# APHICIDAL EFFICACY OF AQUEOUS NEEM SEED EXTRACT AGAINST APHIDS (*Myzus persicae* SULZER) INFESTATION ON SWEET PEPPER (*Capsicum annum* L.)



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**Abstract:** Field trials were conducted to comparatively evaluate the aphicidal efficiency of aqueous neem seed extract against *Myzus persicae* infestation on two varieties of sweet pepper (California wonder and Yolo wonder). Three concentrations of aqueous neem seed extracts (200 g/L, 150 g/L and 100 g/L), Deltrin® and neem Azal were evaluated. Aqueous neem seed extract significantly ($p<0.05$) exhibited good level of aphicidal activity towards eliminating *Myzus persicae* population in comparison to Deltrin® and neem Azal. The efficacy of the extract in reducing *Myzus persicae* infestation increased with increasing concentration and duration of exposure to the extract. The 200 g/L concentration of aqueous neem seed extract was most active in reducing the population of *Myzus persicae* on both varieties of sweet pepper at exposure duration of 168 h. However, at this concentration (200 g/L) phytotoxicity of the extract was observed on the plants. The 100 g/L and150 g/L aqueous neem seed extract improved the vegetative and reproductive traits of sweet pepper with no phytotoxic effects. Thus suggestion that at these concentrations, aqueous neem seed extract is active, safe and could be adopted by local farmers as an efficient and cost effective biopesticide in the elimination of *Myzus persicae* infestation on sweet pepper.

**Keywords:** *Myzus persicae*, sweet pepper, *Azadiracta indica*, neem, biopesticide

## Introduction

Agricultural activities have been faced with the destructive activities of numerous pests, insects, fungi, and weeds which lead to radical decrease in yields. This crisis was reduced to a great extent with the advent of chemical pesticides. Over dependence and uninhibited use of chemical protection has necessitated for alternatives due to environmental concerns (Phoofolo *et al.*, 2013). Efforts have been devoted to the discovery and development of plant extracts and phytochemicals as alternatives to synthetic insecticides for pest management. The use of more biodegradable pest control materials with greater selectivity might help to avoid the disadvantage caused by the use of synthetic pesticides (Michereff *et al.*, 2011). Phoofolo *et al.* (2013) stated that plants have the richest source of renewable natural pesticides and their extracts provide a safe and viable alternative to synthetic pesticides and they are compatible with the use of beneficial organisms, pest-resistant plants, and to preserving a healthy environment in an effort to decrease reliance on synthetic pesticides. Among the wider range of plants, Neem derivatives have shown great potential in controlling insect pests.

Neem, (*Azadirachta indica* A. Juss) (Family: Meliaceae) is native to the arid regions of the Indian sub-continent, from where it spread out to many Asian and African countries as well as Australia and South America (Mahmoud and Maha, 2008). Various Neem products have been studied extensively for their phytochemistry and exploitation in pest control programs and a number of bioactive components have been isolated from various parts of the Neem tree. Azadirachtin is currently considered as Neem's main agent for controlling insects (Dubhashi *et al.,* 2013). Himesh *et al.* (2011) described azadirachtins as a complex tetranortriterpenoid of the limonoid class which are a family of natural phago-repellents and antifeedants found in the Neem tree. Azadirachtin Structurally resembles insect hormones called "ecdysones," which control the process of metamorphosis as the insects pass from larva through pupa to adult. They exert a strong negative influence on behavior, postembryonic development and fecundity of insects resulting in significant reduction of general fitness (Hummel *et al.,* 2012). When insects ingest this compound, their growth and development are inhibited due to the blocking of the biosynthesis of insect hormones, such as ecdysteroids. In addition to blockage of hormone synthesis, the development of reproductive organs such as the ovary and testis, is significantly inhibited, and the fertility and fecundity of the adults are also reduced (Ohn Mar Lynn *et al.,* 2010). More than 450 species of insects have been tested with Neem products in the world and 413 of these are susceptible to neem used at different concentrations (Mesfin, 2007).

