Evaluating the effects of Yeast isolates on the Control of sore shin in Tobacco *(Nicotiana tabacum L.)* seedlings

By

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Declaration

I do hereby declare that this thesis entitled, "Evaluating the effects of Yeast isolates on the Control of sore shin in Tobacco *(Nicotiana tabacum L.)* Seedlings," was written by me and that it is the record of my own research work. It is neither in part nor in whole been presented for another degree elsewhere.

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(Student Name) (**Signature)** **(Date)**

The above declaration is affirmed
Mrs. Makaure B. T ...…… ……
(Supervisor's Name) (**Signature)** **(Date)**

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Abstract

Sore shin aused by *Rhizoctonia solani* is responsible for remarkable economic losses of up to 10% in tobacco (*Nicotiana tabacum)* seedlings grown in float beds, thereby becoming important production limiting factor of tobacco crop. Sore shin is responsible for wiry stem formation, stem lesions, root rots, root discolouration and damping off of tobacco seedlings. Tobacco production in Zimbabwe contributes about 9% GDP and is form of employment to more than 90 000 people in the country. Currently two fungicides Shavit and Azoxystrobin are being used for controlling sore shin on tobacco but recent trends are showing element of resistance with *R. solani* therefore becoming a threat. In a bid to circumvent the tobacco losses due to sore shin using eco-friendly methods the researcher hereby sought to evaluate five yeasts isolates for the control of sore shin in greenhouse produced tobacco seedlings. In this study five isolates were used which are TY5, TY3, TY14, TY17 and TY18, the experiment was arranged in a completely randomised design and two controls were included with the untreated as negative control and Azoxystrobin as positive control. *R. solani* were isolated from the diseased tobacco plants showing sore shin symptoms. The experiment was carried out at Kutsaga Research Station located in Harare Zimbabwe during the September –November 2016 tobacco nursery period. Data was analysed using Genstat 18th edition, on parameters measured the yeast isolates proved to reduce sore shin significantly in comparison with the untreated control but TY18 was similar to the untreated control. The isolates resulted in an increase in shoot fresh weight with the highest recorded on TY3 with an average of 3.76g per seedling, also yeast isolates resulted in an increase root fresh weight, highest weight was recorded on seedlings treated TY5 but TY18 was even less than the untreated control. Significant increase in dry weight was observed on plants treated with yeast isolates seedlings plots treated with TY3 recorded the highest dry weight with an average of 0.39g per seedling. It could be suggested that such yeast isolates might be the promising as alternatives for controlling sore shin on tobacco seedlings and have the ability to be used as bio-fertilisers.

Dedication

I dedicate this project to my two grandmothers and family members.

Table of Contents

List of figures

List of tables

List of Appendices

Chapter 1

1.1 Introduction and Justification

Zimbabwe is amongst major producers and exporters of tobacco (*Nicotiana tabacum*) in the world, being ranked 6th in the world and first in the African countries for producing tobacco (Lamarchand and Schneegans, 2014; FAO, 2016). Zimbabwe has a tobacco world market share of 2.6%, of which the major export is in China where 42% of its tobacco is sold (FAOSTAT, 2016; Tianze, 2016). Tobacco is one of Zimbabwe's most valuable agricultural crop that is contributing about 26% of the agricultural Gross Domestic Product (GDP) and accounting for 60% of agricultural exports (Aneseeuw *et al.*, 2012). The Zimbabwe 2015-2016 tobacco growing season yielded an average annual yield of 190 million tonnes which fetched US$604.7 million export revenue.

Tobacco is grown for its high nicotine leaf, where nicotine a major alkaloid is extracted from (Tayaoub *et al.*, 2015). Some compounds like solanesol and other beneficial alkaloids that have different uses in chemical formation, medicinal industries, preservatives and fuel industries are extracted from the tobacco leaf (Mazarura, 2014*).* Tobacco is amongst few crops that are in world trade entirely based on dry leaf and is worldwide grown commercially as non-food crop (Koga and Rukuni, 2017).Tobacco leaf is also used in food processing industries and cosmetic industry and mainly tobacco industry as a raw material for cigarettes production and other tobacco production system (Kulic *et al*., 2008).

The production of tobacco dates back to the pre-colonial era in Zimbabwe (Mazarura, 2014). Zimbabwe had already established an international reputation for producing quality tobacco with high nicotine content that out compete others on world market. In Zimbabwe from the period 2001 – 2008 tobacco production was increasing rapidly and this was as a result of land reform programme. During the period of 2009 to 2013 tobacco production in the country was

also characterized by rapid increase production (ZTA, 2014), however quality and yield trends of produce start declining in 2014 (FAO, 2016), due to different factors which includes diseases, pests, crop plant management and other abiotic factors (TIMB, 2012) and also noticed was declining of tobacco active growers numbers.

Huge tobacco losses are being experienced in Zimbabwe due to plant disease and pests prevalence, its impact is being noticed from crop production up to storage hence reduction in tobacco production (Cerda *et al.,* 2017). Pests of economic importance to tobacco include tobacco aphids, cutworm, hornworm, fungus gnats, root knot nematodes and budworm (Masukwedza *et al.,* 2013). Tobacco is susceptible to diseases like Angular leaf spot, Alternaria leaf sport, frog eye, black shank, pythium root rot and sore shin (Elliott *et al.,* 2008). Pythium root rot and sore shin are the most seedbed problematic diseases leading to losses of tobacco seedlings (Sigobhodhla *et al*., 2010).

Sore shin is caused by a pathogen called *Rhizoctonia solani,* and is characterised by brown lesions on tobacco seedlings stem and these can be referred to as stem rots. These rots will continue spreading around the stem leading to wirery stems (Elliot *et al.,* 2008). Sore shin is very common in tobacco, and it leads to uneven stunted growth of tobacco transplants (Lamondia, 2012). In seedlings *Rhizoctonia solani* is also responsible for causing damping off of tobacco seedlings as well as root rots (Seema and Devaki, 2012). It is common in both conventional seedbeds and tobacco float beds and can cause huge losses of greater than 10% (Seema and Devaki, 2012). Damaged tissues of seedlings will lead to poor development of the seedlings therefore stunting and delays in transplanting might occur (Seebold, 2011).

Tobacco plant breeders in Zimbabwe are still working on the production of resistant cultivars to *Rhizoctonia solani* but they have not yet found any promising lines (CORESTER, 2015). Methyl bromide a fumigant with methyl bromide 98% as active and chloropicrin 2% was being

used for the control of most plant pathogenic diseases in convectional seed beds including sore shin, but have been phased out due to its toxicity and lack of target specificity, more so it was also causing ozone depletion (Hong, 2014). However due to phasing out of this fumigant and introduction of float beds in trial to minimise losses associated with sore shin, has reduced pathogen pressure but did not completely eradicated the problem (Manyumwa *et al.,* 2013).

Good hygiene is also used to minimise likelihood of sore shin occurrence this is by thoroughly washing the trays and soaking them in disinfectants, however the disease occurrence still remains a challenge as sclerotia the resting propagules of *Rhizoctonia solani* are formed in small crevices of the float trays (Gutierrez, 1997). The recommended chemical for *R solani* disease is Triadamenol with triadamenol 25EC also known as Shavit is being used but the application should be done 48 hours before transplanting that is for the seedlings of above 12 weeks after sowing and Azoxystrobin 25 SC which is used for early sore shin emergence (De Curtis *et al.,* 2010: Lamondia, 2012).

