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Effects of a Foliar Neem Formulation on Colonization and Mortality of Whiteflies (Hemiptera: Aleyrodidae) on Collard Plants

Dana Krzyzaniak[1,2], Alvin M. Simmons[3*], B. Merle Shepard[4]

[1]Academic Magnet High School, North Charleston, SC, USA
[2]School of Chemical and Biomolecular Engineering, Georgia Institute of Technology, Atlanta, GA, USA
[3]USDA-ARS, U.S. Vegetable Laboratory, Charleston, SC, USA
[4]Coastal Research and Education Center, Clemson University, Charleston, SC, USA
Email: *alvin.simmons@ars.usda.gov

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Abstract

A study was conducted to determine the effects of foliar sprays of a selected neem (*Azadirachta indica* A. Juss) product (GOS Neem 7-Way) on colonization and development by the Middle-East Asia Minor-1 (= B-biotype sweetpotato whitefly) *Bemisia tabaci* (Gennadius) on collard (*Brassica oleracea* variety a*cephala* de Condolle) plants. GOS Neem 7-Way is marketed for use as an insecticide in organic and conventional crop production. Caged choice, caged no-choice, and Y-tube olfactometer assays were conducted on oviposition, survival and adult behavioral response to plant treatment with 1.25% azadiractin. In the caged choice experiment, colonization by the whiteflies was reduced and fewer eggs were deposited on neem-treated plants as compared with control plants (only treated with an adjuvant). Similarly, decreased numbers of adult whiteflies and reduced whitefly development were observed in no-choice assays for the neem-treatment, as compared with the untreated control. Both horizontal and vertical-orientated Y-tube olfactometer assays provided complementarily assessments that the neem had a repellency effect on the adult whiteflies. However, the repellency effect primarily dissipated within one day post treatment. Overall, the greatest benefit of the neem treatment appears to have been on whitefly mortality. The findings may be useful in providing a more ecologically sound way to manage populations of the *B. tabaci* whitefly in organic vegetable production.

Keywords

Bemisia tabaci, Vegetable, Biorational, Sustainability, Azadiractin

1. Introduction

Whiteflies in the *Bemisia* complex are problematic on a wide range of crops on a global scale. The Middle-East Asia Minor-1 (MEAM1 = B-biotype sweetpotato whitefly) *Bemisia tabaci* (Gennadius) [1] (also reported as *Bemisia argentifolii* Bellows and Perring [2], is an important member of the *Bemisia* pest complex. This pest is very adaptable to its environment [3], and it has developed resistance to several insecticides [4] [5] [6] [7]. In addition to direct injury by feeding on over 1000 agricultural and wild hosts [8] [9] [10] this whitefly transmits over 300 species of plant viruses [11] which adds to its importance in agricultural production.

Growers commonly use synthetic insecticides to manage whiteflies. One appeal to this approach is that insecticides often suppress whitefly populations quickly. However, repeated use of conventional insecticides in agricultural systems has led to much concern about adverse effects on human health, the environment, insecticidal resistance in the whitefly target, adverse effects on non-target organisms, and other negative effects of insecticides [7] [12] [13]. Therefore, for sustainability, pest management options that incorporate a more compatible approach are desirable. Moreover, when an insecticide application is needed, cost, efficacy, and compatibility within the agricultural system are important considerations. Accordingly, biorational insecticides help fill certain critical needs by some growers, such as in organic agricultural production.

Azadiractin is an important plant-derived limonoid extract from the neem tree, *Azadirachta indicate* A. Juss, and it is used in insect pest management [14] [15] [16]. Azadiractin extracts are most commonly taken from neem seeds, the source for commercial use, but also are taken from leaves and fruits of this plant [17]. Azadiractin has activity against a wide range of insect pests as an insecticide and antifeeding deterrent [16] [18] [19] [20] [21]. However, with different formulations of azadiractin and different modes of applications, it is difficult to compare among studies [16]. Elevated storage temperature (30˚C), duration of storage and decreased concentrations can lower the efficacy of neem against a targeted pest [22]. Azadiractin is the most active compound in neem seed and is registered in the U.S. as a relatively non-toxic pesticide [23]. It is structurally similar to ecdysone which controls metamorphosis. If applied carefully, neem may be virtually less harmful compared to most commonly used pesticides [23].

