An investigation of the susceptibility of three *Acacia* species to *Viscum articulatum* infection

By

MELODY MANDAZA

R157449H

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Department of Applied Biosciences and Biotechnology

Faculty of Science and Technology

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Approval form

This is to certify that the dissertation titled "An investigation of the susceptibility of three *Acacia* species to *Viscum articulatum* infection", submitted in partial fulfillment of the requirements for Bachelor of Science Honors Degree in Applied Biosciences and Biotechnology at Midlands State University, is a record of the original research carried out by MELODY MANDAZA R157449H under my supervision. No part of the dissertation has been submitted for any other degree or diploma.

The assistance received during the course of this research has been duly acknowledged. Therefore, I recommend that I will be accepted as fulfilling the dissertation requirements.

Name of supervisor MR C MAKAKA

Signature ……………………………………...

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ABSTRACT

Acacia species are indigenous trees which are of great importance in Zimbabwe. They can be used for various purposes for example making pulp and tannin, as cattle feed, as fuel (charcoal and wood) and for timber production which can be used for construction purposes. However, *Acacia* species are being affected by *Viscum articulatum* in Zimbabwe especially in Greystone Park, Mazowe district, Glen Lorne and some parts of Midlands province. At present, *Acacia* trees affected by the *Viscum articulatum* are pruned to remove all the infected parts. The removed parts are buried to control the parasite and in heavily infested cases they are cut down and burnt as a way of controlling the *Viscum articulatum* parasite from spreading. A study was carried out at Forestry Commission to investigate the susceptibility of three *Acacia* species (*Acacia sieberiana, Acacia polyacantha* and *Acacia nilotica*) to *Viscum articulatum* infection between mid-January 2018 and mid-February 2019. Samples of *Viscum articulatum* parasite were collected from Hakuna Matata Primary School and Gaydon road in Greystone park form *Acacia* hosts. Inoculation of parasite was done by watering with infected water and by means of air layering for a period of four months. Five seedlings of each of the three *Acacia* species were used. Susceptibility of the species to the infection was determined by the development of the mistletoe and growth retardation of the seedlings. It was observed that *Acacia sieberiana* and *Acacia polyacantha* showed greater retardation by the infection especially on the seedlings which were inoculated by air layering compared to watering. *Acacia sieberiana* which was inoculated by means of air layering developed mistletoe which grew up to a mean height of 19 µm and recorded highest proportion (100%) of seedlings showing signs of infection after a four months period. The controls, which were the untreated seedlings, were not affected. Findings from this study shows that *Acacia nilotica* is less susceptible to *Viscum articulatum* whilst *Acacia sieberiana* and *Acacia polyacantha* are susceptible, therefore growing *Acacia nilotica* which is less susceptible to the infection can be a method of choice as it is environmental friendly other than making use of chemicals.

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DEDICATION

I dedicate this work to Getrude Ndarowa, a huge friend of mine with whom I started this journey in the life sciences.

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CHAPTER ONE

INTRODUCTION

1.1 Background

Acacia trees are a large genus of shrubs and trees in the subfamily Mimosoideae of the pea family Fabaceae commonly known as wattles. *Acacia* species are different in terms of their morphological appearance and are used for different purposes in both economic and medicinal sector. There are many species of *Acacia*s which include *Acacia raddiana*, *Acacia sieberiana*, *Acacia polyacantha* and *Acacia nilotica* (Pedley, 2002).

The *Acacia* tree is used for various purposes, for instance, in ancient Egypt, it was used as an ointment made from the ground leaves of the plant to treat haemorrhoids (Kulkarni and Kumbhojkar, 2002). A number of species, most notably *Acacia mangium* (hickory wattle), *Acacia mearnsii* (black wattle) and *Acacia saligna* (coojong) are economically important and are planted worldwide for wood products, tannin, firewood and fodder. *Acacia melanoxylon* (black wood) and *Acacia aneura* (mulga) supply some of the most attractive timbers in the genus (Ellesmore, 2002).

In Zimbabwe, farmers who focus on growing *Acacia* trees use them for various purposes in agriculture, for example, as fodder (animal feed). The pods are specifically used as cattle feed. In the economic sector, black wattle bark is used for tannin production for further manufacturing of waterproof adhesives. Seeds of some of the *Acacia* species contain as much as 25% more protein than common cereals which store well for long periods due to the hard seed coats. In addition to utilizing the edible seed and gum, people employ the timber for implements, weapons, fuel (wood and charcoal) and musical instruments (Dean, Midgley and Stock, 1994). They are also important sources of building material, fibre, rope, honey and are also used in agro-forestry and for aesthetic purposes (Midgley and Bond, 2001; Van Rooyen, Bezuidenhout and de Kock, 2001; Venter and Venter, 2002). In addition, they play a significant role in nitrogen fixation (Midgley and Bond, 2001; Brockwell, Searle, Jeavons and Waayers, 2005).

A number of *Acacia* species form the basis for multi-million dollar commercial forestry operations, providing pulp for paper and viscose production in various continents mainly in

Australia (Brown, 2000; Midgley and Turnbull, 2003). In this regard, plantations have been established in Africa,

Indonesia, Malaysia, South America and the Indian continent (Brown, 2000; Midgley and Turnbull, 2003). In Africa, the majority of plantations can be found in South Africa and the Mediterranean countries of North Africa (Brown, 2000). They are mainly planted for the production of paper and rayon, fuel wood, tannins, human and animal food, for land rehabilitation, conservation and as windbreaks (Bakshi, 1976; Midgley and Turnbull, 2003).

Large-scale plantations of Australian *Acacia* species in Africa were established during the 1800's up until the early/mid 1900's (Sherry, 1971). Many *Acacia* species were introduced into southern Africa over the years for commercial purposes (Norton and Carpenter, 1998). Australian *Acacia* species are particularly popular for planting, due to their adaptability, fast growth and ease of germination especially *Acacia polyacantha*. It is also known to produce high quality timber and tannins (Bakshi, 1976; Midgley and Turnbull, 2003).

Acacia species are susceptible to *Viscum articulatum* infection as the parasite displays a considerable variation in host-specificity. Susceptibility is the extent to which an ecological community would suffer from a threatening factor if exposed, without regard to the likelihood of exposure (Birkmann, 2012). Therefore, not all *Acacia* species shows susceptibility to *Viscum articulatum.* Different *Acacia* species, for example, *Acacia sieberiana* are attacked by the parasite of the *Viscum* species in Zimbabwe and an attack by *Viscum articulatum* will result in reduced growth and fewer flowers on the twigs carrying the mistletoe. In cases of heavy *Viscum articulatum* infection, the *Acacia* species growth is reduced, leaves turn brownish in colour and start to fall off until the tree dies completely.