Reddy and Nagarajan (2005) alluded that use of chemicals have some toxicological risks, commonly used fumigants and fungicides have drastic effects on biota. Despite the negative effect of chemicals on crops, chemical residues in the plants are harmful to the consumer (Naseby, 2000). In addition, handling chemicals needs great caution, so there must be a reduction on usage of chemicals and find alternatives, among these, use of microbial antagonists was taken to be the most capable way (Spadaro and Gullino, 2004; Liu *et al.,* 2013) thus biological control. Seebold (2011) defines biological control as the use of microbial antagonism in suppression of other microorganisms. *Trichoderma harzianum* an entomopathogenic fungi have been successfully used in the control of sore shin in conventional seedbeds here in Zimbabwe Dimbi and Sigobodhla (2013)

From recent research that was conducted in Egypt for biological control of sore shin in common beans, yeast isolates were used and they were effective in controlling the disease (Mahmoud, 2016). Suppression of *Rhizoctonia solani* on sugar beet by antagonistic yeasts was also successful in research done by EI-Terability (2003). Loganathan (2004) observed that yeasts where very effective in controlling *Pythium aphaidermatum* on tobacco. These yeasts isolates are found in native soils, on fruit surfaces, plant roots and as well as on plant leaves (de Melo Pereira *et al.,* 2015). Yeasts elicits disease resistance on plants, they parasitise other fungi, they excretes antibioses and they out compete pathogens for nutrients hence being good bio-control agents (Mahmoud, 2014). In Zimbabwe there is need for isolation and evaluation of yeasts which might have the potential of controlling sore shin on tobacco hence the aim of this study.

1.2 Objectives

1.2.1 Main objective

To evaluate the effects of different yeasts isolates in the control of sore shin in tobacco seedlings

1.2 Specific objectives

1.2.2 To determine the effects of yeasts isolates on sore shin severity on *Rhizoctonia solani* inoculated tobacco seedlings.

1.2.3. To evaluate yeast isolates effects on seedling growth parameters (root fresh weight, root length, shoot fresh weight, shoot length and seedling dry weight) on *Rhizoctonia solani* inoculated tobacco seedlings.

1.3 Hypotheses

1.3.1 There are significant differences on the effects of yeast isolates on sore shin severity on *Rhizoctonia solani* inoculated tobacco seedlings.

1.3.2. There are significant differences on the effects of yeast isolates on seedling growth parameters (root fresh weight, root length, shoot fresh weight, shoot length and seedling dry weight) on *Rhizoctonia solani* inoculated tobacco seedlings.

Chapter 2

2.0 Literature review

2.1 Origin of tobacco

Tobacco plants are of the genus *Nicotiana* and of the *Solanaceae* (nightshade) family (Gately, 2003). Tobacco (*Nicotiana tabacum*) originated in northen Agentina in the highland Andes, probably Bolivia (Albrecht *et al.,* 2012). This was as a result of the hybridisation of two old species that are the *Nicotiana slyvestris* and *Nicotiana tomentosiformis* (Sierro *et al.*, 2013). Long back before Spanish colonization, tobacco crop had been all around dispersed outside its origins, all through South America, getting into Mesoamerica and Eastern Woodlands of the Northern America not later than ~300 BC. Some scholarly work suggests that some of the varieties have originated in the central of America or southern Mexico (TIMB. 2015).

N tabacum L. is an herbaceous perennial crop that is found and cultivated where other crops can be grown world wide (Gebhardt, 2016). It belongs to the *Solanaceae* family and some of the crops found within the *Solanaceae* family include tomatoes, eggplant and potatoes (Knapp *et al.,* 2002). It can grow to an average height of 1.5m but can even grow even up to 2m (Agyar *et al.,* 2013).

2.2 Economic significance of tobacco

Zimbabwe is among the biggest producers of tobacco leaf in Africa and in 2000 Zimbabwe was fourth-biggest producer of flue cured tobacco in the world, after China, Brazil and United States of America. Recently FAO (2016) indicated that Zimbabwe is the largest producer of tobacco in Africa and is now at 6th position in the whole world wide. FAO (2016) also indicated

that 2.6% of the world tobacco is produced in Zimbabwe. The 98% of the produced tobacco is exported, amongst these exports 42% of its tobacco is exported to China (Tianze, 2016). The crop normally accounts for more than 50 percent of agricultural exports, 30 percent of total exports and nearly 10 percent of GDP (Marowa and Rukuni, 2015).

More so a crop rotation scheme with tobacco crop included is very effective, it can be rotated with Khatambora grass a fodder crop though important at suppressing nematodes (Karavina and Mandumbu, 2012). It can likewise be grown on small scale to create money for on farm tasks and utilization. This crop is the most beneficial business venture in commercial agriculture, although some of the cash crops, including maize and even cotton are more crucial for all most every resettlement and communal farmer, tobacco is still an important crop that offers smallholder grower's unique opportunity for higher producer profits and exceptional marginal returns (ZTA, 2013: Scoones *et al.,* 2017).

In addition, a considerable rural employment is generated from tobacco production, more than 90 000 farmers are prioritising tobacco farming in Zimbabwe (Lown *et al.,* 2016). Also, employment is being created for workers involved in the tobacco research, marketing services and manufacturing. In Zimbabwe tobacco is also being grown on contract this is to boasts tobacco production as well as GDP (Scoones *et al.,* 2017). Moreover, production of tobacco is important for generating government revenue, this is through a levy system whereby tobacco producers and buyers are taxed a fixed percentage for the value of their crop sales as stated by Tobacco Industry and Marketing ACT (18:20) getting millions of dollars yearly (TIMB, 2016: National Budget Statement, 2017)

2.4 Production trends of flue cured tobacco.

In 1980 Zimbabwe start experiencing a drop in production and the number of production units this was prior to the country's independence, in the country there were duality of agriculture and government intervention was high in the Agriculture sector intending stimulating production (FAO, 2003). In the period of 1980 to 1990 the trend was quickly reversed such that by late 1980's the annual production was almost over 120 million kg. From 1990 – 2000 there was a steady increase due to a slow programme of land resettlement, also there were agricultural policies aimed to reduce inequality and support smallholder famers (Woras *et al*, 2008). The area planted to tobacco peaked in 1998 at about 92 000 ha and annual sales reached a record of 237 million kg in 2000. Also the period of 2001 – 2008, the period was dominated by land reform programme, therefore there was rapid increase in the potential tobacco production base.

During the period of 2009 to 2013 tobacco production in the country was characterized by rapid increase production, (ZTA, 2014). The numbers of tobacco active growers were increasing at the same trend up to 2014 in which a decline started to be noticed in 2015 and 2016 as shown on fig 2.1 (TIMB 2016), due to a some production constraints which deter farmers from growing tobacco. More so the area for tobacco production also started declining meaning production is being reduced. The 2015 and 2016 seasons were characterised by uprising of Potato Virus Y (PVY) and sore shin which re-emerged in float-beds system posing seedling loses up to 10% or more and reduction in seedling quality (TRB, 2016). The major challenge of sore shin is that it does not have recommended fungicide. Koga *et al.,* (2016) highlighted that it is more ideal to start with high quality tobacco seedlings as transplants for the farmer to obtain greater yields and quality. Good quality seedlings are as a result of high disease management and application of fertilisers in right amounts and on right time. Tobacco

seedlings ready for transplanting must be well hardened, with length of 15cm and a diameter of 5mm thick (Koga *et al.,* 2016). Hardening in float-bed system implies a well programmed fertilizer application schedule that ensures good hardening of seedlings before transplanting, achieved through nutrient stress (Seebold, 2011). Pythium root rot and sore shin affects the hardening process as these pathogenic diseases interferes with nutrient uptake and flow as by causing rotting of the seedling roots and the stem. However, there are issues with *R. solani* being resistant to Strobilurins and Azoxystrobins, so managing sore shin is becoming a challenge (Gonzalez *et al.*, 2011).

