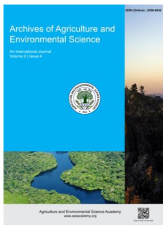
Archives of Agriculture and Environmental Science 8(2): 125-129 (2023)
https://doi.org/10.26832/24566632.2023.080205

e-ISSN: 2456-6632

This content is available online at AESA

Archives of Agriculture and Environmental Science

Journal homepage: journals.aesacademy.org/index.php/aaes



ORIGINAL RESEARCH ARTICLE

Effect of Wolbachia as biocontrol agent on fecundity and survival of cassava whitefly in northwest Nigeria

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ARTICLE HISTORY

Received: 09 March 2023
Revised received: 22 May 2023
Accepted: 10 June 2023

Keywords

Biocontrol
Cassava
Dan Aliero
Farin rogo
Whitefly
Wolbachia

ABSTRACT

Whitefly (*Bemisia tabaci* Genn.) is known to vectored *Cassava mosaic virus* (CMV) and *Cassava brown streak virus* (CBSV), these viruses caused major diseases of cassava in Africa. An experiment was conducted to determine the effect of *Wolbachia* as biocontrol agent on fecundity and survival of whitefly infesting cassava. Whiteflies infesting cassava were captured and reared using rearing cages. *Wolbachia* was isolated from infected cassava whiteflies, cultured and used for the inoculations. The experiment was conducted under control conditions using glass cages. Results of this research revealed that number of eggs lay by *Bemisia tabaci* inoculated with *Wolbachia* significantly (P = 0.05) reduced weeks after inoculation which significantly differ among the cassava genotypes. Number of eggs lay by *B. tabaci* free of *Wolbachia* was significantly higher (964.35) than those inoculated with *Wolbachia* (46.541) at 4 weeks after inoculation (4WAI). The genotype 'Dan Aliero' had the highest number (4WAI 82.33), while, 'Farin rogo' had the lowest number of eggs (4WAI 23.33). Number of whitefly nymphs and adults also significantly reduced when *B. tabaci* was inoculated with *Wolbachia*. 'Dan Aliero' had the highest number of whitefly nymphs and adults (4WAI = 59.00) and (4WAI = 51.00), respectively. 'Farin rogo' recorded the lowest number of whitefly nymphs (17.67) and adults (13.00) at 4WAI, respectively. For the first time, Wolbachia were evaluated for whiteflies management. From the finding of this study, *Wolbachia* can be used as biocontrol agent to suppress whiteflies infestation on cassava and other vegetable crops.

Citation of this article: Magaji, M. D. Y., Mohammed, I. U., Jega, I. Y., & Musa, A. (2023). Effect of *Wolbachia* as biocontrol agent on fecundity and survival of cassava whitefly in northwest Nigeria. *Archives of Agriculture and Environmental Science*, *8*(2), 125-129, https://dx.doi.org/10.26832/24566632.2023.080205

