8.33			9.44			
P value	0.001			0.001			
Cv%	24.6			34.3			

*Numbers with different letters in a column are significantly different at p=0.05 according to Duncan's multiple range test

*Red numbers reflected negative returns

Table 5.4: Cost Benefit Analysis for Micro-dosing Fertilizers and Lime, and Applying a Biofertilizer, *Bacillus megaterium* in the 2013/2014 season on Maize

Treatment	Yield (t ha^{-1})	Gross income per ha	Increased cost of treatment per ha	Net return per ha	Increased return relative to Untreated Control	Increased return relative to Untreated Control (%)	Increased return relative to Fertilized Control
Untreated Control	3fg	R 6,045	R 0	R 6,045bcdef	R 0.00	0%	R 9,308
Fertilized Control plus lime as per soil test**	4.1cdefg	R 8,262	R 11,525	-R 3,263g	-R 9,308.02	-335%	R 0
B. megaterium	3.8defg	R 7,657	R 25	R 7,632abcde	R 1,587.00	12%	R 10,895
Lime	2.8g	R 5,642	R 408	R 5,234def	-R 811.43	-7%	R 8,497
Superphosphate	4.5bcde	R 9,068	R 2,212	R 6,856abcdef	R 810.58	6%	R 10,119
Fertilizer2:3:2 (34)	3.4efg	R 6,851	R 2,396	R 4,455f	-R 1,590.08	-15%	R 7,718
Lime + *B. megaterium*	2.8g	R 5,642	R 433	R 5,209def	-R 836.43	-7%	R 8,472
Superphosphate + *B. megaterium*	3.7efg	R 7,456	R 2,237	R 5,219def	-R 826.42	-7%	R 8,482
Fertilizer2:3:2 (34) + *B. megaterium*	5.8ab	R 11,687	R 2,421	R 9,266a	R 3,220.92	21%	R 12,529
Lime + Superphosphate	3.8efg	R 7,657	R 2,620	R 5,037ef	-R 1,008.35	-9%	R 8,300
Fertilizer2:3:2 (34) + lime	4.6bcde	R 9,269	R 2,805	R 6,464bcdef	R 419.49	3%	R 9,728
Fertilizer2:3:2 (34) + Superphosphate	6.3a	R 12,695	R 4,608	R 8,087abc	R 2,041.50	14%	R 11,350
Superphosphate +lime+ *B. megaterium*	5.2abc	R 10,478	R 2,645	R 7,833abcd	R 1,787.65	13%	R 11,096
Fertilizer2:3:2 (34) + Superphosphate +lime	4.8bcde	R 9,672	R 5,016	R 4,656f	-R 1,389.43	-13%	R 7,919
Fertilizer2:3:2 (34)+lime + *B. megaterium*	5.6ab	R 11,284	R 2,830	R 8,454ab	R 2,409.49	17%	R 11,718
Fertilizer2:3:2(34)+Superphosphate+ *B.megaterium*	4.4bcdef	R 8,866	R 4,633	R 4,233f	-R 1,812.00	-18%	R 7,496
Fertilizer2:3:2(34)+Superphosphate+lime+ *B.megaterium*	5.2abcd	R 10,478	R 5,041	R 5,437cdef	-R 205	-2%	R 8,899
F value	6.21			10.48			
P value	<.001			<.001			
Cv%	19.7			30.3			

*Numbers with different letters in a column are significantly different at p=0.05 according to Duncan's multiple range test

**The yield figure used was obtained from the Dohne Research Institute evaluation results of the land used in the study.

The treatment with *B. megaterium*, lime and 2:3:2 (34) NPK fertilizer micro-dose, increased maize yield, significantly, relative to the Untreated Control in both seasons and the net return on investment was increased significantly in Season 1 by R5,633, but not in Season 2 when it was only R2,409. Relative to the Fertilized Control the increase in the net return was R14,738 and R11,718 in the first and second seasons, respectively.

The treatment that gave the highest net return on investment in Season 1 was the integration of 2:3:2 (34) NPK fertilizer and lime micro-doses. In Season 2, however, the treatment that gave the highest net return on investment was the integration of 2:3:2 (34) NPK fertilizer and *B. megaterium*. This integration of 2:3:2 (34) NPK fertilizer and *B. megaterium* was the treatment that showed consistency of performing significantly higher than the Untreated Control in both seasons for both maize yield and net return.