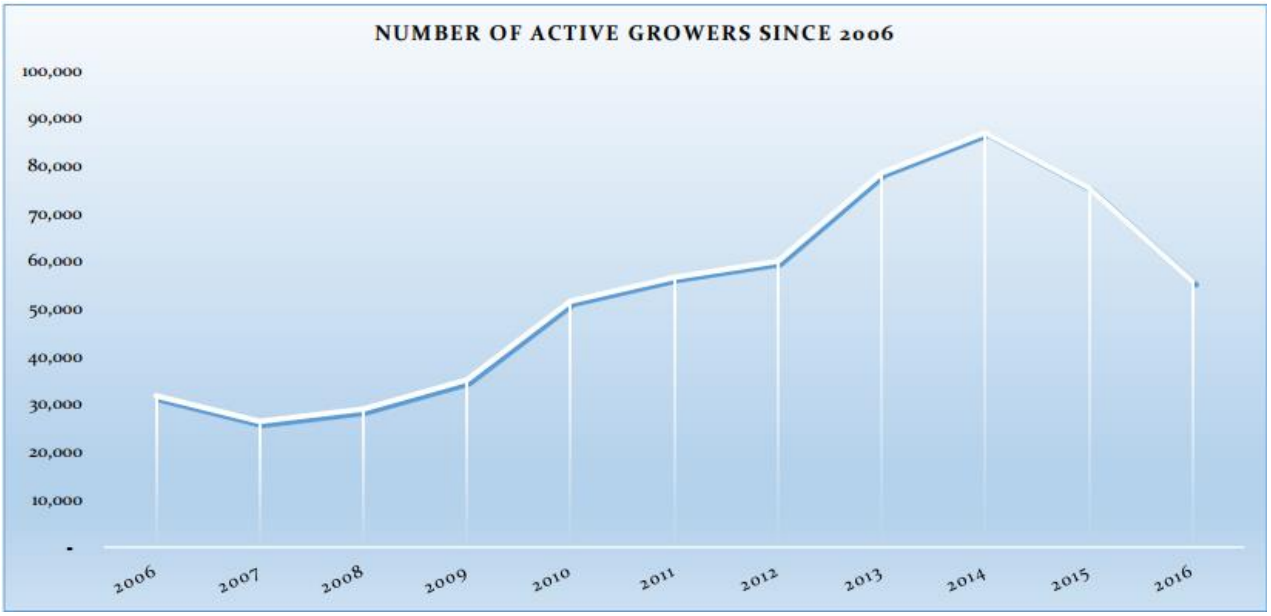

Fig 2.1: Trends of active tobacco farmers (TIMB, 2016)

2.3 Effects of sore shin on tobacco seedlings

Sore shin is caused by a fungal pathogen called *Rhizoctonia solani. R. solani* is a pathogen of economic importance in most of tobacco growing countries. *R solani* is a basidiomycete soil-borne fungal pathogen that can cause diseases in an extensive variety of plants around the world (Gonzalez M *et al.,* 2011). *Rhizoctonia solani* Kuhn (Teleomorph: *Thanatephorus cucumeris*

(Frank) Donk) is a soil borne pathogen responsible for severe damage on many crop species (Campion, 2003 cited in Seema, 2014). *R. solani* have mycelium which is big in diameter and is usually consistent diameter, and can be viewed as septate hyphae which branches at right angles under microscope. The hosts include all the members of solanacea, some of woody species and most of the grasses. This fungus can survive for many years in soil as mycelium, and also reproduce vegetatively by producing sclerotia, which makes the management of the disease using conventional means very difficult (Gutierrez, 1997).

R. solani have been reported as causal agent for diseases like damping-off, stem rot or stem lesions, root rots, foliar blight, sheath blight, and fruit decay on a wide diversity of very important agricultural plants and they either live saprophytic or as mycorhiza (Hyakumachi *et al*., 2005). Primary sources of *R solani* inoculum are infested trays used in the previous season. Sclerotia is formed in the trays in which the disease developed in the previous season, they are formed on styrofoams trays surfaces and also in the crevices, although basidiospores may be carried in air currents from other sources directly outside the seedbed facility (Pfeufer, 2018). *R. species* are placed into anastomosis groups (AGs) according to their capability of fusing their hyphae with other designated AGs members (Kiliçoğlu and Özkoç, 2010). The anastomosis subgroups have been divided on the basis of host range, cultural morphology and biochemical or molecular characteristics.

Tobacco can be infected by *R. solani* from AGs 1 to 5 causing damping-off, sore shin and target spots on tobacco leaves (Tarantino *et al*., 2007; Portieles *et al.,* 2017). In more recent research Garcia *et al.,* (2009) highlighted that BN Rhizoctonia from groups AG-G and AG-R were also reported for causing damping-off in tobacco. Damping-off of tobacco seedlings generally occurs on the early growth developmental stages of the seedlings (Pfeufer, 2018). Firstly, the disease appears at the base of seedling stems as water-soaked lesions. Afterwards,

the lesions dry up and become sunken with brown appearance and girdling of the plant may occur. Infected seedlings will eventually collapse and die (Gutierrez *et al.,* 1997. On young tobacco seedlings discolouration of the whole stem of affected seedlings occurs, chlorosis and necrotic of leaves will occur as well. On float beds system leaves in direct contact with the float trays will get infected and becomes necrotic (Gonzalez *et al.,* 2011).

If *R. Solani* inoculums are in contact with tobacco leaves, target sports will be formed within 48 hours after infection. The spots begin as water-soaked small, round spots with a diameter of about 2 to 3 mm. The lesions expand very fast during warm and high humid periods. Primary lesions where the spot originated will remain distinct even after the lesions have continued expand. Expanded lesions will appear almost transparent light green at first, with irregular shaped margins and will later form huge circular spots of concentric rings.

Sore shin is an important disease in tobacco nursery worldwide Lucas *et al.,* (1992). Sore shin, is a very common disease of tobacco seedlings, it causing stunting of the seedlings and uneven plant growth of greenhouse produced tobacco transplants (Lamondia, 2012). Sore shin development is severe in warm and humid weather (Gonzalez *et al.,* 2011). High moisture conditions present in greenhouses and high plant density of tobacco seedlings produced in float beds provide prolonged leaf wetness thus promoting succulent plant tissues that are conducive for disease development and quick spread of sore shin (Gutierrez *et al.,* 1997). The lesions formed on the plant stem vary from light brown to dark brown. Losses up to 10% of the tobacco seedlings may occur if the disease management is not done on time, also can cause delays in transplanting and reduced quality of tobacco transplants (Seema and Devaki, 2012). Poor quality tobacco transplants affects crop establishment and losses may continue to be incurred even after transplanting. Sore shin becomes more pronounced in the fields especially if infected transplants are used (Cole and Cole, 2008)

2.4 Chemical control of *R. solani* in float beds

Fungicides containing Mancozeb and Azoxystrobin are said to have a better control against sore shin **(**Kirk *et al.*, 2008). These fungicides can also be applied as preventative foliar spray for sore shin and target spot caused by this disease, some trials have been carried out for sore shin control using Azoxystrobin on tobacco and it proved to work (Lamondia, 2012). Some signs of phytotoxicity were noted on tobacco treated with Azoxystrobin 25 SC and disease inhibition decreased as time goes on, in the study carried out by (Lamondia, 2012) However some of these products are still undergoing investigation by Tobacco Research Board which deals with approval of chemicals to be used in tobacco (TRB, 2015).Currently in Zimbabwe the recommended chemical for sore shin disease is Triadamenol with triadamenol 25EC also known as Shavit is being used but the application should be done 48 hours before transplanting that is for the seedlings of above 12 weeks after sowing and Azoxystrobin 25 SC which is used for small seedlings but there should be issues of resistance as the problem of sore shin is re-emerging even treated with the recommended fungicide (Cole and Cole, 2008; TRB, 2015).

