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THE EFFECTIVENESS OF NEEM SEED (*Azadirachta indica*) EXTRACT IN THE CONTROL OF YELLOW SUGARCANE APHID IN THE SOUTH EAST LOWVELD OF ZIMBABWE

BY

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Abstract

The yellow sugarcane aphid (*Sipha flava*) is one of the most economically damaging pests of sugarcane in the South Eastern Lowveld of Zimbabwe. *Sipha flava* was recorded for the first time in Zimbabwe in March 2014. Management of yellow aphid through the use of synthetic pesticides may result in resistance developing due to selection pressure, hence researchers are advocating the use of botanicals such as neem seed extracts (*Azadirachta indica*). A field trial was conducted at Emanzini Farm, Subdivision 07 of Triangle from November 2019 to April 2020 in order to determine the effects of neem seed extracts on the yellow sugarcane aphid. The specific objectives were to; (i) determine the effects of neem seed solution on the infestation by yellow aphids, and (ii) quantify the effect of different neem rates. The sugarcane variety N14 was used. The trial was laid out in a randomized complete block design (RCBD). Fifteen plots were randomly allocated in the field and each plot measured 14m by 6m with inter row spacing of 1.5m and one peripheral discard row on either side of the field. Five treatments namely 100ml neem seed solution per 15 litres of water, 75ml neem seed solution per 15 litres of water, 50ml neem seed solution per 15 litres of water, dimethoate 1litre per 100 litres of water and an untreated control were used. The treatments were replicated three times. Neem seed solution showed great potential on controlling yellow sugarcane aphid population. The 75ml neem seed solution showed significant efficacy by reducing leaf damage and *Sipha flava* colonies from 19 to 12 and 7.13 to 0.64 respectively. The 75 ml neem seed solution resulted in a significantly higher mean Pol % of 13.56%. This is so because if yellow sugarcane aphid is not controlled the content of soluble solids and the apparent percentage of sucrose in the juice of sugarcane plants will be affected greatly. There was a significant difference ($P<0.05$) on the mean Tons ERC among the five treatments. The 50 ml and untreated control had significantly lower cane yields of 13.03 tons and 13.27 tons respectively. The study showed that farmers can adopt the 75 ml neem seed solution as a viable alternative to the use of synthetic insecticides in the control of yellow sugarcane aphid.

Key words: yellow sugarcane aphid, neem seed solution, management.

Declaration Page

I declare that this dissertation is my original work except where sources have been cited and acknowledged. The work has never been submitted, nor will it ever been submitted to another university for the award of a degree.

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Dedication

This dissertation of MSc Crop Production by me is dedicated:

To my parents who sacrificed everything to give me the most and best education possible

To my brother, Simeon, who helped me in many facets in life as well as making this research a success.

To my sister, Tanaka, who has been there for me forever and also sacrificed some of her interests for my benefit.

To my wife, Vivian, who was key and instrumental through love and support throughout our life together.

To my sons, Spencer Jnr. and Justin John, who, someday, when they read their names in this dissertation may be motivated and may feel challenged to aim higher.

List of Acronyms and Abbreviations

ANOVA	Analysis of Variance
AUREC	Africa University Research Ethics Committee
BRIX	Sugar content on an aqueous solution
ERC %	Estimated Recoverable Crystal percentage
IPM	Integrated Pest Management
N14	Natal 14 sugarcane variety
POL %	Polarization Percentage
SELZ	South Eastern Lowveld of Zimbabwe
SD 07	Subdivision 07
SUSCO	Successful Rural Sugarcane Farming Community Project
TONS	Tonnage
YSA	Yellow Sugarcane Aphid

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CHAPTER 1 INTRODUCTION

1.1 Introduction

The Yellow Sugarcane Aphid (*Sipha flava*) (Hemiptera: Aphidae) feeds and damages young spindle leaves of sugarcane and other grasses such as *Digitaria, Chloris, Urochloa* and *Leptochloa* as well as cultivated crops that include sorghum, maize, rice, wheat and barley (Mabveni, 2018). *Sipha flava* is one of the most economically damaging pests of sugarcane in the South Eastern Lowveld of Zimbabwe (SELZ). *Sipha flava* is yellow and adults are 1.3 to 2 mm long. During the year 2013, *Sipha flava* was recorded for the first time in the South African sugarcane industry (Conlong & Way, 2014) and was found attacking sugarcane in Zimbabwe in March 2014 (Mabveni, 2018). *Sipha flava* numbers have shown that there is a recurring seasonal cycle in sugarcane. *Sipha flava* outbreaks are normally experienced during the dry hot months of October all the way to December, extending into the warm wet season (January to April). Aphid numbers during the winter and autumn are generally low but climb sharply from September onwards; hence sugarcane stands are more susceptible to attack in summer (Mabveni, 2018).

The damage caused on sugarcane by *Sipha flava* is often visible on the upper surface of the leaf, although the insects preferentially feed on the lower surface. Sugarcane leaves damaged by *Sipha flava* turn yellow and, after prolonged feeding, turn into red. On close examination red dots are visible where the insect has pierced through the leaf surface. These symptoms are clearly visible in the section of the leaf behind the colony, which has fed and then moved along the leaf. It is assumed that the prolonged feeding will lead to premature senescence of leaves thereby reducing stalk diameter and causing yield loss (Conlong & Way, 2014; Mabveni, 2018). The environmental risks associated with the continuous use of synthetic pesticides have prompted this

experimental study to use plant based insecticidal components of Neem seed extracts (*Azadirachta indica*) that provide selective toxicity to insects with minimum non-target effects. The use of botanical pesticides offers eco-friendly pest control strategies to aid the agricultural practices. Among the various botanicals, neem plant based insecticides have been the most accepted bio-pesticides, due to the presence of multiple limonoids in neem plant extracts and oil that not only provides a sustainable pest control mechanism but also prevents the development of insect resistance to various synthetic insecticides. Additionally, the efficacy of these pesticidal ingredients of neem can be augmented by encapsulating them in nanocarriers that facilitate in providing sustained and controlled release of phytochemicals along with site targeted delivery thus, increasing the crop productivity and yield of crops.

Although aphids seldom kill a mature plant, the damage they cause and the unsightly honeydew they generate sometimes warrant control. Consideration needs to be mainly put on the nonchemical controls as most insecticides will destroy beneficial insects along with the pest.

Insecticides are registered against *S. flava* (Nuessly & Hentz, 2002) but these can have negative knock-on effects on non-target organisms, for example various predator populations, including coccinellids, were reduced for up to 10 weeks by in-furrow applications of nematicide-insecticide aldicarb during the spring (Showler & Reagan, 1991), and fenvalerate (pyrethroid insecticide) as a foliar spray enhanced *S. flava* populations by 63% while substantially suppressing certain predator groups. Management of yellow aphid through the use of synthetic pesticides may result in resistance developing because of the migratory behaviour of the pest hence researchers are advocating the use of botanicals such as neem seed extracts

(*Azadirachta indica*). Use of neem seed extracts will embrace the Integrated Pest Management ideology which aims at minimising use of synthetic insecticides.

This study sought to determine the best levels and rate of application of neem seed solution in controlling *Sipha flava*, to quantify the effect of different neem rates and to determine variability of levels of infestation of yellow aphids with neem seed solution use.

1.2 Background to the Study

During the year 2014, the yellow sugarcane aphid, *Sipha flava* (Hemiptera: Aphididae), was recorded in the Zimbabwe sugarcane industry for the first time. Initially it was discovered at the Zimbabwe Sugarcane Association Experiment Station (ZSAES) in Chiredzi, Masvingo Province of Zimbabwe, and it was subsequently detected across the entire sugarcane industry and more recently in Swaziland and Madagascar. Morphological and molecular taxonomic techniques were employed to confirm its identity (Mabveni, 2018). Native to North America, it currently has a wide geographical distribution across Central and South America, as well as the Caribbean and Hawaiian Islands. It was first reported on sugarcane in Africa in Morocco in 2006. Over the years, extensive use of commercially available synthetic pesticides against phytophagous insects has led to their bioaccumulation in the environment causing increased insect resistance and reduction in soil biodiversity. Further, some 90% of the applied pesticides enter the various environmental resources as a result of run-off, exposing farmers as well as consumers of the agricultural produce to severe health issues. Therefore, growing attention has been given toward the development of alternate environmentally friendly pesticides that would aid an efficient pest management system and also prevent chronic exposures leading to diseases. The experimental study focused on the use of neem plant seed extract

(*Azadirachta indica*) active ingredients which exhibit agro-medicinal properties conferring insecticidal as well as immunomodilatory and anti-cancer properties. The most prominent constituent of neem is azadirachtin, which has been established as a pivotal insecticidal ingredient. It acts as an antifeedant, repellent, and repugnant agent and induces sterility in insects by preventing oviposition and interrupting sperm production in males. Neem seed extract offers immense anti-feedant properties due to its efficacy in suppressing the feeding sensation in insects, at concentrations even less than 1 part per million (Isman, 1991). Seeds from this tree comprise of 40% of oil with azadirachtin as the major active ingredient, that is mainly responsible for the insecticidal activity of neem (Isman, 1991). Neem seed extracts act as an antifertility agent (Nisbet, 2000).

Most importantly an active ingredient of neem known as NLGP has now evolved as a potent immunomodilatory agent (Mallick, 2013), thus making it an ideal agro-medicinal plant This unique attribute of neem makes it an ideal bio-pesticidal agent, as it does not cause non-specific toxicity to mammals. Neem seed extract was first isolated from *A. indica* by Morgan at Keele University, England (Nisbet, 2000). It is a complex tetranortriterpenoid limonoid with repellent and pesticidal properties. Biosynthesis of triterpenoids from *A. indica* initiates with azadirone and a C-ring opening, which culminates in Azadirachtin formation. Azadirachtin, along with other related triterpenoids such as Azadirachtin B, salannin and nimbin, are the active ingredients in neem plant-based bio insecticides and they act by disrupting the growth and development of insects and by deterring their feeding. It is considered as a botanical pesticide with exceptional growth regulating and biocidal efficacy along with deterrent effects on the ovipositing and feeding of insects (Morgan, 2009).

Azadirachtin is structurally similar to the insect hormones known as “ecdysones” which are responsible for metamorphosis in insects. The feeding behaviour in insects is dependent on the neural inputs received from the chemical sensors of the insects, for example, the taste receptors in the mouthparts, tarsi and oral cavity. These sensors integrate a “sensory code” that is delivered to the central nervous system. Manifestation of antifeedancy by *Azadirachta indica* occurs through the stimulation of deterrent cells in these chemoreceptors and by blocking the feeding stimulation in insects by firing the “sugar” receptor cells (Jennifer & Mordue, 1998). In addition to antifeedancy, azadirachtin injection also leads to physiological effects in the insect's midgut, which causes a reduction in the post-ingestive digestive efficiency. This reduction in efficiency is known as “secondary” antifeedancy and is due to disturbances in the hormonal as well as physiological systems. These disturbances include hindrance in the food movement through the insect's midgut and inhibition in production of digestive enzymes (White, 1990). The formation of juvenile stages during each molt is controlled by the juvenile hormone from the corpora allata (Nisbet, 2000). Disruption in these events by azadirachtin, leads to various sterility and moulting defects. Moreover, cellular uptake of azadirachtin inhibits both cell division as well as protein synthesis thus, causing midgut cell necrosis and flaccid paralysis of muscles (Nisbet, 2000). Neem products influence fecundity in female insects in a dose-dependent manner. Azadirachtin prevents oviposition by inhibiting oogenesis and synthesis of ovarian ecdysteroid. In males, azadirachtin acts by interrupting the meiotic process responsible for sperm production (Nisbet, 2000). The effect on final sugarcane yield due to *S. flava* infestations in Zimbabwe as a whole remains to be determined. Management tactics have yet to be developed and, should

natural enemies and weather fail to keep populations in check, then resistant/tolerant varieties and insecticides could be considered.

1.3 Statement of the problem

Management of yellow aphid through the use of synthetic pesticides may result in resistance developing because of the migratory behaviours of the pest. *Sipha flava* has several generations in a year. Most aphid species in Southern parts of Zimbabwe's warm climate reproduce asexually throughout most or all of the year with adult females giving birth to live offspring often as many as 12 per day without mating. Adult aphids can produce up to 80 offspring in a matter of a week and thus aphid populations can increase with great speed (White, 1990). The adverse impact of synthetic insecticides on non-target beneficial organisms implies that alternatives that are friendly to beneficials have to be found. It is in this vein that this trial was conducted to find alternatives to synthetic insecticides in the control of the sugarcane yellow aphid.

1.4 Research Objectives

The objectives of the study were to;

(i) Determine the effects of neem seed solution on the infestation by yellow aphids.

