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BIOLOGICAL ASPECTS AND INSECTICIDE ACTION OF PLANT SPECIES ON EGGS AND NYMPHS OF CITRUS BLACK FLY (*Aleurocanthus woglumi Ashby - Aleyrodidae*) AT LABORATORY LEVEL[1]

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ABSTRCT - *Aleurocanthus woglumi* Ashby (Hemiptera: Aleyrodidae) is an important pest of many plant species in particular citrus species. The aims of this study were: (1) to observe 'in vitro' biological aspects in three hosts and (2) to evaluate the bioactivity of plant species on immature *A.woglum*. Aqueous neem extract (Meliaceae), cassava wastewater, pepper sauce and gravy peduncle floral carnation guinea button and commercial neem were used in the experiment. Egg viability (%), nymphal period duration, nymphal mortality (%), puparium stage duration and pupae viability (%) were evaluated in 50 egg positions, with minimum of five eggs in "Pera" orange, mango and malay apple leaves obtained in the field. The insecticide effect (translaminar action and direct spray) at different concentrations on immature *A.woglumi* was evaluated. The first bioassay conducted to evaluate the bioactivity of species by translaminar action, each sample unit corresponded to three leaves, with minimum of 20 eggs and 20 1st instar nymphs, the variables were unviable eggs and nymph mortality (%); the experiment had a completely randomized design with four replications. In the second bioassay, fully infected branches were sprayed on upper and lower face of leaves with all treatments. Leaves with at least 40 eggs and 40 1st instar nymphs were marked, and mortality (%) and emergence (%) of adults were evaluated for seven days; each sample unit was represented by 40 eggs and 40 1st instar nymphs and the experiment had a completely randomized design with four replications. The average duration of the nymphal period ranged from 7.76 to 24.18 days in "Pera" orange, and from 8.86 to 25.20 days in mango. There was no significant difference between the viability of eggs and nymphs in "Pera" orange and mango or pupae viability between "Pera" orange and malay apple. Treatment efficiency was evaluated using the Abbott's formula [E(%) = T-I/T x 100], where E (%) = efficiency percentage, T = number of live insects in the control treatment and I = number of live insects in treatment with insecticide. Therefore, in the first bioassay conducted to evaluate the bioactivity of species by translaminar action, all treatments showed efficiency greater than 50%. In the second bioassay by direct spraying to cassava wastewater (100% and 50 %) and commercial neem oil (1%), treatment had nymphal mortality efficiency greater than 80%.
Index Terms: *Azadirachta indica.* Botanical insecticide. Manipueira.

ASPECTOS BIOLÓGICOS E AÇÃO INSETICIDA DE ESPÉCIES VEGETAIS SOBRE IMATUROS DA MOSCA-NEGRA-DOS-CITROS (*Aleurocanthus woglumi* Ashby – HEMIPTERA: ALEYRODIDAE) EM LABORATÓRIO

RESUMO - *Aleurocanthus woglumi* Ashby (Hemiptera: Aleyrodidae) é uma importante praga para diversas espécies botânicas, em especial as espécies cítricas. Os objetivos deste estudo foram: (1) observar *in vitro* aspectos biológicos em três hospedeiros (laranjeira-pera, mangueira, jambeiro-vermelho) e (2) avaliar a bioatividade de espécies vegetais sobre imaturos de *A.woglum*. Foram utilizados no experimento extrato aquoso de nim (Meliaceae), manipueira, calda de pimentas, calda do pedúnculo do botão floral do craveiro da índia e o óleo de nim comercial. Foi avaliada a viabilidade de ovos (%), duração do período ninfal, mortalidade de ninfas (%), duração da fase de pupário e viabilidade dos pupários (%) em 50 posturas, com mínimo de cinco ovos, em folhas de laranjeira-pera, mangueira e jambeiro-vermelho obtidas em campo. Foi avaliado o efeito inseticida (ação translaminar e pulverização direta) em diferentes concentrações sobre imaturos de *A.woglumi*. No primeiro bioensaio, realizado para avaliar a bioatividade das espécies pela ação translaminar, cada unidade amostral correspondeu a três folhas, com mínimo de 20 ovos e 20 ninfas de 1º ínstar, as variáveis avaliadas foram inviabilidade de ovos e mortalidade de ninfas (%); o experimento foi em delineamento inteiramente casualizado, em quatro repetições. No segundo bioensaio, ramos infestados foram pulverizados totalmente, nas faces inferior e superior das folhas, com todos oss tratamentos. Folhas com, no mínimo, 40 ovos e 40 ninfas de 1º instar foram marcadas, e durante sete dias foram avaliada a mortalidade (%) e a emergência (%) de adultos; cada unidade amostral foi representada por 40 ovos e 40 ninfas de 1º instar, e o experimento foi em delineamento inteiramente casualizado, em quatro repetições. A duração média do período ninfal variou de 7,76 a 24,18 dias em laranjeira-pera, e de 8,86 a 25,20 dias em mangueira. Não foi observada diferença significativa entre a viabilidade de ovos e ninfas em laranjeira-pera e mangueira nem a viabilidade de pupários entre laranjeira-pera e jambeiro-vermelho. A eficiência dos tratamentos foi avaliada pela fórmula de Abbott [E(%) = T-I/T x 100], onde E (%)= porcentagem de eficiência, T = número de insetos vivos na testemunha e I = número de insetos vivos no tratamento com inseticida. Sendo assim, no primeiro bioensaio realizado para avaliar a bioatividade das espécies pela ação translaminar todos os tratamentos apresentaram eficiência maior que 50%. No segundo bioensaio através da pulverização direta a manipueira (100% e 50%) e o óleo de nim comercial (1%) apresentaram eficiência superior. **Termos para indexação:** *Azadirachta indica*, inseticida botânico, manipueira.