Neem has been studied on several species of sucking insects. For example, in an aqueous state, it can affect the growth of *Clavigralla scutellari* (Westwood) (Hemiptera: Coreidae), a pest of pigeon pea plant (*Cajanus cajan* (L.) Millsp.) [24]. Dipping fourth instars of *C. scutellari* into neem solution and then held for continued development resulted in wing abnormalities that, in some cases, prevented the insect from flying. Aqueous neem also had severe effects on the cotton aphid (*Aphis gossypii* Glover) [25]. In the aphid study, the researchers found that neem extract reduced the fecundity of the aphids, and resulted in nymph mortality. Similar results were found during a study with neem on the southern green stink bug [*Nezara viridula* (Linnaeus)] [26]. In that study, researchers found that neem could affect molting by the nymphs and caused morphological defects in the adult stink bugs. These experiments suggest that neem may also have detrimental effects on the populations of whiteflies. The effects of neem on natural enemies vary [16] [27] [28] [29]. However, the quality among specific formulations of neem may not be consistent. For example, shelf life and storage conditions are factors which may affect efficacy. The objective of this study was to determine the effects of a selected formulation of neem (GOS Neem 7-Way) on the colonization and mortality of *B. tabaci* on collard host plants.

2. Materials and Methods

MEAM1 *B. tabaci* was obtained from a colony maintained on several vegetable crops [30] in a greenhouse. The genetic identity of this whitefly colony was recently reconfirmed [31] by polymerase chain reaction analyses of its DNA. For the assays in this study, adult whiteflies were taken directly from collard, *Brassicaoleracae* variety a*cephala* de Condolle, plants. The whiteflies in the colony had not been exposed to chemicals before being used in the experiments. Likewise, collard plants for the assays were not exposed to any pesticides or other chemical except for liquid fertilizer. Both seedlings and mature plants were used for the experiments. Seedlings were held in a growth chamber at 28˚C, a photoperiod of 14:10 (light: dark) and 60 ± 5% relative humidity. Plants used were similar in size, coloration, and number of leaves. Except where noted, seedlings were established in Jiffy starter pellets (Jiffy Products of America, Batavia, Illinois, USA) and were approximately 5 cm high. Older plants were potted and were about 20 cm tall.

During testing, plants were held in cages to keep them insect-free. Two types of cages were used. The cage used for testing seedlings was an 18-cm-depth by 20-cm-diameter clear plastic cylinder that was covered with mesh on top, secured with rubber bands. Cages for larger plants were 0.61 × 0.61 × 0.61 m and constructed of 70 mesh chiffon screen with aluminum frames (Bio Quip Products, Rancho Dominguez, California, USA).

Neem treatment solution was applied to plants in a concentration of 37.0 mL of azadiractin per 3.79 L of water and an emulsifier of 7.39 mL per 3.79 L. The neem, labeled as GOS Neem 7-Way, and emulsifier were obtained from Georgia Organic Solutions. The azadiractin was stored in a refrigerator at 2˚C until used. The spray treatment was newly mixed each day and it was then applied to the plants using a plastic, handheld mist spray bottle.

A solution of water and emulsifier was sprayed on control plants and used as a comparison with the neem treatment. The control spray was comprised of only the emulsifier at the same concentration (7.10 ml/3.79 liters). The control solution was applied in the same manner as the neem solution. Adult whiteflies were collected and transported in 30 ml plastic vials. Whiteflies were aspirated into the vials, capped with plastic tops and then released onto the collard plants. A compound microscope was used for examining the leaves for immature whiteflies while no magnification was used to observe the adult whiteflies.

Horizontal Y-tube assay.

A horizontal Y-tube olfactometer was used for testing the relative attractiveness/repulsion of adult *B. tabaci* toward neem-treated plants. The olfactometer (as described [32]) was connected to a vacuum line that was calibrated to pulled a total flow of 4.2 L·min-1. Air was pulled across leaf surfaces of the samples in the separate compartments of the olfactometer. The olfactometer contained a neem-treated collard leaf in one arm of the Y-tube and a collard leaf that was only treated with water and the emulsifier in the other arm. Five adult whiteflies were released in the entrance of the olfactometer for each trial. After 25 minutes, the location of the whiteflies were observed and recorded based on which arm they were in or if they were still at the entrance. Four runs were done with the neem treatment in the right chamber of the olfactometer and four runs were done with the neem in the left chamber of the olfactometer. The experiment was repeated four times for a total of 160 insects.

Vertical Y-tube Assay.