Good hygiene is crucial in the management sclerotia to minimise the likelihood of sore shin occurrence, this is achieved by thoroughly washing of the trays and soaking them in disinfectants like sodium hypochlorite, however the disease occurrence still remains a challenge as sclerotia the resting propagules of *Rhizoctonia solani* are formed in small crevices of the float trays (Gutierrez, 1997). Better control of sclerotia was being obtained by fumigation of recently used trays with methyl bromide (Gutierrez, 1997). Methyl bromide has been phased out due to its toxicity and lack of target specificity, more so it was also causing ozone depletion, so infection of *R. solani* from previously used trays is a threat to tobacco seedling production (Hong, 2014; Koga and Khuddu, 2016)..Moreover handling chemicals needs great caution so the use of eco-friendly methods that are less harmful to the ecosystem as well as users is

necessary, the methods include breeding of resistant cultivars and use of biological control method (Liu *et al.,* 2013).

2.5 Biological control of *R. solani*

Biological control is the use of antagonistic microorganisms to suppress other microorganisms in order to minimize their impact on economic and environmental practices (Seebold, 2011). Researches pertaining to biological controls have started for more than one hundred years ago, and have demonstrated that various phylogenetic microorganisms have antagonistic effect to diverse plant pathogens (McSpadden and Fravel, 2002). They have different modes of action for attacking or controlling other organisms including antibiosis, myco parasitism, competing for nutrients and space with phytophatogens, also they secretes antibiotic volatiles and diffusible metabolites which modify soil conditions promoting growth and plant defence mechanisms (Gajera *et al.,* 2013).

Biological disease control is very target specific, whatever antagonistic fungi introduced to plants will manage only the target pathogen population (Moore *et al.,* 2012). Unlike chemicals biological control of diseases have no adverse effects to the environment and on the human health (Charnley *et al.,* 2007). More so biological control agents are self-sustaining so once applied to the plant they keep on perpetuating, so they can be cost effective. The probability of host resistance development is minimised unlike chemicals (Naseby, 2000). Integrated disease management can be compatible with biological control method but limited with fungicides. Most biological control agents enhance plant nutrient uptake therefore outcompeting pathogen (Saba *et al.,* 2012). The biological control method is eco-compatible and environmentally friendly so it can be applied whenever possible with zero environmental pollution (Liu *et al.,* 2013).

Biological control agents for *R. solani* includes *Trichoderma species*, *Beuveria bassianna*, quiet a number of Bacillus species, actinomycetes and yeasts. Some of the BCAs has been standardised and commercialised and already on the market, examples include T77 which contains *Trichoderma harzianum*, Ecco-Tab which constitutes *Trichoderma asprillium, Bacillus subtalis, Beuveria bassianna*and *Bacillus cereus* (McSpadden and Fravel, 2002). Use of *Trichoderma harzianum* has been used in the control sore shin on tobacco seedling and it proved to be effective and increase in biomass was also noticed.

Combination of *T. Harzianum* with shavit was also effective in control and plant growth enhancement of tobacco seedlings (Cole and Zvenyika, 1988 in Cole and Cole, 2008). Sore shin control was achieved with improved tobacco seedling growth as well as increased root density on tobacco seedlings treated with *Bacillus subtalis* (Maketon *et al.,* 2008). Moreso yeasts are potential bio control agents for sore shin control and have been successfully used in the control of pythium root rot as well as sore shin on faba bean (Magri, 2018). More research must as well focus yeast species in control *R. solani* in different plants especially tobacco (Mahomoud, 2016).

2.6 Yeasts as biological control agents

Yeasts species are single celled eukaryotes, which belongs to the fungi kingdom (Ghosh, 2013). Yeasts are divided into two phyla the Ascomycota and the Basidiomycota and some lies into the group of Deuteromycetes. Some yeasts are dimorphic they become filamentous under some environmental conditions. These yeasts isolates are found in native soils, on fruit surfaces, on plant roots as mycorrhizae and as well as on plant leaves (de Melo Pereira *et al.*, 2015). Yeast species are found in association with many plants, on parts which include the phyllosphere, where they will be subjected to harsh environmental conditions (Lee *et al.,* 2017). Most

antagonistic yeasts belongs to the *Saccharomyces species, Albicans, Pichia, Sporobolomyces, Klyveromyces, Clavispora, Yarrowia, Debaryomyces, Candida,* and many others (de Melo Pereira *et al.,* 2015).

Generally yeasts are used in wine, brewing and bakery industries, carotenoids production and recombinant vaccines production (Ghosh, 2013). In recent trends yeasts are now being used as biological control agents against many diseases of plants (Kurtzman *et al.,* 2012). Loganathan (2004) in his study he observed that yeasts where very effective in controlling *Pythium aphaidermatum* on tobacco, yeast isolates were used as seed dressing for the control of damping off incited by *Pythium aphaidermatum*. In this study *Sacharomyces cerevisiae* also reduced the disease incidence by 10.81% which was comparable to Ridomil a chemical. Yeasts foliar application on tobacco plants confirmed that yeast of strains elicits induced plant resistance and defence priming, it controls powdery mildews as well as limited chances of Tobacco Mosaic Virus infection (Lee *et al.,* 2017).

Minimum of ten yeasts genera have been also used to control postharvest diseases, mostly on fruits. Successful usage of yeast isolates was obtained recently in the research that was conducted in Egypt for biological control of sore shin in common beans (Mahmoud, 2016) in this research three *Sacharomyces cerevisiae* yeasts were used and managed to reduce the disease severity by 60%. Suppression of *Rhizoctonia solani* on sugar beet by antagonistic yeasts was also successful in research done in United Arab Emirates this study included *C. valida* and *T. asahii* and these proved to be more effective in controlling of the disease, extensive root colonisation and improved plant growth was noticed on sugar beets (EI-Terability, 2003).

2.6.1 Mode of action of yeasts

According to Khaled (2006) little attention on yeasts as bio-control agents have been received in comparison to actinomycetes, filamentous fungal antagonists and bacterial. Mechanisms of action of these potential antagonistic yeasts in relation to fungal soil borne plant pathogens are expected to be the same with those involved with aerial plant parts pathogens (Khaled, 2006). Many yeasts species have been recognised as endophytes in most plants, with a small number of species promoting plant growth (Farrar *et al.*, 2014). Yeast species produce a number of biologically active compounds that are useful in stimulating plant development and growth as well as helping increase in plant productivity (Pérez-Montaño *et al.*, 2014).

2.6.1.1 Plant growth promoters

Most of the antagonistic entomopathogenic fungi play a significant role in plant growth promotion. Some yeasts isolates are good at producing plant growth regulators indicating the potential to exploit plants as more than biological control agents but also plant growth promoters (Curtis *et al.*, 2007). Some yeast species produces auxin which stimulates plant growth and improved nutrition up take, because they have the ability to produce indole-3-acetic acid (IAA)(Limtong *et al*., 2014). The desirable use of microbial auxins is the initiation and elongation of plant roots and stems (El-Tarabily and Sivasithamparam, 2006). More so a minimum of ten yeasts genera have been also used to control postharvest diseases, mostly on fruits. Suppression of fungal pathogens of foliage and fruits that are similar to those of soil borne fungal root pathogens, greatly suggests that yeasts have potential for the biological control of diseases incited by soil-borne. According to Boby *et al.*, (2008), application of yeasts isolates enhances Phosphorous and Nitrogen uptake by plants increasing fresh weight of plants, dry matter yields of plants, number of leaves and biomass

2.6.1.2 Antimicrobial action

Suppression of fungal pathogens of foliage and fruits that are similar to those of soil borne fungal root pathogens, greatly suggests that yeasts have potential for the biological control of diseases incited by soil-borne plant pathogens, as is evident in reports of certain yeasts in suppressing some soil-borne fungal plant pathogens (Berta *et al .*, 2005). Yeasts have the ability of multiplying rapidly, producing mycocins, phytohomones, vitamins and hydrolytic cell wall degrading enzymes (chitinases andβ-1,3-glucanases) helps in reducing phytopathogenic infection thus inducing resistance in plant tissues (Curtis *et al.*, 2007). Some yeast isolates have the capacity of producing volatile phytotoxins which causes plasma membrane damage, inhibition of cell cycle on first growth interphase (G1 arrest) and increase membrane permeability to ions of the pathogen cell (Marquina *et al.*, 2002).However not all yeasts isolates can be used as biological control agents, some yeasts isolates are saprophytic yeasts and these are normally found on the outer most part the plants, (El-Tarabily, 2004)

2.6.1.3 Competing for space and nutrients

Yeast usually ferment and depletes sugars, inhibiting growth of undesirable phytopathogens (Serena and Restuccia, 2015).Similarly, plant sugary root exudates are essential for enhancement of the yeasts antagonistic activities in relation to competition in colonising plant roots (EI-Terabily, 2003).