(ii) Quantify the effect of different neem rates on yellow sugarcane aphid mortality.

1.5 Hypotheses tested

The hypotheses tested in the study were as follows;

(i) The use of neem seed extract can lead reduced infestation of yellow aphids.

(ii) Yellow aphid responds differently to neem seed solution rates.

1.7 Significance of the study

Almost all sugarcane production in Zimbabwe is concentrated in the south-eastern lowveld parts of the country, predominantly in Chiredzi and Mwenezi districts. Sugar production in Zimbabwe in the 2012/2013 financial year increased by 28 percent to 475 000 tons (2011/2012: 372 000 tons) as cane deliveries from private and third-party farmers grew substantially (Tongaat Hullet, 2016).

Tongaat Hulett embarked on a comprehensive private rehabilitation programme named Successful Rural Sugarcane Farming Community Project (SusCo), with the goal of rehabilitating private farmers, with the support and expertise of Tongaat Hulett, to increase their supply of sugarcane. The SusCo project re-established the private farmer sugarcane production area from just over 11 200 hectares in 2015, to 15 880 hectares, with the direct beneficiaries including hundreds of private sugarcane farmers from the Hippo Valley, Triangle and Mkwasine Mill Group areas (Tongaat Hulett, 2016)

Another new initiative in the form of Project Kilimanjaro (Phase 1) is the first private farmer development project and will entail the development of some 3 300 hectares of new sugarcane land in the Southern Lowveld region. The project will result in 165 new farmers employing some 1 600 employees. Based on the current cane price, this project will result in revenue of some US$18, 5 million flowing into these private farmers and the surrounding rural communities. In light of this importance of the sugarcane crop to private enterprise, local farmers and the Zimbabwean economy at large, it is imperative that sustainable solutions are found towards the control of the emerging threat of the yellow sugarcane aphid problem. Aphids can lead to great

economic losses if not controlled. Neem seed solution leaves no toxic residue on the sugarcane plants so they do not kill or repel natural enemies (beneficial insects) that migrate in afterspray operations. Data generated from the study will be distributed to all stakeholders in the form of publications, as well as farmers meetings among other fora.

1.8 Delimitation of the study

The experimental study was conducted using the sugarcane variety N14. The experimental study commenced from end-November 2019 up to April 2020. The experiment was conducted at Sub division 07, Emanzini Farm in Triangle, Chiredzi district.

1.9 Limitations of the study

Heavy rains intercepted by the leaves led to a cool blast which dislodged yellow aphids. Typically, they were unable to find their way back to the same plant host plant.

Temperatures above 35 degrees Celsius might also have dislodged *Sipha flava* from the sugarcane plants.

CHAPTER 2 REVIEW OF RELATED LITERATURE

2.1 Introduction

Azadirachta indica, or Neem tree belongs to the family of meliaceae. This tree can reach 30 meters in height, but it generally stays smaller (between 5 and 10 meters). Its leaves are evergreen and its flowers are in pinatte, white or yellowish. The fruit is a drupe of 1 or 2 centimetres; yellow at maturity. *Azadirachta indica* has a worldwide distribution mainly in the arid tropical and subtropical zones. Native to India and the South-East of Asia, it had been introduced in Australia, in Africa, in the Antilles and in tropical America (Jennifer & Mordue, 1998). Thanks to its deep roots system, it can easily tolerate the dry season. However it doesn't survive a long cold period. According to recent studies, all the parts of this tree contain substances which have useful pharmacological properties. In fact, about fifty oxidized tetranortriterpenoïds have been discovered in leaves, seeds and bark. This is why the neem tree possesses many biological activities such as insecticidal, nematicidial, bactericidal, spermicidal, antiviral, antifungal, diuretic and antipyretic. Although its use as a medicinal plant is becoming more and more developed in the pharmaceutical industry, it is in the control of insect pests that Neem has the most potential. Farmers and agriculturists also widely use neem oil or neem leaves solution as natural pesticides and insecticides. Some American agro-chemical companies have already "patented" this tree, but following an international campaign which denounced these behaviours, Europe decided on not recognizing the legality of this patent (Conlong & Way, 2014).

Azadirachtin is the principal ingredient used in the manufacture of fertilizers and pesticides. In fact, this active substance is a mixture of seven isomeric compounds (from A to G). Although isomer A is the most abundant, the one which is the most active as pesticide remains the isomer E. It seems that it inhibits the growth and the development of insects by altering their cycle life, and acts such as an anti-nutritional factor. Other compounds of the Neem also have the same insecticide power. A recent study shows that there are at least 24 different compounds. That's why the risk of resistance development by insects decreases. But the most active compounds remain the azadirachtin, the salannin, the meliantriol and the nimbin. Neem is principally effective against chewing and sucking pests on which it has an antifeeding, repellent, deterrent and growth disruptive effect. To summarize Neem is biodegradable, non-toxic and environmentally friendly. But using too much neem oil can harm plants, so it's important to stay vigilant with the quantities, principally during the dry season.

In Kenya yellow Sugarcane aphid was first reported in the Transmara and south Nyanza sugar zones in 2016. It spread out to the western Kenya. Serious concerns emerged in 2018 and 2019 seasons when several Sugarcane farms in Kakamega County began to notice cane withering following yellow sugarcane attack. A survey was conducted over 6 months and revealed that the variety Co 421 which was predominantly grown in the factory zones was severely attacked. Use of pyrethrin lambda-cyhalothrin 17.5g/l at 0.5l in 200-300 l water was recommended as a short-term measure (Mabveni, 2018).

Yellow Sugarcane aphid is a new sugarcane pest in the Shire valley of Malawi. The first observations of yellow sugarcane aphid were in 2013 and it seems that the aphids came from Swaziland. The origin of the first introduction of this pest in Malawi is not known yet. In few years, yellow sugarcane aphid has become a major pest in the Shire

valley. Massive infestation occurs on plantation and on young ratoon cane. Loss in yield of infested field can reach more than 30%. Some fields with new plantation have already been completely destroyed by yellow sugarcane aphid. The aphids are potential vectors of the sugarcane mosaic virus. This virus is not present yet in the Shire valley but a particular attention should be given to this eventuality of the introduction of mosaic virus (Conlong & Way, 2014).

The agricultural department of Illovo Estate has implemented a survey observation system for aphids. Regular visual controls are done in the fields. In case of infestation; chemicals are spread to kill the aphids. The chemicals used are cypermethrin and imidacloprid. The spraying is usually aerial. It can also be done with knapsack sprayer, especially for the young plants in a new plantation.

Illovo Estate is also working on biological method to control aphids. There is work on the identification of entomopathogenic fungi efficient on yellow sugarcane aphid. An entomopathogenic fungus is a fungus that can act as a parasite of insects and kills or seriously disables them. Much hope relies on this method as it will be an organic solution to control this pest (Conlong & Way, 2014).

2.3 Description of Yellow Sugarcane Aphid

Sipha flava is yellow and adults are 1.3 to 2 mm long. These aphids live in dense colonies in sugarcane, usually on the lower leaf surface. The abdomen has two double rows of dusky coloured spots on the dorsal surface and along the lateral margin and it is covered in short stiff black hairs. The pair of cornicles (siphunculi) on the abdomen are reduced in size to elevated pores. Generally, both sexes of adults are wingless, but winged females do occur. Nymphs resemble adults but are wingless and smaller (Conlong & Way, 2014).

2.4 Damage symptoms

The damage caused on sugarcane by *S. flava* is often visible on the upper surface of the leaf, although the insects preferentially feed on the lower surface. Sugarcane leaves damaged by *S. flava* turn yellow and, after prolonged feeding, red. On close examination red dots are visible where the insect has pierced through the leaf surface. These symptoms are clearly visible in the section of the leaf behind the colony, which has fed and then moved along the leaf.

Although Hall & Bennett (1994) report the occurrence of honeydew and a black sooty mould associated with colonies of *S. flava* in sugarcane, this phenomenon has not been recorded in South Africa, Swaziland or Zimbabwe. *Sipha flava* is reported to transmit sugarcane mosaic virus (Blackman & Eastop, 1984); however, this aspect requires further investigation as there is some debate amongst pathologists in the USA regarding the efficiency of the aphid as a vector of the disease.

Nuessly & Hentz (2002) report that prolonged feeding can result in premature leaf senescence and stalk death. In Florida (USA), where *S. flava* is a major pest of economic importance (Reagan,1994), when two leaves are damaged due to aphid feeding when the crop is three months old, there may be a 6% lower final yield. Leaf chlorosis and death of three pairs or more of active growing leaves can result in 19% yield loss (Reagan, 1994). According to Hall (2001), yield reduction and reduced tillering are usually caused by feeding damage to early plant growth stages; however, late season crops may also suffer yield loss.

2.5 Biology of Yellow Sugarcane aphid

Reproduction can be sexual or without mating (asexual) depending on climatic conditions. In Florida (USA), *S. flava* females' mate with wingless males in areas with cold winters (Reagan, 1994). Females produce one to five nymphs each day, and

nymphs develop through four instars. Development from nymph to mature adult takes 18-22 days on sugarcane, compared with only eight days on *Sorgum bicolor*. On the latter hosts, females produce one to five nymphs each day over 22 days. On other grasses fecundity ranges from 16-72 nymphs with a lifespan of 16-36 days. Depending on the host, adults require 10-12 days to reach reproductive maturity (Hentz & Nuessly, 2004). In South Africa there are often small populations of *Melanaphis sacchari* Zehnt. (Hemiptera: Aphididae) found on sugarcane amongst colonies of *S. flava*, and according to Nuessly & Hentz (2002) it is thought that *M. sacchari* emits an alarm pheromone which *S. flava* responds to by dropping off the sugarcane leaf, thereby protecting itself from danger.

2.6 Hosts of Sugarcane Aphid

Sipha flava was first described from specimens collected on *Sorghum bicolor* (L.) in Illinois by Forbes in 1884, and hence was originally referred to as the 'sorghum aphis' (Reagan, 1994). Worldwide many hosts for *S. flava* are reported, including *Sorghum halepense* (Johnson grass), *Pennisetum clandestinum* (kikuyu grass), *Digitaria ciliaris* (crabgrass), *Cymbopogon citratus* (lemon grass), species from the genera *Panicum, Cynodon*, *Paspalum, Hordeum* and the crops such as wheat, barley, maize, *Avena* (oats) and rice. *Carex* and *Cyperus* from the Cyperaceae, and various pasture grasses, are also hosts (Reagan, 1994). In South Africa *S. flava* has been collected from sugarcane, *S. bicolor*, *Tragus berteronianus* Schult, and *Echinochloa colona* (L.).

2.7 Management of Yellow Sugarcane Aphid

It is too early after the invasion to have developed a management strategy for this pest in southern Africa. However, the following tactics reviewed in the Americas warrant consideration. *Sipha flava* is monitored every season in their sugarcane industries

(Nuessly, 2005). Initially numbers are counted and, when excessive, leaf damage is used as an indicator of the effect on growth. The industries find that natural enemies and weather conditions usually maintain *S. flava* densities at low levels (Hall &Bennett, 1994; Hall, 2001). Temperatures above 35°C and heavy summer rainfalls dislodge *S. flava* from the plants, thus causing a degree of control (Nuessly, 2005). In South Africa natural enemies of *S. flava* are present in sugarcane fields and the surrounds. These comprise ladybird species (Coccinellidae), hover flies (Syrphidae), spiders (Araneae), earwigs (Dermaptera) and ants (Formicidae). Current and future farming practices adopted in South Africa must conserve these natural enemies.

American research has provided evidence for at least partial resistance to *S. flava* attack, using pubescent sugarcane varieties (White, 1990; Nuessly, 2010). Varietal resistance has been demonstrated between different sorghum varieties (Strakes & Mirkes, 1979) and grass species (Kindler & Dalrymple, 1999). Furthermore, insecticides are registered against *S. flava* (Nuessly & Hentz, 2002) but these can have negative knock-on effects, e.g., various predator populations, including coccinellids, were reduced for up to 10 weeks by in-furrow applications of nematicidial-insecticide aldicarb during the spring (Showler & Reagan, 1991) and fenvalerate (pyrethroid insecticide) as a foliar spray enhanced *S. flava* populations by 63% while substantially suppressing certain predator groups.

2.8 Summary

Little or no information has been gathered yet on the use of neem seed extracts on yellow sugarcane aphids in Zimbabwe. The research attempted to quantify most efficient neem seed extracts application rate.

CHAPTER 3 METHODOLOGY

3.1 Introduction

The study was carried out at SD 07 Emanzini Farm,Triangle in Chiredzi district. The variety N14 was used because it is widely grown in the South East Lowveld of Zimbabwe and has a history of yellow aphid attack. Neem seed solution was used in controlling yellow aphid in sugarcane because of its pungent smell which disturbs or inhibits the development of the eggs, larvae or pupa, disturb mating and sexual communication, repels larvae and adults, deters females from laying eggs and deters feeding. The study was carried out in from November 2019 up to April 2020.

