**FACULTY OF AGRONOMY AND FORESTRY ENGINEERING**

# EVALUATION OF THE OCCURENCE OF PARASITOIDS ASSOCIATED WITH THE INVASIVE COCONUT WHITEFLY (*Aleurotrachelus atratus*) IN INHAMBANE PROVINCE, MOZAMBIQUE

**BY**

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**A thesis submitted to the Faculty of Agronomy and Forestry Engineering, in fulfilment of the requirements for the award of a Master of Science (MSc) in crop protection at Eduardo Mondlane University, Maputo, Mozambique**

**Maputo, June 2016**

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## DECLARATION BY THE CANDIDATE

I, Ronald Kityo, hereby declare that:

This thesis is a representation of my original research work. All content and ideas drawn directly or indirectly from external sources are indicated as such. The thesis has not been submitted to any other examining body or any other university and has not been published. It does not contain other person's data, pictures, graphs or other information, unless acknowledged as being sourced from such persons. It does not contain text, graphics or tables copied and pasted from the internet unless specifically acknowledged as so and the source being detailed in the thesis reference section.

This work was produced under the supervision of Prof. Domingos Cugala at Eduardo Mondlane University, Maputo, Mozambique.

_______________________________________ ___/___/2016

Signature Date

Ronald Kityo (MSc. candidate)

I confirm that the work reported in this thesis was carried out by the candidate under my supervision.

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_______________________________________ ___/___/2016

Signature Date

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# DEDICATION

This thesis is dedicated to my incredible brother, Godfrey who has been both a brother and father in my life since my childhood and whose examples have taught me the value of hard work and whose hard work and sacrifice made me what I am today.

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# ACRONYMS

ANOVA: Analysis of Variance

CABI: Centre for Agriculture and Bioscience International

CIRAD: Centre de Cooperation Internationale en Recherche Agronomique pour le Dė veloppement (Agricultural Research Centre for International Development)

EdM: Electricidade de Moçambique (Electricity of Mozambique)

FAEF: Faculdade de Agronomia e Engenharia Florestal (Faculty of Agronomy and Forestry Engineering)

FAS: Foreign Agricultural Services

FISP: Farm Income Support Project

GPS: Geographical Positioning System

IAS: Invasive Alien Species

INRAPE: Institut National de Recherche pour l'Agriculture, la Pêche et l'Environnement (The Agriculture, Fisheries and Environment Research Institute).

ISC: Invasive Species Compendium

IUCN: International Union for Conservation of Nature

NIFOR: Nigerian Institute for Oil Palm Research

PAR: Permanent Agriculture Resources

PRPV: "Crop Protection Network for the Indian Ocean"

UEM: Universidade Eduardo Mondlane (Eduardo Mondlane University)

UK: United Kingdom

US: United States

USA: United States of America

WMO: World Meteorological Organization

## ABSTRACT

The coconut whitefly, *Aleurotrachelus atratus* Hempel (Homoptera; Aleyrodidea) is a highly invasive pest of coconut and ornamental palms (Arecaceae). In Mozambique, it was first detected in 2011 and 100% of infested plants have been reported. Currently, biological control is the most preferred, safest and nontoxic method in controlling invasive pest species, such as *A. atratus*. Since its first detection in Mozambique, no parasitoids were known being associated with this pest. A study was conducted to evaluate the occurrence of parasitoids associated with *A. atratus* as a basis for the introduction of classical biological control in Inhambane province. Data from samples collected from five districts of the province in September and December 2015 showed that whitefly infestation was 99.9±0.14% with no significant differences among districts and between sampling periods. Whitefly severity varied between severe to very severe with no significant differences among districts but differed significantly between sampling periods, being higher in September compared to December. Mean whitefly density for the province was 26.5±1.2 larvae per leaflet. There were no significant differences among districts but sampling periods differed significantly in terms of whitefly density, being higher in September compared to December. Four parasitoid species being associated with *A. atratus* were recovered during the study period including; *Encarsia basicincta*, *Eretmocerus cocois*, *Encarsia sp*. and *Signiphora sp*. with parasitism rates of; 4.08%, 0.22%, 5.99% and 0.45% respectively. Overall parasitism was 10.74±2.03% varying significantly among districts. The recovery of *Encarsia basicincta* and *Eretmocerus cocois* from the coconut whitefly is an indication that *A. atratus* was introduced with parasitoids considered efficient for the suppression of its population in its native range and it may constitute potential biological control agents against the invasive whitefly in Mozambique. Therefore, the national phytosanitary authorities should consider development of integrated pest management (IPM) including classical biological control and augmentative approaches to reduce the pest population, crop damage and yield loss.

**Key words**: Coconut palm, *Aleurotrachelus atratus*, parasitoids, parasitism

## RESUMO

A mosca branca do coqueiro, *Aleurotrachelus atratus* Hempel (Homoptera: Aleyrodidae) é uma praga altamente invasiva do coqueiro e plantas ornamentais (Arecaceae). Em Moçambique, foi dectetada pela primeira vez em 2011 e 100% de plantas infestadas foram relatadas. Actualmente, o controlo biológico é o método mais preferido, mais seguro e não tóxico para controlar espécies de pragas invasivas como *A. atratus*. Desde a sua primeira detecção em 2011, não são conhecidos parasitóides associados a esta praga. O estudo foi realizado para avaliar a ocorrência de parasitóides associados à *A. atratus* como base para a introdução de um programa de controlo biológico clássico na província de Inhambane. Os dados de amostras coletados em cinco municípios da província, nos meses de Setembro e Dezembro de 2015 mostraram que a infestação pela moca branca foi de 99.9 ± 0.14%, sem diferenças significativas entre os distritos (P = 0.4243, α = 5%) e entre os períodos de amostragem. O nível de severidade variou de severo a muito severo sem diferenças significativas, mas diferenças significativas entre os períodos de amostragem foram observadas onde valores mais altos foram observados em Setembro comparativamente a Dezembro. A densidade média da mosca branca na provincia foi de 26.5 ± 1.2 pupas por folíolo. Ao nível dos distritos avaliados não houve diferença significativa em termos de densidade media da mosca branca, tendo se verificado apenas diferenças significativas entre os períodos de amostragem que foram mais altas em Setembro comparativamente a Dezembro. Quatro espécies de parasitóides associadas a *A. atratus* foram recuperados durante o estudo: *Encarsia basicincta*, *Eretmocerus cocois*, *Encarsia* sp. e *Signiphora* sp. com taxas de parasitismo de 4.08%, 0.22%, 5.99% e 0.45%, respectivamente. O parasitismo total foi de 10.74 ± 2.03% variando significativamente entre os distritos. A recuperação da *Encarsia basicincta* e *Eretmocerus cocois* a partir da mosca branca do coqueiro é uma indicação de que *A. atratus* foi introduzida com parasitóides considerados eficientes para a supressão da sua população na sua zona de origem e podem constituir potenciais agentes de controlo biológico contra a mosca branca invasiva em Moçambique. Portanto, as autoridades nacionais fitossanitárias devem considerar o desenvolvimento de maneio integrado de pragas (MIP), incluindo controlo biológico clássico e abordagens aumentativas para reduzir a população da praga, os danos nas culturas e perdas de rendimento.

**Palavras-chave:** Coqueiro, *Aleurotrachelus atratus*, parasitóides, parasitismo

# CHAPTER ONE
# 1.0 INTRODUCTION

## 1.1 Background

The coconut palm (*Cocos nucifera* L.: Arecaceae) is a major cash crop that is widely grown in coastal tropical regions of the world including Mozambique, and contributes to the economy, livelihood and food security of millions of rural inhabitants (Bila *et al.,* 2015). Its fruit is one of the most globally and naturally widely spread fruits existing virtually on every continent (Junior & Martins 2011). Because of this dispersion and adaptability, its cultivation and use significantly occurs throughout the world, resulting in a variety of products, both in fresh and industrial forms. This fruit tree is grown mostly by small scale farmers (about 96 % of world production) constituting the main source of income (Pershly, 1992). The role of coconut in food production, foreign exchange earnings, raw materials for industries, income and employment generation to the citizens including women and young people makes it a very crucial crop for national economic development (Uwubanmwen *et al.*, 2011).

Coconuts have for long been an important crop in Mozambique and the copra made from them is an important commodity for export (Donovan *et al.,* 2010). In 2007/8, for example, Mozambique was one of the world's ten leading producers of copra, according to the Foreign Agricultural Services (FAS, 2010), producing 50,000 metric tons of copra a year. In fact, Mozambique is the fourth largest producer of coconut in Africa (after Tanzania, Ghana and Nigeria) and has the third largest coconut growing area (after Benin and Tanzania) (FAO, 2014). The crop is mostly produced in the provinces of Inhambane (67.07%) and Zambezia (18.55%) with the remaining 14.38% being distributed in the other provinces (FISP, 2010) all together providing jobs for more than 80% of the active workforce and contributing about 14-30% in food security for rural families especially those living in the coastal zone (Mondjana *et al*., 2011). Because of the many uses of the coconut palm, several proverbs have been composed to demonstrate its usefulness (Uwubanmwen *et al.,* 2011) and in Mozambique and the Philippines, it is referred to as the tree of life (Chan *et al*., 2006, Cugala *et al*., 2013).

### 1.2 Problem statement and justification

The coconut whitefly, *Aleurotrachelus atratus* Hempel **(**Homoptera: Aleyrodidae) is a highly invasive pest of coconut and ornamental palms (Malumphy, 2013). It is a hemipteran of the family Aleyrodidae originally described from coconut (*Cocos nucifera*) in Brazil (Borowiec *et al.,* 2010) and later recorded on nine other species (Arecaceae) by Howard *et al.* (2001) and Evans (2007). In Africa, *A. atratus* was first detected in 1992 in Nigeria, Congo, Benin, Ghana, Mauritius and Cape Verde in 2000, Seychelles in 2001, and Reunion islands in 2005 (Muniappan *et al*., 2012). It is also known to be invasive in many other tropical countries (Martin, 2005) and on several islands of the Indian Ocean including; Madagascar, Comoros Islands since 2002 (Cugala *et al*., 2013). In some of the affected countries, significant losses in yield of coconut have been recorded. In the Comoros Islands, *A. atratus* accounted for over 55% of economic losses to local producers of coconut (Youssoufa *et al*., 2006). Ferreira *et al*. (2010), reported in Brazil (Paracurú), a reduction in production of around 35.9% two years after the attack of coconut by *A. atratus*.

Currently, in Mozambique, coconut plantations have been damaged due to this invasive pest, with heavy infestation levels since its first detection in 2011. Studies have reported 100% of infestation of coconut plants by *A. atratus* in Inhambane province, with a severity index that ranges from severe to very severe, causing production losses estimated at around 340.13Kg/ha for each 1% severity index of whiteflies (Cugala *et al*., 2013). It has been reported by Cugala *et al*., (2013) being responsible for 70.74% of the total reduction in coconut production in Inhambane and by small scale farmers in the last 6 years, affecting seriously the coconut production in the area. The importance of *A. atratus* as an economic pest has extended continuously, representing a major threat to the production of coconuts and ornamental palms as well as to natural palm ecosystems in the absence of effective parasitoids (Borowiec *et al*., 2010).

Conventional measures for the control of whiteflies which involve the use of insecticides have not been effective in the control of *A. atratus* because of the growth characteristics of coconut plants (which are tall), cost of application that is not affordable to small scale farmers, it associated with environmental problems and difficult to apply in the coconut production system prevalent in Inhambane. Additionally, whiteflies have developed resistance to various groups of insecticides including synthetic pyrethroids (Begum *et al*., 2011) especially where frequent applications have been done.

Currently, classical biological control is considered a potential strategy for effective management of *A. atratus* in Mozambique as the pest is an alien invasive species. There has been no assessment of parasitoids associated with *A. atratus* in Mozambique. Therefore, this study was conducted to evaluate the occurrence of parasitoids associated with *A. atratus* as a basis for the introduction of classical biological control in Inhambane province.

### 1.3 Objectives

#### 1.3.1 General objective

The overall objective of this study was to evaluate the occurrence of parasitoids associated with the coconut whitefly (*Aleurotrachelus atratus*) as biological control agents to suppress its population in Inhambane province, Southern Mozambique.

#### 1.3.2 Specific objectives

i) To determine the current level of infestation, population density and severity of coconut whitefly (*Aleurotrachelus atratus*) in Inhambane province, Mozambique.

ii) To determine the occurrence of parasitoids associated with the invasive coconut whitefly in Inhambane province.

iii) To estimate the rate of parasitism in Inhambane province.

## CHAPTER TWO

## 2.0 LITERATURE REVIEW

### 2.1 The coconut palm

#### 2.1.1 Origin

The coconut palm (*Cocos nucifera* L.) originates from tropical and subtropical regions of the Pacific Ocean, and South East Asia, its centre of origin and diversity (Maturaca, 2014). Currently, the tree is found in more than 200 countries and is found in large plantations between latitudes 23 ° N and 23 ° S which include Latin America, the Caribbean and tropical Africa (Persley, 1992). The coconut palm, coconut, is also called "coco da India, coco da Bahia, coqueiro de Bahia", (Portuguese) and *cocos nucifera,* Arecaceae (Botanical) (Orwa *et al.*, 2009*)*. The name *Cocos* probably comes from a Portuguese word meaning monkey, perhaps because its nut bearing three germinating pores resembles a monkey face. Its specific name derives from Latin, meaning nut-bearing (from *fero* = I bear and *nux-nucis* = nut).