An additional olfactometer assay was conducted using a vertical Y-tube olfactometer assay (as described [33]). This vertical orientation can complement the negative geotaxis and positive phototaxis behavior exhibited by many insects, including whiteflies, which tend not to exhibit movement readily in horizontally oriented olfactometers [32] [33] [34]. A 2-ml centrifuge vial was placed 6.3 cm below the top of an arm of the vertical olfactometer, and the vial was filled with deionized water. A vial of water was likewise setup in the other arm, and the stem of a detached leaf from a collard seedling was placed in the vial. The top of the tubes were covered with fine mesh screen. Thirty adult female whiteflies were allowed to escape from a 30 ml vial that was placed on the floor of the olfactometer. A paired test was set up for two olfactometers, one with a control leaf and water check, and the other with a treated leaf and water check. Four pairs of olfactometers were set up for a given trial. The plants were set up on a laboratory bench with continuous light (1172 Lux at bench top) during the experiment. Temperature was 24 ± 2˚C during the exposure times. Data were taken on whitefly counts for a 90 minute expose to the plants at different time periods after the leaves were sprayed. Exposure times were 1 - 2.5, 5 - 5.5, 22.5 - 24, and 46.5 - 48 hours post spray. After each exposure time, the numbers of live whiteflies of the leaf, in the check arm, in the base of the olfactometer and number of dead whiteflies and number of whitefly eggs on the upper and lower leaf surfaces within each olfactometer were recorded. The experiment was repeated five times for all exposure times except that three replicates were conducted for the 46.5 - 48 exposure.

Caged Choice Tests.

To conduct an additional assessment on the repellant effects of the neem solution on whiteflies, two similar-sized potted collard plants were placed into each of 20 large aluminum cages (as described above). Cages were held in an outdoors environment below a structure of 60% shade cloth (6426 Lux at ground level) which were completely opened on all sides. One plant was treated with neem solution, and the other plant was treated with the control solution, and both plants were placed in the same cage. After allowing the plants an hour to dry following the spray, a 30-ml vial containing 200 unsexed adult whiteflies was placed of the floor inside of the cage and located between the two plants. The two plants were aligned east and west; 20 cages were set up and the cardinal location was alternated between each pair of plants for a given cage. The plants were left in the cage for 24 hours. After that time, each plant was removed and the numbers of adult whiteflies and eggs on each plant were counted and recorded. This procedure was repeated three times with new plants and insects.

Egg Hatch.

A no-choice experiment was set up to assess any neem effect on the incidence of egg hatch. Eight plastic cages (as aforementioned) containing five collard seedlings each were used, and 200 adult whiteflies of undetermined gender were added to each of the containers at 26 ± 2˚C. After 24 hours, the adult whiteflies were removed and the plants were treated with either the control or the neem solution. All plants within a cage were treated with the same treatment; five cages were treated with the control spray and five were treated with the neem spray. After 9 days, the leaves from each plant were removed and bagged. They were then taken to the laboratory and examined for the number of eggs and nymphs on each leaf. The experiment was repeated four times.

Adult Emergence.

A no-choice experiment was conducted to determine the effects of the neem formulation on the development of *B. tabaci* nymphs to the adult stage. Eight plastic cages (as aforementioned) containing five collard seedlings each were used. A total of 200 adult whiteflies were added to each container held at 26 ± 2˚C. After 24 hours, the adult whiteflies were removed. After 9 days were allowed for egg hatch, the plants were treated with either the control spray or the neem spray. All plants within a given cage were treated with the same treatment. Five cages of control plants and five cages with neem-treated plants were used. After 10 more days, the leaves from each plant were removed and bagged. They were then examined for the number of live nymphs, dead nymphs, and adults (based on empty exuviae) on each leaf. The experiment was repeated three times.

All data were analyzed with SAS 9.4 TS 1M2, Window version 6.2.9200 [35]. The independent variable was the neem applied to the collard. The dependent variables were numbers of eggs, nymphs and/or adults. The data were analyzed with ANOVA to assess any significant differences. Percentage data were arcsine-transformed before the analysis, but the results are presented on back-transformed data. Significant differences were determined at $P < 0.05$ unless otherwise noted.

3. Results and Discussion

3.1. Horizontal Olfactometer Assay

A significant difference ($F = 46.65$, $df = 62$; $P < 0.0001$) in whitefly behavior, was observed with the horizontal olfactometer assay. Only 3.7 ± 1.4% of the adult whiteflies resulted in the Y-tube chamber with the neem treatment as compared with the control (96.2 ± 1.4%). This demonstrated a strong, but not absolute, repellency effect of neem on the whiteflies.

