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Spray deposition within *Capsicum chinense* Jacq. plant canopy affects mortality in adult *Bemisia tabaci* (Gennadius) (Hemiptera:Aleyrodidae)

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ABSTRACT

Spray droplet deposition on *C. chinense* leaves was observed to vary according to the vertical position of the leaf on the plant canopy. The mean number of droplets was highest and lowest on leaves located in the upper and lower third of the plant canopy, respectively. *B. tabaci* mortality due to Aza-Direct®-treated leaves was not significantly influenced by cardinal direction. However, the location of the sprayed leaf (upper, middle or lower third of canopy) along the vertical axis had a strong influence on *B. tabaci* mortality. Mean *B. tabaci* mortality on sprayed leaves in the middle third of the canopy was 6.20% higher and significantly different from that on leaves in the lower third. The age of the Aza-Direct® residue also had an influence on the mortality of *B. tabaci* adults, with highest and lowest mortality being recorded on leaves located in the upper and lower third of the plant canopy, respectively. Adult *B. tabaci* mortality generally decreased with increasing Aza-Direct® residue age. Degradation of Aza-Direct® residues was most rapid on the upper leaves and was 1.4 times faster than that on the leaves on the lower third of the plant canopy while it was 1.1 times as fast on the middle leaves.

KEYWORDS: *Bemisia tabaci*, *Capsicum chinense*, droplet distribution, plant canopy, insecticide residue, mortality.

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INTRODUCTION

Bemisia tabaci (Gennadius) (Hemiptera:Aleyrodidae) is a pest of major economic importance worldwide including the Caribbean region, which causes both direct and indirect damage [1]. Additionally, *B. tabaci* has been identified as the most efficient vector of the Gemini virus complex affecting *Capsicum chinense* Jacq. in the Caribbean [2, 3].

Bemisia tabaci has developed resistance to a number of different classes of insecticides and consequently insecticide resistance management by rotation of different classes of insecticides has been done throughout the world [4]. Insecticide resistance to the newer insecticides including neonicotinoids (eg. imidacloprid) and pyriproxyfen, in *B. tabaci* has also been recorded [5, 6]. Resistance as high as 100-fold and 126-fold for imidacloprid has been reported in studies done on *B. tabaci* Q biotype in Spain and B biotype in Guatemala, respectively [7, 8]. As much as 500-fold level of resistance to pyriproxyfen has been reported in studies conducted on cotton [9].

Capsicum chinense has become a crop of great economic importance to the Caribbean through the export of fresh fruit, pepper mash and various hot sauces to markets in the United States, Canada and Europe. Trinidad produced 1.64×10^6 kg *C. chinense* in 2020 valued at US$6.7 million. While there are demands for Caribbean 'Scotch Bonnet' hot peppers and hence room for expansion, there are increasing barriers including stringent pesticide and food standard requirements

(Good Agricultural Practices Protocol (GAP) and Maximum Residue Level (MRL) of pesticides), to trade which can limit this industry. This has resulted in the greater dependency on biological pesticides for the control of *B. tabaci* and other pests of crops of economic importance. The active ingredient azadiracthin obtained from *Azadirachta indica* A. Juss. has been exempted from residue tolerance levels by the US Environmental Protection Agency for food crop applications [10]. The exemption from residue tolerance levels and its different modes of action, which reduces insecticide resistance to *B. tabaci*, would result in insecticides produced from *A. indica* being an essential tool for crop production in the future.

The current study aims to determine the effectiveness and duration of activity of Aza-Direct® ®1.2 EC, an aqueous extract of *A. indica* containing 1.2% azadiracthin, against adult *B. tabaci* on *C. chinense*. Additionally, it seeks to determine if spray distribution on the plant and cardinal direction affects mortality of adult *B. tabaci*.

MATERIALS AND METHODS

Plant material

One hundred and twenty-five *C. chinense* (variety '7-Pot Chili') plants were transplanted from seedling stage into 11 L (23 cm × 28 cm) black plastic pots. All agronomic practices done by farmers were followed. Plants were fertilized similar to field conditions using Fersan® 12-24-12 fertilizer (Fersan, Dominican Republic) at the end of week 1 followed by the applications of BASF® 12-12-17+2 two and four weeks after transplanting. An application of Fersan® 13-13-21 was then done at week eight and repeated at 2-week intervals until the end of the experiment. All plants were enclosed in an insect-free screenhouse. Simultaneously, 100 *C. chinense* plants were grown in a separate screenhouse under the same conditions. These were used to rear *B. tabaci* for use in subsequent bioassays.