INTRODUCTION

Cassava (*Manihot esculenta* Crantz.) is an important root crop that provides starch and used throughout the tropics as a staple food. Its young leaves are also used as vegetables. Africa account for 64.3% of the global cassava production and Nigeria remain the leading cassava producing country globally producing about 80 million tons in 2020 (FAOSTAT, 2020). Cassava production in Africa is constrained by pest and diseases; cassava mosaic disease (CMD) and cassava brown streak disease (CBSD) remain the two most important diseases of cassava in Africa and these diseases are caused by viruses, which are vectored by whiteflies and perpetuated by farmers practices as well. These viruses are transmitted by whitefly (*Bemisia tabaci* Genn.) (Aleyrodidae: Hemiptera). *B. tabaci* has been reported infesting a wide diversity of host plants in sub-Saharan Africa but cassava remain the most important host for *B. tabaci* in Africa and it occurs wherever the crop is cultivated. *B. tabaci* had reported to vectored over 200 plant viruses belonging to *Begomovirus* (*Geminiviridae*), *Crinivirus* (*Closteroviridae*), *Carlavirus* (*Betaflexiviridae*), *Ipomovirus* (*Potyviridae*) and *Torradovirus* (*Secoviridae*) (Polston *et al*., 2014). Continuous infestation of cassava and other vegetable crops by whiteflies had led to frequent use of synthetic chemicals by farmers for management. This had led to high cost of production, air and water pollutions, and loss of biodiversity. In order to mitigate these effects of synthetic chemicals, an eco-friendly measures for whiteflies management need to be discovered. Biocontrol of whitefly and other pests is an eco-friendly and has been pursued through observations and utilization of natural enemies or through analysis of existing agroecosystems, indicating which key factors control the pest (Albajes *et al*., 2003). *Aleurothrixus floccusus* (Maskell) *Aleurodicus dispersus* (Russell) have been successfully managed using biocontrol methods. Although, the two species differ from *B. tabaci*, however, similar biocontrol methods can be tested on *B. tabaci*. Whitefly is a sap sucking insect and feed mostly on phloem sap, the sap is rich in carbohydrate but deficient in amino acids. Symbiotic relationships of sap sucking insect pests with intracellular bacteria was proposed and confirmed to be based on the nutritional need of the hosts (Rao *et al*., 2015). High number of endosymbiotic elements had been reported on *B. tabaci*. Having one obligate and seven different facultative bacteria and these are transmitted vertically (Marubayashi *et al*., 2014). *Wolbachia* is among the secondary endosymbionts and perhaps the most commonly found associated with arthropods and has been estimated to infect about 70% of all species (Hilgenboecker *et al*., 2008). As an endosymbiont, *Wolbachia* can either be mutualistic or parasitic. It acts as a reproductive manipulator that either reduce the reproductive output of uninfected females via cytoplasmic incompatibility (CI), or shifting the sex ratio of offspring in favour of females by either killing male offspring, inducing feminisation in genetic males or parthenogenesis (Werren *et al*., 2008). This research was conducted with aim to determine the effect of *Wolbachia* as biocontrol agent on fecundity and survival of whiteflies infesting cassava. As far our understanding this research was the first study that investigated the effect of *Wolbachia* on whitefly survival on cassava.

MATERIALS AND METHODS

Source of cassava genotypes

Cassava cuttings were sourced from farmers' field and cuttings were taken from disease-free asymptomatic plants of 12 months old. Cuttings of about 15 cm were taken from the middle part of the stems, from these genotypes; Bako-bako, Dan Aliero and Farin rogo. Cuttings were planted in a plastic pot containing a mixture of sterilized sandy soil, compost and sawdust. Cassava plants were maintained in an insect-proof screenhouse under ambient conditions and later three plants from each variety were transferred to glass cages four weeks after planting for establishing whitefly colonies.

Whitefly culture

Three cassava plants of four weeks old were transferred into a cage. The plants were kept in cages for two weeks before adult whiteflies were introduced. Life adult whiteflies were collected from cassava plants using aspirator. The adult whiteflies were introduced into the cages containing test cassava plants of four weeks old under ambient conditions (Figures 1a, b).

Preparation of nutrient agar

One litre of nutrient agar was prepared by dissolving 28.0 g of nutrient agar in 1000 ml of distilled water, the medium was dissolved completely by heating it gently. The medium was sterilized by autoclaving at 121^{0}C for 15 minutes. After the medium was allow to cool down, anti-fungi were added and 10-15 ml of the medium was dispensed in a petri dish as desired.

Isolation of *Wolbachia* from whitefly

Wolbachia isolation was achieved using the protocol of Frydman (2006) with slight modification. Whiteflies feeding on cassava leaves in farmers' fields were collected in 70% ethanol and place on ice, whiteflies were transferred to the centrifugal filter (5 or 0.65 micro meter) which was washed 5 times with 70% ethanol. The tube containing the whiteflies was inverted a couple of times between washes. This was done to collect as much as debris and sterilize whitefly surface as much as possible. The tube was spined for 5-10 seconds using centrifuge machine (1,000 g) to dispose most of the ethanol. The whiteflies were then washed three times with sterile water and then, the filter unit was transferred to a new sterile microcentrifuge tube. Then, it was spined for three minutes at 10,000 g, the content was diluted 100 times with sterile distilled water and spread on nutrient agar plates. The plates were incubated at 30-35°C for 2-3 days. Bacterial colonies were selected and purified by sub culturing on nutrient agar plates.

