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FIRST RECORD OF PARASITOIDS ASSOCIATED WITH THE INVASIVE COCONUT WHITEFLY IN INHAMBANE PROVINCE, MOZAMBIQUE

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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. 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. Coconut leaflets were collected from five districts of the province including; Zavala, Inharrime, Jangamo, Morrumbene and Massinga districts in September and December 2015. Whitefly pupae were counted after which samples were kept in the laboratory for at least 15 days. Emerged parasitoids were identified, counted and recorded. Four parasitoid species 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 for the first time in Mozambique 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. 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 vield loss.

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1.0 INTRODUCTION

The coconut palm (*Cocos nucifera* L.: Arecaceae) is a major cash crop that is widely grown in coastal tropical regions of the world and contributes to the economy, livelihood and food security of millions of rural inhabitants (Bila *et al.*, 2015). 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), making the country the fourth largest producer of coconut in Africa (after Tanzania, Ghana and Nigeria) and having 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).

The coconut whitefly, Aleurotrachelus atratus Hempel (Homoptera: Alevrodidae) is a highly invasive pest of coconut and ornamental palms (Malumphy, 2013). 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. 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. Studies have reported 100% of infestation of coconut plants by A. atratus in Inhambane province, southern Mozambique, 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 being responsible for 70.74% of the total reduction in coconut production in Mozambique and by small scale farmers (Cugala et al., 2013) in the last 6 years, affecting seriously the coconut production in the country. 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).

The current study was conducted to evaluate the occurrence of parasitoids associated with *A. atratus* as the basis for the introduction of classical biological control in Inhambane province. The study was specifically intended to i) determine the current level of infestation, population

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density and severity of coconut whitefly (*Aleurotrachelus atratus*), ii) to determine the occurrence of parasitoids associated with the invasive coconut whitefly and, iii) to estimate the rate of parasitism of recovered parasitoids in Inhambane province, southern Mozambique.

2.0 MATERIALS AND METHODS

2.1 Description of the study area

Inhambane province is located on the coast in the southern part of Mozambique with an area of 68,775 km² covered by 12 districts (EdM, 2013). 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. 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°C and 28.1°C with annual rainfall of 949.8mm (WMO, 2015).

2.2 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 accessibility. 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.

2.3 Whitefly infestation

For estimation of whitefly infestation, 20 plants were randomly selected in each field 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 formula 1.

Percentage of infestation =
$$\frac{\text{number of infested plants}}{\text{total number of plants observed}} \times 100\%$$
 (1)

2.4 Whitefly severity

To determine whitefly severity, all the 20 plants selected in each field for whitefly infestation were observed for estimation of severity levels. On each plant, 5 leaves (sheets) in the directions:

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North, South, East, West and centre were selected to avoid sampling errors and minimize aggregation and dispersion problems. For each selected leaf, the level of coverage of pupae was estimated and assigned a value according to a six-level scale (table 1) modified from Borowiec *et al.*, (2010). The level of severity was estimated using formula 2 described by Borowiec *et al.* (2010).

Level of severity =
$$\frac{\Sigma \text{ (grade of scale*frequency)}}{\text{total number of units* maximum grade of the scale}} \times 100\%$$
 (2)

Table 1: Scale used to estimate the level of whitefly severity in Inhambane Province, Mozambique.

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	Low	Slight	Medium	Severe	Very
	infestation					severe

Modified from Borowiec et al. (2010)

2.5 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 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 using formula 3.

Population density =
$$\frac{\text{number of pupae collected}}{\text{number of leaflets observed}}$$
 (3)

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

Samples of leaflets collected for estimation of whitefly density 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) in Maputo and kept at room temperature for at least 15 days to allow for emergence of parasitoids. The samples were later opened and all emerged parasitoids were counted and recorded.

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

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).

$$P_p = \left(\frac{Tpe}{Tpc}\right) \times 100$$

Where:

Pp: Percentage parasitism

Tpe: Total parasitoids 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.

2.7 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) (STATA 12.0). For percentage parasitism, as normality of the error term could not be reached, the data was log(x+1) transformed after which analysis of variance 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) at 95% confidence level. Linear regression was used to predict the current contribution of parasitoids to reduction in whitefly density.