#### 2.1.2 World production

The coconut palm, *Cocos nucifera* L. is the only species classified in the genus Cocos (Shuckla *et al*., 2012) and also a palm of great economic importance. According to FAO (2011), in 1998 world production of coconut was 49 million tons, a harvested area of 11.2 million hectares, while in 2008 the production was approximately 60.7 million tons in the same area, representing a productivity increase globally. About 80% of the area planted with coconut tree is located in Asia (India, Philippines, Indonesia, Sri Lanka and Thailand) and the rest distributed between Africa, Latin America, Oceania and the Caribbean (Sources & Wanderley, 2010). Indonesia is highlighted as the largest producer of coconut, followed by the Philippines and India, however, in terms of the harvested area, the Philippines stand out for having a larger cultivated area. These countries in addition to being the world's leading producers are also responsible for 30%, 26% and 18% of fruit production, respectively (Malumphy & Treseder, 2010). This crop is considered "Tree of life" in Mozambique (Cugala *et al*., 2013) and in many other countries (Uwubanmwen *et al.,* 2011) because of its many uses. Inhambane province currently has the largest count of coconut palm trees with about 67%, and annually exporting 1,500 tons of coconut derivatives to South Africa, Tanzania and Malawi (Cugala *et al*., 2013).

### 2.1.3 Botanical description

*Cocos nucifera* trees have a smooth, columnar, light grey-brown trunk, with a mean diameter of 30-40 cm at breast height, and topped with a terminal crown of leaves. Tall selections may attain a height of 9-30 m; dwarf selections also exist (Orwa *et al.,* 2009, CABI, 2014). Trunk is slender and slightly swollen at the base, usually erect but may be leaning or curved. Leaves are pinnate, feather shaped, 4-6m long (Uwubanmwen *et al*., 2011), and 1-1.5 m wide at the broadest part. Inflorescence consists of female and male axillary flowers. Flowers are small; light yellow, in clusters that emerge from canoe-shaped sheaths among the leaves, about 25 mm in diameter (Orwa *et al.,* 2009). Male flowers are small and more numerous. Female flowers are fewer and occasionally completely absent; larger, spherical structures. Fruits are drupes, roughly ovoid, up to 30 cm long and 20 cm wide (CABI, 2014), 1-2 kg in weight, composed of a thick, fibrous husk surrounding a somewhat spherical nut with a hard, brittle, hairy shell. Three sunken holes of softer tissue called 'eyes' are at one end of the nut. Inside the shell is a thin, white, fleshy layer known as the 'meat'. The interior of the nut is hollow but partially filled with a watery liquid called 'coconut milk'. The meat is soft and jellylike when immature but becomes firm with maturity. It is a white rich stored food that lines the inside of the seed and is very nutritious and high in calories (Uwubanmwen *et al*., 2011). Coconut milk is abundant in unripe fruit but is gradually absorbed as ripening proceeds. The fruits are green at first, turning brownish as they mature; yellow varieties go from yellow to brown (Orwa *et al.,* 2009).

### 2.1.4 Biology of the coconut palm

The tall varieties reproduce by cross-pollination. Male flowers open first, producing pollen for about 2 weeks. Female flowers are not usually receptive until about 3 weeks after the opening of the inflorescence, making cross-pollination the usual pattern. Wind is the main pollinating agent. Reproduction in dwarf varieties is generally through self pollination. Female flowers are receptive about a week after the male flowers open, both ending at about the same time. The plant flowers approximately after the 6$^{th}$ year (Orwa *et al.,* 2009).

### 2.1.5 Tree ecology

*Cocos nucifera* is unknown in the wild state. In the coastal areas of the tropics and subtropics where it is grown, it requires a hot, moist climate and deep alluvial or loamy soil, thriving especially near the seaboard, but also considerable distance inland, provided that climatic conditions and soil are suitable. Rocky, laterite or stagnant soils are unsuitable (Orwa *et al.*, 2009).

The hot and humid climates are more favourable to the development of this tree. The relative humidity of 80% is suitable for the growth of the plant (Maturaca, 2014). If humidity is less than 60% and is associated with hot, dry winds, there may be damage to the crop. The high moisture reduces nutrient absorption capacity and favours the occurrence of fungal diseases (Ferreira *et al*., 2002). Low humidity reduces the development of crop. The average annual temperature for a good development of coconut is 27$^{o}$C, with fluctuations of 5 to 7$^{o}$C. These conditions are usually found in the tropical zone. Temperatures below 15°C disturb the development of the coconut palm but it tolerates temperatures above the optimal temperature (Ferreira *et al*., 2002).

### 2.1.6 Biophysical limits of coconut palm

The crop grows well at below 1000 ft elevation, with mean annual temperature of 21-30$^{o}$ C, mean annual rainfall 1500-2500 (CABI, 2014) but can also do well in temperature of 20-28$^{o}$ C and 1000-1500 mm (Orwa *et al.*, 2009). *C. nucifera* is tolerant to soil variations but its natural preference is for sandy, well-aerated and well drained soils. It has considerable ability to adapt to soils of heavier texture (Orwa *et al.*, 2009).

### 2.1.7 Species distribution

The crop is native from Brunei, Cambodia, Indonesia, Laos, Malaysia, Myanmar, Philippines, Singapore, Thailand and Vietnam (Orwa *et al.*, 2009*)*.

It is an introduced species in Argentina, Benin, Bolivia, Brazil, Burkina Faso, Cameroon, Chad, Chile, China, Colombia, Cook Islands, Cote d'Ivoire, Ecuador, Fiji, French Guiana, Gambia, Ghana, Guinea, Guyana, Haiti, India, Jamaica, Kenya, Kiribati, Liberia, Madagascar, Mali, Marshall Islands, Mauritania, New Caledonia, Niger, Nigeria, Papua New Guinea, Paraguay, Peru, Samoa, Senegal, Sierra Leone, Solomon Islands, Sri Lanka, Surinam, Togo, Tonga, Uganda, United States of America, Uruguay, Vanuatu, Venezuela, Zanzibar (Orwa *et al*., 2009) and Mozambique.

### 2.1.8 Products and uses

Coconut cultivation is very important in generating income, in food, and in the production of over one hundred (100) products (Maturaca, 2014). It is one of the most important perennial crops, able to generate a self-sustaining system operation. Several products are extracted from different parts of the plant, for example; coal, coke, oil, coconut milk, coconut water, fibre for coconut industry and copra, among others (Ferreira *et al*., 2004).

It is estimated that about 90% of the world coconut production comes from smallholders, with areas of up to 5 hectares, and this production is almost consumed domestically in producing countries (Junior & Martins, 2011). Copra, the dried coconut endosperm is the most important food product from coconut, containing edible cooking oil (coconut oil). The apical region of *C. nucifera* ('millionaire salad') is a food delicacy in areas where it is grown. Other food derivatives of coconut include coconut chips, coconut jam, coconut honey, coconut candy and other desserts. Copra meal and coconut cake, the residues of oil extraction from copra containing approximately 20% protein, 45% carbohydrate, 11% fibre, fat, minerals and moisture, are used in cattle feed rations (Uwubanmwen *et al*., 2011).

### 2.1.9 Pests of coconut palm

The importance of coconut crop to the national economic sector has been seriously affected by pests and diseases. Among pests, the exotic and invasive coconut whitefly (*Aleurotrachelus atratus*) is a serious threat. There are also other pest problems related to the coconut palm. Bird pests include the Hispaniolan woodpecker, which attacks the trunk for nesting sites and damages immature nuts, and the village weaver, which strips the leaves for nest building. The nematode *Rhadinaphelenchus cocophilus* invades the stem and crown base, causing red-ring disease (Orwa *et al.*, 2009). More than 100 species of insects afflict the tree, apart from the coconut whitefly (*A. atratus*), rhinoceros beetle (*Orycetes rhinoceras, O. moceros*), coconut mite (*Aceria guerreronis*) and coconut weevil (*Rhynchoporus cruentatus*) (Chandramohanan *et al*., 2012). Other important species include *Strategus spp.* attacking the softwood and the heart of the tree, *Brontispa spp.* severely damaging leaves and leaf miners (*Promecotheca spp.*) that render leaves non-functional (Orwa *et al*., 2009), mealy bugs, termites and thrips (Miguel, 2014). All these are responsible for damage to the plant, delaying development and early production, reducing productivity, and causing losses in coconut plantations (Ferreira *et al*., 2011).

### 2.2 The coconut whitefly

The coconut whitefly (*Aleurotrachelus atratus*) is a Neotropical whitefly, originally described by Hempel (1922) from specimens collected from coconut (*Cocos nucifera*) in Brazil. This species is assigned to the genus *Aleurotrachelus* in the subfamily Aleurodidae, *Aleurotrachelus* being one of the largest genera of whiteflies and currently containing 74 species (Martin and Mound, 2007). It is a eukaryote belonging to Kingdom: Metazoa, Phylum: Arthropoda, Subphylum: Uniramia, Class: Insecta, Order: Homoptera, Suborder: Sternorrhyncha, Superfamily: Aleyrodoidea, Family: Aleyrodidae, Genus: Aleurotrachelus and Species: Aleurotrachelus atratus.

#### 2.2.1 Biology and ecology

The development of *A. atratus* involves six stages: egg, four larval/pupal instars and adult. It is mainly parthenogenetic (Malumphy and Tresedar, 2011) but some rare males were found in Réunion and Mayotte (Borowiec *et al*., 2010). Its development takes around 48 days from egg to adulthood at 25-27°C (Borowiec *et al*., 2010). It is multivoltine and will breed continuously if environmental conditions are suitable (Malumphy, 2013). With a sex ratio of one male per 1022 females collected in La Réunion, it appears that the whitefly reproduces by thelytoky (Browiec *et al.*, 2010*)*.

#### 2.2.2 Taxonomy and identification

There are no comprehensive diagnostic keys available to *Aleurotrachelus*, which is one of the largest genera of whiteflies, containing 74 species (Martin and Mound, 2007), several of which are quite unrelated morphologically. Whitefly taxonomy is based on the morphology of the fourth-larval instar, commonly known as the pupa or puparium. *A. atratus* pupae require slide mounting and examination for reliable determination (Malumphy, 2013). Field identification of the pest is unreliable as there are similar species with black pupae in the genus *Aleurotrachelus* and other genera such as *Aleurotulus*, *Aleurolobus* and *Tetraleurodes* (Malumphy, 2013). The eggs and early-instar larvae are small and very difficult to detect during plant health inspections. They (eggs and larvae) are only found on foliage, and therefore the trade in palm seeds poses a negligible risk (Malumphy, 2013) of spread. The body of *A. atratus* is dark yellow and all the four larval/pupal stages are black. The first instars have four pairs of wax plumes excreted by glands at the base of dorsal setae. Each dorsal seta has curving longitudinal grooves that guide the wax flakes as they are secreted from the seta base (Delvare *et al*., 2008). When the wax has been removed, each pupa can be seen to have a distinct diagnostic pair of submarginal longitudinal cephalothoracic folds that extend into the abdomen (Malumphy and Tresedar, 2011). *A. tratus* pupae are elliptical, black, 1.0-1.1 mm long with a long marginal white wax fringe and dorsal wax filaments that often completely cover the insect. The pupae often occur in dense colonies that smother the underside of the fronds with pupae wax secretions and honeydew, on which sooty moulds grow (Malumphy, 2013). Feeding ceases during this phase and the insect emerges as a white winged adult (Howard *et al*., 2011) ready to fly. A case from which a whitefly has emerged appears black with a T-shaped exit at the dorsal end.

### 2.2.3 Pest distribution

Before the 1990s this species was only known to feed on coconut in Brazil (Hempel, 1922; Mound & Halsely 1978, cited by Borowiec *et al.*, 2010) but since 2001, it has been reported widely in the tropics and subtropics on more than 100 plant species having spread rapidly, probably due to anthropogenic activities such as trade in ornamental palms (Malumphy, 2013). It is now found in Africa, North and South America, Central America and the Caribbean, Europe and Oceania (Borowiec *et al*., 2010). It is also widely distributed in the Neotropical region including Antigua, Bahamas, Barbados, Bermuda, Brazil, Colombia, Guyana, Nevis, Puerto Rico, Venezuela, Florida and USA (Howard *et al*., 2001).

*A. atratus* has since been recorded in many other countries within the Neotropical region (Evans 2008; Delvare *et al*., 2008) and has spread into other geographical regions (Malumphy, 2013). In Africa, it is now known to be invasive in many tropical countries (Martin, 2005) on several islands of the Indian Ocean: Madagascar and Mauritius (Ollivier *et al*., 2004) and La Reunion (Youssoufa *et al*., 2006); it was also reported in Cape Verde in 2003, Comoros Islands in 2002, Seychelles Islands (Cugala *et al*., 2013) and was quoted from continental Africa; Mozambique in 2011 and Uganda (Gerling *et al*., 2006).

Field surveys in La Re´union, the Seychelles, the Comoros and glasshouses in Paris recorded this whitefly on 56 palm species, some of which are endemic and/or threatened species (Delvare *et al*., 2010). Such a wide host range has facilitated the rapid geographical dissemination of the whitefly world over (Delvare *et al*., 2010). It thrives throughout the islands, and in areas from sea level to 800 m altitude. From all sampled locations in Mozambique, *A. atratus* was commonly found in all sites at <200 m asl where it was widely spread and well established (Cugala *et al*., 2013).

### 2.2.4 Dispersal ability

Although adult *A. atratus* are winged, they are small and fragile, and relatively poor fliers (Malumphy, 2013). Long distance (international) dispersal is most likely to have resulted from the trade of infested ornamental palms for planting; for example, the pest has been intercepted at US ports of entry on palms originating from the Caribbean (Evans, 2008), and in the UK on palms and palm foliage from the Caribbean and Central America, respectively (Malumphy & Tresedar, 2011).