Pepper production in Nigeria has been faced with many biotic and environmental constraints, prominent among such constraints are pests and diseases which reduce yields, quality of marketable fruits and these subsequently results in considerable economic losses and wastage. In the tropics, particularly in Nigeria, many insect pests are associated directly with vegetable damage and yield losses while some others are most important as vectors of diseases (Umeh *et al.*, 2002). Dagnoko *et al.* (2013) have implicated Aphids (*Myzus persicae* Sulzer) as an economically important pest to peppers in West Africa.

*Myzus persicae* (Green Peach Aphid) is a polyphagous and economically important pest with a worldwide distribution (Iguchi *et al*., 2012). It is one of the most significant pests on agricultural crops worldwide (Blackmanand Eastop, 2000). The green peach aphid has been a particularly challenging taxon for over 200 years (Joan, 2004). Prolonged aphid infestation can cause appreciable reduction in plant yield because they consume plant nutrients and their sucking behavior can cause chlorosis, distortion of the leaves and abscission of blooms, plant stunting and wilting. Some species inject toxic salivary secretions into plants during feeding. If left unchecked aphids can stunt plant growth, deform and discolor leaves and fruits or cause gall formation on leaves, stems and roots.In case of heavy attack plants wither resulting in drastic loss in seed yield and oil contents (Akbar *et al.,* 2012). *Myzus persicae* is resistant to most insecticide classes, making chemical control of this species particularly problematic (Radcliffe and Ragsdale, 2002). To combat problem of pest resistance and resurgence, effective chemical control of sucking pests requires an increased number of applications and application doses. Additionally, chemical control of aphid has become less effective due to the evolution of insecticide resistance in natural populations (Michereff*et al*., 2011). Besides being unaffordable to peasant farmers, the use of chemical insecticides in vegetable production presents potential poisonous concerns on human health through dietary exposure (Phoofolo *et al*., 2013). As such botanical pesticides are important alternatives to minimize or replace the use of synthetic pesticides (Anand *et al.,* 2008). Extracts from various part of Neem are among the most useful botanical pesticide and serve as a viable alternative for the management of many sucking insects.

*Azadirachta indica* is known to be active on more than 200 economically important species of insects. The selectivity of azadirachtin toward phytophagous insects with minimal toxicity to beneficial insects increased itspotential value in pest management (Weathersbee and McKenzie, 2005). 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 (Dela *et al.,* 2014). Therefore, this study aim to evaluate the efficacy of aqueous neem seed extracts on *Myzus persicae* population on two varieties of sweet pepper commonly grown in Nigeria.

## Materials and Methods

### *Preparation of aqueous neem seed extract*

Aqueous Neem seed extract was prepared according to methods described by Shafie (2001). Three concentration levels (200 g/L, 150 g/L and 100 g/L) of aqueous neem seed extract (ANSE) were prepared by soaking 1 kg of neem seed powder in 5 liters of water and allowed to stay for twenty four hours, after which the extract was repeatedly filtered through cheese cloth to remove coarser particles. 1 mL 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 100% (200 g/L) aqueous Neem seed extract served as the stock solution. Neem Azal (biopesticidal check), Deltrin® 70% EC (synthetic insecticide check) applied based on manufacturers recommendation and tap water (neutral check) served as control for the evaluations.

### *Establishment of aphid colony*

The colony of green peach aphid (*Myzus persicae*) was established from its apterous forms originally collected from infected host plants (pepper and cabbage) around Bassawa, Zaria, Nigeria. Insect identification was verified at the Insect museum laboratory, Department of crop protection, Ahmadu Bello University, Zaria, Nigeria. Aphids population were maintained on young plants of the test host (sweet pepper) grown in soil-filled plastic pots (25 x 25 cm) under greenhouse conditions at 25 ± 3°C. Plants infested with aphids were kept in cages in a wooden frame of 60 × 60 × 100 cm lined with wire mesh (0.2 cm) on all sides. A continuous supply of new plants was provided as needed for the colony replenishment and multiplication.

### *Experimental design*

A Randomized Complete block Design (RCBD) was adopted for treatment layout. Each experimental site consisted of 18 plots with a plot size of 3 x 2.5 m. Inter and intra plot distance of 50 cm and 25 cm were maintained. Hence, six (6) treatments ($T_1$ - Neem Seed Extract (100%), $T_2$ - Neem Seed Extract (75%), $T_3$ - Neem Seed Extract (50%), $T_4$ - neemAzal (0.7%), $T_5$ - Deltrin® (0.75%), $T_6$ -Distilled Water (neutral control) were replicated thrice and each replicate consisted of two (2) pepper varieties with 10 replicate per plant. Experimental field was covered completely with a wire net of 0.2 cm x 0.2 cm mesh size.