Figure 1. *a, b: Cages used for whitefly colony and one month old cassava plant.*

Cassava genotypes and experimental design

Three (3) commonly cultivated cassava genotypes in Kebbi State were obtained from farmer's field: Bako-Bako, Dan Aliero and Farin rogo. Treatments were *Bemisia tabaci* inoculated with *Wolbachia* (BTIW), *Bemisia tabaci* uninoculated with *Wolbachia* (BTFW) and cassava without *Bemisia tabaci* and *Wolbachia* (CnoBT noW). Treatments were layout using Completely Randomised Design (CRD) and replicated three times. Experiment was conducted under insect free screenhouse conditions.

Wolbachia inoculation

Wolbachia colonies of 4 days old were collected and diluted with sterilized distilled water. At four weeks after planting, cassava plants with 50 introduced whiteflies (25 males and 25 females) were inoculated with suspension of *Wolbachia* inoculum concentration being 428.571×10^3 using hand held sprayer. This was done in early morning (0630-0715 h) when temperature was relatively low.

Data collection

Data were collected on number of whitefly eggs per plant, number of whitefly nymphs per plant and number of whitefly adults per plant.

Data analysis

Data generated from the experiment were subjected to analysis of variance (ANOVA) using SAS Version 9. Means were separated using least significance difference (LSD).

RESULTS AND DISCUSSION

Frequent application of synthetic insecticides for the management of whitefly had resulted to the development of insecticidal resistance by whitefly and this had negative impact on our environment and resurgence of whitefly transmitted viruses. Brelsfoard and Dobson (2009) suggested that, there is need for development of other management strategies that are environmentally friendly to reduce over dependence on synthetic chemicals.

Number of eggs lay by *Bemisia tabaci* inoculated with *Wolbachia* significantly (P = 0.05) reduced weeks after inoculation and it also significantly differ among the cassava genotypes used for the research. Higher number of eggs were recorded one week after inoculation but there was significant reduction in the subsequent weeks. Among the genotypes, 'Dan Aliero' had the highest number of eggs for all the weeks of data collection (Table 1). Number of eggs lay by *Bemisia tabaci* uninoculated with *Wolbachia* was significantly higher than those inoculated with *Wolbachia* and number of eggs significantly increase weeks after introduction of whiteflies. Among the genotypes, 'Dan Aliero' also had the highest number of eggs for all the weeks of data collection. While, 'Farin rogo' had the lowest number of eggs for all the weeks of data collection in both inoculated and uninoculated *Bemisia tabaci* (Tables 1 and 2).

Table 1. Effect of *Wolbachia* on whiteflies eggs, nymphs and adults.

Genotypes	Mean number of eggs 1WAI	Mean number of eggs 2WAI	Mean number of eggs 3WAI	Mean number of eggs 4WAI
Bako-bako	194.00^{ns}	143.33^{ab}	107.33^{ab}	77.67
Dan Aliero	237.67	185.00^{aa}	138.00^{aa}	82.33
Farin rogo	170.33	93.67^{bb}	59.33^{bb}	23.33
Control	0.00	0.00	0.00	0.00
LSD	67.817	57.497	76.615	46.541
Genotypes	**Mean number of nymphs 1WAI**	**Mean number of nymphs 2WAI**	**Mean number of nymphs 3WAI**	**Mean number of nymphs 4WAI**
Bako-bako	167.00^{ab}	102.67	81.67^{ab}	65.33
Dan Aliero	197.33^{aa}	139.00	104.33^{aa}	59.00
Farin rogo	124.33^{bb}	63.33	45.00^{bb}	17.67
Control	0.00	0.00	0.00	0.00
LSD	57.543	64.695	57.981	76.101
Genotypes	**Mean number of adults 1WAI**	**Mean number of adults 2WAI**	**Mean number of adults 3WAI**	**Mean number of adults 4WAI**
Bako-bako	152.00^{ab}	92.67^{ab}	79.33^{ab}	59.33
Dan Aliero	182.00^{aa}	130.33^{aa}	94.33^{aa}	51.00
Farin rogo	109.00^{bb}	56.00	38.33^{bb}	13.00
Control	0.00	0.00	0.00	0.00
LSD	60.094	62.178	52.392	63.161

WAI = weeks after inoculation, LSD = least significant difference.