3.2 The Research Site

The soil type is sandy loamy classified as Lithosols with Total Available Moisture of 64 mm. SD 07 farm irrigates using irrigation and receives rainfall of 625mm per annum, falling predominantly in the hot summer months (October-March).

3.3 The Research Design

The variety N14 was used for the field study because it has a historical infestation of *Sipha flava.* Fifteen plots were randomly allocated in the field study with each plot measuring 14m by 6m with inter row of 1.5m and one peripheral discard row on either side of the field. The field study involved 5 treatments namely 100ml neem seed solution per 15l of water, 75ml neem seed solution per 15l of water, 50ml neem seed solution per 15l of water, dimethoate1l per 100l of water and untreated control.

The treatments were replicated three times and laid out in a Randomised complete block design (RCBD). White plot markers were used to demarcate each and every treatment area. The field experiment was conducted during the 2019-2020 cropping season. The crop was planted in November 2019 and data was collated up to April 2020. The seed source material was taken from an out-grower seed certified by Zimbabwe Sugar Association Experiment Station (ZSAES).

3.3 Population and Sampling

The N14 variety was used in the experimental plots. The cane sets were planted in rows in continuous form. The crop was planted using 2-3 eyed –cane setts at an interrow spacing of 1.5m. The depth of the furrows was 0.3m.

Cane knives were dipped in Jeyes fluid, a disinfectant; during the preparation of seed cane setts in order to prevent the spread of Ratoon Stunting Disease (RSD). The seed cane setts were dipped in Bayfidan to reduce the transmission of disease pathogens.

Phosphorous and Potassium were applied as per soil sampling recommendations. Nitrogen was applied at 4 weeks after emergence as per ZSAES recommendation. The crops were sprayed according to the treatments using knapsack sprayers.

3.4 Data Collection Variables

Data was collected on a number of variables namely;

1) Number of aphids per selected number of clusters per 2 weeks interval.

2) Number of damaged leaves at 2 weeks interval.

3) Number of tillers population at 3 months old at the interval of 2 weeks and thereafter monthly up to 5 months.

4) Leaf damage score at 2 weeks interval.

3.5 Preparation of the neem seed solution

Neem seed solution was prepared and analysed in a laboratory in order to quantify the *Azadirachta indica* active ingredient.

3.6 Data Analysis and Organisation

The data was subjected to analysis of variance using Minitab statistical package and means were compared at probability $p< 0.05$.

3.7 Ethical Considerations

The research proposal was submitted to the Africa University Research Ethics Committee (AUREC) for approval before data collection commenced. Assistants were trained on how to spray insecticide and data collection with all the procedures involved being adequately covered. The experimental study tried all means on minimising systemic insecticides to plants in bloom so as to protect pollinators and beneficial insects. Assistant spray operators were equipped with personal protective equipment. Spraying was done early in the morning to safeguard safety and health of the assistants. Use of appropriate methodologies was done in this research so as to minimise bias related errors through adhering to the scientific processes of research.

3.8 Summary

The Chapter highlighted the research plan that was adopted for the study. A presentation of the data collection strategies and analytical approaches were also done. Due to increased advocacy of Integrated Pest Management (IPM), farming practices need to embrace new technology and use of botanicals as well as improved seed varieties in trying to curb pest infestations and problems.

CHAPTER 4 DATA PRESENTATION, ANALYSIS AND INTERPRETATION

4.1 Introduction

This chapter looks at presentations and analysis of the data obtained from the field, where an experimental study was done. Reference was also made to statistical tests in order to spell out the statistical links between the variables drawn for data analyses. The data collated on number of leaves damaged, number of tillers, leaf damage score and aphid colonies were subjected to analysis of variance (ANOVA) using mini tab and means were compared at probability $P<0.05$. The table 1 shows results of all variables after analysis were done. The P value which is greater than 0.05 is not significantly different and $P<0.05$ is significantly different. Error bars are used on bar graphs to show representations of the variability of data and used on graphs to indicate the error or uncertainty in a reported measurement. The data collected from the experimental study are analysed, presented, and discussed according to set objectives of the study.

4.2 Sugarcane Juice Quality Parameters

The sugarcane juice quality parameters of Pol %, Brix % and ERC % for the five treatments are as outlined in table 1 below.

Table 1: Juice Quality Parameters

Treatment	Pol %	Brix %	Cane Tons	ERC%	Tons ERC
100 ml	13.1200	15.7670	122.1670	11.3630	13.6030
75 ml	13.5600	15.6500	121.8870	11.2470	13.5500
50 ml	13.0400	13.6670	119.2500	11.0300	13.0270
Dimethoate	13.1267	15.6670	122.9170	11.4300	14.2500

		15.567	119.277	11.100	13.267
Untreated	13.1500	0	0	0	0
Lsd	0.1404	1.54	1.933	0.5123	0.2884

Table 2: Number of leaves, Number of tillers and Leaf damage scores at 5, 5.5 and 6 months respectively.

Treatment	No of leaves damaged @ 5months	No of leaves damaged @ 5.5months	No of leaves damaged@ 6months	No of tillers@ 5 months	No of tillers @ 5.5months	No of tillers@ 6 months	Leaf damage score @ 5 months	Leaf damage @ 5.5 months	Leaf damage @ 6 months	Aphid colony@ 5 months	Aphid colony@ 5.5 months	Aphid colony @6 months
100ml	19.67	21.67	12.67	2.00	1.33	2.00	3.00	2.33	2.33	7.13	4.16	0.64
75ml	19.67	19.33	13.00	2.00	1.333	1.667	2.67	2.33	2.32	6.76	6.33	1.85
50ml	16.67	15.33	8.667	1.667	1.000	1.667	3.00	2.00	1.886	6.79	4.603	1.97
Dimethoate	18.00	9.00	4.00	2.667	1.670	1.33	3.00	2.33	2.00	6.02	0.33	0.00
Untreated	17.667	24.33	29.33	2.333	1.330	1.67	3.00	2.00	2.11	6.18	7.63	7.673
P value	0.93	0.053	0.008	0.831	0.833	0.903	0.452	0.737	0.402	0.852	0.04	0.02
Sd	5.02	5.627	6.542	1.095	0.683	0.8165	0.258	0.447	0.321	1.399	2.46	1.658

4.3 Effect of neem seed extract on number of leaves damaged at 5 months

At 5 months after planting there was no significant difference ($p>0.05$) in the mean number of leaves. The mean number of leaves ranged from a low of 17.67 leaves per plant in the untreated control to a high of 19.67 leaves per plant at 100 ml neem and 75 ml neem treatments as shown in Table 2.

4.4 Effect of neem seed extract on number of leaves damaged at 5.5 months

At 5.5 months after planting there was no significant difference ($p>0.05$) in the number of leaves (Table 2). The mean number of leaves ranges from a low of 9.0 leaves per plant in the dimethoate treatment to a high mean of 24.3 leaves per plant in the untreated control. Schmutterer, (1990) stated that neem seed extract offers immense antifeedant properties due to its efficacy in suppressing the feeding sensation in insects, at concentrations even less than 1 parts per million (Isman *et al.*, 1991).

4.5 Effect of neem seed extract on number of leaves damaged at 6 months

At 6 months after planting there was a significant difference ($p<0.05$) in the number of leaves (Table 2). The mean number of leaves ranged from a low of 4.0 leaves per plant in the dimethoate treatment to a high of 29.3 leaves per plant in the untreated control.

Azadirachtin is the active ingredient in neem plant-based bio insecticide and act by disrupting the growth and development of insects and by deterring their feeding thereby minimising leaf damage. Neem seed extract is considered as a botanical

pesticide with exceptional growth regulating and biocidal efficacy along with deterrent effects on the ovipositing and feeding of insects (Morgan, 2009).

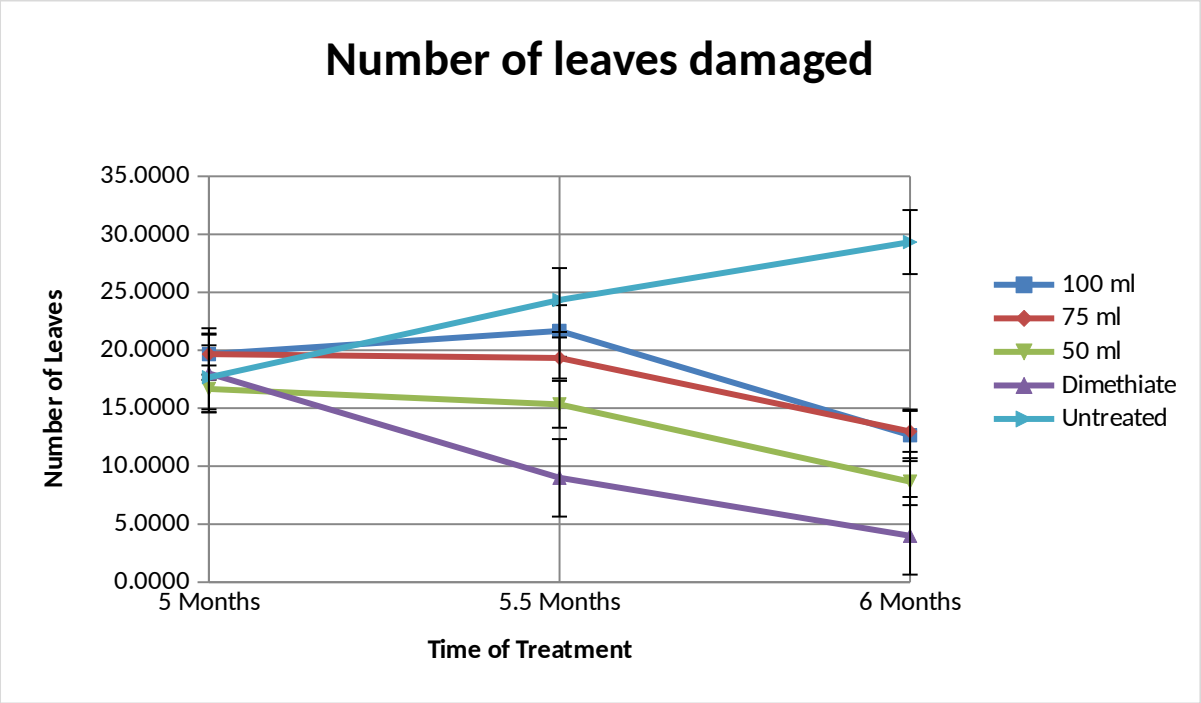

Fig.1: Number of damaged leaves from March to April 2020

Figure 1 shows that number of leaves damaged by yellow sugarcane aphid on the untreated treatment rose drastically from 16 to 29 from 5th to the 6th month of the study whereas the dimethoate and neem seed solution treatments reduced significantly. The neem seed solution treatments showed great potential in controlling aphid attack on sugarcane as number of leaves damaged declined to a low 8 in the 50ml, 12 on both 75 and 100ml neem seed solution treatments. Number of leaves damaged on neem treated plots declined significantly because azadirachtin is structurally similar to the insect hormones known as "ecdysones" which are responsible for metamorphosis in insects. The feeding behavior in insects is dependent on the neural inputs received from the chemical sensors of the insects, for example, the taste receptors in the mouthparts, tarsi and oral cavity. These sensors

integrate a “sensory code” that is delivered to the central nervous system. Manifestation of antifeedancy by azadirachtin occurs through the stimulation of deterrent cells in these chemoreceptors and by blocking the feeding stimulation in insects by firing the “sugar” receptor cells (Mordue, 1998).

Fig.2: Damaged leaves by yellow sugarcane aphid

Figure 2 above shows sugarcane plants with leaves severely damaged by *Sipha flava.* The aphids attacked young sugarcane prior to the development of multiple internodes. The aphids fed on underside of immature leaves causing yellowing of tissues leading

to chlorosis. Yellow sugarcane aphid feeding reduced chlorophyll content in leaves as well as amino acids. As a result, the surface area available for photosynthesis was minimised thus negatively impacting plant growth and carbohydrates assimilation.

4.6 Effect of neem seed extract on tillering at 5 months

At 5 months after planting there was no significant difference ($p>0.05$) in the number of tillers (Table 2). The mean number of tillers ranged from a low of 1.67 tillers per plant in the 50 ml neem treatment to a high of 2.67 tillers per plant in the dimethoate treatment. Tillers constitute the major sink for the products of photosynthesis. Leaf damage indirectly affects tillering in sugarcane. Yellow sugarcane aphid affects tillering and also affect the number of leaves and leaf area index (Shoko, 2007; 2009), thereby indirectly influencing light interception and biomass production.

