



Original Research Paper

Efficacy of different neem-based biopesticides against green peach aphid, *Myzus persicae* (Hemiptera: Aphididae)

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The effects of three commercial neem-based formulations, namely Azatrol (1.2% Azadiractin A and B), Triple Action Neem Oil (70% neem oil) and Pure Neem Oil (100% neem oil), were evaluated on the green peach aphid, *Myzus persicae*, under both laboratory and greenhouse conditions. A leaf disc choice test bioassay demonstrated that none of the formulated neem-based insecticides tested were repellent to green peach aphid at recommended concentrations, but a two-fold increase in the concentration of Azatrol and Triple Action Neem Oil elicited a 50% reduction in the number of aphids settling on treated leaf tissue in comparison with untreated leaf tissue. When aphids were fed foliage containing neem-based insecticides, the rates of honeydew excretion were significantly reduced, to 14-40% of the control, thus demonstrating feeding deterrence. Azatrol also functioned well systemically when applied via the roots, resulting in 50% decrease in the feeding activity of treated aphids compared to that of the controls. Greenhouse evaluation of these products at the recommended concentrations revealed that aphid colonization was reduced to 50-75% of the control one week after neem-based products were applied as a foliar spray, while almost total elimination of aphids was observed by Pure Neem Oil and Azatrol treatments when a second application of these chemicals was applied to the foliage at seven days following the first spray. Results indicate that the neem-based formulations tested were highly effective in suppressing aphid population, but did not act as an efficient repellent at standard application rates, and while suppressing feeding, were not able to completely inhibit food intake.

Key words: Green peach aphid, *Myzus persicae*, biopesticide, neem.

INTRODUCTION

One of the major strengths of Jordanian agriculture is the ability to grow a wide range of vegetables and fruit trees. In fact, Jordan is one of the leading horticultural crop-producing countries in the Middle East, and has increasingly export of horticultural crops in the last decades. In both the internal and export markets,

consumers expect residue free products. However, plant pests form a major constraint in quantity and quality production of horticultural products. The green peach aphid, *Myzus persicae* (Sulzer), is worldwide in distribution, and is one of the most important polyphagous pests, attacking hundreds of plants in over 40 plant families,

including a wide range of vegetables and ornamental crops plants grown in both field and in greenhouses (Blackman and Eastop, 2000). *M. persicae* can attain very high population densities on young plant tissue due to its short generation time and tremendous fecundity. It is capable of inflicting severe injury directly by depriving the plant of its essential nutrients, resulting in a wilting, deformation, premature leaf senescence, and retarded growth rate of the plant. It also acts indirectly by contamination of the fresh products with honeydew and sooty mould (Gray and Gildow, 2003; Girousse et al., 2005; Pegadaraju et al., 2005). In addition, *M. persicae* is an important vector of over 100 plant viruses, greatly exacerbating the damage potential of the aphid (Blackman and Eastop, 2000).

Like other aphid species, *M. persicae* is a significant challenge for agricultural pest management programs. One of the principal options used by growers to protect their crops from aphids, particularly under outbreak situation, is the indiscriminate use of chemical pesticides, which often leads to deleterious effects on beneficial insects and humans, development of resistance, secondary pest outbreaks, excessive pesticide residues, and soil and water pollution. As a result of these critical effects of conventional pesticides, growers have to adopt more environmentally friendly integrated pest management or organic farming approaches (Leake, 2000; Cuthbertson and Murchie, 2003).

Formulation of new bioinsecticides, particularly those based on neem oil extract, is an exciting option for integrated pest management programs, since such plant-derived insecticides have various benefits, including selectivity, greater safety for non-target organisms, and compatibility with biological control organisms (Tang et al., 2002). The primary active ingredient of most neem-based pesticides is azadirachtin, a liminoid compound, which has multiple biological activities on more than 400 insect species from several orders (Schmutterer and Singh, 1995). Besides azadirachtin, there are other active components in some formulations. Azadirachtin-based compounds obviously have insecticidal, feeding deterrent, repellent, antioviposition, and physiological properties. They have an effect on some important physiological processes in insect such as survival, longevity, molting and reproduction (Mordue and Nisbet, 2000; Ulrichs et al., 2001; Tang et al., 2002). Despite the registration of neem formulations for many insect species, their efficacy for several plant pests in field and greenhouse experiments has been reported to be variable (Akey and Henneberry, 1999). Such variations in efficacy are both dose- and time- dependent and oftentimes are caused by the mixture of components in neem extracts (Mordue and Blackwell, 1993). Furthermore, methods of extraction, storage conditions, origin of neem, or contamination with mycotoxins can affect their action (Ermel et al., 2002). The objective of this work was to

evaluate the effects of three commercially available neem seed extracts on repellency, feeding activity, and survival of *M. persicae* reared on pepper plant.