Table 2. Number of whiteflies' eggs, nymphs and adults uninoculated with *Wolbachia*.

Genotypes	Mean number of eggs 1WAI	Mean number of eggs 2WAI	Mean number of eggs 3WAI	Mean number of eggs 4WAI
Bako-bako	1060.7^{a}	1483.7^{a}	1972.0^{a}	2350.7^{b}
Dan Aliero	1517.7^{aa}	2041.3^{aa}	2416.7^{aa}	3374.7^{a}
Farin rogo	436.3^{b}	611.3^{b}	720.0^{b}	857.7^{c}
Control	0.00	0.00	0.00	0.00
LSD	510.48	714.14	975.46	964.35

Genotypes	Mean number of nymphs 1WAI	Mean number of nymphs 2WAI	Mean number of nymphs 3WAI	Mean number of nymphs 4WAI
Bako-bako	638.0^{ab}	1348.3^{a}	1184.0^{ab}	2238.0^{b}
Dan Aliero	1359.3^{aa}	1685.3^{aa}	2300.7^{aa}	3313.7^{a}
Farin rogo	428.7^{bb}	566.7^{b}	692.0^{bb}	811.3^{c}
Control	0.00	0.00	0.00	0.00
LSD	725.84	444.91	1208.6	951.01

Genotypes	Mean number of adults 1WAI	Mean number of adults 2WAI	Mean number of adults 3WAI	Mean number of adults 4WAI
Bako-bako	609.0	1191.0^{a}	1729.7^{a}	2055
Dan Aliero	873.0	1532.0^{aa}	2194.0^{aa}	2295
Farin rogo	393.7	492.3^{b}	572.7^{b}	291973
Control	0.00	0.00	0.00	0.00
LSD	1026.3	389.64	738.34	582071

WAI = weeks after introduction, LSD = least significant difference.

Previous researches suggested that *Wolbachia* can be used as alternative insect pest management strategy by using it as a form of sterility for a mass male release strategy, analogous to a sterile insect technique (Brelsfoard and Dobson, 2009). The reduction of number of eggs laid by whiteflies inoculated with *Wolbachia* as compared with uninoculated whitefly might be the effect of *Wolbachia* on the fecundity of whitefly and hence laid a smaller number of eggs. Effect of *Wolbachia* on the fecundity of whiteflies feeding on tomato and okra was similarly reported by Prasannakumar and Maruthi (2021). Number of whitefly nymphs significantly (P = 0.05) reduced when *Bemisia tabaci* was inoculated with *Wolbachia*. Higher reduction of whitefly nymphs was recorded at fourth week after inoculation (4th WAI). 'Farin rogo' recorded the lowest number of whitefly nymphs for all the weeks of data collection in both inoculated and uninoculated (Tables 1 and 2). There was significant increase in number of whitefly nymphs on cassava leaves of uninoculated *Bemisia tabaci*. 'Dan Aliero' had the highest number of whitefly nymphs for all the weeks of data collection in both inoculated and uninoculated (Tables 1 and 2).

Number of adult whiteflies significantly (P = 0.05) reduced on cassava leaves weeks after inoculation. The lowest number of adult whiteflies was recorded at fourth week after inoculating *Bemisia tabaci* with *Wolbachia*. 'Farin rogo' had the lowest adult whiteflies in first, second, third and fourth weeks after inoculation (Table 1). 'Dan Aliero' had the highest number of adult whiteflies in first, second and third weeks, while, in fourth week, 'Bako-bako' had the highest number of adult whiteflies (Table 1). Number of adult whiteflies significantly increased in first, second, third and fourth weeks after introducing *Bemisia tabaci* uninoculated with *Wolbachia*. 'Farin rogo' had the lowest number of adult whiteflies, while, 'Dan Aliero' had the highest number of whiteflies in first, second, third and fourth weeks after introducing *Bemisia tabaci* uninoculated with *Wolbachia* (Table 2). Also, Greenberg *et al.* (2009), reported that when whitefly fecundity was affected, the nymphal emergence was significantly affected. Number of whitefly adult emerged was also significantly affected by *Wolbachia*. This can be attributed to the smaller number of nymphs that survived. Previous research reported that *Wolbachia* was used for the management of insect-vectors by shortening the lifespan of the insect-vectors and reducing the multiplication of the virus inside the vectors (McMenima and O'Neill). It was found in this study that, *Wolbachia* had effect on whitefly survival, oviposition, nymphal and adult development. However, using Wolbachia in the field requires more research and investigations.