5.3.3. Integrated Management of *Rhizoctonia solani* on Maize

The pathogen reduced yield by 34%, 16% and 10% in Seasons 1, 2 and 3, respectively, relative to the Untreated Control thus, the loss of income was R1,177, R1,304, and R824 per ha in Seasons 1, 2 and 3, respectively, due to the uncontrolled pathogen (Table 5.5, Table 5.6, Table5.7).

All treatments in Season 1 gave a significantly higher yield and net return on investment, relative to the Inoculated Control, with the lowest giving a 39% net return and the highest giving a 65% net return (Table 5.5). In Season 2 none of the treatments resulted in significantly higher yields (Table 5.6). In Season 3 the same scenario as in Season 1 where all treatments resulted in significantly higher yields and net return relative to the Inoculated Control, with the exception of *B. megaterium* in the presence of the pathogen (Table5.7).

Table 5.5: Cost Benefit Analysis for the Integrated Treatments of potassium silicate, *Bacillus megaterium* and *Trichoderma harzianum* in the 2012/2013 season

Treatment	**Yield (t ha^{-1})**	**Gross income per ha**	**Increased costs for disease control per ha**	**Net Income per ha**	**Increased return relative to Untreated Control**	**Increased return relative to Untreated Control (%)**	**Increased return relative to Inoculated Control**	**Increased return relative to Inoculated Control (%)**
Untreated Control	1.739e	R 3,504	R 0	R 3,504f	R 0	0%	R 1,177	20%
Pathogen (*R. solani*) Control	1.155e	R 2,327	R 0	R 2,327f	-R 1,177	-20%	R 0	0%
R. solani+ *T. harzianum*	5.498a	R 11,078	R 25	R 11,053a	R 7,549	52%	R 8,726	65%
R. solani + KSil Slow release + *B. megaterium*	3.421cd	R 6,893	R 1,599	R 5,294de	R 1,790	20%	R 2,967	39%
R. solani + KSil Slow release + *T. harzianum*	5.468a	R 11,018	R 1,599	R 9,419a	R 5,915	46%	R 7,092	60%
R. solani + *T. harzianum* + *B. megaterium*	4.839ab	R 9,751	R 50	R 9,701abc	R 6,197	47%	R 7,373	61%
T. harzianum + *B. megaterium*	3.308cd	R 6,666	R 50	R 6,616de	R 3,112	31%	R 4,288	48%
KSil Slow release + *T. harzianum*	4.409abc	R 8,884	R 1,599	R 7,285abcd	R 3,781	35%	R 4,958	52%
KSil Slow release + *B. megaterium*	4.374abc	R 8,814	R 1,599	R 7,215abcd	R 3,710	35%	R 4,887	51%
T. harzianum	5.205ab	R 10,488	R 25	R 10,463ab	R 6,959	50%	R 8,136	64%
R. solani + KSil Slow release + *T. harzianum* + *B. megaterium*	3.997bcd	R 8,054	R 1,624	R 6,430cde	R 2,926	29%	R 4,103	47%
R. solani + *B. megaterium*	3.475cd	R 7,002	R 25	R 6,977de	R 3,473	33%	R 4,650	50%
KSil Slow release	3.985bcd	R 8,030	R 1,574	R 6,456cde	R 2,952	30%	R 4,128	47%
KSil Slow release + *T. harzianum* + *B. megaterium*	3.266cd	R 6,581	R 1,624	R 4,957de	R 1,453	17%	R 2,630	36%
B. megaterium	2.924d	R 5,892	R 25	R 5,867e	R 2,363	25%	R 3,540	43%
R. solani + KSil Slow release	4.208bc	R 8,479	R 1,574	R 6,905bcd	R 3,401	33%	R 4,578	50%
F value	10.62			10.52				
P value	0.001			0.001				
Cv%	19.6			19.7				

*Values with same letters are not significantly different at p=0.05 according to Duncan's multiple range test

Table 5.6: Cost Benefit Analysis for the Integration of potassium silicate, *Bacillus megaterium* and *Trichoderma harzianum* in the 2013/2014 season