3.0 RESULTS AND DISCUSSION

3.1 Whitefly infestation

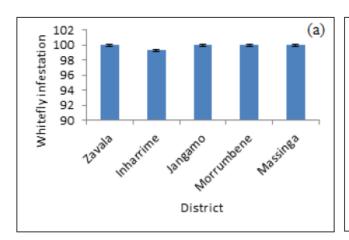
Whitefly infestation was 100% in all districts of study except Inharrime where the infestation

level was 99.3% (Table 2). However there were no significant differences at 95% level of confidence among sampling districts (Figure 1a) based on Kruskal-Wallis rank test (P = 0.4243, $\chi^2 = 3.867$, df = 4). Similarly, all sampled locations registered 100% whitefly infestation except Inhacoongo which had $98\pm0.54\%$ infestation but was also not significantly different from other locations (P = 0.7548, $\chi^2 = 13.600$, df = 18, $\alpha = 0.05$) based on Kruskal-Wallis rank. Among the sampling periods, whitefly infestation of 100% was recorded in September, reducing slightly to 99.6% in December (Figure 1b), however the reduction not being significant (P = 0.3262, $\alpha = 0.05$, N = 73). All sampled locations were in the altitude range between 12 and 152 above sea level.

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

District	Level of	Severity (%±SE)	Whitefly density		
	infestation (%±SE)		(Pupae per leaflet±SE)		
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).



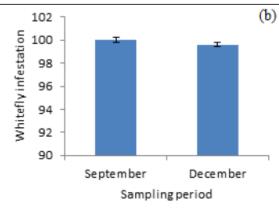


Figure 1: Variation of whitefly infestation: (a) among districts and (b) between sampling periods

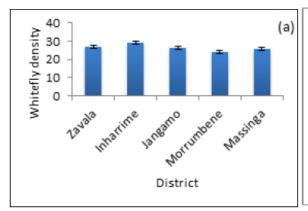
The current high whitefly infestation recorded in the study area implies that *Aleurotrachelus atratus* is well established in Inhambane. This might be due to the invasive nature of the pest, high mobility and dispersion in coastal regions since the sampled districts were bordering the

Indian Ocean, and high range of hosts (Borowiec *et al.*, 2010) for this pest in the study area. It may also be due to favourable agro-climatic conditions including but not limited to; temperature, relative humidity and presence of host plants. These results are 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) also 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 may be responsible for the high whiteflies infestation level in Inhambane.

3.2 Whitefly density

There were no significant differences among districts in terms of whitefly density (P = 0.9554, α = 5%, N=73). Similarly, variation of whitefly density among locations within districts was not significant (P = 0.2206, α = 5%, N=73).

Whitefly density differed significantly between sampling periods (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 2b. Overall whitefly density was 26.4 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).



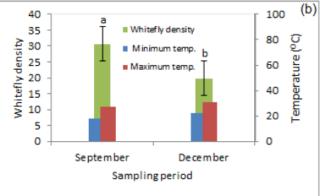


Figure 2: Variation of whitefly density: a) among districts and b) between sampling periods

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Higher population densities were 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. 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 Cugala *et al.*, (2013) 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. However, the density reported 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 cm² registered in Comoros where *Aleurotrachelus atratus* has been effectively controlled (PRPV, 2016).

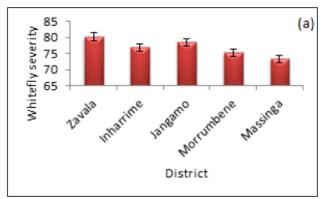
The decrease in whitefly density from 30.6 to 19.8 larvae per leaflet between September and December 2015 might be due to higher temperatures that prevailed in December (22°C-31°C) compared to September which had 18°C-27°C (WMO, 2015). Desai and Gupta (2015) also recorded maximum whitefly density during 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°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 in the study area. 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.

3.3 Whitefly severity

Whitefly severity varied from $73.9\pm2.49\%$ in Massinga district to $80.2\pm2.49\%$ in Zavala district (Table 2). There were no significant differences among the districts studied (P = 0.6882, α = 0.05, N=73) (Figure 3a). Severity differed significantly between sampling periods (P = 0.0299, α =0.05, N=73), being higher in September 2015 at $79.1\pm1.19\%$ compared to December 2015 which had $73.7\pm2.05\%$ (Figure 3b). Overall, whitefly severity varied between 74.75% and 79.19%, which corresponds to a variation from severe to very severe according to the scale described in Table 1.