1.2 Problem statement

Acacia species are indigenous trees used globally for timber and as wind breaks. Their shortage is a problem to farmers, who, of late use *Acacia* species as fodder since the pods are highly palatable to cattle. It can also be used to give strength to soil, therefore their shortage can result in soil erosion and siltation.

Acacia sieberiana are being affected by *Viscum articulatum* parasites in Zimbabwe. The areas mostly affected include Greystone Park, Mazowe district, Glen Lorne and some parts of the

Midlands province. High infestation of *Viscum articulatum* can lead to extinction of *Acacia* species because it decreases growth of the trees since the infection can spread and the trees eventually die. Falling off of leaves and turning brown of the tree take place as the mistletoe (parasite) use up nutrients for the trees for its own growth, therefore the trees do not grow to expected height due to nutrient deficiencies.

1.3 Justification

There are great differences in the host specificity of different parasitic plants. *Viscum articulatum* infection is selective in terms of its host and a number of hard wood species are accepted as hosts. Even among parasites belonging to the same genus, there is often great variation in their host specificity. However, there is also often great uncertainty about the observed host specificity. The uncertainty has several reasons but in particular, there are problems of interpreting the negative observations. If a certain parasite does not grow on a certain host species, the reason may be other than tissue incompatibility (Cooper *et al*., 2012). Thus, this study focuses on helping farmers or even individuals growing *Acacia* trees, so that they have knowledge of which species are less susceptible to *Viscum articulatum* parasite. Hence there will be massive planting of *Acacia* trees without hesitation because farmers will specialize on the species that are less susceptible to *Viscum articulatum* parasite.

At present, *Acacia* species infected by the *Viscum articulatum* are pruned to remove all the infected parts and burnt or buried to control the parasite and in heavily infested cases the tree is cut down and burnt as a way of controlling the *Viscum articulatum* parasite from spreading. Hence, growing of *Acacia* species resistant to *Viscum articulatum* is a possible management strategy which can avoid deforestation.

The demand for timber in Zimbabwe continues to grow, and *Acacia* species are a major source of tannin production, building material, fiber, rope and honey foraging. More importantly and of particular concern of late, is the continued use of fuel (firewood and charcoal) for tobacco curing by newly resettled farmers and for domestic purposes. *Acacia* trees are considered to be ideal to address the demand for pulp production because they are adaptable to a wide range of sites. Commercial companies such as Forestry Commission in Zimbabwe also encourage the establishment of *Acacia* woodlots as an alternative supply of fodder, timber and for enhancing biodiversity. However, *Viscum articulatum* poses a threat to *Acacia* species and the establishment of such woodlots.

1.4 Objectives

1.4.1 Main objective;

- to identify the most preferred host of *Viscum articulatum* amongst three *Acacia* species (*Acacia sieberiana*, *Acacia nilotica* and *Acacia polyacanatha*).

1.4.2 Specific objectives;

- to determine the *Acacia* species which are susceptible to the mistletoe,
- to determine the extent of growth retardation in the different *Acacia* species, and
- to determine the most prevalent way of the spread of the parasite.

CHAPTER 2

LITERATURE REVIEW

2.1 History of *Acacia* species

Acacia was formally adopted by Miller in 1754 in a paper which contained descriptions of 24 African and American species, although there was no attempt to divide these into groups. Miller's generic concepts were very broad such that a number of his species were no longer referable to *Acacia* in its current sense (Maslin, Orchard and West, 2003). Prior to Miller, the name *Acacia* had been used in pre-Linnean literature (Ross, 1980). Linnaeus had placed most Mimosoids known to him in the genus *Mimosa* and of the 39 species that he treated, only six are currently recognised as belonging to that genus, 14 to *Acacia* and the remainder distributed across eleven different genera (Maslin, Orchard and West, 2003).

Two of the species designated by Linnaeus were *Mimosa scorpioides* and *Mimosa nilotica*, these taxa were subsequently transferred to *Acacia* and are now considered conspecific with *Acacia scorpioides* generally regarded as the type of the genus (Maslin, Orchard and West, 2003). Miller later adopted Linnaeus' broad concept of *Mimosa* and the name *Acacia* fell into disuse (Pedley, 1987).

Acacia trees are commonly known as the wattles which are a large genus of shrubs and trees in the subfamily Mimosoideae of the pea family Fabaceae. Initially it comprised a group of plant species native to Africa and Australia, with the first species *Acacia nilotica* described by Linnaeus. Controversy erupted in the early 2000s when it became evident that the genus was not monophyletic, and that several divergent lineages needed to be placed in separate genera (Maslin, Orchard and West, 2003). It turned out that one lineage comprising over 900 species mainly native to Australia was not closely related to the mainly African lineage that contained *Acacia nilotica* the first and type species (Maslin, Orchard and West, 2003). This meant that the Australian lineage has the most number of species need to be renamed. Botanist Les Pedley named this group Racosperma, which was inconsistently adopted (Pedley, 1987).

2.1.1 Acacia sieberiana

Acacia sieberiana is commonly known as the paperbark thorn or paperbark acacia. It is native to southern Africa and introduced into Pakistan later on (Brown *et al.,* 2006). It varies from 3 to 25m in height, with a trunk diameter of 0.6 to 1.8m. *Acacia sieberana* is not listed as being a threatened species (Kyalangalilwa *et al*., 2013).

Acacia sieberiana often has a flattened canopy when it is medium or large in sized tree. Its bark is grey in colour, rough and it peels to reveal a yellowish layer underneath. *Acacia sieberiana* are characterised by their thorns which are paired at the nodes, straight and older trees appear thornless which differentiate them with other species (Brown *et al.,* 2006). The leaves of *Acacia sieberiana* are compound large with 8-20 pairs of pinnae and the leaflets are pale green or yellowish-green in appearance. The flowers in axillary are spherical heads and creamy-white in colour. The pods of *Acacia sieberiana* are large, thick and woody. They are usually found in wooded grassland and woodland, on floodplains and along rivers. They are distributed worldwide, and they are widespread in Africa from tropical West Africa to Sudan and Ethiopia and southwards through East Africa to Namibia, Zimbabwe and South Africa (Brown *et al.,* 2006). The flowers of *Acacia sieberiana* attract a wide array of insects to the trees and these in turn lure a range of insect-eating birds into the vast canopies. Wood hoopoes and woodpeckers forage in the soft flaky bark whilst certain birds, like pied and crested barbets, utilize the stems for nesting holes (Brown *et al.,* 2006).