Treatment	**Yield (t ha^{-1})**	**Gross income per ha**	**Increased costs for disease control per ha**	**Net Income per ha**	**Increased return relative to Untreated Control**	**Increased return relative to Untreated Control (%)**	**Increased return relative to Inoculated Control**	**Increased return relative to Inoculated Control (%)**
Untreated Control	4.1a	R 8,213	R 0	R 8,213ab	R 0	0%	R 1,304	19%
Pathogen (*R. solani*) Control	3.5a	R 6,909	R 0	R 6,909ab	-R 1,304	-9%	R 0	0%
R. solani+ *T. harzianum*	4.9a	R 9,928	R 25	R 9,903a	R 1,690	9%	R 2,993	43%
R. solani + KSil Slow release+ *B. megaterium*	3.9a	R 7,846	R 1,599	R 6,247ab	-R 1,966	-14%	-R 662	-10%
R. solani + KSil Slow release + *T. harzianum*	4.2a	R 8,558	R 1,599	R 6,959ab	-R 1,255	-8%	R 49	1%
R. solani + *T. harzianum* + *B. megaterium*	4.3a	R 8,789	R 50	R 8,739ab	R 526	3%	R 1,830	26%
T. harzianum + *B. megaterium*	3.3a	R 6,627	R 50	R 6,577ab	-R 1,636	-11%	-R 332	-5%
KSil Slow release + *T. harzianum*	3.2a	R 6,541	R 1,599	R 4,942b	-R 3,272	-25%	-R 1,968	-28%
KSil Slow release + *B. megaterium*	4.7a	R 9,402	R 1,599	R 7,803ab	-R 410	-3%	R 893	13%
T. harzianum	4.2a	R 8,519	R 25	R 8,494ab	R 281	2%	R 1,585	23%
R. solani + KSil Slow release + *T. harzianum* + *B. megaterium*	4.3a	R 8,695	R 1,624	R 7,071ab	-R 1,142	-7%	R 161	2%
R. solani + *B. megaterium*	3.7a	R 7,365	R 25	R 7,340ab	-R 873	-6%	R 430	6%
KSil Slow release	4.2a	R 8,532	R 1,574	R 6,957ab	-R 1,256	-8%	R 48	1%
KSil Slow release + *T. harzianum* + *B. megaterium*	3.3a	R 6,754	R 1,624	R 5,130ab	-R 3,083	-23%	-R 1,779	-26%
B. megaterium	4.0a	R 8,068	R 25	R 8,043ab	-R 170	-1%	R 1,134	16%
R. solani + KSil Slow release	4.0a	R 8,052	R 1,574	R 6,478ab	-R 1,735	-12%	-R 432	-6%
F value	1.01			1.01				
P value	0.464			0.461				
Cv%	24.6			24.8				

*Values with same letters are not significantly different at p=0.05 according to Duncan's multiple range test

Table 5.7: Cost Benefit Analysis for Integration of potassium silicate, *B. megaterium* and *T. harzianum* in the 2014/2015 season

Treatment	Yield (t ha^{-1})	Gross Income per ha	Increased costs for disease control per ha	Net Income per ha	Increased return relative to Untreated Control	Increased return relative to Untreated Control (%)	Increased return relative to Inoculated Control	Increased return relative to Inoculated Control (%)
Untreated Control	4.0gh	R 7,979	R 0	R 7,979gh	R 0	0%	R 824	12%
Pathogen (R. solani) Control	3.6h	R 7,155	R 0	R 7,155h	-R 824	-5%	R 0	0%
R. solani+ T. harzianum	7.7bcd	R 15,604	R 25	R 15,579bcd	R 7,600	32%	R 8,424	118%
R. solani + KSil Slow release+ *B. megaterium*	5.9defg	R 11,798	R 1,599	R 10,199defg	R 2,219	12%	R 3,043	43%
R. solani + KSil Slow release + *T. harzianum*	10.6a	R 21,317	R 1,599	R 19,718a	R 11,738	42%	R 12,562	176%
R. solani + T. harzianum + B. megaterium	6.7def	R 13,444	R 50	R 13,394def	R 5,415	25%	R 6,239	87%
T. harzianum + B. megaterium	7.2cde	R 14,589	R 50	R 14,539cde	R 6,559	29%	R 7,383	103%
KSil Slow release + *T. harzianum*	9.0abc	R 18,230	R 1,599	R 16,631abc	R 8,651	35%	R 9,475	132%
KSil Slow release + *B. megaterium*	5.6efg	R 11,340	R 1,599	R 9,741efg	R 1,762	10%	R 2,586	36%
T. harzianum	9.5ab	R 19,126	R 25	R 19,101ab	R 11,122	41%	R 11,946	167%
R. solani + KSil Slow release + *T. harzianum + B. megaterium*	6.3def	R 12,721	R 1,624	R 11,097def	R 3,117	16%	R 3,941	55%
R. solani + B. megaterium	5.4efgh	R 10,877	R 25	R 10,852efgh	R 2,873	15%	R 3,697	52%
KSil Slow release	6.8def	R 13,636	R 1,574	R 12,061def	R 4,082	20%	R 4,906	69%
KSil Slow release + *T. harzianum + B. megaterium*	10.5a	R 21,133	R 1,624	R 19,509a	R 11,530	42%	R 12,354	173%
B. megaterium	4.9fgh	R 9,966	R 25	R 9,941fgh	R 1,962	11%	R 2,786	39%
R. solani + KSil Slow release	6.8def	R 13,636	R 1,574	R 12,061def	R 4,082	20%	R 4,906	69%
F value	11.91			11.82				
P value	0.001			0.001				
Cv%	17.8			17.9				