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INTRODUCTION

The citrus industry is a highly organized and competitive sector and has great representativity in the Brazilian agribusiness. Accounting for 60% of the orange juice world production, Brazil is the main exporter, and up to July 2016, about 254,239 tons were exported (CITRUSBR, 2016; BRASIL, 2016).

In the year 2014, the State of Bahia produced about 1,026,167 tons of orange (62,303 ha), 67,559 tons of lemon (3,405 ha) and 10,288 tons of tangerine (712 ha), having the "Recôncavo Baiano" as the main producing region (IBGE, 2016). Citrus plants are subject to the attack of several pests in their different stages of formation and development: sowing, nursery and orchard (EMBRAPA, 2016). One of the major pests affecting citrus production is the citrus black fly, *Aleurocanthus woglumi* Ashby (Hemiptera: Aleyrodidae), which is located on the underside of leaves. They cause direct damages by the continuous sap sucking and consequent decline in the vigor of plants, as well as indirect damages by favoring the appearance of sooty mold on leaves, branches and fruits, affecting respiration, photosynthesis and the quality of fruits for fresh commercialization, resulting in increased costs due to intensive washing during processing in the packing house.

This pest originated in Asia and is widespread in Africa, North, Central and South America, the Caribbean and Oceania (EPPO, 2008). The first occurrence in Brazil was in 2001, in Belém, state of Pará, and is currently disseminated in several states of the country, including Bahia (SILVA et al., 2010). *Aleurocanthus woglumi* is a pest of agricultural importance because it is polyphagous, with a record of more than 300 botanical species as hosts, including cultivated, wild and ornamental species (BARBOSA, 2007, MAIA, 2008, PENA et al. Sá et al., 2008; SILVA, 2010; SILVA et al., 2011).

Females lay their eggs in the form of a spiral on the abaxial face of leaves, which is a mechanism used in their protection against natural enemies and the action of abiotic factors. Several studies have shown that the number of eggs deposited in the form of a spiral is from 10 to 70.3 eggs (LEMOS et al., 2006; FARIAS et al., 2011; RAGA et al., 2012; MORAES et al., 2014), and the average number of eggs was 1.23 positions / leaf on "Pera" orange (*Citrus sinensis* L.) and 0.25 positions / leaf on tangerine (*Citrus reticulata* L.) (Rutaceae) in the state of São Paulo (IMPERATO et al., 2014).

Depending on the climate, the hatching of 1st instar nymphs occurs between 4 and 12 days, and these nymphs are oval and elongated in shape, are hyaline and have short legs and antennae being the only nymph stage that is mobile, and after 7 to 16 days, they fix the mouth device on the leaf for the beginning of feeding. In laboratory conditions, 2nd instar nymphs showed a duration of 6.94 and 7.36 days, and under field conditions, this period varied from 8 to 11 days, and for 3rd instar nymphs, this period ranged from 6.36 to 9.96 days and the mean period of the puparium stage (4th instar nymphs) was 33.58 days (Oliveira et al., 2001, CUNHA et al., 2003; RONCHI-TELES et al., 2009; LOPES et al. al., 2013).

The development of *A. woglumi* is favored by temperatures between 28ºC and 32ºC, and relative air humidity between 70% and 80%, not surviving at temperatures around 40ºC and altitudes above 1,000 meters (EPPO, 2008).