Figure 1: Image of *Acacia sieberiana* (Venter and Venter, 2002)

2.1.2 Acacia polyacantha

Acacia polyacantha is also known as white thorn. It is a flowering tree which can grow up to 25m tall in height (Norton and Carpenter, 1998). *Acacia polyacantha* is native to Africa, India, the Indian Ocean and Asia, but it has also been introduced to the Caribbean. They are usually medium-sized to large semi-deciduous tree with a typically layered crown. The bark is pale to dark yellowish-brown in colour and pealing to show whitish inner layers. The thorns of *Acacia polyacantha* are hooked and often persisting on the larger branches and the trunk. The leaf petioles have an oblong flattened gland near the base and inflorescences axillary are elongated spikes consisting of creamy white flowers (Bingham, 2012).

Figure 2: An image of an *Acacia polyacantha* (Bingham, 2012).

2.1.3 Acacia nilotica

Acacia nilotica is commonly known as gum arabic tree, babul, thorn mimosa, Egyptian acacia or thorny acacia (Raghavendra, Satish and Raveesha, 2006). It is native to Africa, the Middle East and the Indian subcontinent. It is also a Weed of National Significance and is an invasive species of significant concern in Australia (Raghavendra, Satish and Raveesha, 2006).

Acacia nilotica is a small to medium-sized tree with a dark brown to almost black, deeply fissured in older specimens. The thorns are in pairs, straight and they are usually pointing backwards (Maywald, 2003). The leaves of *Acacia nilotica* are compound with 4-8 pinnae and the flowers are in spherical heads on the new growth and yellow in colour. The pods are distinctive and constricted between the seeds. When *Acacia nilotica* pods are in their early stages, they usually exhibit a green colour which turns black when ripe. The indehiscent and old pods are often still on the tree when the new pods appear (Maywald, 2003). *Acacia nilotica* species is similar to *Acacia karroo* but, it can easily be distinguished by the distinctive pods. When no pods are present, the slightly deflexed thorns and the small, closely spaced leaflets may aid in differentiating them from other *Acacia* species (Maywald, 2003).

Figure 3: Image of *Acacia nilotica* (Maywald, 2003).

2.2 Distribution and habitat of *Acacia* species

Acacia species are present in all terrestrial habitats such as alpine settings, rainforests, woodlands, grasslands, coastal dunes and deserts (Kaplan, 2012). In drier woodlands or forest they are an important component of the understory. In a different place they may be dominant, as in the Brigalow Belt, Myall woodlands and the Eremaean Mulga woodlands (Ross, 1980).

In Australia, *Acacia* forest is the second most common forest type after Eucalypt forest, covering 980,000 square kilometres or 8% of total forest area. *Acacia* is also the nation's largest genus of flowering plants with almost 1000 species found (Ross, 1980).

2.3 Importance of *Acacia* species

2.3.1 Economic importance

Aboriginal Australians have traditionally collected the seeds of some *Acacia* species*,* so that they can be crushed into flour and eaten as a paste or baked into a cake (Midgley and Bond, 2001). The seeds contain as much as 25% more protein than common cereals and they store well for long periods due to the hard seed coats (Venter and Venter, 2002). In addition to utilizing the edible seed and gum, the people employed the timber for implements, weapons, fuel and musical instruments (Midgley and Bond, 2001; Van Rooyen *et al.*, 2001; Venter and Venter, 2002).

Various species especially *Acacia mangium*, *Acacia mearnsii* and *Acacia saligna* are economically important and are widely planted globally for wood products, tannin, firewood and fodder. *Acacia melanoxylon* and *Acacia aneura* supply some of the most attractive timbers in the genus. Black wattle bark supports the tanning industries of several countries and is used to supply tannins for production of waterproof adhesives (Ellesmore, 2002).

The hardened sap of various species of the acacia trees are known as acacia gum. *Acacia* gum is used as an emulsifier in food, a binder for watercolour painting, an additive to ceramic glazes, a binding in gum bichromate photography, a protective layer in the lithographic processes and as a binder to bind together fireworks.

2.3.2 Ecological importance

Acacia is a common food source and host plant for butterflies of the genus *Jalmenus,* as balance to the food chain and enhancing biodiversity as well (Dean, Midgley and Stock, 1994). The imperial hairstreak, *Jalmenus evagoras,* feeds on at least 25 *Acacia* species. All *Acacia* species also have an ecological importance of giving strength to the soil and are used as wing breaks (Bakshi, 1976).

2.3.3 Medicinal value

In ancient Egypt, an ointment made from the ground leaves of the plant was used to treat haemorrhoids (Kulkarni and Kumbhojkar, 2002). *Acacia polycantha*'s roots and bark have medicinal uses as the root extract is useful for snakebites and can also be applied to wash the skin of children who are agitated at night time. The root is also used for treating gonorrhoea, venereal diseases, dysentery and gastrointestinal disorders (Uhlig, 2012).

2.4 History and nature of *Viscum articulatum* infection

Mistletoes belong to the families Loranthaceae and Viscaceae, which both are taxonomically related to each other, and share the order Santalales (Rahmad and Addo-fordjour, 2014b). The family of Viscaceae has seven genera (*Arceuthobium, Dendrophthora, Ginalloa, Korthalsella, Notothixos, Phoradendron, Viscum*) and several hundred species world-wide (Poulin, Krasnov and Mouillot, 2011). *Viscum* is a genus of about 70–100 species of mistletoes, native to temperate and tropical regions of Europe, Africa, Asia and Australasia (Rahmad and Addo-fordjour, 2014b). The genus has been placed in its own family Viscaceae, but recent genetic research by the Angiosperm Phylogeny Group shows this family to be correctly placed within a larger circumscription of the sandalwood family, Santalaceae (Rahmad and Addo-fordjour, 2014a).

Parasitic plants constitute about 1% of the total angiosperms in nature and comprise of 19 families of angiosperms and about 4,100 species (Blick, Burns and Moles, 2012). Among those with various parasitic habits, mistletoes are well known as perennial plant parasitic plants that attack stems or branches of other trees (Pennings and Callaway, 2002). There are two main groups of parasitic plants namely the hemi-parasites and the holo-parasites. Hemi-parasites are green plants with chlorophyll and are able to photosynthesise. They can produce all or the main part of all necessary carbon compounds if they have access to water, light and carbon dioxide and *Viscum articulatum* belongs to this group. The other group, the holo-parasites need to have all water and all nutrients supplied by the host. In each group there are parasites growing on either the roots or the stems of the host and they are called root parasites and stem parasites respectively (Reid, 1991).