*Values with same letters are not significantly different at p=0.05 according to Duncan's multiple range test

The top three treatments that resulted in high net return in the presence of the pathogen in Season 1 were *T. harzianum* (65%); followed by *T. harzianum* plus *B. megaterium* (61%) and lastly potassium silicate plus *T. harzianum* (60%) (Table 5.5). In Season 2, the top three treatments that gave high net return were *T. harzianum* (43%); followed by *T. harzianum* plus *B. megaterium* (26%) and lastly *B. megaterium* (6%) (Table5.6). In Season 3, the top three treatments were potassium silicate plus *T. harzianum* (176%); followed by *T. harzianum* (118%) and lastly *T. harzianum* plus *B. megaterium* (87%) (Table 5.7).

The top three treatments that resulted in high net return in the absence of the pathogen in Season 1 were *T. harzianum* (50%); followed by potassium silicate plus *T. harzianum* (35%) and potassium silicate plus *B. megaterium* (35%) and lastly *T. harzianum* plus *B. megaterium* (31%) (Table 5.5). In Season 2, it was only the *T. harzianum* treatment that gave a positive net return (Table 5.6). In Season 3, the top three treatments that gave high net return were potassium silicate plus *T. harzianum* plus *B. megaterium* (42%); followed by *T. harzianum* (41%); and lastly potassium silicate plus *T. harzianum* (35%) (Table 5.7).

5.4. Discussion

5.4.1. Integration of *Trichoderma harzianum* and potassium silicate (KSil) to improve growth and yield of maize in the presence of *Rhizoctonia solani*

The combination of *T. harzianum* and the liquid formulation of potassium silicate gave the highest returns on investment in both seasons, which was 37% and 23% in Seasons 1 and 2, respectively, in controlling *Rhizoctonia solani*. Relative to the Inoculated Control, the net return was highly significant in both seasons. These treatments individually have been reported to control root pathogens. *T. harzianum* has been reported to control *R. solani* (Kobori et al., 2015; Nezarat and Gholami, 2009). Potassium silicate on the other hand has been reported to restrict penetration of the pathogens to the plant cell thus protecting the plant from pathogen infections. (Meharg and Meharg, 2015). In this study it was also confirmed by the results that *T. harzianum* and potassium silicate individually increased maize yields significantly, relative to the Inoculated Control (Table 5.1 and Table 5.2). All treatments gave a positive return on investment in both seasons. The most cost effective way of controlling *R. solani* was the combination of *T. harzianum* and potassium silicate application.

5.4.2. Integration of a Biofertilizer with Micro-dosed Chemical Fertilizers on Maize Grown in Marginal Soils

In this study on fertilization, the Untreated Control with no soil treatments yielded 2.8 $t.ha^{-1}$, with a value of R5,642, with no risk or requirement for labour to apply the fertilizers (Table 5.3). However, this is not sustainable because nutrients in the soil will become depleted if not supplemented. In Season 2 the Untreated Control plot yielded 3.0 $t.ha^{-1}$, with a value of R6,045 as a return on investment, 6.7% higher than the previous season, which was not significant.

Full fertilization as per an official recommendation gave the highest yield of 4.0 $t.ha^{-1}$. However, the cost of full fertilization was R11,525 per ha because the land was a fallow land with 56% acid saturation and it needed a large quantity of lime to be applied. This cost exceeded the value of the maize yielded by -R3 463 per ha. Relative to the Untreated Control crop, losses of R9,105 and R9,308 per ha, were realized in Seasons 1 and 2, respectively. This confirms the wisdom of small scale farmer who consistently refuse to apply the recommended levels of lime and fertilizers. However, the need remains for alternative strategies of fertilization small scale farmers can afford and which provide a positive return on investment.