CHAPTER 3

3.0 Materials and Methods

3.1 Experimental Site

The research was conducted at Kutsaga Research Station also known as Tobacco Research Board (TRB), in 2016 from September up to November thus during tobacco nursery time. Kutsaga Research Station is stationed in Harare near the Harare international airport. The research station is loated in Natural Region II, which receives an average rainfall which varies from 800-1000mm annually and is received from November up to April. This site is at an altitude of 1 479 meters above sea level and is on 17º55`S latitude and 31º 08`E longitude (FAO, 2009). Mean temperatures experienced at this site are 32˚C and 18˚C in summer and winter

respectively (FAO, 2009). The experiment was set in a greenhouse where the temperature and relative humidity were controlled and maintained at 25˚C and 15% respectively.

3.2 Experimental Design

A completely randomised design with 7 treatments replicated 5 times was used thus giving a total of 35 experimental units. A plot was comprised of one float tray with 11 by 22 cells (242 cells), the tray encompasses 242 plants. The treatments were randomly allocated using the randomisation plan that was generated using excel. The treatments included 5 different yeasts isolates with the codes TY3, TY5, TY14, TY17 and TY18. Untreated control was included to act as negative control and a fungicide Azoxystrobin was also included to act as positive control. Treatments were given codes and described as shown in the table 3.1.

Table 3.1: Table of treatments

Treatments	Description of treatments
1	Untreated (negative control)
2	Azoxystrobin (positive control)
3	TY3 (yeast isolate)
4	TY5 (yeast isolate)
5	TY14 (yeast isolate)
6	TY17 (yeast isolate)
7	TY18 (yeast isolate)

3.3 Experimental Procedure

3.3.1 *Rhizoctonia solani* isolation and mass production

Tobacco seedlings showing sore shin symptoms were scouted from the trays that were brought to the Kutsaga plant clinic by farmers that were experiencing sore shin problem in their tobacco nurseries. The seedlings were in the 4th week after sowing according to the information given by the famers. The diseased tobacco plants were cut into small pieces of about 1cm using a scapel and the pieces were surface sterilised by soaking them in 1% Sodium hypochlorite for a minute and rinsed in sterile water three times and cultured into Selective Medium for Rhizoctonia (SMR). SMR only allows the growth of *Rhizoctonia solani* and inhibits growth of any other fungi therefore obtaining pure cultures.

SMR constitutes potassium phosphate 1g, magnesium sulphate 0,5g, potassium chloride 0,5g, ferrous sulphate 0.01g, Sodium Nitrate 0,2g, (Gallic Acid 0.4g, Dexon 0.09g, Chloramphenicol 0,05g, Streptomycin 0,5g) must be in stock solution so must be added in 50mls of water each and draw 0,1ml of each stock, Agar 20g and Water 1000 ml. The mycelium growing out of the plated pieces was then sub-cultured into Czapeks Agar, which is made from autoclaved Sodium Nitrate 3,0g, Potassium Phosphate, Magnesium sulphate 0,5g, Potassium Chloride 0.5g,

Ferrous Sulphate 0,01g, Sucrose 30g and 5g yeasts extract mixed in 1litre of water. The inoculated bottles containing Czaperks Agar was then incubated for two weeks, spores and mycelium were harvested and dried in oven at 30ºC. The dried inoculum was crushed into fine particles for easy spreading, when inoculating the tobacco seedlings.

3.3.2 Yeast isolates selection and mass production.

Preliminary bioassay *in vitro* testing

The bioassay in vitro testing was used to select best yeast isolates. Yeasts isolates were cultured in the same petri dishes with *R. solani* to investigate if the yeast isolates have the capability of inhibiting the growth of *R. solani in vitro* as shown in fig 1 (Eduardo and Mikkelsen, 2005).

Mass production of yeasts isolates

Nutrient yeasts broth (NYB) which constituted of 20g glucose, 20g peptone and 10g yeast extract per litre were used in this experiment for yeast mass production. The mixture was autoclaved for 15 minutes and was left to cool down. Yeasts from the incubated petri dishes were used to inoculate the NYB each isolate in each flask. These yeasts isolates were isolated from the phyloderm of the health tobacco plants. Five isolates were used and these are TY3, TY5, TY14, TY17 and TY18, the cultures where incubated in a rotary shaker with rotation speed of 120rpm and temperature of 28°C for 48 hours. The cultures were removed from the rotary shaker and where added into centrifuge tubes 10mls per round and were centrifuged at 5000 rounds per minute for 5 minutes. The centrifuged culture will have a pellet of yeasts at the bottom of the centrifuge tube and supernatant was removed, the pellet was then suspended in 1ml of sterile distilled water.

The yeast cells collected were diluted using the serial dilution method in which by 1ml of concentrated yeasts cells was taken and added to 9ml sterile water forming a solution with 10^{-1}cfu concentration, the solution with 10^{-1}cfu concentration was well shaken and 1ml was extracted from it and was added to 9mls of sterile distilled water forming10^{-2}cfu (Reynolds. 2005). This procedure was repeated up to 10^{-9}cfu and the concentrations of 10^{-5}cfu to 10^{-9}cfu was cultured on to Yeast Potato Dextrose Agar (YPDA) by taking 1ml from each concentration and add onto Petri-dishes with YPDA and incubated till the yeasts colons are formed. The colons were count and concentration was established as colon forming units (cfu) (Table 3.2).

Table 3.2: Yeasts culture concentrations

Yeasts Isolate	**Concentration**
TY3	7.18×10^7 cfu/ml
TY5	6.05×10^7 cfu/ml
TY14	6.84×10^7 cfu/ml
TY17	6.10×10^7cfu/ml
TY18	8.42×10^7 cfu/ml

The concentrations were adjusted to 6.05×10^7 cfu such that when applying the colony forming units will be the same using the formula: M1V1=M2V2 where M is concentration and V is volume, (Chabay 1998)

3.3. Trial management.

Thirty five ponds of $0.26m^2$ were made in the plant pathology greenhouse at Kutsaga Research Station. They were made by arranging bricks in rectangular form with inner dimensions of $0.38m$ by $0.7m$ and a black polystyrene plastic was laid into the pond as well on the walls such that they will act as water reservoir for the float trays. The ponds were half filled with water. Float trays with 242 cells were filled with decomposed pine bark (Gromix plus) with a corrected pH of 5.5. KRK 26R raw seed was seeded on the trays and the trays were placed into ponds containing water. After one week first float fertiliser was applied at a rate of 14mls per tray/pond, second was applied after three weeks and 4th after 5 weeks at the rate of 28mls/tray and 42mls/tray respectively. All other activities like checking weeds, controlling algae and pond water topping were done when necessary. Plugging and transfer of tobacco seedlings was done when the seedlings were at three weeks after sowing.

3.4 Inoculation and treatment application

Yeasts and Azoxystrobin a fungicide were applied to the trays on the third week after sowing this was prior to the 4th week at which sore shin was being noticed on the tobacco seedlings. 1ml of yeast culture was added to each cell using a micropipette until the whole tray was done, they were being done as scheduled on the treatment randomisation plan, as for Azoxystrobin it was being applied at a rate of 0.2ml per litre per tray. The untreated control was left without any application done. On the fourth week *Rhizoctonia solani* inoculums was added to the float trays with seedlings at the rate of 0.7g of dried mycelium per tray. The 0.7g of dried mycelium was mixed in 1 litre of borehole water and applied as drench application using watering cans. Use of borehole water will ensures that there is absence of pythium spores which might bring almost similar symptoms with that of sore shin on tobacco seedlings.