Mistletoes are considered as a component of biodiversity (Reid, 1991). They are characterised by the development of a root like absorptive organ called haustorium which forms the host parasite interface and draws nutrients from the host conductive tissues to the parasite. Mistletoes occur mostly on forest, fruit and ornamental host trees preferentially harbouring zones rich in biodiversity and thus are found excessively on mountain ridges provided with favourably optimum light intensities and in few in slopes and plains (Pennings and Callaway, 2002).

Mistletoes do not follow a uniform pattern of distribution which is affected by local environments and effected by habits of seed dispersing avian visitors. They are woody, obligate hemi parasitic shrubs with branches 15–80 centimetres long and their hosts are woody shrubs and trees (Pochhi, no date). The foliage is dichotomously or branching, with opposite pairs or whorls of green leaves which perform some photosynthesis minimal in some species, notably *Viscum nudum*, but with the plant drawing its mineral and water needs from the host tree (Rahmad and Addo-fordjour, 2014b). Mistletoe seed do not have to be in contact with a host plant to germinate because seeds will germinate on a variety of substrates, including soil, fences, gates, dead twigs, thorns, leaves, other mistletoes and telephone wires, as well as on branches of host and none host trees. The parasite can be dispersed by wind making is easy to move from one tree to another within the same area (Rahmad and Addo-fordjour, 2014a).

The flowers of the *Viscum* parasite are inconspicuous, greenish-yellow, 1–3 millimetres (0.039–0.118 in) diameter. The fruit is a berry, containing one or more seed sembedded in very sticky juice. The seeds are dispersed when birds particularly the mistle thrush eat the fruit, and remove the sticky seeds from the bill by wiping them on tree branches where they can germinate (Reid, 1991).

2.5 Spread of *Viscum articulatum* infection

Seed dispersal as well as pollination is usually mediated by the birds that thrive on fruiting body from the *Viscum articulatum* (Carlo and Aukema, 2005). The mistletoe bird also eats insects and therefore has a grinding gizzard. When eating the mistletoe fruiting body the bird is able to close the gizzard and the toxic seeds are usually swallowed as a whole and are shunt through their gut in about 3-4 minutes (Reid, 1991) and because the seed has a sticky coating, the bird applies its vent to the edges of the host tree branches and the seed then sticks onto the branch where they may then germinate (Midgley and Joubert, 1991). These birds also act as vectors for pollination in the hemi-parasite whose flowers bear a mechanism that causes pollen to explosively spray on the plumage of the visiting flower peckers (Blick, Burns, Moles and 2012).

Some seeds are dispersed by the droppings of birds whilst others stick to twigs while the bird is trying to get rid of the outermost part of the fruit shell (Carlo and Aukema, 2005). Due to the behaviour of the birds which may have regular eating places, the seeds are often dispersed in clumps. This implies a risk for greater mortality among the seedlings due to mutual competition when the seeds are germinating almost side by side (Reid, 1991). However, dispersal in clumps also secures that both sexes are within close distance of one another.

When the *Viscum articulatum* parasite invades a tree branch and it becomes the breeding area of the parasite developing into a fruiting body. The birds then eat the fruit, spreading the spores to other parts of the infected species as well as other *Acacia* species in that area. *Acacia* species are susceptible to *Viscum articulatum* whilst others are less susceptible showing that the parasite is selective to certain species (Birkmann, 2012). Before the *Viscum articulatum* parasite invades the trees, the trees would grow perfectly but when the parasite takes over, the whole branch of the tree dies from the tips of the tree as the fruiting body would be absorbing water and nutrients (Reid, 1991). Therefore the transfer of nutrients from the host to the parasites weakens the host to some degree and eventually the tree dies.

2.6 *Viscum articulatum* host specificity

There are great differences in the host specificity of different parasitic plants (Norton and Carpenter, 1998). *Viscum articulatum* infection is selective in terms of its host and a number of hard wood species are accepted as hosts. Even among parasites belonging to the same genus there is often great variation in their host specificity. However, there is also often great uncertainty about the observed host specificity (Birkmann, 2012). The uncertainty has several reasons but in particular there are problems interpreting the negative observations (Norton and Carpenter, 1998). If a certain parasite does not grow on a certain host species, the reason may be other than tissue incompatibility hence, not all *Acacia* species are affected by *Viscum articulatum* infection (Reid, 1991).

2.7 Damage caused by *Viscum articulatum* on *Acacia* species

Widespread and ever increasing host range of *Viscum articulatum* continues to pose heavy losses in economically valuable uses of *Acacia* species and those with medicinal properties whether growing in forests, or domestic homes (Kulkarni and Kumbhojkar, 2002).

Healthy tree hosts can tolerate few branch attachments with mistletoes but a heavily infected host slowly moves towards its decline as the mistletoe feeds upon its nutrients and spreads on all other surrounding parts (Carlo and Aukema, 2005). The *Acacia* species infected becomes more prone to other forms of biotic and abiotic stresses (Rasanen and Lindstrom, 2003). The host branches infected with *Viscum articulatum* show a gradual reduction in growth and diameter as compared to other healthy uninfected branches (Reid, 1991).

The development of mistletoe plants on the host tree is a dynamic process which necessarily leads to the death of the host tree and that the whole process may last for about a decade (Ellesmore, 2002). It is known that mistletoes have higher nutrient titer than their host (Reid, 1991) and this could possibly cause key nutrients to unavailable to the host branches since there will be high competition of nutrients and water between the host and the parasite (Pennings and Callaway, 2002).

2.8 Control measures on *Viscum articulatum*

2.8.1 Mechanical control

Mechanical pruning of the infected host branches is done by cutting the mistletoe attachment in order to completely remove embedded haustoria. This method is used to control *Viscum articulatum* parasite, but in cases of heavily instead the trees is cut down and burnt so to destroy the source of the mistletoe (Pennings and Callaway, 2002). Regrowth of the parasite may be prevented by pruning it and burning or burning the infected parts (Blick, Burns and Moles, 2012).

2.8.2 Biological control

Delias eucharis, a medium sized pierid butterfly (Common Jezebel) commonly occurs in South and South-East Asia (Bingham, 2012) and is particularly known to lay its eggs and feed on leaves of mistletoes (Carlo and Aukema, 2005) and has been suggested in its use to control the parasitic mistletoes (Midgley and Joubert, 1991). Use of biological control strategies including mistletoe targeting fungi has also been reported in Australia (Reid, 1991). However, this research was not done specifically in the *Viscum articulatum* infection on *Acacia* species.