MATERIAL AND METHODS

Plant rearing and aphid culture

A colony of green peach aphid was established from apterous individuals originally obtained from a continuously maintained culture at Department of Entomology and Nematology, University of Florida, Florida, USA. Aphids were maintained on young plants of sweet pepper grown in potting soil-filled plastic pots (15 cm in diameter) under greenhouse conditions at $25 \pm 3^\circ\text{C}$ with a 16:8 (L:D) photoperiod. *M. persicae* was kept in cages with a wooden frame of $60 \times 60 \times 100$ cm covered with fine mesh on all sides and above. A continuous supply of new plants was provided as needed for the colony replenishment.

Pepper seeds were pre-germinated for three days in plastic Petri dish (9 x 1.5 cm) lined on the bottom with wet filter paper before being planted in plastic seedling trays (50 x 30 x .6 cm, 50 seed/tray) containing commercial potting soil. After growing for 10 days under greenhouse conditions, seedlings in the primary leaf stage were individually transplanted into a plastic pot (15 cm diameter) filled with soil. Plants were kept in a greenhouse at $25 \pm 3^\circ\text{C}$, supplemented with grow lights to attain a 16:8 h (L:D) photoperiod. Plants were fertilized weekly using 20-9-20 water-soluble fertilizer (N:P:K) and irrigated as needed.

Neem-based insecticides

Commercially available neem formulations [Azatrol (1.2% azadirachtin), Triple Action Neem Oil (70% neem oil) and Pure Neem Oil (100% neem oil)] were obtained from Pbi/Gordon Corporation, Kansas City, Missouri, USA; Southern Agricultural Insecticides Inc., Palmetto, Florida, USA; and Dyna-Gro, San Pablo, California, USA, respectively. The formulated products were screened at recommended application concentrations of 31.5 ml/l for Azatrol, 7.5 ml/l for Triple Action Neem Oil, and 7.5 ml/l for Pure Neem Oil to evaluate repellent, antifeedant, and toxic effects on *M. persicae* aphids.

Laboratory bioassays

Repellency effects of the three neem-based insecticides were tested under laboratory conditions. For each test, young leaves of the same maturity selected from the upper portion of uninfested plants were excised, dipped for one

minute in solutions of each neem-based product prepared at the aforementioned concentrations, and then left to dry at room temperature for 15 minutes. Control leaves were similarly dipped in tap water and used for comparison.

Separate leaves from each treatment were excised and maintained with their abaxial surface facing up in a plastic container (25 cm diameter x 10 cm height) that was lined on the bottom with moist tissue and covered with a tight lid to avoid early drying of the leaves. In the multiple-choice bioassay, the bottom of the container was divided into four equal parts; each part was occupied with one leaf assigned randomly to each treatment. In the two-choice test, two leaves were treated with the chemical and the others untreated, with their distribution randomized within the container.

Fifty late-instar individuals were randomly collected from a synchronized aphid colony, released at the center of each container using soft hair brush, and left to settle freely for 24 h for both two-choice and multiple-choice bioassays. After a 24 h exposure period, the number of aphids in treated and untreated leaves was recorded. Each test was replicated ten times. Each treatment was replicated three times. All bioassays were carried out in a temperature-controlled room at $25\pm 1^\circ\text{C}$ and a photoperiod of 16:8 (L:D) h.