There are currently only four insecticides registered in the Ministry of Agriculture, Livestock and Supply (MAPA), which are commonly recommended for the control of *A. woglumi* in citrus, three of which having neonicotinoid as active ingredient and the other one is based on pyrethroid and antranilamide (AGROFIT, 2016). The use of plant extracts as insecticides alternative to synthetic chemicals is a form of control that minimizes several problems inherent to the use of synthetic chemicals, which can be more economical and easier to handle, being a good option for small producers. The aim of this study was to observe at laboratory level the following biological aspects: egg viability, nymphal period duration, nymph mortality, puparium phase duration and puparia viability in three hosts and to evaluate the bioactivity of plant species on *A. woglumi* eggs and nymphs.

MATERIAL AND METHODS

Biological aspects of *A. woglumi* in three hosts

"Pera" orange, mango and malay apple leaves, collected on the campus of the State University of Santa Cruz (UESC) (14° 47'41" S and 39° 10' 04" W; 31 m a.s.l.), containing insect eggs were kept in climatic chamber (BOD) at 25 ± 1ºC and 12-hour photoperiod. A total of 50 egg positions of each host were observed, with at least five eggs, and the following biological parameters were evaluated: egg viability (%), duration of nymphal period l, nymph mortality (%), puparium phase duration and puparia viability (%). Results were submitted to analysis of variance (ANOVA) and the means were compared by the Tukey test at 5% of probability.

Bioassays with botanical insecticides on *A. woglumi*

The translaminar action and the direct spray of different treatments on eggs and 1st instar nymphs were evaluated. Treatments used were: aqueous extract of neem leaves (10% and 20%), and gravy peduncle floral carnation guinea button (10% and 20%), pepper sauce (10% and 20%), cassava wastewater (50% and 100%) and commercial neem oil (1%, and 2%).

Aqueous neem extract was obtained by drying leaves in an oven at 50ºC for 48 hours. Then, samples were ground and the powder was placed in distilled water for 24 hours for the extraction of compounds.

The preparation of the extract to obtain the pepper sauce was carried out from the maceration of 200 g of garlic; 50 g of black pepper; 50 g chili pepper; 50 g of cumarí pepper and 2 L of sugarcane brandy. Peppers sauce was obtained with the mixture of 50 mL of pepper extract, 20 g of brown sugar, 10 L of water and 35 mL of vinegar.

The gravy peduncle floral carnation guinea button extract was prepared by immersing 200 g of gravy peduncle floral carnation guinea button in 2 L of sugarcane brandy, and to obtain the gravy peduncle floral carnation guinea button, 50 mL of this extract were mixed with 20 g of brown sugar, 10 L of water and 35 mL of vinegar.

After preparation, sauces were stored in amber glass bottle under refrigeration until their use. Changes in the physical appearance or presence of fungi detectable at the time of application were observed (NEVES et al., 2005; SANTOS et al, 2009).

In order to evaluate the translaminar action, leaves infested with eggs and nymphs collected in the field were detached from plants, and by means of a hand spray, 50 mL of each treatment was applied on the adaxial part of leaves, that is, on the opposite face of the surface containing eggs and nymphs. The petioles of treated leaves were wrapped with moistened cotton to avoid dryness, and each was placed in a disposable Petri dish lined with moistened filter paper and covered with plastic film and conditioned in a climatic chamber (BOD) at 25 ± 1ºC and 12-hour photoperiod. Each sample unit corresponded to three leaves, with a minimum of 20 eggs and 20 1st instar nymphs. Treatments were evaluated every 24 hours for up to seven days after application. The experimental design was completely randomized, with 11 treatments and four replicates. In another bioassay, citrus branches collected in the field were placed in voile cages, and new branches without egg position were placed inside the cages for reinfestation. The re-infested branches were labeled and sprayed with 50 mL of the different treatments. Each sample unit corresponded to three leaves, with a minimum of 40 eggs and 40 1st instar nymphs, and after seven days, egg infeasibility and nymph mortality were evaluated. The design was repeated with 11 treatments and four replicates.

Analysis of obtained data

Data were submitted to statistical analysis (ANOVA) and the means were compared by the Tukey test at 5% probability when significant. The efficiency of treatments (corrected mortality) was evaluated using the Abbott formula [E (%) = TI / T x 100], where E (%) = efficiency percentage, T = number of live insects in control treatment and I = number of live insects in insecticide treatment. The procedure to evaluate treatments under citrus black fly was performed seven days after application.

RESULTS AND DISCUSSION

Biological aspects in three *A. woglumi* hosts under laboratory conditions

Regarding egg viability, no significant difference was observed between “Pera” orange and mango. The average egg viability was 82.39%, in “Pera” orange and 79.79% in mango, and in malay apple leaves, the viability was lower, with mean value of 66.15%.

