



Efficacy of Aqueous Neem Seed Extract in the Control of Green Peach Aphids (*Myzus Persicae Sulzer*) on Chili Pepper (*Capsicum Annum L.*)

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Abstract: The paucity of documented investigations on the effect of aqueous neem seed extract in the control of colour morphs of *Myzus persicae* necessitated the investigations of this study. Experiments were conducted to compare the efficacy of aqueous seed extracts of *Azadirachta indica* with Neemazal and Deltrin against Lemon and Dark green morphs of *Myzus persicae*. The effect of the extract on the aphid's population and on plant biometrics was evaluated at three concentrations (viz 100%, 75% and 50% v/v). The effect of aqueous neem seed extracts were significant ($P < 0.05$) compared to Deltrin and Neemazal. The efficacy of the extract in reducing lemon and dark green morphs of aphids increased with increasing concentration and duration of exposure. Lemon green morphs of *M. persicae* were most susceptible to the neem seed extract at all concentrations. At 168 hours after application of 100% aqueous neem seed extract, reductions in population of the morphs were 91.8% and 84.8% in lemon and dark green morphs respectively. However, this concentration was toxic as localized lesions were observed on the leaves of chili pepper. The application of 50% aqueous neem seed extract was non toxic and proffers an efficacious, ecofriendly, cheap and more effective method of control of the imminent pest

Keywords: *Myzus persicae*; Neem; Biopesticide; Efficacy; Chili pepper; Plant morphometrics.

1. Introduction

Nigeria is known as one of the major producers of Pepper in the world accounting for about 50% of the African production [1]. Although, pepper is widely cultivated throughout Nigeria, yields obtained by peasant farmers are often very low [2]. This is mainly due to infestations by insect pests among which is the green peach aphid (*Myzus persicae*), an important pest of the Solanaceae family reported as one of the most economically important pests of Pepper in West Africa [3].

The use of chemical insecticides for the efficient control of *Myzus persicae* is often unaffordable to peasant farmers and their use is associated with many undesirable and sometimes lethal consequences. In addition, the evolution of insecticide resistance in natural populations of *M. persicae* [4] has made chemical control of this species particularly problematic [5]. To combat problem of pest resistance and resurgence, effective chemical control of sucking pests requires an increased number of applications and application doses. As such, botanical pesticides are important alternatives to minimize or replace the use of synthetic pesticides [6].

Extracts from various parts of Neem are among the most widely used biological pesticides and serve as a viable alternative for the management of many sucking insects. *Azadirachta indica* is often used in this regard. It is known to be active on more than 200 economically important species of insects and the fact that azadirachtin is selective toward phytophagous insects with minimal toxicity to beneficial insects, increases its potential value to pest management [7]. Various scientists have reported that neem products exhibit antifeedant, growth regulatory and repellency effects on insects. Lowery and Isman [8] demonstrated that nine species of aphids were susceptible to the insect growth regulating (IGR) activities of Neem seed oil and the mortality occurred mainly during failed attempts to molt. Similarly, studies by Egbo and Ilondu [9] also showed that Neem seed extract significantly reduced aphid population (*Aphis craccivora*) when compared to the unprotected plots (control). Although several commercial formulations containing azadirachtin are available in the world market for insect control, these refined products are expensive for peasant farmers in developing countries [10].

2. Materials and Methods

2.1. Preparation of Aqueous Neem Seed Extract

Aqueous Neem seed extract was prepared according to methods described by [11]: Three concentration levels (100%, 75% and 50%) of aqueous extracts of neem seed (ANSE) were prepared by soaking 1kg of neem seed

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powder in 5 liters of water and allowing it to stay for twenty four hours, after which the extract was repeatedly filtered through cheese cloth to remove coarser particles. 1ml of Tween 80 was added to the extract as an emulsifier to dissolve the active substances in the Neem powder and to enable stickiness to plant surface. The aqueous Neem seed extract was serially diluted with tap water to obtain the following concentrations: 50% (5:5 v: v), 75 % (7.5:2.5 v: v) and 100% (plant extract stock solution). Neemazal applied at a recommended rate of 3L/ha served as the positive biopesticidal control while a Cypermethrin derivative commercially known as Deltrin® was applied based on the manufacturers' recommendation (7.5ml/L). Tap water and the synthetic insecticide (Deltrin® 70% EC) were used as untreated control (UC) and standard control (SC) treatments respectively.