Conclusion

Findings of this research revealed that *Wolbachia* have some considerable effect on fecundity and survival of whiteflies infesting cassava. This is because number of eggs, nymphs and adult whiteflies reduced when inoculated with *Wolbachia* compared with uninoculated. It can be concluded that, *Wolbachia* can used as biological agents for the management of whiteflies infesting cassava. It can also be integrated with other insect pest management measures as a form of Integrated Pest Management.

REFERENCES

Albajes, R., Sarasúa, M. J., Avilla, J., Arnó, J., & Gabarra, R. (2003). Integrated pest management in a Mediterranean region: the case of Catalonia. In *Integrated Pest Management in the Global Arena*, ed. KM Maredia, D Dakouo, D Mota-Sánchez, pp. 341–355. Oxon, UK: CABI Publishing.

Brelsfoard, C. L., & Dobson, S. L. (2009). Wolbachia-based strategies to control insect pests and disease vectors. *Asia Pacific Journal of Molecular Biology and Biotechnology*, *17*(3), 55-63

FAOSTAT. (2020). FAOSTAT. Food and Agriculture Organization of the United Nations, Rome, Italy. http://faostat.fao.org/site/ 567/default.aspx

Frydman, H. M. (2006). Isolation of live bacteria from adult insects. *Research Square*, https//doi.org/10.1038/nprot.2006.131

Greenberg, S. M., Jones, W. A., Liu, & Xian, T. (2009). Tritrophic interactions among host plants, whiteflies and parasitoids. *Southwestern Entomology*, *34*, 431-445.

Hilgenboecker, K., Hammerstein, P., Schlattmann, P., Telschow, A., & Werren, J. H. (2008). How many species are infected with Wolbachia? – a statistical analysis of current data. FEMS *Microbiology Letters*, *2*, 215–220.

Marubayashi, J. M., Kliot, A., Yuki, V. A., Rezende, J. A. M., Krause-Sakate, R., Pavan, M. A., & Ghanim, M. (2014). Diversity and localization of bacterial endosymbionts from whitefly species collected from Brazil. *PlosOne*, *9*(9), e108363

McMenima, C. J., & O'Neill, S. L. (2010). A virulent *Wolbachia* infection decrease the viability of the dengue vector *Aedes aegypti* during periods of embryonic quiescence. *Plos Neglected Tropical Diseases*, *4*(7), p. e748

Polston, J. E., De Barro, P., & Boykin, L. M. (2014). Transmission specificities of plant viruses with the newly identified species of the *Bemisia tabaci* species complex. *Pest Management Science*, *70*(10), 1547-1552.

Prasannakumar, N. R., & Maruthi, M. N. (2021). Understanding the interactions among the crop plants, a virus, insect vector whiteflies and their endosymbionts. *Phytoparasitica*, *49*,739-750.

Rao, Q., Rollat-Farnier, P. A., Zhu, D. T., Santos-Garcia, D., Silva, F. J., Moya, A., Latorre, A., Klein, C. C., Vavre, F., & Sagot, M. F. (2015). Genome reduction and potential metabolic complementation of the dual endosymbionts in the whitefly (*Bemisia tabaci*). *BMC Genomics*, *16*(1), 226

Werren, J. H., Baldo, L., & Clark, M. E. (2008). Wolbachia: master manipulators of invertebrate biology. *Nature Reviews Microbiology*, *6*, 741–751.