3.5 Assessment and data collection.

Two weeks after disease inoculation assessment was done, this was at 6 Weeks After Sowing (WAS). Ten seedlings were assessed per tray these ten seedlings were randomly sampled using the drawing card method from each tray. The seedlings were uprooted and washed to remove growth media on the seedlings and excess moisture on these seedlings was wiped using bloating paper. The assessment was done for all plots once and the observable and measurable parameters were observed, measured and recorded.

3.5.1 Sore shin severity

Sore shin disease severity was measured using Plant Pathology scale for sore shin damage of 0-5 at six weeks after sowing (Dimbi and Rukuni, 2014), skilled personnel were included during assessment to ensure correct observations.

Table 3.3 Sore shin severity scale

score	Percent damage	Description
0	0%	Absolutely no damage on the stem and roots
1	0-1%	Slight damage on stem and roots slightly decoloured
2	1.1-10%	Two lesions on stem and slight decolouration of roots
3	11-25%	Several lesions on stem, about one third roots discoloured
4	> 26%	Extensive lesions on stem, remains of root discoloured
5	100%	Plant dead

3.5.2 Measurements of plant growth parameters

After disease assessment the same ten seedlings were taken for growth parameters measurements, the parameters that were taken were root fresh weight, shoot fresh weight, root length, shoot height, plant fresh weight and plant dry weight.

3.5.2.1Root length

Root length was observed by measuring the ten tobacco seedling root length from the root crown up to the longest root using a ruler and the values were averaged.

3.5.2.2 Shoot length

Shoot length of ten tobacco seedlings were assessed using was using a ruler, measuring from the crown up to pith of the seedling and averaged

3.5.2.3 Root fresh weight

Root fresh weight of ten tobacco seedlings were obtained by cutting the roots at the crown, weighed on a scale and the average was calculated

3.5.2.3 Shoot fresh weight

Ten tobacco seedling shoot fresh weight was weighed on a scale by first removing the roots using the microtone, this was achieved by cutting the plant on the crown to separate roots from the shoots and averaged

3.5.2.4 Dry weight

Dry weight was obtained by weighing the ten tobacco seedlings that were left in khaki bags in an oven with temperature of 40ºC and an air liner for fair distribution of heat for two days and the mean was calculated.

3.6 Data analysis

All data collected were analysed using one way analysis of variance (ANOVA). Tests for variances, homogeneity and normality were also done. Genstat 18th edition was the statistical tool used for this analysis of data. Fisher's unprotected test was used to separate the treatments means at 5% significant level. Scores were transformed using the square root transformation before analysis.

Chapter 4

4.0 Results

4.1 Effects of yeasts isolates on the sore shin severity of tobacco seedlings inoculated with *R. solani*.

There was a significant difference (P<0.05) on the effects of yeast isolates on the severity sore shin on the tobacco seedlings inoculated with *R. solani*. Azoxystrobin the positive control scored the least score whilst the highest disease damage was observed on the untreated control that is the negative control. Disease score recorded on tobacco seedlings treated with yeast isolate TY18 was statistically similar with the untreated control thus the negative control, but amongst TY3, TY5, TY14, and TY17 there were no statistical differences.

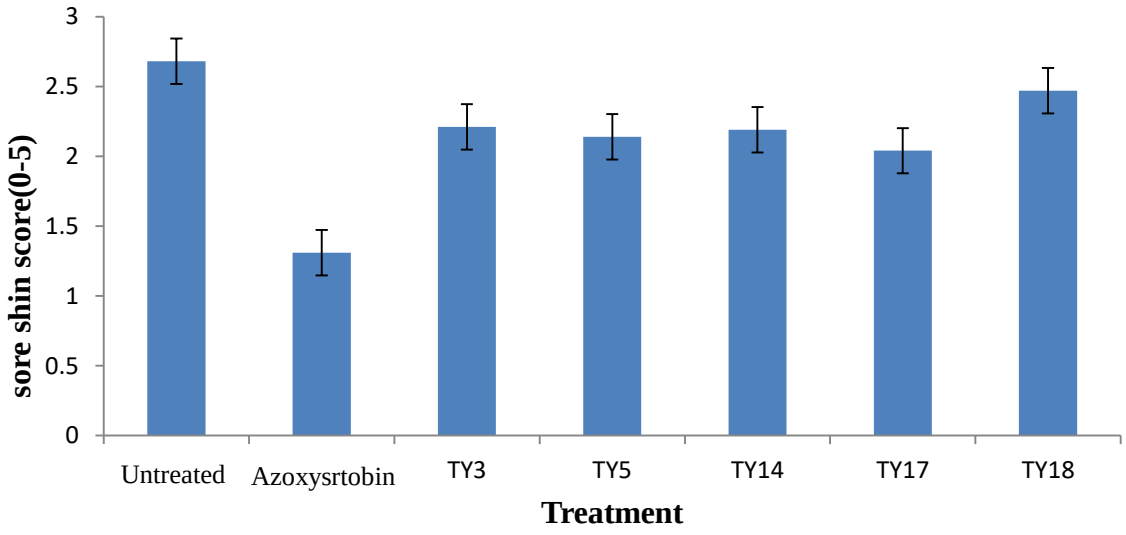

Figure 4.1 Effects of yeasts isolates on the sore shin severity of tobacco seedlings inoculated with *R. solani*

4.2 Effect of yeast isolates on growth parameters of tobacco seedlings inoculated with *R. solani*.

4.2.1 Effect of yeast isolates on the shoot fresh weight of tobacco seedlings inoculated with *R. solani*

There was a significant difference (P<0.05) on the effects of yeast isolates on the seedling shoot fresh weight. TY3 recorded the highest shoot fresh weight seedlings with average of 3.76g per tobacco seedling, the least weight was recorded on the untreated control with 1.386g average shoot fresh weight of one seedling. However, among the yeasts isolates TY3 was significantly different from other isolates which were statistically similar, TY3 and TY5 were statistically similar to the positive control.

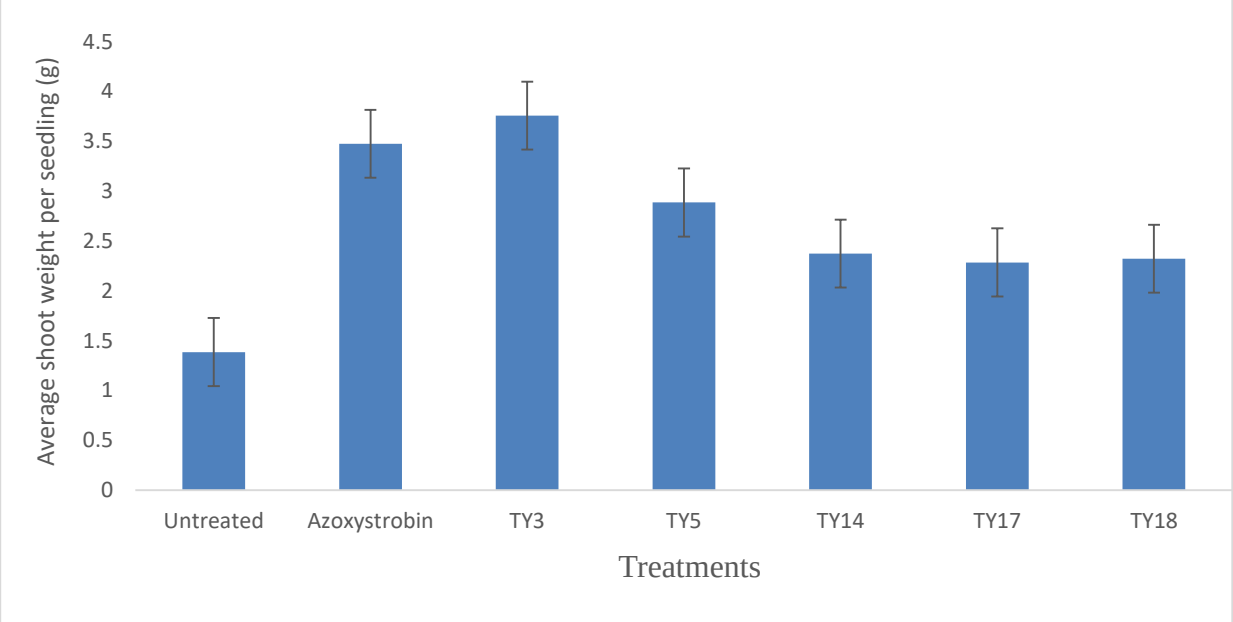

Figure 4.2.1: Effect of yeasts on shoot fresh weight of tobacco seedlings inoculated with *R. solani*