Whole-Plant Foliar Applications

Prior to the aphid release, young sweet pepper plants at the fourth true leaf stage were sprayed until runoff with solutions containing one of the neem formulations at the recommended application concentration using a hand-held sprayer. Control plants received water only. Sprayed plants were left to dry outdoors in the shade for 2 h. Eight plants for each treatment were placed individually in a cage (60 x 60 x 60 cm) covered via a gauze from all sides and above in an greenhouse at $25\pm 3^\circ\text{C}$, and 14:10 (L:D) h photoperiod. Groups of 20 same-aged apterous adult (24 d old) obtained from a synchronized colony were transferred to the upper portion of each treated and control plants and allowed to feed freely and produce offspring. Application of the treatments was repeated 7 days following aphid infestation. The numbers of live adults and offspring per plant were recorded at 7 and 14 d post-application.

Honeydew production

To identify the effect of neem-based insecticides on the feeding activity of aphids, the amounts of honeydew secreted by same-aged aphids were quantified. In this study, eight plants were treated with each neem product until runoff at recommended rates using a hand-held sprayer. Plants sprayed with tap water served as a control.

The treated plants were allowed to dry outdoors in the shade for 2 h. Thereafter, plants at third true leaf stage were infested with 20 late-instar nymphs obtained from a synchronized colony. Introduced aphids were confined on the undersurface of the fully expanded third leaf using a clip-on cage. The cages were made of a plastic Petri dish (6 x 1.5 cm) with two holes on the sides covered with fine mesh cloth for ventilation. Each clip-on cage was lined on the bottom with a spherical piece of aluminum foil precisely fitting its internal bottom surface.

Azatrol was also tested for its systemic effect on feeding activity. Potted seedlings of sweet pepper plants at the third true leaf stage were uprooted from the pot and roots were extracted from the potting soil under gentle tap water flow. Subsequently, the seedlings were transferred into a one litter plastic container (10 cm diameter x 20 cm height) covered with a plastic lid. The stem of the seedling was inserted through a hole in the lid while the roots were immersed in a 200 ml Azatrol solution prepared at recommended field concentration (31.8 ml/l). Plants with their roots immersed in water were used as a control. After 12 h of root immersion, 20 late instar nymphs were confined in clip-on cage lined on the bottom with aluminum foil on the undersurface of the third leaf and left to feed for 24 h. Eight plants were used for both the neem treatment and control, with 20 aphids per plant. The quantity of the honeydew produced by aphids in both experiments was determined by weighing the aluminum foil pieces prior to aphid release and at 24 h after aphid infestation. Measurements were made with a Sartorius cp2p microbalance with ± 0.001 mg accuracy.

Statistics

Data were submitted to analysis of variance using SAS software version 9.2 (2000) and treatment means were compared by the Least Significance Differences (LSD) test at 5% of probability.

RESULTS

Aphids given a choice of young leaves from sweet pepper plants either untreated or treated with each of three commercially available neem-based products in two-choice assays did not display a significant preference for settling on treated and untreated seedlings (Table 1). In the multiple-choice tests conducted with the recommended concentrations of neem, there was no difference in settling behavior on control plants and any of the three foliage treatments. However, increasing the concentration of each neem-derived biopesticide to two times the recommended field concentration induced significantly different levels of

Table 1. Mean number of aphids settled on the leaf discs 24 hours after treatment with three neem-based insecticides using two-choice bioassays.

Treatment	No. aphids	
Control	24.2a	LSD 5.75
Triple Action Neem Oil	18.0a	df 1, F 5.14
Control	16.2a	LSD 6.10
Azatrol	20.0a	df 1, F 1.72
Control	20.9a	LSD 8.51
Pure Neem Oil	21.9a	df 1, F 0.06

Means within column for each instar followed by the same letter are not significantly different at $P \leq 0.05$

Table 2. Mean number of aphids settled on the leaf discs 24 hours after treatment with one of three neem-based insecticides at two concentrations using a multiple-choice bioassay.

