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# Toxicity of Botanic and Synthetic Pesticide Residues to Citrus Psyllid *Diaphorina citri* Kuwayama and *Chrysoperla carnea* (Stephens)

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**Abstract.-** Bioassays were conducted to investigate the toxicity and residual activity of botanic and synthetic pesticides against adult female citrus psyllids, *Diaphorina citri* Kuwayama and second instar larvae of *Chrysoperla carnea* (Stephens). A dose of 1% neem seed oil inflicted 80% mortality on *D. citri* and 100% to *C. carnea* within 48 hours. Neem seed extract at a high dose of 3% resulted in 68.0 and 80% mortality to *D. citri* and *C. carnea*, respectively. Addition of 5% sugar to neem oil or neem water extract as feeding stimulant improved toxicity of the product to about 30 and 50%, respectively. Bioassays with synthetic pesticide such as methomyl and imadicloprid inflicted 97-100% mortality to *D. citri* on day 4, 70% and 34% on day 9 post application. Decline in mortality was gradual in case of methomyl but very quick in imidacloprid and lambda cyhalothrin from 100% on day 1 to 60% and 35% on day 9, respectively. Neem oil and its extract also degraded very quickly and showed decline in mortality from 80 to 20% within 5 days post application period.

**Key Words** Citrus psyllid, *D. citri*, *C. carnea*, pesticide residues, neem, bio-control.

## INTRODUCTION

Citrus cultivars such as sweet oranges, mandarin, grape fruit, lemon and lime are grown commercially in countries with tropical or sub tropical climate. In Pakistan, citrus is grown on an average area of 199.4 thousand hectares with production of 2.3 million tones annually (Anonymous, 2008). Pakistan exports citrus fruit to the Far and Middle Eastern regions. Kinnow stands first in export and is worth Rs. 5 billion annually. The Asian citrus psyllid, (*Diaphorina citri* Kuwayama Homoptera: Psyllidae) is the most destructive pest of citrus in Pakistan and harbors the bacterium *Candidatus Liberibacter* asiaticus (*Ca.* L. a.) responsible for citrus greening disease (Halbert and Manjunath, 2004). Both adults and nymphs of the psyllids feed on young foliage. High populations on young flush can kill the growing tip, while moderate populations can distort shoots and leaves leading to the death of plant within 4-5 years.

Traditionally synthetic chemicals have been used for the control of citrus pests. For example Confidor (Imidacloprid), Evisect (thioclam) and mineral oil Oleosect (Boulahia *et al*., 1996) and mixture of abamectin and mineral oil (Rezk *et al*., 1996) have been shown to reduce pest population in comparison to untreated control. Due to changing scenario and WTO constraints, interest in the use of bio-pesticides with selectivity towards phytophagous insects has increased particularly in cropping systems that rely on natural enemies within IPM system (Heinrichs, 1998; Rausell *et al*., 2000).

The insecticidal properties of neem seed extract and its oil has been reported by Kabeh and Jalingo (2007). The principal active ingredient 'azadirachtin' is concentrated in neem seed but leaves and other parts of the tree also contain significant amounts of this alkaloid. Throughout Asia, azadirachtin has been used successfully used against a variety of rice pests, including brown planthopper, *Nilaparvata lugens* (Stal) (Rao and Ravo, 1979; Saxena and Khan, 1985), white-backed planthopper, *Sogatella furcifera* (Horváth) (Skula *et al*., 1991), green leafhopper, *Nephotettix virescens* (Distant) (Mariappan and Saxena, 1983) and rice water weevil, *Lissorhoptrus oryzophilus* Kuschel (Mochizuki, 1993). Azadirachtin also inhibits the development of citrus root weevil larvae *Diaprepes abbreviatus* (L.), disrupt reproduction in the adults, and thus protect seedlings roots from feeding damage of the pest (Weathersbee III and Tang, 2002). Azadirachtin has also been used for the control of brown citrus aphid, *Toxoptera citricida* (Kirkaldy), which harbors tristeza virus and reported

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safe for its native parasitoid, *Lysiphlebus testaceipes* (Cresson) (Tang *et al.*, 2002). Azadirachtin inhibits development of psyllid nymphs at low dose of 10 ppm concentrations but show little or no effect on adults even at a concentration range of 11-180 ppm (Weathersbee III and McKenzie, 2005).