Also, no significant difference was observed in relation to nymph viability between “Pera” orange and mango hosts. The mean viability was 68.92% in “Pera” orange and 60.67% in mango, while the average viability was 50.28% in malay apple leaves (Table 1). No significant difference was observed between “Pera” orange and malay apple hosts regarding puparia viability. In “Pera” orange, the average viability was 70.02% and in malay apple, the average viability was 52.97%; however, it was observed that in mango leaves, viability was lower, with mean value of 44.75%, similar to that observed by Pena et al. (2009).

In relation to the nymphal period in “Pera” orange, 1st instar of nymphs lasted on average 7.76 days, statistically differing from mango (8.86 days) and malay apple (9.42 days) (Table 1). No significant difference was observed between the mean nymphal period duration of 2nd instar, ranging from 7.78 days in orange, 7.86 days in mango and 10.96 days in malay apple. For 3rd instar nymphs, the average duration was 7.86 days in orange and 8.10 days in mango, with no statistical difference, and in malay apple, it was 10.50 days, corroborating data found in other studies (RONCHI -TELLES et al., 2009; LOPES et al., 2013).

The puparium phase duration was 24.18 days on average in orange and 25.20 days in mango, with no significant difference, and in malay apple, this phase was longer, with mean duration of 26.66 days (Table 1). The puparium phase period was the longest, as found in other studies (CUNHA, 2003).

Toxic effect of botanical extracts on immature *A. woglumi* by the translaminar method.

In relation to egg viability, only commercial neem oil at the two concentrations analyzed (1% and 2%) presented efficiency above 50%. At 1% concentration, the mean egg infeasibility was 61%, and at 2% concentration, the mean value was 60% (Figure 1). The aqueous extract of neem leaves at 10% concentration did not differ statistically from cassava wastewater at both concentrations, with mean of 53.25% of nonviable eggs, and the aqueous extract from neem leaves (20%) turned about 50.50% of eggs unfeasible (Figure 1). Egg infeasibility was higher under the action of neem treatments, probably due to the presence of the azadirachtin substance, which has insecticidal power, as has been observed in other studies (MORDUE; NISBET, 2000).

Gravy peduncle floral carnation guinea button (10%), cassava wastewater (50% and 100%) and pepper sauce (10% and 20%) treatments made infeasible about 45% of eggs (Figure 1). Gravy peduncle floral carnation guinea button at 20% concentration caused the unfeasibility of 32.50% of eggs, and in the control, only 16% of eggs were not feasible (Figure 1).

The low egg unfeasibility on citrus black fly eggs may be indicative that the bioactive compounds present in treatments used found a probable physical resistance of the chorion to the action of extracts, as also observed in other studies (GONÇALVES, 2010; PENA, 2012).

In the translaminar action on 1st instar nymphs, all treatments determined mortality higher than 50.00%. Cassava wastewater (50% concentration) caused average mortality of 84.25%, and at 100% concentration, the average mortality was 78.50% (Figure 2), probably due to the action of cyanogenic compounds (PONTE, 1999). Commercial neem oil at 2.0% concentration had toxic effect of 76.25%, and at 1.0% concentration, the nymph mortality was 68.50% (Figure 2). The aqueous extract of neem leaves at concentration of 20% caused an average mortality of 78.25%, and at 10% concentration, the mean mortality was 72.75%, showing no significant difference (Figure 2). Under the action of pepper sauce, mean mortality ranged from 53.75% (10%) to 61.00% (20%), and gravy peduncle floral carnation guinea button (20% concentration) caused average mortality of 52.20% (Figure 2), and these treatments did not present statistical differences among themselves. Gravy peduncle floral carnation guinea button sauce at 10% concentration caused average mortality of 32.50%, not statistically differing from control treatment (17.50%) (Figure 2).

Toxic effect of botanical extracts on immature *A. woglumi* by the direct spray method.

The unfeasibility of citrus black fly eggs was greater than 85% by the direct spray of commercial neem oil (1% and 2%) and cassava wastewater treatments (100%) (Figure 3). Pepper sauce (10%) caused mean egg unfeasibility of 69.25%, and at 20% concentration, egg unfeasibility was 78.00%. Under the action of cassava wastewater (50%), aqueous extract of neem leaves (20%), and gravy peduncle floral carnation guinea button (20%), egg unfeasibility was higher than 70%, and under gravy peduncle floral carnation guinea button (10%) and aqueous extracts of neem leaves (10%), the mean egg unfeasibility was close to 50% (Figure 3).