2.9 Measures taken in Zimbabwe to control *Viscum articulatum* infection

No chemical or physical control is practised in Zimbabwe to control *Viscum articulatum* infection in Zimbabwe. At the moment, as a way of controlling *Viscum articulatum* infected parts are removed and burnt or buried. Hence, there is need for this research so that farmers have knowledge of the *Acacia* species which is less susceptible to *Viscum articulatum* attack.

Replacing the *Acacia* species which are susceptible to *Viscum articulatum* with less susceptible species can be a method of choice. However, this has not been reported yet specifically for *Acacia* species which are less susceptible for *Viscum articulatum*.

Figure 4: Image of *Acacia* species infected with *Viscum articulatum* (Picture was taken at Hakuna Matata Primary School in Greystone Park).

.

CHAPTER 3

MATERIALS AND METHODS

3.1 Area of study

The investigation was carried out at Forestry Commission (Forest Research Center) located in Highlands, Harare from mid-January 2018 to mid-February 2019. The soil pH in Harare ranges from 4.5 to 8.2. The Forest Research Center has fertile and well-drained soil. FRC is situated at an elevation of 1521 metres above sea level. Highlands has average annual minimum and maximum temperatures of 14°C and 23°C, respectively. It has an average annual minimum and maximum rainfalls of 191.4mm and 805.2mm, respectively. The latitude of FRC Harare, Zimbabwe is 17°80'03.37", and the longitude is 31°08'51.74".

3.2 Source of materials

Acacia sieberiana, *Acacia polyacantha* and *Acacia nilotica* seedlings were grown from seeds taken from the seed laboratory at Forestry Commission, Harare. *Viscum articulatum* samples were taken from Hakuna Matata primary school, 898 Ex Hall and 113 Gaydon road in Greystone Park, Harare from *Acacia* hosts.

3.3 Growing of host

A total of 15 seeds for each of the three *Acacia* species were sown into small pots in the nursery and watered daily until germination. The germinated seedlings were watered to saturate the soil and thereafter, watered daily for 3 months. After three months, prickling was done to separate each seedling into a single pot and liquid manure (folia) was added to enhance the growth of the seedlings. The growth of the three *Acacia* species seedlings was monitored for 10 months.

3.4 Collection and preparation of *Viscum articulatum* seeds

Viscum articulatum samples were collected from *Acacia* host in Harare. The seeds of the *Viscum articulatum* were subjected to abrasion by scouring them using a pestle and mortar, and 500g of the scoured seeds were diluted in 2 litres of water (Abrasion enhances germination by breaking seed-coat induced dormancy).

3.5 Inoculation of the *Viscum articulatum* infection on *Acacia* species

Five seedlings of each of the three species were inoculated by watering with 250 ml of *Viscum articulatum* infected water weekly for 4 months.

Another set of five seedlings of each species were inoculated by means of air layering to simulate natural inoculation by birds. This involved girdling of the seedling stem, packing the area with moist scoured *Viscum articulatum* seeds, covering and tying the area with polyethene plastic. The covered area was injected with 50 ml of *Viscum articulatum* solution weekly for 4 months using a hypodermic syringe.

A control set of five seedlings of each of the three species were left untreated.

3.6 Assessments

3.6.1 Assessment the extent of growth retardation in different Acacia species and the most prevalent way of the spread of the parasite

Assessment of the extent of growth retardation in the *Acacia* species was done every month for 4 months. This was done by measuring of heights of the seedlings. Signs of parasite infection on seedlings were also recorded. Signs of infection include wilting and change in leaf colouration (brown colouration indicates parasitic infection). Assessment of the most prevalent way of spread of the parasite was achieved by recording the time lag between infection and symptom manifestation (in months). The proportion (% age) of seedlings showing signs of infection after the 4 months period was also observed and recorded.

3.6.2 Assessment of the development of the mistletoe

Each of the five seedlings of the three species was assessed if they developed the mistletoe. The length of the mistletoe on each seedling was measured 4 months after inoculation in all treatments using a vernier calliper (series 530 model) and the mean lengths calculated.

3.7 Data analysis

Data were subjected to the software SPSS version 2.1. Two way ANOVA was done to investigate the effect of the two predictor variables towards the response variable. The predictor variables were the inoculation method of the parasite and the *Acacia* species susceptibility to *Viscum articulatum* infection. The height (cm) of the seedlings was the response variable. Two factors which were the methods of inoculating the *Viscum articulatum* infection (watering with infected water and inoculation by means of air layering) were under study.

CHAPTER 4
RESULTS

4.1 Effects of *Viscum articulatum* on seedlings growth

Acacia sieberiana was the most affected species compared to *Acacia nilotica* and *Acacia polyacantha*. It was shown by a greater growth retardation indicated by the change in height of seedlings (Figure 5). *Acacia polyacantha* was also affected as the height growth was stunt. *Acacia nilotica's* growth was least affected indicating that it was less susceptible to *Viscum articulatum* infection. Reduction in height of the *Acacia sieberiana* and *Acacia polyacantha* occurred as a result of wilting and drying of the seedlings (die-back), and this displayed presence of the infection.