The present studies were conducted to see the toxic and residual effect of neem oil and its water extract and other synthetic pesticides on *D. citri* adults and its general predator, the common green lace wing *Chrysoperla carnea.* Addition of sugar as feeding stimulant to neem alkaloids was also investigated. Neem water extract was prepared through local methods due to its wide application and adoptability by farmers.

## MATERIALS AND METHODS

*Preparation of neem seed extract*

Crude extract of neem in water was prepared by crushing dry neem seeds in a blender. One kilogram of the grinded seed was tied in a cotton cloth in the form of a bag and dipped in five liter of hot water at initial temperature of 80C° for 16 hours. The cloth was then squeezed into the container. The freshly prepared suspension thus obtained was taken as 20% stock solution, which was diluted to lower concentrations of 2, 3 and 5% with tap water. Neem oil from the neem seed was extracted with an oil expeller. Neem seeds were sun dried and then feed into the oil extracting machine for mechanical pressing. The neem oil thus obtained was collected in a small container. Further filtration of the oil was done by passing the oil through a muslin cloth to remove the various unwanted particles left in the extracted oil**.** Different concentrations of the crude oil were prepared to lower concentrations with water and 5 gram detergent (surf) was used as surfactant.

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Base line data on efficacy of neem oil and its crude water extract was done through laboratory bioassays at various concentrations. Neem oil was used at 0.25, 0.5, 0.5+ 5% sugar, 0.75 and 1.0% concentration. Its water extract was used at 1.0, 1.5, 1.5 + 5% sugar, 2.0 and 3.0%. Fully expanded leaves from field trees were collected and dipped in the pesticide solutions for 10 seconds. Control leaves were dipped in simple water. After treatment, leaves were allowed to dry at room temperature. Five leaves per treatment were placed individually in Munger cells (Munger, 1942) that exposed 3.2 cm diameter section of the leaf surface. Opposite edge of the cells contained screened holes (5mm diameter) for ventilation. *D. citri* females (12-15) were aspirated from untreated 15 years old sour oranges, *Citrus aurantium* trees into clear plastic straws that were plugged with a cotton swab at one end and cork at the other end. For possible aeration to the collected psyllids, sides of the straws were pin holed. All straws were brought to the laboratory in an ice-chest with lid open. Psyllids in each straw were released over the treated leaf in the Munger cells by gently taping on the close end of the straw. Data on the number of dead and surviving individuals was recorded after 48 hrs exposure. Mortality was corrected using (Abbott, 1925).

Bioassays on *C. carnea* were conducted on second instar *C. carnea* larvae (one larva cell$^{-1}$ to prevent cannibalism) released on pretreated citrus leaves in Munger cells. Eggs of *Sitotroga cerealella* were used as food for larvae confined in the experimental arena. Each concentration was replicated five times. Mortality was recorded after 48 hrs. exposure period.

*Residual effect of field weathered synthetic and bio pesticide on* D. citri *and* C. carea

The residues of three synthetic pesticides; imidacloprid (Imicon 2.5 WP), lambda-cyhalothrin 2.5 EC, and methomyl 250 WP, and a botanical pesticide; neem oil and neem seed water extract were accessed on *D. citri* and *C. carne.* Four branches with fully expanded fresh leaves on the south east quadrant of 15 years old sour oranges, *Citrus aurantium* trees (total 24 trees, 96 branches) were tagged with plastic strips of various colours for each treatment. Pesticide solutions at their doses; imidacloprid at 0,0063 g ai L$^{-1,}$ , lmbda cyhalothrin at 0.0125 g ai L$^{-1}$ , methomyl at 0.005 g ai L$^{-1}$ , neem oil at 1% and neem seed water extract at 5% were applied to the selected branches with a hand sprayer gun till run off. Two liters (0.5 liter per branch) of each pesticide solution was consumed on 4 branches of each tree. Branches on control trees were sprayed

with two liters of simple water. Residue bioassays of field weathered pesticides were done post-treatment (day 1 to day 33) on mortality of adult female *D. citri* and 2nd instar *C. carnea* larvae. For each test on *D. citri* and *C. carnea*, 5 leaves per treatment were harvested and put individually in Mugger cells as done in the previous experiment. Fifteen *D. citri* females and one 2nd instar *C. carnea* per cell were confined in each cell over exposed surface of the leaves. Controls for each test were run during each bioassay using untreated leaves of the uniform size from control trees.