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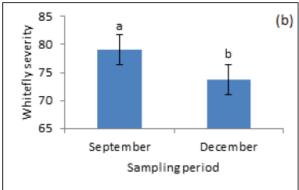


Figure 3: Variation of whitefly severity: a) among districts and b) between sampling periods.

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 high severity.

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.

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

Parasitized whitefly 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, which included; *Encarsia basicincta* Gahan (Hymenoptera, Aphelinidae), *Eretmocerus cocois* Delvare (Hymenoptera, Aphelinidae), *Encarsia sp.* and *signiphora sp.* This is the first time for coconut whitefly parasitoids to be reported in Mozambique.

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 3. Details of percentage parasitism of each parasitoid species in each district are

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shown in table 4.

The rate of parasitism varied significantly among districts (P = 0.0188, α =5%, N=73) with Morrumbene having the highest rate at 28.3±4.35% and Massinga with the lowest at 6.4±4.05% (Figure 4). There were no significant differences among locations in terms of percentage parasitism (P = 0.5709, α =0.05). Percentage parasitism did not differ significantly between sampling periods at 95% confidence level (P = 0.8511, α =0.05).

Table 3: 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).

Table 4: Rate of parasitism of each parasitoid species in each district in Inhambane province

Districts	Parasitism of in				
	Encarsia	Encarsia sp.	Eretmocerus	Signiphora sp.	Overall (district
	basicincta		cocois		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 are not significantly different (P<0.05)

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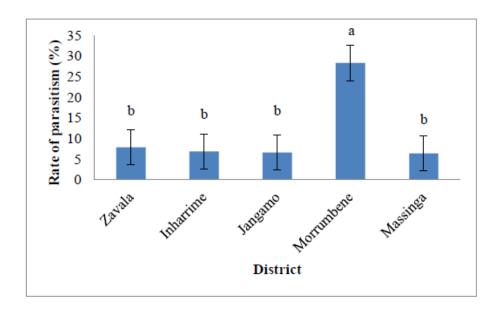


Figure 4: Variation of percentage parasitism among sampled districts in Inhambane

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 in Inhambane province, southern Mozambique 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.

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 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). *Eretmocerus cocois* was reported as the most abundant parasitoid of *A. atratus* in La Réunion. It was thought to have been introduced into the 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, *E. cocois* probably reproduces by parthenogenesis of type thelytoky, which could have several advantages for biological control (Stouthamer, 1993).

A linear regression analysis indicated a negative relationship between percentage parasitism and whitefly density (Figure 5). 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 (R²=0.047) indicates that the current rate of parasitism can only contribute 4.7% to reduction in whitefly density. 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.

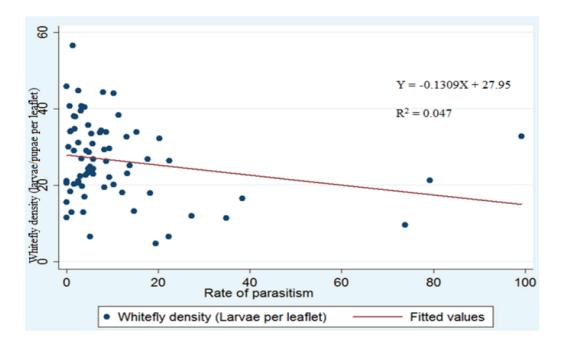


Figure 5: Linear regression of whitefly density and percentage parasitism in Inhambane

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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 parasitoids' population will be fully established, grow and exert a significant impact on the whitefly population in Mozambique.

4.0 CONCLUSION

Whitefly infestation in Inhambane province is high with a high severity index. Although parasitoids are known to reduce pest density, it is only possible when the rate of parasitism is high. The role of the recovered parasitoids in the control of this pest needs to be enhanced as the current rate of parasitism is still low. It is recommended that efforts should be made to increase the rate of parasitism through inundative, conservation and classical biological control approaches.

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