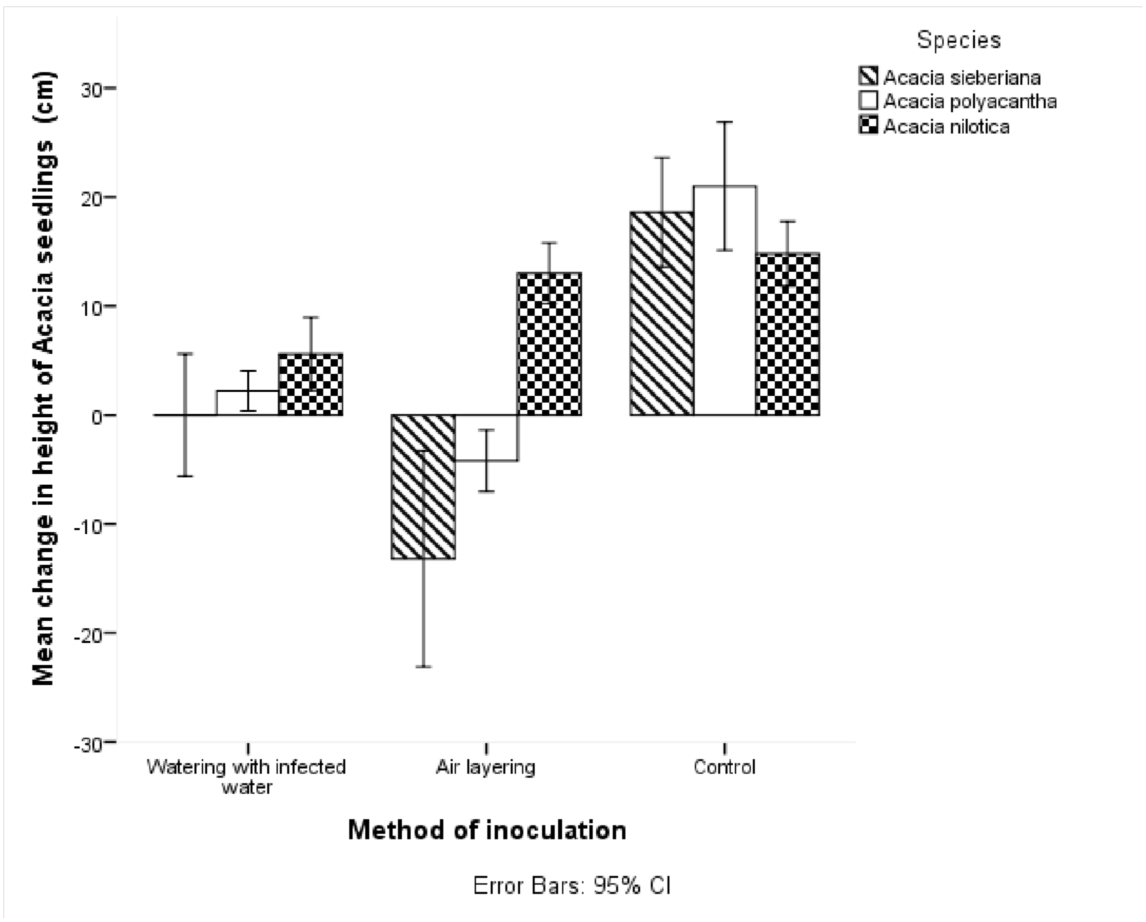

Figure 5: Effects of *Viscum articulatum* method of inoculation on three *Acacia* species growth.

4.2 Effect of the treatments on seedlings

The controls, which were the untreated seedlings showed the highest growth in height compared to the ones which were treated (Figure 5). The seedlings which were inoculated by the parasite by both air layering and watering showed retarded growth due to *Viscum articulatum*. Seedlings of *Acacia nilotica* were least affected as they showed the highest growth amongst all species in all treatments.

Anova results indicated that the method of inoculation significantly affect the potency of the parasite on *Acacia* seedlings ($P < 0.05$) (Appendix 3). Seedlings of *Acacia sieberiana* and *Acacia polyacantha* which were inoculated with *Viscum articulatum* by air layering showed the most retardation resulting from die-back as compared to the ones which were inoculated by watering with infected water. Thus, air layering was the most effective way to inoculate the parasite because it showed greater retardation of growth by parasite (*Viscum articulatum*) as compared to infection through watering. *Acacia sieberiana* which was inoculated by means of air layering showed the most retardation in growth, followed by *Acacia polyacantha*. However, in both treatments, *Acacia nilotica's* growth was least affected showing that it was less susceptible to *Viscum articulatum* infection.

4.3 Development of mistletoe on *Acacia* species

Mistletoe development started to show after 4 months. The mistletoe developed on *Acacia sieberiana* seedlings which were inoculated by means of air layering and it grew up to a mean height of 19 micrometers (Table 1).

Table 1: Average length (μm) of mistletoe four months from time of infection

Treatment/Method of inoculation	Species		
	Acacia sieberiana	*Acacia polyacantha*	*Acacia nilotica*
Watering	*	*	*
Air layering	19	*	*
Control	*	*	*

KEY: * No mistletoe development

4.4 Time taken for infection signs to show and the proportion (% age) of seedlings showing signs of infection after the four months period

The time lag (in months) symptoms developed in the three *Acacia* species are shown in Table 2. The monthly assessments showed that both *Acacia polyacantha* and *Acacia sieberiana* showed signs of infection as the stem turned brown (rust like brownish). *Acacia sieberiana* infected by air layering developed symptoms earliest (after 1 month). This was followed by both *Acacia sieberiana* (infected by watering) and *Acacia polyacantha* (infected by air layering). Infection by watering was the least potent form of inoculation in *Acacia polyacantha* as symptoms of infection took the longest period (3 months) to manifest. However, *Acacia nilotica* did not show any signs of infection for the whole period of monitoring (for 4 months). In all control seedlings, no sign of infection developed.

Table 2: Time lag (months) in symptom development in the three *Acacia* species

Treatment/ Method of inoculation	Species *Acacia sieberiana*	*Acacia polyacantha*	*Acacia nilotica*
Watering	2	3	-
Air layering	1	2	-
Control	-	-	-

KEY: - No symptom development

Table 3: The proportion (% age) of seedlings showing signs of infection after the four months period

Treatment/ Method of inoculation	Species *Acacia sieberiana*	*Acacia polyacantha*	*Acacia nilotica*
Watering	60	40	0
Air layering	100	60	0
Control	0	0	0

The proportion (% age) of seedlings which showed signs of infection after the four months amongst the *Acacia* species are shown in Table 3. The proportion of *Acacia sieberiana* seedlings infected by air layering was 100% as all the 5 seedlings showed signs of infection. In *Acacia polyacantha* seedlings infected by watering, the proportion infected was 60%. *Acacia sieberiana* and *Acacia polyacantha* seedlings infected by watering had a proportion of 60% and 40% respectively. In all control seedlings, there was 0% proportion of seedlings which showed signs of infection after the four months period. In *Acacia nilotica* which were infected by both watering and air layering there were no symptom developments in all the seedlings.