Table-1. List of Treatments used as pesticide

Treatments	Description
T1 - Neem Seed Extract (100%)	100% aqueous Neem seed extract without dilution
T2 - Neem Seed Extract (75%)	750mls of aqueous Neem seed extract diluted in 250mls of tap water
T3 - Neem Seed Extract (50%)	500mls of aqueous Neem seed extract diluted in 500mls of tap water
T4 - NeemAzal (0.7%)	Manufacturers' rate NeemAzal solution
T5 - Deltrin® (0.75%)	A control treated with the standard synthetic insecticide (Deltrin® 70 EC) at field rate
T6 -Tap Water (neutral control)	Untreated control (a control treated only with tap water)

2.2. Establishment of Aphid Colony

Nymphs of Lemon (LG) and dark green (DG) morphs of *M. persicae* used for raising the culture were collected from infected Cabbage and Pepper plantations around Bassawa, Zaria, Nigeria. The insects were collected alongside the plant parts where they were located. Identification of the collected insects, as *Myzus persicae* was done at the Insect museum laboratory, Department of crop protection, A.B.U, Zaria, Nigeria. This was professionally identified by viewing them under a USB Microscope and matching them with available museum specimen. Green peach aphids that were positively identified were used to infest several plants of susceptible pepper cultivars of sweet pepper (California wonder). Stock cultures were maintained on pepper plants in aphid-proof cages. Each morph of *Myzus persicae* was cultured separately to avoid mix up of the aphids. The wooden culture cages (1.5m wide x 1m long) were made from net of small mesh size and kept at 25^oc.

2.3. Experimental Design

Experiments were conducted in the Experimental fields located at the Department of Biological sciences, Ahmadu Bello University, Zaria Botanical garden. The departmental garden coordinates under experimental design with this latitude 11° 84.516" North, Longitude 7° 39. 175" East and on Altitude 2184 ft above sea level. The experimental field used was covered completely with a wire net of 0.2cm x 0.2cm mesh size and polythene bags to act as a barrier to predators and other insects.

Chili pepper were transplanted (after 35 days of nursery raising) in a randomized complete block design (RCBD) with three replicates, each replicate consisted of 6 treatment plots of 3 x 2.5 m including a control plot with five replication per plant. Plant to plant distance was kept at 50 cm. A buffer was maintained between each treatment to avoid spray drift of other insecticides.

2.4. Inoculation of Aphids

Inoculation of aphid was done in two week after transplant, at this stage transplanted pepper plants had fully recovered from transplanting shock and had continued normal growth. Insects were inoculated according to a method described by Ochieng and Nderitu [12]. Approximately 25-30apterous *M. persicae* were introduced on young fully expanded leaves of each plant using a pencil brush. Subsequent re-infestation was done to maintain aphid population.

2.5. Treatment Application and Data collection

After infestation, the aphids were allowed 24hours acclimatization period before treatment was applied. Four rounds of treatment application were done bi-weekly using a 2 liter capacity hand held sprayer. Each plant was sprayed from all sides with the treatment till point of runoff as described by Feng and Isman [13]. Insecticides at the recommended rates (Table 1) were sprayed in morning or late in the evening.

Data on insect population change were collected according to method described by Akbar, et al. [14]; Pre-treatment counts were made 24 hours before treatment application and post-treatment data were recorded after 24, 72, 120 and 168 hours after spraying. The insect population reduction percentage was computed using Henderson-Tilton's formula:

$$\text{Percent (\%) reduction in population} = 1 - \left(\frac{T_a}{C_a} * \frac{C_b}{T_b} \right) * 100 \% \quad [15].$$

2.6. Pepper Growth and Yield Parameter.

Morphological parameters of the chili pepper were taken from two weeks after transplanting until harvest. The following parameters were measured: shoot height (cm), Leaf area (cm²), Number of leaves, number of flowers, and fruit development from flowers.

The data obtained were subjected to Analysis of variance using SAS statistical tool pack. Significant differences among treatment means were tested with Duncan multiple range test (DMRT).

3. Results

3.1. Effect of Aqueous Neem seed extract on *Myzus* population

All concentrations of neem seed extract tested were significant ($P < 0.05$) and highly effective against both lemon and dark green morphs of *Myzus persicae*. The highest percent reduction in lemon green morphs after 24 hours was observed in deltrin® followed by 100%, 75% and 50% aqueous concentrations of neem seed extract. The reduction pattern with aqueous neem seed extract showed that the higher the treatment concentration the higher its efficacy and toxicity on test plants. It was further observed that the longer the duration of the exposure, the greater the reduction of *M. persicae* population. At 168 hours of exposure after treatment application, the neem seed extracts were most efficacious ($P < 0.05$) than other treatments (Table 2).

All concentrations of ANSE showed similar reduction in aphid population when used as insecticide against the dark green morphs of *M. persicae*. The reduction efficacy of the extract increased with increasing concentration of the extract (Table 3).