All the other treatments gave a positive return on investment relative to full fertilization ranging from R7,669 to R16,380 and R7,496 to R12,529 in Seasons 1 and 2, respectively. Although full fertilization corrects the soil nutrient shortages, in the case of small scale farmers, it is not affordable. Correcting the soil pH and nutrient balance needs to be done over several seasons, hence the value of micro-dosing as employed in this study.

The lime micro-dosing treatment gave a 16% net return increase in Season 1 and a 7% decrease in net return, which was not significant relative to the Unfertilized Control ($p > 0.05$). The increase may have resulted from the fact that liming prevents phosphorus from being bound to soil particles, due to acidic soils, thus the P is available for plant use (Mijangos *et al*., 2010). Therefore, lime application to unfertilized soils in Season 1 may have released the previously bound phosphorus because the soils used in the study were highly acidic. In Season 2 there were no more nutrients to be released because they were depleted in the previous season, hence there was a decrease in the return on investment and lower yields. This means that micro-dosing lime must be accompanied by NPK application. Micro-dosing of lime will correct the soil pH over seasons because it is a targeted application. For a small scale farmer, correcting the soil pH gradually would be an affordable, productive option.

Micro-dosing with 2:3:2 (34) NPK fertilizer and lime significantly increased maize yield in both seasons. This treatment gave the highest net return on investment in Season 1. The net return on investment was significantly higher in Season 1 by R8,479 relative to the Untreated Control and R17,584 relative to the Full Fertilization. Although the net return in Season 2 was not significant, it was higher than the Controls. This was expected because it is the option that most farmers use. Relative to the 2:3:2 (34) NPK fertilizer micro-dose, this treatment 2:3:2 (34) NPK fertilizer plus lime micro-dose increased the yield and net return, although it was not significant ($p > 0.05$). However, in Season 2 the yield increase was significantly higher than the 2:3:2 (34) NPK fertilizer only and the net return was higher but not significant. This confirms that the micro-dosing corrects the soil gradually, thus increasing the maize production. This is a more sustainable option for small scale farmers. This is in agreement with Camara *et al.* (2013) and Ousman and Aune (2011) who found that micro-dosing increases productivity and yields, especially for small scale farmers.

The combination of *B. megaterium* and 2:3:2 (34) NPK fertilizer significantly increased both the yield and the net return in both seasons. This means that 2:3:2 (34) NPK fertilizer plus *B. megaterium* was the more sustainable option because its performance was consistent. In Season 2 this treatment performed the best. The improved performance of this combination in Season 2 could be that the *B. megaterium* population increased in Season 2 because the same plot was used in Season 2, which may have resulting in increased activity. Higher populations of *B. megaterium* allow for more bound phosphates to be solubilized and made available to plants (Hassan *et al*. 2012; Xinxian *et al*., 2011).

The treatment with lime, *B. megaterium* and 2:3:2 (34) NPK fertilizer significantly increased maize yields in both seasons but the net return increase was significant only in Season 1. The yield increase in both seasons was not greater than the combination of 2:3:2 (34) NPK with lime or *B. megaterium*. This means that it is not economical to use all three treatments.

The treatment with lime, *B. megaterium*, 2:3:2 (34) NPK fertilizer and superphosphate resulted in a significantly higher maize yield in both seasons. The net return in Season 1 was higher but not significant different from the Untreated Control but was significant higher than the Fertilized Control. In Season 2 the net return was lower than the Untreated Control but not significantly, but was significantly higher than the Fertilized Control. It is a relatively expensive combination, and is not the best option, relative to using two treatments such as 2:3:2 (34) NPK fertilizer plus lime or 2:3:2 (34) NPK fertilizer plus *B. megaterium*.

5.4.3. Integrated Management of *Rhizoctonia solani* on Maize

On the integrated treatment of the maize crop, the option of not controlling *R. solani* reduced yields by 34%, 16% and 10% in Seasons 1, 2 and 3, respectively. The income lost due to the reduced yields was R1,177 per ha, R1,304 per ha and R824 per ha in Seasons 1, 2 and 3, respectively. For a small scale farmer not to control *Rhizoctonia solani* is costly. Avoiding this loss may help his or her family to increase its food security status, to a small scale farmer R1000 increase makes a big difference. In turn if most small scale farmers in the Eastern Cape were to adopt the available technology and control their root diseases such as *Rhizoctonia solani*, they may influence a change in the Eastern Cape status that it is the top Province in the rankings of food insecurity (Labadarios, 2011).