### 2.2.5 Host range

*A. atratus* has been recorded feeding on 114 host plant species belonging to five families, but most (96%) being palms in the family Arecaceae (Malumphy, 2013). Coconut is the most commonly reported host although the pest has been occasionally recorded on non-palm hosts, including two highly important crops, citrus and aubergine (Malumphy, 2013). The pest also attacks many ornamental palms commonly planted in tourist areas, such as the Canary Islands and Seychelles, and therefore has the potential to impact tourism (Borowiec *et al*., 2010). It feeds on 17 palm species that are listed in the International Union for Conservation of Nature (IUCN) red list (Borowiec *et al*., 2010), and therefore is detrimental to biodiversity, particularly in more vulnerable island ecosystems.

### 2.2.6 Economic importance

*Aleurotrachelus atratus* is the most economically important pest of coconut. For example, in Grand Comoros, 90% of coconut palms were severely infested by the whiteflies *A. atratus* and *Paraleyrodes bondari* Peracchi, with *A. atratus* accounting for 90% of the whiteflies found (Streito *et al*., 2004). In Mozambique, 70.74% of the reduction in coconut produced is explained by attack of whiteflies (Cugala *et al*., 2013). The sooty mould that develops on the honeydew excreted by whiteflies significantly affects coconut palm growth and yields. Heavy infestations can even result in death of the palms (Ollivier *et al*., 2004). The economic impact is substantial, with annual lost earnings for producers estimated at 3-5 million euros (Malumphy, 2013). Crops growing under infested coconuts, such as vanilla, banana, physic nut and guava, are also damaged (Ollivier *et al*., 2004).

Both the larval stages and adults of *A. atratus* damage plants directly by feeding on the foliage. The removal of sap reduces plant vigour, causes chlorosis and premature leaf drop and reduces yields. Large infestations of whiteflies, in combination with other stress factors such as lack of water, may result in the death of palms. Indirect damage is caused by the excreted honeydew that serves as a medium for the growth of sooty moulds, which hinder photosynthesis and gas exchange, and reduce yields (Malumphy, 2013). The highly conspicuous flocculent white wax that covers dense groups of pupae and the sooty moulds reduce the aesthetic appearance and market value of ornamentals and crops. Plants growing below infested palms also become covered in sticky honeydew, sooty mould and wax. The honeydew attracts ants, flies and other insects which can be a nuisance. There appears to be no specific data published on the social impact of *A. atratus*; however, it is an economic pest of coconut and has reduced earnings in the Comoros Islands (Streito *et al*., 2004) and Mozambique, where coconut is traditionally used in many areas of life such as food, agroforestry and the construction of traditional/ordinary houses. It has the potential to cause a significant social impact, as coconut is a staple food source for many people in the tropics and subtropics, and plays a role in culture and religion in some countries in Africa (e.g. Comoros Islands) and Asia. Presently the coconut whitefly, *A. atratus*, has gained greater importance in coconut cultivation, mainly in the southern part of Mozambique. The pest is a polyphagous phloem-feeding insect currently causing significant damage in Inhambane (Cugala *et al*., 2013) by direct feeding and indirectly by deposition of honeydew that gives rise to sooty mould growth, blocking light and air from the leaves (Muniappan *et al*., 2012) and reducing photosynthetic productivity (Williams & Granara de Willink, 1992).

### 2.2.7 Control methods

Several methods have been used to control coconut whiteflies, including; cultural, use of crop resistance and biological control (Malumphy & Tresedar, 2011). Cultural control and sanitary measures targeting *A. atratus* in a glasshouse at botanical gardens in the UK include pruning out the worst-infested leaves and, sometimes, the removal of whole plants (Malumphy, 2013). On some of the important specimen plants, the leaves are washed by hand and wiped with alcohol (Malumphy & Tresedar, 2011) while chemical treatments targeting at *A. atratus* in a glasshouse at a botanical garden at the same site are only partially successful; one of the main difficulties being achieving an even application of the pesticides, which is made difficult by the height of the plants and the shape of the palm leaves (Malumphy & Tresedar, 2011).

In the Seychelles archipelago, the department of environment imposed a ban on the movement of all palm species in mid-2007 and a warning was issued to concerned stakeholders against the slash and burn method (removal and burning of badly infected leaves from coconut trees) as this would only encourage the movement of adults to other host plants in different areas (Beaver *et al.*, 2009). Biological control which involves the use of natural enemies is a more effective, safe and environmentally friendly method in pest management.

### 2.2.8 Biological control

Biological control is the use of one type of organism to reduce the population density of another (Soloneski *et al.*, 2013). The term may sound new but the idea of using insects is ancient, dating as far back as the 3rd century when the Chinese placed the nests of a predatory ant (*Oecophylla smaragdina*) in citrus orchards to control insect pests (Konish & Ito, 1973). Similar strategies have been widely accepted and used in pest management since the end of the nineteenth Century (Lenteren *et al.*, 2005). The use of natural enemies is considered an important and effective strategy in pest management, particularly of exotic and invasive pests (Hoy, 1989) which could be through classical, augmentation or conservation biological control. Considerable effort has gone into biological control of pests in many parts of the world, including the use of microorganisms, predators and parasitoids (Bortoli *et al.*, 2013).

**a)** **Parasitoids**: Parasitoids are insects which are only parasitic in their immature stages, kill their host in the process of development, and have free-living adults that do not move their hosts to nests or hideouts. In studies by Borowiec *et al*. (2010) in La Reunion, six species of parasitoids of the coconut whitefly *Aleurotrachelus atratus* were identified, all of Aphelinidae Family: *Eretmocerus cocois* Delvare a neotropical species (Delvare *et al*., 2008) and primary parasitoid of *A. atratus* in La Reunion islands, *Cales noacki* Howard, *Encarsia forster*, *Encarsia cubensis* Gahan, *Encarsia hispida* and *Encarsia basicincta.*

Another study recorded several parasitoids (Chalcidoidea, Aphelinidae) from *A. atratus* including*; Cales noaki* Howard, *Encarsia cubensis* Gahan, *Encarsia brasilensis* (Hempel) (=*hispida* De Santis), *Encarsia lanceolata* Evans and Polaszek, *Encarsia nigricephala* Dozier, *Eretmocerus cocois* Delvare and *Eretmocerus desantis* Rose (Borowiec *et al*., 2010; Evans and Polaszek 1997, 1998; Delvare *et al*., 2008; Noyes, 2012). The use of parasitoids now appears to be the new hope for the sustainable management of the coconut whiteflies in coconut palms in Mozambique.

b) **Predators**: Among the natural enemies of pests, predators appear to be least studied and understood. In most cases, they merely constitute a listing of species. In general, predators have been suggested to be important stabilizing agents for pest populations. It is also suggested that the higher numbers of whiteflies in insecticide treated fields comes because of lower predator numbers, and the large unknown mortalities of whiteflies in life table studies may be attributed to predators; birds inclusive.

c) **Microbials (Entomopathogens)**: The use of microbial agents for controlling pests has been reported being effective among larval stages where it is highly effective. Many commercial formulations are available and used as part of integrated pest management program. Biological control of insect pests in general involves the use of parasites, predators or parasitoids, most commonly insects.

#### 2.2.8.1 Classical biological control

Classical biological control is best described as "the reuniting of old enemies". It recognizes that most serious pests are invaders, usually from another country. These invaders arrive, usually accidentally as part of shipments of plants or food products. When they arrive, they are in a new habitat that lacks the natural enemies (insect predators and parasitoids, and diseases) that were adapted to keep that species population numbers in check. Without these natural enemies, the population of the invasive species becomes very large which is usually why it is a pest. Reuniting old enemies means that the natural enemies that are adapted to kill and control an insect's population are imported from the country of origin and released in the new place the pest has invaded.

In La Reunion, searches revealed the presence of two parasitoids, *Cales noacki* Howard (Hymenoptera, Chalcidoidea) and *E. cocois* Delvare. *Cales noacki* Howard emerged from the second and third instars of *A. atratus*; it had been introduced in La Reunion in 1976 to control the citrus whitefly *Aleurothrixus floccosus* (Quilici *et al*., 2003). It is also known from a number of other hosts including *A. atratus* in the Canary Islands (Hernandez-Suarez *et al*., 2003). The parasitoid *Eretmocerus cocois,* having been reared for the first time in Guadeloupe Island in 1994 was found to effectively parasitize populations of *A. atratus* in Guadeloupe (Neotropics) and in the Indian Ocean islands of Réunion and Mayotte (Delvare *et al.*, 2008), and was introduced to Ngazidja (Comoros Islands) for the biological control of *A. atratus* by CIRAD and the Agriculture, Fisheries and Environment Research Institute (INRAPE) in the Comoros within the Crop Protection Network for the Indian Ocean (PRPV). The parasitoid proved to be an effective bio-control agent of *A. atratus* (Cave, 2008) in Comoros and currently the pest appears to be successfully controlled by this parasitoid (Beaver *et al.*, 2009) in areas where it has been introduced.

# CHAPTER THREE

# 3.0 MATERIALS AND METHODS

## 3.1 Description of the study area

Inhambane province is located on the coast in the southern part of Mozambique with an area of 68,775 $km^2$ covered by 12 districts (EdM, 2013). The provincial capital is also called Inhambane located about 500 km north of Maputo, the country's capital. It is bordered to the North by the Provinces of Manica and Sofala, to the South and East by the Indian Ocean, and to the West by the Province of Gaza, and is considered as perhaps one of the best quality touristic regions in Mozambique. The climate is tropical throughout, more humid along the coast with a lot of mangrove swamps and dryer inland. Annual average temperature ranges between 19.5$^oC$ and 28.1$^oC$ with annual rainfall of 949.8mm (WMO, 2015). The province is known for crop production, being the first in coconut production, second largest grower of cashew nuts (after Nampula), and also producing citrus fruits in significant quantities.

## 3.2 Determination of the infestation of coconut whitefly (*Aleurotrachelus atratus*) in Inhambane province

### 3.2.1 Sampling procedure

Five (5) districts which were major coconut producers were sampled including; Zavala, Inharrime, Jangamo, Morrumbene and Massinga (Figure 1), and in each district three (3) locations were selected based on their coconut production levels and accessibility along the main road. Three (3) fields were selected in each location based on the presence of coconut plants shorter than 3 m. The exact position and altitude of each sampling site was determined using a Garmin GPS. Samples were collected in September and December 2015.

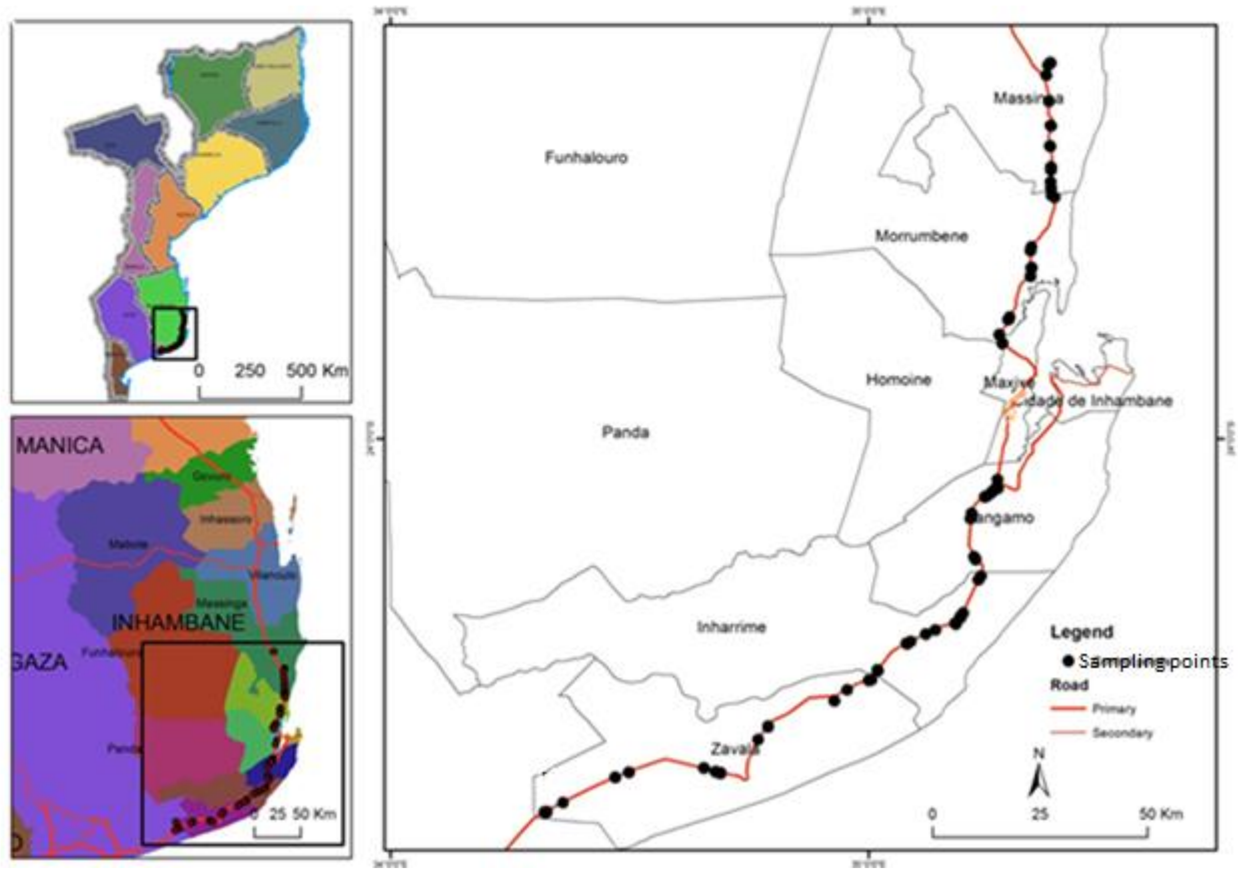


**Figure 1: Map showing sampled districts and sites/fields in Inhambane province**

### 3.2.2 Estimation of percentage of whitefly infestation

In each field, 20 plants were randomly selected and visually inspected, and scored one (1) for infested or zero (0) for non-infested plants. A plant was considered infested when nymphs or whitefly pupae were observed feeding on the leaves to avoid the possibility of vagrant adults leading to false host records (Borowiec *et al*., 2010). The percentage of whitefly infestation was estimated as the ratio of the number of infested plants to the total number of plants observed and expressed as a percentage using the following formula.

$$Percentage\ of\ infestation = \frac{number\ of\ infested\ plants}{total\ number\ of\ plants\ observed} * 100\%$$

### 3.2.3 Assessment of whitefly density

To estimate the population density of whiteflies, 5 infested coconut trees with a height of less than 3 m were purposefully selected in each field for sampling. From each infested plant, three (3) leaflets were removed from the third leaf (counted from the top), well shaken to remove all other organisms and then placed in plastic containers and properly labelled. The samples were taken to a room and the whitefly larvae on the leaflets were counted and recorded. After counting, the samples were kept in plastic containers for subsequent evaluations. The density of whiteflies was estimated as the ratio of the total number of collected individual whitefly pupae in the leaflets to the total number of leaflets observed.

$$\textit{Population density} = \frac{\textit{number of pupae collected}}{\textit{number of leaflets observed}}$$

### 3.2.4 Determination of whitefly severity

All the 20 plants selected in each field in section 3.2.2 were observed to estimate the severity levels. On each plant, 5 leaves (sheets) in the directions: North, South, East, West and centre were selected to avoid sampling errors and minimize aggregation and dispersion problems. In each selected leaf, the level of coverage of pupae was estimated and assigned a value according to a six-level scale modified from Borowiec *et al*., (2010).