4.7 Effect of neem seed extract on tillering at 5.5 months

At 5.5 months after planting there was no significant difference ($p>0.05$) in the number of tillers (Table 2). The mean number of tillers ranged from a low of 1.00 tillers per plant in the 50 ml neem treatment to a high of 1.67 tillers per plant in the dimethoate treatment. There was no significance because light intensity and day length are the most important factors influencing tillering of sugarcane.

4.8 Effect of neem seed extract on tillering at 6 months

At 6 months after planting there was no significant difference ($p>0.05$) in the number of tillers (Table 2 and figure 3). The mean number of tillers ranged from a low of 1.33 tillers per plant in the dimethoate treatment to a high of 2.00 tillers per plant in the 100 ml neem treatment.

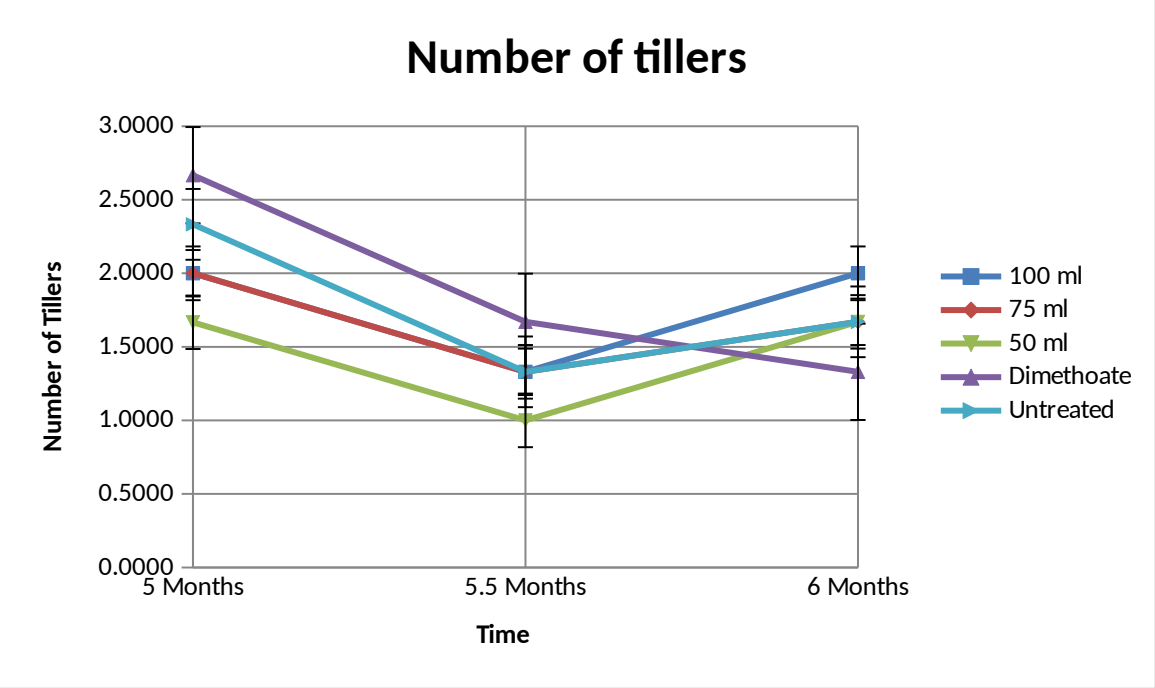

Fig.3: Number of tillers from 5 to 6 months

In the 5th month new seedlings were recorded. New seedlings emerged from the main seedlings of the sugarcane. Cultivation of sugar cane kind gramineous plants has habit of tillering. Tillering is the very important in life growth link of sugarcane, is all of great significance the growth of sugarcane and output. Tillers ranged from 3 in the fifth month to 2 in the sixth month in all neem seed solution treatments. The environmental factors such as illumination, irrigation, fertiliser, temperature, light intensity and day length all can have a great impact tiller formation and productive tiller.

Fig.4: Research assistant counting tillers during data collection period

At each sampling date, numbers of tillers were counted. Sampling was done every 14 days at a specific day and time until end of 6th month. Tillers form the above ground portion of sugarcane and carry leaves and flowers (King, 1965). They constitute the major sink for the products of photosynthesis.

4.9 Effect of neem seed extracts on leaf damage score at 5 months

At 5 months after planting there was no significant difference ($p>0.05$) in the mean leaf damage (Table 2). The mean score for leaf damage ranged from a low of 2.67 score per plant in the 75 ml neem treatment to a high score of 3.00 per plant in the other four treatments. Neem seed extract led to antifeedancy thereby lowering leaf damage score. Moreover, azadirachtin injection also led to physiological effects in the insect's midgut, which causes a reduction in the post-ingestive digestive efficiency.

This reduction in efficiency is known as “secondary” antifeedancy and is due to disturbances in the hormonal as well as physiological systems. These disturbances include hindrance in the food movement through the insect's midgut and inhibition in production of digestive enzymes (Schmutterer, 1985).

4.10 Effect of neem seed extracts on leaf damage score at 5.5 months

At 5.5 months after planting there was no significant difference ($p>0.05$) in the mean leaf damage (Table 2). The mean score for leaf damage ranged from a low of 2.00 score per plant in the 50 ml neem treatment and the untreated control to a high score of 2.33 per plant in the other three treatments.An early study conducted by Nisbet (1996) highlighted antifeedant feature of azadirachtin. It was established that a concentration of 50–100 ppm of azadirachtin caused an insecticidal effect. This insecticidal effect of azadirachtin can dramatically decrease the fecundity in aphids within 48 h of feeding hence maintaining leaf damage score at lower levels.

4.11 Effect of neem seed extracts on leaf damage score at 6 months

At 6 months after planting there was no significant difference ($p>0.05$) in the mean leaf damage score (Table 2). The mean score for leaf damage ranged from a low of 1.89 score per plant in the 50 ml neem treatment to a high score of 2.33 per plant in the 100 ml neem treatment. Nisbert (1996) stated that a diet containing more than 10 ppm azadirachtin led to the production of non-viable nymphs. Thus, it can be concluded that even with a low concentration of azadirachtin, it cannot cause an immediate antifeedancy. Secondary antifeedancy effect as well as a sterility effect can rapidly manifest themselves and aid in providing crop protection by reducing the pest

population without harming non-target or natural predator populations and as a result keeping leaf damage score at lower levels (Nisbet, 1996).

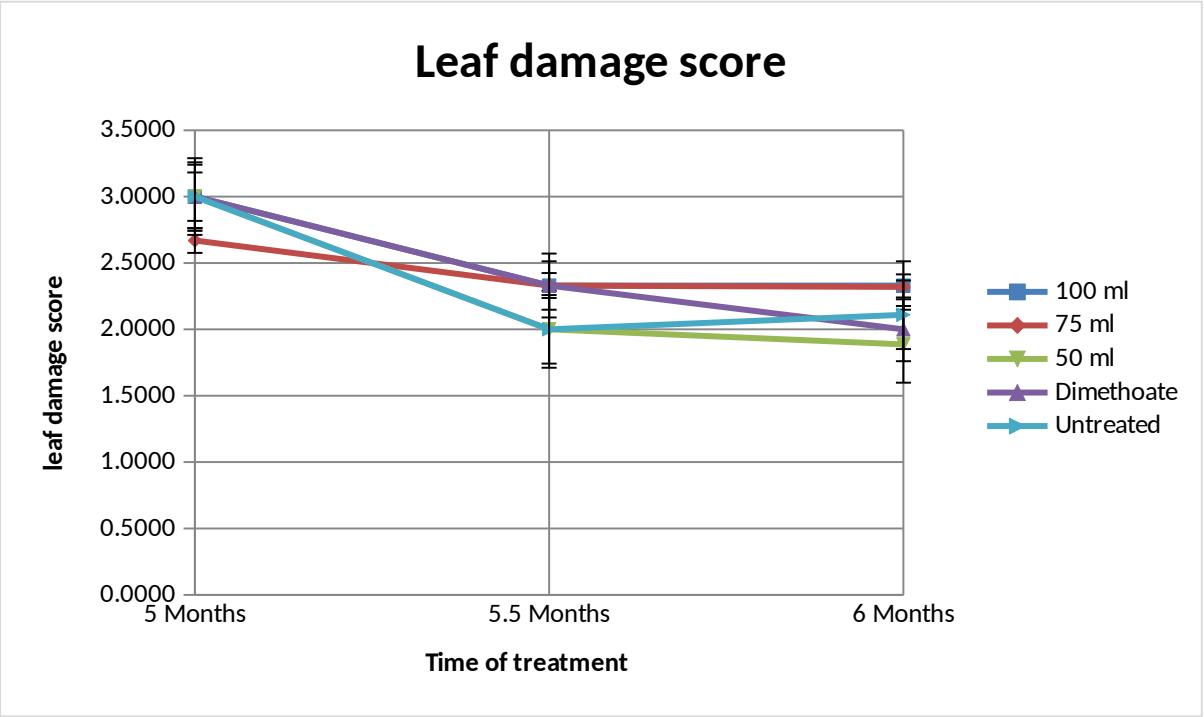

Fig.5: Leaf damage score during the 5th and 6th months of study

The figure 5 above shows that there was a steady decline on the leaf damage score from 3 in the 5th month to around 2 in the 6th month. The 3rd score was awarded to plants with severely damaged leaves while 2nd score was awarded to less damaged plant leaves. Factors considered on scoring leaf damage included leaf color, honeydew and sooty. Yellowish/ reddening of leaves entails chlorosis of leaves due to prolonged sapping of chlorophyll by aphids. Honeydew and black sooty mould is associated with colonies of yellow sugarcane aphids. Figure 2 show that there was improved leaf damage mainly attributed to the pesticidal effects of neem seed solution. Pesticidal effect might have led to repellence of aphids, reduced feeding and sterility in males.

Fig.6: Sugarcane plant recovering from Yellow sugarcane aphid infestation.

After neem seed solution was applied on sugarcane plants there was a marked improvement on the plants. This is evident as shown in figure 6 above. Sugarcane leaves which had turned yellowish started to retain the green color. The reddening/ yellowish of leaves slowly started to turn green as the plant regained its healthy status. The healthy status of sugarcane is mainly attributed to olfaction of *Sipha flava. Azadirachta indica* produced a pungent smell which deterred aphids from sapping cane leaves, oviposition of eggs as well as making male aphids sterile. As a result the once affected plant rejuvenated and amino acids were no longer affected making the process of photosynthesis fruitful since chlorophyll was no longer being sapped.

4.12 Effect of neem seed extracts on aphid colonies at 5 months

At 5 months after planting there was no significant difference ($p>0.05$) in the mean number of aphid colonies. The mean number of aphid colonies ranged from a low of 36.33 colonies per plant in the dimethoate treatment to a high of 53.33 colonies per plant in the 100 ml neem treatment.

4.13 Effect of neem seed extract on aphid colonies at 5.5 months

At 5.5 months after planting there was no significant difference ($p>0.05$) in the mean number of aphid colonies. The mean number of aphid colonies ranged from a low of 0.33 colonies per plant in the dimethoate treatment to a high of 63.00 colonies per plant in the untreated control. However, When the data is transformed, at 5.5 months after planting there was A significant difference ($p<0.05$) in the mean number of aphid colonies. The mean number of aphid colonies ranged from a low figure in the dimethoate treatment to a high figure in the untreated control. Aphid colonies declined significantly after neem seed extracts treatment because azadirachtin interferes with the growth and molting process of insects. Its ingestion leads to abnormal molts, growth reduction and increased mortalities. Azadirachtin interferes with the synthesis of an “ecdysteroid” hormone, which is responsible for the molting in insects. Nisbert (2000) reiterated that azadirachtin affects the neurosecretory system in insects by blocking the release of morphogenetic peptide hormones such as prothoracicotropic hormones that control the prothoracic glands and allatostatins, which in turn control the corpora allata and as a result, aphid colonies declined significantly.

4.14 Effect of neem seed extract on aphid colonies at 6 months

At 6 months after planting there was a significant difference ($p<0.05$) in the mean number of aphid colonies (Figure 7). The mean number of aphid colonies ranged from a low of 0.0 colonies per plant in the dimethoate treatment to a high of 63.78 colonies per plant in the untreated control. Moreover, when the data is transformed, at 6 months after planting there was a significant difference ($p<0.05$) in the mean number of aphid colonies. The mean number of aphid colonies ranged from a low of 0.0 colonies per plant in the dimethoate treatment to a high of 63.78 colonies per plant in the untreated control.