### *Inoculation of aphidsand treatment application*

Aphids were inoculated on the pepper varieties two weeks after transplanting following the method described by Ochieng *et al*. (2011) where approximately 25-30 apterous *M. persicae* were introduced on young fully expanded leaves of each plantwith a pencil brush. Subsequent re-infestation was done to maintain aphid population. Inoculated aphids were allowed 24 h acclimatization period before treatment application. Young sweet pepper plants were sprayed until runoff with treatments with a 2 liter capacity hand held sprayer. Four rounds of treatment application were done bi-weekly using. Control plants were sprayed with tap water.

### *Aphid determination*

Data on insect population were determined according to method described by Akbar *et al.* (2010) where 10 plants were randomly selected per treatment, pre-treatment counts was made 24 h before treatment application and post-treatment data was recorded after 24, 72, 120 and 168 h after spray. Reduction of aphid population was computed in percentage with the formula of Henderson-Tilton's: % Efficacy=[1- Ta/Ca*Cb/Tb]* 100 (Henderson and Tilton, 1955).

### *Pepper growth and yield parameter*

Plant morphological growth parameters were evaluated from two weeks after transplanting till harvest. The following parameters were measured: shoot height (cm), Leaf area ($cm^2$), Number of leaves, number of branches, number of flowers, and fruit development from flowers. Data obtained were subjected to Analysis of variance using SAS statistical tool pack. Duncan's multiple range test (DMRT) was used to separate the meanswhere significant.

## Results and Discussion

### *Effect ofAqueous treatments on the mortality of Myzus persicae*

Progressive and significant ($p<0.05$) decline in the population of *Myzus persicae* was observed with increasing exposure to biopesticides (Table 1 – 2). At 24 h exposure period, Deltrin was most effective in reducing *Myzue persicae* infestation. Aqueous Neem seed extracts (ANSE) had varying effects on *Myzus persicae* population with increasing duration of exposure. At 24 h after spray with 200 g/L ANSE, the control of *M. persicae* was highest in comparison to other ANSE extract. The effect of ANSE at this concentration was comparable to Deltrin. At 72 and 120 h exposure time the efficacy of ANSE, and neemAzal appreciated and hence higher mortality of *M. Persicae* was observed. The increasing efficacy was not duplicated by Deltrin s the efficacy of the chemical insecticide declined (Table 1 – 2). This decline was also observed at 160 h exposure to Deltrin. The efficacy of ANSE and neem Azalon the pepper varieties was highest at 160 h of exposure of *M. persicae*. Tap water was not effective in reducing *M. persicae* infestation on Carlifonia wonder and Yolo wonder. With increasing duration of exposure of the varieties to tap water, the population of *M. persicae* rose and new instars developed (Table 1 – 2). The mean infestation of *M. persicae* per plant suggests that pepper variety might play an important role in the establishment of m. persicae and hence the preference observed at all durations of exposure (Fig. 1).

**Table 1: Effect of aqueous neem seed extracts, neem Azaland Deltrin® on percent reduction of *M. persicae* infestation on California wonder variety of sweet pepper**

| Duration of Exposure (Hrs) | 200 g/L ANSE | 150 g/L ANSE | 100 g/L ANSE | neemAzal (0.7) | Deltrin® | TAP Water |
|---|---|---|---|---|---|---|
| 24 | 89.55$^{b}$ | 61.19$^{c}$ | 58.21$^{d}$ | 88.06$^{b}$ | 99.13$^{a}$ | 44.51$^{a}$ |
| 72 | 91.89$^{b}$ | 82.43$^{c}$ | 71.62$^{c}$ | 91.23$^{b}$ | 97.53$^{a}$ | 42.08$^{a}$ |
| 120 | 98.26$^{a}$ | 94.78$^{b}$ | 93.04$^{b}$ | 93.21$^{b}$ | 97.53$^{a}$ | 33.62$^{b}$ |
| 168 | 100.00$^{a}$ | 97.65$^{a}$ | 97.53$^{a}$ | 98.26$^{a}$ | 93.52$^{b}$ | 26.54$^{c}$ |