Table 1: Scale used to estimate the level of whitefly severity in Inhambane

| Percentage of leaf sheet covered by Whitefly Colonies | 0 | 1-10% | 11-25% | 26-50% | 51-75% | >75% |
|---|---|---|---|---|---|---|
| Severity Level ( scale) | 0 | 1 | 2 | 3 | 4 | 5 |
| Infestation level assessment | No infestation | Low | Slight | Medium | Severe | Very severe |

Modified from Borowiec *et al*. (2010)

The level of severity was estimated using the following formula described by Borowiec *et al*. (2010).

$$\textit{Level of severity} = \frac{\Sigma\,(\textit{grade of scale} * \textit{frequency})}{\textit{total number of units} * \textit{maximum grade of the scale}} * 100\%$$

## 3.3 Assessment of occurrence of parasitoids associated with *Aleurotrachelus atratus* in Inhambane province

### 3.3.1 Collection of samples

Sampling districts and locations were selected as described in section 3.2.1 above. The samples of leaflets collected in section 3.2.3 above were used to assess the occurrence of parasitoids associated with coconut whitefly. These were taken to the Entomology laboratory at the Faculty of Agronomy and Forestry Engineering of Eduardo Mondlane University (FAEF/UEM) and kept at room temperature for at least 15 days to allow for emergence of parasitoids. The samples were later opened and all emerged adult parasitoids (tiny wasps) were counted and recorded.

### 3.3.2 Identification of parasitoid species

All adult parasitoids were first identified at the Laboratory of Entomology, at Faculty of Agronomy and Forestry Engineering of Eduardo Mondlane University in Maputo. For precise identification, the parasitoid specimens were sent to La Reunion (CIRAD) for species confirmation which involved both taxonomic and molecular procedures.

### 3.3.3 Evaluation of parasitism rates

The percentage parasitism of each observed parasitoid species was estimated as the ratio of the total number of parasitoids found of each species to the total number of whitefly pupae collected or observed, considering that the emerged parasitoids are solitary (Rogers, 1974).

$$Pp = \left(\frac{Tpe}{Tpc}\right) * 100$$

Where: *Pp* – Percentage parasitism

*Tp*e – Total parasitoids emerged of each species

*Tp*c – Total of pupae collected

The combined or overall rate of parasitism was a proportion of the sum of all parasitoid individuals of all species and the total pupae collected. The potential of the parasitoids in suppressing *A. atratus* population was predicted using linear regression by plotting whitefly population density against rate of parasitism.

### 3.3.4 Data analysis

For whitefly infestation, as normality of the error term and variance homoscedasticity could not be reached, a non-parametric ANOVA (Kruskal–Wallis test) was used. For whitefly severity and density, data was subjected to analysis of variance (ANOVA) (software STATA 12.0). For percentage parasitism, as normality of the error term could not be reached, the data were log (x+1) transformed after which analysis of variance (ANOVA) using STATA 12.0 software was performed. Means were separated using Tukey test at 95% confidence level. A student t-test was used to compare variations between the two sampling periods (September and December 2015) also at 95% confidence level.  Linear regression was used to predict the current contribution of parasitoids to reduction in whitefly density and correlations between variables were checked using Kendall's rank correlation coefficient. Relative abundance of a parasitoid species was estimated as a ratio of number of individuals of a species to total number of parasitoids observed expressed as a percentage.

## CHAPTER FOUR

## 4.0 RESULTS AND DISCUSSIONS

### 4.1 Determination of the infestation of the coconut whitefly (*Aleurotrachelus atratus*) in Inhambane province

#### 4.1.1 Whitefly infestation

Whitefly infestation was 100% in all districts of study except Inharrime where the infestation level was 99.3% (Table 2). However there were no significant differences at 95% level of confidence among sampling districts (Figure 2a) based on Kruskal-Wallis rank test (P = 0.4243, $\chi^2$ = 3.867, df = 4) (Appendix 2). Similarly, all sampled locations registered 100% whitefly infestation except Inhacoongo which had 98$\pm$0.54% infestation but was also not significantly different from other locations (Table 3) at 95% confidence level (P = 0.7548, $\chi^2$ =13.600, df = 18) based on Kruskal-Wallis rank test (Appendix 3).

Among the sampling periods, whitefly infestation of 100% was recorded in September, reducing slightly to 99.6% in December (Figure 2b), however the reduction not being significant according to the student t-test at 95% confidence level (P = 0.3262, α = 0.05). There was no effect of interaction between sampling period and sampling location on whitefly infestation (F = 0.00, P =1.000, α = 0.05, n = 73) (Appendix 4). All sampled locations were in the altitude range between 12 m and 152 m above sea level.

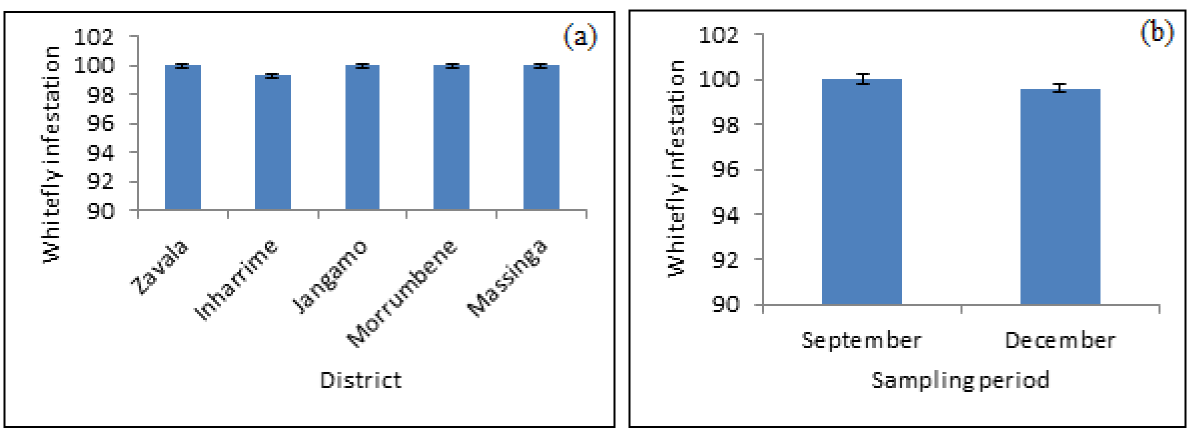


Figure 2: Variation of whitefly infestation: (a) among districts and (b) between sampling periods

Table 2: Level of whitefly infestation, severity and density in sampled districts

| District | Level of infestation (%) | Severity (%) | Whitefly density (Pupae per leaflet) |
|---|---|---|---|
| Zavala | 100±0.3a | 80.2±2.49a | 26.8±2.74a |
| Inharrime | 99.3±0.3a | 76.9±2.49a | 29.2±2.74a |
| Jangamo | 100±0.3a | 78.5±2.49a | 26.5±2.74a |
| Morrumbene | 100±0.3a | 75.3±2.68a | 24.2±2.95a |
| Massinga | 100±0.3a | 73.9±2.49a | 25.7±2.74a |
| P-value | 0.4243 | 0.6882 | 0.9554 |

Means followed by the same letter within a column are not significantly different (P<0.05).

Table 3: Whitefly infestation, density and severity in sampled locations in Inhambane.

| District | Sampling location | Level of infestation (%) | Whitefly density (Pupae per leaflet) | Severity (%) |
|---|---|---|---|---|
| Zavala | Zandamela | 100a | 28.0 ±4.60a | 78.9±2.86a |
| | Quissico | 100a | 18.7±4.60a | 79.5±2.72a |
| | Nyakundela | 100a | 39.4±5.94a | 79.1±0.44a |
| | Mwani | 100a | 25.3±7.27a | 86.4±2.14a |
| Inharrime | Inharrime sede | 100a | 32.7±4.60a | 79.6±3.16a |
| | Dongane | 100a | 25.2±7.27a | 84.3±7.14a |
| | Chongola | 100a | 21.8±5.93a | 86.7±2.78a |
| | Inhacoongo | 98a | 31.8±4.60a | 65.5±6.16a |
| Jangamo | Jangamo Rovene | 100a | 23.2±7.27a | 84.3±1.43a |
| | Bambela | 100a | 35.0±5.94a | 83.1±3.11a |
| | Lindela | 100a | 20.1±4.60a | 67.9±1.70a |
| | Cumbana | 100a | 29.3±4.60a | 83.9±2.71a |
| Morrumbene | Morrumbene sede | 100a | 22.5±3.64a | 74.6±3.78a |
| | Malaia | 100a | 29.4±5.94a | 84.9±5.13a |
| | Mucoduene | 100a | 23.1±7.27a | 63.6±10.71a |
| Massinga | Rio pedres | 100a | 22.3±4.60a | 73.9±4.86a |
| | Massinga sede | 100a | 28.6±4.60a | 69.8±1.94a |
| | Massinga Rovene | 100a | 31.0±5.94 | 82.2±1.78a |
| | Mahocha | 100a | 19.0±7.27a | 71.4±2.86a |

Means followed by the same letter within a column are not significantly different (P<0.05).

The current whitefly infestation recorded in the study area implies that *Aleurotrachelus atratus* is well established in Inhambane. Checo (2014) also reported high levels of infestation by *A. atratus* in Jangamo (98.9%), Inharrime (97.7%) and Morrumbene (97.7%). The slight increase in infestation levels in the current study could have been favoured by the invasive nature of the pest, high mobility and dispersion in coastal regions since the sampled districts were bordering the Indian Ocean (Figure 1), and high range of hosts (Borowiec *et al*., 2010) for this pest in the area of study. It may also indicate that its population is increasing due to favourable agro-climatic conditions including but not limited to; temperature, relative humidity and presence of host plants.

These results are also consistent with the observations made by Cugala *et al*. (2013) who found *A. atratus* in all sampled locations in Inhambane, in all study sites at <200 m altitude where the pest was widely spread and well established. Borowiec *et al*. (2010) observed that the coconut whitefly was commonly found in all regions of La Réunion, from sea level up to 600 m above sea level, the greatest infestations being located on the coast, indicating that high altitude might be a limiting factor for this species. Since all the sampled locations in the current study lied between 12 m and 152 m above sea level, it may indicate that low altitude favours high infestation of *A. atratus*. The continuous availability of the main host plant (coconut) throughout the year and favourable environmental conditions (Cugala *et al*., 2013) may also be responsible for the high whiteflies infestation level in Inhambane.

### 4.1.2 Whitefly density

There were no significant differences among districts in terms of whitefly density ($P = 0.9554$, $\alpha = 5\%$) (Appendix 6). Similarly, variation of whitefly density among locations within districts was not significant ($P = 0.2206$, $\alpha = 5\%$) varying from 19.0 larvae per leaflet in Mahocha to 39.4 larvae per leaflet in Nyakundela (Table 3).

Whitefly density differed significantly between sampling periods at 95% confidence level ($P = 0.0000$, $\alpha = 0.05$). It was found to be higher in September with 30.6 larvae per leaflet and lower in December with 19.8 larvae per leaflet as shown in figure 3b**.** Overall whitefly density was 26.4 larvae per leaflet with a confidence interval of 23.97 – 29.20 larvae per leaflet. There was no effect of interaction between sampling period and sampling location on whitefly density ($F = 1.56$, $P = 0.1716$, $\alpha = 0.05$, $n = 73$) (Appendix 9).