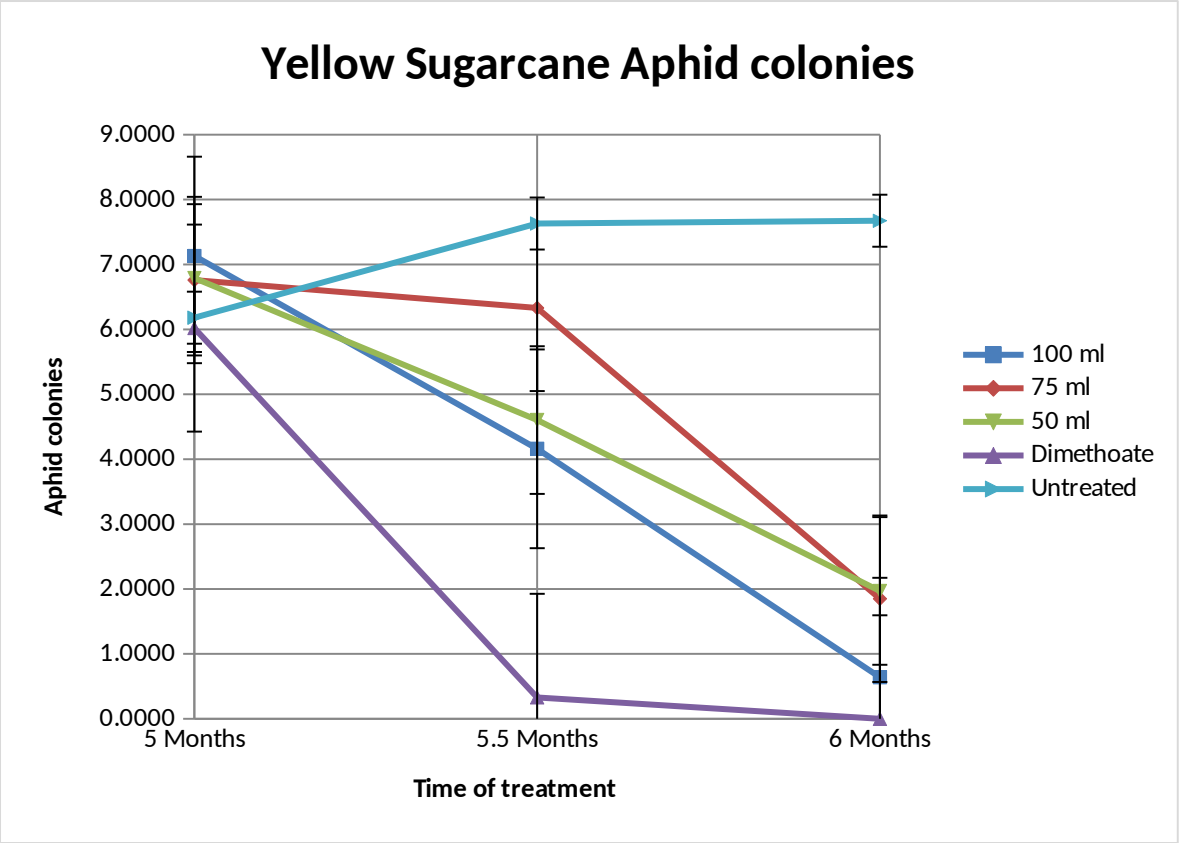

Fig.7: Yellow Sugarcane Aphid colonies on 5th and 6th months of the study

Yellow Sugarcane aphid colonies were high reaching the value of 60 when data was first recorded shortly before spraying was done. After 14 days of spraying there was significant decline in aphid colonies as shown in figure 8. As expected, dimethoate treatment-controlled aphids totally to 0 colonies. 100ml neem seed solution controlled aphid colonies greatly followed by 50ml and 75ml. *Azadirachta indica* induces

sterility in aphids by preventing oviposition and interrupting in sperm production in males. Azadirachtin disturbs oviposition by inhibiting oogenesis and synthesis of ovarian ecdysteroid. Disruption in these events by azadirachtin, leads to various sterility and molting defects. Moreover, cellular uptake of azadirachtin inhibits both cell division as well as protein synthesis thus, causing midgut cell necrosis and flaccid paralysis of muscles (Nisbet, 2000). Neem products influence fecundity in female insects in a dose-dependent manner. The untreated plots show that aphid colonies increased immensely to a high 75 maintaining that colony to the 6th month.

Fig.8: Dead aphids after neem seed extract spray

Azadirachta indica had also knock-on effects on aphids. Aphid colonies deteriorated largely due to cellular uptake of *azadirachtin* which inhibited both cell division as well as protein synthesis thus causing midgut cell necrosis and flaccid paralysis of muscles leading to aphids' death. Figure 8 show that aphids are very susceptible to *azadirachtin*. The figure shows dead aphids turned brown, having a fuzzy, shrivelled texture after succumbing to neem seed solution.

Fig.9: Heavily infested sugarcane plant

Yellow Sugarcane Aphids have many generations a year. They reproduce asexually throughout most or all of the year with adult females giving birth to live offspring often as many as 12 per day without mating. Untreated plots had heavy infestation as

shown in figure 8 because each adult aphid can produce up to 80 offspring in a matter of a week.

4.15 Effect of neem seed extracts on Pol %

There was a significant difference ($p<0.05$) on the pol% among the different treatments (Figure 10). The standard deviation error bars do not overlap, thus a clue that the difference may be significant. Statistical test was done and there was a significance difference ($p<0.05$). The 75 ml neem treatment has a significantly higher mean pol % of 13.56% and this is significantly different from the other four treatments. There is no significant difference in the pol% among the 100ml neem, 50 ml neem, dimethoate and the untreated control.

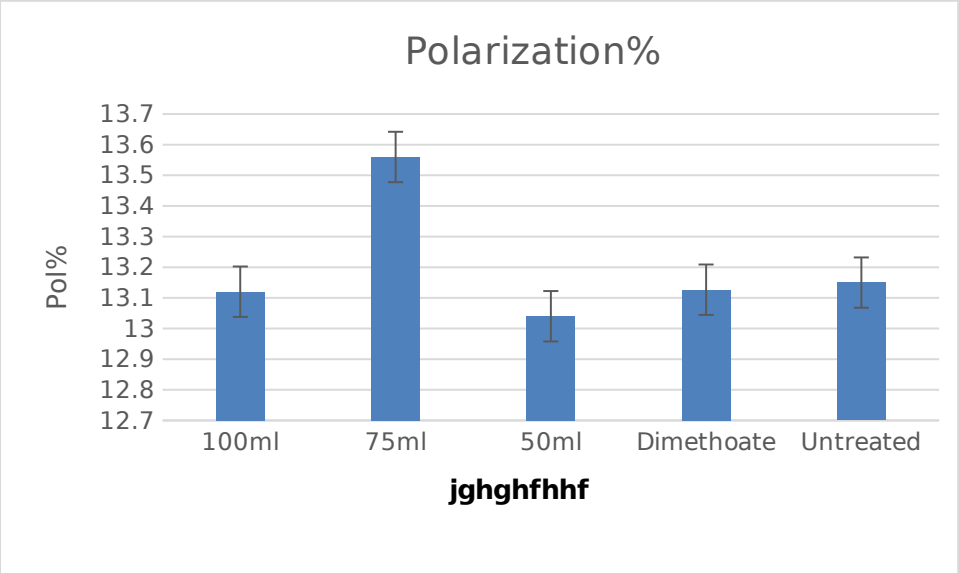

Fig.10: Pol % of sugarcane after harvesting

The 75ml treatment had a highest pol % of 13.56% followed by dimethoate with 13.1267 %. 100ml had 13.2% whilst 50ml had 13.04%. Untreated had a pol % of 13.15% as shown in fig 10 above.

4.16 Effect of neem seed extract on Brix %

There is a significant difference ($p<0.05$) in the Brix% among the five treatments (Figure 11). The error bars do not overlap showing that the difference was significant. The 50 ml neem treatment has a significantly lower mean brix % of 13.67%. The other four treatments have a significantly higher brix % compared to the 50 ml neem treatment. The four treatments however are not significantly different from each other in terms of their mean brix%.

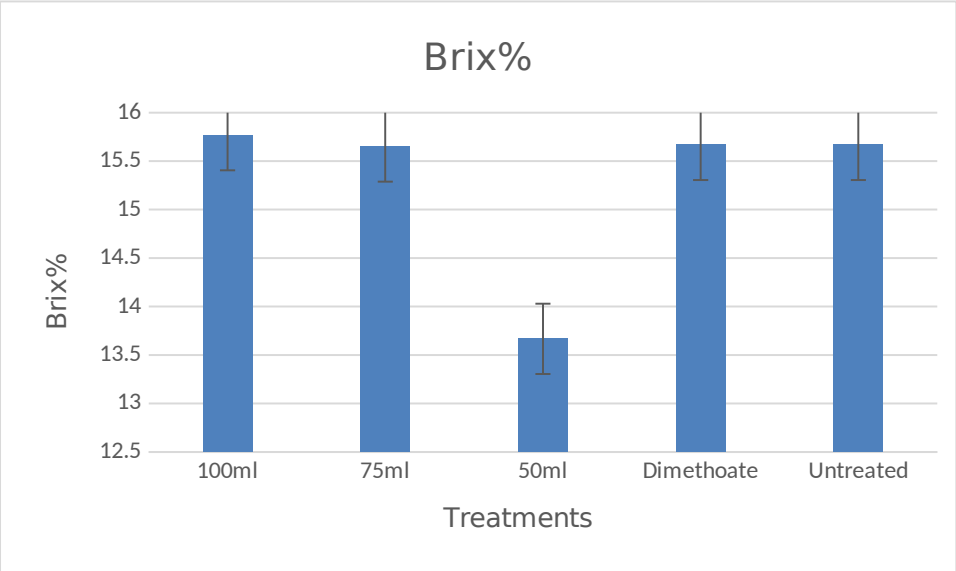

Fig.11: Brix % of sugarcane after harvesting

Dimethoate had a most brix % value of 15.667%. Figure 11 shows that on neem seed solution treatments, 100ml had highest value of 15.767%, followed by 15.65% on 75ml then 50ml had a least value of 13.667%.

4.17 Effect of neem seed extract on Cane Tons

There was a significant difference ($p<0.05$) on the mean cane yield among the five treatments (Figure 12). The 50 ml neem treatment and the untreated control have significantly lower cane yields compared to the other three treatments. The 50 ml neem and untreated control are not significantly different from each other with mean

yields of 119.25 and 119.28 tonnes respectively. 50ml and untreated control have standard deviation error bars overlapping meaning to say that the difference is not statistically significant. The 100 ml neem, 75 ml neem and the dimethoate treatment were not significantly different from each other with mean yields of 122.17, 121.89 and 122.92 tons respectively. However, the dimethoate treatment had a marginally higher yield.

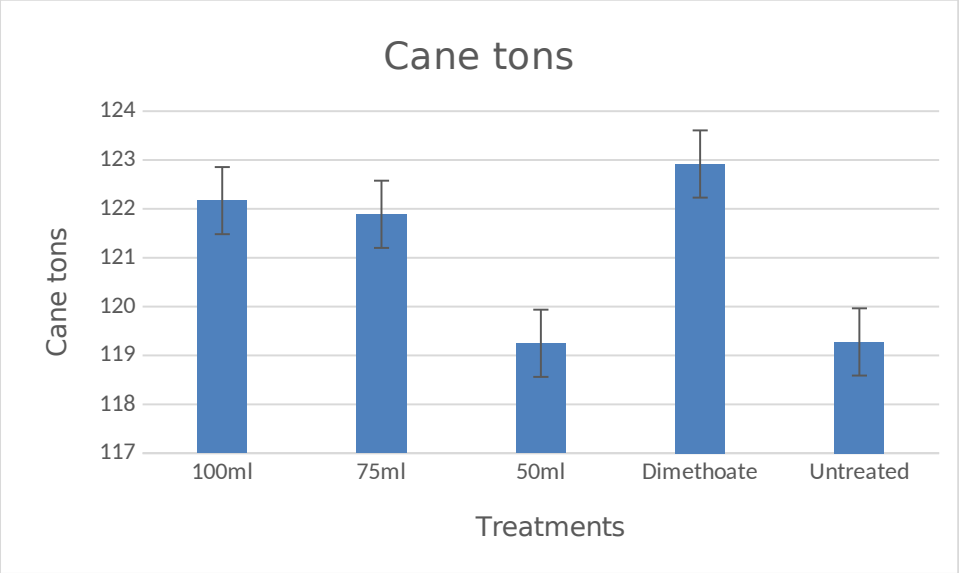

Fig.12: Cane Tons recorded after sugarcane harvest

Dimethoate recorded highest value of cane tons of 122.917 after the sugarcane was harvested. Figure 12 shows that 100ml and 75ml had a slight difference of 122.167 and 121.887 respectively. Untreated plot had a low value of 119.277 followed by 50ml with a value of 119.250.