All treatments in Season 1 resulted in significantly higher yields and significantly higher net return relative to the Inoculated Control. The lowest gave 39% and the highest gave 65% increase in net return, relative to the Inoculated Control. In Season 2 none of the treatments gave a significantly higher net return as in Season 1, although some gave a higher net return. In Season 3 the same scenario as in Season 1 was observed, where all treatments resulted in significantly higher net return relative to the Inoculated Control, with the exception of *B. megaterium* as a treatment on its own. This means that all treatment may control *R. solani*.

The treatments that were consistently in the top three that significantly controlled *R. solani* were *T. harzianum* only and *T. harzianum* plus *B. megaterium*. The application of *T. harzianum* and *B.*

megaterium may control root pathogens (Mghalu *et al.* 2007; Dubey *et al*. 2009), solubilize bound phosphorus (Hu *et al.* 2013), and fix atmospheric nitrogen for the crop (Hassan *et al*., 2012). This could be important for the Eastern Cape because the majority of farmers are small scale farmers and conventional fertilization and disease control is not affordable.

The combination that gave consistently higher return on investment in the control of *R. solani* and also in the provision of nutrients was the combination of *T. harzianum* plus *Bacillus megaterium* plus 2:3:2 (34) NPK and lime micro-doses. Therefore this combination may be recommended for the small scale farmers in the Eastern Cape.

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General Overview

Food insecurity is high in Africa. Enhancing agricultural output is the primary vehicle to reduce food insecurity. Biotic stresses, abiotic stresses and unaffordable input costs all combine to reduce crop outputs for small scale farmers, and increase food insecurity. Small scale farmers in the Eastern Cape of South Africa cannot afford the conventional inputs used to control plant diseases, or to fertilize or lime their soils adequately, resulting in nutrient depleted, highly acidic soils. As a result, there is a large gap in the maize yields achieved by commercial farmers and small scale farmers, a nearly fourfold difference of 5.8 t ha^{-1} versus 1.6 t ha^{-1}(Dredge, 2014)

The overall aim of this study, was find ways to manage the low fertility and disease control issues facing small scale farmers by developing affordable solutions to the control of root diseases, liming and fertilization to enhance maize yields of small scale farmers in the Eastern Cape.

The specific objectives were:

- To evaluate the efficacy of a strain of *Trichoderma harzianum* and fertilization with potassium silicate in order to control *Rhizoctonia solani* root rot;
- To evaluate the performance of micro-dosing of macronutrient fertilizers and lime to ameliorate low soil fertility and extremely acid soil conditions;
- To evaluate the capacity of a selected strain of *Bacillus megaterium* to improve maize yields through nitrogen fixation and solubilization of phosphorus bound to soil particles
- To evaluate the potential of combinations of treatments with *T. harzianum*, *B. megaterium*, potassium silicate, and micro-dosing of fertilizers and lime, to improve maize yields.
- To do a financial analysis on the costs of applying the above technologies versus the increased maize yields and hence income of a putative small scale farmer.

All the above field experiments were conducted successfully. They were all conducted over two maize seasons with the exception of the integration experiments that were conducted over three seasons. The novel discoveries were as follows:

CHAPTER 2: Integration of Trichoderma harzianum and potassium silicate (KSil) to improve growth and yield of maize in the presence of Rhizoctonia solani, the causal agent of root rot

Findings:

- Application of *T. harzianum* plus liquid formulation of KSil significantly controlled *R. solani* on maize thus improving maize yields.
- Potassium silicate controlled *R. solani* on maize field experiments as a single application
- *Trichoderma harzianum* also controlled *R. solani* in the second season (p = 0.018).

Implications:

- The combination of *T. harzianum* and liquid formulation of potassium silicate is an option that small scale farmers may use to control root pathogens such as *R. solani* thus improve maize yields
- The reports in many articles about *T. harzianum* controlling *R. solani* are true.
- Potassium silicate may be used by small scale farmers to control the causal agent of root diseases on maize and other crops.