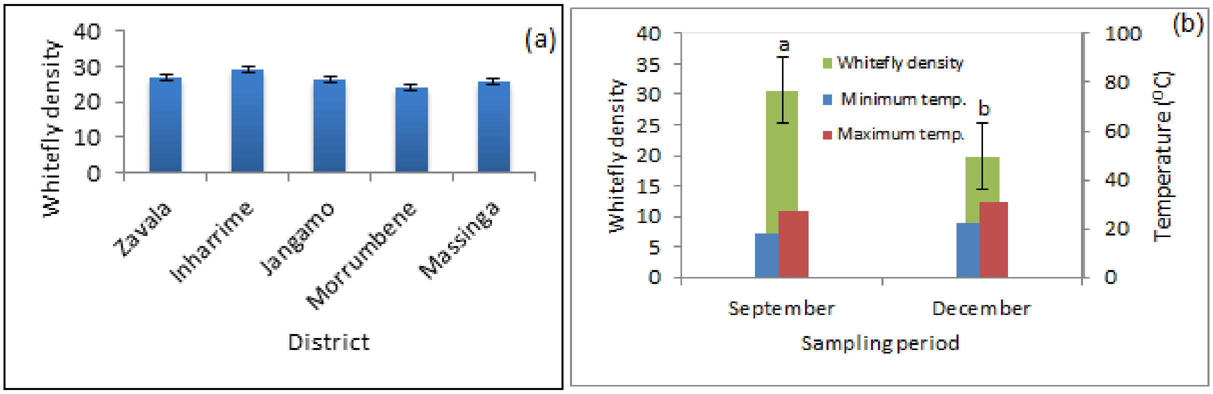


Figure 3: Variation of whitefly density: a) among districts and b) between sampling periods

These results are lower compared to the observations made by Miguel (2014), who observed much higher population densities during her study in Jangamo district (January and July 2014) in the rage of 97.75 to 167.33 individuals per leaflet. The lower densities reported in the current study could be justified by the fact that the whitefly density in the current study was estimated based on the third leaf, while the densities reported by Miguel (2014) were estimated based on the older leaflets. Old plant leaflets may have higher pest (larval/pupal) densities due to greater protection in the older leaves (Ruberson, 1999) desired by some ovipositing whiteflies and also due to longer/larger exposure time and surface for oviposition.

Higher population densities were also reported by Cugala *et al*., (2013) from the same study area (Inhambane), who observed average of 131.7 individuals per leaflet in Inharrime district as highest and 53.7 individuals per leaflet in Massinga as lowest during the 2012 dry season. However, the density registered in the current study (26.4 larvae per leaflet) is still by far high especially that samples were collected from young leaves, compared to 0.34 larvae per $cm^2$ registered in Comoros where *Aleurotrachelus atratus* has been effectively controlled (PRPV, 2016).

The decrease in whitefly density from 30.6 to 19.8 larvae per leaflet between September and December 2015 might be due to higher temperatures that prevailed in December (22$^o$C-31$^o$C) compared to September which had 18$^o$C-27$^o$C (WMO, 2015). Desai and Gupta (2015) recorded maximum whitefly density during a lower temperature season and minimal density in a hot season in India. According to CABI (2015), optimum temperature for *A. atratus* is in the range of 25-27$^{o}$ C, which was occasionally exceeded during the month of December in 2015 in the study area. The decrease in density could also be attributed to higher total precipitation of 115.57mm reported in December compared to 1.27mm reported in September (WMO, 2015) in the same year. Temperature and rainfall influence whitefly population dynamics (Horowitz, 1986). Horowitz (1986) observed in Sudan, that heavy rains were usually followed by a drop in whitefly population levels. Higher precipitation level recorded in December compared to September 2015 could also be responsible for the reduction in whitefly density observed in the current study.

### 4.1.3 Whitefly severity

Whitefly severity varied from 73.9±2.49% in Massinga district to 80.2±2.49% in Zavala district (Table 2). There were no significant differences among the districts studied (P = 0.6882, α = 0.05) (Figure 4a) and among locations with Chongola having the highest (86.7±4.6%) and Mucoduene having the lowest (63.6±5.7%) level of severity (Table 3). Severity differed significantly between sampling periods at 95% confidence level (P = 0.0299), being higher in September 2015 at 79.1±1.19% compared to December 2015 which had 73.7±2.05% (Figure 4b). Overall, whitefly severity varied between 74.75% and 79.19% (Appendix 7), which corresponds to a variation from severe to very severe according to the scale described by Borowiec *et al*. (2010).

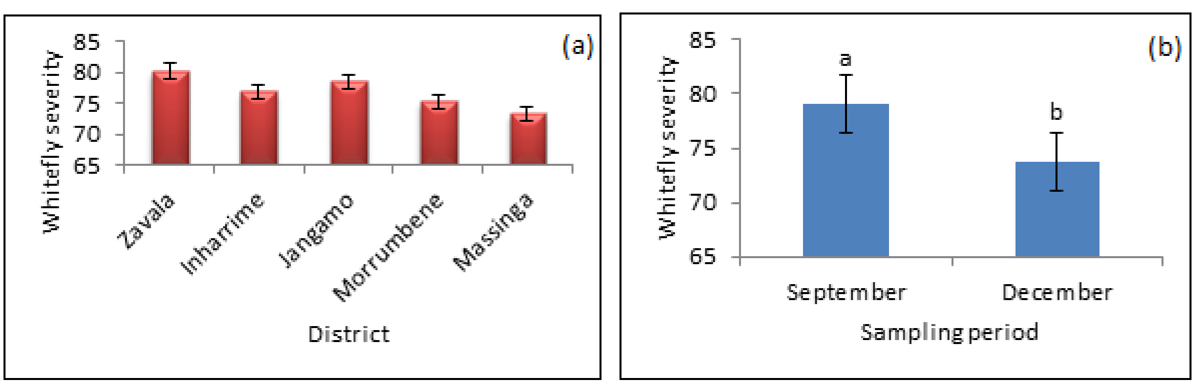


Figure 4: Variation of whitefly severity: a) among districts and b) between sampling periods

Whitefly severity levels reported in this study concur with those reported by Checo (2014) who observed that whitefly infestation levels in the districts of Jangamo, Morrumbene and Inharrime ranged from severe to very severe.

Cugala *et al*., (2013) also reported that whitefly severity in Inhambane province ranged from 4 to 5 which, according to the severity scale (Table 1) corresponds to "severe to very severe". The high whitefly severity in the study area may be due to favourable agro-climatic environmental conditions (Cugala *et al*., 2013) with a mean annual minimum temperature of 19.5°C and maximum of 28.1°C (WMO, 2015) and 70% of relative humidity characteristic of the study area (Cugala *et al*., 2013). Salvador (2004), argued that high temperatures in the appropriate range and high humidity favours the occurrence of *A. atratus* leading to magnified severity.

The observations from the current study regarding whitefly severity agree with the results from Borowiec *et al*., (2010) who reported high levels of infestation in coastal areas where the temperature and relative humidity were high. Therefore, the coastal location of the sampling sites (Figure 1) may explain the observed severity of *A. atratus*. Various insects, respond to abiotic factors such as humidity, thermal effect, and light in different ways. These abiotic factors not only affect the behaviour of insects but also their physiological mechanisms (Karl *et al*., 2011) such as egg production and oviposition. Their populations may thus vary according to these factors.

### 4.1.4 Correlation among whitefly (pest) indicators (density, infestation and severity)

There was a strong positive correlation between whitefly density and whitefly infestation ($\tau$ = 0.6521, n=73, $\alpha$ = 0.05) (Table 4). Correlation between whitefly density and severity was positively weak ($\tau$ = 0.14, n =73, $\alpha$ = 0.05). Similarly, correlation between whitefly infestation and severity was positively weak ($\tau$ = 0.1116, n= 73, $\alpha$ = 0.05).

Table 4: Kendall's rank correlation coefficients between whitefly density, infestation and severity

| n = 73, $\alpha$ = 0.05 | Density | Infestation | Severity |
|---|---|---|---|
| Density | 1.0000 | | |
| Infestation | 0.6521 | 1.0000 | |
| Severity | 0.1473 | 0.1116 | 1.0000 |

**4.2 Assessment of occurrence of parasitoids associated with coconut whitefly and their potential for the control of *Aleurotrachelus atratus* in Inhambane province**

The parasitized pupae appeared slightly swollen and on emergence of the parasitoid, a small round exit hole was observed. Four parasitoid species were recovered from the whitefly pupae, two of which were identified up to species level and the other two were identified only up to genus level. Recovered parasitoids included; *Encarsia basicincta* Gahan (Hymenoptera, Aphelinidae), *Eretmocerus cocois* Delvare (Hymenoptera, Aphelinidae), *Encarsia sp*. and *Signiphora sp*. with relative abundances shown in figure 5 and respective species appearances in figure 6. This is the first time for coconut whitefly parasitoids to be reported in Mozambique. Details of the relative abundance of each parasitoid species in each district are shown in figure 7 and appendix 17.

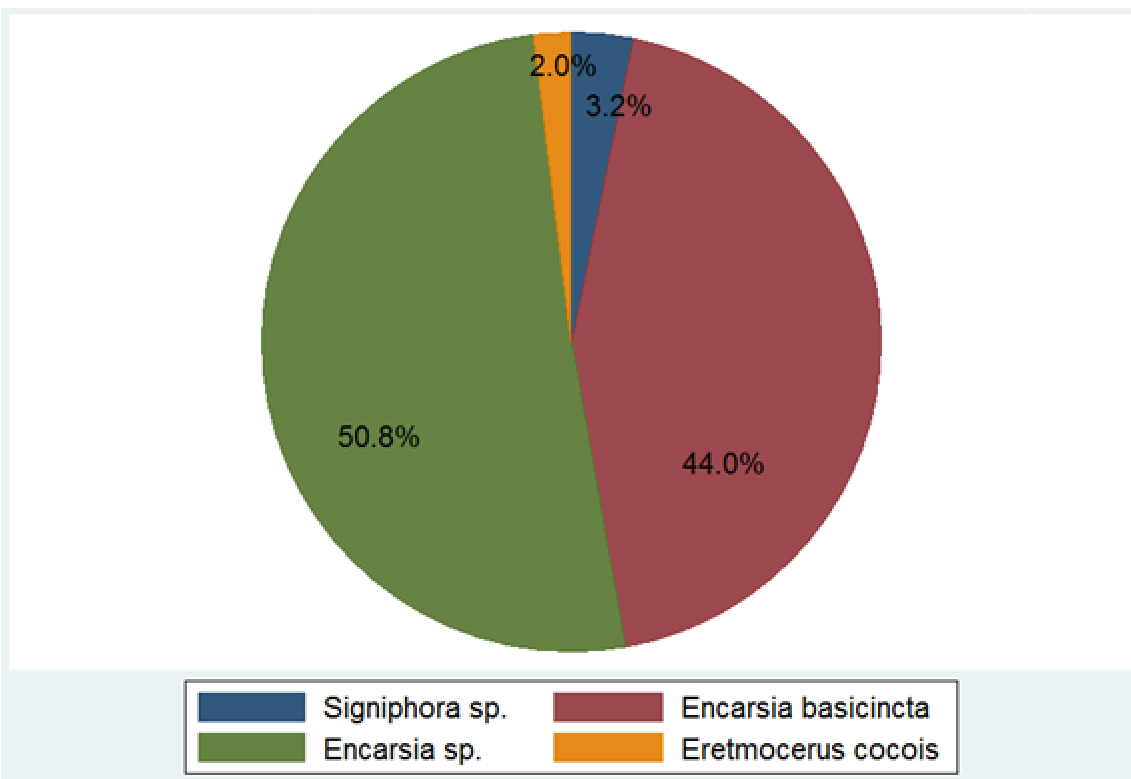


Figure 5: *A. atratus* parasitoid species composition and relative abundances in Inhambane