4.18 Effect of neem seed extracts on ERC %

There was no significant difference ($p>0.05$) among the treatments on the ERC% (Figure 13). There is enough evidence in figure 13 where standard deviation error bars overlap quite a bit to show that the difference is not statistically different. Moreover,

the 50 ml neem and untreated control have lower percentages of 11.03 and 11.1 respectively.

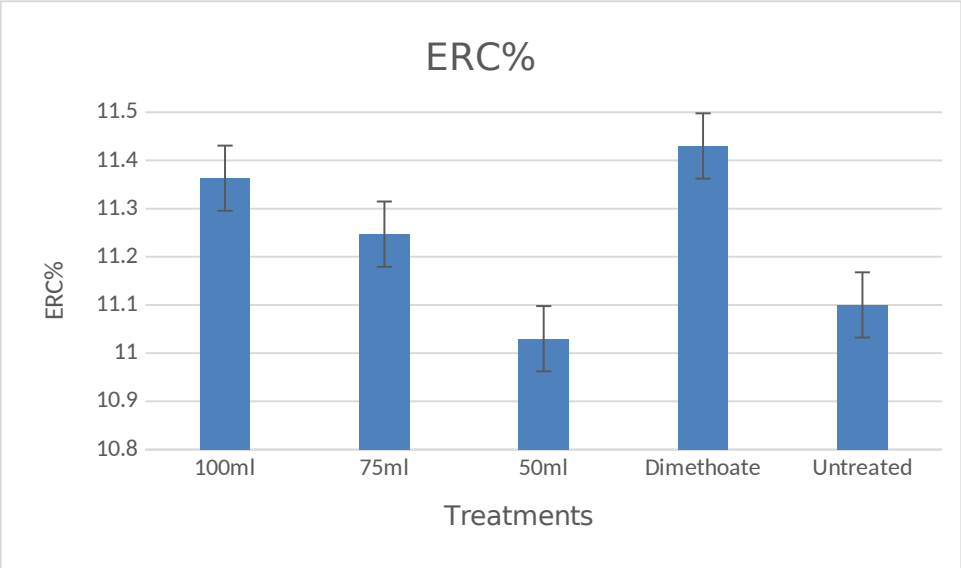

Fig.13: ERC % of sugarcane after harvest

Dimethoate treatment yielded highest value of ERC % with 11.43%. Neem seed solution treatments had 11.36%, 11.25% and 11.05% for 100ml, 75ml and 50ml respectively as shown in figure 13.

4.19 Effect of neem seed extracts on Tons ERC

There was a significant difference ($p<0.05$) on the mean tons ERC among the five treatments (Figure 14). The 50 ml neem treatment and the untreated control have significantly lower cane yields compared to the other three treatments. The 50 ml neem and untreated control are not significantly different from each other with mean yields of 13.03 and 13.27 tonnes respectively. The 100 ml neem and 75 ml neem are not significantly different from each other with tonnage of 13.6 and 13.55 respectively. Standard deviation error bars overlap less giving a clue that the difference is probably not statistically significant. A statistical test was performed to draw the conclusion that there was no significant difference. The dimethoate

treatment is significantly different from the 100 ml neem and 75 ml neem treatments with a mean tonnage of 14.25.

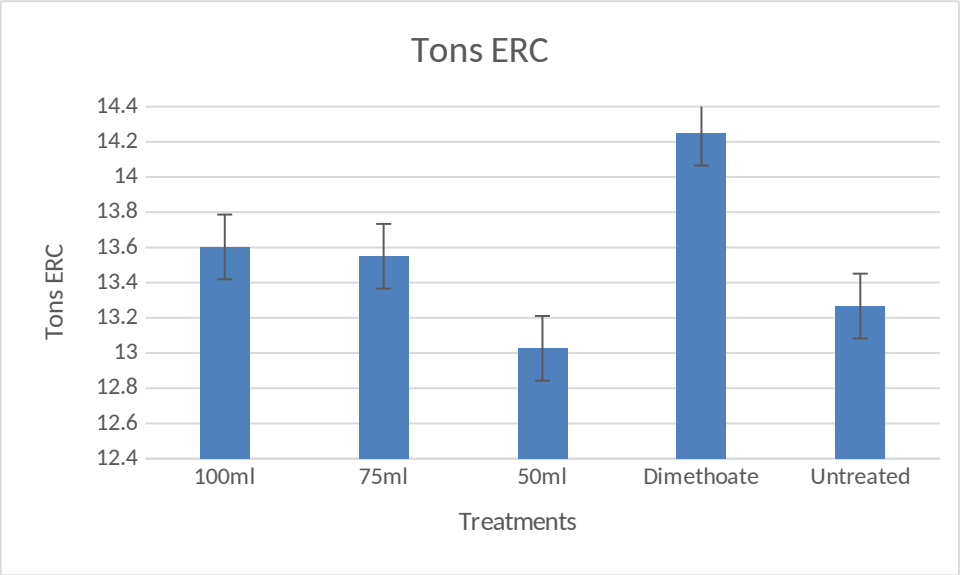

Fig.14: Tons ERC of sugarcane

100ml and 75ml neem seed solution treated sugarcane plants yielded 13.603 t and 13.55t respectively. 50ml yielded 13.027tons of ERC. The untreated sugarcane plants had 13.267t of ERC as shown in fig 14 above.

4.20 Discussion

4.20.1 Effect of neem seed extract on number of leaves damaged

At 6 months after planting there was a significant difference ($p<0.05$) in the number of leaves. The mean number of leaves ranged from a low of 4.0 leaves per plant in the dimethoate treatment to a high of 29.3 leaves per plant on the untreated control. On average the number of leaves damaged on the plots treated with neem seed solutions are 11. Plots treated with dimethoate which is the control have an average leaf damage of 4. This showed that there is potential on neem seed extracts because of low value of 11 leaves versus 4 on the control. Saxena (1989) reported that *Azadirachta*

indica the main pesticidal component of neem extract especially neem seed solution possessed feeding deterrent and repellent properties. Morde & Blackwell (1993) reported that antifeedant, repellent, and insect growth regulatory effects are present in neem product which can be used for insect management in crop production. Similar finding was also reported by Saha (2006). Azadirachtin can act as a feeding deterrent against a number of insect pests including yellow sugarcane aphid (Schumtter, 1990). *Azadirachta indica* reduced the level of the insect hormone Ecdysone by disrupting the insect's molting process so that the immature larvae did not develop into adults. Or the immature larvae and nymphs remain in an immature stage and then die (Stone, 1992). Neem seed solution treatment did not have immediate knockdown effect and insects continued to feed. However, due to its repellent effects, insect feeding was reduced. *Azadirachta indica* properties which include antifeedant, repellence and spermaticide were evident and supported by downward trend of leaf damage from 3 months up to 5 months on plots treated with neem seed solution.

4.20.2 Effect of neem seed extract on aphid colonies

Yellow sugar cane aphid colonies significantly reduced from an average of 7on neem treated plots to an average of 2. Nisbert (2000) found out that *Azadirachtin* induces sterility in yellow sugarcane aphid by preventing oviposition and interrupting in sperm production in males.

Moreover, neem seed solution influenced fecundity in female insects in a dose-dependent manner. *Azadirachta indica* prevented oviposition by inhibiting oogenesis and synthesis of ovarian ecdysteroid. In males, *Azadirachta indica* acted by interrupting the meiosis process responsible for sperm production, Nisbert (2000).

Aphid colonies may also have declined significantly on neem treated plots because, Isman, (1991) suggested that neem seed solution offers immense anti feedant

properties due to its efficacy in suppressing the feeding sensation in insects. Jennifer and Mordue (1998) stated that the feeding behaviour of yellow sugarcane aphid is dependent on the neural inputs received from the chemical sensors of the aphid. Manifestation of antifeedancy by *Azadirachta indica* occurred through the stimulation of deterrent cells in these chemoreceptors and by blocking the feeding stimulation in yellow sugarcane aphids by firing the sugar receptor cells.

Nisbert (2000) also supported neem insecticidal properties by stating that it acts as an anti-fertility agent. Whyte (1990) stated that *Azadirachta indica* injection led to physiological effects in the insect's mid gut which in turn causes a reduction in the post-ingestive efficiency. Reduction in efficiency is as a result of disturbances in the hormonal as well as physiological systems. These hindrances take form of hindrance in the food movement through the insects' mid gut and inhibition in production of digestive enzymes. The formation of juvenile stages during each moult is controlled by the juvenile hormone from the corpora allata (Nisbert, 2000). Disruption in these events by *Azadirachta indica*, led to various sterility and moulting defects. Moreover, cellular uptake of *Azadirachta indica* inhibits both cell necrosis and flaccid paralysis of muscles (Nisbert, 2000).

4.20.3 Effect of neem seed extract on Tons ERC.

Nuessly & Hentz (2002) reported that prolonged feeding can result in premature leaf senescence and stalk death. In Florida (USA), where *S. flava* is a major pest of economic importance (Reagan, 1994), when two leaves are damaged due to aphid feeding when the crop is three months old, there may be a 5% lower final yield. Leaf chlorosis and death of three pairs or more of active growing leaves can result in 15% yield loss (Reagan, 1994). According to Hall (2001) yield reduction and reduced

tillering are usually caused by feeding damage to early plant growth stages; however, late season crops may also suffer yield loss.

Feeding on sugarcane leaves by *M. sacchari* causes minimal symptomology until heavy infestations lead to growth of black sooty mold on leaves covered in aphid-produced honeydew. Devastating impacts of *M. sacchari* infestations on yields of sugarcane are common but direct effect of feeding on sugarcane yields are not well understood. Aphid feeding reduced chlorophyll content in leaves and removes amino acids (Hall, 2001). Formation of sooty mold results from growth of a complex of fungi on honeydew, and is thought to reduce the surface area available for photosynthesis. Presumably, the development of sooty mold reduces photosynthesis negatively impacting plant growth.

Damage by yellow sugarcane aphid on sugarcane plant produced for sugar and ethanol occurs in several ways, depending on the stage of plant development when aphids infest the crop, aphid population increase, and use of the crop. Sugarcane aphids remove plant sap when feeding on the underside of sugarcane leaves and along the stalk. Based on field observations and the literature, plant damage from sugarcane aphid results from a combination of direct loss of plant nutrients and sugars during feeding, which can be exacerbated by plant water stress, and reduction in photosynthetic efficiency due to sooty mold build-up from honeydew excreted by aphids (Singh, 2004)

Symptoms of aphid damage include purpling of young plants, which can lead to stunting, chlorosis, and necrosis of maturing leaves (Singh, 2004). Often during initial field infestation and populations increase, the foliage remains green despite readily detectable aphids on the underside of leaves. As aphid population increase and

feeding injury intensifies, leaves change color to yellow, purple, and, finally, brown as leaf health declines.

Plant mechanisms to reduce stress caused by insects and pests are directly and indirectly related to physiologic processes such as respiration, transpiration, and photosynthesis. Photosynthesis influences plant biomass accumulation, and plants exhibiting high photosynthetic rates may result in higher yields (Haile, 2001). This mean that sugarcane yield (ERC ton) is positively related to pest infestation. In addition to yield reductions, plant injury can also negatively affect yield quality, and reductions in quality have been reported for sugarcane-infested plants (Madaleno, 2008; White, 2008; Ravaneli, 2011). Also, sucking insects, in general, may remove plant tissue affecting physiological processes, release saliva that is toxic to the plants, and cause tissue necrosis (Fewkes, 1969; Haile, 2001).

Therefore, plants under both abiotic and biotics tress or may have a reduction in nutrients and water flow to leaves (Culy, 2001) and result in a decrease of accumulated biomass (Vaadia, 1985). The effect of photosynthetic rate reduction was reflected on yield at harvest. Plants with sugarcane aphids infestation (individually or combined) showed thinner stalks, and some plants completely had dried leaves. Similar results were also reported by Dinardo & Miranda (2003). In this study the diameter and length of stalks were significantly affected when spittlebug was present. This impact in the stalks was caused by the spittlebug nymphs whose feeding injures the roots affecting phloem and xylem flow of water and nutrients such as nitrogen, phosphorus, potassium, calcium, and glucose (Garcia, Botelho & Parra, 2006).