## RESULTS AND DISCUSSION

*Toxicity of neem to* D. citri *and* C. carnea

Toxicity of neem oil and its extract at various concentrations to *D. citri* adults and *C. carnea* larvae are shown in Figures 1 and 2**.** Neem seed oil @ 1.0% concentration resulted in 80.0% mortality to *D. citri* adults and 100% to *C. carnea* larvae. Neem oil at 0.5% caused 31 and 40% mortality to D. citri and C. carnea. At 0.25% oil concentration, mortality was 12 and 30 % to *D. citri* and *C. carnea* respectively. Neem seed extract (Fig. 2) at 3% resulted in 68.0 and 80% mortality to *D. citri* and *C. carnea*, respectively. A dose of 2% resulted in 58 % mortality to *D. citri* and 60% to *C carnea* while at 1.0% mortality was 40 % for both *D. citri* and *C. carnea*.

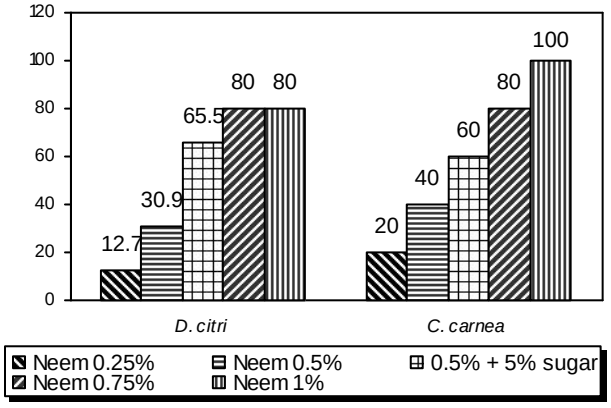


Fig. 1. Percent corrected mortality (48 hrs.) of *D. citri and C. carnea* from various concentrations of neem oil. Control mortality was zero for both *D. citi* and *C. carnea*

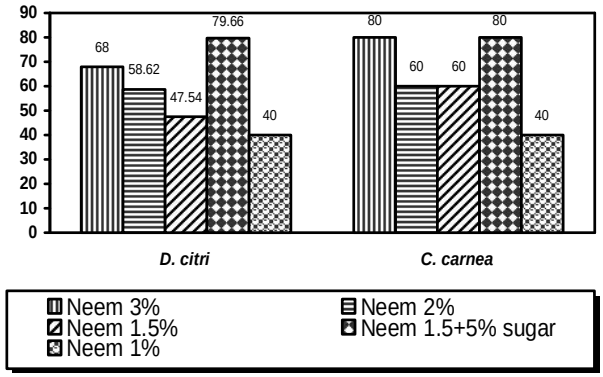


Fig. 2. Percent corrected mortality (48 hrs.) of *D. citri and C. carnea* from various concentrations of neem seed water extract. Control mortality was zero for both *D. citi* and *C. carnea*

A dose of 0.5% neem oil with 5% sugar (Fig.1) resulted in 66% mortality to *D. citri* while the same dose without sugar resulted in 31% mortality to *D. citri* adults. Similarly, 1.5% neem seed water extract with 5% sugar (Fig. 2) resulted in 80% mortality as compared to 47% without sugar. Thus addition of 5% sugar as feeding stimulant to the neem extract and oil improved toxicity of the product and resulted in about 30% (neem oil) to 50% (neem seed extract) increase in mortality.

Studies on addition of sugar as bait to sabadilla alkaloids (Hare and Morse, 1997) for the control of citrus thrips have been investigated and the use of sugar in mixture with plant alkaloids recommended. Other studies on pesticide residues to citrus thrips have also shown improvement in the toxicity of abamectin (Khan and Morse, 1998) Due to similar feeding nature of psyllids and citrus thrips and type of compound (plant alkaloid), we hypothesized that addition of sugar to neem might improve the efficacy of azadirachtin alkaloids too. It is encouraging to see that addition of 5% sugar to neem oil or neem water extract resulted in about 30% increase in mortality with neem oil and 50% with its water extract. The relatively higher mortality seen in the water extract might be due to higher solubility of sugar in the water. Thus addition of sugar to neem oil or its compounds is worth to be tested in future field trials. Sutherland *et al*. (2002) examined the efficacy of crude neem kernel extracts and several commercially available products and

found low contact kill to small rice stinkbug as compared to commercial products. However, they used high concentrations (12.5-50%) of the extract. In our present studies we used low concentration of the neem extract but promising results were obtained through addition of 5% sugar.