CHAPTER 5

DISCUSSION

5.1 Extent of growth retardation in *Acacia* species

The result of this study shows that, *Acacia nilotica* is less susceptible to *Viscum articulatum* infection, since its growth was unaffected as it did not show any reduction in growth. *Acacia sieberiana* was the most susceptible host amongst the three species as it showed the highest reduction in height, followed by *Acacia polyacantha*. This reduction in height displayed presence of the infection, indicating great differences in the host specificity of different parasitic plants (Norton and Carpenter, 1998). This study shows that, *Viscum articulatum* infection is selective in terms of its host and, both *Acacia sieberiana* and *Acacia polyacantha* are accepted as host. However, there is also often great uncertainty about the observed host specificity (Birkmann, 2012). The uncertainty has several reasons but in particular there are problems of interpreting the reason upon susceptibility other than the genetic components of the species which allow it to be susceptible (Norton and Carpenter, 1998). Thus, if a certain parasite does not grow on a certain host species, the reason may be other than tissue incompatibility (Reid, 1991). The controls, which were the untreated seedlings, showed the highest growth indicating that the heights were not affected since the *Viscum articulatum* infection was absent.

5.2 Development of the mistletoe

It was observed that *Acacia sieberiana* is the most susceptible host amongst the three species as it is the only species which developed the mistletoe. The mistletoe developed to an average height of 19 μm on the seedlings which were inoculated by air layering, possibly because the infection was inoculated directly into the plant. *Acacia polyacantha* did not develop any mistletoe in all treatments but growth retardation was observed and it could be explained by the fact that the infection weakens its host to some extent before the mistletoe develops. *Acacia nilotica* did not develop the mistletoe and this could be because it could be less susceptible since its growth was not affected as well.

5.3 The most prevalent spread of the parasite

The seedlings which were inoculated by air layering showed signs of infection the most compared to the ones which were inoculated by watering revealing that the method of *Viscum articulatum* spread has an impact on the infestation levels of the infection. The *Acacia* species which were inoculated by air layering tend to show signs and symptoms of *Viscum articulatum* infection faster compared to those which were infected through watering. This could be because air layering results in direct infection of seedling as opposed to infection by watering. *Acacia sieberiana* had the most growth retardation amongst the species followed by *Acacia polyacantha,* indicating that *Acacia sieberiana* is more susceptible compared to *Acacia polyacantha. Acacia* n*ilotica's* growth was not affected by both watering and air layering and this could be attributed to its lower susceptibility to *Viscum articulatum*. *Acacia sieberiana* which was infected by watering showed signs and symptoms of *Viscum articulatum*, hence this study reveals that the infection can be spread by water dispersal and impact differently at different times on the host.

Mistletoe seed do not have to be in contact with a host plant to germinate because seeds will germinate on a variety of substrates, including soil, fences, gates, dead twigs, thorns and leaves, as well as on branches of host and non-host trees (Rahmad and Addo-fordjour, 2014b), The parasite can be dispersed by wind making it easy to move from one tree to another within the same area. This was shown by the fact that the *Viscum articulatum* can disperse amongst susceptible *Acacia* species when they are watered with infected water. However, air layering as a method of *Viscum articulatum* infection was the quickest way for the *Acacia* to take up the infection since it was inoculated directly on the seedling through the stem. It is similar to the natural way of transmission as *Viscum articulatum* is spread by birds as they move from tree to tree. The birds have a grinding gizzard which can break seed-coat dormancy and the bird applies its vent to the edges of the host tree branches and the seed then sticks onto the branch where they may then germinate (Carlo and Aukema, 2005).

5.4 Time taken for infection signs to develop

Stem turned brown in colour, and this occurred to *Acacia sieberiana* and *Acacia polyacantha* which were inoculated by the *Viscum articulatum* by both watering and air layering. It shows that the infection weakens the root system such that water and nutrients have difficult time reaching all parts of the tree. Due to the weakening of the root system, the plants wilt and dry, and hence dieback causing a reduction of the seedlings height. *Acacia sieberiana* had the most rapid signs of infection followed by *Acacia polyacantha*, and *Acacia nilotica* did not show any signs during the period of monitoring. This results that *Acacia sieberiana* and *Acacia polyacantha* are susceptible to *Viscum articulatum* whilst *Acacia nilotica* is less susceptible to the infection.

Before the *Viscum articulatum* parasite invades the trees, the trees would grow perfectly but when the parasite takes over, the whole branch of the tree dies from the tips of the tree as the infection would be absorbing water and nutrients (Reid, 1991). Therefore the transfer of nutrients from the host to the parasites weakens the host to some degree and eventually the tree dies.

5.5 CONCLUSION AND RECOMMENDATIONS

5.5.1 Conclusion

Viscum articulatum shows host specificity on *Acacia* species. *Acacia sieberiana* and *Acacia polyacantha* shows greater susceptibility to *Viscum articulatum* whilst *Acacia nilotica* shows less susceptibility.

5.5.2 Recommendations To

the farmers:

- To specialize on growing *Acacia nilotica* which are less susceptible to *Viscum articulatum* can be a method of choice as it is safe to the soil and human beings other than making use of chemicals. It is also a cheap resolution to both small scale farmers and commercial farmers. It is also control effectively other than cutting down infected trees which may lead to deforestation.

- In small scale woodlots, farmers or individuals are advised to space out those species which are susceptible, for instance, *Acacia polyacantha* and *Acacia sieberiana* so as to control or reduce spread of the *Viscum articulatum* infection by birds as vectors.

- To make sure that *Viscum articulatum* seeds that fall down are racked and removed because, if they are washed away by water they can infect other trees.

To researchers:

- Further studies on other species other than *Acacia nilotica* which are less susceptible to *Viscum articulatum* infection.
- Interest must be put on other ways to control the *Viscum articulatum* other than cutting down the infected parts or trees and burning, especially making use of herbicides which are environmental friendly.

- To study if the susceptible species can be genetically modified so that they can be less susceptible to the *Viscum articulatum* infection.

- To further study if *Acacia nilotica* is just less susceptible to *Viscum articulatum* or it is resistance.

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APPENDICES

APPENDIX 1 SPSS OUTPUT FOR NORMALITY TEST

Tests of Normality[a]

	Acacia species	Kolmogorov-Smirnov[b]			Shapiro-Wilk		
		Statistic	df	Sig.	Statistic	Df	Sig.
Height of Acacia seedlings growth	A. sieberiana	.213	5	.200*	.885	5	.332

*. This is a lower bound of the true significance.

a. Method of inoculation = Watering with infected water, Acacia species = A. sieberiana b.