3.2. Vertical Olfactometer Assay

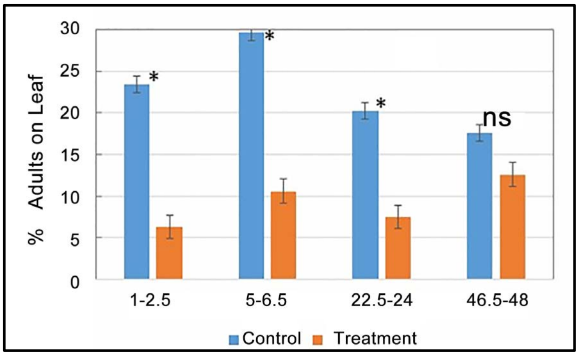
Unlike the horizontal olfactometer, a vertical olfactometer is able to assay parameters beyond relative attractancy/repellency [33]. Significant detrimental impacts were observed on host acceptance and oviposition over time following the spray treatment of collard with neem in the vertical olfactometer assay (**Figure 1(a)**, **Figure 1(c)**). For the three exposure periods within 24 h post treatment, differences were observed in the percentage of released adults that were found on leaves of collard (**Figure 1(a)**). No significant difference between the treatment and the control was observed 2 days post treatment. Thus, the repellency had apparently dissipated by that time. Significantly elevated numbers of dead whiteflies were observed up to the 5 - 5.5 hours post treatment, but mortality was not significantly affected for the 1-day and 2-day post treatment exposures (**Figure 1(b)**). Although data were not taken on the location within the olfactometer where dead whiteflies were found, many of the dead whiteflies were observed on the top surface of the leaf and on the floor of the olfactometer. Significantly fewer eggs were deposited on the treated leaves during the three exposure periods during the first day post treatment, but not 2 days post treatment (**Figure 1(c)**). For a given exposure time, it is not known if the time of the whitefly collection affected oviposition. Insects for a given exposure time was consistently collected either early morning, late morning, or mid-afternoon.

3.3. Outdoors Caged Choice Test

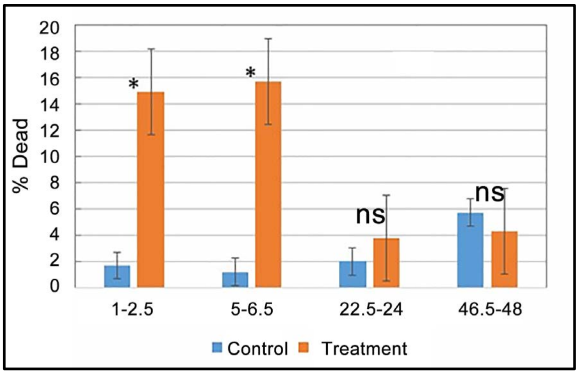
After 24 hours of the spray treatment, when given a choice between control and neem-treated plants, the adult whiteflies most often chose the control (untreated) plants. The average percentage of the whiteflies was significantly greater ($t = 10.49$; $df = 118$; $P< 0.0001$) on the control plants than on the neem-treated plants (**Table 1**). There was an average of 0.78 adults per·cm^2 of leaf on control plants and 0.31 adults per·cm^2 of leaf on plants treated with neem. Moreover, there was a significant reduction ($t = 7.31$; $df = 178$; $P < 0.0001$) in percentage of eggs on the neem-treated plants as compared with the control plants. Leaf area was statistically the same between the treated and control plants (**Table 1**). These data support that *B. tabaci* is less likely to infest plants and deposit eggs on plants treated with the neem formulation. Additionally, these data suggest that plants treated with the neem would be more likely to delay a whitefly population increase. These data complement the two aforementioned olfactometer experiments in further demonstrating the repulsion of the neem sprays to the adult whiteflies. Apparently, the decreased incidence in oviposition on the neem-treated plants may be because of the lower number of adults on those plants.

3.4. No-Choice Egg Hatch Assay

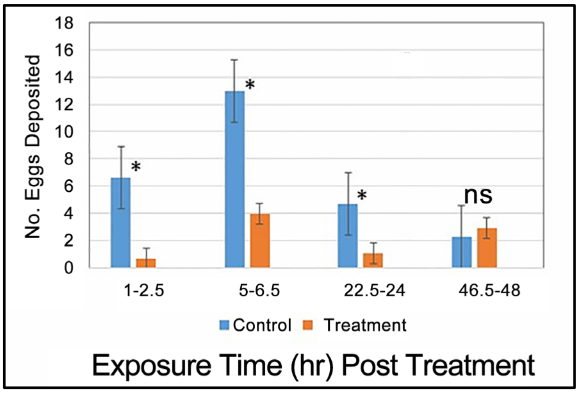
Neem-treated eggs had a significantly lower ($t = 7.82$; $df = 130$; $P < 0.0001$) hatch rate (61%) as compared to the non-treated eggs which hatched at an average rate of 90% (**Table 2**). Reduction in egg hatch is important because this would result in an overall reduction in population level. Because most eggs are deposited on the lower surface of collard leaves, good spray coverage was more challenging on this surface than the top surface.