In direct spray on 1st instar nymphs, treatments that caused mean mortality of 80% were, respectively, cassava wastewater (50% and 100% concentration), with mean of 80.5% and 89%, respectively (Figure 4), showing the efficacy of cassava wastewater on mite mortality (SANTOS et al., 2012) and commercial neem oil (1% concentration), with mean of 86%, not statistically differing from cassava wastewater (Figure 4).

Commercial neem oil (2%) caused mortality of 81.00%, and due to the action of the aqueous extract of neem leaves (20%), mortality was of 77%, not statistically differing from each other (Figure 4). These results are similar to those obtained in another study with application of neem-based commercial products (SILVA et al., 2012).

Pepper sauce (10% and 20% concentration), aqueous extract of neem leaves (10%) and the gravy peduncle floral carnation guinea button (20%) caused mean mortality above 65.00% of 1st instar nymphs of citrus black fly, showing no significant differences. Gravy peduncle floral carnation guinea button at

10% concentration caused mortality of 36%, and in the control treatment, mortality was 24% (Figure 4).

Average efficiency of the different treatments on immature *A. woglumi* under translaminar effect.

It was observed that none of treatments under translaminar action presented ovicidal effect above 80% (Figure 5). Probably, the effect on nymphs has been greater because they are more susceptible and suck sap from leaves (GONÇALVES, 2010; PENA, 2012).

Under translaminar action, it was observed that the aqueous extracts of neem leaves (10% and 20%), cassava wastewater (50% and 100%) and commercial neem oil (1% and 2%) have efficiency higher than 80%, according to the Abboutt formula, and can be considered the best treatments for nymphal phase control of the citrus black fly (Figure 6).

Average efficiency of the different treatments on immature *A. woglumi* by the direct spray method

Only treatments aqueous extract from neem leaves (10% and 20%), cassava wastewater (50% and 100%) and commercial neem oil (1% and 2%) presented efficiency above 80.00%, being considered efficient in the control of citrus black fly in the egg (Figure 7) and 1st instar phases (Figure 8), as was also observed by Gonçalves (2010).

In direct spraying, the efficiency of all treatments exceeded 70%, being evident that the control of the citrus black fly can be carried out with products alternative to synthetic chemicals (Figure 8).

TABLE 1 - Biological aspects of citrus black fly in three host species under laboratory conditions.

Host	Vo(%)	Vn(%)	Vp(%)	Pn1(days)	Pn2(days)	Pn3(days)	Pp(days)
Pera orange	82,39 a	68,92 a	70,02 a	7,76 a	7,78 a	7,86 a	24,18 a
Mango	79,79 a	60,67 a	44,75 a	8,86 a	7,86 a	8,10 a	25,20 a
Malay apple	66,15 b	50,28 b	52,97 a	9,42 b	10,96 b	10,50 b	26.66 a

*Vo% = Egg viability; Vn% = Nymph feasibility; Vp% = puparia viability; Pn1 (days) = Average duration of the 1st instar nymph period; Pn2 (days) = Average duration of the 2nd instar nymph period; Pn3 (days) = Average duration of the 3rd instar nymph period; Pp (days) = Mean duration of the puparium period. Data compared in columns.

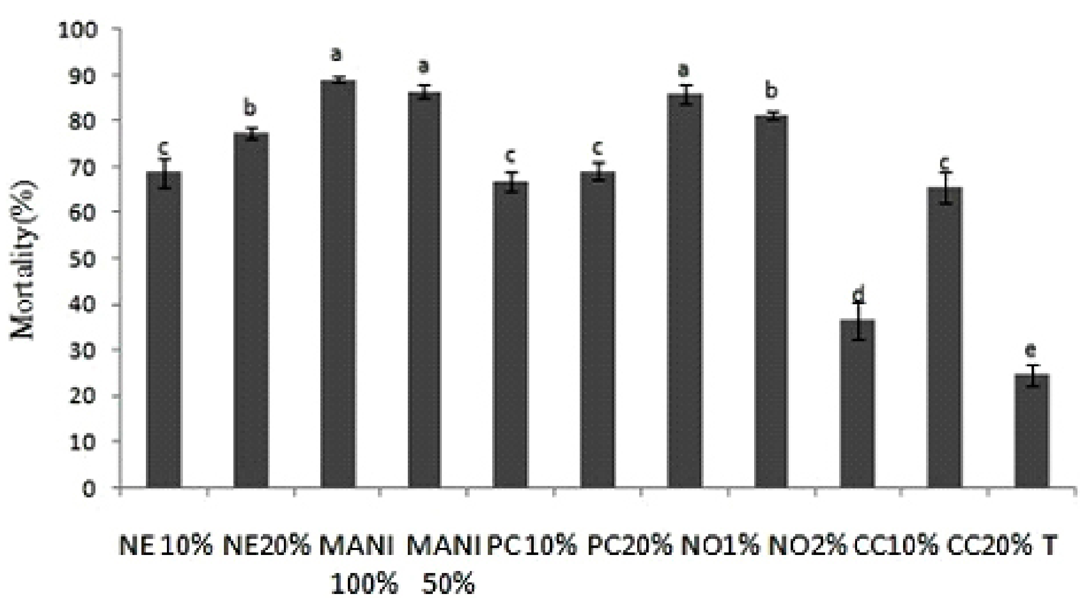

FIGURE 1 – Unfeasibility (%) of citrus black fly eggs by translaminar action of different treatments.