4.20.4 Effect of neem seed extract on Pol% and Brix%

Yellow sugarcane aphid affected the content of soluble solids (Brix) and the apparent percentage of sucrose in the juice (Pol) of sugarcane plants as shown in table 1 causing significant reduction in Brix and Pol on 100ml and 50ml neem extract treated plots, dimethoate and untreated plots. This result is probably due to the fact that neem seed extract acts higher on 75ml neem extract concentrations. Other studies have also pointed out the increase in sugarcane Brix and Pol due to the effects of 75ml treatment whereby yellow sugarcane aphid was restricted from sapping the leaves thereby leading to crop having adequate chlorophyll needed by the crop thus characterising optimum consumption (Franco2008; Megda 2012). Healthy sugarcane crop is usually associated with greater vegetative growth which invariably leads to plants with higher water contents, but with impairment is sucrose accumulation due to nitrogen fertilisation (Megda, 2012). These impairments result from the conversion of sucrose into simple sugars directed to vegetative growth, as a consequence of the nitrogen levels associated with less sap feeding (Bahrani, 2009). Brix is a by-product of photosynthesis. Ball (2006) stated that all other factors being equal a plant with high brix makes more food.75ml neem treated plants yielded more brix and polarization because yellow aphid colonies were controlled significantly. Aphid colonization leads to decrease in the photosynthesis ranging from 25-75% depending on the cultivar and age. Aphid colonization reduces stomatal conductance and then photosynthesis, probably resulting in negative effect on assimilation, biomass accumulation and other important physiological processes (Haile, 1999).

4.21 Summary

Neem seed extracts showed great potential on controlling yellow sugarcane aphid population. Besides controlling aphid population, neem seed solution is also

environmentally friendly. Beneficial insects were not killed or repelled by the solution meaning to say the solution can well be embraced as an integrated pest management tool.

Cane juice quality parameters (Pol, Brix, ERC, and ERC TONS) showed improved percentage as compared to untreated sugarcane. This is because if aphids are not controlled photosynthesis will be negatively affected. Photosynthesis rate is a function of leaf color, leaf size, and total leaf area. This means that leaf sapping by aphids will lead to chlorosis of leaves, leaf senescence thereby reducing rate of photosynthesis and assimilation of biomass.

The significance differences in number of leaves damaged, aphid colonies and leaf damage score showed that the 5 treatments affected sugarcane differently. Results showed that dimethoate followed by 75ml neem seed solution/ 15ml water, 100ml neem seed solution / 15ml water and 50ml neem seed solution/ 15ml water had less aphid colonies, a smaller number of damaged leaves and low leaf damage score. This indicates the potential of neem seed solution on controlling yellow sugarcane aphid and improving sugarcane yield.

CHAPTER 5 SUMMARY, CONCLUSION AND RECOMMENDATIONS

5.1 Introduction

During 2013, yellow sugarcane aphid (Hemiptera: Aphidae) was recorded for the first time in the Southern Africa sugarcane industry (Conlong & Way, 2014). Although N14 variety is susceptible to yellow sugarcane aphids, sugarcane growers still grow N14 because it is one of the highest yielding varieties (Zhou, 2004). Several methods of controlling yellow sugarcane aphids, such as break crops (soybeans), chemical applications such as Malathion, dimethoate have been adopted by sugarcane growers in Zimbabwe (Conlong & Way, 2014). These methods alone cannot control yellow sugarcane aphids. This has led to this research being conducted so as to widen the options for controlling aphids. Neem seed solution has shown great potential in controlling yellow sugarcane aphid. When 75ml neem seed solution was applied at 5 months total number of damaged leaves was 19.67. The number significantly declined to 12 in the 6th month. Leaf damage score significantly declined from 3 to 2 after 75ml neem seed solution application. Aphid colonies also showed how effective neem seed solution is after application. There was a significant drop of aphid colonies from 7.13 to 0.640 signalling the pesticidal effects of neem seed solution. Yellow sugarcane aphids need to be controlled so as to have better cane juice quality that is (Brix%., Pol

%, ERC and ERC Tons). 75 ml neem seed solution resulted in a significant higher mean Pol % of 13.56%. This is so because if yellow sugarcane aphid is not controlled the content of soluble solids and the apparent percentage of sucrose in the juice of sugarcane plants will be affected greatly. There was a significant difference ($P<0.05$) on the mean Tons ERC among 5 treatments. 50 ml and untreated control have significantly lower cane yields of 13.03 tons and 13.27 tons respectively.

However, there is limited literature on the effect of yellow sugarcane aphids on the Zimbabwe Sugar Industry as a whole. This research provides some information which is useful on the fight against yellow sugarcane aphid. The overall objective of this study was to determine the effects of neem seed solution (*Azadirachta indica*) on yellow sugarcane aphid (*Sipha flava*) in the South East Lowveld of Zimbabwe.

5.2 Discussion

The neem seed extracts (*Azadirachta indica*) has been widely studied because it presents a great number of compounds with insecticidal properties and is effective on reducing population of several pest species including Yellow Sugarcane aphid (*Sipha flava*) Hemiptera: Aphidae (Jacobson 1989, Schmutterer 1990, & Saxena 1997. Its main active compound, azadirachtin, is toxic to over 500 insect species and acts mainly as food deterrent and growth disruptor. Martinez &Emden (2001) stated that a dose of azadirachtin induces sterility in insects by preventing oviposition and interrupting in sperm production in males. This insect static effect, also observed in other species (Rembold 1995) favors the neem seed solution, because it extends the exposure time of the pests to natural enemies (Martinez 2002). Another factor favorable to this is the fact that azadirachtin presents higher toxicity by ingestion than by contact (Lowery & Isman 1995, Martinez & Carvalho 1996). This makes this compound potentially less toxic to natural enemies in the field, since these insects

would not feed directly on the plants. Besides, 90% of the azadirachtin is eliminated from the insect body until 7hours after ingestion (Rembold, 1984), thus reducing the concentration ingested by the natural enemies that feed on preys that could have fed on treated plants.

5.3 Conclusion

Although yellow sugarcane aphids seldom kill a mature plant, the damage they do and unsightly honeydew they generate sometimes warrant control. Consideration needs to be mainly put on the non-chemical controls as most insecticides will destroy beneficial insects along with the pest. Nuessly & Hentz (2002) argued that insecticides can have negative knock-on effects, e.g., various predator populations, including coccinellids were reduced for up to 10 weeks by in-furrow applications of nematicide-insecticide aldicarb during the spring and fenvalerate (pyrethroid insecticide) as a foliar spray enhanced *Sipha flava* populations by 63% while substantially suppressing certain predator groups. Destruction of beneficial insects and pests by chemical insecticides has led to the advocacy and promotion of botanicals in the Integrated Pest Management. Botanicals such as Neem Seed *(Azadirachta indica*) easily biodegrades and there is no build up and traces of active chemicals left by the use of neem seed solution hence yellow sugarcane aphid population is controlled while improving sugarcane yield. Management tactics are being slowly developed and should natural enemies, weather and botanicals fail to keep populations in check, then resistant/ tolerant varieties and insecticides could be considered.

5.4 Implications

Schmutterer (1990) stated that the ovicide action of neem oil treatments is common, and the product can obstruct the egg membrane, thus impeding the respiratory changes of the embryo, in a dose-dependent manner. The action of the growth regulators present in the neem seed solution inhibits oogenesis and synthesis of ovarian ecdysteroid. The ovicide effect of neem oil-based products on phytophagous insects was also reported by Souza & Vendramim (2000). Neem seed solution seems remarkably benign to spiders, butterflies, and insects such as bees that pollinate crops and trees, ladybugs that consume aphids and wasps that act as parasites on various crop pests. In the main, this is because neem products must be ingested to be effective. Thus, insects that feed on plant tissues succumb, while those that feed on nectar or other insects rarely contact significant concentrations of neem products (Saxena, 1987).

In Males, neem seed solution's active ingredient *azadirachtin* acts by interrupting the meiotic process responsible for sperm production (Nisbert, 2000). If sperm production is negatively affected by *azadirachtin* yellow sugarcane aphid colonies will decline significantly and as a result yield of sugarcane will be high since rate of photosynthesis will not have been interrupted by sapping of chlorophyll. Sugarcane yield is a function of cane height, stalk diameter, number of stalks, leaf colour, number of plants leaves just to mention a few. Nymphs and adults feed on plant juices and attacking leaves especially succulent and new growth. If *Sipha flava* is controlled early then final yield will be a bumper harvest.

5.5 Recommendations

Recently emerging issues regarding the increasing prevalence of pest resistance has prompted the adoption of alternative strategies with special emphasis on integrated

pest managements. Neem is an ideal alternative candidate as a natural non-synthetic plant pesticide. Over the years, numerous researches have validated its pesticidal activity. It is a cost effective and eco-friendly alternative to the commercial chemically synthesized pesticides.

Schmutterer (1990) stated that neem seed solution, in general, is safe to several species of natural enemies, although some species show higher susceptibility (Hough-Goldstein & Keil 1991). Therefore, studies are necessary to better understand the neem action on natural enemies, to support recommendations of neem use in pest control. The objective of this research was to determine the effect of neem seed solution on yellow sugarcane aphid in the South East Lowveld of Zimbabwe.

5.6 Suggestions for further research

Synthetic insecticides have long been used in the agricultural sector and for example in cotton protection, resulting in pest resistance, toxicity and environmental pollution. Bio pesticides have been suggested as alternatives to synthetic pesticides. Further research needs to show the relationship between yellow sugarcane aphids and the yield. Emphasis needs to be put on which stage of sugarcane is mainly affected by aphids. Other notable area which needs further research include planting dates, tolerance of sugarcane varieties to yellow sugarcane aphids, use of neem tree extracts such as roots, leaves and barks rather than concentrating on the neem seed.

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APPENDICES

APPENDIX 1: Number of Leaves Damaged by Yellow Sugarcane Aphids at 5 months

One-way ANOVA: at 5 months after planting versus Treatment

```
Analysis of Variance for number of leaves damaged at 5 months
Source     DF        SS        MS        F        P
Treatment    4      20.7       5.2     0.20    0.930
Error       10     252.7      25.3
Total       14     273.3
            Individual 95% CIs for Mean
                                  Based on Pooled StDev
Level       N      Mean     StDev----------+---------+---------+------
1           3    19.667     3.786        (------------*------------)
2           3    19.667     3.215        (------------*------------)
3           3    16.667     5.686 (------------*------------)
4           3    18.000     6.245     (------------*------------)
5           3    17.667     5.508    (------------*------------)
                                  ----------+---------+---------+------
Pooled StDev =    5.027                   15.0      20.0      25.0
```

APPENDIX 2: Number of Leaves Damaged by Yellow sugarcane Aphid at 5.5 months

One-way ANOVA: at 5.5 months after planting versus Treatment

```
Analysis of Variance for at 5.5 m
Source     DF        SS        MS        F        P
Treatment    4     430.3     107.6     3.40    0.053
```

```
Error      10     316.7      31.7
Total      14     746.9
                                  Individual 95% CIs For Mean
                                  Based on Pooled StDev
Level       N      Mean     StDev---------+---------+---------+-------
1           3    21.667     8.963               (-------*------)
2           3    19.333     5.859             (------*-------)
3           3    15.333     3.055          (------*-------)
4           3     9.000     3.606   (------*------)
5           3    24.333     4.619                    (------*-------)
                                  ---------+---------+---------+-------
Pooled StDev =    5.627                   10        20        30
```

APPENDIX 3: Number of Leaves Damaged by Yellow Sugarcane Aphid at 6 months

One-way ANOVA: at 6 months after planting versus Treatment

```
Analysis of Variance for at 6 months
Source     DF        SS        MS        F        P
Treatment    4    1095.7     273.9     6.40    0.008
Error      10     428.0      42.8
Total      14    1523.7
            Individual 95% CIs for Mean
                                  Based on Pooled StDev
Level       N      Mean     StDev----+---------+---------+---------+--
1           3    12.667     0.577          (------*------)
2           3    13.000     2.000          (------*------)
3           3     8.667     3.512      (------*------)
4           3     4.000     1.000 (------*------)
5           3    29.333    14.012                       (------*------)
                                  ----+---------+---------+---------+--
Pooled StDev =    6.542               0        12        24        36
```

APPENDIX 4: Number of Tillers

One-way ANOVA: at 5 months after planting versus Treatment

```
Analysis of Variance for at 5 months
Source     DF        SS        MS        F        P
Treatment    4      1.73      0.43     0.36    0.831
Error      10     12.00      1.20
Total      14     13.73
            Individual 95% CIs for Mean
                                  Based on Pooled StDev
Level       N      Mean     StDev--------+---------+---------+--------
1           3     2.000     1.000     (-----------*----------)
2           3     2.000     0.000     (-----------*----------)
3           3     1.667     1.528 (-----------*----------)
4           3     2.667     1.155          (-----------*-----------)
5           3     2.333     1.155        (----------*-----------)
                                  --------+---------+---------+--------
Pooled StDev =    1.095                 1.2       2.4       3.6
```