Values are means SEM, a, b, c = values with different superscripts differ significantly ($P < 0.05$); ANSE = Aqueous Neem Seed Extract

**Table 2: Effect of aqueous neem seed extracts, neem Azal and Deltrin® on percent reduction of *M. persicae* infestation on Yolo wonder variety of sweet pepper**

| Duration of Exposure (Hrs) | 200 g/L | 150 g/L | 100 g/L ANSE | neemAzal (0.7) | Deltrin® | Tap Water |
|---|---|---|---|---|---|---|
| 24 | 80.52$^{b}$ | 78.26$^{b}$ | 64.94$^{c}$ | 54.55$^{c}$ | 100.00$^{a}$ | 45.21$^{a}$ |
| 72 | 88.44$^{b}$ | 85.51$^{b}$ | 79.22$^{c}$ | 75.36$^{b}$ | 100.00$^{a}$ | 44.25$^{a}$ |
| 120 | 97.10$^{a}$ | 90.26$^{b}$ | 84.52$^{b}$ | 86.30$^{a}$ | 98.64$^{a}$ | 32.45$^{b}$ |
| 168 | 99.32$^{a}$ | 98.64$^{a}$ | 97.26$^{a}$ | 88.44$^{a}$ | 94.20$^{b}$ | 27.32$^{c}$ |

Values are means SEM, a, b, c = values with different superscripts differ significantly ($P < 0.05$); ANSE = Aqueous Neem Seed Extract

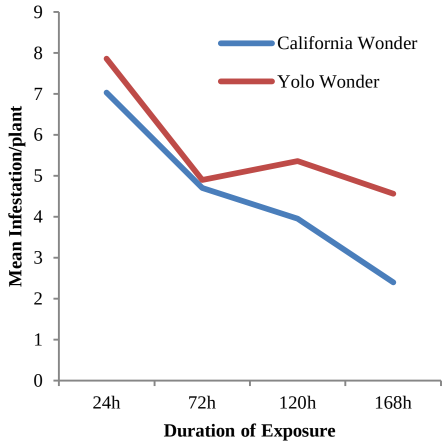


**Fig. 1:** Mean infestation of*Myzus persicae* on Carlifonia wonder and Yolo wonder.

***Effects of ANSE, neem Azal, Deltrin® and tap water on the growth and yield parameters of California and Yolo wonder varieties of sweet pepper***

The spray of the pepper varieties with tap water showed no significant effect in reducing *Myzus persicae* infestation and hence, significantly ($p<0.05$) resulted in poor growth and yield of Carlifornia and Yolo wonder. The spray of ANSE on California wonder did not significantly ($P<0.05$) increase their plant heights compared to spraying with Deltrin. These treatment effects does not hold true on Yolo wonder where plant height was significantly higher upon spray of Deltrin (Table 3). ANSE treated plants showed better development of leaf area. The number of leaves produced was best with the applications of 100 g/L ANSE and neem Azal for both pepper varieties.

Furthermore, the number of branches produced upon treatment with the bio pesticides was comparable with Deltrin. Califonia wonder produced significant ($p<0.05$) higher numbers of flower with the applications of 150 g/L, 100 g/L ANSE and neem Azal. The significant ($P<0.05$) increase in number of flowers produced by Yolo wonder was due to the applications of 150 g/L and 100 g/L ANSE. The number of fruits harvested per plant was highest with 100 g/L and neem Azal application on California wonder. Fruits harvested per plant on Yolo wonder, was highest with the applications of 150 g/L ANSE, 100 g/L ANSE and neem Azal (Table 3). Hence, the use of aqueous neem seed extract decreased the adverse effect of aphids by significant improvement of plant vegetative and reproductive characters especially leaf area, number of flowers and marketable fruits from flower in sweet pepper plants. The effect of 200 g/L ANSE on the measured sweet pepper morphometric characters did not significantly improve growth and yield of the plants compared to Deltrin®.