Insecticide application

On the 70th day after transplanting, *C. chinense* plants were separated into treatment (75 plants) and control (50 plants) groups which were placed up wind from the treatment plants and marked as the control plot. A Cooper-Pegler® CP3 Classic Series 20L Knapsack Sprayer at an operating pressure of 3.2 kg/cm^2 (= 45 psi) was used to apply Aza-Direct® insecticide to *C. chinense* plants at a rate of 0.75 L/ha. The sprayer lance, fitted with a hollow cone nozzle, was held 50 cm directly above each treatment plant and sprayed with insecticide for 5 s. The procedure was repeated for control plants sprayed with water only. Twenty-four hours after Aza-Direct® application, five plants from the treated plot and three plants from the control plot were randomly selected. Each plant was divided into three strata comprising of an upper, middle, and lower third. Further, each third was then divided into the four cardinal points: North, South, East and West. One randomly selected leaf within each third of the plant was picked from each cardinal point and placed adaxial surface up into a 9 cm labeled petri dish. The covers of the petri dishes had 6 cm diameter windows with fine organza mesh to allow for ventilation of *B. tabaci*. Twenty adult *B. tabaci* were placed into each petri dish using an insect aspirator and the mortality and immobility were recorded 24 h post introduction. Minimal movement by *B. tabaci* adults only when gently prodded with a needle was classified as immobile, while no movement by adults was considered as mortality. This procedure was repeated every 24 h for a 10-day period using fresh leaves selected randomly from each plot. Average temperature, humidity and rainfall over the duration of the experiment was 29.1 ± 2.4°C, 85.7 ± 4.1%, 245.2 ± 0.6 mm while wind speed was 6 km/h.

Droplet density determination

Prior to Aza-Direct® application, 10 separate *C. chinense* plants were used to determine the spray droplet density on leaves. Four litres of water and 10 g food-grade red dye (FD&C Red No. 2) were added to the sprayer and thoroughly mixed. White stock card (5 cm × 5 cm) was attached on the adaxial leaf surface of one leaf located on each of the upper, middle and lower thirds of the plant canopy in each cardinal direction (N, S, E and W). Each plant thus had 12 cards attached. The plants were sprayed for 5 s with the water-dye mixture at the previously stated pressure, height above the plant and rate. Cards were removed and the droplets on each plant were counted to determine the mean density/cm^2 at each level and cardinal direction.

Statistical analysis

All statistical analyses were conducted using Minitab 17 (Minitab, LLC, USA). Mortality data were corrected to account for control mortality using Abbott's formula [11]. A first-order degradation curve for the upper, middle and lower leaves sprayed with Aza-Direct® was fitted using the equation: $M_t = M_0^{e-at}$ where M_t represents the corrected mortality of adult *B. tabaci* when exposed to Aza-Direct® residues aged 't' days, M_0 - the corrected mortality of *B. tabaci* adults when exposed to residues of age 0 days, while 'a' represents the rate constant [12]. Determination of the rate constant 'a' was done by plotting graphs of ln (M_t/M_0) against time and then a linear regression equation was derived. The rate constant was determined from this linear regression equation. The LT_{50} (time for 50% mortality) for *B. tabaci* exposed to different aged Aza-Direct® residues on upper, middle and lower leaves was determined using the equation ET_{50} = (ln2)/*a*. Both t- and F-tests were used to compare data where appropriate.

RESULTS

Spray droplet deposition on *C. chinense* leaves varied according to vertical location in the canopy. The mean number of droplets on the upper, middle and lower third leaves was 54.00 ± 0.54, 44.82 ± 0.74 and 8.37 ± 0.09/cm^2, respectively. Leaves in the upper third of the plant canopy had significantly more droplets compared to either those in the middle (t_{18} = 2.33, p = 0.032) or lower third (t_{18} = 83.27, p<0.0001) of the canopy. Similarly, leaves in the middle third of the plant canopy had significantly more droplets compared to those on the lower third (t_{18} = 48.91, p<0.0001). Mean droplet deposition on leaves in decreasing order was thus upper>middle>lower.