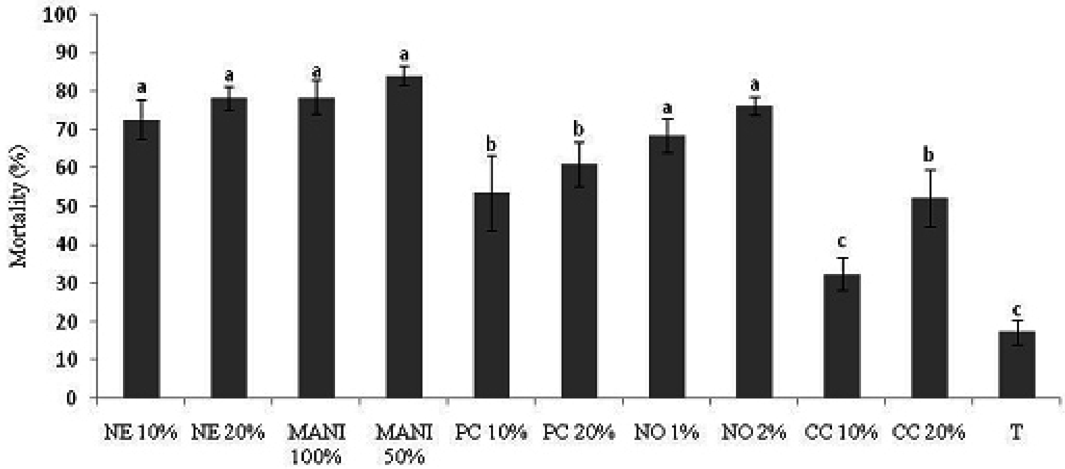

FIGURE 2 - Mortality (%) of 1st instar nymphs of citrus black fly by translaminar action of different treatments.

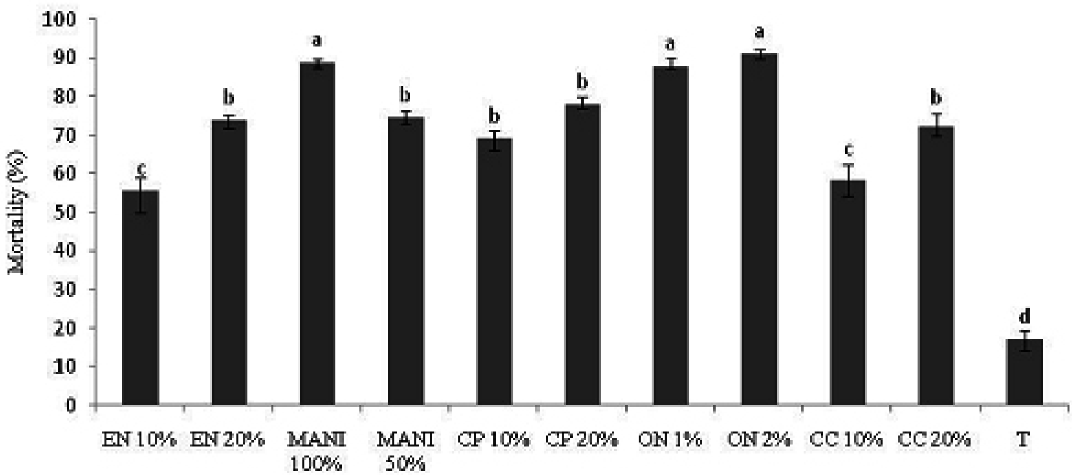

FIGURE 3 - Unfeasibility (%) of citrus black fly eggs by direct spraying of different treatments.