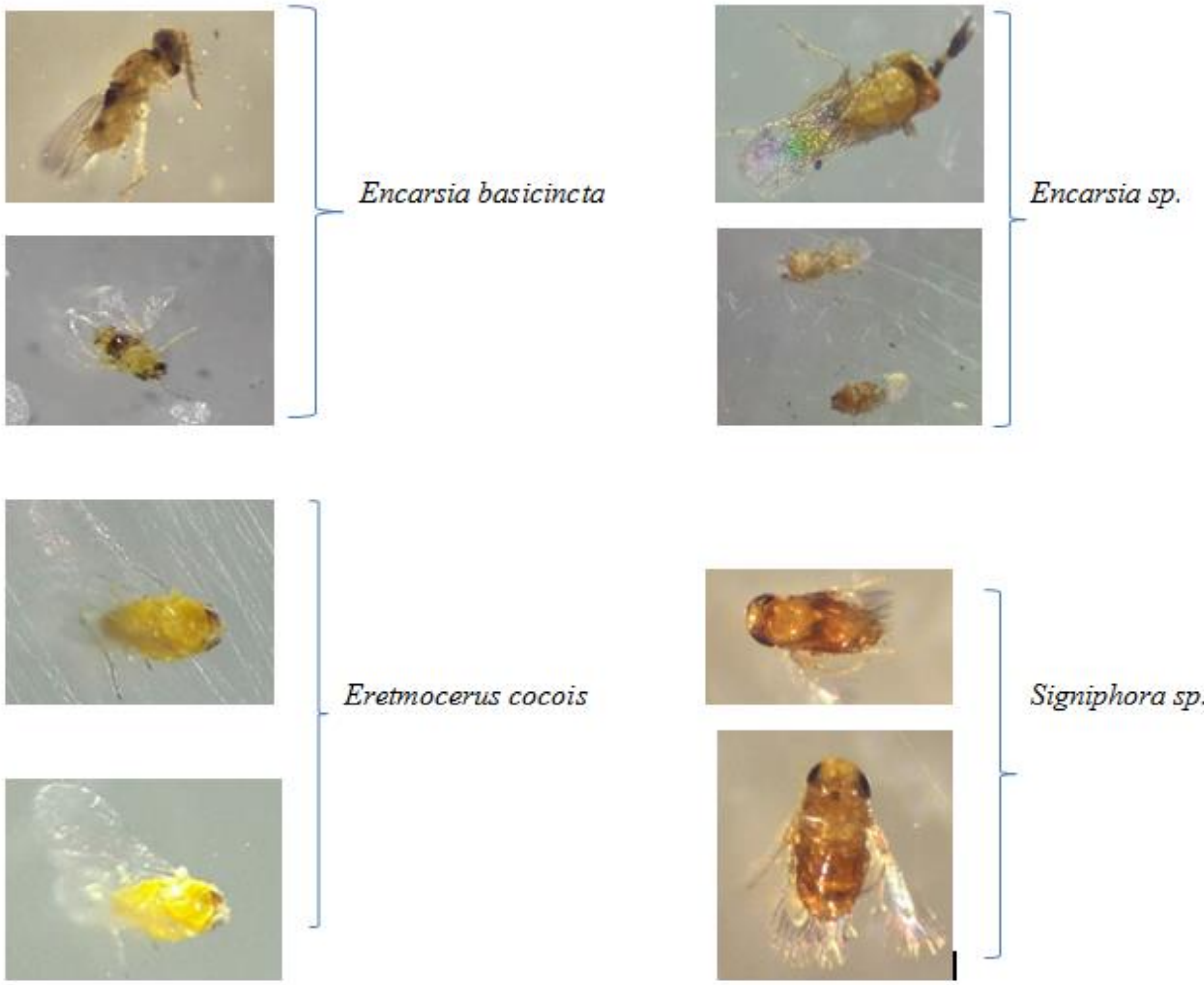


Figure 6: Parasitoid species recovered from *A. atratus* in Inhambane province

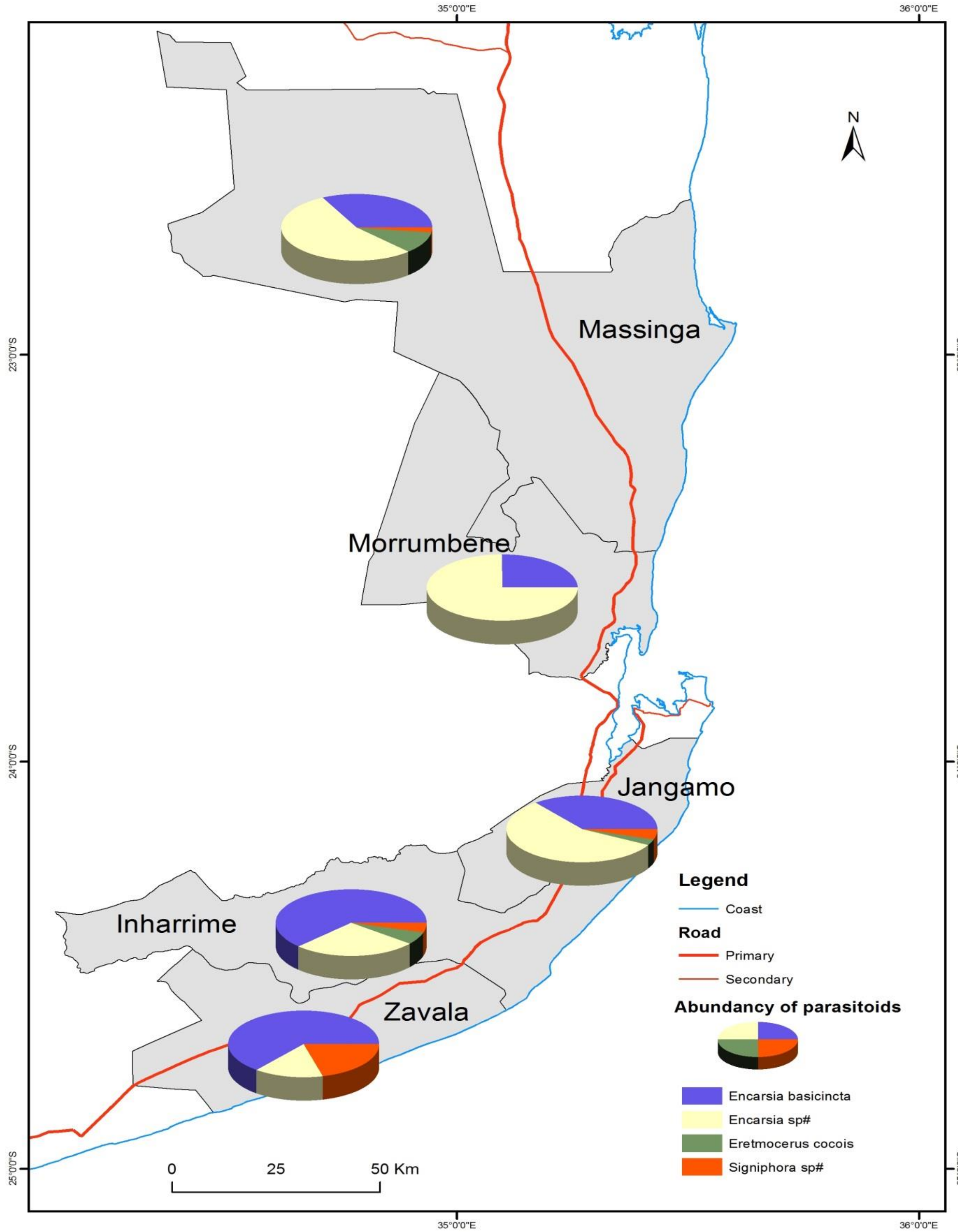


Figure 7: Relative abundance of parasitoid species in the sampled districts in Inhambane

The overall rate of parasitism of the recovered parasitoids was 10.74±2.03% with *Encarsia sp*. having the highest rate of 5.99±1.62%, followed by *Encarsia basicincta* (4.08±0.94%), *Signiphora sp.* (0.45±0.26%) and *Eretmocerus cocois* having the lowest rate of 0.22±0.08 as shown in table 5. Details of percentage parasitism of each parasitoid species in each district are shown in table 7 and appendix 10.

Table 5: Percentage parasitism of the recovered parasitoids in Inhambane province

| Parasitoid species | Percentage parasitism (±SE) |
|---|---|
| *Encarsia basicincta* | 4.08±0.94a |
| *Eretmocerus cocois* | 0.22±0.08b |
| *Encarsia sp.* | 5.99±1.62a |
| *Signiphora sp.* | 0.45±0.26b |
| Total | 10.74±2.03 |

Means followed by the same letter within a column are not significantly different (P<0.05).

The rate of parasitism varied significantly among districts (P = 0.0188, α =5%) with Morrumbene having the highest rate at 28.3±4.35% and Massinga with the lowest at 6.4±4.05% (Figure 8). There were no significant differences among locations in terms of percentage parasitism (P = 0.5709) at 95% confidence level (Table 6). The highest rate was recorded in Mucoduene at 42.2±11.58% and the lowest was in Bambela at 0.63±9.45%. Percentage parasitism did not differ significantly between sampling periods at 95% confidence level (P = 0.8511). There was no effect of interaction between district and sampling period on percentage parasitism (F=1.16, P=0.3351 α =5%, n=73), (Appendix 13). Likewise there was no effect of interaction between location and sampling period on percentage parasitism (F=0.75, 0.6314, α =5%, n=73) (Appendix 14).

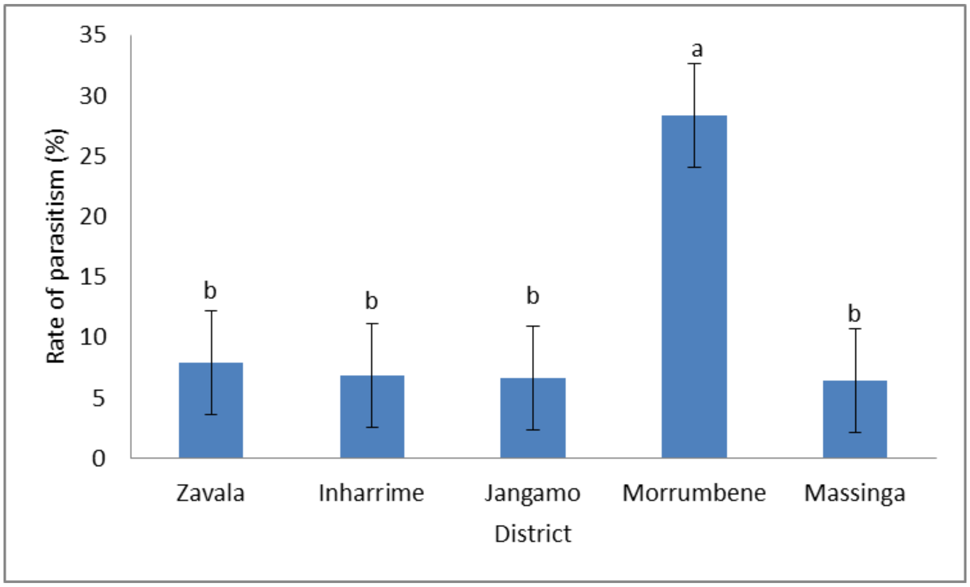


Figure 8: Variation of percentage parasitism among sampled districts in Inhambane

Table 6: Percentage parasitism in sampled locations in Inhambane

| District | Sampling location | Percentage parasitism (±SE) |
|---|---|---|
| Zavala | Zandamela | 5.7±2.71a |
| | Quissico | 14.4±6.32a |
| | Nyakundela | 2.9±0.692a |
| | Mwani | 5.3±2.82a |
| Inharrime | Inharrime sede | 5.7±3.23a |
| | Dongane | 4.0±0.81a |
| | Chongola | 14.3±7.14a |
| | Inhacoongo | 4.4±1.00a |
| Jangamo | Jangamo Rovene | 4.5±0.89a |
| | Bambela | 0.6±0.10a |
| | Lindela | 9.2±3.73a |
| | Cumbana | 8.6±3.51a |
| Morrumbene | Morrumbene sede | 19.9±8.72a |
| | Malaia | 41.34±29.27a |
| | Mucoduene | 42.2±37.11a |
| Massinga | Rio pedres | 5.2±2.54a |
| | Massinga sede | 9.0± 2.29a |
| | Massinga Rovene | 5.4±2.01a |
| | Mahocha | 4.3±4.30a |

Means followed by the same letter within a column are not significantly different ($P<0.05$).

Table 7: Rate of parasitism of each parasitoid species in each district in Inhambane province

| Districts | Parasitism of individual species (Mean ± SE) | | | | |
|---|---|---|---|---|---|
| | *Encarsia basicincta* | *Encarsia sp.* | *Eretmocerus cocois* | *Signiphora sp.* | Overall (district level) |
| Zavala | 4.87±1.66 | 1.52±0.55 | 0.06±0.04 | 1.53±1.22 | 7.99±0.047b |
| Inharrime | 4.05±1.78 | 2.14±1.16 | 0.33±0.20 | 0.23±0.11 | 6.75±4.047b |
| Jangamo | 2.54±0.76 | 3.71±1.60 | 0.15±0.10 | 0.24±0.18 | 6.64±4.047b |
| Morrumbene | 7.12±4.30 | 21.10±7.66 | 0 | 0.07±0.05 | 28.28±4.348a |
| Massinga | 2.23±0.81 | 3.52±0.83 | 0.55±0.33 | 0.11±0.01 | 6.40±4.047b |
| Overall (Province) | 4.08±0.94A | 5.99±1.62A | 0.22±0.08B | 0.45±0.26B | 10.74±2.03 |

Means followed by the same letter within a row or column are not significantly different (P<0.05)

*Encarsia basicincta* and *Eretmocerus cocois* are parasitoids associated with *A. atratus* in its native home (Brazil). The recovery of *Encarsia basicincta* and *Eretmocerus cocois* from the whitefly pupae is an indication that *A. atratus* was introduced with parasitoids considered efficient for the suppression of its population in its native range and it may constitute potential biological control agents against the invasive whitefly in Mozambique.

Borowiec *et al.* (2010) also recovered one unidentified *Encarsia* species in the Comoros Islands and the Seychelles. Therefore, studies should be undertaken in the area of the current study to identify the species name and to specify its role in controlling the population of *A. atratus*.
However, the current status of the coconut whitefly density in the study area indicates that the impact of parasitoids is still low to reduce the whitefly population. In general, the percentage parasitism varied from moderate (in Morrumbene, 28.28±4.348 pupae per leaflet) to low in the other sampling districts. Several hypotheses have been advanced to explain these time lags. One explanation is the alteration of the habitat by the introduced parasitoid to make it more favourable over a long time period (Liebhold and Tobin, 2008). Another hypothesis is the necessity for local adaptation by the individuals in an area, which occurs over a prolonged period (Overholt *et al*., 1997). Similar scenarios were observed in La Reunion Island where *A. atratus* was introduced with its parasitoids *Encarsia basicincta* and *Eretmocerus cocois* which are suppressing the whitefly population (CIRAD, 2011).

Both parasitoids, *Encarsia basicincta* and *Eretmocerus cocois*, are considered efficient natural enemies for the suppression of the coconut whitefly population in its native range in Brazil (CIRAD, 2011). Borowiec *et al.* (2010) argued that the *A. atratus* has never gained the status of a serious pest in its native home, probably, due to the presence of an endemic natural enemy complex.

*Eretmocerus cocois* was reported as the most abundant parasitoid of *A. atratus* in La Réunion. It was thought to be introduced into this island together with its host. It was also found to parasitize *A. atratus* in its area of origin, Central America (Delvare *et al*., 2008). This parasitoid species is considered specific for *A. atratus* as no other whitefly host has been reported for the same (Delvare *et al*. 2008). The fact that 98.5% of individuals are females, this species probably reproduces by parthenogenesis of type thelytoky, which could have several advantages for biological control (Stouthamer, 1993).