4.2.2: Effects of yeasts isolates on root fresh weight of tobacco seedlings inoculated with *R. solani*

There was a significant difference (P<0.05) on the effects of yeast isolates on the seedling root fresh weight. TY5 recorded the highest average root fresh weight per seedling of 0.81g, the least root fresh weight was recorded on the yeast isolate TY18 with 0.21g average root fresh weight per seedlings. The weight recorded in TY18 was even lower than that of the negative control though they were statistically similar. Amongst yeasts isolates TY3, TY14 and TY18 were similar statistically whereas TY5 was statistically different from other isolates.

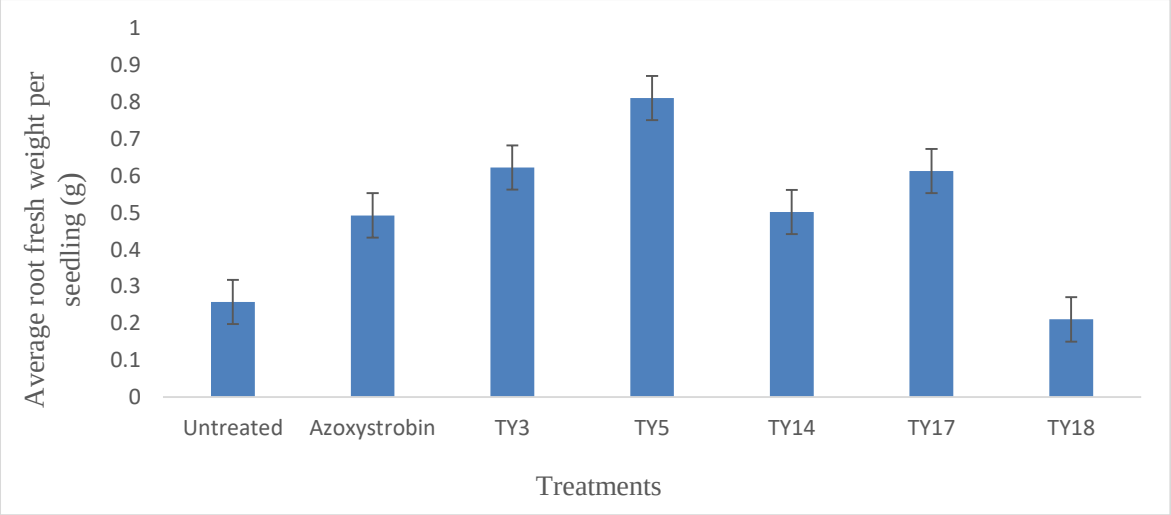

Figure 4.3.2: Effects of yeasts on root fresh weight of tobacco seedlings inoculated with *R. solani*

4.4.3 Effects of yeasts isolates tobacco seedling dry weight of tobacco seedlings inoculated with *R. solani*

There was a significant difference (P<0.05) on the effects of yeast isolates on the dry weight of *Rhizoctonia solani* inoculated tobacco seedlings, TY3 having the highest seedling dry weight with 0.388g average weight per seedling and the least weight was recorded on the

untreated control which had 0.142g average dry weight per seedling. Amongst the yeasts isolates TY3 and TY5 were statically similar whilst TY14, TY17 and TY18 were also statistically the same.

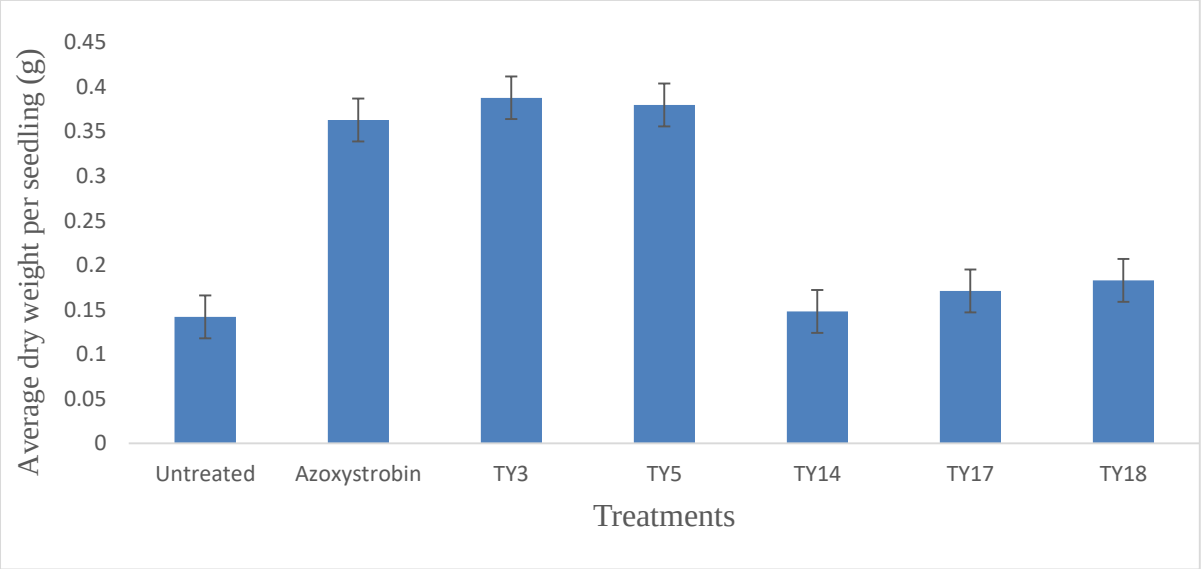

Figure 4.4: Effects of yeast isolates tobacco seedling dry weight of tobacco seedlings inoculated with *R. solani*

Chapter 5

5.0 Discussion

5.1 Effects of different yeast isolates on sore shin severity on tobacco seedlings inoculated with *R. solani*

The effects of different yeast isolates on sore shin establishment and severity on tobacco seedlings inoculated with *R. solani* were signifiantly distinct from those untreated plots, though TY 18 was not statistically different from the untreated control. TY3, TY5, TY14 and TY17 managed to keep the disease establishment and severity low, though not effective as Azoxystrobin. TY3, TY5, TY14 and TY17 were statistically similar on managing sore shin establishment and progression on tobacco seedlings, the score for severity of sore shin recorded on tobacco seedlings treated with these yeasts isolates was much lower compared to the untreated control. This can be attributed to the ability of yeast isolates to release proteinaceous mycotocins that are lethal to phytopathogens therefore inhibiting the establishment and growth of *R. solani* (Curtis *et al.,* 2007; Mahmoud, 2016). Also it can be due to the tendency of some yeast isolates to feed on stimulants root exudates that triggers *R. solani* germination thus reducing disease establishment as well as progression (Basseto *et al.,* 2007).