Treatment	Concentration	No. aphids	Concentration	No. aphids
Control	-	8.6 ab	-	15.1 a
Triple Action Neem Oil	7.5 ml/l	9.1 ab	15 ml/l	7.4 b
Azatrol	31.5 ml/l	6.2 b	33 ml/l	7.5 b
Pure Neem Oil	7.5 ml/l	11.2 a	15 ml/l	10.6 ab
		LSD 4.28		LSD 4.69
		df 3, F 1.89		df 3, F 4.89

Means within column for each instar larva followed by the same letter are not significantly different at $P \leq 0.05$

Table 3. Total amount of honeydew produced by 20 individuals of fourth instar *Myzus persicae* fed for 24 hours on pepper plants treated with different formulated neem-based pesticides

Treatment	Honeydew (mg/20 aphids)
Control	1.005 a
Triple Action Neem Oil	0.649 b
Azatrol	0.597 b
Pure Neem Oil	0.859 c
	LSD 0.075
	df 3, F 49.4

Means within column for each instar larva followed by the same letter are not significantly different at $P \leq 0.05$

settling (repellency). After 24 h of exposure, the minimum number of aphids was recorded in Triple Action Neem Oil and Azatrol at 15 ml/l and 63 ml/l, respectively, compared to that of untreated plants (Table 2). Thus, although some of the neem treatments demonstrated the potential for repellency, at the recommended concentrations they were not functionally effective.

All commercially formulated neem products exhibited

potential antifeedant properties to aphids, but with different magnitudes. Food intake by aphids on plants treated with of Triple Action Neem Oil and Azatrol was significantly diminished by up to 40% of the control, whereas aphids fed on Pure Neem Oil-treated plants excreted 15% less honeydew than the control (Table 3). Immersing the root of pepper seedlings in Azatrol solution induced nearly a 50% reduction in feeding activity of

Table 4. Total amount of honeydew produced by 20 individuals of fourth instar *Myzus persicae* fed for 24 hours on pepper plant seedlings that their roots were immersed in water with and without Azatrol.

Treatment	Honeydew (mg/20 aphids)
Control	0.608 a
Azatrol	0.311 b
LSD	0.075
	df 1, F 63.5

Means within column for each instar larva followed by the same letter are not significantly different at $P \leq 0.05$

Table 5. Effects of neem-based insecticides on aphid population (mean number per plant) 7 and 14 days after initial application of neem products. A second application was made on day 7. Initial population densities were 20 aphids per plant.

Treatment	No. of aphids after	
	7 days	14 days
Control	156.2 a	903.0 a
Triple neem oil	87.5 b	372.5 b
Azatrol	35.5 c	3.1 c
Pure neem oil	42.1 c	0.7 c
LSD	13.05	87.24
	df 3, F 149.51	df 3, F 196.4

Means within column for each instar larva followed by the same letter are not significantly different at $P \leq 0.05$

aphids as compared to controls, demonstrating the potential value of systemic neem-based materials for treatment of aphids (Table 4).

Spraying neem-based products onto potted sweet pepper plants in the greenhouse significantly reduced the densities of aphid by half to three-fourths by 7 days post-treatment. By day 14, control aphid populations in control treatment multiplied about 8-fold. Aphids displayed greater susceptibility to Azatrol and Pure Neem Oil than to Triple Action Neem Oil (Table 5). The second spray, on day 7, produced the same trend as the first insecticide application, but with more pronounced effects. On day 14, aphid abundance on Pure Neem Oil and Azatrol-treated plants was significantly reduced to only about 1% of the control, whereas aphid densities on Triple Action Neem Oil-treated plants were less than half the rate of aphids on untreated plants. The recommended concentration of Pure Neem Oil was caused some phytotoxicity to tender foliage of sweet pepper plants, but this was not observed with the other products.

DISCUSSION

Commercially available neem seed extracts have diverse pest control properties, affecting insect growth, fertility, and metamorphosis in addition to direct toxicity and antifeedant and oviposition-deterrent effects (Naqvi, 1996).

The present study demonstrated that three commercial neem-based formulations are potentially very effective as aphicides, though they differed in their effectiveness. Sprays of different neem preparations to intact pepper plants caused a significant reduction in the numbers of green peach aphids 7 days post-treatment. Even more intense mortality of aphids was attained following a second spray. Pure Neem Oil and Azatrol were superior for suppressing the aphid population when compared to treatment with Triple Action Neem Oil or control plants. Similarly, a number of other neem-based products induced mortality of *M. persicae* on different host plants (Lowery et al., 1993; Coventry and Allan, 2001; Cutler et al., 2009; Akbar et al., 2010). A previous study conducted by Coventry and Allan (2001) revealed that exposure of adult *M. persicae* to neem seed oil and azadirachtin had a significant influence on the survival of offspring produced by treated adults, whereas the survival of adults remained unaffected. In addition to direct toxicity, these authors declared that adults successfully emerging from *M. persicae* nymphs treated with neem were undersized with abnormal wings, legs and stylets. The degree of abnormality varied with both the growth stage of the insect, and the host plant on which it fed. Nisbet et al. (1994) observed that the reproductive potential of apterous *M. persicae* fed on diet containing azadirachtin was less than half the rate of aphids fed on control diet within the first 26 h, whereas nymph production virtually ceased after 50 h, and any nymphs produced were born dead and with undeveloped appendages after this period.