B. tabaci mortality from Aza-Direct®-treated leaves was not significantly influenced by cardinal direction ($F_{3, 36}$ = 2.16, p = 0.11). However, the location of the sprayed leaf (upper, middle or lower third of canopy) along the vertical axis had a strong influence on *B. tabaci* mortality ($F_{2, 9}$ = 128.95, p<0.0001), with significantly higher adult *B. tabaci* mortality occurring on sprayed leaves in the upper third of the canopy compared to the middle and lower thirds (1.65 and 7.85% higher respectively). Mean *B. tabaci* mortality on sprayed leaves in the middle third of the canopy was 6.20% higher and significantly different (p<0.05) than on leaves in the lower third (Table 1).

The age of the Aza-Direct® residue also had an influence on the mortality of *B. tabaci* adults. Generally, mortality was in the decreasing order upper third leaves>middle third leaves>lower third leaves and also decreased with increasing residue age. Whitefly mortality on *C. chinense* leaves in the upper and middle third of the plant canopy did not vary significantly (p>0.05) from days 1 to 3 post treatment and there was no significant difference (p>0.05) in mortality between sprayed leaves in the middle and lower third of the canopy from days 4 to 8. No significant (p>0.05) variation in *B. tabaci* mortality between upper, middle or lower third sprayed leaves from day 9 onward was observed (Table 1).

B. tabaci immobility varied significantly with time in the upper, middle and lower third leaves of the plant canopy (p<0.05) until day 4, while *B. tabaci* immobility was not significantly different between the treated leaves on the middle and lower canopy from day 5 onward (Table 2).

The degradation rate of Aza-Direct® on upper, middle and lower *C. chinense* leaves over a 10-day period is presented in Figure 1. The linear regression equation of ln (M_t/M_0) against time for each leaf strata explained the 89, 98 and 94% degradation variation in the upper, middle and lower leaves, respectively (Figure 1). The half-lives (ET_{50}) of Aza-Direct® residues on upper, middle and lower leaves of *C. chinense* plants, calculated from the rate constants 'a' for each line were 1.7, 1.8 and 2.4 days, respectively. Thus, degradation of Aza-Direct® residues was most rapid on upper leaves and was 1.4 times faster than that of the leaves on the lower third of the plant canopy while it was 1.1 times as fast on the middle leaves.

Table 1. Mean (±SE) corrected percent mortality of *Bemisia tabaci* exposed to differently aged Aza-Direct®-sprayed *Capsicum chinense* leaves from upper, middle and lower third of the plant canopy.

Day	Mean (±SE) corrected mortality (%)*		
	Upper	Middle	Lower
1	34.75 ± 1.31^{aA}	32.25 ± 3.35^{aA}	12.00 ± 1.47^{aB}
2	27.25 ± 1.31^{bA}	31.00 ± 2.35^{bA}	10.00 ± 0.58^{bB}
3	24.25 ± 1.44^{cA}	22.25 ± 1.60^{cA}	9.25 ± 0.85^{cB}
4	15.75 ± 1.70^{dA}	11.00 ± 1.47^{dB}	6.75 ± 0.85^{dB}
5	9.75 ± 1.31^{eA}	7.00 ± 0.71^{eB}	5.75 ± 0.85^{eB}
6	7.50 ± 0.65^{fA}	4.75 ± 0.48^{fB}	4.50 ± 0.65^{fB}
7	6.00 ± 0.41^{gA}	3.50 ± 0.50^{gB}	3.75 ± 0.48^{gB}
8	5.00 ± 0.41^{hA}	3.00 ± 0.71^{hB}	2.00 ± 0.41^{hB}
9	2.50 ± 0.65^{iA}	2.00 ± 0.10^{iA}	0.75 ± 0.48^{iA}
10	0.50 ± 0.29^{jA}	0.00 ± 0.00^{jA}	0.00 ± 0.00^{jA}

***Values followed by the same lowercase letter along a column and the same uppercase letter along a row are not significantly (P>0.05) different from each other based on Tukey-Kramer multiple comparisons test.

Table 2. Mean (±SE) corrected percent immobility of *Bemisia tabaci* exposed to differently aged Aza-Direct®-sprayed *Capsicum chinense* leaves from the upper, middle and lower third of the plant canopy.