The recorded decrease in sore shin severity maybe as a result of direct fusion of yeasts isolates with filamentous mycelium of pathogenic fungi and feed on these thereby reducing the impact of *Rhizoctonia solani* on tobacco seedlings (Serena and Restuccia, 2015). To add on, the results obtained on yeast applied plots might be the yeasts capacity of producing cell wall degrading enzymes (chitinases andβ-1,3-glucanases) which is effective at degrading *R. solani* cells subjecting it to excessive water intake or losing water (Elwakil *et al.,* 2009). Khaled, (2006) alluded that some yeasts mode of action against pathogenic fungi is similar for both folia

pathogens as well as root pathogens. Lee *et al.*, (2017) noticed that yeast foliar applications on tobacco plants confirmed that some yeast strains elicits induced plant resistance and defence priming so is yeast isolates to *R. solani* leading to results that were obtained. Yeasts isolates also out competes other microorganisms on Carbon take up thus suppressing *Rhizoctonia solani* germination and establishment chances so can explain the why TY3, TY5, TY14 and TY17 reduced the sore shin severity (Serena and Restuccia, 2015). However, TY18 did not show virulence against *Rhizoctonia solani* as compared to other yeast isolates though it proved to inhibit *R. solani* growth *in vitro*, similar scenario was observed by El-Tarabily, (2004) in which some yeast isolates failed to colonise plant roots and some successfully colonised the plant roots but were attacked by the *R. solani* antibioses resulting in reduced efficacy of yeast isolates as BCAs against *R. solani* so was the same with the results that were observed on the plots treated with TY18. More so the reduced efficacy of TY18 in controlling sore shin was as a result of saprophytic yeasts isolates which feeds on the plants causing undesirable effects on tobacco seedlings (El-Tarabily, 2004). Similar results for failure of yeast isolates to control fungal diseases were obtained by El-Tarabily and Sivasithamparam, (2006) in their study.

5.2 Effects of different yeast isolates on the growth parameters of tobacco seedlings inoculated with *R. solani*

5.2.1 Effect of yeast isolates on the shoot fresh weight of tobacco seedlings inoculated with *R. solani*

Application of yeasts isolates on tobacco seedlings that were inoculated with *R. solani* significantly increased the fresh and dry weight of tobacco seedlings but did not significantly increase the root and shoot length. TY3 and TY5 recorded the highest shoot fresh weight in comparison with all other treatment, TY14, TY17 and TY18 also increased the shoot fresh

weight of tobacco seedling comparing with the untreated control. This increase in shoot fresh weight was caused by yeasts isolates ability to produce plant growth regulators like indole-3-acetic acid and cytokinins indicating the potential to exploit plants as more than biological control agents but also plant growth promoters and this should significantly increase the fresh weight of tobacco seedling shoots weight (Curtis *et al.,* 2007). This increase in shoot weight was as an attribute of yeast isolates ability of solubilising phosphorous and enhancing Nitrogen uptake thereby giving room for efficient use of fertilisers and assimilation rate, thus increasing shoot fresh weight giving them the potential to be used as biological fertilisers (El-Tarabily and Sivasithamparam, 2006; Boby *et al*., 2008).

Improved shoot fresh weight of tobacco seedlings treated with yeast isolates was as a result of increased production of Chlorophyll A, Chlorophyll B and carotenoids which are the major components of photosynthetic pigments which some yeast isolates are believed to induce thereby increasing photosynthesis rate, similarly in the study where yeast isolates were applied on tomatoes, there was significant increase in plant shoot weight (Abdo *et al*., 2012). More so the increase in weight was due to the ability of some yeasts isolates to produce greater amounts of phytohormones like auxins, vitamins, amino acids, minerals and enzymes, (Sakr *et al*., 2012). These phytohormones indicates stimulatory effects on the cell division, cell enlargements, protein synthesis and nucleic acid synthesis (Neseim *et al*., 2014).

5.2.2: Effects of yeasts isolates on root fresh weight of tobacco seedlings inoculated with *R. solani*

In this study TY3, TY5, TY14 and TY18 significantly increased root weight of tobacco seedlings but TY18 did not perform like other yeast isolates, the increase in root fresh weight can be credited to the successful colonization of plant roots with yeasts isolates which induces rapid cell division, cell elongation by production of cytokinins, auxins and enhanced storage

of photosynthates (Werne *et al*., 2001; Ignatova *et al.*, 2015). Boby *et al.*, (2008) also noticed that cowpea plants that were inoculated with yeasts isolates have improved root weight and root length in comparison with the untreated control. TY18 did not successfully increased seedling root fresh weight in comparison to others as well as to the untreated control this was because plots treated with this isolate exhibited greater damage on the roots which was caused by *R. solani*. Sore shin damages and affects the storage of photosynthates in the roots thereby reducing the root weight of tobacco seedlings (Berta *et al.*, 2005).

5.2.3 Effects of yeasts isolates on dry weight of tobacco seedlings inoculated with *R. solani*

TY3 and TY5 significantly increased the dry weight of tobacco seedlings whilst the rest were statistically similar with the untreated control. The increase in dry weight of tobacco seedlings can be ascribed to increased production of Chlorophyll A, Chlorophyll B and carotenoids the major components of photosynthetic pigments which some yeast isolates believed are to induce (Werne *et al*., 2001). The increase in photosynthetic pigments results in improved photosynthesis rate as these pigments are responsible for photosynthesis and reduced rate of leaf senescence so it means there is increased dry mater accumulation, so explaining the results that were obtained in this study (Jin *et al.*, 2016). In Dry mater accumulation is also as a result of increased growth of tobacco seedlings which is induced by cytokinin and auxins which are not produced at the same level with yeast isolates (Abdo *et al*., 2012).

Chapter 6

6.0 Conclusion and Recommendations.

6.1 Conclusion

Conclusion can be drawn that yeast isolates TY3, TY5, TY14 and TY17 are capable reducing or controlling sore shin on tobacco seedlings, however among these yeast isolates TY18 did not show antagonism against *R. solani* as compared to others and on the negative control. It can be concluded that yeasts isolates applied on tobacco seedlings increases fresh shoot weight of tobacco seedlings. Yeasts (TY3, TY5, TY14 and TY17) application to tobacco seedlings also increased root fresh weight of tobacco seedlings giving yeast isolates the potential to be used as bio-fertilisers, but TY18 cannot lead to increase in root fresh weight. More so the application of yeasts isolates improved the dry matter content of the tobacco seedlings. However it can be concluded that yeasts isolates will not result in differences in shoot height and root length.

6.2 Recommendations

Repetition of the experiment to ascertain if the yeast effects on sore shin severity and growth parameters will remain constant is recommended, also repeating the experiment on different environment to see which suits the yeasts most. The yeast cells that were used to inoculate the seedlings does not have food carriers to feed on so it is recommended to use of food carriers such that when they got applied they will not struggle to survive before colonising the plants. Characterisation of these yeast isolates is strongly recommended.

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List of appendices

Appendix 1 Analysis of variance of sore shin severity on tobacco seedlings inoculated with R. solani.

Variate: sore shin score

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	6	5.4999	0.9167	6.30	<.001
Residual	28	4.0727	0.1455		
Total	34	9.5726			

Appendix 2 Analysis of variance of shoot fresh weight of tobacco seedlings inoculated with *R. solani.*

Variate: shoot_fresh

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	6	199.479	32.246	11.56	<.001
Residual	28	78.121	2.79		
Total	34	277.6			

Appendix 3 Analysis of variance of root fresh weight of tobacco seedlings inoculated with *R. solani*.

Variate: root_fresh

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	6	13.4766	2.2461	21.72	<.001
Residual	28	2.8962	0.1034		
Total	34	16.3729			

Appendix 4 Analysis of variance of dry weight of tobacco seedlings inoculated with *R. solani.*

Variate: plant_dry weight

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	6	4.1234	0.6872	60.28	<.001
Residual	28	0.3192	0.0114		
Total	34	0.4426			