**Table 3: Effects of ANSE, neem Azal, Deltrin® and tap water on the growth and yield parameters of California and Yolo wonder varieties of sweet pepper**

| Variety | Treatment | Plant Height (cm) | Leaf Area (cm) | Number of Leaves | Number of Branches | Number of Flowers | Fruit per plant |
|---|---|---|---|---|---|---|---|
| California Wonder | 200 g/L ANSE | 20.9 ± 1.63$^{b}$ | 16.85 ± 0.02$^{ab}$ | 18.80 ± 1.30$^{c}$ | 3.00 ±0.41$^{b}$ | 7.40 ± 0.53$^{b}$ | 4.00 ±0.12$^{c}$ |
| | 150 g/L ANSE | 23.6 ±0.75$^{a}$ | 18.64 ±0.97$^{a}$ | 23.50 ±1.35$^{b}$ | 3.80 ±0.44$^{b}$ | 10.00 ± 0.18$^{a}$ | 6.60 ± 0.12$^{b}$ |
| | 100 g/L ANSE | 25.8 ± 1.01$^{a}$ | 18.50 ± 1.80$^{a}$ | 26.75 ± 1.22$^{a}$ | 4.20 ± 0.57$^{a}$ | 12.40 ± 0.05$^{a}$ | 7.60 ± 0.07$^{a}$ |
| | neemAzal | 23.6 ± 1.30$^{a}$ | 15.32 ± 0.85$^{b}$ | 29.60 ± 0.63$^{a}$ | 4.40 ± 0.10$^{a}$ | 10.60 ± 0.34$^{a}$ | 9.00 ± 0.05$^{a}$ |
| | Deltrin® | 24.8 ± 1.47$^{a}$ | 14.95 ± 1.63$^{b}$ | 22.00 ± 0.94$^{b}$ | 4.60 ± 0.27$^{a}$ | 9.40 ± 0.49$^{b}$ | 3.40 ± 0.05$^{c}$ |
| | Tap Water | 15.8 ± 1.76$^{c}$ | 12.42 ± 0.62$^{c}$ | 11.20 ± 1.16$^{d}$ | 2.60 ± 0.39$^{c}$ | 3.60 ± 0.12$^{c}$ | 1.20 ± 0.12$^{d}$ |
| Yolo Wonder | 200 g/LANSE | 20.20 ± 1.05$^{b}$ | 13.51 ± 1.58$^{b}$ | 20.00 ± 1.31$^{b}$ | 3.40 ± 0.37$^{b}$ | 8.00 ± 0.38$^{c}$ | 4.80 ± 0.12$^{c}$ |
| | 150 g/L ANSE | 21.43 ± 0.71$^{b}$ | 14.68 ± 0.89$^{a}$ | 26.80 ± 1.35$^{a}$ | 5.20 ± 0.29$^{a}$ | 16.40 ± 0.10$^{a}$ | 8.20±0.10$^{a}$ |
| | 100 g/L ANSE | 21.60 ± 1.26$^{b}$ | 17.52 ± 1.08$^{a}$ | 26.10 ± 1.31$^{a}$ | 7.40 ± 0.29$^{a}$ | 19.40 ± 0.18$^{a}$ | 10.60 ± 0.10$^{a}$ |
| | neemAzal | 22.80 ±1.30$^{b}$ | 15.00 ± 2.45$^{a}$ | 17.00 ± 0.88$^{b}$ | 6.60 ± 0.30$^{a}$ | 13.40 ± 0.00$^{b}$ | 8.80 ± 0.05$^{a}$ |
| | Deltrin® | 26.80 ± 1.85$^{a}$ | 10.53 ±1.03$^{bc}$ | 17.80 ± 1.80$^{b}$ | 4.00 ± 0.49$^{b}$ | 15.00 ± 0.21$^{b}$ | 7.00 ± 0.07$^{b}$ |
| | Tap Water | 16.00 ± 1.36$^{c}$ | 9.95 ± 1.21$^{c}$ | 13.60 ± 0.97$^{c}$ | 2.80 ± 0.31$^{c}$ | 5.00 ± 0.31$^{d}$ | 2.40 ± 0.07$^{d}$ |

Values are means SEM, a, b, c = values with different superscripts differ significantly ($P < 0.05$); ANSE = Aqueous Neem Seed Extract