CHAPTER 3: Integration of a *B. megaterium* (Biofertilizer) with micro-dosed chemical fertilizers and lime on maize grown in marginal soils

Findings:

- The integration of *B. megaterium* and 2:3:2 (34) fertilizer improved maize yield significantly relative to the control and the yield Eastern Cape small scale farmers normally get as per the Economic Review SA Agriculture 2012/13

- Micro-dosing of fertilizers provides the small scale farmer with a technique to fertilize efficiently and affordably, and to enhance the net income derived from farming maize;
- Micro-dosing of lime was an efficient and effective way of managing extreme soil acidity in an affordable way that enhanced maize yields and provided a significant net return on investment.
- Relative to normal maize yields Eastern Cape small scale farmers get of 1.6 $t.ha^{-1}$, microdosing 2:3:2 (34) fertilizer and lime almost double that yield.
- Instead of applying 4.5 $tons.ha^{-1}$ of lime, only 185kg was applied but significant yields were obtained. Up to 61.64% of the expected yield was obtained through this application.

Implications:

- Micro-dosing may afford the small scale farmers an opportunity to fertilize properly, and harvest higher yields using smaller quantities of fertilizers.
- Liming during planting time may save the subsistence farmers cost of mechanization during liming three months before planting and this increases weeds that will need to be removed before planting through discing.
- Micro-dosing of fertilizers and lime is a viable option for small scale farmers as it is affordable yet have benefits
- Liming soils over time is attainable for small scale farmers as they get significantly higher yields, which is almost double what they currently get.

CHAPTER 4: Integrated management of *Rhizoctonia solani* on maize

Findings:

- All selected treatments controlled *R. solani*
- The top three treatments that controlled *R. solani* consistently over the seasons are *T. harzianum*, the combination of *T. harzianum* plus potassium silicate and lastly the combination of *T. harzianum* and *B. megaterium*

Implications:

- All the selected treatments were a good selection for integration
- The small scale farmer may choose any of the combinations mentioned above, depending on affordability

CHAPTER 5: Cost-benefit analysis of integrated management of maize

Findings:

- The combination of *T. harzianum* and the liquid formulation of potassium silicate resulted in the highest control of *R. solani* thus giving highest yield and net return on investment.
- Conventional recommendations from government research stations on liming and fertilization are not financially viable for small scale farmers cultivating low fertility, highly acidic soils. Although full liming and fertilization applications were effective at enhancing maize yields, the input costs substantially exceeded the increased income that they brought. Hence the small scale farmers of the Eastern Cape are perfectly rational in rejecting these recommendations. In contrast, the decision to apply no lime and no fertilizer was rational because the net income was positive, even if it was low.

  A combination of beneficial microbes applied to maize seed, and micro-dosing of planting holes with lime and fertilizers resulted in yield increases, and a significant increase in net financial return on investment for small scale farmers.

Implications:

- The affordable option for small scale farmers to control *R. solani* is the use of the combination of *T. harzianum* and the liquid formulation of potassium silicate.
- Micro-dosing of fertilizers and lime is cost effective for small scale farmers
- The use of the combination of beneficial microbes and microdosing fertilizers and lime is the recommended option for small scale farmers as it gives them an increase in the net financial return on investment.

Hence, the overall aim of the project was achieved in the development of affordable, effective technologies that small scale farmers in the Eastern Cape could adopt to enhance maize yields and income.

In the future, further research is needed in the following areas:

- Designing a small scale, affordable, fertilizer applicator to facilitate the accurate application of micro-doses of fertilizers and lime into maize planting holes. This is needed to reduce the labour intensive nature of manual applications of micro-doses into planting holes. The cap of a soda bottle is a cheap, accessible, indestructible fertilizer dispenser but it is not a scalable technology;
- Designing an attachment for commercial seed planters that will support micro-dosing of fertilizers and lime into the planting holes. This will allow the commercial farmers to utilize the technology of micro-dosing in order to reduce their input costs.
- Formulate a product that contains both *T. harzianum* and *B. megaterium* and uses potassium silicate powder as a carrier. When applied with a sticker, a three product treatment will be attached to the maize seed. This will enable the farmer to use one product to provide plant disease control, priming of the plant's self-defense mechanisms, biological nitrogen fixation, and phosphorus solubilization.
- Technology transfer, or the active communication of the potential of these technologies to assist small scale maize farmers in the Eastern Cape, and South Africa as a whole, presents a significant challenge. However, the impact of enhanced crop yields using these affordable, simple technologies on a national scale could significantly enhance food security in rural areas