During running our bioassays, we experienced unexpected mortality in our laboratory colony of *C. carnea* and we were compelled to use minimum number of *C. carnea.* The reason we observed relatively high mortality of *C. carnea* from neem oil and neem seed water extract was due to this limited number of replicates which resulted in high percent mortality during each bioassay. Studies on three commercial neem preparations sprayed upon eggs, only neem oil have shown to exert a negative impact on the hatching rate of *Coccinella septempunctata* and *Chrysoperla carnea.* Larvae of *C. carnea* proved to be less susceptible, when feeding on neem-sprayed aphids, than *E. balteatus* and *C. septempunctata* Ahmad *et al*. (2003). The findings on the effect of neem oil and neem seed water extract on *C carnea* in the present study may not be conclusive due to limited number of *C. carnea* in each bioassay however, due to zero mortality in the control insects, results are reported here for comparison. We recommend further field and laboratory studies on the evaluation of neem oil and its extract on the *C. carnea*.

*Residual effect of field weathered synthetic and bio pesticides on* D. citri *and* C. carnea

Post treatment mortality of *D. citri* from field weathered residues of pesticides is shown in Figure 3. Methomyl and imidacloprid resulted in 97-100% mortality to *D. citri* up to 4 days post treatment. On day 9, mortality due to methomyl was over 80% while it was 57% to imidacloprid. Decline in mortality was gradual in case of methomyl followed by imidacloprid and lambda cythrin . On day 26, it was 43, 17, and 8% to methomyl, imidacloprid and lambda cyhalothrin respectively. Boina *et al*. (2009) have reported decreased (8 days) adult longevity, nymph survival (12%) fecundity (33%) and fertility (6%) from sub lethal concentration (0.1 µg $mL^{-1}$) of imidacloprid as compared with untreated controls. In a similar study, Gatineau *et al*. (2010) ccompared fenobucarb and imidacloprid treatments with

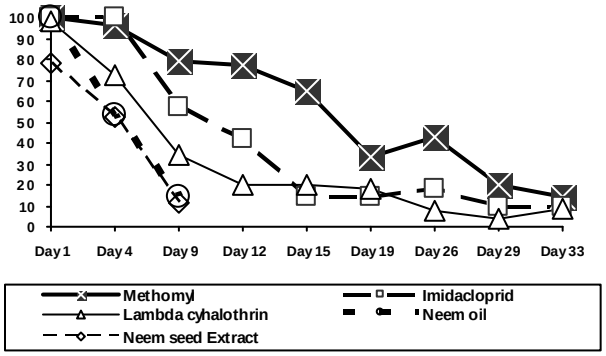


Fig. 3. Percent corrected mortality of *D. citri* on various days post treatment to synthetic and bio pesticides. Control mortality was between 7- 15% for bioassays on *D. citri* and zero for *C. carnea* throughout the experiment.

control, and found both the insecticides effectively reduced *D. citri* populations by killing adults and nymphs and prevented psyllid reproduction in orchards, but neither of the two insecticides totally succeeded in preventing transmission of *Ca. L. asiaticus.* However, due to its long-lasting effect and systemic activity, the imidacloprid treatment provided the best protection against infections, and delayed the spread of the pathogen.

Very sharp decline (from 80% to 20%) in mortality was noticed within a week from neem oil and its extract. Its effect was noticeable only up to 5 days post treatment. Masood and Mamoon-ur-Rashid (2006) reported that neem oil at 1.5% and 2% and neem seed water extracts at 2% and 3% significantly reduced the population of spotted bollworms and American bollworms up to 168 hours (7 days) after treatment. Similarly, neem oil at 2% and neem seed water extract at 3% significantly affected the egg parasitization of *Trichogramma* spp. but did not show any negative effect on the adult emergence of the *Trichogramma* spp. These results are in close association of our studies on *C. carnea* and *D. citri* where its effect was noticeable up to 5 days post treatment. In summary it is concluded that neem oil at 0.5% and its water extract at 1-2% when mixed with sugar resulted in the same control to *D. citri* as its higher doses. Additional benefits to the agro ecosystem might be incurred from application of low doses and safety to the bio control agents.

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