Products containing azadirachtin have been implicated to possess better aphicidal activities (Stark and Walter, 1995). The aphicidal ability of ANSE to reducethe population of *Myzus persicae* and improve the growth and yield of both varieties of sweet pepper was due to the active compounds contained in the extract, doses and duration of exposure of the aphids. Lowery and Isman (1994) reported that crude formulations of neem seed extracts contains limonoids that contribute to it insecticidal properties. Boursier *et al.* (2011) reported thatthe presence of other terpenoids in crude neem seed extractcould act as insecticides. It was evident that neem based treatments affected the aphids via direct toxicity and later acted as feeding repellant which subsequently led to the death of aphids and an increase in percentage mortality observed over time. This observation was in line with the study of Metspalu *et al*. (2000) who reported that aphids are very susceptible to the contact action of neemAzal T/S due to their thin cuticle and that the mode of action was via repellant, deterrent mode and toxicity. Lowery *et al.* (1993) proved the effectiveness of neem seed oil against aphids and found up to 50% mortality with 0.4% neem oil in the field conditions.

Additionally, Dimetry *et al.* (1996) reported that the percentage reduction in population of whitefly larvae reached maximum level one hour after treatment with different formulation of neem seed extract. The differential effect of neem Azal- T/S compared to 200 g/Land 150 g/L ANSE against *Myzus persicae* may be attributed to the variance in the concentration of active ingredient in the extracts. The decline in the efficacy of Deltrin® as the duration of exposure increased could be attributed to increasing tolerance of *M. persicae* to the pesticideor the failure of the pesticide to act as a reproduction deterrent. An increase in the number of aphids on plant treated with Deltrin® at 168 h after spray can be attributed to the development of resistance amongst the aphids and or the failure of the insecticide to act as a reproduction deterrent. Foster *et al.* (2003) reported that some clones of *Myzus persicae* (926B) used in their study were still alive and reproducing 7 days after treatment with the full recommended dose of imidacloprid (2 mg per plant) this demonstrates apotential for aphids with this level of tolerance to withstand field applications. Kerns *et al.* (1998) reported that red and green morphs of green peach aphids show only slight differences in their susceptibility to foliar and systemic insecticides; they also reported that Red-colored aphids were consistently more resistant to dimethoate and lambda-cyhalothrin.

The phytotoxic effect observed in the current study might have been due to the high concentration of the neem seed extract. Although treatment of plants with 200 g/L ANSE resulted in higher percentage reduction of *M. persicae* population it resulted in visible burns of pepper leaves resulting in premature wilting, this was accompanied by defoliation of the affected leaves. This must have caused the low mean number of leaves observed. This observation was in line with the findings of Olaifa and Adenuga (1988) who reportedthat neem products caused yellowing and subsequentshedding of leaves of cassava plants. Appiagyei (2010) also observed that when neem aqueous kernel extract was applied as foliar spray, the leaves of some tomato plant showed symptoms of burning and this caused some of the leaves to wilt prematurely four days after application. Abassi *et al.* (2003) also reported phytotoxic effect of neem oil in green house grown pepper plants.

The growth decline in plant morphological traits with 200 g/L ANSE application could be attributed to the toxicity of the concentration. Phytotoxic effect of neem oil in green house grown pepper plants have been reported by Abassi *et al.* (2003). This must have accounted for the lesion, visible burns, premature wilting of leaves and subsequently poor yield of sweet pepper plants at 200 g/L ANSE application. Similar observations were reported by Appiagyei (2010) that some tomato plant showed symptoms of burning when neem aqueous kernel extract was applied as foliar spray. The differences in growth and yield of the sweet pepper variety may be attributed to the degree of *Myzus persicae* infestation and might be unrelated to the growth habit of the plants.

The differential preferences of the varieties by *Myzus persica* was probably as a result of the differences in the growth habit of the varieties, their branching patterns and leaves number which serves as a canopy and hide out for the aphids against pesticides and predators. Similar reports were presented by Fajinmi *et al.* (2011) who reported that sparse vegetation cover as well as overcrowding has been reported to have impact on aphid population. Hummel *et al.* (2012) reported that pepper cultivars have pronounced effect on the number of *M. persicae*; they also reported in their study that yellow California and red California variety of sweet pepper had the lowest number of aphids. Results herein thus suggests that 100 g/Land 150 g/L ANSE are efficient in reducingthe population of *Myzus persicae* on sweet pepper without phytotoxic effects and could serve as a better and cost effective alternative to Deltrin.

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