APPENDIX 5: Number of Tillers

One-way ANOVA: at 5.5 months after planting versus Treatment

```
Analysis of Variance for at 5.5 m
Source     DF        SS        MS        F        P
Treatment   4     0.667     0.167     0.36    0.833
Error      10     4.667     0.467
Total      14     5.333
            Individual 95% CIs for Mean
                                  Based on Pooled StDev
Level       N      Mean     StDev---------+---------+---------+-------
1           3    1.3333    0.5774       (------------*------------)
2           3    1.3333    0.5774       (------------*------------)
3           3    1.0000    1.0000   (------------*------------)
4           3    1.6667    0.5774            (------------*------------)
5           3    1.3333    0.5774       (------------*------------)
                                  ---------+---------+---------+-------
Pooled StDev =   0.6831                  0.70      1.40      2.10
```

APPENDIX 6:Number of Tillers

One-way ANOVA: at 6 months after planting versus Treatment

```
Analysis of Variance for at 6 months
Source     DF        SS        MS        F        P
Treatment   4     0.667     0.167     0.25    0.903
Error      10     6.667     0.667
Total      14     7.333
            Individual 95% CIs for Mean
                                  Based on Pooled StDev
Level       N      Mean     StDev-------+---------+---------+---------
1           3    2.0000    0.0000           (------------*------------)
2           3    1.6667    0.5774       (------------*------------)
3           3    1.6667    1.1547       (------------*------------)
4           3    1.3333    0.5774   (------------*------------)
5           3    1.6667    1.1547       (------------*------------)
                                  -------+---------+---------+---------
Pooled StDev =   0.8165               0.80      1.60      2.40
```

APPENDIX 7: Leaf Damage

One-way ANOVA: at 5 months after planting versus Treatment

```
Analysis of Variance for at 5 months
Source     DF        SS        MS        F        P
Treatment   4    0.2667    0.0667     1.00    0.452
Error      10    0.6667    0.0667
Total      14    0.9333
            Individual 95% CIs for Mean
                                  Based on Pooled StDev
Level       N      Mean     StDev---+---------+---------+---------+---
1           3    3.0000    0.0000              (----------*----------)
2           3    2.6667    0.5774   (----------*----------)
3           3    3.0000    0.0000              (----------*----------)
4           3    3.0000    0.0000              (----------*----------)
5           3    3.0000    0.0000              (----------*----------)
                                  ---+---------+---------+---------+---
Pooled StDev =   0.2582             2.40      2.70      3.00      3.30
```

APPENDIX 8: Leaf Damage

One-way ANOVA: at 5.5 months after planting versus Treatment

```
Analysis of Variance for at 5.5 m
Source     DF        SS        MS        F        P
Treatment   4     0.400     0.100     0.50    0.737
Error      10     2.000     0.200
Total      14     2.400
            Individual 95% CIs for Mean
              Based on Pooled St Dev
Level       N      Mean     StDev--+---------+---------+---------+----
1           3    2.3333    0.5774         (-----------*----------)
2           3    2.3333    0.5774         (-----------*----------)
3           3    2.0000    0.0000 (-----------*-----------)
4           3    2.3333    0.5774         (-----------*----------)
5           3    2.0000    0.0000 (-----------*-----------)
                                  --+---------+---------+---------+----
Pooled StDev =   0.4472           1.50      2.00      2.50      3.00
```

APPENDIX 9: Leaf Damage

One-way ANOVA: at 6 months after planting versus Treatment

```
Analysis of Variance for at 6 months
Source     DF        SS        MS        F        P
Treatment   4     0.462     0.115     1.12    0.402
Error      10     1.035     0.103
Total      14     1.497
                                  Individual 95% CIs for Mean
                                  Based on Pooled StDev
Level       N      Mean     StDev  --------+---------+---------+--------
1           3    2.3333    0.5774               (-----------*----------)
2           3    2.3200    0.3305              (-----------*-----------)
3           3    1.8867    0.1963  (-----------*-----------)
4           3    2.0000    0.0000     (-----------*-----------)
5           3    2.1100    0.1905        (-----------*-----------)
                                  --------+---------+---------+--------
Pooled StDev =   0.3217                 1.75      2.10      2.45
```

APPENDIX 10: Aphid Colonies

One-way ANOVA: at 5 months after.panting-3 versus Treatment

```
Analysis of Variance for at 5 months
Source     DF        SS        MS        F        P
Treatment   4       578       145     0.41    0.796
Error      10      3509       351
Total      14      4087
            Individual 95% CIs for Mean
                                  Based on Pooled StDev
Level       N      Mean     StDev----+---------+---------+---------+--
1           3     53.33     25.48           (-----------*-----------)
2           3     47.67     24.42        (-----------*-----------)
3           3     47.33     18.04        (-----------*-----------)
4           3     36.33      3.51 (-----------*-----------)
```

```
5           3     39.00     13.08   (------------*-----------)
                                    ----+---------+---------+---------+--
Pooled StDev =    18.73               20        40        60        80
```

APPENDIX 11: Square root transformed data for aphid colonies at 5 months after planting

One-way ANOVA: Transformed 5.0 versus Treatment

```
Analysis of Variance for Transformed
Source     DF        SS        MS        F        P
Treatment    4      2.57      0.64     0.33    0.852
Error       10     19.56      1.96
Total       14     22.14
            Individual 95% CIs for Mean
                                    Based on Pooled StDev
Level       N      Mean     StDev--+---------+---------+---------+----
1           3     7.130     1.935          (-----------*-----------)
2           3     6.763     1.724       (-----------*-----------)
3           3     6.793     1.320       (-----------*-----------)
4           3     6.020     0.291 (-----------*-----------)
5           3     6.177     1.112   (-----------*-----------)
                                    --+---------+---------+---------+----
Pooled StDev =    1.399            4.5       6.0       7.5       9.0
```

APPENDIX 12: Aphid Colonies

One-way ANOVA: at 5.5 months after planting-3 versus Treatment

```
Analysis of Variance for at 5.5 m
Source     DF        SS        MS        F        P
Treatment    4      6464      1616     2.34    0.126
Error       10      6914       691
Total       14     13378
            Individual 95% CIs for Mean
                                    Based on Pooled StDev
Level       N      Mean     StDev  ---------+---------+---------+-------
1           3     27.33     29.74         (--------*-------)
2           3     42.33     21.13             (--------*-------)
3           3     24.00     18.52         (-------*-------)
4           3      0.33      0.58   (-------*--------)
5           3     63.00     42.23                  (--------*-------)
                                    ---------+---------+---------+-------
Pooled StDev =    26.29                    0        40        80
```

APPENDIX 13: Square root transformed data for aphid colonies at 5.5 months after planting

One-way ANOVA: Transformed 5.5 versus Treatment

```
Analysis of Variance for Transformed
Source     DF        SS        MS        F        P
Treatment    4     91.75     22.94     3.79    0.040
Error       10     60.50      6.05
Total       14    152.25
            Individual 95% CIs for Mean
                                    Based on Pooled StDev
Level       N      Mean     StDev--------+---------+---------+--------
1           3     4.160     3.880            (-------*-------)
2           3     6.333     1.850                  (-------*-------)
3           3     4.603     2.062              (-------*------)
4           3     0.333     0.577   (-------*-------)
5           3     7.630     2.681                      (-------*-------)
                                    --------+---------+---------+--------
Pooled StDev =    2.460                  0.0       4.0       8.0
```

APPENDIX 14: Aphid Colonies

One-way ANOVA: at 6 months after planting - 3 versus Treatment

```
Analysis of Variance for at 6 months
Source     DF        SS        MS        F        P
Treatment    4      8931      2233     5.76    0.011
Error       10      3874       387
Total       14     12805
            Individual 95% CIs for Mean
                                    Based on Pooled StDev
Level       N      Mean     St Dev --------+---------+---------+--------
1           3      0.64      0.67   (------*------)
2           3      5.22      7.61    (------*-------)
3           3      6.55      9.93     (------*------)
4           3      0.00      0.00   (------*------)
5           3     63.78     42.19                     (------*------)
                                    --------+---------+---------+--------
Pooled StDev =    19.68                   0        35        70
```

APPENDIX 15: Aphid Colonies at 6 months after planting Square root transformed data

One-way ANOVA: Transformed versus Treatment

```
Analysis of Variance for Transformed
Source     DF        SS        MS        F        P
Treatment    4    111.44     27.86    10.13    0.002
Error       10     27.50      2.75
Total       14    138.94
            Individual 95% CIs for Mean
                                    Based on Pooled StDev
Level       N      Mean     St Dev -------+---------+---------+---------
1           3     0.640     0.586     (-----*-----)
2           3     1.850     1.640        (-----*-----)
3           3     1.973     1.950         (-----*-----)
4           3     0.000     0.000   (-----*-----)
```

```
5           3     7.673     2.630                          (-----*-----)
                                          -------+---------+---------+---------
Pooled StDev =    1.658                  0.0       3.5       7.0
```

APPENDIX 16: One-way ANOVA - Pol% versus Treatment

```
Analysis of Variance for Pol%
Source     DF        SS        MS        F        P
Treat       4   0.50843   0.12711    18.04    0.000
Error      10   0.07047   0.00705
Total      14   0.57889
            Individual 95% CIs for Mean
                                  Based on Pooled StDev
Level       N      Mean     St Dev -----+---------+---------+---------+--
1           3   13.1200    0.0529       (----*----)
2           3   13.5600    0.1706                                (----*----)
3           3   13.0400    0.0200   (----*----)
4           3   13.1267    0.0503       (----*-----)
5           3   13.1500    0.0200        (-----*----)
                                  ----+---------+---------+---------+--
Pooled StDev =   0.0839             13.00     13.20     13.40     13.60
```

APPENDIX 17: One-way ANOVA - Brix% versus Treatment

```
Analysis of Variance for Brix%
Source     DF        SS        MS        F        P
Treat       4     9.621     2.405     3.91    0.037
Error      10     6.152     0.615
Total      14    15.772
            Individual 95% CIs for Mean
                                  Based on Pooled StDev
Level       N      Mean     St Dev -----+---------+---------+---------+-
1           3    15.767     0.153                    (-------*--------)
2           3    15.650     0.350                   (-------*--------)
3           3    13.667     1.528 (--------*-------)
4           3    15.667     0.577                   (--------*-------)
5           3    15.567     0.513                  (--------*-------)
                                  -----+---------+---------+---------+-
Pooled StDev =    0.784              13.2      14.4      15.6      16.8
```

APPENDIX 18: One-way ANOVA - Cane Tons versus Treatment

```
Analysis of Variance for Cane Ton
Source     DF        SS        MS        F        P
Treat       4    35.412     8.853     9.27    0.002
Error      10     9.550     0.955
Total      14    44.961
                                  Individual 95% CIs for Mean
                                  Based on Pooled StDev
Level       N      Mean     St Dev -+---------+---------+---------+-----
1           3   122.167     0.666                  (-----*-----)
2           3   121.887     0.677                (-----*------)
3           3   119.250     1.517   (-----*------)
4           3   122.917     0.580                      (------*-----)
5           3   119.277     1.111   (-----*------)
                                  -+---------+---------+---------+-----
Pooled StDev =    0.977         118.0     120.0     122.0     124.0
```

APPENDIX 19: One-way ANOVA - ERC% versus Treatment

```
Analysis of Variance for ERC%
Source     DF        SS        MS        F        P
Treat       4    0.3446    0.0862     1.36    0.316
Error      10    0.6353    0.0635
Total      14    0.9800
           Individual 95% CIs for Mean
                                  Based on Pooled StDev
Level       N      Mean     StDev----+---------+---------+---------+--
1           3    11.363     0.382              (----------*----------)
2           3    11.247     0.186          (----------*----------)
3           3    11.030     0.020   (----------*---------)
4           3    11.430     0.356               (----------*----------)
5           3    11.100     0.100     (----------*----------)
                               ----+---------+---------+---------+--
Pooled StDev =    0.252             10.80     11.10     11.40     11.70
```

APPENDIX 20: One-way ANOVA - Tons ERC versus Treatment

```
Analysis of Variance for Tons ERC
Source     DF        SS        MS        F        P
Treat       4    2.5393    0.6348    28.47    0.000
Error      10    0.2230    0.0223
Total      14    2.7623
           Individual 95% CIs for Mean
                                  Based on Pooled StDev
Level       N      Mean     St Dev ----+---------+---------+---------+--
1           3    13.603     0.200              (---*---)
2           3    13.550     0.132             (---*---)
3           3    13.027     0.015   (---*--)
4           3    14.250     0.180                            (---*---)
5           3    13.267     0.146       (---*---)
                               ----+---------+---------+---------+--
Pooled StDev =    0.149             13.00     13.50     14.00     14.50
```