Moreover, studies conducted in La Réunion reported that the presence of *E. cocois* in La Réunion where low infestation levels of *A. atratus* occur, and its absence in Comoro Islands where high infestations and damage occur on coconut, indicated that this parasitoid is a potential candidate for the classical biological control of *A. atratus* in the region (Borowiec *et al*., 2010).

A linear regression analysis indicated a negative relationship between percentage parasitism and whitefly density (Figure 8). Reduction caused by parasitoids could be predicted by a linear function; $Y = - 0.1309X + 27.95$. According to this model, the negative coefficient of X indicates a descending slope suggesting that parasitoids could cause a reduction of 0.13 pupae per leaflet for every 1% increase in parasitism. Similar observations were reported by PRPV (2016) in the Grande Comoros where two parasitoids species; *Encarsia basicincta* and *Eretmocerus cocois* were well established reducing the population of *A. atratus* to acceptable levels. Martin (2004) stated that population densities of whiteflies in many parts of the world have been controlled by natural enemies such as predators and parasitoids. However, the low value of the coefficient of determination ($R^2=0.047$) indicates that the current rate of parasitism can only contribute 4.7% to reduction in whitefly density which is not significant ($P = 0.0655$, $\alpha=0.05$) (Appendix 18). This could be the reason why the current level of coconut whitefly in the study area is still alarming. This situation is expected in classical biological control because the parasitoids may need some time to adapt to the local environmental conditions for population build up and only then they produce significant impact on the target pest populations.

Inundative releases of laboratory reared agents may supplement the impact of pre-existing biocontrol agents to suppress pest populations. Once exotic natural enemies have been established, their activities and those of native species as well, may be enhanced by one or a more of a number of environmental manipulations (Horn, 1988). Biological control which involves the use of parasitoids has proven effective in several other parts of the world and certainty warrants increased effort and emphasis in managing the invasive *A. atraus*. It can be more successful against pests of perennial crops, such as coconut palms than in annual cropping systems which are attributed to the relative temporal stability of such habitats, contrasted with the seasonal disruption brought about by ploughing and harvesting (Horn, 1988), characteristic of annual crops production systems.

The parasitoid species recovered in the present study are very promising as biological control agents of *A. atratus* and have been reported, reared and/or released in other African countries including Comoro Islands, La Rèunion, Seychelles, (PRPV, 2016) where *A. atratus* population is now under control due to the activities of the same parasitoids species (*E. basicincta* and *E. cocois*) reported in the present study. Therefore, it is expected that the parasitoid's population will be fully established, grow and exert a significant impact on the whitefly population.

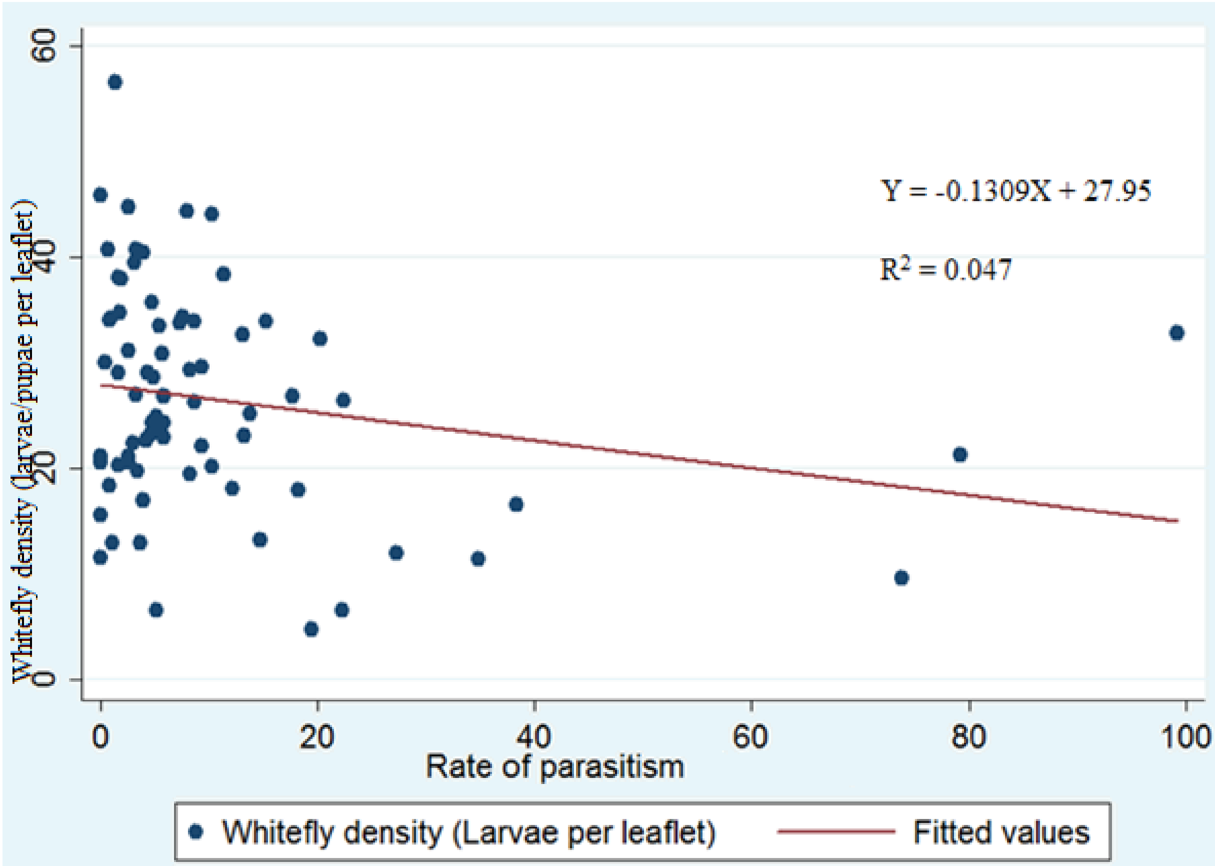


Figure 9: Linear regression of whitefly density and percentage parasitism in Inhambane

## CHAPTER FIVE

## 5.0 CONCLUSIONS AND RECOMMENDATIONS

### 5.1 Conclusions

Whitefly infestation in Inhambane province is very high to the tune of 99.86%. Whitefly severity ranges between severe to very severe being highest in Zavala district. Whitefly density has slightly reduced in Inhambane compared to recent past 26.4 larvae/pupae per leaflet. There are four parasitoid species associated with the coconut whiteflies in Inhambane province. *Encarsia sp*. has the highest rate of parasitism of 5.99%, followed by *Encarsia basicincta* (4.08%), *Signiphora sp.* (0.45%) and *Eretmocerus cocois* having the lowest rate of 0.22%. The rate parasitism observed during the current study is still low (10.74%) and varies significantly among districts.

### 5.2 Recommendations

- ✓ Awareness should be created and raised among farmers and field advisers in Inhambane to conserve the parasitoids with the aim of supporting their natural multiplication and increasing their parasitism.
- ✓ More research should be conducted in other whitefly-infested districts for the availability of these and other parasitoids of *Aleurotrachelus atratus*.
- ✓ Studies should be conducted on the behaviour and interaction of the recovered parasitoids (interspecific) to guide their effective use in the control of *Aleurotrachelus atratus*.
- ✓ A mass rearing program and release should be initiated to increase the numbers/parasitism of the recovered parasitoids in order to enhance their effectiveness, especially for *Encarsia basicincta* and *Eretmocerus cocois* due to their co-evolution with the pest in its native home.
- ✓ The relevant authorities should intervene by financially supporting the introduction of classical biological control programs and through policy and long-term political commitments.

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# APPENDICES

Appendix 1: Districts and locations sampled during the study in Inhambane province.

| District | Location | Latitudes | Longitudes |
|---|---|---|---|
| Zavala | Zandamela | 244549 | 342138 |
| | | 244657 | 341934 |
| | | 244705 | 341929 |
| | | 244660 | 341918 |
| | | 244704 | 341921 |
| | Quissico | 244203 | 344123 |
| | | 244207 | 344122 |
| | | 244158 | 344051 |
| | | 244127 | 343918 |
| | | 244154 | 344045 |
| | Nyakundela | 243020 | 350019 |
| | | 243023 | 350001 |
| | | 243021 | 350014 |
| | Mwani | 243610 | 344723 |
| | | 243615 | 344724 |
| Inharrime | Dongane | 242313 | 351051 |
| | | 242318 | 351055 |
| | Sede | 242545 | 350446 |
| | | 242550 | 350446 |
| | | 242532 | 350517 |
| | | 242911 | 350107 |
| | | 242906 | 350107 |
| | Chongola | 242406 | 350822 |
| | | 242434 | 350710 |
| | | 242437 | 350711 |
| | Inhacoongo | 242231 | 351131 |
| | | 242221 | 351132 |
| | | 242155 | 351151 |
| | | 241747 | 351350 |
| | | 241747 | 351347 |
| Jangamo | Bambela | 241509 | 351325 |
| | | 241512 | 351324 |
| | | 241450 | 351315 |
| | Rovene | 241453 | 351318 |
| | | 241453 | 351315 |
| | Lindela | 240509 | 351611 |
| | | 240508 | 351610 |
| | | 240614 | 351612 |
| | | 240611 | 351559 |
| | | 240631 | 351541 |
| | Cumbana | 240720 | 351435 |
| | | 240710 | 351452 |
| | | 240923 | 351255 |
| | | 240943 | 351255 |
| | | 241004 | 351247 |
| Morrumbene | Sede | 233559 | 352024 |
| | | 233551 | 352026 |
| | | 234459 | 351731 |
| | | 234456 | 351736 |
| | | 233621 | 352019 |
| | | 234440 | 351741 |

| | | | |
|---|---|---|---|
| | | 233827 | 352028 |
| | | 233829 | 352023 |
| | Malaia | 232929 | 352309 |
| | | 232923 | 352258 |
| | | 232936 | 352318 |
| | Mucoduene | 234654 | 351626 |
| | | 234652 | 351622 |
| Massinga | Rio Pedres | 231238 | 352245 |
| | | 231240 | 352248 |
| | | 231413 | 352216 |
| | | 231259 | 352231 |
| | | 231239 | 352247 |
| | Sede | 232033 | 352250 |
| | | 231727 | 352237 |
| | | 232036 | 352253 |
| | | 232312 | 352246 |
| | | 232306 | 352244 |
| | Rovene | 232607 | 352253 |
| | | 232545 | 352253 |
| | | 232739 | 352250 |
| | Mahocha | 232826 | 352253 |
| | | 232830 | 352254 |

Appendix 2: Significance test for whitefly infestation in the sampled districts in Inhambane

```
. kwallis infestation, by(district)

Kruskal-Wallis equality-of-populations rank test
```

| district | Obs | Rank Sum |
|---|---|---|
| Zavala | 15 | 562.50 |
| Inharrime | 15 | 526.00 |
| Jangamo | 15 | 562.50 |
| Morrumbene | 13 | 487.50 |
| Massinga | 15 | 562.50 |

```
chi-squared =     0.157 with 4 d.f.
probability =     0.9971

chi-squared with ties =     3.867 with 4 d.f.
probability =     0.4243
```

Appendix 3: Significance test for whitefly infestation for sampled locations in Inhambane

```
Kruskal-Wallis equality-of-populations rank test
```

| location | Obs | Rank Sum |
|---|---|---|
| Zandamela | 5 | 187.50 |
| Quissico | 5 | 187.50 |
| Nyakundela | 3 | 112.50 |
| Mwani | 2 | 75.00 |
| Inharrime sede | 5 | 187.50 |
| Dongane | 2 | 75.00 |
| Chongola | 3 | 112.50 |
| Inhacoongo | 5 | 151.00 |
| Jangamo Rovene | 2 | 75.00 |
| Bambela | 3 | 112.50 |
| Lindela | 5 | 187.50 |
| Cumbana | 5 | 187.50 |
| Morrumbene sede | 8 | 300.00 |
| Malaia | 3 | 112.50 |
| Mucoduene | 2 | 75.00 |
| Rio pedres | 5 | 187.50 |
| Massinga sede | 5 | 187.50 |
| Massinga Rovene | 3 | 112.50 |
| Mahocha | 2 | 75.00 |

```
chi-squared =     0.551 with 18 d.f.
probability =     1.0000

chi-squared with ties =    13.600 with 18 d.f.
probability =     0.7548
```

Appendix 4: Effect of interaction of sampling period and sampling location on whitefly infestation

```
anova  infestation samplingperiod##location

                          Number of obs =       73     R-squared     =  0.1889
                          Root MSE      = 1.31876     Adj R-squared = -0.2696

                Source | Partial SS    df       MS           F     Prob > F
  ---------------------+----------------------------------------------------
                 Model |  18.630137    26   .71654373       0.41     0.9912
                       |
           samplingp~d |  1.2816e-27     1  1.2816e-27      0.00     1.0000
              location | 17.6772466    18  .982069256      0.56     0.9065
  samplingp~d#location |  9.3463e-27     7  1.3352e-27      0.00     1.0000
                       |
              Residual |         80    46  1.73913043
  ---------------------+----------------------------------------------------
                 Total |  98.630137    72  1.36986301
```

Appendix 5: Analysis of variance (ANOVA) of whitefly severity in Inhambane

```
. anova severity district / location|district /

                          Number of obs =       73     R-squared     =  0.4838
                          Root MSE      = 8.01036     Adj R-squared =  0.3117

                Source | Partial SS    df       MS           F     Prob > F
     ------------------+----------------------------------------------------
                 Model | 3247.18021    18  180.398901       2.81     0.0017
                       |
              district | 469.596131     4  117.399033       0.57     0.6882
     location|district | 2879.38676    14  205.670483
     ------------------+----------------------------------------------------
     location|district | 2879.38676    14  205.670483       3.21     0.0010
                       |
              Residual | 3464.95822    54   64.165893
     ------------------+----------------------------------------------------
                 Total | 6712.13843    72  93.2241449
```