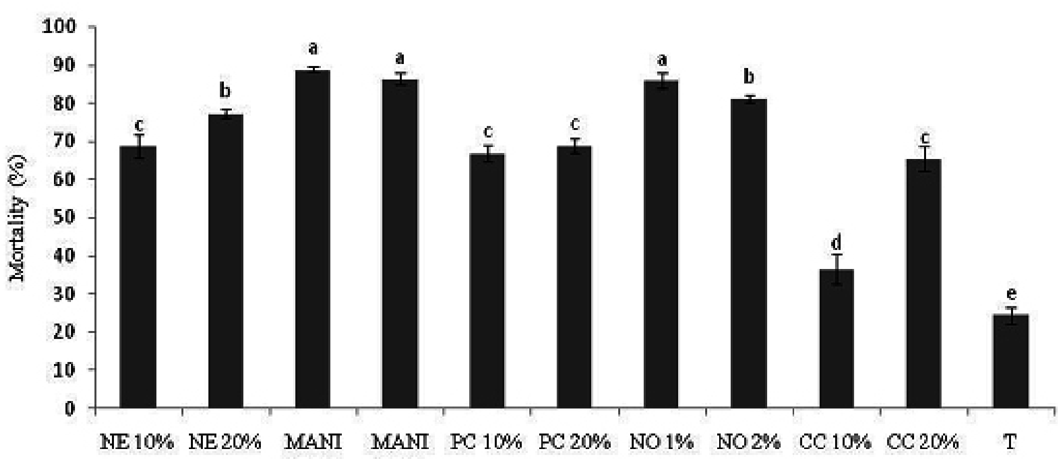

FIGURE 4 - Mortality (%) of 1st instar nymphs of citrus black fly under the effect of direct spraying of different treatments.

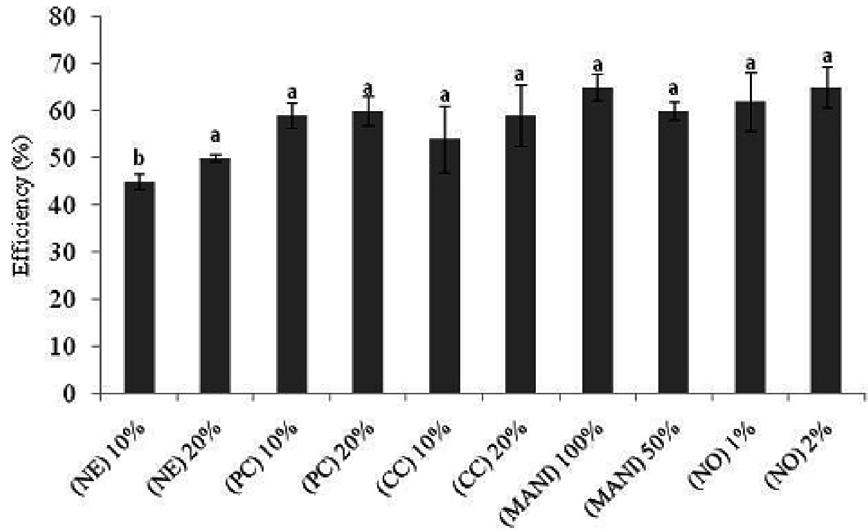

FIGURE 5 - Mean efficiency of the different treatments on *A. woglumi* eggs under the translaminar effect.

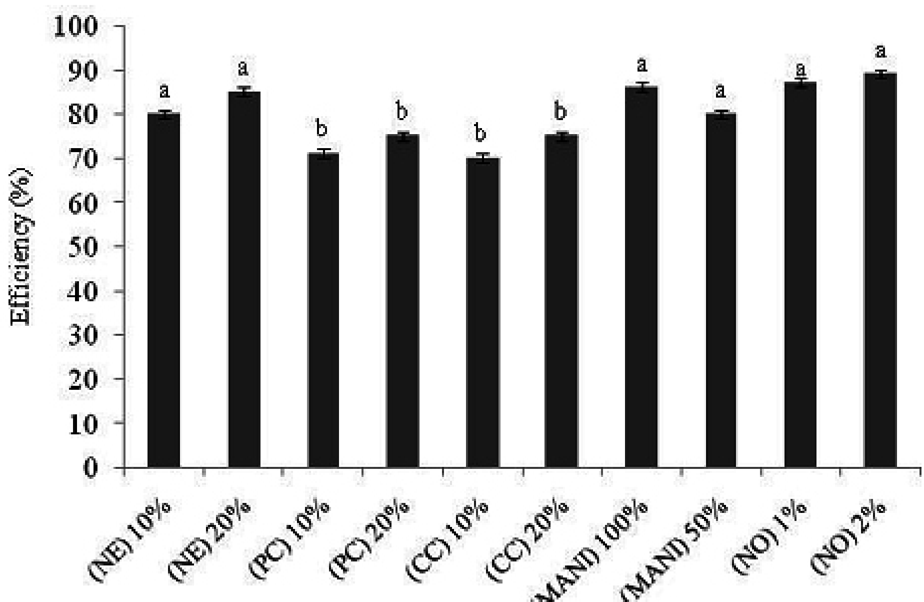

FIGURE 6- Average efficiency of the different treatments by the translaminar action on 1st instar nymphs of *A. woglumi*.