Table-2. Effect of insecticidal concentrations and duration of exposure on Percentage (%) reduction of Lemon green morphs of *Myzus persicae*

Time Interval (Hours)	Treatment Concentrations (%)				
	100 ANSE	75 ANSE	50 ANSE	0.7 NEEMAZAL	0.75 DELTRIN®
24	50.63 ^b	50.63 ^b	32.28 ^c	31.60 ^c	82.91 ^a
72	63.95 ^b	56.98 ^{ab}	62.79 ^b	24.42 ^a	81.40 ^a
120	78.30 ^a	78.30 ^a	68.96 ^b	61.24 ^c	58.92 ^d
168	91.86 ^a	81.90 ^b	77.83 ^b	73.76 ^c	79.19 ^b

Means followed by same letter across rows are not significantly different ($P < 0.05$), Duncan's multiple range test (DMRT). ANSE = Aqueous Neem Seed Extract

Table-3. Effect of insecticidal concentrations and duration of exposure on Percentage (%) reduction of Dark green morphs of *Myzus persicae*

Time Interval (HOURS)	Treatment Concentration (%)				
	100 ANSE	75 ANSE	50 ANSE	0.7 NEEMAZAL	0.75 DELTRIN®
24	61.69 ^b	56.52 ^c	44.29 ^d	28.80 ^e	77.45 ^a
72	62.61 ^c	67.23 ^b	61.74 ^c	46.22 ^d	82.02 ^a
120	84.82 ^a	79.23 ^b	64.86 ^c	62.30 ^c	81.79 ^a
168	85.94 ^a	79.91 ^b	73.66 ^c	73.66 ^c	76.79 ^{bc}

Means followed by same letter across rows are not significantly different ($P < 0.05$), Duncan's multiple range test (DMRT). ANSE = Aqueous Neem Seed Extract

3.2. Effect of Aqueous Neem Seed Extract on Plant Growth and Yield Parameters of Pepper

Highest mean plant height (24.80cm) was observed in plants treated with 50% ANSE followed by 100% concentration of ANSE (20.7cm) and then 75% ANSE (19.4cm), these shoot heights were significantly ($P < 0.05$) lower than that obtained in NeemAzal and Deltrin® (Table 4). For plants infested with lemon green and dark green morphs of *M. persicae*, highest shoot height was observed in plants treated with 50% ANSE (Table 5). Other morphological character measured appeared to decrease as the concentration of the extract increased.

Yield in chili pepper plants treated with 50% ANSE were significantly ($P < 0.05$) higher when compared to that obtained with NeemAzal, Deltrin® and the control (Table 4 and 5). Lowest yield was with 100% ANSE. However, yield obtained in plants treated with 75% concentration of the neem extract were not significantly different when compared to that obtained in Deltrin and NeemAzal.

Table-4. Effect of concentrations of ANSE in comparison with NeemAzal and Deltrin® on growth and yield of chili pepper infested with Dark Green (DG) morphs of *Myzus persicae*.

Treatment Concentration (%)	ShootHeight(cm)	Plant Growth and Yield parameters			Fruit from flower
		Leaf Area (cm ²)	Number of leaves	Number of flower	
100ANSE	20.7± 1.23 ^c	19.40 ± 0.70 ^c	28.60 ± 3.14 ^b	8.20±0.69 ^d	1.00 ± 0.08 ^d
75 ANSE	19.4± 2.40 ^c	24.80 ± 1.02 ^b	32.67±4.98 ^a	27.60±0.64 ^a	5.20 ± 0.00 ^b
50 ANSE	24.8± 1.04 ^b	32.40 ± 1.40 ^a	35.10 ± 3.88 ^a	14.40±0.79 ^c	6.80 ± 0.11 ^a
0.7 NeemAzal	32.4± 1.33 ^a	27.80 ± 0.81 ^b	37.80 ± 4.43 ^a	26.40±1.19 ^a	5.40 ± 0.16 ^b
Deltrin®	27.8± 1.79 ^{ab}	21.16± 1.42 ^{bc}	28.10 ± 1.15 ^b	17.80±0.33 ^b	7.60 ± 0.07 ^a
Water	21.16± 1.49 ^c	19.40 ± 0.94 ^c	21.00 ± 3.47 ^c	14.40±0.84 ^c	0.40± 0.11 ^d

Means followed by same letter within columns are not significantly different ($P < 0.05$), Duncan's multiple range test (DMRT). ANSE = Aqueous Neem Seed Extract

Table-5. Effect of concentrations of ANSE in comparison with NeemAzal and Deltrin® on growth and yield of chili pepper infested with Lemon Green (LG) morphs of *Myzus persicae*.