Day	Mean (±SE) corrected immobility (%)*		
	Upper	Middle	Lower
1	16.75 ± 0.51^{aA}	13.75 ± 0.33^{aB}	6.25 ± 0.20^{aC}
2	15.75 ± 0.49^{bA}	9.25 ± 0.33^{bB}	5.25 ± 0.18^{bC}
3	15.50 ± 0.55^{bA}	8.50 ± 0.31^{cB}	5.00 ± 0.24^{bC}
4	9.25 ± 0.35^{cA}	8.50 ± 0.31^{cB}	4.75 ± 0.22^{cC}
5	5.00 ± 0.20^{dA}	4.25 ± 0.26^{dB}	4.25 ± 0.21^{dC}
6	4.05 ± 0.15^{eA}	3.75 ± 0.18^{eB}	3.75 ± 0.18^{eB}
7	2.60 ± 0.18^{fA}	2.50 ± 0.18^{fB}	2.44 ± 0.17^{fB}
8	1.25 ± 0.16^{gA}	1.00 ± 0.17^{gB}	0.75 ± 0.13^{gB}
9	0.00 ± 0.00^{hC}	0.00 ± 0.00^{hC}	0.00 ± 0.00^{hC}
10	0.00 ± 0.00^{hC}	0.00 ± 0.00^{hC}	0.00 ± 0.00^{hC}

***Values followed by the same lowercase letter along a column and the same uppercase letter along a row are not significantly (P>0.05) different from each other based on Tukey-Kramer multiple comparisons test.

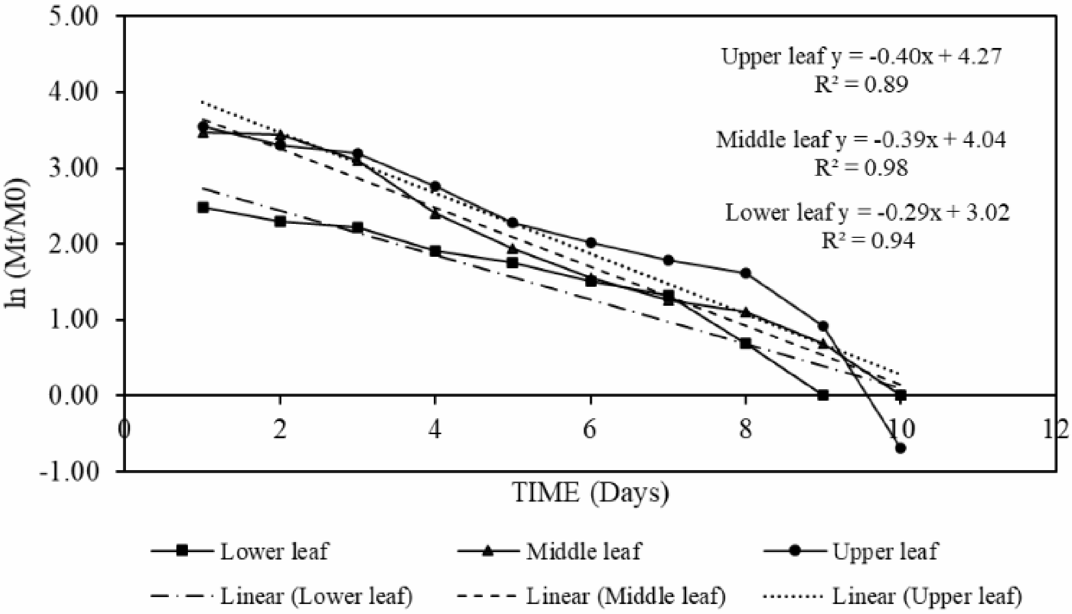

Figure 1. Adult *Bemisia tabaci* mortality on *Capsicum chinense* upper, middle and lower canopy leaves with differently aged Aza-Direct® residues.