Lilliefors Significance Correction

Tests of Normality[a]

	Acacia species	Kolmogorov-Smirnov[b]			Shapiro-Wilk		
		Statistic	df	Sig.	Statistic	Df	Sig.
Height of Acacia seedlings growth	A. nilotica	.246	5	.200*	.956	5	.777

*. This is a lower bound of the true significance.

a. Method of inoculation = Watering with infected water, Acacia species = A. nilotica
b. Lilliefors Significance Correction

Tests of Normality[a]

	Acacia species	Kolmogorov-Smirnov[b]			Shapiro-Wilk		
		Statistic	df	Sig.	Statistic	Df	Sig.
Height of Acacia seedlings growth	A. polyacantha	.241	5	.200*	.903	5	.427

*. This is a lower bound of the true significance.

a. Method of inoculation = Watering with infected water, Acacia species = A. polyacantha

b. Lilliefors Significance Correction

Tests of Normality[a]

	Acacia species	Kolmogorov-Smirnov[b]			Shapiro-Wilk		
		Statistic	df	Sig.	Statistic	df	Sig.
Height of Acacia seedlings growth	A. sieberiana	.237	5	.200*	.917	5	.509

*. This is a lower bound of the true significance.

a. Method of inoculation = Air layering, Acacia species = A. sieberiana

b. Lilliefors Significance Correction

Tests of Normality[a]

	Acacia species	Kolmogorov-Smirnov[b]			Shapiro-Wilk		
		Statistic	df	Sig.	Statistic	df	Sig.
Height of Acacia seedlings growth	A. nilotica	.233	5	.200*	.884	5	.329

*. This is a lower bound of the true significance.

a. Method of inoculation = Air layering, Acacia species = A. nilotica

b. Lilliefors Significance Correction

Tests of Normality[a]

	Acacia species	Kolmogorov-Smirnov[b]			Shapiro-Wilk		
		Statistic	df	Sig.	Statistic	df	Sig.
Height of Acacia seedlings growth	A. polyacantha	.127	5	.200*	.999	5	1.000

*. This is a lower bound of the true significance.

a. Method of inoculation = Air layering, Acacia species = A. polyacantha

b. Lilliefors Significance Correction

Tests of Normality[a]

	Acacia species	Kolmogorov-Smirnov[b]			Shapiro-Wilk		
		Statistic	df	Sig.	Statistic	df	Sig.
Height of Acacia seedlings growth	A. sieberiana	.261	5	.200*	.932	5	.608

*. This is a lower bound of the true significance.

a. Method of inoculation = Control, Acacia species = A. sieberiana

b. Lilliefors Significance Correction

Tests of Normality[a]

	Acacia species	Kolmogorov-Smirnov[b]			Shapiro-Wilk		
		Statistic	df	Sig.	Statistic	df	Sig.
Height of Acacia seedlings growth	A. nilotica	.263	5	.200*	.900	5	.410

*. This is a lower bound of the true significance.

a. Method of inoculation = Control, Acacia species = A. nilotica

b. Lilliefors Significance Correction

Tests of Normality[a]

	Acacia species	Kolmogorov-Smirnov[b]			Shapiro-Wilk		
		Statistic	df	Sig.	Statistic	df	Sig.
Height of Acacia seedlings growth	A. polyacantha	.175	5	.200*	.974	5	.899

*. This is a lower bound of the true significance.

a. Method of inoculation = Control, Acacia species = A. polyacantha

b. Lilliefors Significance Correction

APPENDIX 2 SPSS OUTPUT FOR PAIRWISE COMPARISON ON ACACIA SPECIS SEEDLINGS GROWTH

Pairwise Comparisons

Dependent Variable: Height of Acacia seedlings growth

(I) Acacia species	(J) Acacia species	Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b]	
					Lower Bound	Upper Bound
A. sieberiana	A. nilotica	-4.533*	1.483	.004	-7.540	-1.526
	A. polyacantha	-9.333*	1.483	.000	-12.340	-6.326
A. nilotica	A. sieberiana	4.533*	1.483	.004	1.526	7.540
	A. polyacantha	-4.800*	1.483	.003	-7.807	-1.793
A. polyacantha	A. sieberiana	9.333*	1.483	.000	6.326	12.340
	A. nilotica	4.800*	1.483	.003	1.793	7.807

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

b. Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments).

APPENDIX 3 SPSS OUTPUT FOR TEST BETWEEN SUBJECTS EFFECTS

Tests of Between-Subjects Effects

Dependent Variable: Height of Acacia seedlings growth

Source		Type III Sum of Squares	Df	Mean Square	F	Sig.
Intercept	Hypothesis	1856.022	1	1856.022	1.156	.395
	Error	3209.911	2	1604.956[a]		
Acacia_species	Hypothesis	653.511	2	326.756	1.009	.442
	Error	1295.956	4	323.989[b]		
Method_of_inoculation	Hypothesis	3209.911	2	1604.956	4.954	.083
	Error	1295.956	4	323.989[b]		
Acacia_species * Method_of_inoculation	Hypothesis	1295.956	4	323.989	19.649	.000
	Error	593.600	36	16.489[c]		

a. MS(Method_of_inoculation)

b. MS(Acacia_species * Method_of_inoculation)

c. MS(Error)

APPENDIX 4 SPSS OUTPUT OF MEAN AND STANDARD ERROR FOR ACACIA SPECIES AND METHOD OF INOCULATION

3. Acacia species * Method of inoculation

Dependent Variable: Height of Acacia seedlings growth

Acacia species	Method of inoculation	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
A. sieberiana	Watering with infected water	-1.009E-013	1.816	-3.683	3.683
	Air layering	-13.200	1.816	-16.883	-9.517
	Control	18.600	1.816	14.917	22.283
A. nilotica	Watering with infected water	2.200	1.816	-1.483	5.883
	Air layering	-4.200	1.816	-7.883	-.517
	Control	21.000	1.816	17.317	24.683
A. polyacantha	Watering with infected water	5.600	1.816	1.917	9.283
	Air layering	13.000	1.816	9.317	16.683
	Control	14.800	1.816	11.117	18.483

APPENDIX 5 MULTIPLE COMPARISONS OF *ACACIA* SPECIES SEEDLINGS GROWTH

Multiple Comparisons

Dependent Variable: Height of Acacia seedlings growth
Tukey HSD

(I) Acacia species	(J) Acacia species	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
A. sieberiana	A. nilotica	-4.53*	1.483	.011	-8.16	-.91
	A. polyacantha	-9.33*	1.483	.000	-12.96	-5.71

	A. sieberiana	4.53*	1.483	.011	.91	8.16
A. nilotica	A. polyacantha	-4.80*	1.483	.007	-8.42	-1.18
	A. sieberiana	9.33*	1.483	.000	5.71	12.96
A. polyacantha	A. nilotica	4.80*	1.483	.007	1.18	8.42

Based on observed means.

The error term is Mean Square (Error) = 16.489. *.

The mean difference is significant at the .05 level.