(a)



(b)



(c)

Figure 1. Mean percentage of 30 released adult *B. tabaci* that were: (a) alive on collard leaf; (b) percentage of the released that were dead; and (c) the number of eggs deposited, during 90 minutes at different exposure times after treatments of the leaf with neem or control spray. Asterisk above means for pairs within a given exposure time indicates significant difference at $P < 0.05$ while "ns" above means for pairs indicates no significant difference based on t-test.

3.5. Nymphal Survival

Overall estimated adult emergence was low (25%) for those on the control plants which was 8-fold greater than the rate (3%) for nymphs subjected to the neem treatment (Table 3). Considering the proportion of surviving nymphs and emerged adults provide a better comparison between treatments than counts of the whiteflies because the number of individuals in each cohort was not the same. Although some whiteflies in both treatments remained in the nymphal stage, high nymphal mortality (71%) was observed for the neem treatment as compared with the check (8.8%) (Table 3). Because the total number of eggs deposited was not determined, the assessment of percent adult emergence is probably an underestimation. In concert with results from the experiment on egg hatch, data in this experiment demonstrate the negative impact of the neem spray on survival of the immature whiteflies. Lower emergence levels would further decrease the population of whiteflies and slow future growth.

Table 1. Mean percentages of adult *B. tabaci* and eggs deposited on collard plants 24 hours after releasing 200 adult whiteflies in a cage containing a neem-treated plant and a control plant.

Treatment	% of total number of adults on plant pair ± SEM (mean count)	% of total number of eggs on plant pair ± SEM (mean count)	Leaf area (cm^2)
Control	69.7 ± 2.7 a (56.4 ± 4.3)	72.6 ± 4.4 a (98.6 ± 11.0)	107.9 ± 9.0 a
Neem	30.1 ± 2.7 b (25.5 ± 3.2)	28.7 ± 4.1 b (37.8 ± 6.4)	112.7 ± 10.3 a

Means within columns with different letters are significantly different (P < 0.0001).

Table 2. Mean percent egg hatch by *B. tabaci* after treatment with neem-sprays on collard in non-choice laboratory cages.

Treatment	% Egg hatch ± SEM
Control	92.0 ± 1.8 a
Neem	61.1 ± 3.7 b

Means are significantly different (P < 0.0001) according to ANOVA.

Table 3. Mean estimated incidence of adult emergence and incidence of live nymphs of *B. tabci* 10 days after collard plants containing first instar *B. tabaci* nymphs were treated with neem-sprays and control sprays in non-choice laboratory cages.

Treatment	Average Incidence of Whiteflies		
	% of cohort as live nymphs ± SEM (mean count)	% of cohort as dead nymphs ± SEM (mean count)	% adult emergence ± SEM (mean count)
Control	66.2 ± 4.4 (26.3 ± 4.6)	8.8 ± 4.4 (20.2 ± 2.0)	25.0 ± 3.4 (14.4 ± 4.6)
Neem	25.9 ± 5.5 (5.1 ± 0.1)	71.1 ± 5.5 (6.2 ± 4.9)	3.0 ± 1.4 (0.3 ± 0.1)

Means within columns are significantly different (P < 0.0001).

4. Conclusions

The collective data from the different experiments in this study support that the evaluated neem formulation can have a significant detrimental effect on the population of *B. tabci*. Namely, the research suggests that spray application of the neem can repel the whiteflies as well as have a detrimental effect on egg hatch and nymphal development. This study only concerned a single application of the insecticide in confined environments. Multiple applications would be expected to provide greater efficacy against whiteflies. Further testing including assessment of efficacy of the neem against whiteflies in the field environment is needed to define frequency of application and how this biorational material could best fit in an integrated pest management program for whiteflies.

A disadvantage of using a foliar neem application for insect treatments is that it can have a negative effect on the populations of natural enemies [27] [29]. Various impacts of several biorational insecticides, including a neem formulation, have been demonstrated to have a negative impact on natural enemies (predators and parasitoids) of *B. tabaci* in vegetable crops [27] [29] [36]. However, delivery of neem through a soil or seed application may have less negative impact on predators and parasitoids as compared with a spray treatment [37] [38].

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