Appendix 6: Analysis of variance (ANOVA) of whitefly density among study districts and locations

```
. anova whiteflydensity district / location|district /

                           Number of obs =       73     R-squared     =  0.2745
                           Root MSE      =  10.2852     Adj R-squared =  0.0327

                  Source |  Partial SS    df       MS           F     Prob > F
       ------------------+----------------------------------------------------
                   Model |  2161.45858    18  120.081032       1.14     0.3466
                         |
                district |  89.7470798     4    22.43677       0.16     0.9554
       location|district |  1971.28342    14  140.805959
       ------------------+----------------------------------------------------
       location|district |  1971.28342    14  140.805959       1.33     0.2206
                         |
                Residual |   5712.4192    54  105.785541
       ------------------+----------------------------------------------------
                   Total |  7873.87778    72  109.359414
```

Appendix 7: Comparison t-test for whitefly severity for September and December 2015.

```
. ttest severitysept == severitydec, unpaired unequal

Two-sample t test with unequal variances
------------------------------------------------------------------------------
Variable |     Obs        Mean    Std. Err.   Std. Dev.   [95% Conf. Interval]
---------+--------------------------------------------------------------------
severi~t |      45    79.05185    1.191532    7.993038    76.65048    81.45323
severi~c |      29    73.74384    2.047999    11.02881    69.54871    77.93898
---------+--------------------------------------------------------------------
combined |      74    76.97169    1.114821     9.59005    74.74985    79.19352
---------+--------------------------------------------------------------------
    diff |            5.308009    2.369398                .5407351    10.07528
------------------------------------------------------------------------------
    diff = mean(severitysept) - mean(severitydec)                 t =   2.2402
Ho: diff = 0                     Satterthwaite's degrees of freedom =  46.7548

    Ha: diff < 0                 Ha: diff != 0                 Ha: diff > 0
 Pr(T < t) = 0.9851         Pr(|T| > |t|) = 0.0299          Pr(T > t) = 0.0149
```

Appendix 8: Comparison t-test for whitefly density for September and December 2015.

```
. ttest whiteflydensitysept == whiteflydensitydec, unpaired unequal

Two-sample t test with unequal variances

Variable |     Obs        Mean    Std. Err.   Std. Dev.   [95% Conf. Interval]
---------+--------------------------------------------------------------------
wh~ysept |      45    30.63852    1.463758    9.819189    27.68851    33.58853
whi~ydec |      29    19.81379    1.442336    7.767216     16.8593    22.76828
---------+--------------------------------------------------------------------
combined |      74     26.3964     1.21678    10.46713    23.97136    28.82143
---------+--------------------------------------------------------------------
    diff |            10.82473    2.054975                6.725037    14.92441
------------------------------------------------------------------------------
    diff = mean(whiteflydensit~t) - mean(whiteflydensit~c)        t =   5.2676
Ho: diff = 0                     Satterthwaite's degrees of freedom =  68.8808

    Ha: diff < 0                 Ha: diff != 0                 Ha: diff > 0
 Pr(T < t) = 1.0000         Pr(|T| > |t|) = 0.0000          Pr(T > t) = 0.0000
```

Appendix 9: Effect of interaction between sampling period and location on whitefly density

```
 anova  whiteflydensity samplingperiod##location

                         Number of obs =      73     R-squared     =  0.4639
                         Root MSE      =  9.5796     Adj R-squared =  0.1609

                  Source |  Partial SS    df       MS           F     Prob > F
              -----------+----------------------------------------------------
                   Model |  3652.51796    26   140.48146       1.53     0.1019
                         |
              samplingp~d |  399.928992     1  399.928992       4.36     0.0424
                location |  1450.33566    18  80.5742035       0.88     0.6054
     samplingp~d#location |   1001.9815     7  143.140214       1.56     0.1716
                         |
                Residual |  4221.35982    46  91.7686917
              -----------+----------------------------------------------------
                   Total |  7873.87778    72  109.359414
```

Appendix 10: Percentage parasitism of each parasitoid in each district in Inhambane province

| Over | Mean | Std. Err. | [95% Conf. | Interval] |
|---|---|---|---|---|
| signiphorasp | | | | |
| Zavala | 1.533177 | 1.224307 | -.9074342 | 3.973789 |
| Inharrime | .2294477 | .1112152 | .0077443 | .4511511 |
| Jangamo | .2382224 | .179727 | -.1200568 | .5965016 |
| Morrumbene | .0663767 | .0477754 | -.0288619 | .1616153 |
| Massinga | .105563 | .0554705 | -.0050156 | .2161415 |
| encarsiabasicincta | | | | |
| Zavala | 4.874805 | 1.660909 | 1.563844 | 8.185766 |
| Inharrime | 4.050646 | 1.780605 | .5010746 | 7.600217 |
| Jangamo | 2.538933 | .7597996 | 1.0243 | 4.053566 |
| Morrumbene | 7.117694 | 4.300311 | -1.454819 | 15.69021 |
| Massinga | 2.225461 | .8108619 | .6090373 | 3.841885 |
| encarsiasp | | | | |
| Zavala | 1.520203 | .5508024 | .4221983 | 2.618207 |
| Inharrime | 2.140418 | 1.156228 | -.1644806 | 4.445316 |
| Jangamo | 3.707089 | 1.599306 | .5189308 | 6.895247 |
| Morrumbene | 21.0971 | 7.661506 | 5.824166 | 36.37003 |
| Massinga | 3.518584 | .831264 | 1.86149 | 5.175679 |
| eretmoceruscocois | | | | |
| Zavala | .0571781 | .0436672 | -.0298707 | .144227 |
| Inharrime | .3327708 | .1955823 | -.0571153 | .7226569 |
| Jangamo | .1533411 | .0975418 | -.0411049 | .347787 |
| Morrumbene | 0 | 0 | . | . |
| Massinga | .5461523 | .3333721 | -.1184129 | 1.210717 |

Appendix 11: Analysis of variance (ANOVA) for percentage of parasitism

```
. anova tgeparasitism district location|district

                         Number of obs =      73     R-squared     =  0.3076
                         Root MSE      = 1.00199     Adj R-squared =  0.0769

                  Source | Partial SS    df       MS           F     Prob > F
              -----------+----------------------------------------------------
                   Model | 24.0894342    18   1.3383019       1.33     0.2054
                         |
                district |   12.98927     4  3.24731751       3.23     0.0188
       location|district | 12.5470034    14  .896214525       0.89     0.5709
                         |
                Residual |  54.214756    54  1.00397696
              -----------+----------------------------------------------------
                   Total | 78.3041902    72   1.0875582
```

Appendix 12: Comparison t-test for percentage of parasitism between September and December 2015

```
. ttest geparasitismsept == geparasitismdec, unpaired unequal

Two-sample t test with unequal variances
------------------------------------------------------------------------------
Variable |     Obs        Mean    Std. Err.   Std. Dev.   [95% Conf. Interval]
---------+--------------------------------------------------------------------
gepara~t |      45    10.98603    2.380982    15.97211    6.187478    15.78459
gepara~c |      29    10.17521    3.577131    19.26344    2.847786    17.50263
---------+--------------------------------------------------------------------
combined |      74    10.66828    2.000861    17.21206    6.680566    14.65599
---------+--------------------------------------------------------------------
    diff |            .8108251    4.297085               -7.812573    9.434223
------------------------------------------------------------------------------
    diff = mean(geparasitismsept) - mean(geparasitismdec)         t =   0.1887
Ho: diff = 0                     Satterthwaite's degrees of freedom =  51.8319

    Ha: diff < 0                 Ha: diff != 0                 Ha: diff > 0
 Pr(T < t) = 0.5745         Pr(|T| > |t|) = 0.8511          Pr(T > t) = 0.4255
```

Appendix 13: Effect of interaction between district and sampling period on percentage parasitism

```
anova geparasitism  district##samplingperiod
```

Number of obs = 73 R-squared = 0.2796
Root MSE = 15.7147 Adj R-squared = 0.1767

| Source | Partial SS | df | MS | F | Prob > F |
|---|---|---|---|---|---|
| Model | 6038.48705 | 9 | 670.943006 | 2.72 | 0.0097 |
| district | 5739.76626 | 4 | 1434.94156 | 5.81 | 0.0005 |
| samplingp~d | 15.4874309 | 1 | 15.4874309 | 0.06 | 0.8031 |
| district#samplingp~d | 1150.05665 | 4 | 287.514163 | 1.16 | 0.3351 |
| Residual | 15558.0031 | 63 | 246.95243 | | |
| Total | 21596.4901 | 72 | 299.951252 | | |

Appendix 14: Effect of interaction between location and sampling period on percentage parasitism

```
anova geparasitism location##samplingperiod
```

Number of obs = 73 R-squared = 0.3983
Root MSE = 16.8074 Adj R-squared = 0.0582

| Source | Partial SS | df | MS | F | Prob > F |
|---|---|---|---|---|---|
| Model | 8602.01989 | 26 | 330.846919 | 1.17 | 0.3130 |
| location | 7819.13632 | 18 | 434.396462 | 1.54 | 0.1202 |
| samplingp~d | 13.2784377 | 1 | 13.2784377 | 0.05 | 0.8293 |
| location#samplingp~d | 1482.80256 | 7 | 211.828937 | 0.75 | 0.6314 |
| Residual | 12994.4702 | 46 | 282.488483 | | |
| Total | 21596.4901 | 72 | 299.951252 | | |

Appendix 15: Comparison t-test for whitefly density between sampling methods

Two-sample t test with unequal variances

| Variable | Obs | Mean | Std. Err. | Std. Dev. | [95% Conf. | Interval] |
|---|---|---|---|---|---|---|
| white~ya | 73 | 26.54795 | 1.223958 | 10.45751 | 24.10803 | 28.98786 |
| whitef~b | 27 | 59.51852 | 5.97667 | 31.05569 | 47.2333 | 71.80374 |
| combined | 100 | 35.45 | 2.343606 | 23.43606 | 30.79978 | 40.10022 |
| diff | | -32.97057 | 6.10071 | | -45.46315 | -20.478 |

diff = mean(whiteflydensitya) - mean(whiteflydensityb)    t = -5.4044

Ho: diff = 0    Satterthwaite's degrees of freedom = 28.2086

| Ha: diff < 0 | Ha: diff != 0 | Ha: diff > 0 |
|---|---|---|
| Pr(T < t) = 0.0000 | Pr(\|T\| > \|t\|) = 0.0000 | Pr(T > t) = 1.0000 |

Appendix 16: Comparison t-test for percentage parasitism between sampling methods

Two-sample t test with unequal variances

| Variable | Obs | Mean | Std. Err. | Std. Dev. | [95% Conf. | Interval] |
|---|---|---|---|---|---|---|
| gepara~a | 73 | 10.74295 | 2.027047 | 17.3191 | 6.702101 | 14.78379 |
| gepara~b | 27 | 7.984622 | 1.751099 | 9.098976 | 4.385187 | 11.58406 |
| combined | 100 | 9.998199 | 1.553719 | 15.53719 | 6.915283 | 13.08111 |
| diff | | 2.758324 | 2.678669 | | -2.566372 | 8.08302 |

diff = mean(geparasitisma) - mean(geparasitismb)    t = 1.0297

Ho: diff = 0    Satterthwaite's degrees of freedom = 86.3655

| Ha: diff < 0 | Ha: diff != 0 | Ha: diff > 0 |
|---|---|---|
| Pr(T < t) = 0.8470 | Pr(\|T\| > \|t\|) = 0.3060 | Pr(T > t) = 0.1530 |

Appendix 17: Relative abundance of *A. atratus* parasitoids recovered from Inhambane province.

| District | Parasitoid species | Relative abundance (%) |
|---|---|---|
| Zavala | *Encarsia basicincta* | 60.95 |
| | *Encarsia sp.* | 19.02 |
| | *Eretmocerus cocois* | 0.75 |
| | *Signiphora sp.* | 19.15 |
| Inharrime | *Encarsia basicincta* | 60.00 |
| | *Encarsia sp.* | 31.70 |
| | *Eretmocerus cocois* | 4.89 |
| | *Signiphora sp.* | 3.41 |
| Jangamo | *Encarsia basicincta* | 38.25 |
| | *Encarsia sp.* | 55.87 |
| | *Eretmocerus cocois* | 2.26 |
| | *Signiphora sp.* | 3.61 |
| Morrumbene | *Encarsia basicincta* | 25.18 |
| | *Encarsia sp.* | 74.61 |
| | *Eretmocerus cocois* | 0.00 |
| | *Signiphora sp.* | 0.25 |
| Massinga | *Encarsia basicincta* | 34.84 |
| | *Encarsia sp.* | 55.0 |
| | *Eretmocerus cocois* | 8.59 |
| | *Signiphora sp.* | 1.72 |

Appendix 18: Regression analysis for whitefly density against percentage parasitism

```
. regress whiteflydensity geparasitism

      Source |       SS       df       MS              Number of obs =      73
-------------+------------------------------           F(  1,    71) =    3.50
       Model |  369.925122     1  369.925122           Prob > F      =  0.0655
    Residual |  7503.95266    71  105.689474           R-squared     =  0.0470
-------------+------------------------------           Adj R-squared =  0.0336
       Total |  7873.87778    72  109.359414           Root MSE      =  10.281

------------------------------------------------------------------------------
whiteflyde~y |      Coef.   Std. Err.      t    P>|t|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
geparasitism |  -.1308776   .0699559    -1.87   0.065    -.2703657    .0086105
       _cons |   27.95396   1.418662    19.70   0.000     25.12522    30.78269
------------------------------------------------------------------------------
```