Previous research has indicated that neem-based pesticides could control a number of other aphid species infesting plants under laboratory and field circumstances. However, there were differences in the insecticidal activity among commercially available products of which the majority showed to be toxic to aphids, including those on many different crops (Koul, 1999; Ahmed et al., 2007; Kraiss and Cullen, 2008; Duchovskiene and Karkleliene, 2008). In addition, neem products reduce the fecundity and fertility of adults, and molting of nymphs (Lowery and Isman, 1996; Tang et al., 2002), along with increasing development time of nymphs surviving to adulthood (Kraiss and Cullen, 2008). The effect of neem-based pesticides on the reproductive potential of aphids has been attributed to blocking the neurosecretory cells by the active ingredient, azadirachtin, which disrupts adult maturation and egg production (Vimala et al., 2010). Other studies,

however, revealed that azadirachtin and neem seed oil had no influence on the survival (Lowery and Isman, 1994), fecundity (Kraiss and Cullen, 2008), and the length of the development period of immature stages of different aphid species (Pavela et al., 2004). Such contrary findings in the literature suggest that the growth regulatory effects of formulated neem-based products may rely on the host plant, aphid species, treated aphid instar, or the climate conditions (Ermel et al., 1987). Alternatively, azadirachtin and other constituents in neem extracts may vary in their efficacy depending on geographic origin and yearly variations in environmental growing conditions of the neem tree. There is also evidence that the method of neem extraction affects the effectiveness of the insecticide formulation, and thus may vary considerably between manufacturers (Liu and Liu, 2005).

All neem-based pesticides used in this study failed to significantly deter the settling behavior of *M. persicae* on treated leaf disks. This is consistent with the results obtained by Griffiths et al. (1989) for the same aphid species. However, aphids fed on treated plants produced significantly less honeydew during the first 24 h after neem application relative to the control, including on plants with their roots immersed in Azatrol solution. This suggests that these products are acting more as a feeding deterrent rather than as a repellent. Interestingly, Nisbet et al. (1994) determined that honeydew production of apterous *M. persicae* on azadirachtin-treated diets was unaffected during the first 26 h period, but then was only one-third that of control diets during the subsequent 24 h period. This differs somewhat from results of the present study, presumably due to the difference in biopesticides tested. However, electronic recording of feeding activity of *M. persicae* on *Nicotiana clevelandii* seedlings demonstrated that azadirachtin applied systemically reduced the time of aphid feeding on phloem to one third that of control (Woodford et al., 1991), which is more consistent with our findings.

With regard to the antifeedant action of neem extracts or azadirachtin, some neem-based pesticides exhibited feeding inhibition in *Acyrtosiphon pisum* (Hunter and Ullman, 1992), *Ropalosiphum padi* and *Sitobion avenae* (West and Mordue, 1992). Other neem-seed oil and neem extracts were merely highly deterrent or displayed growth regulator actions, but the same products did not disrupt the feeding of other aphid species (Koul, 1999). Lowery and Isman (1994) observed that formulated azadirachtin disturbed feeding of *Chaetosiphon fragaefolii* aphids on greenhouse-grown strawberry within the first hour after application, but its antifeedant activity was lost within 24 h.

In conclusion, results of the study indicated that the antifeedant properties of neem-based products, though significant by some measures, did not appear to contribute

greatly to protection of the plant from aphid feeding. Rather, the benefits accrued from treating plants resulted mostly from toxicity and/or inhibition of adult reproduction, and the failure of nymphs to molt. Clearly, the present results and the variable responses in bioactivity associated with neem-derived insecticides revealed in the literature, suggest caution in making assumptions about the effects of different neem-derived insecticides.

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