DISCUSSION

Most of the studies of *Azadiracta indica*-based insecticides have shown that they are very effective in suppressing both the nymphal and adult stages of *B. tabaci* [13, 14]. However, most of these studies were conducted under laboratory conditions and may not be a true reflection of field conditions. A comparison of the efficacy of Neemix® and Mpede® on *Myzus persicae* (Homoptera:Aphididae) found that to achieve a 90% mortality, the concentration required was much higher than what was recommended in the field and consequently the level of mortality in the field would be very low [15]. A similar conclusion was made by [16] who recorded a 30.85% mortality of *B. tabaci* nymphs under field conditions. Apart from concentration of the insecticide, spray droplet distribution on the plant surface can also influence insect pest mortality. Several factors are known to affect the spray droplet distribution and coverage on plants including the canopy structure as well as leaf surface texture, size, structure and orientation [17, 18]. In the current study, spray droplet deposition on *C. chinense* plants was shown to vary according to leaf location along a vertical axis with the canopy. There was a 9, 37 and 46-fold difference between the mean number of droplets on leaves in the upper and middle, middle and lower, and upper and lower thirds of the plant canopy, respectively. Similar results were obtained on upper leaves of *Zea mays* L. where there was significant reduction in lambda cyhalothrin droplet deposition on lower leaves [19] but the percent reduction was not quantified. Lower leaves of soybean, *Glycine max* L. also had reduced droplet deposition on lower compered to upper leaves when sprayed with a tracer dye [20].

Aza-Direct® does have a low level of efficacy against adult *B. tabaci* within each third of the plant canopy of *C. chinense*. The greatest effect on mortality observed in *C. chinense* occurred in the upper third of the plant canopy and was recorded one-day post treatment at 34.75 ± 1.31%. Similarly, reduction in lambda cyhalothrin droplet deposition on lower leaves of *Zea mays* resulted in a concomitant reduction in mortality of the Asian corn borer, *Ostrinia furnacalis* (Guenee) (Lepidoptera:Crambidae) on the lower leaves compared to those on the upper leaves [19]. The positive efficacy of Aza-Direct® on *B. tabaci* adult mortality reported is supported by research done in Germany on Neem Azal® (aqueous extract) which was found to not only have good effect against *B. tabaci* nymphs and pupae but also had weak effect on adults [21].

Apart from being a potent antifeedant and also causing mortality, the tetranortriterpenoid azadirachtin, has also been reported to cause immobility in some animals including nematodes and ticks [22, 23]. Aza-Direct® caused immobility of adult *B. tabaci* during the course of this study and appeared to follow the same general trend as mortality.

The efficacy of Aza-Direct® was maintained for the first three days post treatment to greater than 20% for the upper and middle third before declining significantly. Both the upper and middle thirds had 97% and 92% higher mean mortality than the lower third on day one post treatment, respectively. From Day 4 post treatment the difference between the mean mortality in the upper, middle and lower third increased significantly. The difference in mortality during this period may be as a result of insufficient coverage (droplet deposition) to the middle and lower thirds of the canopy with the knapsack sprayer.

The degradation of neem oil on plant leaves has been reported to range from 1-3 days on plant leaves [24]. This rapid breakdown is primarily as a result of photodegradation [25] and explains the short half-life of 1.7 days on the leaves of the upper third of the plant canopy compared with the increased half-lives of 1.8 and 2.4 days on the leaves of the middle and lower thirds of the plant canopy.

Greater penetration into the middle and lower thirds of the *C. chinense* plant canopy may be achieved by the use of smaller droplets and alternative spray equipment (eg. motorized mist blower). This could result in higher adult *B. tabaci* mortality in the lower leaves of the plant as smaller insecticide droplets in the diameter range 20-100 µm are known to be more efficient at controlling insect pests and penetrating the plant canopy compared to larger diameter droplets [26-28]. Apart from determining the droplet deposition of botanical insecticides (eg. Aza-Direct®) on different levels of the plant canopy, the information from the current study may also prove useful in the application of other pesticides including fungicides and herbicides as well as entomopathogenic fungi [29, 30] and also in conservation of natural enemies of pests on agricultural crops [31].

CONCLUSION

Spray droplet deposition from a knapsack sprayer on *C. chinense* leaves was highest on the leaves in the upper third of the plant canopy compared to the middle and lower third. Aza-Direct® caused immobility and mortality of adult *B. tabaci* when applied to *C. chinense* plants. Both immobility and mortality were strongly influenced by the location and residue age of the treated leaves in the canopy but not by cardinal direction. Mortality and immobility decreased over time, with highest and lowest values occurring in leaves in the upper and lower thirds of the plant canopy.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflict of interest with the research presented in this manuscript.

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