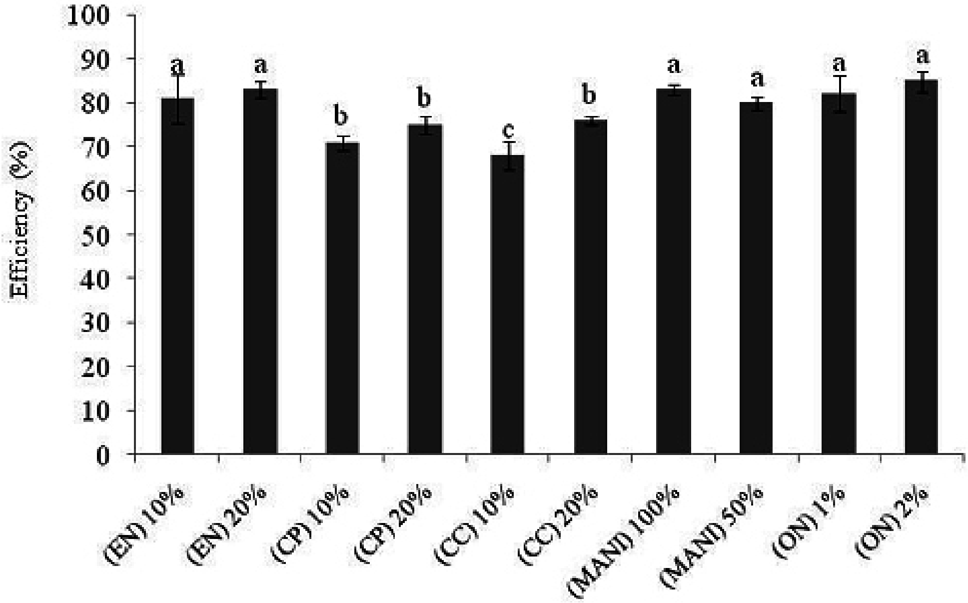

FIGURE 7 - Mean efficiency of the different treatments on 1st instar eggs of *A. woglumi* by the direct spraying method.

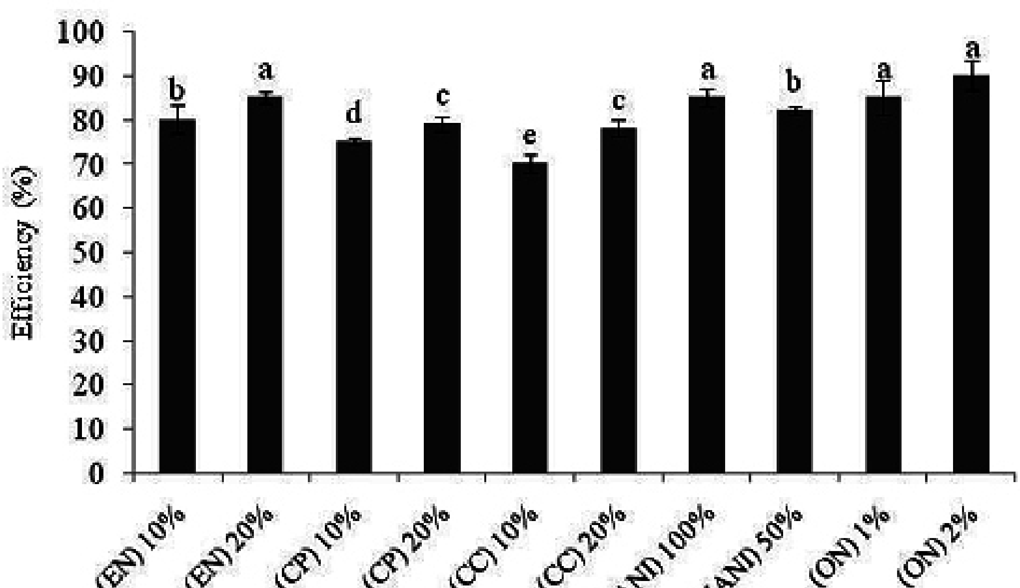

FIGURE 8 - Mean efficiency of different treatments directly sprayed on 1st instar nymphs of *A. woglumi*.

CONCLUSIONS

Azadirachta indica and cassava wastewater are efficient in the control of the citrus black fly.

The method of direct application of treatments is efficient in the control of eggs and 1st instar nymphs of the citrus black fly.

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