Treatment Concentrations (%)	Plant Height(cm)	Plant Growth and Yield parameters			Fruit from flower
		Leaf Area (cm ²)	Number of leaves	Number of flowers	
100 ANSE	13.10± 0.95 ^b	6.29 ± 0.80 ^c	10.60 ± 1.52 ^d	4.20± 0.76 ^c	1.60±0.00 ^c
75 ANSE	14.28 ± 0.76 ^b	8.64 ± 0.97 ^b	16.40 ± 1.40 ^c	5.40±1.36 ^c	2.00 ± 0.29 ^{bc}
50 ANSE	17.02 ± 0.67 ^a	11.14 ± 1.40 ^a	17.15 ± 1.12 ^b	16.20±0.98 ^a	7.60±0.31 ^a
0.7 NeemAzal	16.70 ± 0.59 ^a	11.33 ± 0.81 ^a	18.15 ± 2.38 ^b	8.80±1.88 ^b	3.80 ± 0.39 ^b
Deltrin®	18.30 ± 0.93 ^a	13.26 ± 1.42 ^a	23.25 ± 4.69 ^a	7.20±1.70 ^{bc}	3.20 ± 0.53 ^b
Water	12.50 ± 0.62 ^c	6.90 ± 0.94 ^c	13.40 ± 3.47 ^d	2.00±1.35 ^d	0.60 ± 0.32 ^d

Means followed by same letter within columns are not significantly different ($P < 0.05$), Duncan's multiple range test (DMRT). ANSE = Aqueous Neem Seed Extract

4. Discussion

The concentrations of ANSE tested proved as effective as NeemAzal and the synthetic pesticide in the control of both Lemon and Dark green morphs of *Myzus persicae*. The highest percent reduction was obtained when the extract was used without dilution indicating that the extract acted in a concentration dependent manner. It can be assumed that the high mortality of aphids was due to the various active components contained in the extracts, the dosage and processing time of the aphids. Lowery, *et al.* [16] reported that crude formulations of neem seed extracts contain limonoids that contribute to its insecticidal properties. This observation was contrary to the findings of Kelany, *et al.* [17] who reported that NeemAzal formulation was more effective than neem kernel powder and aqueous neem kernel powder extract when used as surface protectants against potato tuber moth, the contrary observation in their study might have been due to a lower concentration of stock solution of the aqueous extract.

The lower percentage reduction of aphids observed in NeemAzal- T/S at 24 hours after application when compared to 100% and 75% concentration of ANSE, could be attributed to the differences in the concentration of active ingredient in the water extract, as such, products containing azadirachtin in combination with other ingredient (e.g. Nimbin, Salanin) such as the crude neem extract exhibit better aphicidal activities than either ingredients alone [18]. Our finding was in line with the observation of Boursier, *et al.* [19] who reported that the presence of other terpenoids in crude neem seed extract could act as insecticides and or improve the effectiveness of azadirachtin through a synergistic effect of co-extracted compounds. The slower effect of the neem products represented by the lower percentage reductions observed at 24 hours after spray as compared to Deltrin might probably have resulted from its growth regulator effect, mainly due to the abundant presence of azadirachtin, which blocks the synthesis and release of molting hormones [20].

All morphological character measured appeared to decrease as the concentration of the extract increased. During this study it was observed that when 100% concentration of ANSE was used as aphicide, visible burns were observed on the leaves of the plants resulting in premature wilting of the leaves. Our observation was in line with the reports of Abbasi, *et al.* [21] who also reported phytotoxic effect of neem oil in green house grown pepper plants. Similar observations were made by Appiagyeyi [22] who reported that some tomato plant showed symptoms of burning when neem aqueous kernel extract was applied as foliar spray. Excellent growth and yield performance observed with 50% ANSE concentration could be attributed with low toxicity of ANSE constituents at those concentrations. Rouf and Sardar [23] reported a 68.49% increase in pod yield of beans plants when treated with neem seed extract applied 150g/l at 7 days intervals. Similar results obtained by Alabi and Olorunju [24], showed that groundnut plants sprayed with neem seed extract gave higher yields than the plants that received other treatments (black soap and cow dung), apart from the un-treated plants.

5. Conclusion

The results of the present research suggest that aqueous neem seed extract at a 50% concentration (200g/l) could be used to reduce population of Lemon and dark green morphs of *Myzus persicae* without phytotoxic effects on chili pepper plants. From this study the significant reduction in *Myzus persicae* numbers on pepper plants treated with aqueous neem seed extract is an indication that they can be used as alternatives to chemical insecticides and can play a more prominent role in integrated pest management programs in the future.

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